CHAPTER I

INTRODUCTION & REVIEW OF LITERATURE

Therapeutic properties of plant derived compounds have been extensively studied from time immemorial and are even now used in the treatment of variety of ailments. The earliest use of medicinal plants for the prevention of disease and cure of ailments can be traced back to ‘Rig Veda’ written between 4500 and 1600 BC (Singh, 1995). Flavonoids have been intensively investigated during the past years due to their possible protective effect against chronic diseases and herbal remedies containing flavonoids have been used in folk medicine around the world. Because of their widespread occurrence in edible plants they are an integral part of human diet. Therefore the effects of these substances in human nutrition and well being are of considerable importance. Flavonoids have probably existed in the plant kingdom for over one billion years. This long interaction between plant flavonoids and humans stimulated much interest in the biochemical and physiological activities of these chemicals.

Flavonoids

Dr. Szent – Gyorgyi, a pioneer and Nobel laureate in biochemistry discovered flavonoids in 1935 from lemon juice. He named it citrin and vitamin P. From 1940 onwards researchers from many countries began studying the biochemical effects of flavonoids such as catechins, proanthocyanidins, rutin etc. There seems to be resurgence of interest in flavonoid research in recent years owing to the highly potential benefits of these compounds in alleviating many disorders commonly found in man.
Cereals, potatoes, bulbs, roots, peanuts, nuts, vegetables, herbs, fruits, fruit juices, coffee, beer and wine contain fair amounts of these compounds. Perhaps, Szent- Gyorgyi was technically correct in saying that certain bioflavonoids, as a group, should have vitamin status.

Flavonoids are a class of naturally occurring organic phytochemicals derived from 2-phenyl chromones. They are biosynthesized via a combination of shikimic acid and acyl poly malonate pathways. Phenyl propane, synthesised from shikimic acid, acts as the starting compound in a polyketide synthesis, in which an additional three acetate residues are incorporated into the structure. This undergoes ring closure. Through subsequent hydroxylations and reductions, plants are able to form different classes of flavonoids (Samuelsson, 1993) [Fig. I].

**Fig 1: Biosynthesis of flavonoids**

Flavonoids can be grouped according to ring substituent patterns and the degree of benzopyrone ring saturation. The immediate family members
include flavone, isoflavone, 3- hydroxy flavone or flavonol and the 2, 3-dihydro derivatives of flavans namely flavonones, other members from within each group originate from differences in oxygenation of rings and from derivatisation reactions such as O- methylation and O- and C-glycosylation (Das, 1994). Flavonoids per se are compounds with substituent at the 2 position; compounds with substituent at 3 position are termed isoflavonoids. With over 4000 known flavonoid compounds; the structure can vary considerably [Table I, Fig. 1a]. The biochemical activity of many flavonoids and their metabolites depends primarily upon the structure and relative orientation of the various moieties in the molecule as shown from structure activity data (Cody, 1988).

**Table I : The major known classes of flavonoid**

<table>
<thead>
<tr>
<th>Class</th>
<th>Number of known structures&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Biological properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthocyanins</td>
<td>256</td>
<td>Red to blue pigments</td>
</tr>
<tr>
<td>Chalcones</td>
<td>197</td>
<td>Yellow pigments</td>
</tr>
<tr>
<td>Aurones</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Flavones, flavonols and their O-glycosides</td>
<td>1660</td>
<td>Copigments in flowers; UV protectants in leaves</td>
</tr>
<tr>
<td>C-Glycosylflavonoids</td>
<td>303</td>
<td></td>
</tr>
<tr>
<td>Flavanones</td>
<td>319</td>
<td>Some have bitter tastes</td>
</tr>
<tr>
<td>Dihydrochalcones</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>Dihydroflavonols</td>
<td>110</td>
<td>Often present in woody tissues</td>
</tr>
<tr>
<td>Flavans, leucoanthocyanins and proanthocyanidins</td>
<td>309</td>
<td>Astringent substances with tannin properties</td>
</tr>
<tr>
<td>Biflavonoids</td>
<td>134</td>
<td></td>
</tr>
<tr>
<td>Isoflavonoids</td>
<td>630</td>
<td>Oestrogenic or fungitoxic</td>
</tr>
<tr>
<td>Neoflavonoids</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Miscellaneous structures</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Taken from Harborne (1988)
Flavone

5 6 7 4'
Apigenin OH - OH OH
Baicalein OH OH OH -
Chrysin OH - OH -

Flavone glycosides

5 6 7
Baicalin OH OH R
R-β-glucopyranuronosyl

Flavonol

5 7 3' 4' 5'
Quercetin OH OH OH OH -
Kaempferol OH OH - OH -
Myricetin OH OH OH OH OH

