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THE ROLE OF CLINICAL PHARMACY IN THE TREATMENT OF HYPERTENSION IN THE STATE OF KUWAIT

presented by

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SCHOOL OF PHARMACY

BRADFORD UNIVERSITY

2012
THE ROLE OF CLINICAL PHARMACY IN THE TREATMENT OF HYPERTENSION IN THE STATE OF KUWAIT

An analysis of the current treatment of hypertension in Kuwait and the role of the clinical pharmacist in advancing treatment strategies.

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ABSTRACT

The role of clinical pharmacy in the treatment of hypertension in Kuwait.

The thesis investigated nicotine levels and their effects on hypertensive subjects and whether aspirin could be used in the treatment of hypertension to bring about not only an anti-thrombotic effect but reduce the systemic blood pressure especially in those individuals who smoke cigarettes. The study, which also audits the use of aspirin, was conducted in Kuwait and so provides an insight of hypertensive patients very rarely considered in the literature.

The thesis begins in Chapter One with an extensive literature review which analyses the properties and problems that nicotine causes and its ability to cause hypertensive changes along with its multitude of other events. The physiological and pathological problems caused by nicotine are reviewed on the basis of its chemistry and pharmacological properties using a worldwide perspective rather than just focus on Kuwait.

The second Chapter uses extensive analysis of the literature to determine the pharmacological properties of aspirin and its use in cardiovascular disease. The pharmacokinetics and therapeutic effects are presented with emphasis to its inhibitory effects on platelet activation which is central to the development of serious cardiovascular consequences such as stroke and myocardial infarction.

The third Chapter returns to consider the literature in detail and why nicotine has specific effects on the cardiovascular system in terms of receptor stimulation and how aspirin may be able to reduce nicotine’s cardiovascular effects and concludes with the Aims and Objectives of the thesis.

The fourth Chapter investigates urinary nicotine levels in smokers from cigarettes available in Kuwait to indicate the actual levels which could be achieved by smokers in this study. This established that the levels would cause pharmacological effects demonstrating also the effects of passive smoking. The number of cigarettes smoked per day has an unpredictable effect on metabolism and urinary output of nicotine.

The fifth Chapter is the major investigational section of the thesis and considers if aspirin ability to reduce cardiovascular effects, may be useful in terms of diastolic blood pressure and lipid levels in the
blood. The effects were suggestive that aspirin did reduce the blood pressure in hypertensive subjects but was not universal and was limited to those suffering from mild - moderate hypertension. It was determined that aspirin should be sued at the earliest age possible in these patients.

The sixth Chapter involved a large scale trial of the effectiveness of aspirin treatment in hypertensive patients over a one year period in Kuwait. This used ambulatory blood pressure measurements to determine the effectiveness of daytime and nighttime changes in blood pressure in patients with and without aspirin treatment. The overall conclusion was a reduced relative risk of suffering cardiovascular events in mild to moderate hypertension when aspirin (75mg/day) was administered. Specifically in smokers, aspirin lowers the systolic daytime BP and diastolic nighttime BP.

To support this work a comprehensive audit is provided of the use of the current use of aspirin in Kuwait hospitals

**Key words:** Kuwait, hospital pharmacy, treatment strategies for hypertension, aspirin, smoking, nicotine, blood pressure
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DEDICATION

To my father, Mizher Habeeb Al-shammari, my mother, my brothers and sisters, who always encouraged me and prayed for me.

To my wife and Sons Mohammad, Saleh, Kahlid, my daughters Nawal, Dalal, Noura, and Fatema, who pray for me and have been patient with their dad who was busy with studies.

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Chapter One

Literature review part 1 - nicotine, its chemistry, metabolism and pharmacological effects with reference to Kuwait.

1.0 Introduction

This study was carried out in the hospitals of Kuwait where the problems of cigarettes and cardiovascular disease are seen every day. The structure of the health care system has developed over the years and a central feature of the system are the primary health centres (PHC) which patients attend on the first signs of any disease. In Kuwait cardiovascular diseases are a common presenting factor. The role of clinical pharmacy in such settings is in its infancy and as yet has not really made an impact on patient care. The topic of cigarettes causing health problems is one that could be addressed by clinical pharmacy as smoking cessation has been very successful elsewhere in the world especially in the UK where it has become a major role of community pharmacists in promoting and helping people to overcome this addictive practice. The strategy was developed by National Institute for Health and Clinical Excellence (NICE, 2008) and has produced some major reductions in the number of people smoking in the UK. The strategy was entitled ‘Smoking cessation services in primary care, pharmacies, local authorities and workplaces, particularly for manual working groups, pregnant women and hard to reach communities’. The role of the community pharmacist was paramount in implementing this system. The reduction in smoking of course reduces one of the risk factors for
cardiovascular disease which will of course have consequences for both the individual and the health care system of the country, for the purposes of this thesis, Kuwait. So what is the present structure of Kuwait’s health care system?

Kuwait has 77 of these primary health centres (PHCs), staffed by 4 PHC physicians for each 10,000 of the population. Each PHC has a nursing staff which number, between 4 and 6 for each centre. When a patient is first diagnosed with hypertension a file is started which is added to over the years as the disease develops and is treated. This means that every hypertension patient has an accurate and ‘complete’ medical file which documents the progression of their condition and most importantly the drugs which have been prescribed for the condition for example aspirin. In addition to the hypertension treatment some of these PHC centres also have obesity clinics which give advice and possibly treatment for this condition.

All hypertensive patients are provided with treatment cards and educational material to try and encourage the patient to change their lifestyle and behaviour. Such changes are very necessary because the prevalence of cardiovascular disease is the leading cause of death in Kuwait followed by motor vehicle and domestic accidents, neoplasms and diabetes mellitus (Al-Otaibi et al., 2008). This is shown by a newspaper article in the Kuwait Times for the 28th August 2007 (Anon, 2007) which read:

**Heart disease 'main killer' in Kuwait**

KUWAIT: According to a recent study made by the Ministry of Health on the causes of deaths in Kuwait, the three main 'killers' which were detected in 2006 are still the three main killers for 2007 as well.

In reference to the study, heart disease was the prime cause of deaths in Kuwait wherein the study states that 1,497 men and women have died due to heart diseases in 2006 with an increase of 100 compared to 2005.
The second killer was cancer diseases that were responsible for 677 deaths in 2006 including citizens and expatriates.

The third killer, according to the study, was road accidents where 482 were killed in 2006 with an increase of 30 people compared to 2005. Notably, Kuwait has a high rating among countries with high traffic related deaths worldwide.

Hypertension is generally thought to affect 7% of the population with a significant relationship to body mass index (BMI), physical inactivity and smoking. The mortality rate from cardiovascular diseases is 6.6% of total deaths suffered in Kuwait.

It is a truism which is attributed to smokers that once they start smoking they are badly addicted to nicotine (Chapman, 1995). This form of addiction is extremely complex and in order to help such people in their circumstances it is very important to know about the factors which make them addicted, to the pharmacologically active agents contained within tobacco smoke. The pharmacological effects of smoking are multiple but one major consequence is to increase their blood pressure. This increase can cause changes in the cardiovascular system (CVS) and the central nervous system (CNS) both of which have serious consequences (Benowitz, 2008).

The consequences of inhalation of cigarettes or in some cases chewing tobacco products are multiple. A summary of all the reported cardiovascular effects are shown in Table 1 and its effects on other physiological systems are given in Appendix 1.
Table 1. Reported cardiovascular effects of tobacco products in both men and women (Placezek et al., 2004).

<table>
<thead>
<tr>
<th>Cardiovascular diseases</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal aortic aneurysm</td>
<td>&quot;Death more common in cigarette smokers.&quot;</td>
</tr>
<tr>
<td>Atherosclerosis</td>
<td>&quot;Smoking the most common factor to atherosclerotic peripheral vascular disease.&quot;</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>&quot;Smoking is a major cause of cerebrovascular disease (stroke).&quot;</td>
</tr>
<tr>
<td>Coronary heart disease</td>
<td>&quot;Smoking is cause coronary heart disease.&quot;</td>
</tr>
</tbody>
</table>

"The evidence is sufficient to infer a causal relationship between smoking and abdominal aortic aneurysm."

"The evidence is sufficient to infer a causal relationship between smoking and subclinical atherosclerosis."

"The evidence is sufficient to infer a causal relationship between smoking and stroke."

"The evidence is sufficient to infer a causal relationship between smoking and coronary heart disease."
1.01 Literature Review - History of Nicotine.

The use of tobacco began with the native people of North and South America. Early Europeans visiting the New World observed Native Americans smoking cigars and chewing tobacco. When introduced by the Spanish to Europe in the early 1500s, tobacco was thought to hold medicinal properties (Orsinii, 2001). For example, smoke was blown into all openings of the head to treat diseases of the ear, eyes, nose, and mouth. In fact, the tobacco plant was named *Nicotiana tabacum* in honor of Jean Nicot, who promoted it use for its medicinal values. In 1828, two French scientists isolated nicotine as being the active ingredient in tobacco (Jain et al., 2003).

Nicotine, at low to moderate doses, is a central nervous system stimulant. Effects include tremors, an increase in behavioural activity, increased alertness, and facilitation of memory and release of epinephrine from the adrenal glands (Rusted et al., 1994) In addition to these central effects of nicotine, increasing heart rate and blood pressure, it inhibits stomach secretions and stimulates bowel activity. At high doses nicotine produces convulsions and death. Low dose stimulation of the central nervous system is followed by depression at high doses, and death results from failure of respiration due to paralysis of muscles involved in breathing (Einstein et al., 1989).

Chronic use of nicotine leads to changes in the brain and behavior. One of the first changes is tolerance, a decrease in the stimulatory effects of nicotine. Tolerance occurs rapidly and produces some of the unpleasant effects, including dizziness, nausea and vomiting. At low to moderate doses, because nicotine is a cholinergic agonist, it acts by stimulating nicotinic acetylcholine receptors. In fact, as the name suggests, nicotinic
receptors were named after the drug as it was found that they were selectively and potently activated by nicotine (Guillem et al., 2005).

Nicotine can be absorbed through most of the body's membranes. After nicotine is absorbed it is distributed by the blood to a number of sites of pharmacological action. The effects of nicotine are observed rapidly, with its distribution half-life is only eight minutes it can have a rapid and acute effect (Swan, 1998).

Of all the many complex constituents of tobacco it is generally assumed that nicotine has potentially the most significantly pharmacological effects in man. If a consideration is made of nicotine’s effects then perhaps it provides an explanatory basis on which to consider the effect of cigarette use on an individual’s health.

Nicotine is considered to be involved in increasing systemic blood pressure of smokers. By analyzing the levels of nicotine in detail medicine is in a better position to consider the claims made about the various actions of nicotine in the body (Siegmund et al., 2008). A major problem exists, if a person's addiction to the nicotine in cigarettes is impossible to stop, as may be the case for many individuals (Martin, 2008), and this problem centers around the cardiovascular system, see Table 1 (see also Appendix 1 for a more comprehensive range of other effects on the body). One major problem is reported to be the increase in thrombosis risk in people who smoke. This risk could be due to an increase in the development of atherosclerosis, This condition makes the platelets more likely to adhere to the endothelium or for more complex reasons which are grouped together known as the "atherogenic potential" (Weisberg, 2000; Brain, 2010). Consequently, one approach is to target the "adhesiveness" or adherence potential of platelets by using the drug acetylsalicylic acid (aspirin). The use of this drug is central to this thesis.
One of the most important uses of aspirin is the reduction in the ability of platelets to be activated and so prevent platelet aggregation, this effect can have the property of inhibiting aggregates forming (Smith and Willis, 1971) and so prevent the consequences of these aggregates breaking up which may result in the formation of thrombus which block vessels and so cause embolism classically in lungs.

In this thesis it will only be the effects of nicotine in cigarette smoke which are considered. When cigarette smoke is inhaled the constituents of the smoke are capable of being absorbed through the vascularity of any of the mucous membranes of the mouth, nose, pharynx or lung. This drug absorption is reported to be rapid and some studies have found nicotine in the bloodstream within 6-10 seconds of inhalation (Julia, 1991; Lunell et al., 2000). Once in the bloodstream, since it is not plasma bound, its effects can be very rapid and of course widespread throughout the CVS.

One important contributory effect within the CVS is to stimulate nicotinic receptors in the adrenal medulla. This stimulation causes the rapid release and passage into the adrenal blood vessels of both adrenaline and noradrenaline and these agents when in the systemic circulation stimulate both α₁ and β₁ receptors found on the smooth muscle of the heart/blood vessels resulting in both an increase in heart rate and peripheral resistance. This double effect will of course raise the systemic blood pressure by increasing both contributory factors in the fundamental equation concerning blood pressure, namely:

\[ BP = \text{cardiac output} \times \text{peripheral resistance} \]

This equation shows that if one of the factors of cardiac output is heart rate then an increase in β₁ receptors stimulation will change this factor in the equation. The peripheral
resistance is of course due to the tone of the vascular smooth muscle and if this changes in addition to cardiac output the change in blood pressure will be substantial.

1.02 Why should nicotine have such a profound and rapid pharmacological effect?

Nicotine normally makes up about 5% of a tobacco plant by weight. Cigarettes usually contain between 8 to 20 milligrams (mg) of nicotine, the variation being dependent upon the brand. Of this variable quantity, on average, approximately 1 mg is actually absorbed into the body when someone smokes a single cigarette (Gamborne, 2007). Once in the bloodstream, nicotine flows almost immediately to the brain. Although nicotine has multiple actions throughout body (see Table 1 and see Appendix 1), what it produces in the brain is responsible for both the good feelings an individual gets from smoking, as well as the irritability a smoker gets when trying to quit. Within 10 to 15 seconds of inhaling, most smokers are in the "throes of nicotine's effects" (Siegmund et al., 2008).

The plasma half life of nicotine in the human body is, as compared with many other drugs, relatively brief and it has a half-life of about 60 minutes. However due to repetitive smoking of cigarettes the half life effect is more complex than when therapeutic drugs are taken and there is of course the urge to smoke due to the cravings it induces in the individual and so the plasma concentrations can be kept at a higher level than would be the case for therapeutic drugs. During its presence it is of course capable of undergoing metabolism (see Figure 1). This metabolism occurs in the liver and results in metabolites which have a longer half than nicotine but they have no pharmacological action.
Figure 1. Primary pathways of (S)-nicotine metabolism to (S)-cotinine and *cis*- and *trans*-nicotine-1′-*N*-oxide in humans Liver (source: Kyerematen, 1991).

Six hours after smoking of a single cigarette, only about 31 micrograms of the 1 mg of nicotine inhaled remains in body (Grunenberg, 1994). The elimination half-life of nicotine averages 2 hours. The half-life of a drug is very useful in determining the rate of accumulation of that drug in the body but of course is made more complicated due to the repetitive "puffing" and the number of cigarettes smoked both of which modify the time course of decline of plasma levels after the cessation of a period of smoking. Consistent with a half-life of 2 hours, an accumulation of nicotine over 6 to 8 hours during regular smoking and a persistence of significant levels of nicotine in the blood for 6 to 8 hours overnight after cessation of smoking have all been observed (Benowitz, 1996).
Consequently, cigarette smoking represents a situation where the smoker is exposed to significant concentrations and possibly the pharmacological effects of nicotine for 24 hours a day. It has been reported that there is an apparent acute tolerance to nicotine, as determined on the basis of observations of the relationship between venous blood levels and effects (Benowitz, 1996). This may be due to distribution disequilibrium between venous and arterial blood. It has been found that venous blood levels substantially underestimate concentrations of nicotine in arterial blood and at potential sites of action (Henningfield et al., 1993).

True tolerance does, however, develop rapidly, with a half-life of development and regression of about 35 minutes. The complex kinetics of tolerance may be another determinant of cigarette smoking particularly when the smoker smokes his next cigarette (Stolerman et al., 1974).

1.03 The Chemistry of Nicotine.

The chemical formula for nicotine is C\(_{10}\)H\(_{14}\)N\(_2\), with a molecular mass of 162.23. In chemical nomenclature, nicotine is 3-(1-Methyl-2-pyrrolidinyl) pyridine. Nicotine’s structure was deduced by Pinner, and is shown in Figure 2:-

**Figure 2.** Chemical structure of nicotine (Martindale online, 2011).
Nicotine is a hygroscopic, oily liquid that is miscible with water in its base form. As a nitrogenous base, nicotine forms salts with acids that are usually solid and water soluble. Nicotine easily penetrates the skin as shown by the physical data, and its use in nicotine replacement skin applied patches. The free base of nicotine will burn at a temperature below its boiling point, and its vapors will combust at 308 K (35 °C; 95 °F) in air despite a low vapor pressure. Because of this, most of the nicotine is thermally destroyed when a cigarette is smoked; however, enough is inhaled to provide the desired effects. The amount of nicotine inhaled with tobacco smoke is a fraction of the original amount contained in the tobacco leaves.

Nicotine is optically active, having two enantiomeric forms. The naturally-occurring form of nicotine is levorotatory, with \((\alpha)\ D = -166.4^\circ\). The dextrorotatory form, (+)-nicotine, has only one-half the physiological activity of (−)-nicotine. It is therefore weaker in the sense that a higher dose is required to attain the same effects. The salts of (+)-nicotine are usually dextrorotatory (William et al., 1984).

The amount of nicotine absorbed by the body from smoking depends on many factors, including the type of tobacco, whether the smoke is inhaled, and whether a filter is used. For chewing tobacco, dipping tobacco and snuff, which are held in the mouth between the lip and gum, or taken in the nose, the amount released into the body tends to be much greater than with smoked tobacco. Nicotine is metabolized in the liver by cytochrome P450 enzymes (mostly CYP2A6, and also by CYP2B6). A major metabolite is cotinine (see Figure 1). Other primary metabolites include nicotine N'-oxide, nornicotine, nicotine isomethonium ion, 2-hydroxynicotine and nicotine glucuronide. Gluconuration and oxidative metabolism of nicotine to cotinine are both inhibited by menthol, an additive to mentholated cigarettes, thus increasing the half-life of nicotine in vivo. Cotinine is a
byproduct of the metabolism of nicotine which remains in the blood for up to 48 hours. It can therefore be used as an indicator of a person's exposure to nicotine (Donald et al., 2007).

Additionally considering its function within the plant, nicotine is an alkaloid found in the nightshade family of plants (Solanaceae) where it constitutes approximately 0.6–3.0% of the dry weight of tobacco. Biosynthesis takes place in the roots and accumulates in the leaves. It is thought to function as an antiherbivore chemical with particular specificity to insects. Consequently, nicotine was widely used as an insecticide in the past, and currently nicotine analogs such as imidacloprid continue to be widely used for such a purpose (Siegmund et al., 2008).

1.04 Various actions of nicotine.

In low concentrations (an average cigarette yields about 1 mg of absorbed nicotine), the substance acts as a stimulant in mammals and is the main factor responsible for the dependence-forming properties of tobacco smoking. According to the American Heart Association "nicotine addiction has historically been one of the hardest addictions to break". The pharmacological and behavioral characteristics that determine tobacco addiction are similar to those that determine addiction to drugs such as heroin and cocaine. Against this background it is very surprising to read that the nicotine content in cigarettes has actually slowly increased over the years, and one study found that there was an average increase of 1.6% per year between the years of 1998 and 2005. This was found for all major market categories of cigarettes. The detrimental consequences for the average smoker can easily be appreciated.
As nicotine enters the body, it is distributed quickly through the bloodstream and can cross the blood-brain barrier. On average it takes about seven seconds for the substance to reach the brain when inhaled the half life of nicotine in the body is around two hours (Benowitz et al., 1983).

As mentioned above, nicotine acts on the nicotinic acetylcholine receptors, specifically the ganglion type nicotinic receptor and one CNS nicotinic receptor. The former is present in the adrenal medulla and elsewhere, while the latter is present in the central nervous system (CNS). In small concentrations, nicotine increases the activity of these receptors. Nicotine also has effects on a variety of other neurotransmitters through less direct mechanisms (Villegier et al., 2003). By binding to nicotinic acetylcholine receptors, nicotine increases the levels of several neurotransmitters - acting as a sort of "volume control". It is thought that increased levels of dopamine in the reward circuits of the brain are responsible for the euphoria and relaxation and eventual addiction caused by nicotine consumption (Nguyen et al., 2003; Pons et al., 2008). A single amino-acid difference between brain and skeletal muscle acetylcholine receptors explains why nicotine activates the CNS but does not activate skeletal muscles and cause ‘instant death’. Nicotine addiction is therefore a biological oddity and has some strange effects.

A further complication is that tobacco smoke contains the monoamine oxidase inhibitors (MAOIs) harmann, norharman, anabasine, anatabine, and nornicotine. These compounds significantly decrease MAO activity in smokers. MAO enzymes break down monoaminergic neurotransmitters such as dopamine, norepinephrine, and serotonin, so its inhibition will result in the epinephrine release by adrenal medulla having an even greatest effect.
Chronic nicotine exposure via tobacco smoking up-regulates alpha4 beta2 nAChR in cerebellum and brainstem regions but not habenulopeduncular structures Alpha4 beta2 and alpha6beta2 receptors, present in the ventral tegmental area which play a crucial role in mediating the reinforcement effects of nicotine.

Nicotine also activates the sympathetic nervous system, acting via splanchnic nerves to the adrenal medulla, stimulates the release of epinephrine. This stimulates receptors, and causes cell depolarization and an influx of calcium through voltage-gated calcium channels. Calcium triggers the exocytosis of chromaffin granules and thus the release of adrenaline/epinephrine into the bloodstream (Nolley et al., 2006). The release of epinephrine causes an increase in heart rate, blood pressure and respiration, as well as higher blood glucose levels.

Acetylcholine released by preganglionic sympathetic fibers of these nerves acts on nicotinic acetylcholine receptors, causing the release of epinephrine (and norepinephrine) into the bloodstream. Nicotine also has an affinity for melanin-containing tissues due to its precursor function in melanin synthesis or the irreversible binding between melanin and nicotine and this has been suggested to underlie the increased nicotine dependence and lower smoking cessation rates in darker pigmented individuals (King et al., 2009).

Nicotine's mood-altering effects are said to be brought about by complex series of actions. It is both a stimulant and a relaxant. First causing a release of glucose from the liver and adrenaline from the adrenal medulla, it causes stimulation, of smokers and they report feelings of relaxation, sharpness, calmness, and alertness. By reducing the appetite and raising the metabolism, some smokers may lose weight as a consequence.
When a cigarette is smoked, nicotine-rich blood passes from the lungs to the brain within few seconds and immediately stimulates the release of many chemical messengers including acetylcholine, norepinephrine, epinephrine, vasopressin, arginine, dopamine, autocrine agents, and beta-endorphin. This release of neurotransmitters and hormones is responsible for most of nicotine's effects. Nicotine appears to enhance concentration and memory due to the increase of acetylcholine. It also appears to enhance alertness due to the increases of acetylcholine and norepinephrine. Arousal is increased by the increase of norepinephrine. Pain is reduced by the increases of acetylcholine and beta-endorphin (Einstein et al., 1989; Margaret, 2004). Anxiety is reduced by the increase of beta-endorphin. Nicotine also extends the duration of positive effects of dopamine and increases sensitivity in brain reward systems. Most cigarettes (in the smoke inhaled) contain 0.1 to 2.8 milligrams of nicotine.

Research by Stolerman et al., (1995) suggests that, when smokers wish to achieve a stimulating effect, they take short quick puffs, which produce a low level of blood nicotine. This stimulates nerve transmission. When they wish to relax, they take deep puffs, which produce a high level of blood nicotine, which depresses the passage of nerve impulses, producing a mild sedative effect. At low doses, nicotine potently enhances the actions of norepinephrine and dopamine in the brain, causing a drug effect typical of those of psychostimulants. At higher doses, nicotine enhances the effect of serotonin and opiate activity, producing a calming, pain-killing effect. Nicotine is unique in comparison to most other drugs, as its profile changes from stimulant to sedative/pain killer in increasing dosages and use. Technically, nicotine is not significantly addictive, as nicotine administered alone does not produce significant reinforcing properties. However, only after co-administration with an MAOI, such as those found in tobacco, as discussed...
previously, nicotine produces significant behavioural sensitization, a measure of addiction potential. This is similar in effect to amphetamine (Okamoto et al., 1994).

Other research by Bevins and Caggiula, in 1992, showed that nicotine acts on the brain to produce a number of effects. Specifically, its addictive nature has been found to show that nicotine activates reward pathways - the circuitry within the brain that regulates feelings of pleasure and euphoria. Dopamine is one of the key neurotransmitters actively involved in the brain, by increasing the levels of dopamine within the reward circuits in the brain, nicotine acts as a chemical with intense addictive qualities. In many studies it has been shown to be more addictive than cocaine and heroin, though chronic treatment has an opposite effect on reward thresholds. Like other physically addictive drugs, nicotine causes down-regulation of the production of dopamine and other stimulatory neurotransmitters as the brain attempts to compensate for artificial stimulation (Andrew et al., 2004). In addition, the sensitivity of nicotinic acetylcholine receptors decreases. To compensate for this compensatory mechanism, the brain in turn up-regulates the number of receptors, convoluting its regulatory effects with compensatory mechanisms meant to counteract other compensatory mechanisms. The net effect is an increase in reward pathway sensitivity, opposite of other drugs of abuse such as cocaine and heroin, which reduce reward pathway sensitivity. This neuronal brain alteration persists for months after administration ceases (Nestler et al., 1998).

Due to an increase in the reward pathway sensitivity, nicotine withdrawal is said to be relatively mild as compared to alcohol or heroin withdrawal. Man is not unique to its effects as nicotine also has the potential to cause dependence in many animals such as mice where the animals administered nicotine exhibit withdrawal reactions when its administration is stopped (Benowitz et al., 1998). Another study in mice found that
nicotine exposure in adolescent mice retarded the growth of the dopamine system, thus increasing the risk of substance abuse during adolescence (Smith, 2003).

1.05 Toxicity of nicotine.

The LD50 of nicotine is 50 mg/kg for rats and 3 mg/kg for mice. In contrast 40–60 mg (0.5-1.0 mg/kg) can be a lethal dosage for adult humans. Nicotine therefore has a higher toxicity in comparison to many other alkaloids such as cocaine, which has an LD50 of 95.1 mg/kg when administered to mice. It is impossible however to overdose on nicotine through smoking alone, though a person can overdose on nicotine through a combination of nicotine patches, nicotine gum, and/or tobacco smoking at the same time. Applying an extremely high concentration of nicotine onto the skin can result in intoxication or even death since nicotine readily passes into the bloodstream from dermal contact, via the process of transdermal diffusion.

The carcinogenic properties of nicotine by itself as compared to when present in tobacco smoke, have not been evaluated by the IARC (International Agency Research on Cancer), and it has not been assigned to an official carcinogen group. The currently available literature indicates that nicotine, on its own, does not promote the development of cancer in healthy tissue and has no mutagenic properties. However, nicotine and the increased cholinergic activity it causes have been shown to impede apoptosis, which is one of the methods by which the body destroys unwanted cells (programmed cell death). Since apoptosis helps to remove mutated or damaged cells that may eventually become cancerous, the inhibitory actions of nicotine may create a more favorable environment for cancer to develop, though this also remains to be proven (Cohen et al., 2001).
The teratogenic properties of nicotine have not yet been adequately researched, and while the likelihood of birth defects caused by nicotine is believed to be very small or nonexistent, nicotine replacement product manufacturers recommend consultation with a physician before using a nicotine patch or nicotine gum while pregnant or nursing. However women who use nicotine gum (see below) and patches during the early stages of pregnancy face an increased risk of having babies with birth defects, as was reported in a study that looked at 77,000 pregnant women in Denmark (Wisburg et al., 2000). The study found that women who used nicotine-replacement therapy in the first 12 weeks of pregnancy had a 60 percent greater risk of having babies with birth defects, as compared to women who were non-smokers. The controversial findings were published in the authoritative Journal Obstetrics and Gynecology in 2000 (Wisborg et al., 2000).

As an alternative to smoking, many products are available (see Table 2, page 33) and of these perhaps nicotine gum which is the most popular is available in 2-mg or 4-mg ‘doses’. As an alternative, nicotine patches are available which slowly release, 7mg /24 hours (NiQuitin), 7mg /24 hours (Nicopatch), 5mg /16 hours (Nicorette). ‘Smokeless tobacco’ is also available which lacks all the other ingredients in smoked tobacco.

1.06 Metabolism of nicotine and its physiological effects.

Chemically nicotine is a tertiary amine which consists of a pyridine and a pyrrolidine ring (see Figure 1& 2). Nicotine is subjected to a large first pass effect during which 80-90% is metabolized. To a lesser extent, the lung is also able to transform it. The major metabolic product of nicotine, which is also pharmacologically active, is cotinine (see Figure1). Nicotine-1’-N-oxide is also produced but is a minor metabolite. Cotinine is also rapidly metabolized into trans-3’-hydroxy-cotinine which becomes a major metabolite. Consequently, the most commonly found metabolite of nicotine in the blood is trans-3’-
hydroxy-cotinine, which accounts for almost 40% of the nicotine absorbed from the cigarettes, whereas cotinine itself accounts for only about 15% of the quantity of nicotine absorbed in smoking. Surprisingly, the levels of cotinine found in various biological fluids are used to estimate the total absorption of nicotine in tobacco users. The usefulness of cotinine as a quantitative marker of nicotine intake is limited because of an individual’s variability in the percentage conversion of nicotine to cotinine and in the actual rate of elimination of cotinine itself. The alternative measurement, because it accounts for a much greater percentage of nicotine, would be to measure the levels of trans-3'-hydroxycotinine measurement, either alone or in combination with measurement of other metabolites, as it is considered to be a better quantitative indicative of nicotine intake but this has only been carried out in recent times and the literature usually uses the older methods of assessment (Henningfield et al., 2009).

The elimination of the absorbed Nicotine and its metabolites (cotinine and nicotine 1-N-oxide) is usually by passage into the urine. At a pH of 5.5 or less, 23% is excreted unchanged in the urine. At a pH of 8, only 2% of nicotine and its metabolites are excreted in the urine. Consequently, the urinary pH is very important for excretion, as variations in property will modify renal clearance. Nicotine is also secreted into other body fluids, for example, saliva. The saliva containing the secreted nicotine passes into the stomach, combined with the ‘trapping of nicotine’ in the acidic gastric fluid and re-absorption from the small intestine, provides a major route for enteric nicotine recirculation. This recirculation of nicotine also accounts for some of the fluctuations in the final decline phase of nicotine blood levels after intravenous nicotine infusion or cessation of smoking.

The ability of nicotine to enter any body fluid is shown by the finding that nicotine almost freely crosses over the placental membranes and has been found in both the
amniotic fluid and the umbilical cord blood of neonates in mothers who smoke. Nicotine is also found in breast milk (Ilett et al, 2003) and even in the breast fluid of non-lactating women. It has also been found in cervical mucus secretions clearly demonstrating that nicotine does in fact go into every type of body fluid.

As has been explained above, about 80% of nicotine is metabolised to cotinine by liver enzymes. Perhaps surprisingly, nicotine is also metabolized in the lung where it is transformed into cotinine and nicotine oxide. Whatever the source of their production, Cotinine and other metabolites are excreted in urine. Cotinine has a 24-hour half-life, which is greater than the nicotine's half life, so quite usefully it can be tested for to determine whether or not someone has been smoking in the past day or two by screening his or her urine for cotinine. The remaining un-metabolized nicotine is filtered from the blood by kidneys and excreted in the urine.

Nicotine, because it is a normal constituent of plants has also been found in other species and genus of plants. For example, nicotine has also been determined in several frequently consumed vegetables from the family Solanaceae i.e., tomatoes, potatoes, aubergines, and peppers, as well as in some of their processed products. The edible Solanaceae fruits analyzed in a reported study investigation were found to contain relatively consistent amounts of nicotine in the range of 2-7 μg/kg for fresh fruits (Siegmund et al., 1999).

However, these levels are not constant as the nicotine concentrations of the investigated tomato varieties decreased significantly with increasing degree of ripening of the fruits. Another comparison which has been made of nicotine content is between varieties of black tea as compared to green teas. Conflicting results are found in the literature concerning nicotine concentrations in black tea (Sheen, 1988; Davis et al., 1991;
Domino et al., 1993). Concentrations that were found in the dry tea leaves were said by Siegmund et al., (1999) to be surprisingly high, ranging from 163 to 1600 μg kg$^{-1}$. Large variations were found within the types of black tea, whereas the concentrations were more or less consistent within the green teas. For an estimation of the dietary nicotine intake from tea, the nicotine concentration of the tea leaves is less relevant than that in brewed tea. The results showed that nicotine was not efficiently extracted by conventional brewing techniques. Even tea with very high nicotine concentrations in the leaves did not show high amounts in the brewed tea. If detectable, the extraction yield was in a range of 20–25%. Perhaps surprisingly, the nicotine content in tea leaves, although it was found to be highly variable, was sometimes much larger than in the Solanaceae fruits. But since Davis et al. (1991) found nicotine in some tea samples, reporting rather high nicotine concentrations in brewed tea (mean 69 μg L$^{-1}$) and Domino et al. (1993) failed to detect any nicotine in tea the topic stills remains controversial.

On the basis of the observed concentrations and the respective food consumption data for different countries, a distributive analysis of the results suggests that the mean daily dietary nicotine intake for the population of the countries for which consumption data were available was approximately 1.4 μg / day, 2.25 μg / day at the 95th percentile (Tyroller et al., 2000; Zulickenpflug et al., 2006).

The problem is made more difficult to study since different people metabolize nicotine at different rates. Some people even have a genetic defect in the enzymes in their liver which are responsible for quick metabolism of nicotine, whereby the mutant enzyme is much less effective at metabolizing nicotine than the normal variant. If a person has this gene, their blood and brain nicotine levels stay higher for longer after smoking a cigarette. Normally, people keep smoking cigarettes throughout the day to maintain a steady level of
nicotine in their bodies. Nicotine changes how your brain and your body function. The net results are somewhat of a paradox. Nicotine can both invigorate and relax a smoker, depending on how much and how often they smoke. Nicotine initially causes a rapid release of adrenaline, the "fight-or-flight" hormone and this has multiple actions (Zulickenpflug et al., 2006). For example, adrenaline stimulates the use some of glucose which has been stored to be liberated into the blood. This makes sense if we remind our self that the "fight-or-flight" response is meant to help us either defend ourselves from a hungry predator or escape from a dangerous situation. Running away or fighting off an aggressor requires plenty of energy to supply the skeletal muscles during their stimulation.

Nicotine itself may also block the release of the hormone insulin. Since insulin helps the cells to take up excess glucose from blood, the nicotine can make people somewhat hyperglycemic, having more sugar than usual in their blood. Some people think that nicotine also reduces their appetite so that they eat less. This hyperglycemia could be one possible explanation, as nicotine blocks the release of insulin which is responsible for the breakdown of glucose fulfilling the energy requirement of body. This down-regulation of the hormones results in excess concentration of glucose in blood (Stefan et al., 2000).

Nicotine is also considered to increase the catabolic metabolic rate too which means to some extent it is also involved in the breakdown of stored glucose which result in burning of more calories, which over time will cause a reduction in bodyweight. This aspect of smoking may result in ‘the burning of calories’ but this weight reduction is not considered a health benefit of smoking as physical exercise can achieve more, with far less risk to the individual. Nicotine also has an effect on increasing the level of the LDL cholesterol, the result of which causes an accumulation of lipid in the arteries and the
development of atherosclerosis. This pathological change is one of the main contributory factors for heart attacks or a stroke (Zulickenpflug et al., 2006).

The brain is the key player in nicotine's action. Like a computer, our brain processes, stores and uses information. In a computer, information travels in the form of electricity moving through wires; information transfer is a binary process, with switches being either "on" or "off." In our brain, neurons are the cells that transfer and integrate information. Each neuron has thousands of inputs from other neurons throughout the brain. Each of these signals is included in the calculation of whether or not the neuron will pass the signal it receives on to other neurons in the pathway. While signals are conducted through individual neurons as electric current, communication between neurons is mediated by chemical messengers, called neurotransmitters. Neurotransmitters traverse the physical space between two neurons and bind to special protein receptors on the postsynaptic cell. Once bound, these receptors set in motion physiological changes within the neuron that allow it to send the signal on ‘down the line’ (Stefan et al., 2000; Xinan et al., 2009).

Each neurotransmitter has its own specific family of receptors. Nicotine acts by binding to or antagonizing a subset of receptors that bind the neurotransmitter acetylcholine. Acetylcholine is the neurotransmitter that, depending on what region of the brain a neuron is in, stimulates a variety of physiological events such as muscle in the heart and respiration and plays a major role in learning and memory.

Due to its stimulatory properties just like acetylcholine, nicotine can lead to a burst of receptor activity in the CNS. However, unlike acetylcholine, nicotine stimulation is not regulated by the normal feedback regulatory mechanisms. While neurons typically release small amounts of acetylcholine in a regulated manner, nicotine can simultaneously activate
cholinergic neurons (which mainly use acetylcholine to communicate to other neurons) in many different regions throughout the brain this stimulation leads to an increase in the release of acetylcholine from the neurons, leading to heightened activity in cholinergic pathways throughout the brain. This cholinergic activity is the ‘wake-up call’ that many smokers use to re-energize themselves at the beginning and throughout the day. Through these pathways, nicotine can improve the reaction time and a person’s ability to be more attentive to a task in hand and so makes an individual feel they can work better.

Stimulation of cholinergic neurons promotes the release of the neurotransmitter dopamine in the reward pathways of our brain. This neural circuitry is supposed to reinforce behaviors that are essential to our survival, like eating when we are hungry. Stimulating neurons in these areas of the brain brings on pleasant, happy feelings that encourage us to do these things again and again. When drugs like cocaine or nicotine activate the reward pathways, it reinforces a person’s desire to use them again because it produces feelings of ‘peace and happiness’ (Xinan et al., 2009). The release of glutamate, by nicotine which is a neurotransmitter involved in learning and memory, enhances the connections between sets of neurons. These stronger connections may be the physical basis of what we know as memory. When we use nicotine, glutamate release may create a memory loop of the good feelings we get and further drive the desire to use nicotine.

