Evaluation of the putative anti-atherosclerotic effect of epigallocatechin gallate (EGCG) by evaluation of various biochemical parameters in rats fed an atherogenic diet

1. INTRODUCTION

Hypercholesterolemia is one of the most important risk factors for atherosclerosis and subsequent CVD (Steinberg, 2002). Exogenous hypercholesterolemia causes deposition of fat in the liver and depletion of the hepatocyte population; it can also cause malfunctioning of the liver, which apparently follows microvesicular steatosis due to the intracellular accumulation of lipids (Gupta et al., 1976; Assy et al., 2000). Feeding animals excessive amounts of cholesterol has often been used to elevate serum and tissue cholesterol levels in order to study the etiology of hypercholesterolemia-related metabolic disturbances (Bocan, 1998). Hypercholesterolemia-induced microvascular alterations can be demonstrated in animal models within a few days after feeding a diet enriched with cholesterol, i.e., long before the appearance of fatty streak lesions in large arteries (Scalia et al., 1998; Stokes et al., 2001).

Serum cholesterol is carried by several lipoprotein particles that perform the complex physiologic task of transporting dietary and endogenously produced lipids (Witztum and Steinberg, 1995). In the intestine, dietary triglycerides and cholesterol are absorbed into the lymphatics and are incorporated into chylomicrons. The chylomicrons then enter the blood through the thoracic duct and most of the triglycerides are hydrolysed by lipoprotein lipase (LPL), an enzyme bound to the external surface of the capillary endothelium. Free fatty acids, which are the products of hydrolysis, enter either the adjacent muscles where they are used for energy, or the adipose cells where they are used for resynthesis of triglycerides for storage of energy (Brown et al., 1981). Dietary cholesterol derived from the receptor-mediated uptake of chylomicron
remnants is insufficient, hence, the liver synthesises its own cholesterol by increasing the activity of the rate-limiting enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA-reductase) (Brown et al., 1981). The endogenously-made triglycerides and cholesterol are then packed into VLDL, which enters the systemic circulation (Havel, 1994). VLDL triglycerides are hydrolysed at the endothelial cell surface by lipoprotein lipase to produce smaller, more cholesterol-enriched particles, which are then metabolized to intermediate density lipoprotein (IDL). Liver clears the VLDL remnants and the IDL is ultimately converted into LDL (Grundy, 1986). LDL, a major cholesterol-carrying lipoprotein fraction in human blood plasma, is known to be a risk factor for the development of atherosclerosis (Steinberg et al., 1989) and has also been shown to accumulate within the arterial wall. It is well-established that increased levels of LDL cause an increased risk for atherosclerosis (Goldstein and Brown, 1977).

Cholesterol is an important constituent of tissues by virtue of its dual role as a structural component of biological membranes, and as a precursor for steroid hormones and bile acids. In general, homeostasis of cellular cholesterol is maintained by regulation of its de novo synthesis, uptake from plasma and formation of bile acid in the liver. Catabolism of cholesterol to bile acids is an important route for its elimination from the body and accounts for about 50% of the daily elimination of cholesterol. A high-cholesterol diet is known to induce cholesterol 7α-hydroxylase, the rate-limiting enzyme for synthesis of bile acids, which, in turn, stimulates the conversion of cholesterol to bile acids; these events facilitate disposal of excess cholesterol (Jones et al., 1993).

The dietary intake of phenolic compounds in red wine (Frankel et al., 1995), green tea (Vinson et al., 2004), and olive oil (Aviram and Kassem, 1993) have been found to inhibit oxidation of LDL-cholesterol and thereby reduce risk factors for CVD. Recently, green tea has received much attention as a protective agent against oxidative stress-induced CVD (Aneja et al., 2004). EGCG, the principal constituent present in green tea (Giakoustidis et al., 2006), has been reported to possess anti-tumorigenic
(Mukhtar and Ahmad, 2000) and anti-inflammatory (Luo et al., 1997) properties. The present chapter describes a multi-pronged approach to determining the putative antioxidant role of EGCG in ameliorating atherogenesis in experimental animals. It has been executed by evaluating the lipid profile, analysing free fatty acids, and by quantification of lipid metabolizing enzymes and hepatic marker enzymes; the results obtained have been correlated with histopathological observations.

2. MATERIALS AND METHODS

2.1. Isolation and purification of EGCG from Camellia sinensis

The isolation and purification of EGCG from green tea, C. sinensis, has been described in Chapter I.

2.2. Experimental design

Male albino rats of the Wistar strain (150-200 g) were housed under conditions of controlled temperature (25 ± 2°C) with a 12/12 h day-night cycle, during which time they had free access to food and water ad libitum. Animals were maintained per national guidelines and protocols approved by the Institutional Animal Ethical Committee.

This phase of the study was performed on rats that had been fed an atherogenic diet (ATH) ad libitum for 30 days and then treated for 7 or 15 days with EGCG (100 mg/kg b.w.). The dosage of EGCG 100 mg/kg b.w. was selected per the data reported by Kao et al. (2000a), where EGCG was dissolved in saline and administered intraperitoneally.

The rats were divided into three main groups, I, II and III. Groups II and III were in turn, each subdivided into 2 subgroups, namely IIa and IIb and IIIa and IIIb. Thus:
(i) Group I rats (6 rats) were fed the commercial feed powder (normal diet) *ad libitum* for 30 days and were then administered 200 µl of saline by intraperitoneal injection once daily for 15 days (common control).

(ii) Group IIa rats (6 rats) were fed the atherogenic diet *ad libitum* for 30 days and were then administered 200 µl of saline by intraperitoneal injection once daily for 7 days (group II control).

(iii) Group IIb rats (6 rats) were fed the atherogenic diet *ad libitum* for 30 days and were then administered EGCG dissolved in saline (100 mg/kg body weight) by intraperitoneal injection once daily for 7 days.

(iv) Group IIIa rats (6 rats) were fed the atherogenic diet *ad libitum* for 30 days and were then administered 200 µl of saline by intraperitoneal injection once daily for 15 days (group III control).