Flavonol glycosides

3 5 7 3' 4'
Rutin R OH OH OH OH
R-Rhamnosyl glucoside

Flavanone

5 7 4'
Naringenin OH OH OH

Isoflavone

5 7 4'
Genistein OH OH OH

Flavan-3-ol

5 7 3' 4'
Catechin OH OH OH OH

Flavanolol

5 7 3' 4'
Taxifolin OH OH OH OH

Fig 1a: Chemical structure of some flavonoids
There are many reports on the absorption of flavonoids in humans. The liver is largely responsible for the metabolism of absorbed flavonoids (Hackett, 1986). Absorbed flavonoids are present in the common circulation in the form of glucuronide, sulfate and methylated conjugates and are excreted via urine or bile. Microorganisms are responsible for hydrolysis of flavonoid conjugates as well as for the formation of easily absorbed flavonoid ring fission products. A number of reports on flavonoid absorption in humans and rats have been published recently. These include absorption of quercetin (Hollman et al., 1996; Manach et al., 1997; Paganga and Rice-Evans, 1997; de Vries et al., 1998), naringin (Fuhr and Kummert, 1995), (-) epigallocatechin gallate (Unno et al., 1996) and tea catechins (Okushio et al., 1996). Piskula and Terao (1998) showed that ingested epicatechin is absorbed from the alimentary tract and is present in the rat common blood circulation in the form of various conjugates. The intestinal wall and kidney are the secondary sites of the metabolism. A recent study reported that the flavonoid glycosides were metabolized to phenolic acids via aglycones by intestinal microflora producing α- rhamnosidase, exo β-glucosidase and beta- glucuronidase. Rutin, hesperidin, naringinin and poncirin were transformed to their aglycones by bacteria producing α- rhamnosidase and β-glucosidase (Kim et al., 1998). The glucuronides and sulfates are excreted in the bile. Flavonoids once absorbed influence many biological functions including protein synthesis, cell proliferation, differentiation and angiogenesis making them beneficial in a variety of human disorders.

Phenolic compounds are included among secondary metabolites of plants and are associated with different processes such as UV- B filters, plant – microorganism interactions, defense against pathogen attack and insect
attract for pollination and seed dispersal (Harborne and Grayer, 1992). In addition, flavonoids are involved in photosensitization and energy transfer, morphogenesis and sex determination, levels of respiration and photosynthesis, action of plant growth hormones and regulators, gene expression and behavior (Djordjevic et al., 1987; Firmin et al., 1986; Peters et al., 1986; Smith and Bank, 1986; Zaat et al., 1987). There is much data concerning a wide range of biological activities of these compounds in humans. Flavonoids are described to exert a large array of biological activities, which are mostly ascribed to their radical scavenging, metal chelating and enzyme modulation ability. They have been shown to exhibit a strong affinity towards various membrane bound enzymic activities and to interact with biological or model membranes (Havsteen, 1983). Flavonoids are utilized in medicine as protective agents for vascular integrity (Beretz and Cazenave, 1988), as antiosteoporotic agents (Eaton – Evans, 1994) and drugs against hepatotoxicity (Soike and Leng – Peschlow, 1987, Farombi et al., 2000). They were also reported to act in the gastrointestinal tract as either antiulcer (Iizzo et al., 1994; Saito et al., 1998), antispasmodic (Capasso et al., 1991; Capasso et al., 1991), antisecretory or antidiarrhoeal (Di Carlo et al., 1993) agents. Flavonoids and anthocyanidins were used in certain pathological conditions in which there is a defect in vascular permeability. Mian et al. (1976) reported that the protection of capillary wall was mediated through a dual mechanism, in particular by an increase in the endothelium barrier – effect through a stabilisation of the membrane phospholipids caused by an increase in the biosynthetic processing of the acid mucopolysaccharide of the connective tissue ground substances and by a restoration of the affected mucopolysaccharidic capillary sheath. Flavonoids extracted from Silybum marianum have been used in folk medicine for the treatment of liver diseases (Magliulo, 1973). Several reports were available on the antihepatotoxic effects of flavonoids (Iwu, 1985; Robbers et al., 1996;
Gilani et al, 1997). Many researchers have conducted in vitro studies on the potential antitumor activity of flavonoids (Bracke et al, 1988; Kandaswami et al, 1991; Huang et al, 1994). Some flavonoids (quercetin, epigallocatechin) and green tea extract, which is rich in flavonoids, inhibit tumor growth by inhibiting some phase of the cell cycle and by blocking or competing for hormone receptor sites (Komori et al, 1993; Larocca et al, 1994; Scambia et al, 1990). Other mechanisms by which flavonoids may inhibit tumor growth include: stabilising collagen, altering gene expression and reducing free radicals (Scutt et al, 1987; Makimura et al, 1993; Avila et al, 1994; Tanaka et al, 1997). Flavonoids were demonstrated as possessing in vivo antiinflammatory properties (Ferrandiz & Alcaraz, 1991; Pelzer et al, 1998; Chan et al, 1998). Some reports suggested that they had good antiinflammatory activity without the ulcerogenic side effects of other antiinflammatory drugs. Several flavonoids exert antiaggregatory effects through the inhibition of phosphodiesterase (Landolfi et al, 1984) as well as by the inhibition of thromboxane B2 formation (Tzeng et al, 1991). Antiviral and antimicrobial properties of flavonoids against different bacterial strains have also been demonstrated (Chu et al, 1992; El Gammal and Mansour, 1986). The effect of flavonoids on the immune system is complex and still unclear. In high concentrations they inhibit lymphocyte functions, but in lower concentrations they may act as immunostimulants in immune deficient individuals (Boik, 1996; Liu et al, 1998).