Nicotine also increases the level of other neurotransmitters and chemicals that modulate how our brain works. For example, our brain makes more endorphins in response to nicotine. Endorphins are peptides that are often called the body's ‘natural pain killer’. The chemical structure of endorphins in terms of receptor stimulation is very similar to that of the classical opiate analgesics such as morphine and codeine. Endorphins can lead to feelings of euphoria (Stevenson, 1999).
As has been described previously, nicotine is a highly addictive substance. The effects on our body can last between 40 minutes to a couple of hours (Zulickenpflug et al., 2006). It leads smokers to consume more and more cigarettes or other tobacco products so as to reach the same degree of relaxation and euphoric state, thus explaining why a smoker graduates from a 3 cigarettes per day to smoking one or two packets a day. Quitting smoking is easier said than done, as only 10% of those who attempted to become free of the habit have succeeded. (http://www.stopsmokingtoday.com/dyn/127/Nicotine-Withdrawal.html)

Eventually many return to their old bad habit. For those who have succeeded it is said to take one to two months to ‘clean out’ the ‘systems’ from nicotine addiction. But this time frame is the most excruciating for nicotine addicts. Since our system is adapted to having nicotine on a regular basis, sudden withdrawal or "cold turkey" causes our body to function differently in the absence of the drug. Quitters experience extreme craving for nicotine, irritability, anxiety and depression (Tyrolier et al., 2000). After one month, these physiological cravings are said to subside significantly. After a further month, all the nicotine ‘residues’ are stated to have been completely eliminated. But for many smokers, every day without a single cigarette is a struggle and many who try to quit even after just one week, return to their smoking habit. This just shows the irresistible and addictive pull of nicotine. (See Figure 3) (Shaffer et al., 2010).
Nicotine mimics the effects of the neurotransmitter acetylcholine acting primarily on the autonomic nervous system. For example in the adrenal gland there are nicotinic receptors which on stimulation liberate adrenaline and noradrenaline. These catecholamines of course have many effects, one of which is to stimulate adrenergic receptors on the smooth muscles of the vascular system, and due to this can exert a hypertensive effect and in extremes this can be dangerous. Nicotine can also cause respiratory failure and paralysis at doses of less than 50 mg and smaller amounts can cause nausea, dizziness, lowered blood pressure, and heart palpitations (Herraiz & Chaparro, 2005). A smoker inhales approximately 3mg of nicotine per cigarette. Even this small amount constricts blood vessels, increases the heart rate, and acts on the central nervous system. It also confers a feeling of well-being and alertness on the smoker.

People who use tobacco products in any form will develop a physiological addiction to nicotine, (Fowler et al., 1998) as due to the increased flow of dopamine, a neurotransmitter, nicotine creates pleasurable feelings in the user, along with a desire to maintain them. When nicotine levels in the blood drop, smokers will develop withdrawal symptoms within 48 to 72 hours after the last cigarette. The symptoms are so unpleasant that most smokers will resume smoking in an effort to raise nicotine levels in the blood to

Figure 3. Diagrammatic representation of how acetylcholine produces its effects by activation the cholinergic receptors on postsynaptic neurons (source- Shaffer et al., 2010).
the point where the symptoms subside which is the basis of nicotine replacement therapy used in patches, lozenges and chewing gum in smoking cessation (see Table 2).

**Table 2.** Anti-smoking preparations available in the UK, the type of formulation and nicotine content.

<table>
<thead>
<tr>
<th>Trade name</th>
<th>Formulation</th>
<th>Nicotine content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicopass</td>
<td>Lozenges</td>
<td>1.5mg per lozenge</td>
</tr>
<tr>
<td>Nicopatch</td>
<td>Patch</td>
<td>7mg, 14mg and 21mg 'patches’</td>
</tr>
<tr>
<td>Nicorette</td>
<td>Microtab*</td>
<td>2mg</td>
</tr>
<tr>
<td></td>
<td>Chewing gum</td>
<td>2mg and 4mg</td>
</tr>
<tr>
<td></td>
<td>Patch</td>
<td>5mg, 10mg and 15mg 'patches’</td>
</tr>
<tr>
<td></td>
<td>Invis patches</td>
<td>10mg, 15mg and 25mg 'patches’</td>
</tr>
<tr>
<td></td>
<td>Nasal spray</td>
<td>500µg/metered dose spray</td>
</tr>
<tr>
<td></td>
<td>Inhalator</td>
<td>10mg cartridge in inhaler</td>
</tr>
<tr>
<td>Nicotinell</td>
<td>Chewing gum</td>
<td>2mg and 4mg</td>
</tr>
<tr>
<td></td>
<td>Mint lozenge</td>
<td>1mg and 2mg</td>
</tr>
<tr>
<td></td>
<td>TTS patches</td>
<td>10mg, 20mg and 30mg 'patches’</td>
</tr>
<tr>
<td>NiQuitin</td>
<td>Chewing gum</td>
<td>2mg and 4mg</td>
</tr>
<tr>
<td></td>
<td>Lozenges</td>
<td>2mg and 4mg</td>
</tr>
<tr>
<td></td>
<td>Patches</td>
<td>7mg, 14mg and 21mg</td>
</tr>
</tbody>
</table>

*sublingual

Finally, nicotine itself is not considered carcinogenic but it may also enhance tumour growth caused by carcinogens so acting as a co-carcinogen. However, it is likely that it contributes to the increased incidence of heart disease in smokers and this will be considered in the next section.
1.07 Smoking and the increase of high blood pressure (hypertension).

There are several ways, it is thought, as to how nicotine in cigarettes can lead to hypertension:

1. Nicotine as a constituent of cigarettes or tobacco has the tendency to constrict blood vessels and arteries, which can encourage the formation of plaque and so atherosclerosis. This progressively builds up leading to an increased potential for eventually a fissure and the atheroma and so promote blood clots on the surface of the vessel.

2. As nicotine passes through the cardiovascular system, the endothelial linings of the blood vessels are damaged and these hasten the development, within the arterial walls, of atherosclerosis.

3. As tobacco smoke is inhaled, the smoker also inhales one of the many combustion products, namely carbon monoxide which because it irreversibly binds to hemoglobin decreases oxygen the carrying capacity of the blood and so this decreases the oxygen supply to the heart, brain and every other vital organ (Pons et al., 2008).

4. Nicotine has the ability to stimulate the production of the epinephrine also known as adrenaline, which acts as a vasoconstrictor.

Cigarettes are said to contain 4000 chemical compounds and 400 toxic substances the exact physiological consequences of which remain unknown.
The damaging products of smoking are multiple but the major ones are considered to be:

- Tar, which is a potential carcinogen (Wynder et al., 1953)
- Nicotine, which is considered addictive and has the capacity to increase cholesterol levels and so encourage atherosclerosis.
- Carbon monoxide, which forms carboxyhaemoglobin which decreases the effective red cell mass for oxygen transport around the body.
- Other combustion components e.g. free radicals which can cause chronic obstructive pulmonary disorder due to irritation, inflammation and tissue destruction

Based on medical studies, it is said that smoking tobacco or cigarettes can cause our blood pressure level to increase by 5 to 10mm Hg during the day. It is actually the systolic blood pressure that is largely affected since the systolic blood pressure increases by at least 20mm Hg (Chobahian et al., 2003).

Persons already afflicted with hypertension - for example essential hypertension which is the commonest form - are greatly advised to quit smoking as it can cause further arteriosclerosis. Hardening of the arteries and can lead to an even greater potential for heart attacks. However the situation in complex as there is no direct link between smoking and the increase of high blood pressure, rather an association between hypertension disorders to cardio vascular diseases (Nguyen et al., 2003). Consequently, nicotine, together with cholesterol and other fat deposits contributes to the hardening of the arteries, which as the person ages combined with poor blood circulation leads to high blood pressure, and an increased risk of blood clots stroke and heart attacks. It is actually the
systolic blood pressure that is largely affected since the systolic blood pressure count can increase by up to 20 mm Hg (Thomas et al., 2003).

Consequently people already afflicted with hypertension are greatly advised to quit smoking as it can cause further hardening of the arteries and can lead to heart attacks and although there is no direct link between smoking and the increase of high blood pressure, it is known that a blood pressure rise can result in a secondary hypertensive disorder(s) in a range of cardiovascular diseases (Placezek et al., 2004). This combination along with elevated levels of cholesterol and other fats can bring about deposits in the sub-endothelial structures of the blood vessels which contributes to the arterial disease, which progressively develops through the years, leading to poor blood circulation, induction of a higher risk of the formation of blood clots, which may lead to both strokes and heart attacks.

The consequences of cigarette smoking as the most important proven cause of premature death have been shown in the United States. About 35 percent of all smokers die prematurely of a smoking-related disease. Smokers are three times more likely to die of cancer than nonsmokers and it has been found that men who smoke are 10 times as likely to experience sudden death from cardiac arrest as are nonsmokers.

Among women smokers, the mortality rate is five times greater. Furthermore, the combination of cigarette smoking and hypertension increases the risk of stroke and heart attack considerably (Placezek et al, 2004; King et al., 2009).

It has already been discussed that by puffing a cigarette, nicotine immediately enters the bloodstream and reaches the brain within six seconds, where more than 15 percent of it is absorbed. When nicotine reaches the brain, it signals the adrenal glands to
release noradrenaline and adrenaline. This direct stimulation of the adrenal gland, because of the very rapid passage of the agent from the adrenal medulla into the blood vessels increases both the systolic and diastolic pressure. Nicotine is absorbed in the mouth as well as the lungs. Therefore, even if we don't inhale, large amounts of nicotine can still potentially enter the bloodstream. Here the heart rate increases and so, as it pumps more blood, the arteries have to compensate for the blood flow through the body and so this elevates the blood pressure and contributes to blood vessel atherosclerosis (Therapeutic Letters, 1997).

It has further been discussed that in addition to directly causing an increase in heart and vessel activity, cigarette smoking also contributes to the advance of atherosclerosis, hypertension's deadly partner, as nicotine is known to raise the amount of fats and cholesterol circulating in the bloodstream by releasing some of the stored body fat (See appendix 1 and table 1) and this increase in circulating fats also contributes to the process that forms plaque within the artery walls. Cigarette smoking also causes chemical changes in the blood itself, causing it to become more viscous, or sticky, which encourages the chance for the formation of large blood clots, in the process usually known as thrombosis. Emboli from these clots can cause strokes, pulmonary embolism and heart attacks (Grobbee et al., 2002)

Cigarette smoking may also increase the risk of developing renovascular hypertension, a form of secondary hypertension involving the kidneys. Tobacco smoke also contains cadmium, a substance known to contribute directly to the development of high blood pressure possibly via an effect on the kidneys, because when cadmium is inhaled through smoking, it tends to be retained in the kidneys, and so damaging them. This damage can further increase the hypertensive state (Garovic and Textor, 2005).
1.08 Summary of Nicotine and its effects on the cardiovascular system.

Nicotine has been speculated to increase the risk of blood clots by increasing the levels of plasminogen activator inhibitor-1, though this has not been actually proven. However, what has been more strongly suggested is that the plasma fibrinogen levels are elevated in smokers and are further elevated during acute COPD exacerbation (Antonio et al., 2007). Also, Factor XIII, which stabilizes fibrin clots, is increased in smokers. But neither of the two previous effects have been absolutely proven to be caused by nicotine. Whatever the mechanism(s) of inducing an increased risk of blood clots forming the consequences are without question - the clot will at best reduce blood flow in the worst case arrest it and then the tissue supplied by the vasculature, loses its source of oxygen and nutrients and dies within minutes (Green et al., 2000).

In the peripheral circulation, arteries going to the extremities are also highly susceptible to the vasoconstrictor effects of nicotine as well as the increased risk of clots and clogging (UPI, 2001).

1.09 Basis of the thesis (1).

After a consideration of the pharmacological effects of nicotine and its diverse properties in the body and its complicated effects on the blood coagulation system it is now appropriate to consider the potential of using aspirin to study if some of the nicotinic effects can be modified in the presence of this drug. This is a central part of the thesis as a study to determine the interaction between nicotine and aspirin has not been the focus of much investigational interest in either the wider scientific community or in Kuwait.

So it is appropriate to consider next the use of aspirin for the use in cardiovascular diseases such as hypertension and this will form the basis of the next chapter.
Chapter Two

Literature review part 2 - Aspirin’s use in cardiovascular medicine

2.01 Literature review - Aspirin’s use in various diseases.

Aspirin is widely used drug for both its ant-inflammatory and analgesic properties. In reality it has multi aspects to its usage (See Table 3).

Table 3. Aspirin’s use against various diseases.

<table>
<thead>
<tr>
<th>Clinical Outcomes</th>
<th>75 to 81 mg (n=1224)</th>
<th>325 mg (n=2877)</th>
<th>P Value</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cause mortality</td>
<td>9.9</td>
<td>10.7</td>
<td>0.566</td>
<td>(Trutte et al., 1964)</td>
</tr>
<tr>
<td>Cardiovascular composite</td>
<td>11.0</td>
<td>11.7</td>
<td>0.186</td>
<td>(Spranger et al., 1989)</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>6.5</td>
<td>7.3</td>
<td>0.005</td>
<td>(National Health Institute, 1980)</td>
</tr>
<tr>
<td>Stroke</td>
<td>4.1</td>
<td>4.5</td>
<td>0.818</td>
<td>(Escolar et al., 1986)</td>
</tr>
<tr>
<td>Cardiovascular mortality</td>
<td>4.0</td>
<td>4.3</td>
<td>0.876</td>
<td>(Ridker et al., 2005)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>14</td>
<td>16</td>
<td>0.334</td>
<td>(Eidelman et al., 2002)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>60</td>
<td>63</td>
<td>0.001</td>
<td>(Groeneveld et al., 2003)</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>132</td>
<td>133</td>
<td>0.022</td>
<td>(Williams et al., 2003)</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>72</td>
<td>73</td>
<td>0.001</td>
<td>(Stafford, 2000)</td>
</tr>
<tr>
<td>Angina</td>
<td>60</td>
<td>57</td>
<td>0.001</td>
<td>(Harpaz et al., 1996)</td>
</tr>
<tr>
<td>Percutaneous revascularization</td>
<td>17</td>
<td>21</td>
<td>0.001</td>
<td>(Higashi et al., 2004)</td>
</tr>
<tr>
<td>Peripheral artery disease (PAD)</td>
<td>8.1</td>
<td>9.3</td>
<td>0.301</td>
<td>(Baigent et al., 2008)</td>
</tr>
<tr>
<td>Congestive heart failure (CHV)</td>
<td>5.3</td>
<td>5.8</td>
<td>0.250</td>
<td>(Quinn et al., 2004)</td>
</tr>
</tbody>
</table>
Aspirin was the first discovered member of the class of drugs which were later to be called the non-steroidal anti-inflammatory drugs (NSAIDs). Not all NSAIDs are salicylates, although they all have similar pharmacological effects as they inhibit the enzyme cyclo-oxygenase as their primary mechanism of action. Today, aspirin is one of the most widely used medications in the world, with an estimated 40,000 metric tons of it being consumed each year (Michael et al., 2007).

Aspirin is also more correctly known as acetylsalicylic acid (ASA) as it is a derivative of salicylic acid. It is often used as an analgesic to relieve minor aches and pains, as an antipyretic to reduce fever and as an anti-inflammatory medication. Its structural formula is shown below (Figure 4).

![Aspirin structural formula](source - Jinno Laboratory, 1996).

Aspirin causes its antiplatelet effect by inhibiting the production of thromboxane A2, by the irreversible acetylation of the enzyme cyclo-oxygenase, as shown in Figure 5, below.

Thromboxane production under normal circumstances is highly controlled as it is a very potent pro-aggregatory agent which binds platelets together to repair endothelial or more severe damage to blood vessels. Consequently, when ASA is used as a long-term treatment at a low dose, a dose below that usually used for analgesia, the low dose
selectively block the production of thromboxane and so prevents heart attacks, strokes, and blood clot formation in people at high risk for developing these platelets + blood coagulation factor complexes. It has also been established that low doses of ASA may be given immediately after a heart attack to reduce the risk of another heart attack or of the death of cardiac tissue (Danchin et al., 2006).

**Figure 5.** Flow diagram for the generation of Thromboxane (TxA2) from arachidonic acid and its subsequent metabolism to TxB2. Aspirin blocks an enzyme called cyclooxygenase (shown on the diagram as COX) which is involved with the ring closure and addition of oxygen to arachidonic acid converting to prostaglandins (Rapoport, 2008).

Aspirin is the salicylate ester of acetic acid. The compound occurs as a white, crystalline powder or tabular or needle-like crystals. It is a weak acid with a pKa of 3.5.
Aspirin is slightly soluble in water and is freely soluble in alcohol and each gram of aspirin contains approximately 760 mg of salicylate (Brik, 2004).

![Chemical structure of aspirin](image)

**Figure 6.** The acid dissociation reaction of aspirin. (Jinno Laboratory, 1996).

The synthesis of aspirin is classified as an esterification reaction. Salicylic acid is treated with acetic anhydride, an acid derivative, causing a chemical reaction that turns salicylic acid's phenol group into an acetyl group, \((R-OH \rightarrow R-OCOCH_3)\). This process yields aspirin and acetic acid, which is considered a byproduct of this reaction. Small amounts of sulfuric acid (and occasionally phosphoric acid) are almost always used as a catalyst. This method is so straightforward that it is commonly employed in undergraduate teaching labs for the purposes of showing medicinal substance synthesis. The mechanism of synthesis is shown in figure 7.

![Synthesis of aspirin via esterification](image)

**Figure 7.** Synthesis of aspirin via esterification (reaction between alcohol and carboxylic acid for formation of ester) method (Paterson et al., 2008).

Formulations containing high concentrations of aspirin often smell like vinegar. This is because aspirin can decompose through hydrolysis in moist conditions, yielding
salicylic acid and acetic acid (Rapoport, 2008). Aspirin is stable in dry air, but readily hydrolyzes to acetate and salicylate when exposed to water or moist air. It will then exude this strong vinegar-like odor. The addition of heat will speed the rate of hydrolysis. In aqueous solutions, aspirin is most stable at pH of 2-3 and least stable at pH’s below 2 or greater than 8 (Brik, 2004).

As an effective treatment, low dose aspirin has been prescribed by doctors since the 1890’s. Although it has practical benefits, some are concerned about its long term use, especially if used in a person who has been diagnosed with high blood pressure or other cardiovascular disease(s). To receive the drug they have to be informed that they will need to take it throughout their lifetime to maintain control of their symptoms (Awtry et al., 2000).

2.02 A brief history of aspirin and its use to prevent platelet aggregation.

Why is low dose aspirin 75mg or 100mg rather than the normal 300mg dose preferred? Where does the use of this low dose actually come from? How was it discovered? A brief look at the history of Aspirin will give us a sense of appreciation for advantages of aspirin, which is balanced by also mentioning its disadvantages.

A daily aspirin dose of 75gm is an example of a normal prescribed amount for low dose aspirin therapy. Cyclo-oxygenase (COX), which catalyzes the production of prostaglandins is the target of the drug. However prostaglandins are molecules produced normally in cells to carry out many physiological effects. One of these functions is to make the lining of the stomach thick enough to stop stomach acid from irritating and penetrating through and so prevent ulcers from forming in the stomach wall. Prostaglandins have many other basic functions include inhibiting blood clotting, the 6 oxo derivative, known
as prostacyclin, is a very powerful vasodilator and a platelet anti-aggregatory agent and has a role in kidney function. They are also involved with bronchiolar airway tone and may reduce inflammation in some inflammatory states and in one very special case bring about the closure of the ductus arteriosus.

This thesis is however concerned with the role this drug may play in cardiovascular disease. For example when repairing tissue injury, it is necessary for a fresh blood supply to get to the injured area. Prostaglandins facilitate this process by dilating these blood vessels and at the same time they may encourage an inhibition of blood clotting (prostacyclin), to a degree which is enough to keep the blood flowing into the damaged area. Inflammation is also reduced by the action of this type of prostaglandin, which works in a similar sense, but can produce mixed reactions as the body needs it to modulate infections and other problems (Chandrasekharan et al., 2002).

However with more serious injuries, the body chemistry changes so that the prostaglandin E comes into action at the injured site, as in the case of an open wound, where the blood needs to form a blood clot. To do this the prostaglandins and thromboxane causes platelets to stick together by facilitating adhesion to the blood vessel wall and then other prostaglandins induced aggregation swelling and as a consequence inflammation develops and progressively increases. This can be dangerous if we have an imbalance and the prostaglandins E are in excess which has been linked to one of the causes of high blood pressure (Thomas et al., 2003).

Aspirin is prescribed at a low dose for its effectiveness in "thinning" the blood and stopping clotting from excessive thromboxane production and prostaglandin E, which can cause blood clots to block vital oxygen supply to the tissues and if this is in the heart, then this will lead to a heart attack. In addition, low dose aspirin can be prescribed for people
suffering from high blood pressure, as well as other cardiovascular problems, for example post myocardial infarction to decrease the risk of future platelet activation and clotting.

2.03 Mechanism of action of aspirin.

Correlation between inhibitory and anti-inflammatory activity of aspirin.

For many decades after its discovery the real mechanism of action of aspirin like that of many other anti-inflammatory drugs remained unknown. It was known however that as compared to the anti-inflammatory effects which had been produced by steroids, these drugs were different in terms of potency and side effects. They were also different in terms of the analgesia they produced which was different from those produced by opiates. Even today aspirin like drugs have not the same analgesic action as opiates such as morphine. This did not really matter as these aspirin like analgesic drugs have been found to be very effective for low or moderate severity clinical pain but are not, and probably never will be able to be, effective against high intensity pain such as postoperative pain, osteoarthritis, rheumatoid arthritis, ankylosing spondylitis and for some types of the more severe types of headache (Beaver, 1965).

Aspirin like drugs were found to be effective in experimental models involving the induction of a previous inflammatory state and blocked the delayed stretching response induced with an intraperitoneal injection of phenylbenzo-quinone or dilute acetic acid in mice. Aspirin-like drugs were not found effective against nociception (mechanical, thermal and chemical stimuli) which are detected by nerve endings called nociceptors, which are found in the skin and on internal surfaces such as the periosteum or joint surfaces. The concentration of nociceptors varies throughout the body, mostly found in the skin and less so in deep internal surfaces. All nociceptors are free nerve endings that have their cell bodies outside the spinal column in the dorsal root ganglia and are named according to
their appearance at their sensory ends. Nociceptors have a certain stimulatory threshold, that is, they require a minimum level of stimuli before they trigger a signal. Once this threshold is reached a signal is passed along the axon of the nerve into the spinal cord of short duration which may be induced by stimuli such as pinching or stimulating the tail or toes of mouse, rat or guinea pig (Vane, 1964).

It has also been suggested by Guzman et al. (1964) and Lim et al. (1964) how the peripheral analgesic activity of aspirin-like drugs may have worked. Many biochemical effects of aspirin-like drugs had been previously extensively documented but theories based on these effects have now been discounted. However such theories included the uncoupling of oxidative phosphorylation and the induced inhibition of dehydrogenase enzymes, particularly those dependent on pyridine nucleotides (Guzman et al. 1964; Lim et al., 1964).

Some aminotransferases and decarboxylases were also found to be inhibited by these aspirin-like drugs. The inhibition of these enzymes hampered many important body functions as several of these enzymes involved in protein and RNA biosynthesis. Consequently it was through such mechanism the side effects of the drugs were thought to exert themselves.

One major theory was that because the drug could affect Oxidative phosphorylation which is a metabolic pathway that uses energy released by the oxidation of nutrients to produce adenosine triphosphate (ATP) it could bring about its side effects this way. During oxidative phosphorylation, electrons are transferred from electron donors to electron acceptors such as oxygen, in redox reactions. These redox reactions release energy, which is used to form ATP. All of these inhibitory actions of aspirin were for some time the basis of the explanation of the therapeutic action of aspirin. There was a problem with such theories because the inhibitions of many important enzymes were all observed only when
high concentration of these drugs had been taken. Due to this problem of specificity the
correlation between the ability of these drugs to inhibit particular enzymes and their anti-
inflammatory activities was a real problem in explaining their mode of action. The most
serious problem of these theories was that they could not provide valid reason why
inhibition of any of these enzymes should produce the anti-inflammatory, analgesic and
antipyretic effects of aspirin. In other words, if aspirin was involved in inhibition of many
enzymes then how could these drugs show a relatively potent anti-inflammatory action. By
controlling the potency of aspirin by lowering the dose, many of its side effects could be
reduced, because many of these enzymes were only inhibited when there was a high
concentration of salicylates (Whitehouse, 1965).

**Aspirin inhibits prostaglandin production.**

Aspirin may also cause an anaphylactic-like reaction when people are first exposed
to the drug. These reactions are not the same as the immune system response that occurs
with "true" anaphylaxis. However, the symptoms, risk for complications, and treatment are
the same for both types of reactions. It means that histamine and SRS-A (slow reacting
substance of anaphylaxis) are involved with this effect of aspirin as are perhaps, other
possible mediators or stimulators of anaphylaxis (Vane, 1971).

Anaphylaxis is a severe, whole-body allergic reaction to a ‘chemical’ that has
become an allergen. After being exposed to a substance such as bee sting venom, the
person’s immune system becomes sensitized to it. On a later exposure to that allergen, an
allergic reaction may occur. This reaction happens very quickly after the exposure, is
severe, and involves the whole body. Tissues in different parts of the body release
histamine and slow-reacting substance (SRS-A), which is a mixture of the leukotrienes -
LTC4, LTD4 and LTE4. Mast cells can secrete these agents during the anaphylactic
reaction, all of which are capable of inducing inflammation. It can be produced by basophiles. These leukotrienes exhibit a prolonged, slow contraction of smooth muscle and they have a major bronchoconstrictor role in asthma, a view suggested three decades before by Brocklehurst (1962).

As compared to histamine, the leukotrienes are 5000 times more potent and have a slower onset but longer duration of action. This causes the airways to constrict and leads to other symptoms. Leukotriene substances were detected as SRS-A by Piper and Vane who conducted their studies on isolated lungs perfuse with Krebs solution from sensitized guinea pigs (Piper and Vane, 1969). During the detection of these substances, Piper and Vane used the technique which was described as a continuous bioassay with a cascade bioassay system which was developed by Vane (1964). He used this system with blood or artificial salt solution to demonstrate the nature of the products released in anaphylaxis and also the speed of their release (Vane, 1964).

During anaphylaxis in addition to histamine and slow-release substance (SRS-A), being produced there were found to be other substances produced such as prostaglandins which were mainly PGE₂ and some PGF₂α.

It was also observed that another material, known originally as RCS (rabbit aorta contracting substances) was produced which were also known as ephemeral substances (short lived or transitory substances), and which were identified in the cascade bioassay assay tissues. It has also found that RCS had a half-life of about 20 minutes in lung perfusate only when it was cooled to few degrees above its freezing point. At normal laboratory temperatures the RCS was very short lived – a matter of seconds – and even under ideal controlled conditions it decomposed within 20 minutes, which was then called an ephemeral substance. This RCS was chemically identified and characterized by Hamberg et al., (1975) as Thromboxane. This new molecule had never previously been
found and which because it came from platelets, alternatively known as thrombocytes, and had within the molecule an oxane ring, which was given the name, Thromboxane A$_2$. This was the agent which had been present in RCS and it when its synthesis could be prevented with aspirin then this provided the first foundation of establishing the relationship between aspirin and prostaglandin system.

In some further research involving RCS, by Palmer et al., (1973) and Hamburg et al., (1975) they found that RCS was also released by the peptide, bradykinin. This suggested that aspirin has the ability to minimize, some of the effects of bradykinin by blocking off or inhibiting the release of RCS (Hamberg et al., 1975). The experimental evidences presented by Palmer et al., (1973) and Hamburg et al., (1975) confirmed that the release of RCS from isolated guinea pig lungs, in which anaphylaxis had been induced by sensitizing the animal to egg albumen, RCS production was blocked by aspirin. These experiments also indicated that when aspirin inhibited the releasing of RCS, it also caused the reduction in production of prostaglandins (Crutchely et al., 1977).

The amount of prostaglandin which can be decreased as a result of its inhibition by aspirin, is rather complex. The enzyme cyclo-oxygenase catalyzes the production of prostaglandins in response to a variety of mechanical, electrical, chemical or immunological stimuli. The study of pharmacological mediators released from isolated lungs during anaphylactic reaction has proven that aspirin also acted like these mediators and blocked the production of prostaglandin (PG). The concept that aspirin inhibition of PG synthesis was correct was the finding that aspirin’s injury of the wall of stomach was prevented if prostaglandins were added to the mucosa. Clearly, if the production of PG was blocked by aspirin the protection of the stomach wall had been lost and so that is why it damaged the stomach walls, which was, and still is, one of the major side effects of aspirin (Hamberg et al., 1975).
In one of the experiments which were conducted by Samuelsson and Anggard (1965), they had detected the production of PGE$_2$ and PGF$_{2\alpha}$ in the supernatant of broken cell homogenate from guinea pig lungs rather than from ram seminal vesicles which was the site from where the synthetase enzyme had been obtained in all their previous studies. These experiments proved that there was not only one tissue that can contain/produce the enzymes which stimulated the production of prostaglandins, rather, in many other tissues the enzyme was also present. Today, all tissues of the body are thought to contain this enzyme system.

The work on lung homogenates had established that if aliquots of the supernatant from these homogenates were incubated with arachidonic acid, the precursor of all the prostaglandins and thromboxanes of the 2 series, at 37°C the metabolites could be collected after 30 min of incubation. The production of the prostaglandins was measured using bioassay with especial interest being paid to the production of PGF$_{2\alpha}$. When various concentrations of anti-inflammatory drugs such as aspirin, indomethacin or sodium salicylate were added their effect on prostaglandin synthesis could be assessed. The experiments clearly proved that a concentration-dependent inhibition of PG synthesis occurred in the presence of these anti-inflammatory drugs. Indomethacin was found to be the most potent in blocking the production of prostaglandins whereas other drugs such as sodium salicylate were less potent in their inhibitory action. Aspirin was potent with a little higher concentration as compared to indomethacin. In similar experiments the effect of opiate analgesic drugs e.g.; morphine, a steroidal ant-inflammatory drug (hydrocortisone) and H1-antihistamine (mepyramine) were also tested to determine their effects on prostaglandin synthesis. Here it was found that these drugs had very little or no effects on the PG’s synthesis (Anggard and Samulesson, 1965).
At the same time as the above experiments were reported, Smith and Willis studied the effect of aspirin on platelets. In their experiment they collected the blood samples of three people one hour before taking 600mg of aspirin and then one hour after taking of 600mg of aspirin orally. Platelets were isolated, washed and incubated with the enzyme thrombin. The thrombin in addition to being involved with blood coagulation, where the enzyme is involved in the conversion of a substance called fibrinogen to fibrin, also stimulates platelets to contract. The experiment was tested in the presence of various substances. It was found that there was a major difference between the ability of platelets to make PGs before and after their exposure to aspirin. In contrast there were no significant changes in concentration of any other substances found in platelets. This shows that platelets activity to form the clots or stick together to form a clot is due to ‘hyper secretion of prostaglandin’, and as a result of aspirin exposure the production of PGs had been decreased which showed the anti-clotting potential action of aspirin (Smith & Willis, 1971).

The great importance of this last study was that it showed for the first time the action of aspirin on platelets, a cell type which is central to this thesis.

Ferreira also demonstrated in the same year that aspirin and aspirin-like drugs blocked the production of prostaglandin (PG) in the perfused, isolated dog spleen preparation (Ferreira et al., 1971). Finally in the same momentous year, 1971, Collier and Flower also studied the ability of aspirin in blocking the prostaglandin (PG) which has been produced by human seminal fluid. These researchers also proved the diversity of tissues producing the same range of prostaglandins and the blocking by aspirin of their synthesis, irrespective the type of tissues (Collier & Flower, 1971).
Inhibition of cyclo-oxygenase and correction

Cyclo-oxygenase (COX) is an enzyme that is responsible for the formation of important biological mediators called prostanoids, including prostaglandins, prostacyclin and thromboxane. Pharmacological inhibition of COX can provide relief from the symptoms of inflammation and pain. Non-steroidal anti-inflammatory drugs, such as aspirin and ibuprofen, exert their effects through inhibition of COX. The names "prostaglandin synthase (PHS)" and "prostaglandin endoperoxide synthesize (PES)" are still occasionally used to refer to COX (Hemler et al., 1976).

COX converts arachidonic acid to prostaglandin H$_2$ (PGH$_2$), the precursor of the series-2 prostanoids. The enzyme contains two active sites: a heme with peroxidase activity, responsible for the reduction of PGG$_2$ to PGH$_2$, and a cyclo-oxygenase site, where arachidonic acid is converted into the hydroperoxy endoperoxide prostaglandin G$_2$ (PGG$_2$). The reaction proceeds through H atom abstraction from arachidonic acid by a tyrosine radical generated by the peroxides active site. Two oxygen molecules then react with the arachidonic acid radical, yielding PGG$_2$ (Smith, 1986). Different tissues express varying levels of COX-1 and COX-2. Although both enzymes act basically in the same fashion, selective inhibition can make a difference in terms of side-effects. COX-1 is considered a ‘constitutive enzyme’, being found in most mammalian cells. COX-2, on the other hand, is undetectable in most normal tissues. It is an ‘inducible enzyme’, becoming abundant in activated macrophages and other cells at sites of inflammation. In 1975 it was shown to be ‘up regulated’ in various carcinomas and to have a central role in tumorigenesis (Roth et al., 1975).

The main way to inhibit COX is to use drugs. The now ‘classical drug inhibition of prostaglandin and thromboxane synthesis’ has the effect of reduced inflammation, as well
as antipyretic, antithrombotic and analgesic effects. Aspirin specifically acetylates the hydroxyl group of serine residue located 70 amino acids from the C-terminus of the enzyme (Roth et al., 1975). The acetylation of the enzyme by aspirin places a bulky substituent on Ser530 which inhibits the binding of arachidonic acid and its conversion to endoperoxides (Raz et al., 1988). The acetylation which is achieved by aspirin is an irreversible process of COX inhibition and it takes place only in one direction as it can never be reversed via deacetylation. This means for the production of more PGs, new enzyme has to be synthesized. When this enzyme was acetylated in its pure isolated, it has been found that only COX enzyme not its hydroperoxide ability is blocked. The inhibition of enzyme by aspirin is also its concentration depending activity (De Witt et al., 2002).

When the aspirin concentration is low, aspirin rapidly i.e. within a few minutes, specifically acetylates the COX. But if the concentration of aspirin are higher for a longer period of time, it behaves entirely differently from the low concentration as with a higher concentration over long period of time, aspirin non-specifically acetylates a variety of protein and nucleic acids.

After it was established that aspirin irreversibly acetylated COX, major attention was given towards the synthesis of COX. It has been found that synthesis of COX could be stimulated by growth factors, tumour promoters, interleukin-1, lipopolysaccharides and tumour necrosis factor (TNF) (Raz et al., 1988). The synthesis of COX by Interleukin-1 is induced by exerting its effects via transcriptional phase rather than translation phase (Raz et al., 1988). The induction of COX gene expression is rapid and using serum factors it takes place after approximately two hours in mouse 3T3 (fibroblast derived) cells in which PGs are essential for cell division. The report of "Simmons" described the final stages of the theory which related the activation of COX, as it reported that a distinct COX gene
could be induced with mitogens like growth factors, tumour promoters and lipopolysaccharides, the inhibition of which could be inhibited with glucocorticoids. This gene expression elaboration with PGs is best expressed by COX-2 during inflammatory reactions rather than COX-1 which produce PGs and these PGs involved in physiological processes such as protection of the stomach mucosa, platelet aggregation and kidney function.

Both COX-1 and COX-2 are isoenzymes and are 60% homologous between amino acid structures of COX-1 and COX-2. Aspirin binds to the active site of COX-2 which is Ser 516 in the same the way it binds to Ser530 the active site of COX-1. The only difference between both isoenzymes is that the active site of COX-2 is slightly larger than the active site of COX-1 (Simmons et al., 1991). Any anti-enzyme activity can cause a reduction in the production of both prostaglandins and thromboxanes. This inhibitory effect shows the way aspirin acts to produce analgesia, antipyrexia, and reduces the clotting formation ability of platelets (Vane et al., 1998).

The effect of aspirin on platelets is irreversible and to meet the ‘new situation’; the body has to produce new COX enzyme which in turn produces PG for forming the clots in injured and wounded conditions. Platelets cannot produce new COX, which may explain why aspirin is effective for the life of the platelet which is generally considered to be 8 -10 days. In contrast other cells, for example the mast cells, a nucleated resident cell found in many types of tissues which contain many granules rich in histamine and heparin, can synthesis new COX. Although best known for their role in allergy and anaphylaxis, mast cells play an important protective role as well as being intimately involved in wound healing and with defence against pathogens. It has also been observed, in one very
important experiment, that aspirin decreased the clinical symptoms of induced anaphylaxis (Piper and Vane, 1969).

Prostaglandins and thromboxanes have a variety of effects on the body which are not only limited to sending impulses of pain to the brain, modulation of the hypothalamic thermostat and inflammation but also responsible for the aggregation of platelets that facilitate the formation of blood clots. It has been proven that one of major reasons for a heart attack is the increased risk of blood clot formation. Aspirin is found to inhibit the aggregation of platelets, and in this way brings about the anti-clotting effect for which aspirin is now famous (Roth et al., 1975). However, this effect is not without its risks.

As one of the major side effects of aspirin is the ability to inhibit platelet function so severely that excessive bleeding may occur. The newer NSAIDs called COX-2 selective inhibitors were developed to specifically inhibit COX-2 with the hope that although they would still reduce the platelet effect, whilst reducing the risk of causing the gastrointestinal side effects such as gastro-intestinal haemorrhage (Silverstein et al., 2000). The early success has been met with removal from the market due to problems with side effects.

Finally, using the more recent technique of X-analysis has elucidated the structure of cyclo-oxygenase in a much better way than was previously possible. This has provided additional information about the formation of PG by this enzyme. X-rays of COX shows the positions of atoms in tiny crystals of enzyme. The analysis showed the presence of a tunnel running in the middle of the enzyme. The arachidonic acid must pass through this tunnel to reach the core of the enzyme where its oxidation results in its conversion into prostaglandin (Fiorucci et al., 2003). When aspirin is taken, it passes into the tunnel of COX where it occupies the same active site where the precursor of PG formation, arachidonic acid, is itself attached i. In this way the active site of COX enzymes is blocked
by aspirin and acts as a gate effectively blocking the access of arachidonic acid. The researchers further showed that this gate where aspirin can attached itself is present in two positions in the enzyme, either fully or partially closed and the position of the gate in both enzymes COX-1, COX-2 is different. The X- analysis, highlighting these differences, was said to be very helpful to bring about the improvement in NSAIDs (Chandrasekharan et al., 2002).