(v) Group IIIb rats (6 rats) were fed the atherogenic diet *ad libitum* for 30 days and were then administered EGCG dissolved in saline (100 mg/kg body weight) by intraperitoneal injection once daily for 15 days.

At the end of the experimental period, all the animals were sacrificed by cervical decapitation. From each animal, blood samples were collected for separation of serum, and the hepatic tissue and aorta were excised. Faecal matter was collected from the animals for the analysis of bile acids. The serum and hepatic tissue samples were stored at -80°C until analysis.

2.3. **Determination of lipid profile in serum**

Serum lipid profile (levels of total cholesterol (TC), TG, LDL-cholesterol, VLDL-cholesterol and HDL-cholesterol) was determined by using standard assay kits (Diasys, Germany). The units were expressed as mg/dl.
2.4. Determination of activities of hepatic marker enzymes in serum

The hepatic marker enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH), were analysed in the serum samples of experimental animals.

*Alanine aminotransferase (ALT)*

The activity of ALT was determined by the method of King (1965a). The method is based on the ability of ALT to convert glutamate and pyruvate with the help of a substrate containing alanine and 2-oxoglutaric acid. The pyruvate formed was made to react with dinitrophenyl hydrazine (DNPH) reagent. A pyruvate standard was also treated in a similar manner. The colour developed was read spectrophotometrically at 540 nm after the addition of NaOH. The enzyme activity was expressed in terms of μmoles of pyruvate liberated/min/mg protein.

*Aspartate aminotransferase (AST)*

The activity of AST was determined by the method of King (1965a). The method is based on the ability of AST to convert glutamate and oxaloacetate with the help of a substrate containing aspartic acid and 2-oxoglutaric acid. The oxaloacetate formed was made to react with DNPH reagent. A pyruvate standard was also treated in a similar manner. The colour developed was read spectrophotometrically at 540 nm after the addition of NaOH. The enzyme activity was expressed as μmoles of pyruvate liberated/min/mg protein.

*Alkaline phosphatase (ALP)*

ALP was determined by the method of King (1965b). The activity of ALP was determined by measuring the liberated phenol from the reaction of disodium phenyl phosphate with Folin's reagent under alkaline conditions. The colour developed was read spectrophotometrically at 410 nm against a suitable blank. The activity of ALP was expressed as μmoles of phenol liberated/min/mg protein.
Lactate dehydrogenase (LDH)

LDH was assayed by the method of King (1965c). The method is based on the ability of LDH to convert lactate to pyruvate with the help of the coenzyme nicotinamide adenine dinucleotide (NAD\(^+\)). The pyruvate formed was made to react with DNPH in HCl. The hydrazone formed turned into an orange-coloured complex in alkaline medium, which was measured at 420 nm. The activity of LDH was expressed as μmoles of pyruvate formed/min/mg protein.

2.5. Preparation of hepatic tissue samples for analysis

Prior to biochemical analysis, hepatic tissue (100 mg tissue/ml buffer) was homogenized in 50 mM phosphate buffer (pH 7.2); the homogenate was then centrifuged at 10,000 rpm for 15 min and the supernatant was used for biochemical analysis. The protein concentration in each fraction was determined by the method of Bradford (1976), using crystalline bovine serum albumin as a standard.

2.5.1. Assay of lipoprotein lipase (LPL) in hepatic tissue samples

The activity of LPL was assayed by the method of Baginsky (1981). The colour developed was read spectrophotometrically at 430 nm. The assay was standardized against a glycerol solution of known molarity. Activity of LPL was expressed as μmoles of glycerol liberated/h/mg protein.

2.5.2. Assay of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA-reductase) in hepatic tissue sample

The ratio between HMG CoA and mevalonate in the hepatic tissue was taken as an index of the activity of HMG CoA-reductase as described by Rao and Ramakrishnan (1975). Equal volumes of fresh 10% hepatic tissue homogenate and diluted perchloric acid (5%) were mixed, kept for 5 min and centrifuged at 3000 rpm for 10 min. To 1.0 ml of filtrate, 0.5 ml of freshly prepared 50% hydroxylamine reagent (alkaline hydroxylamine reagent in the case of HMG CoA) was added and mixed. After 5 min,
1.5 ml of ferric chloride was added and then shaken well. Readings were taken after 10 min at 540 nm against a similarly-treated saline-arsenate (0.1%) blank. The ratio of HMG CoA to mevalonate was calculated with a low ratio indicative of high enzyme activity and vice-versa.

2.6. Extraction of hepatic tissue lipids

Hepatic tissue lipids were extracted by the method of Folch et al. (1957). Briefly, the tissue was homogenized with chloroform/methanol solvent mixture (2:1, v/v) and the volume of solvent mixture was adjusted to 20 times the weight of the hepatic tissue sample (1 g in 20 ml of solvent mixture). After dispersion, the whole mixture was agitated for 15-20 min in an orbital shaker at room temperature. The homogenate was filtered through Whatmann No.1 filter paper into a conical flask. The solvent was washed with 0.9% NaCl solution. After vortexing for a few seconds, the mixture was centrifuged at 2000 rpm for 5 minutes to separate the two phases. The upper aqueous phase containing gangliosides and other water-soluble compounds was removed. The lower chloroform phase containing lipids was evaporated to dryness and the lipid extract was then used for the estimation of fatty acids.

Determination of fatty acid composition by gas liquid chromatography

The fatty acid composition of the lipid samples was analyzed after converting to methyl esters. Fatty acid methyl esters (FAME) were separated and extracted by the method of Kates (1972).