A. Role of flavonoids in dyslipidemia

Cardiovascular diseases threatens the world as it is the major cause of disability and death. A large body of evidence supports the notion that oxidation of lipids and lipoproteins may contribute to the pathogenesis of atherosclerosis. Cardiovascular diseases, including coronary heart disease,
stroke and peripheral vascular disease is the clinical expression of advanced atherosclerosis. Atherosclerosis is characterised by the focal development of atherosclerotic lesions in the large arteries. Atherosclerotic lesions are thought to be initiated by accumulation of lipoproteins within the intima, adhesion of monocytes into the arterial endothelium, emigration of monocytes into the arterial intima and accumulation of cholesterol within macrophages (Ross, 1993; St.Clair, 1997; Quinn, 1987). In advanced atherosclerotic lesions (atherosclerotic plaques), cholesterol is present extracellularly as cholesterol crystals, and evidence of calcification, necrosis and hemorrhage can be found (Stary et al, 1995). High plasma cholesterol has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart disease (Grundy, 1986; Neaton et al, 1984). However other parameters such as serum triacyl glycerol concentration show similar correlation (Giugliano et al, 1995). Variation in the atherosclerosis between individuals can be partially explained by a collection of "risk factor", with plasma cholesterol concentration, distribution of cholesterol among lipoproteins, blood pressure and smoking status contributing significantly to risk of atherosclerosis related diseases (Hjermann et al, 1981; Berenson et al, 1992; Pekkanen et al, 1990). It is evident from the above reports that synthesis and utilisation of lipids, cholesterol in particular, must be tightly regulated in order to prevent over accumulation and abnormal deposition within the body. An overview of regulatory mechanisms involved in the synthesis and turnover of cholesterol has been depicted as follows:

The reaction catalysed by HMG CoA reductase is the rate limiting step of cholesterol biosynthesis, and this enzyme is subject to complex regulatory controls. Normal healthy adults synthesise cholesterol at a rate of approximately 1 g / day and consume approximately 0.3 g / day.
A relatively constant level of cholesterol in the body (150 - 200 mg / dl) is maintained primarily by controlling the level of *de novo* synthesis. The level of cholesterol synthesis is regulated in part by the dietary intake of cholesterol. Cholesterol from both diet and synthesis is utilized in the formation of membranes and in the synthesis of steroid hormones and bile acids. The greatest proportion of cholesterol is used in bile acid synthesis.

The cellular supply of cholesterol is maintained at a steady level by three distinct mechanisms

1. Regulation of HMG CoA reductase activity and levels.
2. Regulation of excess intracellular free cholesterol through the activity of acyl CoA: cholesterol acyl transferase (ACAT)
3. Regulation of plasma cholesterol levels via LDL receptor mediated uptake and HDL mediated reverse transport. Regulation of HMG CoA reductase activity is the primary means for controlling the level of cholesterol biosynthesis.

Cholesterol is transported in the plasma predominantly as cholesteryl esters associated with lipoproteins. Dietary cholesterol is transported from the small intestine to the liver within chylomicrons. Cholesterol synthesised by the liver, as well as any dietary cholesterol in the liver that exceeds hepatic needs, is transported in the serum within LDLs. The liver synthesises VLDLs and these are converted to LDLs through the action of endothelial cell associated lipoprotein lipase. Cholesterol found in plasma membranes can be extracted by HDLs and esterified by the HDL associated enzyme LCAT. The cholesterol acquired from peripheral tissues by HDLs can then be transferred to VLDLs and LDLs via the action of cholesteryl ester transfer protein (apo- D) which is associated with HDLs. There is an inverse
relationship between HDL concentration and CHD and the most predictive relation is the LDL: HDL cholesterol ratio. The relationship is explainable in terms of the proposed roles of LDL in transporting cholesterol to the tissues and of HDL acting as the scavenger of cholesterol in reverse cholesterol transport. HDL promotes efflux of excess cholesterol from the artery wall into the blood, where cholesterol is esterified and transported to the liver for its excretion. Reverse cholesterol transport allows peripheral cholesterol to be returned to the liver in LDLs. Ultimately, cholesterol is excreted in the bile as free cholesterol or as bile salts following conversion to bile acids in the liver. Bile acid synthesis and subsequent excretion in the feces represent the only significant mechanism for the elimination of excess cholesterol.

Dietary triacylglycerols and cholesterol as well as triacylglycerol and cholesterol synthesised by the liver, are solubilised in lipid – protein complexes. These complexes contain triacylglycerol lipid droplets and cholesteryl esters surrounded by the polar phospholipids and proteins identified as apolipoproteins. In the capillaries of adipose tissue and muscle, the fatty acids of chylomicrons are removed from the triacylglycerols by the action of lipoprotein lipase (LPL). In the circulation VLDLs are converted to LDLs through the action of lipoprotein lipase. LDLs are the primary plasma carriers of cholesterol for delivery to all tissues. HDLs are synthesised de novo in the liver and small intestine, as primarily protein rich disc shaped particles.

Lecithin: cholesterol acyl transferase (LCAT) transfers a fatty acid from the C-2 position of lecithin to the C-3- OH of cholesterol, generating a cholesteryl ester and lysolecithin. Cholesterol rich HDLs return to the liver, where they are endocytosed. LDLs are the principle plasma carriers of cholesterol from the liver (via hepatic synthesis of VLDLs) to peripheral
tissues. Excess cholesterol tends to be deposited in the skin, tendons and within the arteries leading to atherosclerosis. Kuo and associates found that lipid lowering therapy stabilised the basic lesions and reduced the frequency of new lesion production (Kuo et al, 1979). Lipid lowering therapy depletes lesions of “large lipid core and macrophage clusters” to stabilise arterial lesions and decrease clinical event rates (Brown et al, 1993).