2.04 Absorption of aspirin

Aspirin is rapidly absorbed from the stomach and the proximal small intestine in monogastric animals. The rate of absorption is dependent upon many factors such as: the quantity and nature of the stomach contents, gastric emptying times, tablet disintegration rates and gastric pH. In contrast to man, who is a ‘monogastric animal’, in cattle with their many stomachs absorption is slow. In man, approximately 70% of an oral dose will be absorbed in the stomach, which may reflect the fact that the gastric mucosa is permeable to the non-ionized form of aspirin, which passes through the stomach wall by a passive diffusion process. Optimum absorption of the salicylate in the human stomach occurs in the pH range of 2.15 to 4.10. Absorption in the small intestine occurs at a significantly faster rate than in the stomach. After an oral dose of 650 mg Aspirin, the plasma acetylsalicylate concentration in man usually reaches a level between 0.6 and 1.0 mg% in 20 minutes after ingestion and drops to 0.2 mg% within an hour. Within the same period of time, half or more of the ingested dose is hydrolyzed to salicylic acid by esterases in the gastrointestinal mucosa and the liver. The total plasma salicylate concentration reaches a peak between one or two hours after ingestion, averaging between 3 and 7 mg%. Many factors influence the speed of absorption of ASA in a particular individual at a given time; tablet disintegration, solubility, particle size, gastric emptying time "psychological state"
physical condition, nature and quantity of gastric contents, all affect absorption (Levy, 1961).

There are some factors that influence the absorption of a drug and the major two are: Firstly, ionization (the unionized drugs are absorbed better, ionization is affected by the pH) and secondly, the surface area of the gastric lining.

In the stomach where the pH can range from between 1.5-3.5 the aspirin (which is acidic) will not ionize, and because of that, the absorption of aspirin in the stomach is much better than in duodenum. In the duodenum the pH is 6.8 so the aspirin will ionize, therefore the absorption is not as good. However, because the surface area is very large, the time of absorption is greater, so the aspirin although is it absorbed better in the stomach, achieves a greater concentration because of its absorption in the duodenum. This is shown by small intestinal absorption studies of aspirin at pharmacological concentrations in the unanesthetized rat by using a single-pass perfusion technique. The rate of aspirin absorption remained linear with its concentration (0.5mM to 10 mM). Intestinal aspirin absorption increased as the concentration of hydrogen ions, sodium taurocholate, and ascorbic acid in the perfusate increased. Aspirin absorption did not change after ethanol addition. At pH 3.5 or 6.5, intestinal absorption of aspirin was greater than gastric absorption of the compound. Aspirin was not absorbed by the stomach at pH 6.5. These experiments indicate that aspirin can be absorbed to an appreciable extent in its ionized form by the small intestine but not by the stomach.

Once absorbed the aspirin is quickly metabolised. Aspirin is partially hydrolyzed to salicylic acid where it is distributed widely throughout the body. The highest levels may be found in the liver, heart, lungs, renal cortex and plasma. The amount of plasma protein binding is variable, depending on species, serum salicylate and albumin concentrations. At
lower salicylate concentrations, it is 90% protein bound, but only 70% protein bound at higher concentrations. Salicylate is excreted into milk, but levels appear to be very low. Salicylate will cross the placenta, and foetal levels may actually exceed those found in the mother (Levy, 1961a and b)

The excretion of salicylate occurs with first-order kinetics with a half-life between 2 and 19 hours, depending on the dose of aspirin administered. Salicylate is metabolized in the liver primarily by conjugation with glycine and glucuronic acid via glucuronyl transferase. Minor metabolites formed include gentisic acid and 2, 3-dihydroxybenzoic acid, and 2, 3, 5-trihydroxybenzoic acid. Gentisic acid appears to be the only active metabolite, but because of its low concentrations, it appears to play a therapeutically insignificant role. The rate of metabolism is determined by both first order kinetics and dose-dependent kinetics depending on which metabolic pathway is looked at. Generally, steady-state serum levels will increase to levels higher (proportionally) than expected with dosage increases. Salicylate and its metabolites are rapidly excreted by the kidneys by both filtration and renal tubular secretion. Significant tubular reabsorption occurs, which is highly pH dependent. Salicylate excretion can be significantly increased by raising urine pH to 5-8. Salicylate and metabolites, in cases of overdose and poisoning by aspirin, may be removed using peritoneal dialysis or more rapidly by using haemodialysis. About 50–80% of salicylate in the blood is bound by plasma proteins while the rest remains in the active, ionized state. The level of protein binding is concentration-dependent. Saturation of binding sites leads to more free salicylate and increased toxicity. The volume of distribution is 0.1–0.2 litres/kg. Acidosis increases the volume of distribution because of enhancement of tissue penetration of salicylates (Zommermann, 1981).
As much as 80% of therapeutic doses of salicylic acid are metabolized in the liver. Conjugation with glycine forms salicyluric acid and with glucuronic acid forms salicyl acyl and phenolic glucuronide (see Figure 8). These metabolic pathways have only a limited capacity. Small amounts of salicylic acid are also hydroxylated to gentisic acid. With large salicylate doses, the kinetics switch from first order to zero order, as metabolic pathways become saturated and renal excretion becomes increasingly important.

Salicylates are excreted mainly by the kidneys as salicyluric acid (75%), free salicylic acid (10%), salicylic phenol (10%) and acyl (5%) glucuronides, and gentisic acid (< 1%). When small doses (less than 250 mg in an adult) are ingested, all pathways proceed by first order kinetics, with an elimination half-life of about 2 to 4.5 hours. When higher doses of salicylate are ingested (more than 4g), the half-life becomes much longer (15–30 hours) because the biotransformation pathways concerned with the formation of salicyluric acid and salicyl phenolic glucuronide becomes saturated (Barsom et al., 2005). Renal excretion of salicylic acid becomes increasingly important as the metabolic
pathways become saturated, because it is extremely sensitive to changes in urinary pH. There is a 10 to 20 fold increase in renal clearance when urine pH is increased from 5 to 8. The use of urinary alkalinization exploits this particular aspect of salicylate elimination in cases of over dosage or poisoning (Proudfoot, 1983).

2.05 Interference of Laboratory Tests by aspirin

Aspirin is used in patients with cardiovascular disease and some of these have diabetes. It is of concern to note that the aspirin can modify the results of blood sugar determinations in such patients. These difficulties arise when aspirin is being used at high doses, as aspirin may cause false-positive results for urinary glucose if using the traditional cupric sulfate method (Benedict’s solution) and false-negative results if using the more modern method of glucose oxidase (Tes-Tape). Furthermore, urinary ketones measured by the ferric chloride method (Gerhardt) may be affected if salicylates are in the urine (reddish-color produced) (Saeid et al., 1996).

If the more sophisticated hydroxyindoleacetic acid (5-HIAA) determinations by the fluorometric method are being used to assess the 5HT profile in patients then these tests may also be interfered with by salicylates in the urine. Finally falsely elevated vanillylmandelic acid (VMA), used to assess catecholamine turnover, may be seen with most methods used if salicylates are in the urine. In contrast, falsely lowered VMA levels may be seen if using the Pisano method.

Finally, aspirin also causes complications in the assessment of xylose when urinary excretion of this sugar may be decreased if aspirin is given concurrently and aspirin can also cause falsely elevated serum uric acid values if a colorimetric method is used for its measurement (Caraway et al., 1972).
2.06 The therapeutic difficulties caused by the rapid metabolism of aspirin.

Once aspirin has been taken by the oral route and absorbed it goes via the hepatic portal vein to the liver. The liver inactivates the aspirin by deacetylation by the process of first pass metabolism. One recent suggestion to overcome this problem is that a transdermal 'Patch' may be suitable as this would minimize first metabolism and so exert its therapeutic effect more rapidly (Shamsher, et al., 2010) How such aspirin could be formulated to achieve such an effect when it does not compare in potency to drugs usually used in transdermal patches such as nicotine, female sex steroids, opiate - like analgesics e.g. buprenorphine, is not at all clear (Nestler, et al., 1998).

Aspirin also has a unique usefulness among the family of NSAIDs in that it is widely considered to be able to reduce the risk of cardiovascular disease. Low doses (100-300mg per day) can reduce by 30% the risk of myocardial infarction and stroke among patients who already have a history of these disorders. Similarly lower doses of between 75mg - 150mg per day were also said to be showing promise in reducing the risk of cardiovascular disease in patients with a pre-existing condition (Raming, et al., 1996) but this is still an area that requires further research. The prevention of cardiovascular disease is thought to be related to thromboxane inhibition in platelets leading to a reduced risk of potentially dangerous blood clots forming in the cardiac and cerebral blood vessels.

In 1995 the American cancer society epidemiologists (Wannamethee, et al., 1995) found that while low dose aspirin use had no effect on cancers of most organ systems, the risks were greatly reduced for fatal cancers of the oesophagus, stomach, rectum and colon. These four digestive tract cancers were approximately 40% lower among men and women who used aspirin 16 times per month or more for at least one year compared to those who
used no aspirin. Very recently (Rothwell et al., 2012) the use of aspirin was again suggested to be useful in preventing cancer occurrence. Time will tell if such effects are more commonly found in the future. Aspirin has also been shown to decrease the incidence of gastrointestinal cancer and gall bladder disease, to improve diabetes, premenstrual syndrome (PMS) symptoms, and pregnancy outcomes.

However, aspirin has a proven clear benefit in people with a current or past history of heart attack, angina, ischemic stroke, transient ischemic attack, peripheral artery disease (claudication), as well as people with a 10-year risk of having a coronary event of at least 6% to 10%. Aspirin is likely to have a benefit in people with a moderate to high risk of heart attack or ischemic stroke (stroke caused by blockage of arteries that supply blood to the brain).

In contrast he benefits of aspirin may not outweigh the risks in people who have a low risk of heart attack or ischemic stroke. The benefits of aspirin have been studied in a wide range of patients and these will now be considered in some detail below (Placezek et al., 2004).

2.07 Prevention of heart attack or stroke

Several large trials, primarily among men, have shown that aspirin can prevent a first heart attack in people who have no signs or symptoms of cardiovascular disease. This is called primary prevention. However, these trials could not detect the effects of aspirin on the risk of stroke and death related to cardiovascular disease. In one trial of women, aspirin reduced the risk of a first stroke and also decreased the risk of a first heart attack among those aged 65 years and over (Ridker et al., 2005). However, the risk of a first heart attack or stroke in healthy men and women is quite low. As a result, the benefit of reducing
the risk of a first heart attack must be weighed against potential risks, such as gastrointestinal bleeding and other side effects.

Expert groups have recommend aspirin to prevent heart attack or stroke for healthy men and women when the benefits outweigh the risks, and this includes people with a 10-year risk of a coronary event of at least 6% to 10%. The recommended daily dose of aspirin for prevention of heart attack and stroke is generally between 75mg and 100 mg, as shown in Table 3 (Harpaz et al., 1996).

2.08 The problems and consequences which occur during a heart attack

The heart, like all other organs and tissues in the body, requires a constant supply of blood. The blood supply to the heart is rather unusual as oxygen extraction is of a very high order and blood flow to the cardiac muscle only occurs during diastole. If the vessels are compromised for any reason, but primarily atherosclerosis, then blood flow decreases and oxygen availability is less. The structure of the coronary arteries is complex but their passage through the cardiac muscle and its restriction of flow during systole means that any decrease in the vascular lumen diameter for a given heart rate may cause problems. Of course, when the heart rate rises or the pressure increases then the flow is compromised to an even greater extent.

One consequence of a sudden restriction of flow is to cause myocardial infarction (M I) which is commonly known as a "heart attack". The cessation in the coronary arteries prevents blood flow and so causes serious oxygen depletion and this causes either damage or death to the cardiac muscle which the particular vessels supplies. In the majority of cases in which MI occurs there is an underlying condition of coronary heart disease (CHD)
whether this be genetic or related to aging and lifestyle including diet (Amarenco et al., 2010).

2.09 Atherosclerotic plaque ruptures and clot formation

In the development of atherosclerosis, lipid containing plaque builds up in the subendothelial space. This deposition, as it increases in size may displace the endothelium into the lumen of the vessel. This protrusion, by effectively decreasing the lumen diameter, reduces the blood flow. These endothelial covered projections in the artery wall may sometimes develop cracks – i.e. fissures, in their normally perfectly smooth surfaces. If that happens, the platelets within the bloodstream rapidly detect such changes, rapidly adhere and secrete thromboxane to aggregate more platelets in the bloodstream so forming a platelet aggregation on which blood coagulation is facilitated (Delud, 2005).

Normally, blood coagulation within a vessel is a ‘good thing’, because endothelial damage is caused by events which usually mean potential blood loss and so bleeding in an uncontrolled manner is prevented. Unfortunately, when clots form inside the coronary artery damaged by atherosclerosis, they develop in such a way that they may partially or completely block the flow of blood. This is what happens during a heart attack (Penz et al., 2005). When a blood clot forms within a coronary artery, the area of heart muscle fed by that artery no longer receives enough blood flow. This lack of blood flow has serious consequences as cardiac muscle cannot suffer an oxygen debt and so dies. The death of heart muscle in such circumstances is termed an “infarction.”

In some cases aspirin can be life-saving for people who are actively having a heart attack. Healthcare providers recommend that anyone who believes they may be having a heart attack should immediately take 162mg to 325 mg of aspirin (one half to one whole
adult aspirin tablet). This will prevent platelet involvement and so reduce the risk of the platelets contributing to an even greater effect (Harpaz et al., 1996).

2.10 Coronary heart disease

In people with coronary heart disease, sometimes called coronary artery disease or simply, coronary disease, the coronary arteries become narrowed by the fatty deposits due to atherosclerosis. These plaques inside the coronary arteries limit blood flow to the heart muscle, which may cause pain or tightness in the chest especially on exertion when oxygen demand increases. This pain or tightness is called angina pectoris, commonly referred to as "angina". Aspirin is recommended for people who have a history of several conditions (Harpaz, et al., 1996).

As already described, most heart attacks develop when a cholesterol-laden plaque, in a coronary artery, fissures and once this occurs platelets detect the fissure and adhere. There is then a rapid release of thromboxane which causes aggregation of more platelets to produce a surface on which coagulation can occur. The thrombus which forms can actually rupture and the relatively small plaques, which produce only partial blockages, are the ones most likely to rupture. When they do rupture, they attract even more platelets to their surface. Platelets and the associated thrombus, then builds up on the ruptured plaque. The thrombus grows because blood coagulation is stimulated by the platelets contributing factors for the clotting process and the growth may be such as to cause a blockage in the artery. If the blockages completely stop the flow in a vessel then it deprives a portion of the heart muscle of oxygen. As a result, the muscle cells die. This sequence of events is usually described as heart attack.
In these cases aspirin is recommended because by effectively inhibiting platelets ability to produce thromboxane. Only a tiny amount of aspirin is needed to inhibit all the COX in the platelets in the blood stream. In fact, small doses are better than high doses to achieve the degree of selectivity which is required. But since the thrombus grows minute by minute, time is of the essence (Penz, et al., 2005).

Aspirin is also recommended for percutaneous coronary intervention (angioplasty) and post surgically for the treatment of coronary bypass grafted patients (CABAG). For this condition most health care providers recommend that people in this group take between 75mg and 100 mg of aspirin daily (Harpaz, et al., 1996). For heart disease, studies have shown that taking low doses of aspirin and maintaining a diet low in fat and high in fiber such as grains, fruits and vegetables can reduces the risk of cardiovascular complications (Lichtenstein et al., 2006).

Aspirin has benefits in people who are actually having or have recently had an ischemic stroke or transient ischemic attack (TIA). The recommended dose for this group can be between 81mg to 325 mg per day. (see table 2 – Escolar et al., 1986)

2.11 Aspirin used to treat gastrointestinal tract (GTI) problems

When cholesterol-saturated bile accumulates and becomes lodged in the cystic duct, gallstones are formed. During acute cholecystitis, usually a bacterial inflammation associated with gallstones, the production of prostaglandins is increased as part of the normal inflammation and repair processes. This process involves much pain, increased fluid secretions, muscle contraction and decreased bile, all of which exacerbate and perpetuate the inflammation. Several studies have been conducted to show that prostaglandin inhibitors such as aspirin and non-steroidal anti-inflammatory drugs can
prevent the formation of gallstones, as well as reduce the biliary pain associated with it (Truitte and Morgan, 1964).

2.12 Aspirin and pregnancy induced hypertension

Many studies have concluded that low dose aspirin reduces the risks of pregnancy induced hypertension, toxaemia and severe low birth weight (Steve et al., 1998; Robert et al., 2008). In another study by another group aspirin was also thought to be helpful in cases of hypertension induced by pregnancy (Diejomaoh et al., 2002). This hypertension is responsible for significant prenatal morbidity and mortality. In further studies conducted in this area, low dosages of aspirin were effective in reducing this type of hypertension to the extent of 65%. It can be reasonably concluded that although aspirin is not a first line of defense against such causes of hypertension, it plays an additional role in preventing the health problems caused by this disease (Randall, 2000; Williams et al., 2003).

2.13 Pharmacological properties of Aspirin and its major metabolites

Aspirin due to its rapid metabolism can also have active metabolites which may bring about some of its pharmacological actions. The table below shows the effect of the major metabolite namely, salicylic acid expressed as salicylate.
### Table 4. Properties of aspirin and its principle metabolite on analgesic, antipyretic, anti-inflammatory and antiplatelet actions (Murnion, 2010).

<table>
<thead>
<tr>
<th>Pharmacological Property</th>
<th>Salicylate</th>
<th>Aspirin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analgesic</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Antipyretic</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Anti-inflammatory</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Anti-platelet</td>
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#### 2.14 The use of aspirin in hypertension – considerations that aspirin may alter systemic blood pressure in the hypertensive state.

A relatively recent study has shown that systemic blood pressure is affected by taking low dose aspirin which is further related to time. More recent findings have suggested how low aspirin dosage may facilitate the control of blood pressure.

If a patient has been diagnosed with hypertension, it has been suggested by some physicians that they should take low dose aspirin each day to potentially reduce the risk of a heart attack. Aspirin has been shown in some studies to have a positive effect not only on blood pressure but also on the endothelial cells that line both the chambers of the heart and the coronary blood vessels (Randall, 2000; Williams et al., 2003).

The actual use of aspirin for this purpose is interesting to consider. If someone has hypertension and takes low dose aspirin, it is recommended that they take the aspirin at night. This has not always been the case as baseline blood pressure readings through home monitoring were not always made before changes, to the timing of the dose aspirin, were
made. The literature shows a positive association between the dose timing of aspirin and hypertension in terms of overall coronary ‘health’ (Larry et al., 1997). In fact, according to the British Hypertension Society, small dosage of aspirin should be routinely given to persons above 50 years of age. Various studies indicate that people of this age had a lower risk of Coronary Heart Disease (CHD) if their blood pressure was also under control (Aronow et al., 1989).

Very importantly, in another study, which was called the hypertension optimal study (HOT), this current time conducted in a Chest Hospital in Kuwait, the country in which this current study was to be carried out, revealed that taking the aspirin before bedtime decreased the cardiovascular risk by 15% and reduced the chances of myocardial infarction by as much as 36% (Al-Shammari et al., 2005: Al-Otaibi et al., 2008). However there were drawbacks and the major one, as perhaps could be expected based on previous evidence, was that aspirin contributed to increased chances of gastric bleeding by as much as 65%. For this reason aspirin is best taken with or after food (BNF, 2011) Due to its inhibition of prostaglandins in some individuals it also may cause respiratory problems and allergic reactions.

This study and others were the first studies to reveal that taking aspirin before bedtime as opposed to upon waking in the morning was “an effective strategy to lower blood pressure and cost effective way to individualize treatment regimes in pre-hypertensive patients,” said lead investigator Prof. Ramon C. Hermida, Director of Bioengineering and Chronobiology at the University of Vigo in Spain. He also went on to say that ”These findings therefore have vital treatment implications for these at-risk patients throughout the world” (Hermida et al., 2003).
Although researchers are unsure as to the reasons for the critical nature of the timing of the dose of aspirin to pre-hypertensive patients, being in the evening rather than the morning, suggestions have been made that it slows down the production of "hormones" and other substances in the body that cause clotting. Many of those are produced while the body is at rest. The thinking behind such an idea has provided the basic concept for the research carried out for the research in this thesis.

In addition, Professor Ramon C. Hermida had important conclusions about the timing of the dose of aspirin.

"These results show us that we cannot underestimate the impact of the body's circadian rhythms" (Hermida et al., 2003).

They also stated:

"The beneficial effects of time-dependent administration of aspirin have, until now, been largely unknown in people with prehypertension. Personalizing treatment according to one's own rhythms gives us a new option to optimize blood pressure control and reduce risk of cardiovascular disease down the line."

This recorded effect is perhaps not simply related to aspirin’s analgesic effects as men who used acetaminophen (in the UK known as Paracetamol) six to seven days a week for pain control actually increased their risk of incident hypertension by 31% as compared with non-users (P=0.05 for trend) while for other non-steroidal anti-inflammatory drugs the increase in relative risk was 33% (P=0.02 for trend) (Hart et al., 1996; Guillem et al., 2005).
The effect of aspirin is seemingly complex. Somewhat surprisingly, men who used aspirin for pain relief, 300mg, on a nearly daily basis had a 26% increase in risk for new-onset hypertension compared to non-users (P<0.001 for trend). In contrast aspirin has escaped any suggestion of cardiovascular toxicity and has been used for its ability to prevent heart attacks via its effects on platelets. For example, since early 2005 the Food and Drug Association (FDA) of the United States of America has required all NSAIDs to carry warnings about cardiovascular risks associated with prolonged, high dose use of the drugs. But aspirin was exempted from that labeling requirement because the FDA said "It has clearly been shown to reduce the risk of serious adverse cardiovascular events in certain patient populations".

Patients who take aspirin to prevent heart attack should continue to take aspirin unless advised to stop doing so by their cardiologists. However, there are many people who use these medications on a regular basis and do not realize there are potential side effects (Lipton et al., 1987). Consequently, physicians should ask all their patients about their use of these medications and determine if there are alternatives to use. For example, physical therapy for those with arthritis or back pain may reduce symptoms and need for medication (Henningfield et al., 2009). For individuals with recurrent headaches, the cause of the headaches should be explored. If the headaches can be prevented, then medication is not needed. The prospective analysis of the problems of headaches included only men with a mean age 64.6, who had with no history of hypertension or blood pressure-lowering medication use at baseline (Forman et al., 2007). They completed a questionnaire on frequency of use and number of " pills" consumed per week of acetaminophen, NSAIDs, and aspirin. The questionnaire did not actually specify the types of NSAIDs. This was a great pity since different NSAIDs may have had different effects.
However, Dr. Forman reported that the data from the cohort's female counterpart in the previous Nurses' Health Study (Forman et al., 2005) showed "the majority of NSAIDs used were principally ibuprofen followed by Naprosyn." On a follow-up questionnaire four years later, 1,968 men reported a new diagnosis of hypertension. Despite similar baseline blood pressure across medication use groups, the researchers found significant increases in hypertension incidence with more frequent analgesic use. Among the findings: For acetaminophen, elevated risk was found for six to seven days per week of use (1.34, 95% CI 1.00 to 1.79, P=0.01 for trend). For NSAIDs, significantly elevated risk for daily or near daily use (RR 1.38, 95% CI 1.09 to 1.75 P=0.002). For aspirin, the risk was greatest when used two or three times a week (RR 1.36, 95% CI 1.14 to 1.61) and actually decreased with increased use, so that when taken six to seven days a week the RR was 1.26, 95% CI 1.14 to 1.40 P<0.001).

When the data were analyzed by number of tablets/capsules consumed the results were similar to the findings based on the frequency of use. Interestingly, the body mass index (BMI) did appear to have an effect on the relationship between hypertension and analgesic use. For acetaminophen, the risk of the incidence of hypertension was greater among users with a BMI less than 25, whereas the association dropped out for "heftier" men (BMI>25) (P=0.01 for interaction). For NSAIDs, risk of hypertension was greater among overweight and obese men but dropped into non significance for BMI less than 25 (P=0.01 for interaction), for aspirin, the BMI did not appear to modify the aspirin-hypertension association (P=0.94 for interaction).

The mechanisms by which analgesics impact on blood pressure may include:

A- An inhibition of vasodilatory prostaglandins.

B- An increase of renal tubular sodium reabsorption with NSAIDs.
C- An increase in cellular oxidative stress with acetaminophen, and,

D- An impairment of endothelial function with both acetaminophen and NSAIDs.

The researchers concluded that the "contribution of non-narcotic analgesics to the hypertension disease burden merits further study," if not more caution in their use.

2.15 Benefits and harms of low-dose aspirin in well-treated hypertensive patients on different baseline cardiovascular risks.

The use of aspirin in subjects without existing and proven cardiovascular diseases is controversial. In the intensively treated patients of the Hypertension Optimal Treatment (HOT) Study, randomization to low-dose aspirin (75 mg daily) versus placebo significantly reduced cardiovascular events (-15%) and myocardial infarction (-36%), but increased major bleedings (+65%). The analyses of the HOT Study data aimed at identifying subgroups of hypertensive with different benefit-to-harm ratios from aspirin, in order to provide recommendations about the use of aspirin in hypertension.

2.16 Low-Dose Aspirin Therapy.

Aspirin, the common pain reliever, has been available for almost a century. Its recent history has been proved to prevent a first and second heart attack in people who have coronary artery disease. Furthermore, when taken during and after a heart attack, aspirin can reduce the chances of dying. It can also reduce the risk of having a stroke in those who have had a previous stroke or a transient ischemic attack (TIA) - a temporary interruption of blood flow to the brain, which is often a warning sign of an impending stroke. It has been well documented that aspirin reduces the risk of heart attack in people with known CAD. It is also now understood that aspirin lowers the risk of having symptoms for people who have a higher risk for the disease. People who are at higher risk
for coronary artery disease or who already have coronary artery disease benefit the most
from aspirin therapy. Instructions on how to take aspirin in appendix 2.

So, exactly how much aspirin should be taken? To answer this simple question is not easy
and some may say ‘This is where it gets confusing’. Aspirin comes in a wide
range of dosages and in many forms. The optimum dose of aspirin has not been
established. Using aspirin correctly gives us the best chance of getting the greatest
benefits with the fewest unwanted side effects. Some doctors recommend taking low-dose aspirin
(at least 75 mg/day), because a low dose seems to be as effective in preventing heart
attacks as higher doses. However, one low-dose aspirin preparation which is available in
the USA which contains 81 mg and this compares with a one adult-strength aspirin
contains about 325 mg. Some doctors recommend taking 100 mg every other day. Also,
some drug companies combine aspirin at different strengths, of different salts and they
contain other ingredients, such as caffeine. These formulas should not be used for daily
aspirin therapy.

2.17 Low-dose aspirin suppresses aggregation of blood platelets in both sexes.

The study by Hopkins et al., in 2006 showed that low-dose aspirin suppressed
clumping of blood platelets in both sexes. A once-daily tablet of low-dose aspirin helped to
lower the potential for clot-forming blood cells - in both men and women - to stick
together in narrowed blood vessels (Hopkins et al., 2006). A daily dose of 81mg
acetylsalicylic acid, has been found to prevent platelet clumping (See Table 4). However,
while the drug’s overall effects on blood cell function were the same for men and women,
the investigators found that women’s platelets reacted somewhat more ‘strongly’ to aspirin
before the start of therapy, and remained so even after treatment.
This study by Hopkins et al., appeared in the Journal of the American Medical Association (JAMA) online on March 21, 2006. In this paper they challenged the conclusions from several other then recent studies, including the Federal Women’s Health Study, which showed low-dose aspirin had no effect in preventing heart attacks in women, even though it worked in men (Becker et al., 2006). Previous results, the researchers said;

“were not likely caused by the failure of aspirin to prevent platelets from clumping together and forming blood clots in women, but rather due to family history. Aspirin has been proven by all previous studies to lower the risk of stroke and, as the latest findings show, it also reduces platelet aggregation that can lead to potentially fatal clots in blood vessels.”

The controversy therefore still remains and the true effect on women is yet to be established.

2.18 Practical study on how platelet aggregation can be effected by aspirin in both sexes.

Using an electrical measure of how well platelets stick together, researchers found that in aspirin-treated men, clumping decreased by 15.1 ohms. The decrease was statistically the same in aspirin-treated women, at 17.3 ohms. In this test, an ohm is the measure of electrical resistance caused by platelets as they impede the flow of electricity in a wire probe inside a test tube filled with blood.

Moreover, platelet aggregation was largely suppressed in at least three other key pathways related to their function when platelets were stimulated with substances that normally trigger clot formation (Katzung et al., 2006; Benowitz, 2008). Each of these tests involved mixing whole blood, or platelet-rich plasma, from aspirin-treated men and
women with various concentrations of each of the main chemical compounds involved in the pathways; collagen, adenosine diphosphate, and adrenaline - to see how platelets responded. For example, in aspirin-treated men, platelet clumping went down by 14.6 ohms when 1 microgram of collagen per milliliter was added to whole blood, and decreased by 2.4 ohms when exposed to a higher dose of 5 micrograms per milliliter. In treated women, reductions were the same, at 14.9 ohms and 2.42 ohms, respectively.

When 10 micromoles per liter of adenosine diphosphate were added to whole blood, platelet aggregation decreased the same amount, 0.19 ohms in men and 0.21 ohms in women. Addition of 2 micromoles per liter of epinephrine to platelet-rich plasma produced significantly greater reductions in platelet clumping in treated women, a drop of 36.9 percent, while it was less of a reduction for men, at 31.5 percent. Again, the researchers say, these changes would have been zero if aspirin had had no effect.

Further analysis of these results highlighted two main factors:

A- Platelet reactivity levels before therapy starts.

B- Gender, as having played a significant role in predicting the effects of aspirin therapy on platelet clumping.

Other factors, such as age, race or known risk factors for heart disease, including smoking, obesity and high blood pressure, were not found to be good predictors of aspirin’s beneficial effects (Henningfield, et al., 2009).

More than 500 men and 700 women participated in the Kuwaiti study, which was called the Genetic Study of Aspirin Responsiveness (GeneSTAR). Conducted solely at the Kuwait Chest hospital from June 2004 to November 2005, the study enrolled participants from across the country who ranged in age from 21 to 80 years, 31% were non-kuwaiti and
the rest were Kuwaiti. None had previous histories of heart problems, such as a heart attack, but all were considered to be at slightly increased risk of heart disease because of a family history. 50% of women participants were postmenopausal.

Blood testing was conducted both before and after treatment. In total, more than 200 different tests of platelet reactivity were performed and analyzed in the study. Because whole blood contains other cells that can affect platelet aggregation, testing was repeated using a purified version of test samples made up of strictly platelet-rich plasma. At the start of the experiment, laboratory tests of blood platelets in women were found four times more likely than in men to aggregate when exposed to arachidonic acid, a pro-aggregation inducing chemical in the pathway that is most suppressed by aspirin.

While taking aspirin, participants maintained a strict and consistent dietary and exercise regimen, with no smoking or consumption of foods that by themselves affect platelet activity, such as caffeine, chocolate, wine or grapefruit juice. Physical examinations and pill counts were conducted to ensure that all participants adhered to the study protocol. Because aspirin reaches its maximal effect in the body at five days, the researchers say a longer study testing period was not required to determine the drug’s effects on platelet function.

2.19 Studies utilizing aspirin which have found that long-term heart attack survival rates rise when "cardiac drugs" are used.

The use of medicines to fight cardiovascular disease has been a primary focus of research in this area for the past several decades, as combinations of interventions and medicinal therapy have gradually begun to increase long-term survival rates. Two recent studies presented at the American College of Cardiology's 56th Annual Scientific Session look at the measurable impact of the use of aspirin and other maintenance therapies. One
study by Dr Zipes, (2006) demonstrated that lower doses of therapies may prove to be just as beneficial while also the lowering side effects induced by the drugs.

Today cardiovascular disease is the leading worldwide cause of death, and a major focus of research is to find better ways to help these patients through prevention, immediate intervention and long-term treatment regimens. As we continue to discover the benefits of these therapies, we expect to see continued and measurable improvements in overall survival and quality of life. This is demonstrated in a study by Jolly et al., (2009) entitled ‘Effects of Aspirin dose on Ischemic Events and Bleeding after Percutaneous Coronary Intervention (PCI): Insights from the PCI-CURE Study’. After patients with acute coronary syndromes (ACS, a group of symptoms related to acute ischemia, or chest pain related to arterial damage) undergo percutaneous coronary interventions (PCI, including stenting), a significant concern among cardiologists is the risk of major internal bleeding. Aspirin (ASA) acts as an anti-platelet agent which prevents clotting complications, but high dose levels can cause potentially serious bleeding. While PCI trials have traditionally used high-dose ASA (more than 200 mg) in combination with other medicines to prevent thrombosis and ischemic events, a sub-analysis from a clinical trial presented by researchers at McMaster University in Hamilton, Ontario suggests that low-dose aspirin may be just as effective as high doses to prevent thrombosis while reducing the risk of major bleeding in patients who have undergone PCI.

As a sub-analysis of the PCI-CURE study, researchers compared the safety and efficacy of varying doses of aspirin: low (less than 100 mg), intermediate (101-199 mg) and high (more than 200 mg). A total of 2,658 patients with ACS undergoing PCI were divided according to the most commonly used dose, and each dose group was evaluated for event rates relating to cardiovascular (CV) death, MI or stroke as well as major bleeding (Levine et al., 1987).
The researchers found similar rates of CV death, MI or stroke in all of the aspirin dose groups at 30 days and 8 months. While the incidence of major bleeding was not significantly different between the groups at 30 days, the rate of major bleeding was noticeably reduced with low-dose aspirin after 8 months, an important factor in the practice of aspirin dosing for patients in this population. In this large observational analysis, low-dose aspirin appeared to be just as effective as high-dose aspirin in preventing recurrent cardiac events in ACS patients after PCI, while reducing the long-term risk of major bleeding (Tlisara et al., 2003; Henningfield et al., 2009). These data are intriguing, since low-dose aspirin is most commonly prescribed in Europe, but in the United States, higher doses are most commonly used.” Our data suggests that lower doses may be safer, but this finding needs confirmation in a dedicated randomized trial,” said Shamir R. Mehta, Associate Professor of Medicine at McMaster University and the principal investigator in many of the relevant studies.

As we continue to make progress in the area of cardiovascular medicine, including new technologies, better therapies and ultimately improved patient survival, researchers are taking a look back to determine the major factors that have contributed to growing survival rates in cardiovascular patients (Lipton et al., 1987). This was a reference to a large observational study conducted by researchers from Brigham Womens Hospital in Boston which suggests the value of the use of maintenance therapies, and in particular, the increasing use of statins, beta-blockers, and angiotensin-converting enzyme-inhibitors (ACEI) or angiotensin-II-receptor blockers (ARB) after a heart attack. To determine the actual impact of the increasing use of cardiovascular medicines, the research team retrospectively studied Medicare and pharmacy assistance programs records from 1995 through 2004 to identify nearly 22,000 patients who had been hospitalized due to a heart attack and survived more than 30 days after discharge. These patients were followed from
the index date (30 days after discharge) until they either died or were still alive at the end of the study period. (Henningfield et al., 2009). Of the 21,848 patients in the study, about half eventually died during the 74,982 person-years of follow-up (Tlisara et al., 2003). After adjusting for demographics and comorbidities, the post-heart attack mortality rates showed significant decline from 1995 to 2004 (adjusted HR=.97), corresponding to a three percent reduction in mortality each year. After introducing the therapy variables, the team found strong indications that the improvement in mortality after heart attack may be explained primarily by the increasing use of these drugs.

**Aspirin use - summary**

Long-term prognosis in elderly patients with MI has improved considerably over time, and this literature review supports evidence that this can be attributed to the increasing use of cardiovascular medicines, including aspirin, after discharge from MI. As the rate of heart risks continues to increase among the general population, treatment regimens that include the use of cardiovascular medicines will have considerable benefits in overall long-term survival rates.

**Basis of the thesis (2).**

So, in conclusion, the use of low dose aspirin in a range of cardiovascular conditions is both promising and yet remains somewhat controversial. This thesis will examine one aspect of such medication in Kuwait namely the use of aspirin in hypertensive patients to determine if its addition to established anti-hypertensive medication can bring about an improvement in their condition.

**Chapter Three**
Nicotine’s cardiovascular actions and the problems they may cause -

Background to the thesis – Aims and Objectives

3.01 Smoking and hypertension.

Smoking can temporarily raise blood pressure and can increase the risk of blood vessels becoming hardened (arteriosclerosis) which because they cause an increase in peripheral resistance may lead to high blood pressure (See Table 1) (Placzek et al, 2004; Benowitz et al., 2008).

Cigarettes and cigars both contain variable amounts of nicotine. This nicotine by acting on nicotinic receptors in the adrenal medulla causes the release of adrenaline which by acting on alpha receptors causes narrowing of the arterioles/arteries in the body, which in turn raises the systemic blood pressure. Consequently, it has been considered that smoking can cause such elevations of blood pressure, and so is potentially dangerous for the individual. Smoking and hypertension are both risk factors for heart failure. In other words, both raise the chances of developing "heart attacks". Each of these factors – smoking and hypertension- is bad in its own right but when combined, the development of heart failure is accelerated.

Smoking can also increase the chances of developing a stroke. Smoking promotes arteriosclerosis, which means hardening of blood vessels and an effective narrowing of the arterioles, so as a consequence, these can cause a reduction in blood flow. When this affects the blood vessels that form the coronary vessels, the left circumflex and descending coronary arteries, it can also lead to significant alterations of blood flow to the cardiac muscle which ultimately may result in tissue necrosis and ultimately destruction of
sections of the heart muscle supplied by the compromised arterial vessels (Benowitz et al., 2004).

