Summary and Conclusion

Atherosclerosis, a chronic progressive disease, involves multiple processes, including endothelial dysfunction, vascular proliferation, oxidative stress, inflammation and apoptosis. It develops in response to damage to the blood vessel wall, which is characterized by the infiltration of mononuclear blood cells into the intima, foam cell formation, the proliferation of arterial smooth muscle cells and accumulation of connective tissue components in the inner lining of the arteries, resulting in thickening and hardening of arterial walls that may eventually block the arteries. Hypercholesterolemia is one of the most important risk factors for atherosclerosis, and also an important underlying pathology of cardiovascular disease (CVD), the leading cause of morbidity and mortality in developed countries.

Increasingly, reactive oxygen species (ROS) are being found to contribute to the pathogenesis of atherosclerosis, where antioxidant defence systems are impaired. It would seem logical to neutralise the deleterious effects of ROS by boosting such antioxidant defence systems. This may explain the current focus on experiments dealing with natural antioxidants to alleviate atherosclerosis induced by lipidemic-oxidative stress. The dietary intake of phenolic antioxidants in red wine and olive oil, and various compounds with known antioxidant properties such as ascorbic acid, α-tocopherol and ellagic acid have been evaluated for their anti-atherogenic potential. The experimental rodent model of atherogenic diet-induced atherosclerosis is frequently used for such an evaluation.

Green tea contains many biologically active polyphenolic flavonoids, commonly known as catechins, which include epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG). The natural
product, EGCG, is the major polyphenolic constituent found in green tea and has been characterized as an antioxidant with anti-cancerous, anti-mutagenic, anti-neurodegenerative and anti-colitic activities. However, the oral bioavailability of green tea catechins is less than 2% in vivo. Various factors associated with the gastrointestinal tract, such as limited membrane permeability, transporter-mediated intestinal secretion or gut wall metabolism, may contribute to the significantly lower bioavailability of green tea catechins after oral intake; in addition, since the pH of the intestinal tract ranges from 5 to 8, degradation of EGCG and EGC may occur in the intestinal lumen, therein contributing to presystemic loss. Therefore, the intraperitoneal mode of administration of EGCG was preferred in the present study; the green tea extract, of which EGCG constituted 66%, was used at a dose of 100 mg/kg b.w. In the present thesis, the putative anti-atherogenic potential of EGCG was investigated at several levels (and the thesis is composed of the following chapters):

a. Determination of fluorescence-quenching and ferric ion-chelating properties of green tea extract in an in vitro system.

b. Induction of atherosclerosis in an experimental animal model by feeding an atherogenic diet.

c. Comparison of various biochemical markers in an experimental animal model of saline-treated atherosclerosis and of EGCG-treated atherosclerosis.

d. Comparison of antioxidant status and markers of oxidative stress in an experimental animal model of saline-treated atherosclerosis and of EGCG-treated atherosclerosis.

e. Comparison of mRNA expression of C-reactive protein (CRP) and other inflammatory markers in an experimental animal model of saline-treated atherosclerosis and of EGCG-treated atherosclerosis.

f. Comparison of mRNA expression of acyl-CoA:cholesterol acyltransferase (ACAT) and caspase-3 genes in an experimental animal model of saline-treated atherosclerosis and of EGCG-treated atherosclerosis.
Male albino rats of the Wistar strain were used in the experiments. There were three basic experimental groups.

a. Group I (normal) rats were fed a normal diet.

b. Group II (atherogenic diet-fed, saline-treated) rats were fed an atherogenic diet and treated with physiological saline.

c. Group III (atherogenic diet-fed, EGCG-treated) rats were fed an atherogenic diet and treated with EGCG.

The animals were sacrificed at the end of the experiments; serum was separated and the organs were excised, and the tests were performed.

In the first phase of the study, the extract of green tea, *Camellia sinensis*, was purified, and the purity of the resulting compound was evaluated. The resulting compound was found to be composed of EGCG (66%) and other catechins (33%) [ECG (16.43%), EGC (11.22%), EC (3%), and catechin (0.62%)]. The antioxidant activity of the purified compound was determined by employing *in vitro* model systems; the ability of EGCG to chelate ferrous ions, and the fluorescence-quenching effect of EGCG on singlet-excited 2,3-diazabicyclo[2.2.2]oct-2-ene (DBO), was assayed. EGCG was found to exhibit concentration-dependent antioxidant activity by virtue of chelating ferrous ions and quenching DBO, when compared with the established standard.

In the second phase of the study, induction of atherosclerosis was evaluated in Wistar rats. When those rats were fed an atherogenic diet, atherosclerosis resulted. The induction of atherosclerosis was evaluated by two different feeding regimens: a) by feeding the rats a restricted atherogenic diet (10g/rat/day) for 30 and 60 days, and b) by feeding the rats an atherogenic diet *ad libitum* for 30, 45 and 60 days. Serum lipid profile parameters were higher in rats that had been fed the atherogenic diet *ad libitum*
than in rats fed the restricted-atherogenic diet. Histopathological studies revealed that thickening of the aortic intima was more pronounced in rats that had been fed the atherogenic diet ad libitum. However, no significant differences in the lipid profile parameters and only minimal histological variations were observed between rats fed the atherogenic diet for 30, 45 and 60 days. Therefore, rats fed the atherogenic diet ad libitum for 30 days were taken for further investigation.