Current epidemiological evidences suggest that inadequate intake of certain nutrients predispose humans to chronic degenerative diseases (Knekt et al, 1996). Several workers demonstrated the protective role of dietary factors in heart disease. In particular it was demonstrated that consumption of diets rich in whole grains is associated with reduced risk of coronary artery disease. These dietary factors are rich in vitamin E and C and polyphenolic compounds including flavonoids. These compounds are hypothesised to intervene in the development of atherosclerosis and coronary artery disease. One of the effects of phenolic compounds of interest in nutrition is their influence on lipid metabolism. Several epidemiological studies have shown an inverse correlation between flavonoid intake and coronary heart disease. The Zutphen elderly study, the seven countries study and a Finnish study (Hertog et al, 1993 and 1995; Knekt et al, 1996) showed that there was a significant inverse association between dietary flavonoid intake and mortality from coronary heart disease (CHD) and a weaker inverse relation with incidence of myocardial infarction. Flavonol / Flavone intake also was associated with a decreased risk of stroke in a Dutch cohort (Keli et al, 1996). There is an increasing interest in green tea as a protective agent against cardiovascular disease (Chisaka et al, 1988; Imai and Nakachi, 1995; Kono et al, 1992). Increased consumption of green tea has been associated with decreased serum triacyl glycerol (TG), total cholesterol (Kono et al, 1992) and LDL cholesterol and increased HDL cholesterol
concentration (Green and Harari, 1992; Imai and Nakachi, 1995). This beneficial effect of green tea is attributed to the presence of epicatechins. Total flavonoids from clover red and nut decreased the content of cholesterol and triglycerides in intact rats, inhibit the development of hyperlipidemia caused by triton WR-1339, prevented accumulation of triglycerides in rat liver and blood caused by ethanol (Leont'eva et al., 1979). Hesperidin, the most important flavanone of Citrus sp. significantly increases HDL and lowers cholesterol, LDL, total lipid and triglyceride plasma levels in normolipidemic rats and in rats with diet and triton induced hyperlipidemia (Monforte et al., 1995). Administration of the methanol extract of Prunus davidiana for 3 days produced a significant decrease of blood triglycerides and total cholesterol and the atherogenic index was also improved (Choi et al., 1991). Dietary quercetin has also been shown to decrease serum total cholesterol in rats fed a cholesterol-enriched diet (Igarashi and Ohmuma, 1995) or rats with alloxan diabetes (Nuraliev and Avezov, 1992). Vitamin E deficient chicks fed quercetin had greater tissue essential fatty acid levels than controls (Jenkins and Atwal, 1995). Morin also showed similar effects. Hypolipidemic activity of catechin and some flavonoid preparations from certain natural sources have been reported from this laboratory (Valsa et al., 1995; Sudheesh et al., 1997).

B. **Flavonoids and defense mechanism against lipid peroxidation.**

Free radicals and other oxidants are involved in several disease states including arthritis, infection with malaria or other parasites, neurological damage, diabetic cataract, silicosis, atherosclerosis, immune injury to kidney, liver and lungs (Halliwell et al., 1988; Vennerstrom and Eaton, 1988; Halliwell, 1989; Wolff, 1987; Vallyathan, 1988; Halliwell, 1998). Approximately 2 to 3 % oxygen used by cells is reduced by addition of
single electrons which sequentially generates superoxide anion (O$_2^-$), hydroxyl radicals (OH), singlet oxygen (O$_2$) and hydrogen peroxide (H$_2$O$_2$). These free radicals readily permeate cellular membranes and can enter virtually in all cellular compartments. The generation of the various O$_2$ species occurs commonly under normal and pathologic circumstances including ageing and inflammatory reactions. Although cells contain an elaborative network of antioxidative defenses reactive oxygen metabolites are detected in normal conditions. Cells are probably under continuous oxidative stress because of an innate imbalance between the oxidants and antioxidants, in favor of the former, leads to many biochemical changes and is an important contributing factor in several human chronic diseases such as atherosclerosis and cardiovascular disease, mutagenesis and cancer, several neurodegenerative disorders and likely the ageing process per se. The O$_2^-$ is generated within aerobic biological systems during both enzymatic and nonenzymatic oxidations. The various reactions known to produce substantial amounts of O$_2^-$ are autooxidation of hydroquinones, leukoflavins, catecholamines, thiols, haemoglobin and myoglobin (Misra and Fridovich, 1972; Misra, 1974; Gotoh and Shikama, 1976). Active oxygen species have been proposed as the attacking agents on polyunsaturated fatty acids in cell membranes.