It has also been universally considered that smoking offers no advantages to an individual whatsoever and certainly no medical benefit of smoking has ever been discovered. In contrast, it has been found to be a major contributor to many severe and chronic medical conditions and diseases such as chronic obstructive pulmonary disease (COPD), functional anaemia, heart failure and cancers. To ensure a good state of health, all health authorities around the world try to promote the idea that every smoker should be both advised and helped to stop smoking as a matter of urgency. After stopping smoking, it has been observed that the chances of developing heart failure start reducing immediately. By the end of the first year after stopping, the chances of developing heart failure are said to be reduced by 50% (Katzung et al., 2006)

One major problem smokers face is the carbon monoxide content in cigarette smoke which is one of the more important components of smoke as it is considered a potentially lethal gas. It is damaging effect centers on its interaction with hemoglobin in the red cell mass. Carbon monoxide interferes with the process of oxygen which has been breathed in attaching itself to hemoglobin and forming a compound called oxyhemoglobin which is necessary for the oxygen to be released into the tissues of the heart and other organs. Carbon monoxide preferentially, because it has a greater affinity, attaches itself to hemoglobin instead of the oxygen. This attachment is irreversible for the entire life of the red cell, 120 days, so it produces an effective reduction in oxygen carrying capacity which because of the stimulation of the oxygen sensor in the kidney which causes the release of erythropoietin which in turn stimulates the formation of new red cells in the bone marrow. These new cells add to the existing cell mass and so actually increase blood viscosity. This
increase in viscosity requires the heart to work harder to pump the same volume of blood and so this can compromise the load on the heart, increase the oxygen requirement of the cardiac muscle whilst at the same time the coronary vessels are compromised by atherosclerosis and arteriosclerosis which results in oxygen starvation of the myocardium. This anoxia is potentially damaging and of course can result in the muscle suffering anoxia and death (Benowitz et al., 2004; Katzung, 2006; Henningfield et al., 2009).

If these effects were not serious enough, another finding is that cigarettes have a problematic effect on blood pressure which is because some of the other constituents of the smoke, for example the high number of free radicals, can accelerate the rate of advancement of atherosclerotic arterial disease much more rapidly and so cardiovascular disease occurs much more quickly in smokers than non-smokers (Xinan et al, 2009).

Other problems associated with smoking and high blood pressure can be the effects on the brain. The brain constantly requires high levels of oxygen supply because it has a high rate of usage. If the arteries in the brain have a high level of plaque formation and this will decrease the effective ‘flow rate into the brain’ this will reduce tissue oxygen availability. There is also the problem that these atherosclerotic changes increases the potential for blood clots for be initiated in the cerebral vessels. Since atherosclerotic disease can advance rapidly the possibility of clot formation in smokers increases their risk of suffering a stroke and as a consequence of this, death. These increased risks are dependent on a number of factors including: the number of cigarettes which are actually smoked by an individual per day, the depth of inhalation and the type of cigarettes smoked as all these factors modify the actually level of nicotine in blood. As these levels rise on a consistent basis then the chances of hypertension will progressively increase.

3.02 Absorption and metabolism of nicotine.
Numerous studies have shown that smoking or chewing tobacco increases blood pressure and blood pressure falls when an individual stops smoking. Nicotine is easily absorbed from the respiratory tract, and also through tissues within the oral cavity and can even pass through the intact skin as is shown by transdermal drug delivery systems.

The routes of metabolism of the absorbed nicotine, by whatever route it gains access, are well documented. Approximately 80% to 90% of nicotine is metabolized in the liver, kidneys and lungs. The lungs metabolize a major portion of inhaled nicotine. The major metabolites of nicotine are cotinine and (see above) nicotine-N-oxide. The half-life of nicotine after inhalation or injection administration is about 2 hours. The kidney eliminates both nicotine and its by-products. The most important aspect about elimination of nicotine via the kidney is that its excretion is pH dependent. The rate of urinary excretion of nicotine is dependent on the pH of the urine. Excretion is reduced when the urine is alkaline and its excretion is increased when urine is acidic. Nicotine is also excreted in the milk of lactating women who smoke. Mammary milk of heavy smokers may contain 0.5 mg of nicotine per liter of milk (Herraiz et al., 2005).

Absorption of nicotine through cellular membranes depends on the pH within the tissue. If the pH is acidic, nicotine is ionized and does not easily pass through cellular membranes. At physiological pH (pH = 7.4), 31% of nicotine is not ionized and easily passes through membranes (Walsh, et al., 2008; Wullner et al., 2008).

Tobacco smoke has an acidic pH, and this acidity only allows a little absorption in the mouth. Inhalation is therefore necessary to allow nicotine to be absorbed by the huge surface area of alveolar epithelium. In the lungs, nicotine is quickly absorbed into the systemic circulation. This absorption is easy because the blood flow is high in the lung capillaries: a volume equal to the total blood volume of the body flows through each
minute. So, the rate of nicotine progressively increases when a cigarette is smoked. Absorbed nicotine in the systemic circulation is then rapidly distributed among all the organs and it reaches the brain within only ten seconds.

3.03 Active form of nicotine.

The active form of nicotine is a cation whose charge is located on the nitrogen of the pyrrolidine ring not on the pyridine ring (see Figure 1 page 19). This active form is very close in structural terms to acetylcholine (chemical formula CH₃COOCH₂CH₂N⁺(CH₃)₃ and its systematic name, 2-acetoxy-N,N,N-trimethylethanaminium.). It has been demonstrated that nicotine mimics the effects of acetylcholine, which is one of the major neurotransmitters of the brain. Acetylcholine can bind to two different kinds of receptors: nicotinic receptors, which derive their name from being activated by nicotine, and muscarinic receptors, which derive their name from being activated by muscarine. Nicotine and muscarine are thus specific agonists of the two kinds of cholinergic receptors - namely nicotinic and muscarinic (Nguyen et al., 2003).

Nicotine competitively binds to nicotinic cholinergic receptors. The binding of the agonist to the nicotinic receptor triggers off a conformation change of the architecture of the receptor, which opens an ion channel for a few milliseconds. This channel is selective for cations, especially sodium. The opening of the ion channel leads to a brief depolarization. (see Figure 9 (a) and (b) below) Then, the channel closes and the receptor transitionally becomes refractory (a) and (b) to agonists. This is the state of desensitization. Then, the receptor usually goes back to a state of rest, which means that it is closed and is again sensitive to the agonists. In case of continuous exposure to agonists, even in small doses, this state of desensitization will last a longer time, where it is known as - long-term inactivation (Pons et al., 2008).
3.04 Operating cycle of a nicotinic receptor.

Figure 9a. Processes involved in nicotine stimulation of a nicotinic receptor.

In physiologically normal conditions after the opening of the channel by its binding of the neurotransmitter acetylcholine, the receptor becomes desensitized before it goes back to the state of rest or it is regenerated.

Figure 9b. Operating cycle of a nicotinic receptor. (Website, 2011)

If the receptors are exposed ‘continuously’ to nicotine in tobacco the nicotine molecules ‘substitute’ for acetylcholine and this high concentration then over stimulates
the nicotinic receptors. Then, the receptor is subjected to long-term inactivation and its effective regeneration is prevented by nicotine (Prinz, 1988).

3.05 Nicotine causes a ‘pleasurable effect’ in the smoker.

As explained above nicotine causes the stimulation of nicotinic receptors. If this stimulation is excessive then the chronic activation of these receptors is balanced by a down-regulation in the number of active receptors. This effective reduction of the number of active receptors reduces the psychotropic effect of nicotine. Due to the additional phenomenon of tolerance, the smoker needs to smoke more and more cigarettes to keep a constant stimulatory effect.

Nicotine activates the complex arrangement of the dopamine systems within the brain. Dopamine is a neurotransmitter which is directly responsible for mediating many effects in brain, one of which is the ‘pleasure response’. Nicotine triggers the production of dopamine in the nucleus accumbens. A prolonged exposure of these receptors to nicotine reduces the efficiency of dopamine by cutting down the number of available receptors. Consequently, more and more nicotine is needed to give the same pleasurable effect (Kenny et al., 2006).

After a brief period of abstinence, overnight for instance during the hours of sleep, the brain concentration of nicotine declines due to its metabolism and excretion and this effective reduction in concentration allows a part of the nicotinic receptor population to recover their sensitivity. The return to an active state raises the ability of the neurotransmission to function and this is what the smoker feels to be an ‘abnormal state’ and because of this the smoker feels uncomfortable, and so to return to the more pleasurable state it induces him to smoke again. It is said that the first cigarette of the day
is the most pleasant because the sensibility of the dopamine receptors is maximal. Then, the receptors are soon desensitized the pleasurable feelings ‘wear off’. This is best summarized as the vicious circle of smoking.

3.06 Chemical changes induced by nicotine – potential carcinogenic effects.

Nicotine is mainly transformed in the liver, but is can also occur in the lungs and the kidneys. The primary metabolites of nicotine are nicotine N-oxide and cotinine (see figure 10 below), which are some of the products of the hepatic oxidation of nicotine by cytochrome P-450.

**Figure 10.** The structures of the nicotine metabolites – nictotine N oxide and cotinine, as compared against cytochrome P 450. (Guillem et al., 2005)

A very worrying aspect of nicotine and its metabolites is that it is considered by many to be a strong carcinogen. In fact, nicotine can undergo several kinds of metabolic transformation such as the pyrrole ring opening. The methyl group on this open ring structure can then become a very powerful alkylating agent when removed from the cycle (Guillem et al., 2005).

An additional potential carcinogenic effect may be caused by the amine function of nicotine which may react with nitrogen monoxide or with nitrous acid in order to form a
"nitrosonium" type molecule. This compound may then be transformed by the body, which means oxidized and opened. This opening leads to two isomers, two "nitrosamino" type molecules (R2N-N=O) where one of the two R group is a methyl (Oshida et al., 1994). This reaction occurs as shown in Figure 11.

**Figure 11.** The pathway of metabolism of nicotine during the transformation of the molecule to form the open pyrrole ring structure which may act as an alkylating agent. (Oshida et al., 1994)

A = 4 (N-methyl-N-nitrosamino)-1-(3-pyridyl)-butan-1-one

B = 4 (N-methyl-N-nitrosamino)-4-(3-pyridyl)-butanal
In acidic medium, the oxygen of the "nitrosamino" group is protonated and the double bond moves to the central nitrogen, which becomes positively charged. This new molecule is a methyl source. The "nitrosamino" group can then react with another amine, which removes the positive charge from the nitrogen. If the amine that reacts is a part of the structure of the DNA, then the irreversible alkylation of the DNA occurs as shown in Figure 12.

**Figure 12.** The pathway of metabolism of nicotine during the transformation of the molecule to form the "nitrosamino" group which can react with another amine (Richard, 1994).

This alkylation is potentially really damaging and may help in the development of cancer as it prevents the normal development of the cell. (Leoppky, 1994; Villegier et al., 2003)
3.07 Other pharmacological effects of nicotine and its metabolites.

As nicotine enters the body, usually by the pulmonary circulation, it is distributed quickly through the bloodstream and can rapidly cross the blood-brain barrier. On average it takes about seven seconds for the substance to reach the brain when it has been inhaled in the form of cigarette smoke. The half life of nicotine in the body is around two hours (Cohen et al., 2001; Benowitz et al., 2004; Hukkanen et al. 2005; Nolley et al., 2006).

The amount of nicotine actually absorbed by the body from smoking cigarettes depends on many factors, including.

A- The type of tobacco and its nicotine content – or even added content.

B- How the smoke is inhaled and the depth of actual inhalation.

C- Whether a filter is used on the cigarette

For chewing tobacco, dipping tobacco, sinus and snuff, which are held in the mouth between the lip and gum, or taken in the nose, the amount released into the body tends to be much greater than smoked tobacco.

3.08 Nicotine metabolism.

Once absorbed the nicotine is metabolized in the liver by cytochrome P450 enzymes (mostly CYP2A6, and also by CYP2B6). A major metabolite is cotinine (See figure 10). (Green et al., 2000). Other primary metabolites include nicotine N’-oxide, nornicotine, nicotine isomethonium ion, 2-hydroxynicotine and nicotine glucuronide. It is interesting to note that the processes of gluconuration and oxidative metabolism of nicotine to cotinine are both inhibited by menthol, which is used as an additive to ‘mentholated cigarettes’, thus actually increasing the half-life of nicotine in vivo.
Nicotine acts on the nicotinic acetylcholine receptors, specifically the ganglionic type nicotinic receptor and one CNS nicotinic receptor. The former is present in the adrenal medulla and elsewhere throughout the ganglia of the autonomic nervous system, while the latter is present in the central nervous system (CNS). In small concentrations, nicotine increases the activity of these receptors. Nicotine also has complex effects on a variety of other neurotransmitters for example: acetylcholine, serotonin, dopamine, norepinephrine, L-dopa through less direct mechanisms (Hjern et al., 2001).

By binding to nicotinic acetylcholine receptors, nicotine increases the levels of several neurotransmitters - acting as a sort of "volume control". It is thought that increased levels of dopamine in the reward circuits of the brain are responsible for the euphoria and relaxation and eventual addiction which is caused by nicotine consumption. Nicotine has a higher affinity for acetylcholine receptors in the brain than those in the skeletal muscle which is very fortunate as the stimulation of these receptors at what are toxic doses of nicotine can induce unwanted contractions and eventually respiratory paralysis. Nicotine's selectivity in these two receptor types is thought to be due to a particular amino acid difference on these receptor subtypes (Fratiglioni et al., 2000).

In addition to the nicotine in tobacco smoke there are also other perhaps less expected constituents. For example it contains a range of molecules which are able to inhibit monoamine oxidase and these include: harman, norharman, anabasine, anatabine, and nornicotine. These compounds significantly decrease MAO activity in smokers (Gillbert et al., 2001; Jean, 2001). Since MAO enzymes break down monoaminergic neurotransmitters such as dopamine, norepinephrine, and serotonin, nicotine can affect a multiplicity of physiological systems (Aggarwal et al., 2006).
Nicotine also activates the sympathetic nervous system, acting via splanchnic nerves to the adrenal medulla, which when stimulated releases epinephrine. Acetylcholine released by preganglionic sympathetic fibers of these nerves acts on nicotinic acetylcholine receptors, causing the release of epinephrine (and norepinephrine) into the bloodstream. By binding to the ganglion type nicotinic receptors in the adrenal medulla nicotine increases the release of epinephrine (adrenaline), which acts as a stimulating agonist throughout the body. By binding to nicotinic receptors it causes cell depolarization and an influx of calcium through voltage-gated calcium channels. Calcium triggers the exocytosis of chromaffin granules and thus the rapid release of epinephrine and norepinephrine into the bloodstream. The release of epinephrine (adrenaline) causes an increase in heart rate, blood pressure and respiration, as well as higher blood glucose levels (Suemaru et al., 2008).

Nicotine also has an affinity for melanin-containing tissues due to its precursor function in melanin synthesis or its irreversible binding of melanin and nicotine. This has been suggested to underlie the increased nicotine dependence and lower smoking cessation rates in darker pigmented individuals (Aguilar et al., 2005).

In contrast to the brief half life of nicotine, cotinine one of the metabolites of nicotine, remains in the blood for up to 48 hours. It can therefore be used as a very useful indicator of a person’s exposure to nicotine and can be used as a check to see if an individual is in fact currently taking nicotine containing products.

3.09 **Stimulant, relaxant and addictive effects of nicotine.**

There are many reports of how nicotine's mood-altering effects are experienced in different people as it can be both a stimulant and a relaxant (Okamoto et al., 1994).
First by causing a release of glucose from the liver and epinephrine (adrenaline) from the adrenal medulla, it causes stimulation. Users report feelings of relaxation, sharpness, calmness and alertness. By reducing their appetite and raising the overall metabolic rate, some smokers may lose weight as a consequence (De Luca et al., 2004) and when they cease smoking the well documented problem of weight gain has been reported.

When a cigarette is smoked, nicotine rich blood passes from the lungs to the brain and within seven seconds gains access to the brain where it immediately stimulates the release of many chemical messengers including: acetylcholine, norepinephrine, epinephrine, vasopressin, arginine, dopamine, autocrine agents, and beta-endorphin. This release of a complex range of neurotransmitters and hormones is responsible for most of nicotine's effects. Amongst many other effects nicotine appears to enhance concentration and memory due to the increase of acetylcholine release (Aguilar et al., 2005). It also appears to enhance alertness due to the increases of both acetylcholine and norepinephrine.

Arousal is increased by the increase of norepinephrine. Pain is reduced by the increases of acetylcholine and beta-endorphin. Anxiety is reduced by the increase in the release of beta-endorphin. Nicotine also extends the duration of positive effects of dopamine and increases sensitivity in brain reward systems. Research suggests that, when smokers wish to achieve a stimulating effect, they take short quick puffs, which produce a low level of blood nicotine. This stimulates nerve transmission. When they wish to relax, they take deep puffs, which produce a high level of blood nicotine, which depresses the passage of nerve impulses, producing a mild sedative effect. At low doses, nicotine potently enhances the actions of norepinephrine and dopamine in the brain, causing a drug effect typical of those of psychostimulants. At higher doses, nicotine enhances the effect of
serotonin and opiate activity, producing a calming, pain-killing effect. Nicotine is unique in comparison to most drugs, as its profile changes from stimulant to sedative/pain killer in increasing dosages and use, another drug that behaves in a similar way is ethanol (Suemaru et al., 2008).

Theoretically, nicotine is not significantly addictive, as nicotine administered alone does not produce significant reinforcing properties. However, after co-administration with an MAOI, such as those found in tobacco, nicotine produces significant behavioral sensitization, which is a measure of addiction potential. This is similar in effect to amphetamine (De Luca et al., 2004).

Modern research shows that nicotine acts on the brain to produce a number of effects. Specifically, its addictive nature has been found to show that nicotine activates reward pathways the circuitry within the brain that regulates feelings of pleasure and euphoria. (Reuters Health, 2001)

Dopamine is one of the key neurotransmitters actively involved in the brain. Research has shown that by increasing the levels of dopamine within the reward circuits in the brain, nicotine acts as a chemical with intense addictive qualities. In many studies it has been shown to be more addictive than cocaine and heroin, though chronic treatment has an opposite effect on reward thresholds (Fratiglioni et al., 2000; Aguilar et al., 2005; Fallon et al., 2005).

In a similar way to other physically addictive drugs, nicotine causes down-regulation of the production of dopamine and other stimulatory neurotransmitters as the brain attempts to compensate for artificial stimulation. In addition, the sensitivity of nicotinic acetylcholine receptors decreases. To compensate for this 'compensatory
mechanism’, the brain in turn ‘up regulates’ the number of receptors, convoluting its regulatory effects with such compensatory mechanisms is meant to counteract other compensatory mechanisms. The nett effect is an increase in reward pathway sensitivity, opposite of other drugs of abuse such as cocaine and heroin, which reduce reward pathway sensitivity. This neuronal brain alteration persists for months after administration ceases. Due to an increase in reward pathway sensitivity, nicotine withdrawal is relatively mild compared to alcohol or heroin withdrawal.

It is interesting to note that nicotine also has the potential to cause dependence in many animals other than humans. Mice which have been administered nicotine exhibit withdrawal reactions when its administration is stopped. A study found that nicotine exposure in adolescent mice retards the growth of the dopamine system, thus increasing the risk of substance abuse during adolescence (Alok, 2005; Lampl et al., 2007; Tfelt, 2008).

3.10 Nicotine and its effects on the cardiovascular system.

Nicotine via ‘indirect mechanism’ has very powerful effects on arteries throughout the body. Nicotine is clearly a stimulant, as it raises blood pressure by releasing vasoconstrictors which increases total peripheral resistance, increasing the work load on the heart to effectively pump through the ‘constricted arteries and arterioles’. It also causes the body to release its stores of fat and cholesterol into the bloodstream (Steiner et al., 2003; Oldstein et al., 2006; Lampl et al., 2007).

Nicotine also has a series of complex effects on the blood coagulation system. It has been speculated that nicotine increases the risk of blood clots by increasing plasminogen activator inhibitor-1, though this has not been actually proven. Plasma
fibrinogen levels are certainly elevated in smokers and are further elevated during acute COPD exacerbation, which may be one of the consequences of cigarette smoking. Also Factor XIII, which stabilizes fibrin clots, is increased in smokers. But neither of these two effects has been shown to be a direct cause blood clot formation in smokers (Hukkanen et al., 2005). However, peripheral circulation in arterioles going to the extremities is also highly susceptible to the vasoconstrictor effects of nicotine and this combined with the potential increased risk of alterations of factors within the blood coagulation or fibrinolytic system is a serious cause for concern (Gaciong, 2003).

The effects of nicotine need to be taken into account when considering the renin-angiotensin-aldosterone system which is a series of reactions designed to help regulate blood pressure. When blood pressure falls, for systolic, to 100 mm Hg or lower, the kidneys release the enzyme renin into the bloodstream, renin splits angiotensinogen, from a large plasma protein that circulates in the bloodstream. This angiotensinogen is then acted upon by by angiotensin-converting enzyme (ACE) to produce a peptide which is called angiotensin I. This is then quickly converted into angiotensin II, primarily via the ACE found in the pulmonary bed. Angiotensin II is extremely potent on many physiological systems including causing the muscular walls of small arteries (arterioles) to constrict via stimulation of angiotensin receptors, so potently increasing blood pressure. It also triggers the release of the hormone aldosterone from the adrenal cortex which causes the kidneys to retain salt (sodium) and excrete potassium. It liberates antidiuretic hormone from the pituitary gland, and so conserves circulating volume as it causes sodium to be retained, thus increasing blood volume and blood pressure (Hersh et al., 2002).
3.11 **Background to the use of aspirin in cardiovascular disease.**

In terms of cost, availability, and usefulness, aspirin is one of the greatest triumphs of medical science. First isolated from willow bark hundreds of years ago, aspirin is now available as a simple over-the-counter formulation. Aspirin is effective as an analgesic, an anti-inflammatory, and has actions against platelets that provide protection against heart attack and stroke. While there is as no clear definitive study which proves that aspirin has a beneficial effect on blood pressure reduction, its protective benefits are said to be so large that routine, daily administration of aspirin is now recommended by the American Heart Association as a standard component of maintaining a healthy heart (Baigent et al., 2009).

Aspirin is a type of chemical called a "salicylate". Simple salicylates have been used as pain and fever reducers since the time of the ancient Greeks, more than 1,500 years ago. While aspirin has a large number of potential actions in the body, those related to 'heart health' are straight-forward and well-understood. In the body, the major effect is thought to be via the inhibition of thromboxane formation and so preventing platelet adhesion and aggregation and so limiting platelet plugs and stimulation of coagulation (Wolff et al., 2009). Since a large number of heart attacks and strokes are directly caused by small, "spontaneously" forming blood clots, the ability of aspirin to prevent the formation of these small clots means that heart attacks and strokes become less likely. Aspirin may help to prevent blood clots forming via its action of inhibiting platelets adhesion onto atheromatous plaques. A clot in an artery may stop blood flowing to the tissues 'downstream'. If a blood clot forms in an artery in the heart or brain, it may cause a heart attack or stroke (Wolff et al., 2009).

The usual dose to prevent thrombus formation is 75 mg each day. This is a significantly less than the dose for pain relief which is 300mg. Taking more than the
recommended does not make the aspirin work any better as to achieve its selective effect on thromboxane production it is said a greater dose reduces the production of prostacyclin which acts as an ‘anticogulant prostanoid’. The higher doses also increase the risk of side-effects developing. If low-dose aspirin is taken to prevent blood clots to achieve analgesia for other purposes for example, headaches, paracetamol is the drug of choice, rather than a higher dose of aspirin (Bachert et al., 2005).

People with known cardiovascular diseases usually suffer diseases of the heart or vasculature, and in practice, this means those diseases caused by the formation of vascular atheroma. The consequences of atheroma are well known and include: heart attack, angina, stroke, transient ischemic attack (TIA) and peripheral vascular disease (Eccles et al., 2003). Everybody has potentially some risk of developing atheroma that may cause one or more of the above cardiovascular diseases. However, certain "risk factors" increase the risk for individual. These include: high blood pressure, high cholesterol level, smoking, lack of exercise, obesity, an unhealthy diet, excess alcohol, a strong family history, certain ethnic groups, and being male. All these factors are compounded if a person also has diabetes as they have an increased risk of developing cardiovascular diseases for factors other than those listed above. If someone has diabetes he/she will normally be advised to continuously take low dose aspirin.

Although it is usually for atheromatous disease that aspirin is taken, it is not exclusively for this disease. For example, in atrial fibrillation (AF), people have an increased risk of forming a blood clot which can cause a stroke, so some people with AF take warfarin to prevent blood clots forming and some also take aspirin (Hashkes et al., 2003).
For people with existing cardiovascular diseases, and those at high risk of developing cardiovascular diseases, there is said to be great benefits from taking aspirin. Several studies involving thousands of people have proved that the risk of having a heart attack or stroke is much reduced in these people if they take aspirin. For example, the risk of having a non-fatal heart attack is reduced by about a third. The risk of having a non-fatal stroke is reduced by about a quarter. The risk of dying is reduced by about a sixth (Bosetti et al., 2006).

There is a small risk of developing serious side effects, when taking aspirin but the benefits far outweigh the small risk. However, for healthy people with a low health risk, even the small risk of side effects from aspirin is perhaps not always acceptable. The side effects of aspirin may sometimes be over emphasized since most people do not have any side effects with low dose aspirin. Consequently the benefits of taking aspirin usually outweigh the small risk of developing side effects.

As a rule, 75mg of aspirin is usually the preferred dose of drug to prevent thrombus formation. Sometimes an alternative such as lower doses, 25mg of aspirin, is used if there is a problem with using aspirin. Sometimes, aspirin plus another antiplatelet drug are taken together. For example, after having a stroke a combination of low-dose aspirin plus dipyridamole is often prescribed for up to two years. In this situation, after two years the dipyridamole is stopped and the antiplatelet treatment is continued with low-dose aspirin alone. The use of dipyridamole is interesting since its effects are not due to the inhibition of prostaglandin formation but rather an inhibition of adenosine uptake and cGMP phosphodiesterase activity. It has little effect by itself and so should always be given in combination with aspirin. In such formulations it can be administered at a dose as low as 25mg of aspirin (Menezes et al., 2006).
3.12 Effects on Platelets.

Aspirin acetylates a serine hydroxyl group near the active site, of the cyclo-oxygenase enzyme which prevents arachidonate binding. The inhibition by aspirin is irreversible as its acetylates the active site. However, in most body cells re-synthesis of PGH$_2$ synthesis would restore cyclo-oxygenase activity (see figure 13) but this is impossible in platelets which do not have a re-synthesis capability (Baron et al., 2003).

Thromboxane A$_2$ is an extremely potent stimulatory factor in the process of blood platelet aggregation, which is essential to the role of platelets in thrombus formation prior to blood clotting actually occurring. Many people take a daily aspirin for its anti-clotting effect, attributed to inhibition of thromboxane formation in blood platelets. This effect of aspirin is long-lived, because platelets cannot make supplies of the inhibited enzyme since they lack a nucleus and so the ability to make new enzyme is impossible. Since they only 'live' for 8-10 days this inhibition is usually not a major problem since new platelets are produced on a continuous basis.

![Figure 13](image.png)

**Figure 13.** Aspirin acetylates the hydroxyl group of the cyclo-oxygenase which inhibits the binding arachidonate and so inhibiting the generation the hydroperoxides which are then converted to thromboxane and prostanoids.
Two isoforms of PGH₂ synthesis are designated COX-1 and COX-2 (Cyclo-oxygenase 1 & 2) (see Figure 13). COX-1 is constitutively expressed at low levels in many cell types. (Baron et al., 2003; Andrew et al., 2004). COX-2 expression is highly regulated as the transcription of the gene for COX-2 is stimulated by growth factors, cytokines, and endotoxins. COX-2 expression may be enhanced by cAMP, and in many cells PGE₂ is produced as a result of COX-2 activity which itself leads to changes in cAMP levels.

Though both cyclo-oxygenase isoforms catalyze PGH2 formation, differing localization within a cell, and localization of enzymes that convert PGH2 into particular prostaglandins or thromboxanes, may result in COX-1 and COX-2 yielding different ultimate products (see fig. 13) (Akhmedkhanov et al., 2002). COX-1 is essential for thromboxane formation in blood platelets, and for maintaining integrity of the gastrointestinal epithelium. Inflammation is associated with up-regulation of COX-2 and increased formation of particular prostaglandins. COX-2 levels increase in inflammatory diseases such as arthritis (Moysich et al., 2002).

An increased COX-2 expression is seen in some cancer cells. Angiogenesis (blood vessel development), which is essential to tumor growth, requires COX-2. Over expression of COX-2 leads to increased expression of vascular endothelial growth factor (VEGF). Regular use of NSAIDs has been shown to decrease the risk of developing colorectal cancer. (Baron et al., 2003; Andrew et al., 2004).

3.13 Aspirin and its site of action.

Aspirin’s ability to suppress the production of prostaglandins and thromboxanes is due to its irreversible inactivation of the cyclo-oxygenase (COX) enzyme. Cyclo-oxygenase is required for prostaglandin endoperoxide, thromboxane and prostanoid
synthesis (Sanda et al., 2009). Aspirin covalently acetylates a serine residue at COX enzyme active site where arachidonic acid has to attach itself for a cyclization process in presence of molecular oxygen for converting itself to PG endoperoxides (see Figure.13). Aspirin is different from other NSAIDs, for example, diclofenac and ibuprofen, which are reversible inhibitors of the active site of COX-2 whereas aspirin is irreversible. This irreversible inhibition blocks the production of prostaglandins, molecules which have both positive and negative effects in the body. Prostaglandins, also, have protective effects against the development of stomach ulcers, but they can also mediate inflammation as well as the pain response. Therefore, ibuprofen and aspirin both inhibit COX-1 and COX-2, but in different ways. Ibuprofen does not covalently bind to COX enzymes and competes with the enzyme natural substrate, this is called ‘ reversible inhibition’. On the other hand, aspirin forms a covalent bond to a serine residue in the enzyme, and this bond cannot be broken, this is "called irreversible inhibition" (Anggard et al., 1965).

Figure 14. (a) Structure of serine residue present at the active site of COX-2
(b) Structure of aspirin
(c) Structure of tyrosine residue present at the active site of COX-2
(d) Structure of ibuprofen (Mehta et al., 2005).
The mechanism of both residues of amino acids serine and tyrosine (Figure 14) with aspirin and ibuprofen is the same because the reactive site of both enzymes is the same and that is the \(-\text{OH}\) group. Although the reaction of binding with tyrosine is slow due to the aromatic ring, here the electron donating effect of \(-\text{OH}\) decrease the availability of electron or free radical for esterification reaction and also the ‘big structure’ causes steric hindrance which also decreases the binding to tyrosine. The platelet prostaglandin G/H synthase-1 (cyclooxygenase-1) is depicted as a dimer. The arachidonic acid substrate gains access to the catalytic site (red area) through a hydrophobic channel that leads into the core of the enzyme. (Figure 15a) aspirin blocks the access of arachidonic acid to the catalytic site by irreversibly acetylating a serine residue at position 529 in platelet cyclooxygenase-1, near but not within the catalytic site. (Figure 15b) interpolation of the bulky acetyl residue prevents metabolism of arachidonic acid into the cyclic endoperoxides PGG2 and PGH2 for the lifetime of the platelet. Because PGH2 is normally metabolized by thromboxane synthase into thromboxane A2, aspirin prevents the formation of thromboxane A2 by the platelets until new platelets are generated. Nonsteroidal anti-inflammatory drugs, such as ibuprofen, are reversible, competitive inhibitors of the catalytic site (Figure 15c) whose use results in the reversible inhibition of thromboxane A2 formation during the dosing interval. Prior occupancy of the catalytic site by ibuprofen prevents aspirin from gaining access to its target serine (Francesca et al., 2001).
Figure 15. Effect of Aspirin Alone and of Ibuprofen plus Aspirin on Platelet (source: Francesca et al., 2001).

3.14 Aspirin induced bronchospasm

Cysteinyl-leukotrienes (LTs) are derived from arachidonic acid via the 5-lipoxygenase (LO) pathway (Figure 16). The cellular biosynthesis of LTs involves 5-LO activating protein, which transports arachidonic acid into the cytosol to be acted on by the enzyme 5-LO. The sequential catalytic action of 5-LO on arachidonic acid yields LTA4, which is further hydroxylated to LTB4 or is converted into the first of the cysteinyl LTs, LTC4, by LTC4 synthesis (Figure 16). LTC4 is exported to the extracellular space where it forms LTD4, which in turn is cleaved to form the 6-cysteinyl analog of LTC4 known as LTE4. The cysteinyl LTs exert their biological action by binding to two types of G-
protein-coupled 7-transmembrane receptors, cysteinyl-leukotrienes (LT1) and CysLT2 (Kathrina et al., 2005).

Figure 16. Leukotriene synthesis (Sala et al., 2009).

Aspirin-induced bronchoconstriction is thought to be caused by the shunting of the arachidonic acid metabolism away from the cyclooxygenase (COX) pathway toward the lipoxygenase (LO) pathway (Hopkines et al., 1989). This results in the increased production of cysteinyl-leukotriene (LTs) with the resultant bronchoconstriction.
Consistent with this finding, bronchoconstriction in patients with aspirin induce asthma (AIA) can be inhibited by cysteinyll– leukotriene (LT) receptor antagonists (Delanay et al., 2007; Dorsh et al., 2007; Chan et al., 2009).

Provocations with aspirin in (AIA) patients produces airflow obstruction accompanied by the release of cysteinyll LTs into the urine and blood alcohol level (BAL) fluid. LTC4 synthesis is the rate-limiting enzyme for the synthesis of cysteinyll LTs. Bronchial biopsy studies have revealed an over expression of LTC4 synthesis in patients with AIA as compared to aspirin-tolerant asthma (ATA) patients (Guitton et al., 2003; Benowitz et al., 2004; Hukkanen et al., 2005; Gorelick, 2009).

The gene for LTC4 synthesis has been localized to chromosome 5q, telomeric to other candidate genes, including interleukin (IL)-3, IL-4, IL-5, and granulocyte macrophage colony-stimulating factor, which also have been implicated in asthma pathogenesis. A genetic variant of LTC4 synthesis gene promoter has been described, which is over expressed in the AIA population. However, 30% of patients with AIA do not have a predisposing variant of the LTC4 synthesis gene, whereas 25% of the control subjects do have it without any consequence to their health (Moysich et al., 2002). Although this will not explain the pathophysiology of AIA in all patients in the population, such a finding is common in conditions with multifactorial inheritance and is predictable on the basis of nonmendelian low inheritance of AIA. The normal expression of 5-LO in patients with AIA precludes 5-LO as a contributing factor in the pathogenesis of AIA.

Over expression of the LTC4 synthesis in the bronchial wall may be the single most important determinant of acute respiratory reactions to aspirin in subjects with AIA. In addition, the removal of the prostaglandin (PG) E2 brake in all subjects by NSAIDs, as described later, leads to exaggerated cysteinyll LT synthesis only in AIA patients due to the
altered threshold activity of LTC4 synthetase in their bronchial wall. Some common medicines for example and their advantages over aspirin have been described to provide a clear view on the limitations of aspirin in treating high blood pressure (Vane et al., 2003; Paul-Clark et al., 2004; McCarthy et al., 2006).

3.15 Diuretic effect of aspirin.

Diuretics are meant to remove sodium (ions) and with then the necessary osmotic equivalent of water from the body which is responsible for the increase of blood volume in the body. As far as it is known aspirin does not have a reported diuretic action in man.

3.16 The audit the current effective of aspirin and hypotensive agents as compared with aspirin.

Beta-blockers help to reduce blood pressure by reducing heart rate, which is completely different from the reported effects of aspirin. Similarly, there are ACE, alpha blockers, and calcium blockers which have specific purposes in reducing blood pressure. All the above-mentioned medicines are prescribed to target known factors in the systems which elevate blood pressure. So, the perhaps on the available evidence the answer to the question ‘will aspirin lower blood pressure’ is a guarded “no”. But, it may be prescribed to have an effect on elements within the blood, namely clotting factors and platelets which may facilitate the actual effectiveness of antihypertensive drugs. In a report almost ten years ago it was thought that aspirin achieved such an ‘antihypotensive effect’ (Dargan et al., 2002).
3.17 Aims and objectives.

So, taking all these factors into consideration the basis of our thesis can be determined.

**Aim:** To determine if the use of aspirin in cardiovascular disease is beneficial to those people who suffer hypertension in Kuwait especially those who smoke cigarettes

**Objectives:**

1. To undertake a series of experiments investigating nicotine levels and their effects in hypertensive subjects (Chapter 4)

2. To investigate the effectiveness of aspirin in reducing risks (Chapter 5)

3. To investigate the ambulatory blood pressure of hypertensive patients and their treatment with aspirin (Chapter 6)

4. To audit the curative effect of aspirin and a range of cardiovascular drugs in the state of Kuwait hospitals (Appendix 1)
Chapter Four

An experimental investigation of nicotine levels in smokers.

Experimental investigation of nicotine levels and their effects in hypertensive subjects the objectives of which is to use the urinary levels as an index as to what levels of nicotine can be currently found in smokers in Kuwait (see page 104)

4.01 Background.

The relation of nicotine and circulatory diseases is extremely complex because of its (nicotine) ability to stimulate a rise in blood pressure, act as a vasoconstrictor, as well as increasing the utilization of stored body fats and so stimulating the appetite so amongst many other things, the smoker feels hungry (Dominguezdes et al., 1981).

Another of the very important aspects induced by nicotine, which has been observed in the blood of smokers, is the enhanced ability of their blood to clot. Central to blood clotting is the role of an important regulatory protein known as named as plasminogen activator inhibitor-1, stimulation of which prevents the normal activation of plasminogen which breaks down the fibrin formed in the coagulation process in a very precise negative feedback mechanism which enables the clot to be dynamically modified during the complex process and blood coagulation and dissolution by the process of fibrinolysis induced by plasmin formed from plasminogen. The suggestion has been made that nicotine has the ability to trigger the faster release of plasminogen activator inhibitor-1 and also increasing the production of fibrinogen. A combination of these two prominent procoagualant effects will potentially cause an increased risk of clotting to occur and an inability of the plasminogen-plasmin system to be activated which would normally bring
about the resolution of the clot. This is not just a hypothetical possibility as this change of a faster rate of blood clotting has frequently been observed in blood of smokers (Linberg et al., 1998). The situation is even more complex since plasma fibrinogen levels which are elevated in smokers are further elevated during acute COPD exacerbation which as has been described above occurs due to cell damage caused by the constituents of cigarette smoke (see above).

Furthermore, the problems of coagulation are made worse by the finding that Factor XIII, which stabilizes monomeric fibrin into cross linked polymeric fibrin, is also increased in smokers the greater activity of which may increase the stability of any clot which is formed. This would make it more difficult to resolve by the plasminogen-plasmin system. All these potential blood clotting problems can influence the way in which blood flow can be compromised and this may feed back to cause elevations in the systemic arterial pressure due to restrictions of flow caused by thrombi formations.