Sample preparation for fatty acid analysis

The lipid extract was subjected to saponification after solvent evaporation. 1.0 ml of saponification reagent (45 g of NaOH, 150 ml CH₃OH and 150 ml distilled water) was added to the tubes and vortexed for 5-10 sec and kept in a boiling water bath. After 5 min, the tubes were removed from the water bath and vortexed for 5-10 sec, and again kept in a boiling water bath for 25 min. The tubes were removed and cooled in tap water at room temperature. Two millilitres of methylating reagent
(325 ml of 6.0 N HCL and 275 ml of CH3OH) were added to all the tubes, then heated in a water bath at 80°C for 10 min and cooled quickly to room temperature. The FAME thus formed were removed from the aqueous phase and transferred to an organic phase by liquid-liquid extraction procedure. Extraction solvent (hexane and diethyl ether, 1:1) was added to all the above tubes, mixed well and the aqueous lower phase was discarded. To the upper solvent phase, 3.0 ml of base wash solution (10.8 g NaOH and 900 ml distilled water) were added, mixed well and subjected to centrifugation. The upper solvent layer was removed and placed in a GC vial for analysis.

**FAME analysis**

FAME were analyzed by gas chromatography (Shimadzu GC-17A equipped with flame ionization detector) using a capillary column BPX70, 30 m length x 0.25 mm internal diameter x 0.25 μm film thickness at a programmed temperature with nitrogen as a carrier gas (1.0 ml/min). The oven temperature was maintained at 50°C for 3 min, followed by an increase of 30°C/min upto 170°C and followed by a second increase of 3°C/min upto 230°C and finally held for 10 min. Individual peaks of FAME were identified by comparing retention times with that of FAME standards (FAMQ-005) obtained from AccuStandard, USA. The relative content of individual fatty acids was expressed as % of total fatty acids.

2.7. **Analysis of cholesterol and bile acids in faecal matter**

The faecal matter collected was pooled, dried, powdered and finally weighed and aliquots were used for the assay of sterols and bile acids.

2.7.1. **Estimation of cholesterol**

Faecal sterols and bile acids were extracted according to the method of Jayakumari and Kurup (1979). About 500 mg of the dried powdered faecal samples were extracted with 1 N NaOH in 95% ethanol at 80°C for 2 h. The residue was extracted once with 95% ethanol. The extracts containing sterols were pooled and diluted with an equal volume of water. This mixture was shaken well with petroleum
ether (boiling point 80°C) and the extraction was repeated twice. The petroleum ether extracts were combined, evaporated to dryness and redissolved in a known volume of chloroform. Aliquots of the chloroform extracts were used for the estimation of cholesterol by the above-mentioned method. The cholesterol levels were expressed as mg/day/rat.

2.7.2. *Extraction of faecal bile acids*

Faecal samples were first extracted with petroleum ether; the residual ethanol was removed by heating in a boiling water bath and then it was acidified to a pH of 1.0. Bile acids were extracted with chloroform:methanol (2:1 v/v) from the acidified solution. The extract was then concentrated to a known volume and the aliquots obtained were used for the estimation of bile acids.

*Estimation of faecal cholic acid*

Cholic acid was estimated by the method of Levin *et al.* (1961). A standard solution of sodium cholate at a concentration of 50-200 μg/dl and aliquots of faecal bile acid extracts were evaporated to dryness. After cooling, 5 ml of 16 N H₂SO₄ were added slowly followed by 2.0 ml of freshly distilled 0.5% furfural solution. The contents were stirred well and incubated at 65°C for 15 min. The tubes were then cooled and 5.0 ml of glacial acetic acid were added and stirred well. The colour intensity was measured at 640 nm against a suitable blank. The cholic acid extracted was expressed as mg/day/rat.

*Estimation of faecal deoxy cholic acid*

Deoxy cholic acid was estimated by the method of Levin *et al.* (1961). The standard deoxy cholic acid in the range of 50-250 μg/dl and liquorits of bile acids extracts were evaporated to dryness. Four millilitres of freshly distilled benzaldehyde solution were added. The tubes were kept at 3-5°C for 120 min and stirred vigorously for the initial 15 min. At the end of this period, 4.0 ml of cold ethyl acetate were added.
slowly and stirred. The intensity of the colour that developed was read spectrophotometrically at 660 nm against blank. The amount of deoxycholic acid extracted was expressed as mg/day/rat.

2.8. Histopathological studies

Histopathological studies were performed by the methods similar to those described in Chapter II. From slices of fresh hepatic tissue and aorta, sections of 6-8 μm thickness were cut, and stained by aqueous haematoxylin and alcoholic-eosin.

2.9. Statistical analysis

The values are expressed as mean ± SD for six animals in each group. Differences between groups were assessed by one-way ANOVA using SPSS software package for Windows (Version 11.5; SPSS Inc., Chicago, IL, USA). Post-hoc testing was performed for intergroup comparisons using the least significance difference test. Values of \( P<0.001, 0.01 \) and 0.05 have been denoted by distinct symbols in the tables.

3. RESULTS

3.1. Parameters of the lipid profile in serum samples

Serum lipid profile parameters in the different groups of rats are shown in Table 6. The levels of serum TC, TG, LDL-cholesterol, VLDL-cholesterol, HDL-cholesterol and the cardiac risk ratio values were significantly \( (P<0.001) \) higher in rats fed the atherogenic diet and treated with saline (groups IIa and IIIa) than those in rats fed a normal diet (group I). However, in rats in groups IIb and IIIb (treated with EGCG for 7 and 15 days, respectively), there was a significant \( (P<0.001) \) decrease in TC, TG, LDL-cholesterol, VLDL-cholesterol and cardiac risk ratio values and a significant \([(group \ IIb) \ P<0.05 \ and \ (group \ IIIb) \ P<0.001]\) increase in the level of HDL-cholesterol, when compared with values in groups IIa and IIIa rats respectively. Group IIIb rats exhibited significantly lower \( (P<0.01 \ for \ TC, \ P<0.001 \ for \ TG \ and \ P<0.05 \ for \ LDL) \) values and significantly \( (P<0.05) \) higher HDL cholesterol values than those in group IIb rats.
Table 6. Effect of epigallocatechin gallate (EGCG) on serum lipid parameters in atherogenic diet (ATH)-fed rats