Several lines of defense mechanisms against oxidative damage are present within the cells. The oxidatively modified biomolecules were removed by repair enzymes including peroxidases that reduce lipid hydroperoxides to their corresponding alcohols and glycosylases that remove specific DNA lesions. In addition to repairing oxidatively damaged biomolecules another layer of defense against oxidative stress and resultant damage is to prevent formation of reactive oxygen and nitrogen species or to scavenge these species before they can cause oxidative damage to
biomolecules. Among these defenses are antioxidant enzymes, which are mostly intracellular and include several forms of superoxide dismutase (SOD) and catalase.

\[
O_2 \rightarrow O_2^- \rightarrow H_2O_2 \rightarrow OH \rightarrow H_2O
\]

Superoxide dismutase (SOD) catalyses the disproportionation of two molecules of superoxide to form molecular oxygen and hydrogen peroxide.

\[
O_2^- + O_2^- \rightarrow H_2O_2 + O_2^-
\]

Catalase scavenge hydrogen peroxide.

\[
H_2O_2 \rightarrow H_2O + O_2.
\]

The enzyme glutathione peroxidase (GSH Px) catalyses the reduction of hydroperoxides of fatty acids (ROOH) and other compounds to their corresponding alcohols (ROH) (Flohe, 1979) and required reduced glutathione as hydrogen donor.

\[
ROOH + 2 GSH \rightarrow ROH + G-S-S-G + H_2O.
\]

Glutathione reductase regenerates GSH from GSSG formed in the peroxidase reaction

\[
GSSG + NADPH + H^+ \rightarrow 2 GSH + NADP^+
\]
and maintain the high ratio of reduced to oxidised glutathione intracellularly.

Glutathione-S-transferase (GST) is involved in the detoxification of an extensive array of compounds. GST has been reported to possess peroxidase activity and participate in the reduction of fatty acid hydroperoxides to non-toxic alcohols (Aruna et al, 1990). In its process of reacting with fatty acid hydroperoxides, GST requires glutathione as the primary reactant.
It has been proposed that the oxidative modification of low density lipoproteins (LDL) is an important initiating event in atherosclerosis. Atherosclerosis is characterised by a local thickening of the intima or innermost part of the vessel. Atherosclerosis begins with damage to the endothelium (Ross, 1986) followed by attachment of monocytes from circulation (Quinn et al, 1988) which develop into macrophages within the vessel wall. Activated monocytes and macrophages could injure neighbouring cells by secreting $\text{O}_2^-$, $\text{H}_2\text{O}_2$ and hydrolytic enzymes and factors released by macrophages (Mitchinson and Ball, 1987) can stimulate the proliferation of smooth muscle cells. The oxidation of esterified polyunsaturated fatty acids within LDL particles give rise to many modification of the lipoprotein that render it atherogenic. Normal macrophages possess some LDL receptors, but if LDL is peroxidised it is recognised by separate receptors known as acetyl LDL receptors or the scavenger receptors (Haberland and Fogelman, 1987). LDL bound to these receptors is taken up with enhanced efficiency so that cholesterol rapidly accumulates within the macrophage and may convert it to a foam cell (Mitchinson and Ball, 1987; Haberland and Fogelman, 1987). Arterial endothelial cells, smooth muscle cells and macrophages are themselves known to be capable of oxidising LDL so that macrophages will internalise it faster. Products formed in the peroxidised LDL, such as lysophosphatidyl choline, might act as chemotactic factors for blood monocytes, encouraging their recruitment into an atherosclerotic lesion (Quinn et al, 1987 & 1988).

There have been several speculations that oxidation products of cholesterol might also be involved in atherogenesis (Bernheimer et al, 1987). Cholesterol is oxidised to a variety of products in peroxidising lipid systems and oxidised cholesterol has been reported to be toxic to arterial smooth muscle cells (Bernheimer et al, 1987; Sevanian and Peterson, 1986). Therefore elevated blood lipid concentration could lead to elevated lipid
peroxide concentrations contributing to endothelial injury and accelerating the whole process of atherogenesis. If oxidants do initiate atherosclerosis or contribute to its pathology, then an increased intake of antioxidants might be expected to have beneficial effect. Epidemiological studies have demonstrated an association between increased intake of antioxidant vitamins and reduced mortality due to coronary artery disease.

Antioxidative action of flavonoids has been attracted attention of many investigators and good deals of reports are available. Epidemiological studies have shown an inverse correlation between flavonoid rich food consumption and mortality from coronary heart disease (Hertog, 1998). These observations are supported by numerous data on the inhibition of low density lipoprotein (LDL) oxidation and the reduction of platelet aggregation (Frankel, 1997). This inhibition in lipid peroxidation can be brought about either by chelating of transition metals (Clemetson and Andersen, 1966; Afnas’ev et al, 1989) or by scavenging of free radicals with the formation of less reactive flavonoid aroxyl radicals (Afnas’ev et al, 1989; Cotelle et al, 1992). At present radical scavenging is clearly the favoured mechanism as evidenced by the lopsided ratio of reports on scavenging versus chelating properties of flavonoids (Bors et al, 1994). Flavonoids are reported to scavenge HO•, O2•−, LOO2•− and NO2•. Structure – activity relationships have been studied in various models leading to similar results (Brown et al, 1998; Bors et al, 1997; Arora et al, 1998). The inhibition of LDL oxidation by pure isolated flavonoids has been shown in numerous in vitro studies (Teissedre, 1996; Kerry and Abbey, 1998; De Whalley et al, 1990). Antioxidant activity of flavonoids from tea is well documented (Vinson et al, 1995; Ho, 1997; Rice – Evans et al, 1997). Pignol et al reported antilipoperoxidative properties of flavonoids purified from Ginko biloba extract (Pignol et al, 1988). Antioxidant activity of flavonoids from Solanum melongena and
Solenostemon rotundifolius have been reported from this laboratory (Sudheesh *et al*., 1999; Sandhya & Vijayalakshmi, 2001). Polyphenolics are supposed to have an action in the aqueous phase of plasma and at the surface of lipoprotein particles. The surface location of polyphenols can explain the enhancing sparing effect of supplementation on LDL vitamin E (Lairon and Amiot, 1999). Recent studies also demonstrated that flavonoids elicit their antioxidant properties by enhancing the activities of antioxidative enzymes (Miyake *et al*., 1998; Sudheesh *et al*., 1999; Nagata *et al*., 1999). The above reports demand further experimentation in this field. Attention was focussed on carrying out investigations regarding the effect of flavonoids from *G. cambogia* on the above aspects.