In addition to the serious coagulation problems, the arteries which supply the extremities are also highly susceptible to the vasoconstrictor effects of nicotine as well as the increased risk of blood clots and cessation of flow. A transient increase in blood pressure, followed by bradycardia, paroxysmal atrial fibrillation, or cardiac standstill have all been observed in smokers (Johnson et al., 1995). Many of these effects reflect problems with the blood vessels of the heart and nicotine contributes both to the atherosclerotic process and to acute coronary events by several mechanisms. Nicotine could promote atherosclerotic disease by its actions on lipid metabolism and enhanced coagulation, by modification of hemodynamic effects and/or by causing endothelial injury. Nicotine may act by releasing free fatty acids, enhancing the conversion of VLDL (very low density lipoproteins) to LDL (low density lipoproteins), so impairing the clearance of LDL and/or
by accelerating the metabolism of HDL. Nicotine could also affect platelets by increasing the release of epinephrine, which is known to enhance platelet reactivity by inhibiting the production of the anti-aggregatory hormone, prostacyclin, which is normally secreted by endothelial cells, or perhaps by a direct action. Alternatively, by increasing heart rate and cardiac output and thereby increasing blood turbulence nicotine may promote endothelial injury.

Amongst the other cardiac effects of nicotine it also facilitates AV nodal conduction which could result in an increased ventricular response during atrial fibrillation. Nicotine could aggravate peripheral vascular disease by constricting small collateral arteries and/or by inducing local thrombosis. Patients with coronary or peripheral vascular disease are likely to suffer some increase in risk when taken nicotine. Nicotine could contribute to the progression of chronic hypertension by aggravating vasoconstriction either in sympathetic activation or inhibition of prostaglandin synthesis. Based on its pharmacological actions, it is likely that nicotine plays a role in causing or aggravating acute coronary events. Finally, myocardial infarction can be due to one or more of these precipitating factors: excessive demand for oxygen and substrates; thrombosis and coronary spasm all of which are potentially induced by nicotine. Finally, nicotine by increasing heart rate and blood pressure stimulates myocardial oxygen consumption which in a myocardium where there are ‘restricted’ vessels makes the problems even worse (Weber et al., 2001).

These coronary effects of nicotine have been able to be more objectively studied over recent years as nicotine, consumed in the form of nicotine chewing gum, has been used in patients with and without coronary artery disease. Chewing gum containing 4mg of nicotine – usually used in those patients used to more than 20 cigarettes a day – when used
in ‘control’ people who were without coronary artery disease, increased their myocardial contractility. However, in patients with coronary artery disease, nicotine gum decreased the contractility in the ischemic regions of the myocardium, consistent with aggravation of ischemia. In the most severe cases of coronary artery disease, overall contractility decreased after nicotine gum. This supports the idea that nicotine contributes to the induction of myocardial ischemia in susceptible smokers.

Nicotine may also induce coronary spasm by sympathetic activation or inhibition of the vasodilatory effects of prostacyclin. This would agree with the findings that coronary spasm has been observed during cigarette smoking. Sudden cardiac death in smokers might result from ischemia, combined with the arrhythmogenic effects of increased amounts of circulating catecholamine released by nicotine. In addition to creating an imbalance between myocardial oxygen supply and demand, nicotine may actually promote thrombosis.

4.02 Testing of nicotine and its metabolites.

The problem with cigarette use is that although most of its effects may be attributed to nicotine it may be more complicated since this agent is metabolized by the liver into more than 20 compounds, which are excreted by the kidneys into the urine. The primary metabolite is cotinine, and this is widely distributed in the body. Consequently, cotinine is the major metabolite of nicotine and is usually the metabolite of choice to evaluate tobacco use or exposure to tobacco smoke in an individual, because it is stable and is only produced when nicotine is metabolized. Cotinine has a half-life in the body of between 7 and 40 hours, while nicotine has a half-life of 1 to 4 hours. Blood and/or urine cotinine tests may be ordered along with nicotine tests. In some cases, other nicotine metabolites, such as nicotine-1´-N-oxide, trans-3´-hydroxycotinine, or nornicotine, or other tobacco
chemicals, such as anabasine in urine, may also be tested for but cotinine is the most commonly carried out. Clearly the presence of nicotine and/or cotinine in an individual’s biological sample may indicate the use of tobacco products or exposure to environmental tobacco smoke. Testing may be used in a number of situations to evaluate the possible use of tobacco products such as in smoking cessation programs, prospective employment assessments and evaluations of applicants for health or life insurance (Weber et al., 2001; Nishi et al., 2008).

Nicotine and cotinine testing may also be ordered in cases of suspected nicotine poisoning. Acute overdoses of nicotine, such as might happen if a child ingests nicotine lozenges or gum, are relatively rare but generally require immediate medical attention. Symptoms can include a burning mouth, nausea, abdominal pain, salivating (drooling), diarrhea, sweating, confusion, dizziness, agitation, increased heart rate, rapid or difficult breathing, convulsions, coma, and even death. All these are predictable from knowledge of the pharmacological properties of nicotine.

4.03 Collection of samples for nicotine analysis

A venous blood sample is obtained in the usual way by inserting a needle into a vein in the arm and/or a random urine sample is collected. Occasionally, a saliva sample may be obtained – directly or by soaking a collecting ‘cloth’ or swab with saliva. Rarely, a hair sample may be collected. In this study urine was the source used.

4.04 Nicotine test requirement.

In the blood, nicotine levels can rise within a few seconds of a puff on a cigarette. The quantity depends on the amount of nicotine in the cigarette and the manner in which a person smokes, such as how deeply they inhale. Test results are not interchangeable and
are complicated to interpret as concentrations will be higher in urine than in blood or saliva because of the rapid removal and metabolism of the agent. There is also some variability from person to person and some genetic differences in the rate that nicotine is metabolized and also in the rate that cotinine is cleared from the body. When someone stops using tobacco and nicotine products, it can take more than two weeks for blood levels of cotinine to drop to the level that a non-tobacco user would have and several weeks more for urine levels to decrease to very low concentrations (Henningfield et al., 2006).

In general, high levels of nicotine or cotinine indicate active tobacco use or nicotine replacement use. Moderate concentrations indicate a tobacco user who has not had tobacco or nicotine for two to three weeks. Lower levels may be found in a non-tobacco user who has simply been exposed to ‘environmental smoke’ sometimes known as ‘passive smoking’. Very low to non-detectible concentrations are found in non-tobacco users who have not been exposed to ‘environmental smoke’ or a tobacco user who has refrained from tobacco and nicotine for several weeks (Johnson et al., 1995; Linberg et al., 1998).

In Patients who have suffered a nicotine overdose, for whatever reason, the use of the nicotine or cotinine test is somewhat problematic as although the concentrations would be increased the levels do not necessarily correlate with the severity of a person’s physiological symptoms. This makes prediction of the likely events, cardiac or otherwise, somewhat difficult if not impossible in cases of nicotine ‘overdosage’. However nicotine or its primary metabolite, cotinine are most often tested for to measure/evaluate tobacco or its replacement usage. This is useful as it is well documented as has been described above that the long term use of tobacco products can increase the risk of developing many diseases including lung cancer, COPD, stroke, heart disease, and respiratory infections, or
exacerbate asthma, and blood clot formation. Finally, in pregnant women, smoking can retard fetal growth and lead to low birth weight babies (Benowitz et al., 1983).

Although the measurement of blood/urine levels of nicotine is somewhat controversial because the use of tobacco products can greatly affect the health of individuals, companies may use nicotine/cotinine testing to evaluate prospective employees tobacco use. Today, many health and life insurance companies test applicants for nicotine or cotinine as a part of their assessment for insurance cover.

Nicotine and cotinine can both be measured qualitatively or quantitatively. Quantitative testing can help distinguish between active smokers, tobacco users who have recently quit, non-tobacco-users who have been exposed to significant environmental tobacco smoke, and non-users who have not been exposed (Hukkanen et al., 2005). Cotinine may also be measured in saliva and in hair, although hair testing is primarily used in a research setting, such as a study of non-smokers exposure to tobacco smoke. Blood or urine nicotine levels may be ordered by itself or along with cotinine if a doctor suspects that someone is experiencing a nicotine overdose.

When a patient has reported that they are using nicotine replacement products but are no longer smoking, nicotine, cotinine, and urine anabasine measurements may sometimes be ordered. Anabasine is present in tobacco but not in commercial nicotine replacement products. If a sample tests positive for anabasine, then the person is still using tobacco products (Benowitz et al., 2004). Cotinine and/or nicotine may be ordered whenever an evaluation of tobacco use status or tobacco smoke exposure is required. When a person enters a smoking cessation program, blood or urine cotinine tests may be ordered to evaluate compliance. Urine, blood, or saliva testing may be performed as a screen for tobacco use when someone is applying for life or health insurance, or applying
for work with an employer that prohibits smoking. Testing may also be ordered by a court for child custody purposes. Since smoking can increase the risks of medical complications, testing may be performed prior to the start of some drug therapies or surgical procedures (Xinan et al., 2009).

Against this background a series of experiments have been carried out in smokers to determine their urinary levels of nicotine.

4.05 Experimental analysis

Smoking is major health problem throughout the world and to know the exact consequences of smoking it is very important to collect the best and accurate data for experimental analysis because inaccurate, insufficient and poor source of analysis may lead towards to draw wrong conclusions (Fowler et al., 1998). Clinicians and epidemiologists often need an accurate assessment of whether and to what extent a person smokes or otherwise uses nicotine, as relying upon self-reporting by nicotine users regarding their nicotine habits is often inaccurate. In addition, clinicians and epidemiologists may require information concerning inhalation of secondary smoke by non-smokers as well as concerning other passive exposure to nicotine as a result of environmental conditions. It means some people who are not smokers but simply by living close to those people who smoke can develop the same symptoms which have been shown by smokers.

Various assays have been developed to independently obtain such information (Wullner et al., 2008). The most convenient way to analyse the amount or concentration of nicotine in the body of smoker, is to prepare and carry out an analysis of various samples of their urine. The amount of nicotine and/or its metabolites in the urine will reveal a clear
picture of their exposure to this chemical. High performance liquid chromatography (HPLC) has been used to specifically determine the level of a nicotine metabolite(s), in the urine of subjects as the technique will identify and quantify in the same procedure.

4.06 Objectives of using chromatography techniques for the analysis of nicotine and its metabolites.

The following are the objectives of chromatography methods for nicotine analysis as described by Weber et al., (2001).

1. To differentiate active nicotine users from those who do not use nicotine.
2. To differentiate heavy users of nicotine from light users.
3. To detect passive exposure to nicotine such as by secondary smoke inhalation.
4. To detect extremely low levels of nicotine and/or its metabolites in the urine or other body fluids.
5. To detect nicotine and/or nicotine metabolites without the use of any complex instruments.
6. To provide a colorimetric assay system whereby the presence of nicotine can be determined visually.
7. To provide an assay system whereby the presence of nicotine and/or nicotine metabolites can be detected in unprocessed urine.

4.07 Use of the assay for nicotine in subjects who smoke and the Bioavailability of Nicotine

The rapid absorption of nicotine from cigarette smoke through the lungs occurs because of the huge surface area of the alveoli and small airways, and because of dissolution of nicotine at physiological pH (approximately 7.4) which facilitates transfer
across cell membranes. Chewing tobacco, snuff, and nicotine polacrilex gum are formulated at alkaline pH as a result of the selection of appropriate tobacco and/or buffering with additives by the manufacturers. The alkaline pH facilitates absorption of nicotine through mucous membranes (Nishi et al., 2008). Nicotine is a water and lipid soluble drug which, in the free base form, is readily absorbed via respiratory tissues, skin, and the gastrointestinal tract. Nicotine may pass through skin or mucous membranes when in alkaline solution (in which nicotine is largely unionized). When tobacco smoke reaches the small airways and alveoli of the lung, the nicotine is rapidly absorbed (Newton et al., 2006).

After absorption, nicotine enters the blood where, at pH 7.4, it is about 70% ionized and binding to plasma proteins is less than 5%. Studies showed that, after intravenous administration, the distribution of C14-labeled nicotine is immediate, reaching the brain of mice within 1 min. after injection. Similar findings based on positron emission tomography of the brain, were seen after injection of 11C-nicotine in monkeys. Nicotine inhaled in tobacco smoke enters the blood almost as rapidly as after rapid I.V. injections. Because of delivery into the lung, peak nicotine levels may be higher and lag time between smoking and entry into the brain shorter than after IV injection. Consequently, after smoking, the action of nicotine on the brain is expected to occur very quickly (Benowitz et al., 1982; Henning Field et al., 2006).

This rapid onset of effects after inhalation of cigarette smoke is believed to provide optimal reinforcement for the development of drug dependence. The intensity of the effect of nicotine declines as it is distributed to other tissues. The distribution half-life, which describes the movement of nicotine from the blood and other rapidly perfuse tissues, such as the brain, to other body tissues, is about 9 min. Distribution kinetics, rather than
elimination kinetics (half-life about 2hr) determine the time course of the CNS actions of nicotine after smoking a single cigarette. The apparent volume of distribution in animals is approximately 1.0 L/kg whereas in one clinical study it was 2.0 L/kg in smokers and 3.0 L/kg in nonsmokers.

The effective biological half-life of nicotine averaging 2 hours is useful in predicting the rate of accumulation of the drug in the body with repetitive dosing and the time course of decline after cessation of dosing. Consistent with a half-life of 2 hours, accumulation of nicotine over 6 to 8 hours during regular smoking and persistence of significant levels of nicotine in the blood for 6 to 8 hours after cessation of smoking, i.e. overnight, has been observed (Benowitz et al., 2004; Hukkanen et al., 2005). Thus, heavy cigarette smoking represents a situation where the smoker is exposed to significant concentrations and possibly pharmacological effects of nicotine for 24 hours a day. Apparent acute tolerance to nicotine, determined on the basis of observations of the relationship between venous blood levels and effects, may be due to distribution disequilibrium between venous and arterial blood; venous blood levels substantially underestimate concentrations of nicotine in arterial blood and at potential sites of action. True tolerance does, however, develop rapidly, with a half-life of development and regression of about 35 minutes (Xinan et al., 2009). The kinetics of tolerance may be another determinant of cigarette smoking particularly when the smoker smokes his next cigarette.
4.08 Elimination of nicotine and the effect of other factors on the urinary level of this drug.

Nicotine and its metabolites (cotinine and nicotine 1-N-oxide) are excreted in the urine. At a pH of 5.5 or less, 23% is excreted unchanged. At a pH of 8, only 2% is excreted in the urine. The effect of urinary pH on total clearance is due entirely to changes in renal clearance. Nicotine is secreted into saliva. Passage of saliva containing nicotine into the stomach, combined with the trapping of nicotine in the acidic gastric fluid and reabsorption from the small bowel, provides a potential route for enteric nicotine recirculation. This recirculation may account for some of the oscillations in the terminal decline phase of nicotine blood levels after IV nicotine infusion or cessation of smoking.

As has been described above nicotine is an agonist at nicotinic receptors in the peripheral and central nervous system. In man, as in animals, nicotine has been shown to produce both behavioural stimulation and depression. Pharmacodynamic studies indicate a complex dose response relationship, due both to complexity of intrinsic pharmacological actions and to rapid development of tolerance (Nguyen et al., 2003; Walsh et al., 2008).

As stated in the Objectives - the urinary levels were used as an index as to what level of nicotine can be currently found in smokers in Kuwait.
Methodology

4.09 Experimental investigation of urine samples taken from patients in this study using a solid phase reactant system.

These experiments used an analytical system for detecting nicotine and/or nicotine metabolites. The detection system includes a solid phase containing assay reagents including a color determinant, a buffer, a cyanogen releasing agent and a cyanogen halide forming agent (Pons et al., 2008; Xinan et al., 2009). Since this system, is relatively new, a description of the various stages will be given. The method is capable of assaying unprocessed urine from smokers for the presence of nicotine and/or nicotine metabolites (DeNoble et al., 1982).

The term solid phase is intended to include any solid material that is capable of binding the assay reagents and allowing contact of these reagents with a test sample via capillary action. Examples of solid phases include paper, porous membranes, capillary tubes, resin bed (ionic and non-ionic type columns) and the like. The preferred solid phase is paper. Examples of ‘paper’ which may be used for such a procedure can include cellulose, fiberglass and tuff glass. The preferred paper is 100% cellulose (Oshida, 1994). The solid phase is selected for various functions. The solid phase may be adapted to bind reagents that are applied to the solid phase in solutions. Preferably, the solid phase is adapted to permit movement of liquid through the solid phase by capillary action (Schwartz et al., 1982).

A porous solid phase will capture and bind the assay reagents onto the solid phase, and also is adapted to facilitate movement of the reagents through the solid phase by capillary action. The solid phase is selected based on its capacity to hold the reagent...
volumes applied and on its capillary action properties. (Gotti et al., 1982; Jean, 2001; Gilbert et al., 2001) This method entails contacting the liquid sample with a solid phase impregnated with assay reagents including a color determinant, a buffer, a cyanogen releasing agent and a cyanogen halide forming agent, and detecting the formation of color as indicative of the presence of nicotine and/or nicotine metabolites. The terms nicotine and/or nicotine metabolites, solid phase, impregnated, color determinant, buffer, cyanogen releasing agent and cyanogen halide forming agent are used as defined above (Chang et al., 1974).

**Application of the reagents.**

The reagents may be applied to the solid phase so that they are arranged in a sequence which maximizes production of the end product in the color reaction assay. The preferred sequences are: color determinant, buffer, cyanogen releasing agent and cyanogen halide forming agent, or color determinant, cyanogen releasing agent, buffer and cyanogen halide forming agent. In either of these arrangements, immersion of one end of the solid phase into a liquid test sample results in the liquid initially making contact with the color determinant (Reynolds et al., 1978; Hukkanen, 2005; Nishi et al., 2008). The liquid then sequentially passes through the other reagents in the order that they are present on the solid phase, by capillary action. If nicotine or nicotine metabolites are present in the liquid sample, the reactions described above take place, resulting in a colored end product.

In order to achieve the maximal advantages of this experiment, the amount of sample applied to the strip and the manner in which the sample is applied to the strip are specified. It is preferable that the sample contact only a portion of the strip, with capillary action drawing the sample through the strip and into contact with the various reagents of the strip. In the preferred strip (see examples), the end of the strip containing the color
determinant is immersed in the sample with approximately 1/2 of the color determinant region being immersed. The remaining portions of the strip are not immersed. Also in the preferred assay (see examples), a sample of about 0.5 ml is contacted with the color determinant end of the strip. This is a sufficient volume to permit the sample to contact the reagents such that a color end product is produced, but not so much for example to reduce color intensity due to dilution, to reduce the effects of the buffer due to dilution, or to cause any of the reagents to be washed from the strip (Mansour et al., 1977).

**Color detection.**

The term detecting the formation of color is intended to include any process whereby the presence or absence of colour is determined. Such processes include visual detection and detection with instruments. The preferred method for detecting colour formation is visual detection. The formation of color can be detected on the solid phase or in the liquid test sample. Preferably, colour is detected on the solid phase. Depending upon the colour determinant that is selected for the reaction assay, a particular colour is formed if nicotine and/or nicotine metabolites are present in the test sample. When the preferred colour determinant, diethyl thiobarbiturate, is used in the reaction assay, the presence of nicotine and/or nicotine metabolites in the test sample results in the formation of a pink color (Conti-Tronconi et al., 1982).

The assay can also be used as a qualitative assay, in that the formation of any color indicates the presence of nicotine and/or nicotine metabolites. The assay can also be used as a semi-quantitative or quantitative assay, in that the intensity of color formed on the solid phase is indicative of the amount of nicotine and/or nicotine metabolites present in the test sample (Hall, 1981).
This would be desirable for distinguishing between a light or heavy smoker. Preferably, the intensity of color formation from the reaction assay is compared to a standard in which a known quantity of nicotine and/or nicotine metabolites has been contacted with a duplicate solid phase. The term duplicate solid phase is used reagents. The standard may be generated by performing an assay reaction with a solution containing a known concentration of nicotine and/or nicotine metabolites (Aceto et al., 1982). The standard may also be a reference color chart which is generated to simulate the intensity of color formed when an assay reaction with a solution containing a known concentration of nicotine and/or nicotine metabolites is performed. For example, a standard representing 5-6 μg/ml and 12-15 μg/ml of nicotine and/or nicotine metabolites in urine can be used to identify light to moderate and heavy smokers, respectively. A negative control with 0 μg/ml of nicotine and or nicotine metabolites also may be included. Alternatively, a semi-quantitative or quantitative determination of nicotine and/or nicotine metabolites may be made by measuring the optical densities in a spectrophotometer of the liquid test samples themselves, which also turn color as a result of the solid phase of this invention being immersed into the liquid test samples (Abood et al., 1981).

4.10 Use of this assay in non smokers.

This assay is also capable of assaying urine for the presence of nicotine and/or nicotine metabolites from nonsmokers who have been exposed to secondary smoke inhalation/ingestion if their urine sample is concentrated prior to the assay reaction. The urine may be concentrated using any art-recognized concentration protocol (Tripathi et al., 1982). For example, the urine may be concentrated using an XAD-2 resin column as described in Mule et al. (1971), Yamasaki et al., (1977) and Kullberg & Grodezky (1974).
4.11 Application of the samples.

Application of the reagents is accomplished manually or mechanically. Preferably, the reagents are applied such that the reagents are arranged in the particular sequences as previously discussed. The term drying is intended to include air drying and any type of mechanical drying process. (Xinan et al., 2009) The dried solid phase is packaged in a moisture proof material in the form of polypropylene coated aluminum foil, polypropylene and polyethylene (Abood et al., 1980; Martin et al., 1981).

4.12 Experiments on smoker’s urine for nicotine content for this thesis.

Tests were performed on urine of samples from smokers who had developed an elevated blood pressure.

1) A patient who was asked to smoke a cigarette had his urinary level of nicotine monitored over 0-4 hours which was used to establish the validity and sensitivity of the experimental procedure in the Kuwait laboratory. I carried out all these myself.

2) Urine samples from groups of nonsmokers (n=8), light smokers (n=8) moderate smokers (n=9) and heavy smokers (n=10) were collected and tested within identical time frames for the presence of nicotine and/or nicotine metabolites. They were all volunteers, to whom the ethics of the tests were explained and were chosen at random from patients in the hospital. The samples from smokers were performed on those individuals who had developed an elevated blood pressure, so that their levels of nicotine in their urine could be compared to their blood pressure. The experiments I carried out were in conjunction with the hospital laboratories under the supervision of the appropriate technical and scientific staff.
The procedure was as described below:

4.13 Steps in the preparation of the paper strips.

Step 1 - preparation of Paper Strips (see above for a sample of a strip)

Absorbent Paper Grade 222 was cut to 7.5×25 cm size with letters "DG" printed at 0.5 cm intervals along the length of the strip with an arrow pointing to the bottom of the 7.5 cm length. This paper was then cut to 0.5×7.5 cm size strips using a sharp razor blade in a strip cutter. Only strips with sharp edges were used.

Step 2 – preparation of the required reagents which were then applied to Paper Strips.

The reagents were prepared in the following way.

40% chloramine-T was prepared by dissolving 40 g of chloramine-T in warm water to make 100 ml final volume. This solution was kept warm at 65°-70° C. during dispensing.

100% potassium thiocyanate was prepared by dissolving 100 g of potassium thiocyanate in water to make a final volume of 100 ml, while shaking under warm tap water.
2M citrate buffer was prepared by combining 23 ml of 2M citric acid with 27 ml of 2M trisodium citrate to give a pH of 4.2.

2.5% diethyl thiobarbiturate was prepared by dissolving 2.5g of diethyl thiobarbiturate in 100-80% ethanol to a final volume of 100 ml.

The blank strips from step 1 were arranged on a strip support provided with shallow grooves to hold strips in place, with all the "DG" facing one side. Using an eight channel multipipetter, the reagents were applied onto the strips starting with chloramine-T, followed by potassium thiocyanate, citrate buffer, and finally, diethyl thiobarbiturate. The volumes of each of the reagents and the spacing of each on the strip were as follows: 40% chloramine-T-31.5 μl at the top end where "DG" was printed; 100% potassium thiocyanate-15 μl approximately 2 cm from the top; 2M citrate buffer pH 4.2- 20 μl approximately 4 cm from the top; and 2.5% diethyl thiobarbiturate--20 μl at the bottom end of the strip.

After all the reagents were dispensed onto the strips, they were transferred onto a mesh tray and dried in a box attached with an exhaust fan. To enhance the drying process, warm air was blown into the box from the opposite end of the fan. The strips were dry in one hour. Since moisture is an important factor in the stability of the strips, they were generally dried for longer than 1.5 hours.

**Step 3 - Packaging of Paper Strips**

After the paper strips were dried, they were packaged individually in a moisture proof material such as polypropylene coated aluminum foil, polypropylene or polyethylene. The strips were packaged in small numbers, e.g., in sets of 5 per sleeve, each strip in its own pouch. Perforations were made between individual pouches so that single
strips could be used without affecting the rest of the strips. On the side of the box containing the above package, a panel of colors was included to indicate one negative and two positive control colors. One of the positive colors corresponded to light to moderate smoker urine and the second color corresponded to heavy smoker urine. The packaged strips remain stable in the original packaging for at least a year at refrigerator temperature.

**Step 4 - Use of paper strips for assaying urine samples for the presence of nicotine and nicotine metabolites.**

Approximately 0.5 ml of urine was pipetted into a 13×100 mm tube. The package containing the reagent impregnated paper strip was cut open at the top and the strip was taken out with forceps and dropped into the urine sample with the arrow pointing downwards. It was allowed to stand for 10-15 minutes. Any color changes were observed. Urine containing no nicotine or nicotine metabolites did not change the color of the strip. The presence of nicotine and its metabolites in a urine sample gave a pink color, both on the strip and in the solution. The presence or absence of a nicotine/metabolite was independently verified using an enzyme immunoassay (EIA). The color was stable for almost an hour, after which it started to fade. Closing the tubes with a cap improved the final color intensity slightly.

**4.14. ELISA assay method.**

Enzyme-linked immunosorbent assay (ELISA), also known as an enzyme immunoassay (EIA), is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample. In simple terms, in ELISA, an unknown amount of antigen is affixed to a surface, and then a specific antibody is applied over the surface so that it can bind to the antigen. This antibody is linked to an enzyme, and in the
final step a substance is added that the enzyme can convert to some detectable signal, most commonly a colour change in a chemical substrate takes place.

Performing an ELISA involves at least one antibody with specificity for a particular antigen. The sample with an unknown amount of antigen is immobilized on a solid support (usually a polystyrene or polyvinylchloride acts as binding site on solid phase) either non-specifically (via adsorption to the surface) or specifically (via capture by another antibody specific to the same antigen, in a "sandwich" ELISA). After the antigen is immobilized, the detection antibody is added, forming a complex with the antigen. The detection antibody can be covalently linked to an enzyme or can itself be detected by a secondary antibody that is linked to an enzyme through bioconjugation.

Between each step, the plate is typically washed with a mild detergent solution to remove any proteins or antibodies that are not specifically bound. After the final wash step, the plate is developed by adding an enzymatic substrate to produce a visible signal, which indicates the quantity of antigen in the sample (Engvall et al., 1971, Vrolix et al., 2010).

It can be understood by following diagram (after Van Weemen et al., 1971).
1. Plate is coated with a capture antibody;
2. Sample is added, and any antigen present binds to capture antibody;
3. Detecting antibody is added, and binds to antigen;
4. Enzyme-linked secondary antibody is added, and binds to detecting antibody;
5. Substrate is added, and is converted by enzyme to a detectable form due to the formation of a complex which has a certain color which in case of nicotine is a pink color.

Figure 17. Enzyme Immunoassay (ELISA) of nicotine from a urine sample.

4.15 Statistics.

The nicotine concentrations (μg/ml) in the urine samples were compared between non-smokers and the light, moderate and heavy smokers groups using the Mann Whitney U test. A P value of less than 0.05 was considered significant.
4.16 Results

Results found from urine samples taken at various time intervals from a person who had smoked one cigarette are shown in Table 5.

These results established that the method was both feasible and also sensitive and reproducible. Duplicate measurements were made and the results show the mean of these measurements.

**Table 5** - ELISA and paper strip analysis of a smokers urine after smoking a single cigarette. Nicotine values are expressed as microgrammes of nicotine per ml of urine and for the paper strip is reported as showing either (+) the presence or (-) the absence of detectable nicotine.

<table>
<thead>
<tr>
<th>Specimen No.</th>
<th>Time Elapsed after Smoking</th>
<th>Paper Strip Test</th>
<th>ELISA Nicotine concentration (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 hour</td>
<td>-</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>Three hours</td>
<td>-</td>
<td>0.23</td>
</tr>
<tr>
<td>3</td>
<td>Four hours</td>
<td>+</td>
<td>0.16</td>
</tr>
<tr>
<td>4</td>
<td>Five and half hours</td>
<td>+</td>
<td>0.12</td>
</tr>
</tbody>
</table>

**Samples from non smokers and different levels of smoking activity**

Urine samples from nonsmokers (n=8), light smokers (n=8), moderate smokers (n=9) and heavy smokers(n=10) were tested for the presence of nicotine and/or nicotine metabolites by using both a) Paper strip assays were performed as described in 4.18 and b) ELISA assays which were performed as described in 4.14.
4.17 Qualitative test in the paper strip assay.

The Light (L), Medium (M) and Heavy (H) designations were determined by observing the intensity of the pink colour which appeared during the analysis of nicotine in urine. The more intense the pink color developed on strip indicated that the smoker was a heavier smoker where as a light pink colour indicated that the smoker was a light smoker. An intensity of color lying somewhat between light and more intense pink color indicated that the smoker was a moderate smoker.

4.18 Comparison of paper strip and ELISA Assays.

These results were quantified using the very sensitive method of the ELISA assay. This comparison, on the same samples as used for the paper strip method helped to make comparison between light, moderate and heavy smokers far more accurate and without the subjective basis of colour estimation.

The ELISA method used a Solid-phase immunoassay which involved fixing antibody to the antigen on a polyvinylchloride sheet, by putting on a drop of urine and washing off after the antigen-antibody complex has had time to form, and then adding a second labeled or fluorescent antibody, this time specific to a different epitope [specific region where change take place] of the antigen. The amount of the second antibody that binds is proportional to the amount of antigen present. This can convert a colorless substance which is non-fluorescent to a fluorescent product because the second antibody contains a fluorescent component.

These results were quantified using the very sensitive method of the ELISA assay. This comparison (table 6) helped to make comparison between light, moderate and heavy smokers far more accurate and without the subjective basis of colour estimation.
Table 6 - Reagent impregnated paper strip test and ELISA nicotine levels (µg/ml) in the urine of non-smokers and three types of smokers – light, medium and heavy smokers. Key: Known secondary smoke exposure L = light; M = moderate; and H = heavy.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>I Non Smokers</th>
<th>II Light smokers</th>
<th>III Moderate smokers</th>
<th>IV Heavy Smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strip Test ELISA</td>
<td>Strip Test ELISA</td>
<td>Strip Test ELISA</td>
<td>Strip Test ELISA</td>
<td>Strip Test ELISA</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>0.08</td>
<td>L</td>
<td>0.70</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>0.12</td>
<td>L</td>
<td>2.20</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>0.028</td>
<td>L</td>
<td>0.64</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>*0.07</td>
<td>L</td>
<td>0.20</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>N.D</td>
<td>L</td>
<td>2.00</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>0.07</td>
<td>L</td>
<td>1.20</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>0.0</td>
<td>L</td>
<td>0.70</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>0.0</td>
<td>L</td>
<td>1.40</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
<td>M</td>
<td>2.3</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>H</td>
</tr>
</tbody>
</table>

4 out of 8 subjects self reported consumption of 3-5 cigarettes /day
6 out of 9 subjects self reported consumption of 1 pack/day
8 out of 10 subjects self reported consumption of 1-2 packs/day
The bar chart presented in Figure 18 which indicates the concentration of nicotine which was found in the urine of respective subjects. The subjects are grouped together in arbitrary groups of four to facilitate their presentation. They were not linked together in any other way.

**Figure 18.** Bar chart of ELISA results for nicotine levels in the urine of non-smokers as compared to three levels of smoking activity namely - light, medium and high. Each bar represented the levels found in one individual arbitrarily grouped together in groups of four subjects. These groups are designated subject 1-10.
4.19. Discussion.

The values of nicotine in the urine of patients who do not smoke and with those who had with different levels of smoking activity are shown in Figure 18. The findings showed some interesting effects.

For Non smokers.

In some of the non smokers, five out of eight of the subjects, there were actually detectable levels of nicotine in their urine. This value, since they stated they were non smokers, must have been obtained from either the diet (see above) or from passive smoking. The detection and quantification of such low levels clearly established the sensitivity of the assay which was used.

Smokers groups.

In the ‘smoking groups’ the levels of urinary nicotine were clearly dependent on the individuals exposure to cigarettes.

Light smoking group (n=8) designated as being 3-5 cigarettes a day. If the patterns of excretion are examined (Figure 18) for the light smoking group they had a median value of 0.85 µg/ml with a range of 0.20 – 2.2 µg/ml. If these patterns of excretion are statistically compared (Figure 18) for the light smoking group versus the non smoking group they had a significantly (Mann Whitney U test, P<0.01), greater level of excretion than the non smokers. For the light smoking group their median value was 0.85 µg/ml with a range of 0.20 – 2.2 µg/ml as compared to a median value of 0.07 µg/ml for the non smokers with a range of 0 – 0.12 µg/ml. This tenfold range of excretion suggested a
greater variability in this group than the other groups, perhaps reflecting different patterns of smoking than for the other groups.

**Moderate smokers (n=9) designated as up to 20 cigarettes a day.** The moderate group smoked on average between four and six times the number of cigarettes than the light group but did not have four to six times the nicotine excretion in their urine. In fact, the median value was 1.6 µg/ml with a range of 1.0 – 2.3 µg/ml. The range was therefore only a doubling, rather than a tenfold range seen in the light smokers. Although these levels were not significantly different from the light smokers (P>0.05) it may have been anticipated that the levels would have been much greater because they smoked up to 20 cigarettes per day. This was not the case. There was however more uniformity in the levels which were found in the urine.

**Heavy smokers (n=10) designated as being between 20-40 cigarettes a day.** In the final group – heavy smokers - the nicotine excretion was the greatest of the three groups measured and significantly different (P<0.01) from control, light and moderate smoker groups.. In this group the median was 3.85 µg/ml and the range was 2.1 – 8.0 µg/ml. This fourfold range may reflect the differences between individuals smoking between 20 – 40 cigarettes per day. If these urine levels reflect plasma levels, then at this high consumption rate the individual may experience the profound pharmacological effect on the cardiovascular system and other systems of nicotine.

There were differences between members of the same group which may be expected as each member of the group did not smoke exactly the same number of cigarettes and there method of smoking – the depth of inhalation and the rapidity of their use – were all variables which could not be controlled. Also the measurement occurred at different times after smoking cigarettes so the values were bound to be variable as it was
excretion which was measured rather than plasma values. However despite all these ‘uncontrollable variables’ some interesting values were determined.

4.20 Comments and general conclusions about the urinary levels of nicotine found in this study.

The results of the strip test and assay were very informative. The sensitivity and correlation of the strip test with the ELISA assay show that the strip test could be used in a semi-quantitative way of low, medium and high cigarette consumption with some degree of certainty that the results were correct. The ELISA is of course a very precise, but far more complex and costly method to determine nicotine levels. It did however establish that the urinary levels were not simply related to the actually number of cigarettes smoked. This may simply be that urinary volume outputs vary between individuals so the calculation on a per ml basis would need to be extrapolated for 24 hour collections – but even then this assumes that the output would be constant which may be an unwarranted assumption. Perhaps the data which was obtained is sufficient without resorting to extremely complex urinary collection systems as it shows a number of findings. There is also the problem of the variability between individuals of the metabolic products of nicotine which this assay does not detect.

General conclusions

1. Nicotine absorption can occur in individuals exposed to passive smoking.
2. Smoking 3-5 cigarettes a day produces measurable urinary levels of nicotine.
3. Smoking 20 cigarettes gives only twice the urinary output of the light smokers.
4. Smoking 20-40 cigarettes a day gave more than twice the output of those people who smoked 20 cigarettes a day perhaps indicating that metabolism may be
exceeded and more free nicotine is excreted unchanged or in this group there was a more diverse range of metabolic products in the individuals studied.
Chapter Five

Investigation into the effectiveness of aspirin in reducing risks.

5.01 A The objective of this part of the study I to investigate the effectiveness of aspirin with regard to age of the person who is suffering from CHD.

5.02 Methodology.

All aspects of this study were read by and approved by the authorites in Kuwait who give ethical approval for the such studies to be undertaken.

All male patients who were admitted to the Chest Hospital in Kuwait at the time at which this trial was being undertaken (2009-2010) were screened for cardiovascular disease. In the time period of this screening, there were found to be 197 patients who were admitted to the hospital and who were smokers, and also had coronary heart disease of the severity required in the trial.

When these 197 patients were examined in greater detail, including: their smoking behavior, systolic/diastolic pressures, cholesterol levels and coagulation factor levels, it was found that of the 197 patients only 150 patient were considered to be suitable for treatment with aspirin, as the other 43, although they had coronary heart diseases also had many other complicating factors such as because of their age they had a greater potential to bleed if taking aspirin and they were also receiving complex drug therapies for their hypertension which it was thought may have made the analysis of the effects of aspirin extremely difficult to determine. However this group of non aspirin treated patients were not simply rejected but rather used as a ‘control’ for the aspirin treated group as they did
not receive the aspirin but did, as for the aspirin treated group have coronary vascular disease are were receiving their usually medications for their existing diseases. It was a very useful control to have.

This meant they acted as a control against which the pharmacological effects of aspirin could be compared with those who did receive the drug as this was the only difference between the groups. This is a major complicating factor as the therapies in both groups included drug classes such ACE inhibitors, calcium channel blockers and/or beta-blockers which, although treating their hypertension may actually lower the risk of recurrent myocardial infarction. For ethical reasons the patients could not be taken off their existing drugs despite the fact that this made interpretation of the results extremely complicated. Nevertheless, the 150 patients who actually took aspirin also continued on their existing medications so the two groups were comparable in the terms of not changing their existing therapy. Simply, one group of patients suffering from coronary disease problems was compared to another group, the only additional factor in their treatment being the inclusion of low dose aspirin therapy.