<table>
<thead>
<tr>
<th>Serum lipid parameters</th>
<th>Mean levels (in mg/dl) in group I (normal rats)</th>
<th>Mean levels (in mg/dl) in group II rats (ATH for 30 days, then treatment for 7 days)</th>
<th>Mean levels (in mg/dl) in group III rats (ATH for 30 days, then treatment for 15 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean levels (in mg/dl)</td>
<td>IIa (ATH + saline)</td>
<td>IIb (ATH + EGCG)</td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>54.41 ± 3.14</td>
<td>462.55 ± 11.16  *</td>
<td>143.37 ± 2.83  *  b</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>88.63 ± 3.12</td>
<td>182.98 ± 6.37  a</td>
<td>137.13 ± 5.57  a  b</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>18.41 ± 2.28</td>
<td>205.46 ± 13.26  a</td>
<td>80.61 ± 6.15  a  b</td>
</tr>
<tr>
<td>VLDL-cholesterol</td>
<td>15.61 ± 3.24</td>
<td>47.95 ± 6.35  a</td>
<td>23.48 ± 4.35  a  b</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>27.34 ± 0.80</td>
<td>44.68 ± 2.51  a</td>
<td>53.74 ± 3.80  a  b</td>
</tr>
<tr>
<td>Cardiac risk ratio</td>
<td>1.98 ± 0.13</td>
<td>10.27 ± 0.56  a</td>
<td>2.67 ± 0.23  b</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six rats in each group

* not for cardiac risk ratio
Statistical analyses (*, †, and ‡ represent P <0.001, P<0.01 and P<0.05, respectively)

Group I vs. groups IIa, IIb, IIIa, IIIb values
Group IIa vs. group IIb values
Group IIIa vs. group IIib values
Group IIb vs. group IIIb values

**Abbreviations**

LDL- low density lipoprotein
VLDL- very low density lipoprotein
HDL- high density lipoprotein
3.2. Activities of hepatic marker enzymes in serum

The mean serum activities of AST, ALT, ALP and LDH were found to be significantly (P<0.001) higher in rats fed the atherogenic diet and treated with saline (groups IIa and IIIa) than those in rats fed the normal diet (group I) (Table 7). Group IIb rats exhibited significantly lower mean levels of AST (P<0.05), ALT (P<0.05), ALP (P<0.001) and LDH (P<0.001) than did group IIa rats. The mean activities of all the hepatic marker enzymes in serum were significantly (P<0.001) lower in group IIIb rats than those in group IIIa rats. The mean activities of the hepatic marker enzymes in the serum samples of group IIIb rats was significantly (P<0.05) lower than those in group IIb rats.

3.3. Activities of LPL and HMG CoA-reductase in hepatic tissue

The mean activities of the lipid-metabolizing enzyme (LPL) and rate-limiting enzyme of cholesterol biosynthesis (HMG CoA-reductase) in the hepatic tissues of the experimental rats are listed in Table 8. The mean activities of LPL and HMG CoA-reductase were significantly (P<0.001) lower in hepatic tissue of group IIa and group IIIa (fed the atherogenic diet) rats compared with values in group I (fed the normal diet) rats. The mean activity of LPL in hepatic tissue samples was significantly (P<0.05) higher in group IIb (treated with EGCG for 7 days) rats than that in group IIa rats. However, no significant difference in the mean activity of HMG CoA-reductase in the hepatic tissue samples was noted between rats of groups IIb and IIa. Group IIIb (treated with EGCG for 15 days) rats exhibited significantly (P<0.001 for LPL, P<0.01 for HMG CoA-reductase) higher mean activities of the enzymes than did group IIIa rats. However, no such significant differences were observed between the mean activities of LPL and HMG CoA-reductase in hepatic tissue samples of rats of groups IIb and IIIb.
Table 7. Effect of epigallocatechin gallate (EGCG) on activities of hepatic marker enzymes in serum samples of atherogenic diet (ATH)-fed rats

<table>
<thead>
<tr>
<th>Parameters studied</th>
<th>Mean activities in group I (ATH 30 days, then treatment for 7 days)</th>
<th>Mean activities in group II rats (ATH 30 days, then treatment for 7 days)</th>
<th>Mean activities in group III rats (ATH 30 days, then treatment for 15 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IIa (ATH + saline)</td>
<td>IIb (ATH + EGCG)</td>
<td>IIIa (ATH + saline)</td>
</tr>
<tr>
<td>Aspartate amino- transferase (AST)</td>
<td>0.93 ± 0.08</td>
<td>1.91 ± 0.12 a*</td>
<td>1.67 ± 0.12 a*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.67 ± 0.12 a*</td>
<td>1.41 ± 0.10 a* c d†</td>
</tr>
<tr>
<td>Alanine amino- transferase (ALT)</td>
<td>1.19 ± 0.10</td>
<td>2.19 ± 0.13 a*</td>
<td>1.85 ± 0.25 a b t</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.34 ± 0.17 a*</td>
<td>2.34 ± 0.17 a*</td>
</tr>
<tr>
<td>Alkaline phosphatase (ALP)</td>
<td>2.28 ± 0.27</td>
<td>4.31 ± 0.54 a*</td>
<td>3.00 ± 0.20 a b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.90 ± 0.31 a*</td>
<td>4.90 ± 0.31 a*</td>
</tr>
<tr>
<td>Lactate dehydrogenase (LDH)</td>
<td>7.18 ± 0.48</td>
<td>12.99 ± 0.46 a*</td>
<td>10.08 ± 0.43 a b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.04 ± 0.45 a*</td>
<td>14.04 ± 0.45 a*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six rats in each group.