**Influence of flavonoids on platelet aggregation and haematological parameters**

Blood and vessels can respond to vessel injury by interacting immediately in order to stop bleeding following activation of hemostatic process. The first physiologic response is primary hemostasis involving blood components (platelets, leukocytes, plasma proteins), the blood vessel (endothelium, smooth muscle cells), hemodynamic forces and red cells. Primary hemostasis leads to closing of the wound in the vessel wall by a platelet thrombus. This thrombus is then strengthened through activation of the coagulation system and formation of a polymeric network of fibrin. After proliferation of cells in the vessel wall and wound healing, the clot is dissolved by plasmin generated through activation of the fibrinolytic system and the repaired blood vessel wall return to its normal state. Abnormal forms of injury to the vessel wall or perturbed flow conditions induce pathological interactions of blood and the vessel wall leading to thrombosis. Thrombosis probably occurs when there is a delicate balance between (i) thrombogenic factors, damage to the vessel wall, stimulation of platelets and leukocytes,
activation of coagulation, stasis and (ii) natural protective mechanisms, non thrombogenic properties of the endothelium, protease inhibitors, blood flow and fibrinolysis.

Atherosclerosis is closely linked to thrombosis. According to Ross (1986) it is an element in the response of the vessel wall to injury. The initial endothelial injury triggers the interactions of monocytes and platelets with the vessel wall. All these cells secrete growth factors leading to proliferation of smooth muscle cells. Increased monocyte adherence, subendothelial migration and accumulation of lipids to form foam cells and gradual accumulation of smooth muscle cells leads to the formation of atherosclerotic lesions. The thrombogenicity of the lesion can then cause thrombotic complications. The formation of a thrombus in atherosclerotic coronary arteries gives rise to acute ischemic heart disease, and coagulation and fibrinolysis factors play a key role in the control of thrombus formation (Badimon et al, 1993; Ross, 1993). Several studies indeed showed that plasma fibrinogen concentration is an independent risk factor for ischemic heart disease (Meade et al, 1996; Meade et al, 1993; Hamsten et al, 1987) however, it is suspected that not only fibrinogen concentration but also the quality of fibrin network may contribute to CHD risk (Blomback et al, 1992). Changes in fibrinogen concentration directly affect fibrin network structure (Blomback et al, 1992; Nair et al, 1991). Lowering fibrinogen levels may therefore protect against the onset of CHD and perhaps other diseases associated with atherosclerosis (Vorster et al, 1988). Factor VII and plasminogen activity were also associated with ischemic heart disease risk (Meade et al, 1993) and plasma plasminogen activator inhibitor 1 (PAI – 1) activity was associated with increased risks of myocardial reinfarction (Hamsten et al, 1987; Munkvad et al, 1990). Fibrinogen acts as a bridging ligand between platelets and plays a vital role in allowing platelet
aggregation (Peerschke et al, 1980). In some subjects the role of lipids in atherogenesis may not be related to their intraarterial accumulation to give rise to fatty streak like lesions, but to their influence which enhances the procoagulant activities of endothelial cells, variations in the production of plasminogen activator inhibitor, factor V, thrombospondin and von willebrandts factor may all be related to atherogenesis (Loskutoff and Mussoni, 1983; Loskutoff et al, 1983). Unaltered endothelial cells have the ability to release an enzyme that binds to and locally catalyses the lysis of fibrin and this modulation may be critical in atherogenesis. Atherosclerotic lesions are not merely myocytic proliferation foci. They are highly complex lesions that consist of many and variable components such as increased endothelial permeability, infiltration of plasma monocytes and lipids from blood to arterial wall, lipid uptake by myocytes and monocytes, myocytic proliferation, the disintegration of lipid laden cells, the development of extracellular partly crystalline lipid pools, focal wall necrosis, fibrosis, ulceration, thrombosis, incorporation of organised thrombi, capillarisation and hemorrhage and other components (Smith and Geer, 1983). The prevalent change in atherosclerosis is the adhesion of low density lipoproteins (LDL) to the endothelial fibrin lining and / or to the fibrinogen gel thrombus at the site of developing early atheroma (Lie et al, 1977). Atherogenesis is characterised by low levels of plasma fibrinolytic activity (Barboriak, 1978).