**Grouping the patients**

To facilitate analysis it was necessary to put the patients into different age groups as it would have been ‘unfair’ to compare the effects of aspirin in individuals who had been suffering their disease for 1 or 2 years as against those who had been suffering it for 30+ years.

The 150 patients were first of all divided into ‘age range groups’ (Figure 19) in order to see if aspirin exerted an age related effect. The number in the first age range 45-49 years out of a total number of screened coronary disease patients (n=197) was 28. However of these, only 14 patients were judged to be suitable for the trial. Similarly, 33
patients who belonged to age group 50-54 years were found on admission to the hospital to be suffering from coronary problems but of these only 26 patients of this group were actually included in the aspirin trial. Likewise 34 patients of age group 55 to 59 years were treated with aspirin out of a total number of 37 patients of this age group who had coronary heart disease. For patients with ages between 60-64 years, only 36 patients out of 55 coronary heart patients were treated with aspirin. The last age group of patients were those in the 65-69 years group and of the 44 patients who were detected on admission the vast majority i.e. 40 patients, were considered suitable for treatment with aspirin despite their age and therefore included in this trial.

**Figure 19.** Bar chart showing the age groups of patients who were used in which to study the effects of aspirin who were suffering coronary heart disease. In this chart the pink colored bar shows the number of patients in the five age groups at time they were admitted with coronary heart diseases before aspirin treatment and the blue colored bar for number of patients in five groups who actually received aspirin treatment (75mg/day).
This division of the total number of patients detected with coronary heart disease with respect to their age groups and those adjudged suitable for treatment with aspirin is shown in Table 7.

**5.03 Results**

The use of Table 7 attempts to show the effectiveness of aspirin in all the different age related groups. To show the effectiveness of the aspirin therapy the value for the relative risk has been used for all the groups. Briefly, in this experiment relative risk is the probability of an event occurring in the aspirin group as compared to the non aspirin treated group. This is the classical use of such an analysis where the risk of developing/making worse existing coronary disease in those receiving aspirin is compared against those non aspirin treated. A relative risk of 1 indicates there is no difference between the two groups. A value less than 1 means the event is less likely to occur in the treated group rather than the non aspirin treated group. A value greater than 1 indicates that the event is more likely to occur in the treated group rather than the non aspirin treated group.
Table 7. Age group distribution and the number of patients selected to receive aspirin involved in a study on its cardiovascular effects in patients with coronary heart disease as compared with non aspirin treated patients. The relative risk is shown in the last column.

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Total patient number - before selection for aspirin treatment (n=197)</th>
<th>Selected for aspirin treatment (n=150)</th>
<th>Relative risk (to show the benefit of aspirin treatment as compared with those who did not receive aspirin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>45-49</td>
<td>28</td>
<td>14</td>
<td>0.48</td>
</tr>
<tr>
<td>50-54</td>
<td>33</td>
<td>26</td>
<td>0.77</td>
</tr>
<tr>
<td>55-59</td>
<td>37</td>
<td>34</td>
<td>0.73</td>
</tr>
<tr>
<td>60-64</td>
<td>55</td>
<td>36</td>
<td>0.61</td>
</tr>
<tr>
<td>65-69</td>
<td>44</td>
<td>40</td>
<td>1.29</td>
</tr>
</tbody>
</table>

Examination of the relative risk values in Table 7 shows some interesting findings. In the first four groups the risk-ratios are all less than 1. This indicates that for all these four groups the treatment with aspirin has reduced the risk of patients suffering complications of their coronary heart disease. The final age group 65-69 years - with a risk ratio value of 1.29 shows the aspirin treatment has in fact increased the risk of suffering the disease they were first diagnosed with.

These results show that aspirin had been effective for the majority of the age groups studied, all of whom had at the beginning of the study, coronary heart disease. Clearly, aspirin benefited 110 patients in the trial. For the remaining 40, in the age group
65-69 the results were not favourable for the use of aspirin. It is interesting to speculate and give possible reasons for the lack of aspirin’s effect in the final group.

5.04 Discussion/Conclusion

International statistics provide ample evidence that the number of patients who have problems due to coronary heart disease increases with age. This is said to be the major disease of the century in the majority of countries around the world, including Arabic nations. This means that within any group of males as their age increases the chances of coronary heart diseases also increases. Consequently, the aspirin treatment may reflect the finding that in the age group 65-69, where the coronary problems would be expected to be at a high level, the use of aspirin which provided a relative risk of 1.29, perhaps reflects the inability of aspirin to reverse a cardiovascular condition(s) which had gone untreated for many years prior to the trial and/or their existing treatment simply maintained rather than reversed. This is not a failure of the aspirin to bring about changes but rather a reflection that changes to the coronary arteries and indeed other vascular components in such patients may have reached a level where reversing the future potential for increased risk is simply impossible.

The main conclusion is that aspirin should be started as early as possible in younger age groups to avoid such a problem of the lack of a suitable response occurring.
5.05. Part B. The objective of this part of the study were to investigate
‘Does aspirin bring about its beneficial effects by modification of diastolic blood
pressure’?

5.06 Methodology.

To try and find out why the aspirin had exerted such a beneficial effect seen in Part
A, some of the major risk factors which contribute to the risk of heart attacks and strokes
were examined in the subjects who took part in the trial in more detail.

The first of these was to determine if their diastolic pressure had been modified
during their treatment with aspirin.

To examine the effect of aspirin over the whole range of diastolic blood pressures,
the same cohort of patients (n= 150) were placed into three groups made according to their
limits of diastolic blood pressure. This rather arbitrary grouping was not expected to
produce identical numbers in each group but was rather to make comparisons between non
aspirin treated and aspirin treated patients more comparable – in terms of one factor –
diastolic pressure.

Of the 152 patients, those patients whose diastolic pressure was 70mmHg or less
numbered 56 of which 35 were patients were included in the trial of aspirin (Figure 20).
The next group was those in which the diastolic pressure was between 70-80 mmHg. In
this group of patients (n=57) 40 patients were treated with aspirin. The final group of
patients (n=79) were those who had a diastolic blood pressure greater than 80 mmHg and
these patients have been categorized due to its severity as being in Stage 1 or Stage 2
hypertension. Of these 79 patients, 77 received aspirin therapy. The effectiveness of the
aspirin as compared to non treated aspirin for a seven day dosing period (75mg/day) in
these three groups are shown in Table 7
5.07 Results.

**Table 8.** The distribution of patients into three ‘levels of diastolic pressures’, and the numbers treated by aspirin (75mg/day for 7 days) from the total number which was studied. The differences between the total number of patients and the treated group are shown in the table.

<table>
<thead>
<tr>
<th>Diastolic blood pressure (mm Hg)</th>
<th>Total patients (n=192)</th>
<th>Aspirin treated (n=152)</th>
<th>Relative risk (to show the benefit of aspirin treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤70</td>
<td>56</td>
<td>35</td>
<td>0.55</td>
</tr>
<tr>
<td>70-80</td>
<td>57</td>
<td>40</td>
<td>0.75</td>
</tr>
<tr>
<td>≥80</td>
<td>79</td>
<td>77</td>
<td>0.94</td>
</tr>
</tbody>
</table>

**Figure 20.** Bar chart of the numbers of subjects in each group as defined by their diastolic pressures ≤ 70 mmHg, 70-80 mmHg and ≥ 80 mmHg.

The number of subjects who received either aspirin (the blue block) or non treated (the pink block) for each of the three diastolic pressure groups which were studied. The aspirin treated groups received 75mg which was given for 7 days.
5.08 Discussion/conclusion.

From this data it can be seen that the relative risk is favourable for aspirin therapy at all levels of diastolic pressure. It could be argued that the first two diastolic levels are more successful than the third levels, namely over 80mmHg. Again this may be possible to explain as these patients have usually had cardiovascular disease for a longer period of time and the reversal of long standing problems may be simply too great for the aspirin to overcome.

After observing such improvement these patients were followed up for the next 30 days and after that aspirin is suggested on a regular basis and they were advised to continue use low dose of aspirin

5.09 Part C. The objective of this part of the study was to investigate ‘Does aspirin bring about its beneficial effects by modification of plasma cholesterol concentrations?’

5.10 Aims and methodology.

The aim of this study was to determine the effectiveness or otherwise of aspirin treatment the 186 patients who were divided into smokers and non smokers and then divided into three groups made on the basis of their original plasma cholesterol concentration. The patients were placed into three groups namely: a) ≥5.9, b) 5.9-6.7 and c) ≥6.7 mmol/L. This resulted in the number of patients who smoked in each of these groups being, 48, 39 and 58 respectively.
The total number of patients used in this part of the study was 186 (Figure 21) of whom 145 were smokers. These are a different study group to those reported in Part A and B.

5.11 Results.

Figure 21. Bar chart of the numbers of subjects in each group as defined by their plasma cholesterol concentration of i) ≥5.9, ii) 5.9-6.7 and iii) ≥6.7 mmol/L. The number of subjects who received aspirin is shown as blue blocks and the number of patients before aspirin treatment as shown pink block for each of the three cholesterol plasma level groups which were studied. In this experiment based on the results found in Part B The aspirin treated groups received 75mg per day for one and half months (45 days).
Table 9. The distribution of patients into three ‘plasma cholesterol levels’, and the numbers treated by aspirin (75mg/day for 45 days). The difference between the total number of patients and the treated group are those that have received aspirin treatment.

<table>
<thead>
<tr>
<th>Plasma cholesterol concentration (mmol/L)</th>
<th>Total patients (n=186)</th>
<th>Aspirin treatment (n=145)</th>
<th>Relative risk (to show the benefit of aspirin treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤5.9</td>
<td>53</td>
<td>48</td>
<td>0.96</td>
</tr>
<tr>
<td>5.9-6.7</td>
<td>63</td>
<td>39</td>
<td>0.55</td>
</tr>
<tr>
<td>≥6.7</td>
<td>70</td>
<td>58</td>
<td>0.95</td>
</tr>
</tbody>
</table>

Of the 48 patients who smoked and had a cholesterol concentration of less than 5.9 mmol/L, the aspirin treatment brought about a very small reduction in the plasma concentration and this was reflected in a relative risk of 0.96 (see Table 9). In the second group, of 39 patients who had a cholesterol concentration between 5.9mmol/L to 6.7mmol/L, aspirin brought about an even greater reduction in plasma cholesterol levels which altered their risk of cardiovascular problems as shown by the relative risk factor of 0.55. In the final group the 58 patients treated with aspirin and whose initial cholesterol concentration was greater than 6.7 mmol/L a similar relative risk to those below 5.9 mmol/L was recorded.
5.12 Discussion/Conclusion

Perhaps the last finding indicates that the changes induced by a high plasma cholesterol concentration are such, that reversal of the long lasting effect of cholesterol, in terms of cardiac risk factors, is extremely difficult to overcome simply by the use of aspirin.

Plasma cholesterol levels are considered to be one of the major risk factors along with high blood pressure to make heart attacks and strokes more likely to occur. The finding that aspirin can reduce this level in the group 5.9 – 6.7 mmol/L is potentially of great significance.

5.13 Part D The objective of this part of the study is to investigate the effects of aspirin on patients and its placebo equivalent with respect to the age of the patients.

5.14 Methodology.

In Part A of these studies it was found that on detailed examination of the data from 150 patients who took aspirin it had a range of measurable effects on the relative risk of suffering cardiovascular events. During this detailed examination of all the patients who had taken both aspirin and placebo it was found that 26 patients who took the placebo treatment had actually benefitted from the placebo in terms of their coronary problems. These individuals are shown in Table 10.
### 5.15 Results

**Table 10.** Age group distribution and the number of patients selected to receive aspirin involved in the study and those who had the same age but only (Non aspirin) received placebo. Please note - The number of patients in each age range who positively responded to the placebo was variable.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Aspirin (n=150)</th>
<th>Placebo treated with a positive outcome (n=variable depending on age group)</th>
<th>The effectiveness of placebo treatment as compared to aspirin treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>45-49</td>
<td>14</td>
<td>1</td>
<td>NIL</td>
</tr>
<tr>
<td>50-54</td>
<td>26</td>
<td>0</td>
<td>NIL</td>
</tr>
<tr>
<td>55-59</td>
<td>34</td>
<td>11</td>
<td>0.40</td>
</tr>
<tr>
<td>60-64</td>
<td>36</td>
<td>9</td>
<td>0.99</td>
</tr>
<tr>
<td>65-69</td>
<td>40</td>
<td>5</td>
<td>0.59</td>
</tr>
</tbody>
</table>

Although a positive placebo effect was seen in four out of the five groups the numbers in some of the study groups were very small. However in one group, the 55-59 there was placebo response of 11 out of 34 patients (see Figure 22).
Figure 22. Bar chart of the numbers of subjects in the age ranges shown who were treated with aspirin (pink bars) and the number of subjects who positively responded to the placebo or non aspirin treatment (blue bars).

5.16 Discussion/Conclusion.

These results show some positive effects with the placebo. This may be by a chance observation but this age group might be favorably stimulated to ‘look after themselves’ during the study period as this age group should be aware of the serious consequences of their cardiovascular disease. They may have started to eat more wisely, take more exercise or even reduce their consumption of cigarettes but these factors were not recorded in this study. It would be interesting to study what steps people take when on a trial to get the maximum benefit for themselves.
5.17 Part E The objective of this part of the study is to investigate the effect of placebo with respect to diastolic blood pressure.

5.18 Methodology
Again it was observed when the data was being collected that in patients receiving the placebo there were changes in some of their diastolic pressures. In total 26 patients were found to be in this group spread throughout all three diastolic pressure groups. The details are shown in Table 10.

5.19 Results.
Table 11. Diastolic blood pressure distribution and the number of patients selected to receive aspirin involved in the study and those who in the same diastolic pressure range but received (non aspirin) only the placebo. Please note - The number of patients in each age range who positively responded to the placebo was variable.

<table>
<thead>
<tr>
<th>Diastolic blood pressure (mm Hg)</th>
<th>Aspirin (n=152)</th>
<th>Positive Placebo responders ( n=26)</th>
<th>Relative risk (to show the benefit of aspirin treatment compared to placebo treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤70</td>
<td>35</td>
<td>6</td>
<td>0.41</td>
</tr>
<tr>
<td>70-80</td>
<td>40</td>
<td>10</td>
<td>0.21</td>
</tr>
<tr>
<td>≥80</td>
<td>77</td>
<td>10</td>
<td>1.42</td>
</tr>
</tbody>
</table>
Figure 23. Bar chart of the numbers of subjects in the diastolic blood pressure ranges shown who were treated with aspirin (pink bars) and the number of subjects who positively responded to the placebo (blue bars).

5.20 Discussion/Conclusion.

The implications for the findings made in this part of the study are very interesting. Although there was a measurable placebo response which is not too unexpected the effect of aspirin was quite pronounced. What was unexpected was the high rate of placebo effect in the higher diastolic ranges. In the diastolic range 70-80 mmHg aspirin had a measurable effect which was different to the other two groups. The higher risk in the group over 80mmHg is unexpected and perhaps worrying and may reflect the problem that pre existing coronary vascular disease has caused a lack of response to the aspirin.

Whatever the reason(s) it is certainly worth further investigations.
5.21 Part F The objective of this part of the study is to investigate the effect of Aspirin and Placebo with respect to Cholesterol concentration (m mol/L)

5.22 Methodology.

The data for 145 patients treated with aspirin into the three arbitrary plasma levels groups were found to have variable responses to aspirin (See Table 8 above). When the placebo results were studied it was found that some could be classified as ‘positive placebo responders’ (See Table 13).

5.23 Results

Table 12. Plasma cholesterol concentrations and the number of patients selected to receive aspirin involved in the study and those who in the same cholesterol range but received (non aspirin) only the placebo.

<table>
<thead>
<tr>
<th>Cholesterol concentration (mmol/L)</th>
<th>Aspirin (n=145)</th>
<th>Positive Placebo responders (n=26)</th>
<th>Relative risk (to show the benefit of aspirin treatment compared to placebo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤5.9</td>
<td>48</td>
<td>12</td>
<td>0.20</td>
</tr>
<tr>
<td>5.9-6.7</td>
<td>39</td>
<td>8</td>
<td>0.90</td>
</tr>
<tr>
<td>≥6.7</td>
<td>58</td>
<td>6</td>
<td>1.53</td>
</tr>
</tbody>
</table>
Figure 24. Bar chart of the numbers of subjects in the plasma cholesterol levels in the ranges shown who were treated with aspirin (pink bars) and the number of subjects who positively responded to the placebo (blue bars).

5.24 Discussion/Conclusion

The greatest placebo effect was seen at the lowest plasma levels i.e. below 5.9 mmol/L. Perhaps this reflects that the people who took part in the trial may have over the months of the trial period changed their behavior in terms of smoking and diet. If they changed both these factors then the lipid levels may well reduce and such an effect is not just a psychological effect but rather a real effect brought about by behavioral changes. This of course will never be known as it dependent on patients in the trial maintaining a constant behavioral and dietary pattern. This shows how complex a trial lasting over this length of time actually turns out to be.
5.25 Part G The objectives of this study was to investigate any additional factors studied for studying the role of aspirin in controlling diastolic blood pressure.

5.26 Methodology

In this study 239 patients who were having regular disturbances in their diastolic blood pressure were physically examined and their blood pressure measured. On examination it was observed that these patients all had some form of coronary artery problems and/or other heart problems. These changes it was thought were affecting their control of diastolic pressure. It was decided to carry out a study in which these patients received aspirin (75mg/day) to determine if it could be beneficial for the control of diastolic pressure. When the 239 patients were screened for the use of aspirin, as might perhaps be expected, not all were suitable because of the risk of gastric haemorrhage and so the actual number which received the drug was 189.

The patients who were to receive aspirin were divided into three diastolic ranges, the same as which had been used in the previous studies i.e. \( \leq 70\text{mmHg}, 70-80 \text{mmHg} \) and \( \geq 80 \text{mmHg} \). (Figure 25)

5.27 Results

When the data was examined (See Table 13) it was observed that those patients who had a diastolic blood pressure greater than 80mmHg did not benefit from the aspirin treatment. The other 90 subjects did however benefit from the treatment to a significant degree (See Table 13).
Table 13. This table shows the results which have been formulated about those people who when treated with aspirin who have actually developed some form of coronary heart diseases, combined in some cases with heart stock due to changes in the diastolic blood pressure.

<table>
<thead>
<tr>
<th>Diastolic blood pressure (mm Hg)</th>
<th>Total patients before aspirin treatment (n=239)</th>
<th>Aspirin treatment (n=189)</th>
<th>Relative risk (to show the benefit of aspirin treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤70</td>
<td>64</td>
<td>42</td>
<td>0.59</td>
</tr>
<tr>
<td>70-80</td>
<td>64</td>
<td>48</td>
<td>0.68</td>
</tr>
<tr>
<td>≥80</td>
<td>111</td>
<td>99</td>
<td>1.08</td>
</tr>
</tbody>
</table>

Figure 25. Bar chart of the numbers of subjects in the diastolic blood pressure ranges shown who were not treated with aspirin (pink bars) and the number of subjects who positively responded to the aspirin treatment (blue bars).
5.28 Conclusion

The effect of aspirin seems to have been more marked on the lower levels of diastolic pressure rather than the levels above 80 mmHg. This is similar to other findings where the lower diastolic values seem to respond to aspirin therapy whereas the higher levels do not. This may reflect that the hypertension in the third group has simply existed for a longer period of time and may be associated with structural and/or humoural changes which it is impossible in the short term to reverse with aspirin treatment. It would be of great interest to carry out the study for much longer and determine if with longer term treatments there are any more successful results.

Overall conclusions

These results show the effectiveness of aspirin in reducing the risks caused by those cardiovascular conditions which are both complex and potentially very serious in outcome resulting from of a number of different possible diseases. The effectiveness of aspirin appeared to increase as the diastolic blood pressure increased but only to a certain limits. The possible limitations of aspirin therapy with the higher levels of diastolic pressure. is a point which may limit the widespread use of the drug in all severities of hypertension. However for the lower diastolic values the treatment looks as though there is a distinct possibility and this may result in a greater number of hypertensive patients getting relief of their cardiovascular problems by the use of a simple, cheap therapy. Similar results were obtained with systolic blood pressure when we studied the range of blood pressure from greater than 130mm Hg and less than 145mm Hg.

There is of course a worry about the widespread use of aspirin in all age groups as during this treatment the major dominant side effect of aspirin was reported to be gastric
irritation and some bleeding due to the well known antiplatelet effect which in some individuals caused small scale blood coagulation problems.

However as was demonstrated in Part A of this study, aspirin should be started as early as possible in younger age groups to avoid CHD developing.
Chapter Six

An investigation of aspirin on ambulatory blood pressure of hypertensive smokers and non smokers.

6.01 Objectives.

The objective of this part of the thesis was firstly to establish the ambulatory blood pressure of hypertensive, non-smokers and smokers. They would then be divided into two groups. One group would receive placebo or non aspirin treated and another, 75mg/day of aspirin. Their blood pressure would be monitored throughout a 24 hour period. The effects of aspirin and placebo would then be determined over two study periods namely – daytime (7am and 11pm) - and night time (11pm – 7am).

6.02 Background.

It has been reported that smoking causes a temporary rise in blood pressure levels for both hypertensive and normotensive individuals. Individuals can be assessed by an isolated blood pressure measurement technique according to the recommendations of national and international guidelines (JNC 7, ESH 4, and I V DBHA) and these are the guidelines which have been used in this study. They have previously been used and validated by other workers for the determination of blood pressure in smokers. (Cristal-Boneh etal., 1997; Kumar .etal. 2004)

It has been observed that smokers when submitted to ambulatory blood pressure monitoring (ABPM) present a higher mean blood pressure reading than in non-smokers. Consequently, this technique provides a better understanding of

a) The 24-hour effect of smoking on the hour by hour blood pressure trends,
b) The actual systemic blood pressure which is produced,

c) By extrapolation of these values the impact which these may have on organs
which are known to be ‘targeted’ by elevated blood pressure.

Ambulatory blood pressure monitoring (ABPM) is the non-invasive diagnostic tool
which provides the basis for this type of analysis, and also provides a profile which
indicates the variations in blood pressure within the whole 24-hour period.

Consequently, this test provides a better understanding of hypertension for both
diagnosis and for treatment purposes. The object of this study was to determine the effects
on blood pressure caused by smoking throughout 24 hour periods - day and night so the
whole cycle of ambulatory blood pressure parameters could be determined. The
effectiveness of aspirin was also designed to be investigated in these hypertensive patients.

6.03 Methods

At the beginning of this study 200 hypertensive patients volunteered to take part in
the research project and these included both men and women. These two hundred
ambulatory blood pressure monitoring tests were ‘self selected’ from a wider population of
patients. Their blood pressures were measured and any measurements which produced
‘substandard tests’ were repeated in order to ensure the accuracy of the measurements
made. The study group of 200 patients included 147 non-smokers and 53 smokers.

However, despite their initial willingness of both males and female patients to
participate in the study, very unfortunately all the women, 48 in number, were reluctant to
follow through with their agreement to participate in the full study, due to many family
pressures in the Islamic country of Kuwait. Consequently 152 males remained but of these
the final number who agreed to all aspects of the study was eventually only 72.
These 72 patients were then classified as smokers or non-smokers according to the information given when the device was fitted. Anyone who smoked one or more cigarettes per day was classified as a smoker (Kawasaki et al., 1996). These 72 males were then divided into two groups – smokers and non-smokers. Their ambulatory blood pressure (ABPM) was monitored over a 24 hour period in which the following parameters were considered and calculated:

1- Changes in both daytime and night time systolic and diastolic blood pressure readings.

2- The changes in systolic and diastolic pressures during the night time as compared to the day time.

3- The data from the patients was then classified in eight further sub-groups according to whether or not they received aspirin (75mg/day).

The following ABPM parameters were analyzed

1- Change in the average daytime and night time systolic and diastolic blood pressure readings.

2- The variations in systolic and diastolic pressures during the night time.

These measurements were carried out using the Dyna-MAPA 24-hour oscillometric ABP-Monitor which has previously been validated by the American Association for the Advancement of Medical Instrumentation (AAMI) and the British Hypertension Society (BHS). The programming and report generation software was also Dyna-MAPA. The program automatically produced the statistical report without calculation/interpretation by any professional. (Walker et al. 2001: Diretrizes, 2001)
The patients were fitted with the device during normal day time working hours by a trained technician. Each patient wore the device for 24-hours and returned to the clinic the following day for removal and the downloading of the data. The monitoring was performed on a week day that was thought to reflect the patient's normal daily activities. The cuff was placed on the non-dominant arm two fingers widths above the elbow crease. Adequate sized cuffs according to arm circumference were always used. A previously established set of instructions was given to the patient when the device was fitted. (Leon et al. 2000)

The electronics within the device measured data every 15 minutes during the day and every 20 minutes during the night. The normal daily activities of patients, including what medicines they take, both during the day time and night time were also noted by the patient. (Kool M., et al. 1993)

Information about medicines was thought to be central to the study and the patients were asked to record the time they took their aspirin, the dosage actually taken and the time(s) they were taken. Additionally they were asked to record if they had any unexpected symptoms and they were to record such events, noting the hour of onset and the time at which they became absent. Day time was considered as the period between 7a.m. and 11p.m.- a sixteen hour period- and night time between 11p.m. and 7a.m – an eight hour period following the recommendations of the 2nd Brazilian Consensus for ABPM. So, initially the test sample was divided into two groups smokers and non-smokers, these two groups were then further subdivided into smokers taking aspirin and non-smokers taking aspirin or not taking aspirin as follows (Poulter N., et al., 1999). By virtue of the numbers involved, the total for each group was such that comparisons could be made. The groups were:
<table>
<thead>
<tr>
<th>Group 1 with aspirin treatment:</th>
<th>Night time systolic BP (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 2 without aspirin treatment:</td>
<td>Day time systolic BP (n=9)</td>
</tr>
<tr>
<td>Group 3 with aspirin treatment:</td>
<td>Day time systolic BP (n=9)</td>
</tr>
<tr>
<td>Group 4 without aspirin treatment:</td>
<td>Night time systolic BP (n=9)</td>
</tr>
<tr>
<td>Group 5 without aspirin treatment:</td>
<td>Daytime diastolic BP (n=9)</td>
</tr>
<tr>
<td>Group 6 with aspirin treatment:</td>
<td>Daytime diastolic BP (n=9)</td>
</tr>
<tr>
<td>Group 7 without aspirin treatment:</td>
<td>Night time diastolic BP (n=9)</td>
</tr>
<tr>
<td>Group 8 with aspirin treatment:</td>
<td>Night time diastolic BP (n=9)</td>
</tr>
</tbody>
</table>

To express the data in the most suitable way the median and the minimum, and maximum i.e. the range - were calculated as were the arithmetic mean and standard deviation. To show the nature of the numerical results the values were calculated and used for expressing all the values found in the data recordings. Since such a study has not been carried out in Kuwait patients it was thought both essential and appropriate to calculate this data in terms of both non-parametric and parametric data. This could then be used to provide a comparison between the groups and to determine the best sort of values to use. It should be stressed that the values in the tables are the summary data for an individual throughout either the day or night time. No attempt is made to provide hour by hour values as it was thought to be valuable to show the summary data.

Univariate analysis was used for comparing the smoking and nonsmoking groups, the significantly different variables from the groups were selected using multivariate analysis details of which can be found.

Minimum, maximum, median, mean and standard deviation values were used for expressing all variables. The Mann Whitney U test was used for the analysis of comparing
the smoking and non-smoking groups, the significantly different variables from the groups were determined (Chernoff et al. 1954).

6.04 Results

Please note

All the bar charts presented below show the mean values for an individual for the time period under study – day time or night time. They are presented in terms of ascending numerical order as members of the group are paired only in terms of ascending numerical order. The pairs were not matched for any other value except the rank order of value for the blood pressure. This somewhat unusual choice of presentation has been made as it shows perhaps in a very simple, yet informative way, the variability within the group, and the comparison of mean values for each individual in the group and the span of values found for each group can be easily seen.

6.05 Figures tables and calculations

6.06 Group 1. Patients who smoked and non-smokers who received Aspirin treatment - Night time Systolic BP

From the bar chart (Figure 26) it can be seen that the mean systolic blood pressure for individuals was variable within the groups of both smokers and non-smokers. No two patients actually had the same mean pressure and so a range of nine values can be seen for each group. These values are perhaps best seen in Table 14 where the individual values are shown as is the standard deviation. The trend appears to be that the non smokers had a lower overall systolic pressure that the smokers. However when the median values were compared using the Mann Whitney U test then there was found to be no significant difference (P>0.05) between them.
Figure 26. Mean systolic night time blood pressure of male, smokers and non-smokers over an 8 hour period (11pm-7am), who received 75mg of aspirin on a daily basis.

Table 14. Numerical values from which Figure 26 was constructed. For both Aspirin and placebo treatment the figures represent the mean night time systolic BP mmHg.

<table>
<thead>
<tr>
<th></th>
<th>Mean Night time systolic BP mmHg</th>
<th>Mean mmHg</th>
<th>Median mmHg</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non smokers</td>
<td>114,115,118,123,127,129,132,133,139</td>
<td>125</td>
<td>127</td>
<td>8.239</td>
</tr>
<tr>
<td>Smokers</td>
<td>119,122,124,128,132,138,144,148,150</td>
<td>133.8</td>
<td>132</td>
<td>10.9</td>
</tr>
</tbody>
</table>
Specimen Calculations

Mean (non-smokers) = 114+115+118+123+127+129+132+133+139/9 = 125 mmHg

Mean (smokers) = 119+122+124+128+132+138+144+148+156/9 = 133.8 mmHg

Standard deviation calculation

Step 1-find the standard deviation of each number from the mean,

For non-smokers
114-125= -11
115-125= -10
118-125= -7
123-125= -2
127-125= 2
129-125= 4
132-125= 7
133-125= 8
139-125= 14

For smokers

119-133.8= -14.8
122-133.8= -11.8
124-133.8= -9.8
128-133.8= -5.8
132-133.8= -1.8
138-133.8= 4.2
144-133.8= 10.2
148-133.8= 14.2
150-133.8= 16.2

Step 2-square each of the deviations, which amplifies large deviations and makes negative values positive 121, 100, 49, 4, 4, 16, 49, 64, 196

Step 3-find the mean of those squared deviations,

=121+100+49+4+4+16+49+64+196/9 = 611/9 =67.89

Step 4-take the non-negative square root of the quotient (converting squared units back to regular units),

=√67.89 =8.239 So, the standard deviation of the calculated was 8.23
For non smokers

Step 2 - square each of the deviations, which amplifies large deviations and makes negative values positive

219.04, 139.24, 96.04, 33.64, 3.24, 17.64, 104.04, 201.64, 262.44

Step 3 - find the mean of those squared deviations

=219.04+139.24+96.04+33.64+3.24+17.64+104.04+201.64+262.44/9

=1076/9 =119

Step 5 - Take the non-negative square root of the quotient (converting squared units back to regular units), =√119 = 10.9

So, the standard deviation of the set was 10.9

6.07 Group 2 Patients who smoked and non-smokers who received placebo treatment -
day time Systolic BP

Patient smokers and non-smokers in this group received placebo rather than aspirin treatment and their mean daytime systolic BP is shown in (Figure 27). Again the values have been paired simply for graphical presentation, the actually values, mean, median and standard deviation is shown in Table 15.
**Figure 27.** Patients’ daytime (7am-11pm) systolic blood pressure in those male patients smokers and non-smokers who received placebo rather than aspirin treatment.

**Table 15.** Patients on placebo or (non aspirin) therapy (mean day time systolic BP)

<table>
<thead>
<tr>
<th></th>
<th>Day time systolic BP mmHg</th>
<th>Mean mmHg</th>
<th>Median mmHg</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-smokers</strong></td>
<td>100,116,120,123,127,132,135,139,141</td>
<td>125</td>
<td>127</td>
<td>12.20</td>
</tr>
<tr>
<td><strong>Smokers</strong></td>
<td>112,118,125,131,137,143,147,150,156</td>
<td>135</td>
<td>137</td>
<td>14.18</td>
</tr>
</tbody>
</table>
Specimen calculations

Mean (non-smokers) = $\frac{100+116+120+123+127+132+135+139+141}{9}$

$=\frac{1133}{9} = 125 \text{ mmHg}$

Mean (Smokers) = $\frac{112+118+125+131+137+143+147+150+156}{9}$

$=\frac{1219}{9} = 135 \text{ mmHg}$

Standard deviation calculation –

Step 1 - Find the deviation of each number from the mean

For non-smokers

<table>
<thead>
<tr>
<th>Number</th>
<th>Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>-25</td>
</tr>
<tr>
<td>116</td>
<td>-9</td>
</tr>
<tr>
<td>120</td>
<td>-5</td>
</tr>
<tr>
<td>123</td>
<td>-2</td>
</tr>
<tr>
<td>127</td>
<td>2</td>
</tr>
<tr>
<td>132</td>
<td>7</td>
</tr>
<tr>
<td>135</td>
<td>10</td>
</tr>
<tr>
<td>139</td>
<td>14</td>
</tr>
<tr>
<td>141</td>
<td>16</td>
</tr>
</tbody>
</table>

For smokers

<table>
<thead>
<tr>
<th>Number</th>
<th>Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>112</td>
<td>-23</td>
</tr>
<tr>
<td>118</td>
<td>-17</td>
</tr>
<tr>
<td>125</td>
<td>-10</td>
</tr>
<tr>
<td>131</td>
<td>-4</td>
</tr>
<tr>
<td>137</td>
<td>2</td>
</tr>
<tr>
<td>143</td>
<td>8</td>
</tr>
<tr>
<td>147</td>
<td>12</td>
</tr>
<tr>
<td>150</td>
<td>15</td>
</tr>
<tr>
<td>156</td>
<td>21</td>
</tr>
</tbody>
</table>

Step 2 - Square each of the deviations, which amplifies large deviations and makes negative values positive, 625, 81, 25, 4, 4, 49, 100, 196, 256
Step 3. Find the mean of those squared deviations

\[
\frac{625+81+25+4+4+49+100+196+256}{9} = 148.8
\]

Step 4. Take the non-negative square root of the quotient (converting squared units back to regular units),

\[
\sqrt{148.8} = 12.20
\]
So, the standard deviation of the set is 12.20

For smokers

Step 2. Square each of the deviation, which amplifies large deviations and makes negative values positive

529, 289, 100, 16, 4, 64, 144, 225, 441

Step 3. Find the mean of those squared deviations,

\[
\frac{529+289+100+16+4+64+144+225+441}{9} = 201
\]

Step 4. Take the non-negative square root of the quotient (converting squared units back to regular units)

\[
\sqrt{201} = 14.18
\]

The standard deviation of the set is 14.1
6.08 **Group 3** Patients who smoked and non-smokers who received aspirin treatment - day time Systolic BP.

With Aspirin treatment the daytime systolic BP From the bar chart (Figure 28) it can be seen that the mean systolic blood pressure for individuals was variable within the groups of both smokers and non-smokers. No two patients actually had the same mean pressure and so a range of nine values can be seen for each group. These values are perhaps best seen in Table 15 where the individual values are shown as mean, median and the standard deviation. The trend appears to be that the non smokers had a lower overall systolic pressure than the smokers. However when the median values were compared using the Mann Whitney U test then there was found to be no significant difference (P>0.05) between them.

**Figure 28.** Effect of aspirin on day time systolic blood pressure of smokers and non smokers.
Table 16. Effect of aspirin on day time systolic blood pressure of smokers and non-smokers.

<table>
<thead>
<tr>
<th></th>
<th>day time systolic BP</th>
<th>Mean</th>
<th>Median</th>
<th>Standard deviation</th>
</tr>
</thead>
</table>

Mean (non-smokers) = \( \frac{90+94+98+104+108+112+114+118+126}{9} \)

= \( \frac{964}{9} \) = 107

Mean (Smokers) = \( \frac{100+108+114+116+119+127+134+138+140}{9} \)

= \( \frac{1096}{9} \) = 121

Standard deviation calculation

Find the deviation of each number from the mean

For non-smokers

\[
egin{align*}
90-107 &= -17 \\
94-107 &= -13 \\
98-107 &= -9 \\
104-107 &= -3
\end{align*}
\]

For smokers

\[
egin{align*}
100-121 &= -21 \\
108-121 &= -13 \\
114-121 &= -7 \\
116-121 &= -5
\end{align*}
\]
For non-smokers – the steps are the same as used above for both non-smokers and smokers for all the calculations shown below.