**Units:**
- Aspartate and alanine amino transferase: μmoles × 10^2 of pyruvate liberated/min/mg protein
- Alkaline phosphatase: μmoles × 10^2 of phenol liberated/min/mg protein
- Lactate dehydrogenase: μmoles × 10^1 of pyruvate formed/min/mg protein

Statistical analyses (*, †, and ‡ represent P < 0.001, P < 0.01 and P < 0.05, respectively)

* Group I vs. groups IIa, IIb, IIIa, IIIb values
† Group IIa vs. group IIb values
‡ Group IIIa vs. group IIIb values
§ Group IIb vs. group IIIb values
Table 8. Effect of epigallocatechin gallate (EGCG) on levels of lipid-metabolizing enzyme (lipoprotein lipase) and rate limiting enzyme of cholesterol biosynthesis (3-hydroxy-3-methylglutaryl coenzyme A-reductase [HMG CoA-reductase]) in hepatic tissue of atherogenic diet (ATH)-fed rats

<table>
<thead>
<tr>
<th>Parameters studied</th>
<th>Mean activities in <strong>Group I</strong> (normal) rats</th>
<th>Mean activities in <strong>Group II</strong> rats (ATH for 30 days, then treatment for 7 days)</th>
<th>Mean activities in <strong>Group III</strong> rats (ATH for 30 days, then treatment for 15 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipoprotein Lipase</td>
<td>13.75 ± 1.02</td>
<td>9.12 ± 1.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.50 ± 0.78&lt;sup&gt;a, b, c&lt;/sup&gt;</td>
</tr>
<tr>
<td>+HMG CoA-reductase</td>
<td>3.17 ± 0.52</td>
<td>1.92 ± 0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.05 ± 0.21&lt;sup&gt;a, NS&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.05 ± 0.21&lt;sup&gt;a, NS&lt;/sup&gt;</td>
<td>1.48 ± 0.47&lt;sup&gt;a&lt;/sup&gt;</td>
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<td></td>
<td></td>
<td>8.57 ± 0.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.24 ± 0.69&lt;sup&gt;a, c, e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.24 ± 0.69&lt;sup&gt;a, c, e&lt;/sup&gt;</td>
<td>2.30 ± 0.25&lt;sup&gt;a, c, e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six rats in each group. 

Units: Lipoprotein lipase - μmoles of glycerol liberated/h/mg protein 
HMG CoA-reductase - ratio of HMG CoA to mevalonate 

+ Lower ratio indicates higher enzyme activity and vice versa 

Statistical analyses (*, †, ‡) represent *P*<0.001, †*P*<0.01 and ‡*P*<0.05, respectively; NS: not significant) 

- Group I vs. groups IIa, IIb, IIIa, IIIb values 
- Group IIa vs. group IIb values 
- Group IIIa vs. group IIIb values
3.4. **Levels of fatty acids in hepatic tissue**

The mean levels of various fatty acids in the hepatic tissues of the experimental animals are listed in Table 9. The mean hepatic levels of lauric (C12:0), tridecanoic (C13:0), myristic (C14:0), pentadecanoic (C15:0), palmitic (C16:0), heptadecanoic (C17:0), stearic (C18:0) and palmitoleic (C16:ln7) acids in group IIa and group IIIa rats were significantly ($P<0.001$) higher than those in group I rats. Similarly, group IIa rats exhibited significantly ($P<0.01$) higher mean hepatic levels of pentadecanoic acid (C15:0) than did group I rats. Significantly lower ($P<0.01$) mean levels of gamma-linoleic (18:3n6) acid and significantly lower ($P<0.001$) mean levels of octadecanoic (C18:1n7), oleic (C18:1n9) and arachidonic (C20:4n6) acids were noted in the hepatic tissue samples of groups IIa and IIIa (fed the atherogenic diet) rats compared with values in group I (normal) rats. Similarly, group IIa rats exhibited significantly ($P<0.05$) lower mean hepatic levels of oleic acid (C18:2n6) than did group I rats. However, group IIb rats showed significantly ($P<0.01$) lower mean values of tridecanoic (C13:0), myristic (C14:0) and stearic (C18:0) acids and significantly ($P<0.05$) lower mean levels of palmitic (C16:0) acid than those in group IIa rats. Similarly, significantly ($P<0.001$) lower mean hepatic levels of lauric (C12:0), tridecanoic (C13:0), myristic (C14:0), pentadecanoic (C15:0), palmitic (C16:0), heptadecanoic (C17:0), stearic (C18:0) and palmitoleic (C16:1n7) acids were noted in group IIIb rats than in group IIIa rats. Group IIb rats showed significantly higher mean values of hepatic octadecanoic (C18:1n7), oleic (C18:1n9) and arachidonic (C20:4n6) acids ($P<0.05$ for octadecanoic, $P<0.01$ for oleic, $P<0.001$ for arachidonic) than did group IIa rats. Similarly, the mean levels of hepatic octadecanoic (C18:1n7), oleic (C18:1n9), linoleic (18:2n6) and arachidonic (C20:4n6) acids were significantly ($P<0.001$) higher in group IIIb rats than those in group IIIa rats.

The mean levels of saturated fatty acids in the hepatic tissue samples from rats of groups IIa and IIIa were significantly ($P<0.001$) higher than the level in group I rats (Table 9). However, the mean levels of saturated fatty acids in hepatic tissue of rats of groups IIb and IIIb were significantly lower ($P<0.001$) than those in group IIa and
Table 9. Effect of epigallocatechin gallate (EGCG) on levels (percentages) of fatty acids in hepatic tissues of rats fed an atherogenic diet (ATH)