Platelets play a very important role in the hemostatic process and their aggregation induced by collagen, arachidonic acid (AA), adenosine diphosphate (ADP) and restocetin will cause atheromatic plaque formation. In recent years, many potent antiplatelet agents have been isolated from plants (Orsini et al, 1997; Dong et al, 1998; Gleitz et al, 1997). Rusznyak and Szent-Gyorgyi (1936) reported the isolation of citrin from lemon juice.
which normalised the pathologically low capillary resistance of vascular purpura patients and likewise normalised the elevated vascular wall permeability. With these investigations, Rusznyak and Szent-Gyorgyi (1936) first drew attention to the therapeutic effects of plant flavonoids. Many flavonoids have already been studied for their inhibitory effects on platelet aggregation (Ten Cate et al., 1973; Beretz et al., 1982; Landolfi et al., 1984; Beretz et al., 1985; Gryglewski et al., 1987). One general mechanism by which platelet function can be initiated is through an elevation of the cAMP levels (Packham and Mustard, 1977). Beretz and coworkers (1982) have shown that the structural features for flavonoids, required to inhibit platelet aggregation are similar to those required to inhibit cyclic nucleotide phosphodiesterase. Flavonoids apparently do not inhibit platelet activation by interfering with single biochemical mechanism. They are potent inhibitors of cAMP phosphodiesterase in addition to that there exists a close relationship between inhibition of the metabolism of arachidonate by cyclooxygenase and the activity of flavonoids (Corvazier and Maclouf, 1985). However this effect cannot be responsible for the inhibition by flavonoids of the activation of platelets by thromboxane dependant stimuli such as ADP (Beretz et al., 1986). Flavonoids interfere with several aspects of leucocyte activation that might be involved in blood- vessel wall interactions. They inhibit various aspects of neutrophil activation such as arachidonic acid metabolism, respiratory burst and secretion (Showel et al., 1981; Corvazier and Maclouf, 1985; Berton et al., 1980; Pagonis et al., 1986; Middleton, 1986). Flavonoids modify some parameters affecting blood rheology; they lower erythrocyte adhesion and aggregation and accelerate erythrocyte sedimentation rate (Robbins, 1976 & 1977), lower blood viscosity (Thulesius and Gjores, 1972) and increase blood filtratability (Millet et al., 1984). Flavonoids seem to influence various steps both in coagulation and fibrinolysis. High concentration of baicalein and baicalin isolated from Scutellaria baicalensis (0.1-1 M) prolong the clotting time of fibrinogen by
thrombin (Kubo et al, 1985). Procyanidin fractions of cider (0.25 μg/ml) inhibit urokinase induced clot lysis, the amidolytic activity of plasmin, urokinase and tissue activator on fibrin plates (Ogston et al, 1985). Indeed plasma fibrinolytic activity is reduced after drinking cider (Anderson et al, 1983). Micronised purified flavonoid fraction (MPFF) could possibly inhibit collagen induced aggregation by affecting certain pathways leading to platelet aggregation. Collagen induced aggregation implicates a first phase in which adhesion receptors (GP I α / II a, CD 36 and GP VI) interact with collagen. This is followed by a second phase in which fibrinogen and the complex GP II b / III a take over. MPFF interfere with the binding of fibrinogen to the glycoprotein complex (GP II b/ III a) and as a result inhibit platelet aggregation (Mc Gregor et al, 1999). MPFF consisting of 90% diosmin and 10% hesperidin, is currently used to treat patients with venous insufficiency and hemorrhoidal attack (Mc Gregor et al, 1999). The inhibition of platelet aggregation by hispudilin, a natural flavone was correlated to an increase in cyclic adenosine- 5’ – monophosphate (Bourdillat et al, 1988). In addition, the antiaggregatory properties of some flavonoids appeared to be linked to an inhibition of thromboxane formation and of thromboxane receptors on platelets (Tzeng et al, 1991). In as much as platelet – vessel wall interactions are important in the development of thrombosis and atherosclerosis, compounds possessing strong antiplatelet and vasorelaxing effects may hold potential for the treatment of cardiovascular diseases including thrombosis and atherosclerosis.

**Protein Kinase C**

The cellular processes that require some form of transmembrane signalling are diverse and complex, ranging from cellular growth and differentiation to nerve cell communication, learning and memory. Signal transduction at the cellular level refers to the movement of signals from outside the cell to inside. The movement of signals can be simple, like that
associated with receptor molecules of the acetylcholine class. More complex signal transduction involves the coupling of ligand – receptor interactions to many intracellular events. These events include phosphorylations by tyrosine kinases and / or serine/ threonine kinases. Protein phosphorylation is widely recognised as an important event in transmembrane signal transduction in eukaryotic cells. Extracellular signals, so called ligands, either penetrate the cellular membrane or bind to the extracellular domain of receptors. Activated receptors as such or in association with the so called transducers are capable of activating effectors either directly or by changing the amount or intracellular distribution of so called second messangers. All identified second messanger system somehow impinge on a protein kinase, in other words protein kinases are the targets which hormones acting outside the cell are attempting to reach. Two prominent serine / threonine specific kinases, both activated by second messanger action play a central role in signal transduction, cyclic AMP dependent protein kinase (PKA) and the Ca\(^{2+} / \) PL activated protein kinase C (PKC). The latter emphasises its role as a key enzyme in signal transduction by the fact that it represents the direct receptor protein of phorbol esters, substances known to interfere dramatically with proliferative and differentiation events by promoting oncogenic transformation of cells \textit{in vivo} and \textit{in situ}. Members of the PKC super family play key regulatory role in a multitude of cellular processes ranging from control of fundamental cell autonomous activities, such as proliferation, to more organismal functions such as memory. However, understanding of mammalian PKC signaling systems is complicated by the large number of family members (Mellor and parker, 1998). Protein kinase C (PKC) was originally identified as a serine / threonine kinase that was maximally active in the presence of diacyl glycerols (DAG) and calcium ion. It is now known that there are at least 10 proteins of the PKC family. Each of these enzymes exhibits specific patterns of tissue expression and activation by lipid and calcium. PKCs are involved in the signal transduction pathways initiated by certain hormones, growth factors and neurotransmitters.
The phosphorylation of various proteins by PKC can lead to either increased or decreased activity. Phospholipases and phospholipids are involved in the process of transmitting ligand – receptor induced signals from the plasma membrane to intracellular proteins. The primary protein affected by the activation of phospholipases is PKC which is maximally active in the presence of DAG. The generation of DAG occurs in response to agonist activation of various phospholipases. The principal mediators of PKC activity are receptors coupled to activation of phospholipase C-γ (PLC-γ). Activation of PLC-γ leads primarily to the hydrolysis of membrane phosphatidyl inositol bisphosphate (PIP₂) leading to an increase in intracellular DAG and inositol tri phosphate (IP₃). The released IP₃ interacts with intracellular membrane receptors leading to an increased release of stored calcium ions. Together, the increased DAG and intracellular free calcium ion concentrations lead to increased activity of PKC. It has been established that PKC is not a single molecular entity and that many closely related PKC isotypes exists, perhaps providing an explanation for the range of processes in which PKC has been implicated (Nishizuka, 1992; Asaoka et al., 1992; Parker et al., 1989; Hug and Sarre, 1993) The ubiquitous tissue distribution of PKC indicates that it plays a role in the regulation of many enzymes and other proteins. Therefore inhibition or stimulation of PKC would have important physiologic consequences.