Square each of the deviation, which amplifies large deviations and makes negative values positive,

289, 169, 81, 9, 1, 25, 49, 121, 361

Find the mean of those squared deviations

=289+169+81+9+1+25+49+121+361/9 =1105/9 =122.7

Take the non-negative square root of the quotient (converting squared units back to regular units)

=√122.7 =11. So, the standard deviation of the set is 11

For smokers

Square each of the deviation, which amplifies large deviations and makes negative values positive
find the mean of those squared deviations,

\[
\frac{441 + 169 + 49 + 25 + 4 + 36 + 169 + 289 + 361}{9} = \frac{1543}{9} = 171
\]

Take the non-negative square root of the quotient (converting squared units back to regular units),

\[
\sqrt{171} = 13. \text{ So, the standard deviation of the set is 13}
\]

6.09 Group 4 Without Aspirin treatment Night time systolic BP

Figure 29. Night time systemic blood pressure (mmHg) of smokers and non smokers.
**Table 17.** Patient without Aspirin treatment (night time systolic BP mmHg)

<table>
<thead>
<tr>
<th>Night time systolic BP mmHg</th>
<th>Mean mmHg</th>
<th>Median mmHg</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-smokers 96,99,105,108,111,114,120,123,125</td>
<td>111</td>
<td>111</td>
<td>9.68</td>
</tr>
<tr>
<td>Smokers 100,108,111,113,115,124,135,145,159</td>
<td>123.3</td>
<td>115</td>
<td>18.215</td>
</tr>
</tbody>
</table>

Mean (non-smokers) = \(\frac{96 + 99 + 105 + 108 + 111 + 114 + 120 + 123 + 125}{9}\)

\[= \frac{1001}{9} = 111\]

Mean (Smokers) = \(\frac{100 + 108 + 111 + 113 + 115 + 124 + 135 + 145 + 159}{9}\)

\[= \frac{1110}{9} = 123.3\]

Standard deviation calculation

Find the deviation of each number from the mean,

For non-smokers

-96 - 111 = -15
-99 - 111 = -12
-105 - 111 = -6

For smokers

-100 - 123.3 = -23.3
-108 - 123.3 = -15.3
-111 - 123.3 = -12.3
For non-smokers

Square each of the deviation, which amplifies large deviations and makes negative values positive,

225, 144, 36, 9, 00, 9, 81, 144, 196

find the mean of those squared deviations

=\frac{225+144+36+9+00+9+81+144+196}{9} = \frac{844}{9} = 93.77

Take the non-negative square root of the quotient (converting squared units back to regular units),

=\sqrt{93.77} = 9.68. So, the standard deviation of the set is 9.68

For smokers

Square each of the deviation, which amplifies large deviations and makes negative values positive
Find the mean of those squared deviations,

$$\frac{542.89 + 234.09 + 151.29 + 106.09 + 68.89 + 0.49 + 136.89 + 470.89 + 1274.5}{9} = \frac{2986.1}{9} = 331.79$$

Take the non-negative square root of the quotient (converting squared units back to regular units) $= \sqrt{331.79} = 18.215$. So, the standard deviation of the set is 18.215

**6.10 Group 5 Without Aspirin treatment Daytime Diastolic BP**

**Figure 30.** Day time diastolic blood pressure (mmHg) of smokers and non smokers.
Table 18. Patient without Aspirin treatment (mean day time diastolic BPmmHg)

<table>
<thead>
<tr>
<th></th>
<th>Mean daytime Diastolic BP</th>
<th>Mean</th>
<th>Median</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-smokers</strong></td>
<td>68,72,76,79,82,84,86,87,90</td>
<td>70.88</td>
<td>82</td>
<td>4.35</td>
</tr>
<tr>
<td><strong>Smokers</strong></td>
<td>72,74,78,82,86,88,90,94,100</td>
<td>84.88</td>
<td>86</td>
<td>8.746</td>
</tr>
</tbody>
</table>

Mean (non-smokers) = \( \frac{68+72+76+79+82+84+86+87+90}{9} = 638/9 = 70.88 \)

Mean (Smokers) = \( \frac{72+74+78+82+86+88+90+94+100}{9} = 764/9 = 84.88 \)

Standard deviation calculation

Find the deviation of each number from the mean

For non-smokers

<table>
<thead>
<tr>
<th>Deviation</th>
<th>Number</th>
<th>Deviation</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>68-70.88</td>
<td>-2.88</td>
<td>72-84.88</td>
<td>-12.88</td>
</tr>
<tr>
<td>72-70.88</td>
<td>1.12</td>
<td>74-84.88</td>
<td>-10.88</td>
</tr>
<tr>
<td>76-70.88</td>
<td>5.12</td>
<td>78-84.88</td>
<td>-6.88</td>
</tr>
<tr>
<td>79-70.88</td>
<td>8.12</td>
<td>82-84.88</td>
<td>-2.88</td>
</tr>
<tr>
<td>82-70.88</td>
<td>11.12</td>
<td>86-84.88</td>
<td>1.12</td>
</tr>
<tr>
<td>84-70.88</td>
<td>13.12</td>
<td>88-84.88</td>
<td>3.12</td>
</tr>
<tr>
<td>86-70.88</td>
<td>15.12</td>
<td>90-84.88</td>
<td>5.12</td>
</tr>
</tbody>
</table>
87-70.88= 16.12
94-84.88= 9.12
90-70.88= 19.12
100-84.88=15.12

For non-smokers

Square each of the deviation, which amplifies large deviations and makes negative values positive,


Find the mean of those squared deviations


= 1251.5/9 =139

Take the non-negative square root of the quotient (converting squared units back to regular units)

=√139 =4.35 So, the standard deviation of the set is 4.35

For smokers

Square each of the deviation, which amplifies large deviations and makes negative values positive

165.589, 118.374, 47.334, 8.294, 1.254, 9.734, 26.214, 83.174, 228.614

Find the mean of those squared deviations,

=\frac{688.6}{9} =76.509

Take the non-negative square root of the quotient (converting squared units back to regular units),

=\sqrt{76.509} =8.746. So, the standard deviation of the set is 8.746

6.11 Group 6 With Aspirin treatment Daytime Diastolic BP.

Figure 31. Effect of aspirin treatment on day time diastolic blood pressure (mmHg) of smokers and non smokers.
Table 19. Patient with aspirin treatment (mean day time diastolic BPmmHg)

<table>
<thead>
<tr>
<th></th>
<th>daytime Diastolic BP</th>
<th>Mean</th>
<th>Median</th>
<th>Standard deviations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-smokers</strong></td>
<td>70,74,76,78,81,82,83,84,88</td>
<td>79.55</td>
<td>81</td>
<td>5.25</td>
</tr>
<tr>
<td><strong>Smokers</strong></td>
<td>72,76,80,84,87,90,92,94,96</td>
<td>85.66</td>
<td>87</td>
<td>7.83</td>
</tr>
</tbody>
</table>

Mean (non-smokers) = \( \frac{70+74+76+78+81+82+83+84+88}{9} = \frac{716}{9} = 79.55 \)

Mean (Smokers) = \( \frac{72+76+80+84+87+90+92+94+96}{9} = \frac{771}{9} = 85.66 \)

Standard deviation calculation

Find the deviation of each number from the mean,

For non-smokers

- 70-79.55= - 9.55
- 74-79.55= -5.55
- 76-79.55= -3.55
- 78-79.55= -1.55
- 81-79.55= 1.45

For smokers

- 72-85.66= -13.66
- 76-85.66= -9.66
- 80-85.66= -5.66
- 84-85.66= - 1.66
- 87-85.66= 1.34
For non-smokers

square each of the deviations, which amplifies large deviations and makes negative values positive,

91.202, 30.802, 12.602, 2.402, 2.102, 6.002, 11.902, 19.802, 71.402

Find the mean of those squared deviations


=248.218/9 =27.548

Take the non-negative square root of the quotient (converting squared units back to regular units),

=√27.548 =5.25. So, the standard deviation of the set is 5.25

For smokers

Square each of the deviations, which amplifies large deviations and makes negative values positive

186.595, 93.315, 32.035, 2.755, 1.795, 18.835, 40.195, 69.555, 106.915
Find the mean of those squared deviations,

\[= \frac{186.595 + 93.315 + 32.035 + 2.755 + 1.795 + 18.835 + 40.195 + 69.555 + 106.915}{9}\]

\[= \frac{551.995}{9} = 61.33\]

Take the non-negative square root of the quotient (converting squared units back to regular units), \(= \sqrt{61.33} = 7.83\). So, the standard deviation of the set is 7.83

6.12 Group 7. Without aspirin treatment (mean night time diastolic BP)

Figure 32. Night time Diastolic blood pressure (mmHg) of smokers and non smokers
Table 20. Without aspirin treatment (mean night time diastolic BPmmHg)

<table>
<thead>
<tr>
<th></th>
<th>Mean night time Diastolic BP</th>
<th>Mean</th>
<th>Median</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-smokers</td>
<td>61, 64, 65, 66, 68, 70, 72, 74, 76</td>
<td>68.44</td>
<td>68</td>
<td>4.669</td>
</tr>
<tr>
<td>Smokers</td>
<td>67, 69, 71, 72, 74, 78, 80, 86, 92</td>
<td>76.55</td>
<td>74</td>
<td>7.8</td>
</tr>
</tbody>
</table>

Mean (non-smokers) = \( \frac{61 + 64 + 65 + 66 + 68 + 70 + 72 + 74 + 76}{9} = \frac{616}{9} = 68.44 \)

Mean (Smokers) = \( \frac{67 + 69 + 71 + 72 + 74 + 78 + 80 + 86 + 92}{9} = \frac{689}{9} = 76.55 \)

Standard deviations

Find the deviation of each number from the mean,

Non-smokers

\begin{align*}
61 - 68.44 &= -7.44 \\
64 - 68.44 &= -4.44 \\
65 - 68.44 &= -3.44 \\
66 - 68.44 &= -2.44 \\
68 - 68.44 &= -0.44 \\
70 - 68.44 &= 1.56 \\
72 - 68.44 &= 3.56 \\
74 - 68.44 &= 5.56 \\
76 - 68.44 &= 7.56
\end{align*}

Smokers

\begin{align*}
67 - 76.55 &= -9.55 \\
69 - 76.55 &= -7.55 \\
71 - 76.55 &= -5.55 \\
72 - 76.55 &= -4.55 \\
74 - 76.55 &= -2.55 \\
78 - 76.55 &= 1.45 \\
80 - 76.55 &= 3.45 \\
86 - 76.55 &= 9.45 \\
92 - 76.55 &= 15.45
\end{align*}
For non-smokers

Square each of the deviation, which amplifies large deviations and makes negative values positive,

55.354, 19.714, 11.834, 5.954, 0.194, 2.434, 12.674, 30.914, 57.154

Find the mean of those squared deviations

\[
= \frac{55.354 + 19.714 + 11.834 + 5.954 + 0.194 + 2.434 + 12.674 + 30.914 + 57.154}{9}
\]

\[
= \frac{196.226}{9} = 21.803
\]

Take the non-negative square root of the quotient (converting squared units back to regular units),

\[
= \sqrt{21.803} = 4.669. \text{ So, the standard deviation of the set is } 4.669
\]

Smokers

Square each of the deviation, which amplifies large deviations and makes negative values positive,

91.202, 57.002, 30.802, 20.702, 6.502, 2.102, 11.902, 89.302, 238.702

Find the mean of those squared deviations

\[
= \frac{91.202 + 57.002 + 30.802 + 20.702 + 6.502 + 2.102 + 11.902 + 89.302 + 238.702}{9}
\]

\[
= \frac{548.218}{9} = 60.913
\]

Take the non-negative square root of the quotient (converting squared units back to regular units),
So, the standard deviation of the set is 7.8

6.13 Group 8 With Aspirin treatment night time Diastolic BP.

Figure 33. Effect of aspirin on night time diastolic blood pressure (mmHg) of smokers and non smokers.

Table 21. With aspirin treatments (mean night time diastolic BP mmHg)

<table>
<thead>
<tr>
<th></th>
<th>Night time Diastolic BP</th>
<th>Mean</th>
<th>Median</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-smokers</strong></td>
<td>61,63,65,67,69,71,72,75,76</td>
<td>60.33</td>
<td>69</td>
<td>9.774</td>
</tr>
<tr>
<td><strong>Smokers</strong></td>
<td>68,70,71,73,75,77,79,81,83</td>
<td>75.22</td>
<td>75</td>
<td>4.871</td>
</tr>
</tbody>
</table>
Mean (non-smokers) = \(61+63+65+67+69+71+72+75+76 = 619/9 = 68.77\)

Mean (Smokers) = \(68+70+71+73+75+77+79+81+83/9 = 677/9 = 75.22\)

Standard deviation calculation

Find the deviation of each number from the mean,

<table>
<thead>
<tr>
<th>For non-smokers</th>
<th>For smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>61-60.33= 0.67</td>
<td>68-75.22= -7.22</td>
</tr>
<tr>
<td>63-60.33=2.67</td>
<td>70-75.22= -5.22</td>
</tr>
<tr>
<td>65-60.33=4.67</td>
<td>71-75.22= -4.22</td>
</tr>
<tr>
<td>67-60.33= 6.67</td>
<td>73-75.22= -2.22</td>
</tr>
<tr>
<td>69-60.33= 8.67</td>
<td>75-75.22= -0.22</td>
</tr>
<tr>
<td>71-60.33= 10.67</td>
<td>77-75.22=1.78</td>
</tr>
<tr>
<td>72-60.33= 11.67</td>
<td>79-75.22=3.78</td>
</tr>
<tr>
<td>75-60.33= 14.67</td>
<td>81-75.22= 5.78</td>
</tr>
<tr>
<td>76-60.33= 15.67</td>
<td>83-75.22=7.78</td>
</tr>
</tbody>
</table>

For non-smokers

Square each of the deviations, which amplifies large deviations and makes negative values positive,
Find the mean of those squared deviations

\[=\frac{0.449+7.129+21.809+44.489+75.169+113.849+215.209+245.549}{9}\]

\[=\frac{859.84}{9} = 95.537\]

Take the non-negative square root of the quotient (converting squared units back to regular units)

\[=\sqrt{9.774} = 9.774\] So, the standard deviation of the set is 9.774

For smokers

Square each of the deviations, which amplifies large deviations and makes negative values positive

52.128, 27.248, 17.808, 4.928, 0.0484, 3.168, 14.288, 33.408, 60.528

Find the mean of those squared deviations,

\[=\frac{52.128+27.248+17.808+4.928+0.0484+3.168+14.288+33.408+60.528}{9}\]

\[=\frac{213.553}{9} = 23.728\]

Take the non-negative square root of the quotient (converting squared units back to regular units),

\[=\sqrt{23.728} = 4.871\] So, the standard deviation of the set is 4.87
6.14. Comparison between the groups of patients treated with aspirin (75mg/day) and the placebo controls (no aspirin)

**Table 22.** Comparison between the groups of patients treated with aspirin (75mg/day) and the placebo controls (no aspirin).

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood pressure in day/night period</th>
<th>Smokers Mean ±SD mmHg</th>
<th>Non-Smokers Mean ±SD mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Aspirin</td>
<td>No Aspirin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aspirin</td>
<td>No Aspirin</td>
</tr>
<tr>
<td>Day time systolic BP</td>
<td>121±13</td>
<td>134±14</td>
<td>107±11</td>
</tr>
<tr>
<td>Day time diastolic BP</td>
<td>85.6±7.8</td>
<td>84±8.7</td>
<td>79.55±5.25</td>
</tr>
<tr>
<td>Night time systolic BP</td>
<td>133.8±10.9</td>
<td>123±18</td>
<td>125±8.2</td>
</tr>
<tr>
<td>Night time diastolic BP</td>
<td>75.22±4.8</td>
<td>76.55±7.8</td>
<td>60.3±9.7</td>
</tr>
</tbody>
</table>

For all the groups shown there were 9 values determined. The statistical comparisons between groups were made using the Mann Whitney U test.

The data shown in table above are the actual mean values recorded for all the patient groups using the constant 24 hour monitoring device. If the values in each of the
individual columns are examined the mean blood pressure both systolic and diastolic are shown for the day and night periods.

If this figures are closely examined it may for the reader be surprising that none of the values show blood pressure readings which are normally classed as being hypertensive.

This is because the patients in all groups were receiving effective antihypertensive therapy and this study was focused on determining if the addition of aspirin had a further beneficial effect on blood pressure reduction.

**6.15. Experimental tests used during analysis**

Experimental tests used Comparing the mean systolic and diastolic pressure measurements, the mean daytime systolic and diastolic blood pressure measurements as mentioned in Table 22 were significantly higher for the smokers. The rise in blood pressure is a clear indication that smoking causes the elevation of blood pressure. There was also a clear indication of controlling blood pressure by aspirin. It has also been noted that the use of aspirin during nighttime as far more effective than daytime.

**Multivariate analysis** - is based on the statistical principle of multivariate statistics, which involves observation and analysis of more than one statistical variable at a time. In design and analysis, the technique is used to perform studies across multiple dimensions while taking into account the effects of all variables on the responses of interest. (Chernoff et al., 1954)

The variables that presented a significant difference in the univariate analysis between the groups of smokers and non-smokers were used in the multivariate analysis.
Univariate analysis - the ABPM parameters were expressed as minimum, maximum, median, mean and standard deviation values. Univariate analysis was conducted using the smoking variable for the groups that used and did not use aspirin. The data with a statistical significance of p less than 0.05 (p < 0.05) were selected.

Mean systolic and diastolic blood pressure measurements, the mean daytime systolic and diastolic blood pressure measurements (as mentioned in above graphs) were significantly higher for the smokers which have been shown in above given data. The rising in blood pressure is clear indication that smoking causes the rising in blood pressure. There was also clear indication of controlling of blood pressure by aspirin. It has also been note that use of aspirin during night time is far effective then day time. What real mechanism is involve in this is not clear but assumption is this that during day time body's almost all function are active and may be effective absorption of aspirin is reduced due to complex activities during day time. In resting condition during night time, many activities of body may ceased or slow down, and when we take aspirin in resting condition we are stimulating only active absorption of aspirin to blood.

The mean daytime systolic and diastolic blood pressure measurement was significantly higher for the smokers and the smokers which don’t use aspirin.

In the group of patients that did not use aspirin, the daytime diastolic blood pressure load was significantly higher (p = 0.0107) in the smokers. Similarly in the group of patients that did not use aspirin, the day time systolic blood pressure load was significantly higher (p< 0.0001) in the smokers.

Mean daytime systolic and diastolic blood pressures were both significantly higher in the smokers. Mean 24-hour systolic blood pressure readings were significantly higher in
the smokers; Blood pressure loads were consistently and significantly higher in the smokers.

The non-smoker which already has blood pressure, it has been observed in that group a great reduction in blood pressure as result of use of aspirin.

The smokers which did not have symptoms of blood pressure before smoking, they have blood pressure as result of regular smoking, their blood pressure has been controlled effectively after use of aspirin.

Summary of findings.

i) mean daytime systolic and diastolic pressures were both significantly higher in smokers

ii) mean 24 hour

iii) blood pressure loads

iv) the non smoker group who already suffered from blood pressure showed a great reduction in blood pressure as a result of the use of aspirin

v) the smoker group which did not have symptoms of blood pressure before smoking developed blood pressure as a result of regular smoking but their blood pressure was controlled effectively after the use of aspirin.
6.16 Discussion.

For diagnostic purposes as well as therapeutic and prognostic evaluation monitoring 24hrs blood pressure, ABPM analysis is powerful tool. Mean systolic and diastolic blood pressure measurements are essential data for this study. The variables that are more closely related to cardiovascular events such as acute myocardial infarction and encephalic stroke were the mean night time systolic blood pressure followed by the mean 24-hour systolic blood pressure and the mean daytime blood pressure measurements (See Table 23).

**Table 23.** Division of the group into non-smokers and smokers with placebo or aspirin treatment.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 with aspirin</th>
<th>Group without aspirin</th>
</tr>
</thead>
<tbody>
<tr>
<td>A non-smokers</td>
<td>120</td>
<td>83</td>
</tr>
<tr>
<td>B smokers</td>
<td>35</td>
<td>39</td>
</tr>
</tbody>
</table>

The results (Table 22) showing that the mean daytime systolic and diastolic blood pressure measurements were significantly higher in smokers, particularly, systolic blood pressure were found to be higher in night time as compare to diastolic blood pressure.

It is a known fact that smoking one cigarette increases the heart rate by 14% and blood pressure by 6%. This reaction is probably caused by the increased plasma concentrations of adrenaline and noradrenaline while smoking (Yugar et al., 2002).
Norepinephrine levels progressively increase within a 12.5 minute period and peak after 15 minutes before returning towards initial levels after a further 30 minutes. This alteration causes a maximum blood pressure increase which although it reduces towards the previous level after 30 minutes, the level does remain higher than the values registered before smoking (Barua et al., 2002).

This may result in a ‘staircase effect’ when cigarettes are smoked in quick succession. These effects are not irreversible however as it has been recorded that in patients during the first week after they had quit smoking that there a significant decrease in these variables. These patients also demonstrated a significant decrease in the plasma and urinary levels of norepinephrine and epinephrine after one week of not smoking (Mikkelsen et al., 1997).

The effects of smoking are obviously complex. A previous reported study evaluated in ten normotensive smokers who smoked one cigarette every fifteen minutes during an hour and demonstrated that blood pressure and heart rate increased immediately after smoking the first cigarette and remained at that level while the other three cigarettes were smoked. This of course is not a classical ‘staircase effect’ but rather some complex process of elevation of a level which is then maintained. The initial blood pressure increase was approximately 12 mmHg for the systolic reading and 15 mmHg for the diastolic reading. The pressure effect caused by smoking decreased during an hour demonstrated that blood pressure changes caused by smoking probably is affected due to an unknown compensatory mechanism. In this same study, six other normotensive smokers smoked one cigarette every thirty minutes during eight hours and once again there was an increase in blood pressure and heart rate soon after smoking the first cigarette and
they remained elevated during the entire period of exposure to tobacco. The greatest blood pressure variations for both groups occurred while they smoked (Whincup et al., 1995).

Chronically, nicotine diminishes the sensitivity of baroreceptors which are neural receptors located in both the aortic arch and carotid sinuses. They detect the systolic blood pressure and if the pressure is too high, as is the effect of circulating catecholamines, this causes these receptors cause the vasomotor centre to be stimulated which brings about the vasodilatation in both arterioles and veins causing a fall in the overall pulse pressure. There is also an increased parasympathetic activity and a decrease in the cardioacceleratory centre which both reduce the heart rate and so effectively lower the systemic blood pressure. The baroreceptors are an example of a short term blood pressure regulation mechanism. Baroreceptors detect the amount of stretch of the blood vessel walls, and so act as mechanoreceptors sending their signals to the central nervous system in response to this stretch. Baroreceptor sensitivity and stimulation also increases the production of thromboxane A2, a powerful vasoconstrictor. This increase in TxA2 production has been detected, not directly because of its very short half life but rather by measuring the levels of thromboxane B2, the metabolite of TxA2, which have been found in hypertensive smokers. It has also been demonstrated that smoking increases angiotensin II production (Berecek et al., 1982) the potent vasoconstrictor effect of which is widely documented. In contrast to the stimulation of vasoconstriction, nicotine inhibits the synthesis of endothelin, an extremely potent vasoconstrictor which is a potential beneficial effect on the cardiovascular system. Paradoxically, nicotine also inhibits the production of nitrous oxide by endothelial cells which acts as a local vasodilator, which could add to the vasoconstrictor response. This last effect is very curious as the reduction of nitrous oxide is not specifically related to the number of cigarettes smoked.
Contrary to the results of the study in which normotensive patients, monitored by ABPM, were submitted to the effects of nicotine smokers, did not have the same effects on the cardiovascular system.

The smokers presented lower mean day time systolic BP and diastolic BP mean readings. It has been suggested that this outcome is caused by an adaptive effect in the sympathetic nervous system after numerous years of exposure to nicotine. They also report that smokers are less affected by "white coat" hypertension and confirm that the chronic stimulation by nicotine and its metabolites eliminate the power of any other stimuli to provoke further reaction. The authors emphasize that smoking also helps to reduce stress, which could be associated with the lowering of blood pressure. (Lida et al., 2006)

For smokers their mean night time diastolic blood pressure did not present a significant difference between the groups in relation to smoking, as seen previously in other studies with both normotensive and hypertensive patients.

However, the mean night time systolic blood pressure was significantly higher in the smokers that did not use aspirin. It is believed that the acute pressure effect caused by smoking does not exist during the night when most individuals are sleeping and therefore justifies the similar blood pressure measurements of smokers and non-smokers. For both normotensive aspirin taking patients and those who were hypertensive, the physiological blood pressure trend has a circadian rhythm, which reaches lower levels when the person is sleeping and returns to higher levels when the person wakes up.

Most of the ambulatory devices, including those used in this study, register night time hours as the period between 11p.m. and 7 a.m.. This obviously this could lead to inaccurate results for any patients that do not strictly abide by these hours for going to
sleep and waking up. There is a device called the ‘Timepiece’ which is just a concept at the moment – it is called *Escape Clock* and features a black finish and big red digits. When you want to go back to sleep for a while, simply give it a good hit with your fist.

**Figure 34.** The timepiece – which is just a concept at the moment – is called *Escape Clock* and features a black finish and big red digits (website, 2008).

Encouraging the patients to use the sleep/activity button could result in more accurate data for hours of sleep. However, we have observed that some patients become confused when they have to adjust the device distorting the registers. Sleep quality could interfere in the physiological decrease and this information should also be recorded on the test report.
The most interesting and evident fact of this study is that blood pressure levels for smokers increase during the day. If we continue to exclusively use simple in-office blood pressure measurements for smokers it is possible that regular evaluations could be made that do not reflect the actual systemic blood pressure and inadequately estimate the homodynamic effect of target organ lesions. Some theories try to explain the epidemiological findings of blood pressure levels in smokers that are less than or equal to the casual blood pressure measurement. The theory with the highest acceptance rate is that there is a reduction of the acute effect which has been caused by smoking due to tobacco abstinence for a few minutes or hours before the office measurement. Another attempt to explain this phenomenon is that the prolonged and chronic use of tobacco increases heart rate that would lead to a decrease in left ventricular end-systolic volume and consequently lower blood pressure. The use of ABPM exclusively during the daytime can be a valid consideration of future studies for both diagnostic purposes and the assessment of aspirin in smokers.

An alternative to ABPM for smokers could be the repetitive in-home blood pressure measurements taken over a period of a few days using validated equipment. This method has been recommended, since it helps in treatment adherence and is superior to the casual measurements taken at the doctor’s office in regard to target organ lesions. In comparison to both the in-office measurements and the in-home blood pressure measurements the ABPM offers a greater number of readings, better reproducibility and data storage in the unit’s memory chip. However, a greater number of more economical units need to be validated and specific diaries should be created for these patients to perhaps understand the data which they collect. A series of in-home readings recording the times of cigarette smoking can be useful for these patients, avoiding interpretations based on unreal situations like a programmed abstinence from smoking.
All hypertensive patients are advised to quit smoking as a control measure for cardiovascular risk factors. This advice is part of the non-medicinal hypertension treatment that is being incorporated which also includes a low sodium diet, moderate alcohol consumption, weight loss and periodic physical activity.

Short-term longitudinal studies using ABPM to evaluate patients that have quit smoking reveal that the blood pressure measurements of these patients are substantially lower than when they were smoking. These observations suggest a possible aetiopathogenic link between the effect of smoking and hypertension. Prospective studies with long-term follow-up and the control of variables such as weight gain may be able to confirm and quantify this effect.

It appears that the smoking population is a special group in which ABPM is the most adequate method for blood pressure assessment, avoiding the isolated measurement at the doctor's office, a procedure which could incorrectly diagnose hypertensive patients or under medicate patients using aspirin, the ABPM is a really useful technique to eliminate changes on a minute by minute to those over a 24 hour cycle and so provided evidence for a much better rational basis of treatment.

6.17 Valid Conclusion for future studies

It should be stressed that the mean daytime systolic and diastolic blood pressure readings were consistently higher in the smokers when compared to non-smokers. The low dose aspirin was effective treatment in controlling blood pressure of smokers for systolic in the daytime and diastolic in the nighttime. So, perhaps instead of using the more conventional and perhaps more expensive treatment using antihypertensive drugs aspirin could have a central role in the therapy of hypertension.
Chapter 7

General conclusions

Bringing together the conclusions of the individual studies within this thesis, highlights the following key points:

Passive smoking results in a measurable absorption by non smokers perhaps indicating the potential hazards of this process. Any absorbed nicotine has to be both metabolised and excreted in both smokers and non smokers and the level of nicotine in the body reflects the abilities of these two systems. This has been considered in chapter 4.

It is considered that aspirin should be introduced as early as possible into a hypertensives’ treatment so as to prevent the onset of CHD. This has been considered in chapter 5.

Aspirin lowers the systolic daytime BP and diastolic night time BP of smokers and this is a really interesting finding and is considered at length in Chapter 6.

However, this investigation proved to be both challenging and interesting for a number of reasons. The first challenge was the scale and the diverse nature of the world literature on hypertension. It was major challenge to ‘get to grips’ with the wealth of detail which was available. But after a comprehensive reading of the literature it could be clearly established that cardiovascular disease and hypertension in particular is not limited to any one country and there were problems with its treatment. For example in 2003 the WHO considered in the WHO/ISH Hypertension guidelines that:

Hypertension is already a highly prevalent cardiovascular risk factor worldwide because of increasing longevity and prevalence of contributing factors such as obesity. Whereas the
Treatment of hypertension has been shown to prevent cardiovascular diseases and to extend and enhance life, hypertension remains inadequately managed everywhere. (my italics)

Furthermore, two other findings from the literature were that smokers had twice as high a mortality than non-smoking hypertensives and that intensive blood pressure treatment was beneficial to all but the smoking hypertensives (Lund-Johassen, 2003). This provides a clear focus for this thesis to try and develop a better strategy for drug treatment of this sub-group of hypertensives. So the strategy for the thesis had clear and obvious aims and objectives which have been achieved.

Another complicating factor is that most studies on hypertension have been carried out in North America and Europe and the results extrapolated to the rest of the world. When this is considered for Kuwait then there are many differences illustrated by different – genetics, diet, physical activity, and the use of medical guidelines.

A further complicating factor is of course the cigarettes which are available in Kuwait are similar to those elsewhere in the world and it is interesting to note that:

The nicotine content in several major brands is reportedly on the rise. Harvard University and the Massachusetts Health Department revealed that between 1997 and 2005 the amount of nicotine in Camel, Newport, and Doral cigarettes may have increased by as much as 11 percent.

The American brands Marlboro, Kool, Camel and Kent own roughly 70% of the global cigarette market.

The unexpected findings that aspirin showed a hypotensive effect in hypertensives is of great interest. In a publication the atypical response of hypertensives who smoked was briefly described as:

‘the results of the smoking cohort are puzzling in many respects and some are not easy to explain. (Lund-Johansen, 2003)

These hypertensives failed to respond to normal antihypertensive therapy and were resistant to the conventional therapy:

The new subanalysis showed that the incidences of death and cardiovascular events per 1000 patient-years were much higher in the aggressive treatment groups. When the two treatment goal groups (intensive versus less intensive) were compared, the relative risk (RR) was increased in the most intensively treated group for total death, cardiovascular death, sudden death, major cardiovascular diseases, and stroke (RR of 2.03, 2.67, 2.93, 1.74 and 2.44, respectively). The baseline characteristics in the different treatment groups were super imposable.

This makes the effects of aspirin look even more interesting as the drug can hardly be classed as ‘aggressive’ and was more active in the less severe forms of hypertension which suggests that as for conventional therapy the aspirin cannot modify whatever ‘centre’ is responsible for the increased mortality of the severe hypertensives who smoke. One possible explanation is suggested in the paper:

It is well established that smoking induces pathological alterations in the thrombogenic mechanism, and that fibrinogen increases, platelet adhesiveness is increased and fibrinolysis is impaired. Interestingly, some of the same type of abnormalities are seen in hypertensive patients. Thus, the combination of hypertension and smoking could be
particularly harmful for the high pressure compartment vessels, making them more sensitive to reductions in perfusion pressure than in subjects with just moderate hypertension.

From the audit data presented in Appendix 1 it is clear that Kuwait uses a considerable quantity of aspirin in its hospital dispensaries. The strength of the tablets establishes that it not for analgesic doses but rather for its anti-thrombotic effects. Perhaps the use of aspirin could be extended due to the evidence from this study. This would of course necessitate the study of aspirin in hypertensives who do not smoke but that could be done by someone else as a useful project.

**Future studies**

Future studies could be undertaken to develop the findings made in the individual studies of this thesis and produce useful and interesting data to extend them.

1. extend the prophylactic use of aspirin in hypertensives who are non-smokers
2. target recently diagnosed hypertensives both smokers and non-smokers and monitor them using ambulatory methods to plot the development of their hypertension.
3. study the effect of aspirin in women as well as men
4. determine the exact levels of nicotine in the cigarettes commonly sold in Kuwait to establish their relative risk to the smoking population
5. try and introduce the NICE guidelines for the cessation of smoking so as to reduce one of the risk factors in the hypertensive patient which may reduce thrombotic events
Future clinical pharmacy developments (Appendix 4) which may impact on the role of clinical pharmacy in the treatment of hypertension in the State of Kuwait, could consider these as a course of action.

The study contained a limitation which should also be mentioned.

The culture of Kuwait does not allow contact between a male and a female who are not related and so inclusion of women in this study was not therefore possible. This is unfortunate as the effect of aspirin in women would be a very interesting aspect to study to see if the effect was male gender specific or not.
References


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Jinno Laboratory (1996) Acetylsalicylic acid, School of Materials Science, Toyohashi University of Technology.


Yamasaki, E. and Ames, B. N. (1977) Concentration of mutagens from urine by absorption with the non polar resin XAD-2: Cigarette smokers have mutagenic urine. PNAS, 74, pp.3555-3559.


# Appendix 1

Reported effects of tobacco products in both men and women (Placezek et al., 2004).

<table>
<thead>
<tr>
<th>Disease</th>
<th>Consequences</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cancers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bladder cancer</td>
<td>&quot;Smoking cause of bladder cancer.&quot;</td>
<td>&quot;The evidence is sufficient to infer a causal relationship between smoking and bladder cancer.&quot;</td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>&quot;Smoking increase cervical cancer.&quot;</td>
<td>&quot;The evidence is sufficient to infer a causal relationship between smoking and cervical cancer.&quot;</td>
</tr>
<tr>
<td>Esophageal cancer</td>
<td>&quot;Smoking cause esophageal cancer.&quot;</td>
<td>&quot;The evidence is sufficient to infer a causal relationship between smoking and cancers of the esophagus.&quot;</td>
</tr>
<tr>
<td>Kidney cancer</td>
<td>&quot;Smoking is factor in the development of kidney cancer.&quot;</td>
<td>&quot;The evidence is sufficient to infer a causal relationship between smoking and renal cell, and renal pelvis cancers.&quot;</td>
</tr>
<tr>
<td>Laryngeal cancer</td>
<td>&quot;Smoking is cause cancer of the lung, larynx, oral cavity, and esophagus.&quot;</td>
<td>&quot;The evidence is sufficient to infer a causal relationship between smoking and cancer of the larynx.&quot;</td>
</tr>
<tr>
<td>Leukemia</td>
<td>&quot;Leukemia has recently been implicated as a smoking-related disease.&quot;</td>
<td>&quot;The evidence is sufficient to infer a causal relationship between smoking and cancer of the larynx.&quot;</td>
</tr>
<tr>
<td>Disease</td>
<td>Consequences</td>
<td>Conclusion</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>smoking and acute myeloid leukemia.</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>&quot;Smoking causes lung cancer.&quot;</td>
<td>&quot;The evidence is sufficient to infer a causal relationship between smoking and lung</td>
</tr>
<tr>
<td>Oral cancer</td>
<td>&quot;Smoking cause oral cancers.&quot;</td>
<td>&quot;The evidence is sufficient to infer a causal relationship between smoking and cancers of the oral cavity and pharynx.&quot;</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>&quot;Smoking reduces the risk of pancreatic cancer, compared with continued smoking.&quot;</td>
<td>&quot;The evidence is sufficient to infer a causal relationship between smoking and pancreatic cancer.&quot;</td>
</tr>
<tr>
<td>Stomach cancer</td>
<td>&quot;Data on smoking and cancer of the stomach are unclear.&quot;</td>
<td>&quot;The evidence is sufficient to infer a causal relationship between smoking and gastric cancers.&quot;</td>
</tr>
<tr>
<td><strong>Cardiovascular disease</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal aortic aneurysm</td>
<td>&quot;Death more common in cigarette smokers.&quot;</td>
<td>&quot;The evidence is sufficient to infer a causal relationship between smoking and abdominal aortic aneurysm.&quot;</td>
</tr>
<tr>
<td>Atherosclerosis</td>
<td>&quot;Smoking the most common factor to atherosclerotic peripheral vascular disease.&quot;</td>
<td>&quot;The evidence is sufficient to infer a causal relationship between smoking and subclinical disease.&quot;</td>
</tr>
<tr>
<td>Disease</td>
<td>Consequences</td>
<td>Conclusion</td>
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<td>-----------------------------</td>
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</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>&quot;Smoking is a major cause of cerebrovascular disease (stroke).&quot;</td>
<td>&quot;The evidence is sufficient to infer a causal relationship between smoking and stroke.&quot;</td>
</tr>
<tr>
<td>Coronary heart disease</td>
<td>&quot;Smoking is cause coronary heart disease.&quot;</td>
<td>&quot;The evidence is sufficient to infer a causal relationship between smoking and coronary heart disease.&quot;</td>
</tr>
<tr>
<td>Respiratory diseases</td>
<td></td>
<td>&quot;The evidence is sufficient to infer a causal relationship between active smoking and chronic obstructive pulmonary disease morbidity and mortality.&quot;</td>
</tr>
<tr>
<td>Chronic obstructive pulmonary disease</td>
<td>&quot;Smoking is the most important of the causes of chronic bronchitis.&quot;</td>
<td>&quot;The evidence is sufficient to infer a causal relationship between active smoking and chronic obstructive pulmonary disease morbidity and mortality.&quot;</td>
</tr>
<tr>
<td></td>
<td>&quot;Smoking cessation reduces rates of respiratory symptoms such as cough, sputum production, and wheezing, and respiratory infections such as bronchitis and pneumonia, compared with continued smoking.&quot;</td>
<td>&quot;The evidence is sufficient to infer a causal relationship between smoking and acute respiratory illnesses, including pneumonia, in persons without underlying smoking-related chronic obstructive lung disease.&quot;</td>
</tr>
<tr>
<td>Pneumonia</td>
<td></td>
<td>&quot;The evidence is sufficient to infer a causal relationship between smoking and acute respiratory illnesses, including pneumonia, in persons without underlying smoking-related chronic obstructive lung disease.&quot;</td>
</tr>
<tr>
<td>Respiratory effects in utero</td>
<td>&quot;In utero exposure to maternal smoking is associated with reduced lung function among infants.&quot;</td>
<td>&quot;The evidence is sufficient to infer a causal relationship between maternal smoking during pregnancy and respiratory effects in utero.&quot;</td>
</tr>
<tr>
<td>Disease</td>
<td>Consequences</td>
<td>Conclusion</td>
</tr>
<tr>
<td>------------------</td>
<td>------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Respiratory</td>
<td>&quot;Cigarette smoking during childhood and adolescence produces significant</td>
<td>&quot;The evidence is sufficient to infer a causal relationship between active</td>
</tr>
<tr>
<td>effects in</td>
<td>health problems among young people, including cough and phlegm production,</td>
<td>smoking and impaired lung growth during childhood and adolescence.&quot;</td>
</tr>
<tr>
<td>childhood and</td>
<td>an increased number and severity of respiratory illnesses, decreased</td>
<td>&quot;The evidence is sufficient to infer a causal relationship between active</td>
</tr>
<tr>
<td>adolescence</td>
<td>physical fitness, an unfavorable lipid profile, and potential retardation</td>
<td>smoking and the early onset of lung function decline during late</td>
</tr>
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<td></td>
<td>in the rate of lung growth and the level of maximum lung function.&quot;</td>
<td>adolescence and early adulthood.</td>
</tr>
<tr>
<td>Respiratory</td>
<td></td>
<td>The evidence is sufficient to infer a causal relationship between active</td>
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<tr>
<td>effects in</td>
<td></td>
<td>smoking and respiratory symptoms in children and adolescents, including</td>
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<tr>
<td>adulthood</td>
<td></td>
<td>coughing, phlegm, wheezing, and dyspnea. The evidence is sufficient to</td>
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<td></td>
<td></td>
<td>infer a causal relationship between active smoking and asthma-related</td>
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<td></td>
<td></td>
<td>symptoms (i.e., wheezing).&quot;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&quot;Cigarette smoking accelerates the age-related decline in lung function</td>
</tr>
<tr>
<td></td>
<td></td>
<td>that occurs among never smokers. With sustained abstinence from smoking,</td>
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<tr>
<td></td>
<td></td>
<td>the rate of decline in pulmonary function among former smokers returns to</td>
</tr>
<tr>
<td></td>
<td></td>
<td>that of never smokers.&quot;</td>
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</tbody>
</table>

"The evidence is sufficient to infer a premature onset of and an"
<table>
<thead>
<tr>
<th>Disease</th>
<th>Consequences</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other respiratory effects</td>
<td>&quot;Smoking cessation reduces rates of respiratory symptoms such as cough, sputum production, and wheezing, and respiratory infections such as bronchitis and pneumonia, compared with continued smoking.&quot;</td>
<td>&quot;The evidence is sufficient to infer a causal relationship between active smoking and all major respiratory symptoms among adults, including coughing, phlegm, wheezing, and dyspnea.&quot;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&quot;The evidence is sufficient to infer a causal relationship between active smoking and poor asthma control.&quot;</td>
</tr>
<tr>
<td>Other Diseases</td>
<td></td>
<td>&quot;The evidence is sufficient to infer a causal relationship between sudden infant death syndrome and maternal smoking during and after pregnancy.&quot;</td>
</tr>
<tr>
<td>Reproductive effects</td>
<td></td>
<td>&quot;The evidence is sufficient to infer a causal relationship between sudden infant death syndrome and maternal smoking during and after pregnancy.&quot;</td>
</tr>
<tr>
<td>Fetal death and stillbirths</td>
<td>&quot;The risk for prenatal mortality both stillbirth and neonatal deaths and the risk for sudden infant death syndrome (SIDS) are increased among the offspring of women who smoke.&quot;</td>
<td>&quot;The evidence is sufficient to infer a causal relationship between sudden infant death syndrome and maternal smoking during and after pregnancy.&quot;</td>
</tr>
<tr>
<td><strong>Other Diseases</strong></td>
<td>Fertility</td>
<td>Other effects</td>
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</tr>
<tr>
<td>during pregnancy.&quot;</td>
<td>&quot;Smoke has increased risks for conception delay and for both primary and secondary infertility.&quot;</td>
<td>&quot;Women who smoke have an increased risk for cataract.&quot;</td>
</tr>
<tr>
<td></td>
<td>&quot;The evidence is sufficient to infer a causal relationship between smoking and reduced fertility in women.&quot;</td>
<td>&quot;The evidence is sufficient to infer a causal relationship between smoking and nuclear cataract.&quot;</td>
</tr>
<tr>
<td><strong>Low birth weight</strong></td>
<td>&quot;Smoke during pregnancy has a lower average birth weight.&quot;</td>
<td>&quot;Relationships between smoking and cough or phlegm are strong and consistent; they have been amply documented and are judged to be causal.&quot;</td>
</tr>
<tr>
<td></td>
<td>&quot;The evidence is sufficient to infer a causal relationship between maternal active smoking and fetal growth restriction and low birth weight.&quot;</td>
<td>&quot;The evidence is sufficient to infer a causal relationship between smoking and diminished health status that may be diminished.&quot;</td>
</tr>
<tr>
<td><strong>Pregnancy complications</strong></td>
<td>&quot;Smoking during pregnancy is associated with increased risks for preterm premature rupture of membranes, abruptio placentae, and placenta previa, and with a modest increase in risk for preterm delivery.&quot;</td>
<td>&quot;The evidence is sufficient to infer a causal relationship between maternal active smoking and preterm delivery and shortened gestation.&quot;</td>
</tr>
<tr>
<td></td>
<td>&quot;The evidence is sufficient to infer a causal relationship between maternal active smoking and premature rupture of the membranes, placenta previa, and placental abruption.&quot;</td>
<td></td>
</tr>
</tbody>
</table>
### Other Diseases

"Consideration of evidence from many different studies has led to the conclusion that cigarette smoking is the overwhelmingly most important cause of cough, sputum, chronic bronchitis, and mucus hypersecretion."  

manifest as increased absenteeism from work and increased use of medical care services."