<table>
<thead>
<tr>
<th>Carbon chains of fatty acids</th>
<th>Mean levels in group I (normal) rats</th>
<th>Mean levels in group II rats (ATH for 30 days, then treatment for 7 days)</th>
<th>Mean levels in group III rats (ATH for 30 days, then treatment for 15 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IIa (ATH + saline)</td>
<td>IIb (ATH + EGCG)</td>
<td>IIIa (ATH + saline)</td>
</tr>
<tr>
<td>12:0 (Laurie)</td>
<td>0.64 ± 0.06</td>
<td>1.84 ± 0.41*</td>
<td>2.38 ± 0.41*</td>
</tr>
<tr>
<td>13:0 (Tridecanoic)</td>
<td>0.69 ± 0.19</td>
<td>3.48 ± 0.42*</td>
<td>2.16 ± 0.65**</td>
</tr>
<tr>
<td>14:0 (Myristic)</td>
<td>0.75 ± 0.17</td>
<td>1.58 ± 0.11*</td>
<td>0.93 ± 0.24†</td>
</tr>
<tr>
<td>15:0 (Pentadecanoic)</td>
<td>1.85 ± 0.40</td>
<td>2.77 ± 0.27†</td>
<td>2.34 ± 0.20</td>
</tr>
<tr>
<td>16:0 (Palmitic)</td>
<td>22.58 ± 1.81</td>
<td>27.57 ± 0.85*</td>
<td>25.16 ± 2.44‡</td>
</tr>
<tr>
<td>17:0 (Heptadecanoic)</td>
<td>0.66 ± 0.08</td>
<td>1.72 ± 0.25*</td>
<td>1.88 ± 0.13*</td>
</tr>
<tr>
<td>18:0 (Stearic)</td>
<td>09.55 ± 1.35</td>
<td>28.62 ± 0.97*</td>
<td>25.71 ± 0.73†</td>
</tr>
<tr>
<td>16:1 n7 (Palmitoleic)</td>
<td>2.96 ± 0.39</td>
<td>6.88 ± 0.71*</td>
<td>6.71 ± 0.85*</td>
</tr>
<tr>
<td>18:1 n7 (Octadecanoic)</td>
<td>0.22 ± 1.19</td>
<td>6.00 ± 0.58*</td>
<td>7.53 ± 0.70*</td>
</tr>
<tr>
<td>18:1 n9 (Oleic)</td>
<td>10.17 ± 0.98</td>
<td>3.15 ± 0.50*</td>
<td>5.21 ± 1.37**</td>
</tr>
<tr>
<td>18:2 n6 (Linoleic)</td>
<td>12.56 ± 0.46</td>
<td>11.56 ± 0.36*</td>
<td>10.74 ± 0.42†</td>
</tr>
<tr>
<td>18:3 n6 (Gamma-linoleic)</td>
<td>2.93 ± 0.29</td>
<td>2.43 ± 0.08*</td>
<td>2.50 ± 0.13*</td>
</tr>
<tr>
<td>20:4 n6 (Arachidonic)</td>
<td>24.48 ± 2.79</td>
<td>2.83 ± 0.27*</td>
<td>6.33 ± 0.82**</td>
</tr>
<tr>
<td>SFA</td>
<td>36.72 ± 3.38</td>
<td>67.55 ± 0.95*</td>
<td>60.03 ± 3.02*</td>
</tr>
<tr>
<td>PUFA</td>
<td>63.31 ± 3.65</td>
<td>32.84 ± 0.93*</td>
<td>39.01 ± 3.45*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six rats in each group.
Statistical analyses (*, †, and ‡ represent P <0.001, P<0.01 and P<0.05, respectively).
* Group I vs. groups IIa, IIb, IIIa, IIIb values; † Group IIa vs. group IIb values; ‡ Group IIIa vs. group IIIb values.

Abbreviations
SFA- Saturated fatty acids
PUFA- Polyunsaturated fatty acids
group IIIa rats, respectively. Similarly, group IIIb rats exhibited significantly \((P<0.001)\) lower mean hepatic levels of the saturated fatty acids than those in group IIb rats. Interestingly, the mean hepatic levels of PUFAs in group IIa and group IIIa rats were significantly \((P<0.001)\) lower than those in group I rats. Significantly \((P<0.001)\) higher mean hepatic levels of PUFAs were noted in rats of groups IIb and IIIb than in rats of groups IIa and IIIa, respectively. Similarly, group IIIb rats exhibited significantly higher \((P<0.001)\) mean hepatic levels of the PUFAs than did group IIb rats.

3.5. Levels of cholesterol and bile acids in faecal samples

Table 10 lists the levels of cholesterol and bile acids in faecal samples of the experimental animals. The mean faecal concentrations of cholesterol in rats of groups IIa and IIIa were significantly \((P<0.001)\) higher than those in group I rats. Similarly, significantly \((P<0.01)\) higher mean faecal concentrations of cholic acid and deoxycholic acid were noted in group IIa and group IIIa rats than in group I rats. Significantly higher mean concentrations of faecal cholesterol \((P<0.001)\) and of deoxycholic acid \((P<0.01)\) were noted in group IIb and group IIIb rats than in group IIa and group IIIa rats, respectively. Similarly, rats in groups IIb \((P<0.01)\) and IIIb \((P<0.001)\) exhibited significantly higher mean levels of cholic acid than did rats in groups IIa and IIIa, respectively. Interestingly, the mean concentrations of faecal cholesterol and cholic acid were significantly \((P<0.001)\) higher in group IIIb rats than those in group IIb rats.

3.6. Histological examination

When haematoxylin and eosin-stained sections of segments of the thoracic aorta from the experimental groups of rats were examined, the intima of the aorta was found to be thicker in the rats that had been fed the atherogenic diet (group IIa and group IIIa) than that in normal (group I) rats (Fig. 10). However, in rats receiving EGCG treatment (group IIb and group IIIb), the intima of the aorta was found to be less thick than that of rats that had been fed the atherogenic diet and treated with saline.
Table 10. Effect of epigallocatechin gallate (EGCG) on levels of faecal cholesterol and bile acids parameter in atherogenic diet (ATH)-fed rats

<table>
<thead>
<tr>
<th>Parameters studied</th>
<th>Mean levels in <strong>Group I</strong> (normal) rats</th>
<th>Mean levels in <strong>Group II</strong> rats (ATH for 30 days, then treatment for 7 days)</th>
<th>Mean levels in <strong>Group III</strong> rats (ATH for 30 days, then treatment for 15 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IIa (ATH + saline)</td>
<td>IIb (ATH + EGCG)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>20.72 ± 1.94</td>
<td>189.81 ± 9.14 *</td>
<td>276.18 ± 19.86 *</td>
</tr>
<tr>
<td>Cholic acid</td>
<td>8.63 ± 0.97</td>
<td>15.52 ± 1.99 †</td>
<td>22.75 ± 3.38 †</td>
</tr>
<tr>
<td>Deoxycholic acid</td>
<td>7.59 ± 1.78</td>
<td>12.75 ± 2.38 †</td>
<td>18.98 ± 2.48 †</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six rats in each group

**Units:** mg/day/rat

Statistical analyses (*, †, and ‡ represent P<0.001, P<0.01 and P<0.05, respectively)