* See last paragraph of this chapter

A brief description of *Garcinia cambogia* and an overview of the available literature. *Garcinia cambogia* (Gaertn.) Desr.; *Garcinia gummi-gutta* (Linn.) Robs.

*Garcinia cambogia* is seen abundantly in the evergreen forests of Konkan, stretching southwards to Kerala and the Western Ghats. It is known as 'Kodampuli' in vernacular. *G. cambogia* fruit is yellow or red when ripe with 6-13 inches deep horizontal furrows. It has an extremely sharp but pleasant acidity. Though edible, the fruit is rarely eaten raw, but many
traditional recipes in Kerala use it for its distinct flavor. The fruit is listed in indigenous medicine as having high therapeutic value and are even now used as remedies for various diseases. *G. cambogia* is a moderate sized handsome evergreen tree with rounded crown and horizontal or drooping branches, leaves simple, opposite, dark green, elliptic ovate. The leaves and fruits are sour, astringent, thermogenic, constipating and digestive. They are useful in vitiated conditions of *vata* and *kapha*, ulcers, inflammations, haemorrhoids, diarrhoea, dysentery, flatulent colic, dyspepsia and hyperdipsia (Varier, 1996). *Garcinia L.*, containing about four hundred species is the largest genus of the tropical family *Guttiferae* (Willis, 1973). The fruits of several species are edible. *Garcinia* species are also characterised by the production of a yellow or occasionally white latex in the endocarp of the fruit, in the bark and perhaps also in the wood. The biflavonoids isolated from *Garcinia* sp. can be divided in to two subgroups, those made up of one flavone and one flavonone subunit and those made up of two flavonone units (Waterman & Hussain, 1983). Biflavonones with C- 3,8" linkage are the major metabolites occuring in the genus *Garcinia*. *Garcinia* species are employed in traditional medicine for the treatment of various diseases including hepatitis, laryngitis and mouth infections (Iwu & Igboko, 1982). Antioxidative, antiglycation and antiulcer activities of garcinol from *Garcinia indica* fruit rind have been reported recently (Yamaguchi et al, 2000; Yamaguchi et al, 2000). Pharmacological activities of *Garcinia* biflavonones include antiinflammatory, antihepatotoxic, anticholesterolemic etc (Iwu, 1986; Farombi, 2000). Maniflavone isolated from *Garcinia manii* bark is used for the treatment of diseases resulting from disorders of vascular permeability and fragility and in the prevention of the complications of diabetes mellitus (Iwu, 1986). Intraperitoneal administration of kolaviron significantly inhibited carrageenan, yeast and cotton pellets induced oedema. The *Garcinia* biflavonoids inhibit the growth of gram-positive and gram-
negative microorganisms both *in vitro* and *in vivo*. The biflavonones of *Garcinia* are pharmacologically active compounds with several pharmacokinetic advantages over simple monomeric flavonoids in that they survive first pass metabolism, which inactivates most flavonoids. Reports are available on the inhibitory effect of isoflavonones isolated from *Garcinia kola* on lipid peroxidation (Adegoke, 1998).

Keeping in mind the above facts a few plants, traditionally used for various ailments were selected and a screening study was conducted in order to test their biological activity. Flavonoid rich extract from four sources namely *Cocos nucifera* (Coconut inflorescence), *Myristica fragrans* (Nutmeg seed), *Saraca asoca* (Asoka flower) & *Garcinia cambogia* (fruit rind) were administered to rats to assess hypolipidemic and hypoglycemic activities. As *Garcinia cambogia* was proved to be the best among the four for its highly significant beneficial activity (hypolipidemic), it was selected for the study. This thesis embodies the results of investigation of the beneficial effects of flavonoids from *Garcinia cambogia*. 
Garcinia cambogia fruit (Kodampuli)