In the third phase of the investigation, an attempt was made to determine whether EGCG could prevent the occurrence of atherosclerosis by an effect on various biochemical parameters in an experimental model. In rats fed the atherogenic diet: there were alterations in some parameters of the serum lipid profile; there was an increase in saturated fatty acids and a decrease in polyunsaturated fatty acids in hepatic tissue; there was also a significant decrease in the mean activity of the lipid metabolizing enzyme, lipoprotein lipase (LPL), and an increase in activity of the rate-limiting enzyme of cholesterol biosynthesis, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA-reductase), in hepatic tissue; there were increases in the serum levels of hepatic marker enzymes namely alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH). Elevated levels of cholesterol and bile acids were noted in the faecal content of rats that had been fed the atherogenic diet, when compared to normal rats. Furthermore, histopathological studies revealed thickening of the intima of the aorta and marked fatty changes in the hepatocytes of rats that had been fed the atherogenic diet. Administration of EGCG (100 mg/kg, b.w, i.p) for 7 or 15 days to rats that had been fed the atherogenic diet prevented such alterations in the biochemical parameters in the samples of serum, tissue and faecal matter. In addition, thickening of the aortic intima and fatty changes in hepatic tissue appeared to be prevented. The results of the present study strongly suggest that EGCG, the major component of green tea, confers protection against the development of atherosclerosis by several mechanisms: by
correcting abnormalities in the lipid profile, fatty acid levels and activities of hepatic marker enzymes; by replenishing the lipid metabolizing enzyme and rate-limiting enzyme of cholesterol biosynthesis; by enhancing the faecal excretion of cholesterol and bile acids.

In the fourth phase of the study, an attempt was made to determine whether EGCG enhances the antioxidant status and reduces markers of oxidative stress in an experimental model of atherosclerosis. In rats fed the atherogenic diet, decreased mean activities of the antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (Gpx) and glutathione-S-transferase (GST), decreased levels of the non-enzymatic antioxidants reduced glutathione (GSH), and vitamins C and E and elevated mean concentrations of malondialdehyde (MDA) were observed in haemolysate, and in hepatic and cardiac tissue. In addition, a decreased staining intensity of isoenzymes of antioxidant enzymes was also noted in hepatic tissue. Treatment with EGCG for 7 and 15 days resulted in significant elevations in antioxidant components and a decline in MDA (marker of lipid peroxidation). Since treatment for the extended duration of 15 days was found to be more effective than that for 7 days, further analyses were confined to animals receiving 15 days treatment. The results of the present study strongly suggest that EGCG protects against oxidative damage to important tissues in Wistar rats fed an atherogenic diet by preventing excessive lipid peroxidation and by maintaining enzymatic and non-enzymatic antioxidants at near normal concentrations. In addition, administration of EGCG brought about an increase in the staining intensity of isoenzymes of antioxidant enzymes in the hepatic tissue. This study has also demonstrated that the expression of the catalase gene is decreased in the hepatic tissue of atherogenic diet-fed, saline-treated rats, when compared to normal rats, but maintained at near normal levels in the hepatic tissue of atherogenic diet-fed, EGCG-treated rats.
In the fifth phase of the investigation, the regulatory effect of EGCG on expression of CRP and other inflammatory markers in an experimental model was investigated. Significantly higher mean concentrations of CRP were observed in untreated rats (fed the atherogenic diet alone) compared to normal and EGCG-treated rats. The results of the present study suggest that green tea catechins, and particularly EGCG, reduced inflammatory markers in rats fed an atherogenic diet. The expression of CRP protein and levels of its mRNA transcript were found to be decreased in atherogenic diet-fed, EGCG-treated rats, when compared to atherogenic diet-fed, saline-treated rats. These findings strongly suggest that EGCG possesses anti-inflammatory properties, and that these characteristics may play a role in the observed anti-atherogenic effect in rats fed an atherogenic diet.

The final phase of the investigation focused on expression of ACAT and caspase-3 genes in an experimental model of atherosclerosis. The efficacy of EGCG at the molecular level was evaluated by assessing the expression of mRNA of ACAT and caspase-3 in the hepatic tissue. Increased expression of mRNA of ACAT and caspase-3 in hepatic tissue was prevented in rats that had been fed the atherogenic diet and then treated with EGCG, suggesting that EGCG has a protective effect at the mRNA level. The results of the present investigation appear to suggest that EGCG prevents atherogenic diet-induced atherosclerosis by protecting against abnormal expression of genes involved in esterification of cholesterol and in apoptosis.

In conclusion, administration of EGCG appeared to prevent atherosclerosis in an experimental model. EGCG treatment not only prevented abnormalities in serum lipid profiles and fatty acids but also enhanced the enzymatic and non-enzymatic antioxidants and also reduced the levels of oxidative markers. These results strongly suggest that EGCG may play a preventive role in atherosclerosis by suppressing the production of free radicals and by protecting the hepatic and cardiac tissue from...
lipidemic-oxidative stress. In addition, EGCG appeared to reduce the levels of CRP protein and to downregulate the expression of the gene responsible for inducing CRP. The present investigation also recorded the potency of EGCG in downregulating the expression of certain select genes which are involved in the process of atherosclerosis (ACAT) and in apoptosis (caspase-3). Thus, the findings in this study show that EGCG is effective in preventing atherosclerosis in rats, possibly due to both anti-oxidative and hypolipidemic properties. The results suggest considerable promise for EGCG as a protective agent in atherosclerosis induced by intake of an atherogenic diet. An extract of the leaves of green tea, C. sinensis, could serve as an easily accessible item of beverage/food rich in natural antioxidants, as a possible beverage supplement with food or even as a pharmaceutical agent to treat oxidative stress-induced diseases.