"The evidence is sufficient to infer a causal relationship between smoking and increased risks for adverse surgical outcomes related to wound healing and respiratory complications."  

"The evidence is sufficient to infer a causal relationship between smoking and hip fractures."

**Hip fractures**  

"Women who currently smoke have an increased risk for hip fracture."

**Low bone density**  

"Postmenopausal women who currently smoke have lower bone density than do women who do not smoke."  

"In postmenopausal women, the evidence is sufficient to infer a causal relationship between smoking and low bone density."  

**Peptic ulcer disease**  

"The relationship between cigarette smoking and death rates from peptic ulcer, especially gastric ulcer, is confirmed. In addition, morbidity data suggest a similar relationship exists with the prevalence of reported disease from this cause."  

"The evidence is sufficient to infer a causal relationship between smoking and peptic ulcer disease in persons who are *Helicobacter pylori* positive."

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**Appendix 2**  

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Instructions for patients on how to take low dose aspirin are shown by the following examples

For example, ‘briefly mention and you can take aspirin to help you during a heart attack. After you call 911 or other emergency services, chew 1 adult-strength aspirin (325 mg) if you are not allergic to aspirin and if there is no other reason that you can't take aspirin. Aspirin slows blood clotting, so a blood clot that is causing the heart attack stays smaller.’

‘You may also take low-dose aspirin (81 mg) every day to help lower the risk of a heart attack or stroke.

Low-dose aspirin may be used:’

After a heart attack, to prevent another one.

By people who have coronary artery disease.

By people with stable angina.

By people with unstable angina.

After bypass surgery or angioplasty.

By people who have had a stroke and or transient ischemic attack (TIA).

After surgery to prevent a stroke (carotid endarterectomy).

By healthy men over age 40 who have one or more risk factors for heart disease, as long as their blood pressure is controlled and the benefits of aspirin are greater than the risks.

By healthy women over age 65, or women under 65 who have one or more risk factors for
heart disease as long as their blood pressure is controlled and the benefits of aspirin are greater than the risks.

If you have atrial fibrillation and cannot take or choose not to take warfarin, you may take an adult-strength aspirin (325 mg) every day to help lower the risk of a stroke.

Aspirin protects you from having a clot-related stroke in the same way it protects you from having a heart attack.

Aspirin slows the blood's clotting action by reducing the clumping of platelets. Platelets are cells that clump together and help to form blood clots. Aspirin keeps platelets from clumping together, thus helping to prevent or reduce blood clots.

During a heart attack, blood clots form in an already-narrowed artery and block the flow of oxygen-rich blood to the heart muscle (or to part of the brain, in the case of stroke). When taken during a heart attack, aspirin slows clotting and decreases the size of the forming blood clot. Taken daily, aspirin's anti-clotting action helps prevent a first or second heart attack.

Aspirin should not be taken if you think you are having a stroke, because not all strokes are Aspirin should not be taken by people who are at risk for or who have had a hemorrhagic stroke, which is a type of stroke that is not caused by a blood clot but rather by bleeding into and around the brain. Aspirin can trigger asthma attacks in some people who have sensitivity to it. Also, don't take aspirin without first talking to your doctor if you're already taking prescribed blood thinners, such as Coumadin. The combined effect could cause bleeding problems.
Aspirin should not be taken with many prescription and over-the-counter drugs, vitamins, herbal remedies, and supplements. So, before you start aspirin therapy, talk to your doctor about all the drugs and other remedies you take. Because aspirin reduce your blood's ability to clot, your doctor may want you to stop taking aspirin at least 5 days before any surgery or dental procedure that may cause bleeding. Do not suddenly stop taking aspirin without talking to your doctor first. Talking to your doctor first is especially important if you have had a stent placed in a coronary artery.

Tell your doctor if you notice that you bruise easily, have bloody or black stools, or have prolonged bleeding from cuts or scrapes.

Although nonsteroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen and naproxen, relieve pain and inflammation much like aspirin does, they do not affect blood clotting in the same way that aspirin does. Do not substitute NSAIDs for aspirin because they will not decrease your risk of another heart attack.

So, information is available to patient from many sources to give them an insight as to how aspirin should be taken and its possible consequences.

Finally here is a comment about what patients should do if they have take analgesics as well as aspirin.

If both aspirin and a pain reliever every day have been used, doctor's suggestion about what pain reliever should take must be considered. If we take uncoated aspirin and ibuprofen at the same time, the aspirin may not work as well to prevent a heart attack. We may be able to use acetaminophen instead of ibuprofen to treat our pain. But if ibuprofen is our only option, avoid taking it during the 8 hours before and the 30 minutes after our aspirin dose. For example, we can take ibuprofen 30 minutes after our aspirin dose. If we take ibuprofen
once in a while, it does not seem to cause problems. Experts do not know if NSAIDs other than ibuprofen interfere with uncoated aspirin. Also, experts do not know if people who take a daily coated aspirin should be concerned about ibuprofen or other NSAIDs interacting with the aspirin (Thomas et al., 2006).
Appendix 3

An audit of drugs used to treat hypertension and other cardiovascular diseases

Objective:

To audit the current use of aspirin and a range of categories of cardiovascular drugs in the state of Kuwait hospitals in order to contribute to the clinical pharmacy developments of the country

Background

Data has been obtained from the centralized official records of the pharmaceutical supply system of Kuwait for the period January 2007 – January 2011. Written permission to both obtain and use this data was granted by Dr Omar A. Al-Sayed Omar Assistant Undersecretary for Drugs and Medical Supplies Affairs. A copy of this official permission is shown in Appendix 4.

Methodology

This data was examined for the quantities of aspirin tablets of 100mg strength which had been used in this time period and the quantities of this drug were compared with other drugs used for a variety of conditions for both anticoagulation and cardiovascular conditions.

Results

The current use of aspirin in the state of Kuwait hospitals in comparison to other anti-platelet drugs.

The first group to be studied was to compare low dose aspirin usage against the widely used standard treatment for an extension of blood clots in those patients who have an increased risk of blood coagulation, namely warfarin. The relative quantities are shown in Figure A with the actual data being shown in Table A. It can be seen that the total use is in millions of tablets – approximately 18 million warfarin tablets have been used in this four
year period as compared with aspirin where 131 536 400 tablets were used. This means that 7.2 times more aspirin has been used than all strengths of warfarin combined. This may reflect the actual prophylactic use of aspirin as against the use in warfarin in proven greater risk – for example in atrial fibrillation or to inhibit the extension of a clot which has already formed. It could be argued the use of aspirin may have prevented the problems of blood clots otherwise the use of warfarin would have been even greater.
Figure A. The comparative use of 100mg aspirin as compared against three formulations of warfarin – 1, 2 and 5mg tablets during the time period 2007-2011. The actual figures are shown in the table A below.

Medical Group: anticoagulation

![Graph showing the comparative use of 100mg aspirin against three formulations of warfarin.]  

Table A - Actual numbers of tablets dispensed by the central medical stores in the time period January 2007 – January 2011 for three strengths as warfarin – 1, 2 and 5mg and the 100mg strength of aspirin.

<table>
<thead>
<tr>
<th>Basic Description</th>
<th>Unit</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subclass: Anti Coagulants warfarin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WARFARIN 1MG</td>
<td>TAB</td>
<td>5 540 500</td>
</tr>
<tr>
<td>WARFARIN 2MG</td>
<td>TAB</td>
<td>7 148 100</td>
</tr>
<tr>
<td>WARFARIN 5MG</td>
<td>TAB</td>
<td>5 448 500</td>
</tr>
</tbody>
</table>
next comparison which was made was between two other drugs used to modify platelet function so as to decrease the potential of platelet activation and subsequent clot formation.

The drugs in this class were clopidogrel 75mg (Plavix) and dipyridamole 75mg (Persantin). From Figure B and table B it can be seen that the use of both these agents are orders of magnitude less than that for aspirin. They are often prescribed for patients who have an aspirin allergy or are intolerant of aspirin which usually means that the gastric irritation is too serious to warrant further use of the drug.

**Figure B.** The comparative use of 100mg aspirin against two well established drugs clopidogrel and dipyridamole, both of 75mg strength, during the time period 2007-2011. The actual figures are shown in table B below.

![Graph showing the comparative use of aspirin, clopidogrel, and dipyridamole.](image)

**Table B.** Actual numbers of tablets dispensed by the central medical stores in the time period January 2007 – January 2011 for clopidogrel and dipyridamole and the 100mg strength of aspirin.

<table>
<thead>
<tr>
<th>Subclass: Anti platelet</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ACETYL SALICYLIC ACID 100MG</td>
<td>TAB</td>
</tr>
<tr>
<td>CLOPIDOGREL 75MG(PLAVIX)</td>
<td>TAB</td>
</tr>
<tr>
<td>DIPYRIDAMOLE (PERSANTIN) 75MG</td>
<td>TAB</td>
</tr>
</tbody>
</table>
The figures suggest that 9.2 times more aspirin has been used than the other anti-platelet drugs. This would suggest that the aspirin is not used in simple combination with the other two drugs but rather as the primary treatment for conditions in which platelets are involved and in which it exert a prophylactic effect.

The comparative use of aspirin compared to a range of other cardiovascular drugs.

**Digoxin**

The first comparison is the use of aspirin against formulations of digoxin (Figure C and Table C). This is rather a difficult comparison to make as the drug is used for its positive inotropic effects and reduce conductivity within the atroventricular node rather than an antiplatelet effect and the drug is usually used for controlling the ventricular response in persistent and permanent atrial fibrillation rather than a specific effect on platelets. However, atrial fibrillation is known to stimulate platelets to be pro-coagulant which is one of the reasons that warfarin is used in this condition to prevent the occurrence of emboli forming which may lead to vascular occlusions causing myocardial infarctions and stroke. Interestingly, the use of warfarin is greater than for the use of digoxin showing that the warfarin is used for other conditions rather than potential atrial fibrillation which is treated by digoxin. The data shown below provides the relative usage of this drug and shows its use to be small, as would perhaps be expected for a drug which has therapeutic limitations due to its narrow therapeutic index.

**Figure C.** The comparative use of three strengths of digoxin, 0.0625, 0.0125 and 0.25mg as compared with the use of 100mg aspirin tablets both of 75mg strength, during the time period 2007-2011. The actual figures of usage are shown in the table below.
Table C. The three strengths of Lanoxin which are used in the hospital services in Kuwait and their number in the period Jan 2007 – Jan 2011.

<table>
<thead>
<tr>
<th>Subclass: Positive inotropic drugs</th>
<th>TAB</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>LANOXIN 0.0625MG (DIGOXIN)</td>
<td>TAB</td>
<td>1 268 900</td>
</tr>
<tr>
<td>LANOXIN 0.0125 (DIGOXIN)</td>
<td>TAB</td>
<td>2 491 500</td>
</tr>
<tr>
<td>LANOXIN 0.25MG (DIGOXIN)</td>
<td>TAB</td>
<td>1 829 600</td>
</tr>
</tbody>
</table>

Diuretics

Diuretics used in the treatment of Hypertension
In the treatment of cardiovascular disease, especially hypertension, the use of diuretics is in most guidelines of the world as the first line in therapeutic treatments after an increase in exercise and restrictions of salt intake and abolition of smoking. The most frequent drug of choice in most of the international guidelines is to use bendroflumethazide 2.5 mg.

The data shown in Figure D and Table D below do not feature this drug at all. The four major drugs actually dispensed during the period 2007 – 2011 were in terms of their rank order and the quantities used were:

- Furosemide (Lasix) 21 047 600,
- Indapamide SR (Natrilix), 13 202 010,
- Amiloride + hydrochlorothiazide (Moduretic) 7 011 760
- Spironolactone, 6 544 500

This rank order is perhaps surprising, as the commonest was a high ceiling diuretic, namely furosemide which is usually added to existing therapy of hypertension to achieve better control with those who prove to have ‘resistant hypertension’ rather than a first line drug. It is true, that the drug is also used for hypertensives that also have impaired renal function or heart failure but the numbers of patients in this group will be limited in any national group of essential hypertensives.

Indapamide is chemically related to the diuretic chlortalidone and is claimed to lower blood pressure especially in diabetic subjects where it causes less metabolic disturbance. This is a very useful function and perhaps may reflect the problem mentioned in earlier sections that many causes of hypertension in Kuwait are present in patients who also have diabetes. This may explain perhaps its popularity.

The use of the combination of amiloride and hydrochlorothiazide is interesting since it helps compliance to have the two drugs in one preparation and of course. The conservation of
potassium is facilitated by this combination but one dangerous problem is that when this drug is used in combination with ACE inhibitors of angiotensin II receptor antagonists it may cause severe hyperkaleamia

The extensive use of spironolactone is perhaps surprising. In the UK the guidelines for the use of this drug are limited to the treatment of oedema and ascites in liver cirrhosis in addition to malignant ascites, nephrotic syndrome, congestive heart failure and primary hyperaldosteronism. In the UK its use is restricted because of a suggested risk of one of the metabolic products of the drug being a potential carcinogen. This has only been proved in rodents and its risk in man is unknown. In the elderly it can cause hyperkalaemia to occur which of course can cause serious cardiac problems. There is also the problem that it potentiates the effects of thiazide and loop diuretics by antagonising the effect of aldosterone and so acts as a potassium sparing diuretic. Low doses of spironolactone are also used in the treatment of severe heart failure which again is a very restricted group of this large utilization.

Figure D. The comparative use of a range of diuretics during the time period 2007-2011. The actual figures of usage are shown in Table D below.
**Table D.** The diuretics and their respective strengths which were used in the hospital services in Kuwait and the total number dispensed in the period Jan 2007 – Jan 2011.

<table>
<thead>
<tr>
<th>Subclass: Diuretics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>BUMETANIDE (BURINEX) 1 MG TAB</td>
<td>117 964</td>
</tr>
<tr>
<td>BUMETANIDE + POT. CHLORIDE (BURINEX K) TAB</td>
<td>26 356</td>
</tr>
<tr>
<td>CHLOROTHIAZIDE (SALURIC) 500MG TAB</td>
<td>164 400</td>
</tr>
<tr>
<td>CHLOROTHIAZIDE (HYGROTON) 50MG TAB</td>
<td>668 260</td>
</tr>
<tr>
<td>FRUSEMIDE 40MG (LASIX) TAB</td>
<td>21 047 600</td>
</tr>
<tr>
<td>FRUSEMIDE 500MG (LASIX) TAB</td>
<td>335 364</td>
</tr>
<tr>
<td>HYDROCHLOROTHIAZIDE (ESIDREX) 25MG TAB</td>
<td>506 820</td>
</tr>
<tr>
<td>INDAPAMIDE (NATRILIX) SR TAB</td>
<td>13 202 010</td>
</tr>
<tr>
<td>MODURETIC TAB</td>
<td>7 011 760</td>
</tr>
<tr>
<td>SPIRONOLACTONE (ALDACTONE) 100MG TAB</td>
<td>19 450</td>
</tr>
<tr>
<td>SPIRONOLACTONE (ALDACTONE) 25MG TAB</td>
<td>6 544 500</td>
</tr>
</tbody>
</table>
Anti Arrhythmic Drugs

The treatment of cardiac arrhythmias is both complex and difficult. It first of all requires a very accurate diagnosis of the type of arrhythmia and to do this usually requires some form of ECG analysis. The arrhythmia can be due some times to heart failure and so this requires treatment in addition to the arrhythmia which makes overall treatment complex.

The commonest drug in this classification used for this purpose was Telmisartan (Micardis) 40mg, 4 626 636. This was followed by Moxonidine (Physiotens) 0.2mg Tablets, 3 427 636. This was followed by Bisoprolol 5mg 1 383 810 and then Amiodarone 200mg, 1 331 250.

If the drugs are examined in the official data for this group some interesting observations can be made.

Some of these drugs in this listing are not usually included in a group labeled ‘Anti Arrhythmic Drugs’ (Figure E and Table E).

For example, Telmisartan is an angiotensin II receptor antagonist and is usually used for the treatment of hypertension. It can be used for the treatment of heart failure or diabetic nephropathy but not as an anti Arrhythmic Drug.

Another unusual drug to include is monoxidine. This drug is usually classed as a centrally acting antihypertensive which in the UK is licensed for use in mild to moderate essential hypertension and has a useful role when thiazides, calcium channel blockers, ACE inhibitors and beta blockers have not controlled the blood pressure or are simply inappropriate.

Bisoprolol can be used for the treatment of hypertension, angina, as an adjunct to severe heart failure but not as an anti Arrhythmic Drug. In Table E there are two beta blockers which can be used as anti arrhythmic drugs. These drugs do belong in this classification as there are anti arrhythmic drugs because they can attenuate the effects of stimulation of the
sympathetic nerves to the heart by a combination of reducing conductivity and automaticity within the cardiac tissues.

Finally there is Amiodarone which is a drug which is clearly used for arrhythmias. According to the current BNF, (March 2010) it can be used for all forms of arrhythmias including paroxysmal supraventricular, nodal and ventricular tachycardias, atrial fibrillation and flutter, and ventricular fibrillation. The formulation given in Table E is the oral form and this has a very long half life and the attainment of a steady state plasma concentration may require several weeks or even months.

Some of the other drugs in the list are clearly used as anti arrhythmic drugs.

Flecainide – this drug is usually reserved for the treatment of serious symptomatic ventricular arrhythmias. It belongs to the same class as lidocaine and can also be used for the treatment of what is termed ‘junctional re-entry tachycardia’ and for paroxysmal atrial fibrillation. Sometimes it has the unwanted effect of actually causing arrhythmias to occur.

Propanfenone - can slow atrial flutter and can be used along with beta blocker, diltiazem, or verapamil. It can be used in a prophylactic way and also as a form of treatment for ventricular arrhythmias and for some forms of supraventricular arrhythmias. It does have as well some beta blocking properties which make it useful for such a treatment.

**Figure E.** The frequency of use of drugs, over the period Jan 2007 – Jan 2011, which were classified on the central computer of the medical stores as anti - arrhythmic drugs.
Table E. The anti arrhythmic drugs and their respective strengths which were used in the hospital services in Kuwait and the total number dispensed in the period Jan 2007 – Jan 2011.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Strength</th>
<th>Form</th>
<th>Quantity (Dispensed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amiodarone HCL (CORDARON)</td>
<td>200MG</td>
<td>TAB</td>
<td>1 331 250</td>
</tr>
<tr>
<td>Bisoprolol Fumarate (CONCOR)</td>
<td>5MG</td>
<td>TAB</td>
<td>1 383 810</td>
</tr>
</tbody>
</table>
Beta adrenoceptor blocking drugs

In cardiovascular medicine the use of beta blockers has saved an untold number of lives and it is clear from the data provided by the central medicine stores that Kuwait uses a range of these drugs in considerable quantities. This is not unsurprising and unlike the table for ‘anti-arrhythmic drugs’ the vast majority of those included in the list are beta blockers (Figure F and Table F).

**Figure F.** The frequency of use of beta blocking drugs, over the period January 2007-January 2011, which were classified on the central computer of the medical stores as beta blockers.

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Type</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>BISOPROLOL FUMARATE (CONCOR) 10MG</td>
<td>TAB</td>
<td>550 680</td>
</tr>
<tr>
<td>FLECAINIDE ACET. 100MG (TAMBOCOR)</td>
<td>TAB</td>
<td>447 060</td>
</tr>
<tr>
<td>MOXONIDINE (PHYSIOTENS) 0.2MG</td>
<td>TAB</td>
<td>3 427 636</td>
</tr>
<tr>
<td>MOXONIDINE (PHYSIOTENS) 0.4MG</td>
<td>TAB</td>
<td>576 184</td>
</tr>
<tr>
<td>PROPafenONE (RYTONORM) 150MG</td>
<td>TAB</td>
<td>173 850</td>
</tr>
<tr>
<td>PROPafenONE (RYTONORM) 300MG</td>
<td>TAB</td>
<td>42 450</td>
</tr>
<tr>
<td>QUINIDINE BISULPHATE (KINIDIN)</td>
<td>TAB</td>
<td>2 000</td>
</tr>
<tr>
<td>SOTALOL 40MG</td>
<td>TAB</td>
<td>1 540</td>
</tr>
<tr>
<td>TELMISARTAN (MICARDIS) 40MG</td>
<td>TAB</td>
<td>4 626 636</td>
</tr>
<tr>
<td>TRIMETAZIDINE D1 HCL 20MG (VASTAREL)</td>
<td>TAB</td>
<td>180</td>
</tr>
</tbody>
</table>
Table F. The frequency of use of beta blocking drugs, over the period Jan 2007 – Jan 2011, which were classified on the central computer of the medical stores as beta adrenoceptor blocking drugs.

### Subclass: Beta Adrenoceptor Blocking Drugs

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Formulation</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATENOLOL (TENORMIN) 100MG</td>
<td>TAB</td>
<td>18 770 900</td>
</tr>
<tr>
<td>ATENOLOL (TENORMIN) 50MG</td>
<td>TAB</td>
<td>40 080 300</td>
</tr>
<tr>
<td>BOSENTAN 125MG (TRACLEER)*</td>
<td>TAB</td>
<td>39 872</td>
</tr>
<tr>
<td>BOSENTAN 62.5MG (TRACLEER)</td>
<td>TAB</td>
<td>17 304</td>
</tr>
<tr>
<td>CARVEDILOL (DILATREND) 25MG</td>
<td>TAB</td>
<td>2 294 460</td>
</tr>
<tr>
<td>CARVEDILOL (DILATREND) 6.25MG</td>
<td>TAB</td>
<td>3 368 820</td>
</tr>
<tr>
<td>LABETALOL HCL (TRANDATE) 100MG</td>
<td>TAB</td>
<td>488 300</td>
</tr>
<tr>
<td>LABETALOL HCL (TRANDATE) 200MG</td>
<td>TAB</td>
<td>1 292 800</td>
</tr>
<tr>
<td>METOPROLOL TARTRATE (LOPRESOR) 100MG</td>
<td>TAB</td>
<td>2 613 680</td>
</tr>
<tr>
<td>METOPROLOL TARTRATE (LOPRESOR) 50MG</td>
<td>TAB</td>
<td>15 813 120</td>
</tr>
<tr>
<td>NADOLOL (CORGARD) 80 MG</td>
<td>TAB</td>
<td>5 544</td>
</tr>
<tr>
<td>PROPRANOLOL 10MG (INDERAL)</td>
<td>TAB</td>
<td>10 594 000</td>
</tr>
<tr>
<td>PROPRANOLOL 40MG (INDERAL)</td>
<td>TAB</td>
<td>2 948 000</td>
</tr>
</tbody>
</table>
Key *Bosentan
– is not a beta blocker but rather a vasodilator antihypertensive drug

The beta blocker which seems to be used above all others is atenolol whether alone or in combination with the diuretic chlortalidone. In fact 84 078 200 tablets were dispensed in the period Jan 2007 – Jan 2011 for a population is about 2 million. Of the other beta blockers the most commonly used was metoprolol – approximately 17 million tablets - which because of its relatively cardioselective action has merits over the other non-selective beta blockers of which propranolol was the next most commonly used. The combined preparations of propranolol amounted to 13.5 million tablets.

It is impossible from the collective data to know exactly what percentage of the total was used for different conditions. However the combined use for antihypertensive effects, anti-anginal effects as well as for its anti-arrhythmic effects is a substantial number of tablets and yet still does reach the total for the aspirin tablets taken which was 131 536 400. This may indicate that since the aspirin was once a day the majority of sufferers of these
cardiovascular diseases received aspirin in addition to their antihypertensive, and all the other therapies for cardiovascular disease.

Drugs affecting the renin-angiotensin system - either as ACE inhibitors or as angiotensin receptor antagonists.

This well used and understood group of drugs is well documented in the data which was available (Figure G and Table G). Some are of an earlier date of introduction with limitations to effective half life in contrast to the later introductions. From the data the most frequently used ACE inhibitor captopril – 100 million doses in total – which is one of the older group of agents. Of all the other drugs lisinopril is the next most commonly used drug. It is interesting to note that the angiotensin receptor antagonist valsartan was dispensed for 18 million tablets. This perhaps was due to the problems of persistent cough or intolerance to an ACE inhibitor. Some of these doses would have been taken along with aspirin if the risk factors in hypertension were such as to merit its use.
**Figure G.** The frequency of use of ACE inhibitors or as angiotensin receptor antagonists, over the period Jan 2007 – Jan 2011, which were classified on the central computer of the medical stores as rennin–angiotensin drugs.
Table G. The frequency of use of renin-angiotensin drugs, over the period Jan 2007 – Jan 2011, which were classified on the central computer of the medical stores as drug affecting renin – angiotensin.

<table>
<thead>
<tr>
<th>Drug Affecting the Renin-angiotensin</th>
<th>Dosage</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CANDESARTAN (ATACAND) 8MG **</td>
<td>TAB</td>
<td>5 889 400</td>
</tr>
<tr>
<td>CAPTOPRIL (CAPOTEN) 25MG*</td>
<td>TAB</td>
<td>59 240 200</td>
</tr>
<tr>
<td>CAPTOPRIL (CAPOTEN) 50MG*</td>
<td>TAB</td>
<td>39 282 000</td>
</tr>
<tr>
<td>CILAZAPRIL (INHIBACE) 2.5MG*</td>
<td>TAB</td>
<td>522 340</td>
</tr>
<tr>
<td>LISINOPRIL (ZESTRIL) 10MG*</td>
<td>TAB</td>
<td>16 216 844</td>
</tr>
<tr>
<td>LISINOPRIL (ZESTRIL) 20MG*</td>
<td>TAB</td>
<td>13 952 512</td>
</tr>
<tr>
<td>LISINOPRIL (ZESTRIL) 5MG*</td>
<td>TAB</td>
<td>11 468 184</td>
</tr>
<tr>
<td>PERINDOPRIL (COVERSYL) 4MG*</td>
<td>TAB</td>
<td>3 027 810</td>
</tr>
<tr>
<td>VALSARTAN (DIOVAN ) 160MG **</td>
<td>TAB</td>
<td>1 425 760</td>
</tr>
<tr>
<td>VALSARTAN (DIOVAN ) 80MG **</td>
<td>CAP</td>
<td>17 404 520</td>
</tr>
<tr>
<td>ZESTORETIC 20MG ***</td>
<td>TAB</td>
<td>11 121 152</td>
</tr>
</tbody>
</table>

Key * ACE inhibitor ** Angiotensin receptor antagonist ***ACE inhibitor plus hydrochlorothiazide
Vasodilator drugs

Drugs in this class are usually based on some form of ‘nitrate’ from the simple glyceryl trinitrate structure to the more complex isosorbide structure. They have a major role in the treatment of anginas as they are potent coronary vasodilators. Their major function seems to be to reduce venous return which as a consequence reduces left ventricular work and so reduces the oxygen demand of the heart. This allows blood flow which may be compromised by atherosclerotic vessels to be able to allow the heart to receive sufficient blood flow for its needs.

Glyceryl trinitrate

\[
\begin{align*}
\text{O}_2\text{N} & \quad \text{O} \\
\text{O} & \quad \text{NO}_2
\end{align*}
\]

Isosorbide
The range order of use for the nitrate derivatives was Glyceryl trinitrate at 29 733 000 was greater than isosorbide 10mg (19 002 000) followed by 40mg (9 284 250) and 60mg (5 560 800) of isosorbide. Both drugs are used sublingually where the glyceryl trinitrate achieves a quicker effect than the isosorbide although the later has a longer duration of action (Figure H and Table H).

**Figure H.** The frequency of use of vasodilatory drugs, over the period Jan 2007 – Jan 2011, which were classified on the central computer of the medical stores as vasodilators as compared with the use of low dose aspirin tablets.

**Table H.** The frequency of use of vasodilatory drugs, over the period Jan 2007 – Jan 2011, which were classified on the central computer of the medical stores as drug affecting renin – angiotensin.
Trimetazidine is an anti-anginal agent which is currently unlicensed in UK, so perhaps a few words about this drug may be useful.

Marzilli stated in 2001: It has been recently demonstrated that trimetazidine, known for years to be an effective antianginal agent, shifts cardiac energy metabolism from fatty acid oxidation to glucose oxidation by inhibiting mitochondrial long-chain 3-ketoacyl coenzyme A thiolase. By decreasing fatty acid oxidation, trimetazidine stimulates glucose utilization, restoring coupling between glycolysis and carbohydrate oxidation and leading to adenosine triphosphate production with lesser oxygen consumption. The antianginal properties of this agent are independent of haemodynamic changes, and dramatically improve recovery of mechanical function after ischaemia. Several studies have tested the efficacy of trimetazidine and have demonstrated this agent to be at least as effective as and better
tolerated than haemodynamic agents. In stable effort angina, trimetazidine improves exercise
tolerance and elevates the ischaemic threshold to an extent comparable with beta-blockers
and calcium channel blockers. The combination of trimetazidine and a beta-blocker appears
more effective than the combination of nitrates and a beta-blocker, and the addition of
trimetazidine improves symptoms in patients resistant to diltiazem (Marzilli et al., 2001).

**Sympathomimetics**

Etilefrine is an α-agonist agent with a potent vasoconstrictor effect, which is
potentially useful in preventing vasovagal syncope by reducing venous pooling and/or by
counteracting reflex arteriolar vasodilatation. **This drug is not licensed for use in the UK.**

A study carried out by Raviele et al., in 1999 perhaps shows why the product was not
licensed in the UK. They stated that:

The main finding of the VASIS trial is the lack of superiority of etilefrine over placebo
in preventing spontaneous syncopal recurrence. Indeed, the 63 patients treated with
etilefrine at the dosage of 25 mg 3 times a day showed exactly the same recurrence rate
during the first year following enrolment as did the 63 patients treated with placebo
(24%). Furthermore, the time to the first syncope after the start of treatment, as well as
the incidence and the number of presyncopal episodes, were not significantly different
between the 2 study groups.

**Figure I.** The frequency of use of Etilefrine, over the period Jan 2007 – Jan 2011, as
compared to aspirin usage over the same time period.
Table I. The number of tablets of Etilefrine against the use of aspirin for the period Jan 2007 – Jan 2011.

| ETILEFRINE HYDROCHLORIDE (EFFORTIL) | TAB | 104 560 |

Anti Fibrinolytic Drugs and Haemostatic
These drugs both affect problems in the cardiovascular system. The number of drugs in this class is very small, namely two – tranexamic acid and etamsylate (Figure J and Table J).

Tranexamic acid is an inhibitor of fibrinolysis brought about by plasmin. It is generally considered to be a plasmingogen inhibitor. Its uses usually limited to bleeding associated with excessive fibrinolysis as classically seen in operations on the prostate gland, operations on the urinary bladder and in some individuals after dental extractions who suffer haemophilia. It is also used in the management of menorrhagia and for epistaxis. Because of these selective uses it would not be expected to be used in any condition where platelets would be directly involved with fibrinolysis. The relative use is only 0.48% that of the aspirin.

Etamsylate. This drug again has very uses for specific cardiovascular conditions but have some involvement with platelets. It acts as a haemostatic agent and is said to reduce capillary bleeding in the presence of a normal number of platelets. It does not require the mechanisms of fibrin stabilisation but may act via correcting normal platelet adherence. There is some controversy as to whether the drug is modified in the presence of aspirin but they seem to work by very different mechanisms.

**Figure J.** The frequency of use an anti fibrinolytic drug and a haemostatic over the period Jan 2007 – Jan 2011, as compared to aspirin usage over the same time period.
Table J. The number of tablets of anti fibrinolytic drug and haemostatics against the use of aspirin for the period Jan 2007 – Jan 2011.

<table>
<thead>
<tr>
<th>Subclass: Anti Fibrinolytic Drugs and Haemostatics</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETAMSYLATE 500MG (DICYNONE) TAB 271 060</td>
</tr>
<tr>
<td>TRANEXAMIC ACID (CYCLOKAPRON) 500MG TAB 575 880</td>
</tr>
</tbody>
</table>

Lipid lowering drugs

The final group of drugs for which data is available is collectively known as the lipid lowering drugs. These drugs have a history behind them as they were developed over many years and so it is no surprise to find seven different types listed. See Figure 46 for the full display of all the drugs found in the data (Figure K and Table K).
As perhaps may be expected in 2011 the most commonly used drug turned out to be one of the statins, and that it was Atorvastatin is also no surprise as this is the biggest selling ‘lipid lowering drug’ in the world in 2011. If the drug is treated as a single entity, although two different strengths were used then over 65 million doses were used in the period Jan 2007 – Jan 2011. The fibrates as a group then followed, namely Bezafibrate, Fenofibrate and Gemfibrozil. In terms of numbers of usage they were collectively even greater than for the statins.

**Figure K.** The frequency of use Lipid lowering drugs over the period Jan 2007 – Jan 2011, as compared to aspirin usage over the same time period.

**Table K.** The number of tablets of lipid lowering drugs against the use of aspirin for the period Jan 2007 – Jan 2011.

<table>
<thead>
<tr>
<th>Subclass: Lipid Lowering Drugs</th>
<th>ATORVASTATIN 10MG (LIPITOR)</th>
<th>ATORVASTATIN 20MG</th>
<th>TAB</th>
<th>28 389 990</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ATORVASTATIN 20MG (LIPITOR)</td>
<td>TAB</td>
<td>42 031 710</td>
<td></td>
</tr>
</tbody>
</table>
The important finding in the graph is that when the dose of Atorvastatin is increased from 10mg to 20 mg, its effectiveness in ‘dissolving the lipids’ which are deposited within the arterial walls may enhance and so the passage of flow of blood increases. Simvastatin is least effective among these selected agents of this sub class and this shown by it limited use.

**Conclusions**

In conclusion, the data for drug consumption allows us to see that aspirin is very widely used, perhaps it is the most commonly prescribed drug of any in the entire formulary of Kuwait. Its widespread use would suggest all prescribers are aware of its very useful
potential to decrease the risk of stroke in those who have elevated risk factors, including those people who smoke or who used to smoke.

It is hoped this analysis will facilitate the new directions which are planned for the Pharmacy in Kuwait.