* Group I vs. groups IIa, IIb, IIIa, IIIb values
† Group IIa vs. group IIb values
‡ Group IIIa vs. group IIIb values
d Group IIb vs. group IIIb values
Fig. 10. Effect of epigallocatechin gallate (EGCG) treatment on the thickness of the intima in the thoracic aorta of Wistar rats (haematoxylin-eosin, x 200)

(A) Normal diet, (B) ATH, Atherogenic diet-fed rats and (C) EGCG, epigallocatechin gallate-treatment
Sections of hepatic tissue from the different groups of rats were stained by haematoxylin-eosin and then subjected to histopathological examination (Fig. 11). Marked fatty changes were observed in the hepatic tissue sections from rats that had been fed the atherogenic diet and then treated with saline; such changes were not observed in hepatic tissue sections from normal (control) rats. In the sections of hepatic tissue of rats that had been fed an atherogenic diet and then treated with EGCG, only minimal fatty changes were noted; these were less intense than those seen in the samples from rats that had been fed the atherogenic diet and treated with saline (Fig. 11).

4. DISCUSSION

Hypercholesterolemia and atherosclerosis have been implicated in the pathophysiology of CAD and myocardial ischemia (Eisenberg, 1998). Increased levels of total blood cholesterol and LDL are found to be associated with increased risk of developing coronary diseases (Fredrickson et al., 1967). Therefore, much research has been oriented towards supplementation of the diet with natural antioxidant compounds. As regulators of cholesterol homeostasis, these compounds have therapeutic importance for the treatment of hyperlipidemia and oxidative stress-induced pathogenesis of disease. The role of EGCG from green tea in combating the aberrations accompanying atherogenic-diet induced atherosclerosis has been investigated in this chapter.

The oral bioavailability of green tea catechins was reported to be less than 2% in animal studies (Chen et al., 1997). Various factors associated with the gastrointestinal tract, such as limited membrane permeability, transporter-mediated intestinal secretion or gut wall metabolism may contribute significantly to the low bioavailability of green tea catechins after oral intake (Cai et al., 2002); 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 (Cai et al., 2002). Kao et al. (2000a; 2000b) preferred to administer EGCG by the intraperitoneal route in order to clearly assess the possible physiological effects of green tea
Fig. 11. Haematoxylin-eosin stained (x 200) histoarchitecture of hepatic tissue.

a) Group I (normal diet) rats
b) Group IIIa (fed atherogenic diet, treated with saline for 15 days) rats
c) Group IIIb (fed atherogenic diet, treated with EGCG for 15 days) rats
consumption. They concluded that parenteral administration of EGCG may inhibit the growth and regression of prostate and breast tumors in athymic mice treated with EGCG. Hence, for these reasons, the intraperitoneal mode of administration of EGCG was preferred in the present investigation. The dose of 100 mg/kg b.w. was chosen based on the analysis of the serum lipid profile at various treatment doses; there was no significant variation in values of lipid profile parameters when concentrations between 100 and 125 mg were tested. Moreover, Kao et al. (2000a; 2000b) used a dose of 85 mg/kg b.w. of purified EGCG at which concentration they were able to obtain the desired effects. Hence, 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 investigation, administration of EGCG for 7 days to rats that had been fed an atherogenic diet appeared to bring about a decrease in mean serum levels of total cholesterol, TG, LDL-cholesterol and VLDL-cholesterol and an increase in the mean serum HDL-cholesterol level, when compared to rats that had been fed the atherogenic diet and treated with saline. The recovery was more pronounced when EGCG was given for a period of 15 days. The efficacy of EGCG in improving the serum lipid profile in this study is similar to the effect previously noted in ellagic acid-treated hyperlipidemic rabbits (Yu et al., 2005). Sohn et al. (2005a) reported that the administration of a methanolic extract of Sorbus commixta cortex (MSC) to atherogenic diet-fed rats resulted in markedly lowered plasma total cholesterol, TG and LDL-cholesterol concentrations while the HDL-cholesterol concentration remained unchanged. Another group of researchers reported that serum TC and TG increased significantly in rabbits receiving a high fat and cholesterol diet but decreased in rabbits receiving the same diet supplemented with ellagic acid (Yu et al., 2005). Thus, the present study demonstrates that the administration of EGCG results in near normal values of lipid profile parameters in rats fed an atherogenic-diet. The results of the present study suggest that these parameters can be considered for the therapeutic evaluation of anti-atherogenic drugs.
Gupta et al. (1976) demonstrated that hypercholesterolemia causes malfunctioning of the liver through microvesicular steatosis, due to the intracellular accumulation of lipids. The extent of hepatic damage can be assessed by noting the mean activities of serum transaminases and alkaline phosphatase (Molander et al., 1955). In the present study, the mean activities of serum AST, ALT, ALP and LDH were significantly higher in atherogenic diet-fed, saline-treated rats than those in normal rats. Senthilkumar and Nalini (2004) reported that administration of glycine to alcohol-induced hepatotoxic rats resulted in reduced levels of serum hepatic marker enzymes. In the present investigation, the mean activities of serum AST, ALT, ALP and LDH were significantly lower in atherogenic diet-fed, EGCG-treated (groups IIb and IIIb) rats than in samples from rats of groups IIa and IIIa, respectively.

The conversion of HMG CoA to mevalonate by the enzyme, HMG CoA-reductase, using reduced nicotinamide adenine dinucleotide phosphate (NADPH) as a reducing equivalent, is the rate-limiting step in cholesterol biosynthesis. LPL plays an important role in the metabolism of plasma lipoproteins and, thus, the transport of lipids to peripheral tissues. Lower activity of LPL causes marked hyperlipidemia and hypertriglyceridemia (Bensadoun, 1991, Reymer et al., 1995). Earlier studies have shown significantly lowered activity of LPL in rats fed a high-cholesterol diet (Kavitha and Nalini, 2000; Sudhahar et al., 2006b). In the present investigation, the mean activities of LPL and HMG CoA-reductase in hepatic tissue were found to be significantly lower in atherogenic diet-fed, saline-treated rats than the values in normal control rats. However, the mean activity of LPL in hepatic tissue was found to be significantly higher in atherogenic diet-fed, EGCG-treated (group IIb and group IIIb) rats than in atherogenic diet-fed, saline-treated (group IIa and group IIIa) rats. Vijayakumar and Nalini (2006a) reported that the administration of piperine to rats fed a high fat diet, and to hypercholesterolemic rats resulted in increased activities of LPL and HMG CoA-reductase in the hepatic and cardiac tissues. The inhibition of HMG-CoA-reductase decreases cholesterol synthesis and its inhibitors are very effective in lowering serum cholesterol in most animal species, including humans (Kleemann and Kooistra, 2005). Shimada et al. (1996) reported that plasma LPL,
directly or indirectly, may promote or protect against atherosclerosis. The results of the present study suggest that EGCG appeared to act by reducing hypercholesterolemia / lipemic-oxidative stress, therein allowing the restoration of LPL and HMG CoA-reductase enzyme activities in EGCG-treated rats to near normal.

Epidemiological and experimental data indicate that dietary manipulation, especially changes in the source of lipid consumed in the diet, may modify the fatty acid composition of different tissues and many cell types (Zurier, 1993; James et al., 2000). In the present investigation, the mean levels of saturated fatty acids, lauric, tridecanoic, myristic, pentadecanoic, palmitic, heptadecanoic, stearic and palmitoleic acids, were found to be significantly higher, whereas the mean levels of PUFAs, octadecanoic, oleic, linoleic, gamma-linoleic and arachidonic acids were found to be significantly lower in hepatic tissue of atherogenic diet-fed, saline-treated rats than the mean levels in normal rats. Interestingly, administration of EGCG for 7 days to rats that had been fed the atherogenic-diet brought about a decrease in mean hepatic levels of saturated fatty acids, lauric, tridecanoic, myristic, pentadecanoic, palmitic, heptadecanoic, stearic and palmitoleic acids; however, mean hepatic levels of PUFAs, octadecanoic, oleic, linoleic and arachidonic acids were found to be higher than the levels in rats that had been fed an atherogenic-diet and treated with saline. The recovery was more pronounced when EGCG was given for a period of 15 days. Senthilkumar and Nalini (2004) reported that the administration of glycine to rats with alcohol-induced hepatotoxicity resulted in markedly lowered levels of saturated fatty acids and an increase in the levels of PUFAs. Elevated levels of PUFAs are considered to be optimal for health and are associated with a significantly low cardiovascular risk (Sellmayer and Weber, 2002; Dyerberg et al., 2004). Saturated fatty acid has been shown to inhibit the activities / expression of lipogenic enzymes, but PUFAs, especially n-3 fatty acids, upregulate the expression of genes encoding proteins involved in fatty acid oxidation and downregulate the genes encoding proteins for lipid synthesis (Jump et al., 1996; Clarke, 2001). Thus, the data from the present investigation suggest that EGCG administration corrected imbalances in the fatty acid composition of plasma and tissues induced by intake of the atherogenic diet so that the levels in atherogenic
diet-fed, EGCG-treated rats were lower than those in atherogenic diet-fed, saline-treated rats. EGCG possibly possesses the potential to modulate membrane dynamics by altering the lipid composition of membranes and the activity of enzymes involved in the fatty acid metabolism.

In general, the serum/plasma concentration of cholesterol in the body is regulated by cholesterol biosynthesis, removal of circulating cholesterol, absorption of dietary cholesterol and the excretion of cholesterol via the bile and feces. In the bile acid pathway, cholesterol is metabolized to bile acids in the liver and excreted in the bile. After entering the small intestine, a major portion of the excreted bile acids is reabsorbed, and a smaller portion excreted in the feces. Aoyama et al. (1999) suggested that the bile acid pathway is quantitatively important, and that changes in the rate of this pathway influence cholesterol levels in tissue. Increased excretion consequently reduces the pool of bile acids in the liver and enhances the catabolism of cholesterol to bile acids in the liver (Havel, 1988). In the present study, rats that had been fed an atherogenic diet and then treated with saline alone (groups IIa and IIIa) demonstrated increased excretion of cholesterol and bile acids in faecal samples, whereas rats that had been fed an atherogenic diet and then treated with EGCG (groups IIb and IIIb) exhibited a further rise in the excretion of cholesterol and bile acids in faecal samples. Similarly, Sudhahar et al. (2006b) reported that the administration of lupeol and lupeol linoleate to hypercholesterolemic rats resulted in increased excretion of cholesterol and bile acids in faecal matter. Thus, the combined effect of both decreased biosynthesis and increased excretion of cholesterol may have been responsible for the decreased serum levels of total cholesterol, observed in EGCG-treated rats. Overall, EGCG treatment may suppress hepatic cholesterol synthesis remarkably, thus forcing the liver to rely on LDL-cholesterol for synthesis of bile acids. This mechanism of action may also explain the reduced serum levels of LDL-cholesterol observed in rats that had been fed an atherogenic diet and then treated with EGCG in the present investigation.

In the present study, histological examination of sections of aorta from rats fed the atherogenic diet revealed a thickening of the aortic intima (when compared to the
appearance in normal rats), suggesting the onset of atherosclerosis. This intimal thickening of the aorta correlated with the biochemical parameters observed in the atherogenic diet-fed, saline-treated rats. However, in rats that received EGCG treatment, the thickness of the intima was found to be near normal. Sohn et al. (2005a) reported that the administration of a methanolic extract of *Sorbus commixta cortex* to atherogenic diet-fed rats resulted in marked reduction in the thickening of the aortic intima while Mitani et al. (2003) reported that rabbits that had been fed a high cholesterol diet showed a reduction in the thickening of the aortic intima following fluvastatin treatment.

Hypercholesterolemia-induced hepatic abnormalities can be further confirmed by histopathological findings. In the present study, rats fed the atherogenic diet and treated with saline alone showed marked fatty changes in the hepatocytes. Deepa and Varalakshmi (2004) observed similar fatty changes in hepatic tissue of hypercholesterolemic rats. The marked fatty changes in the hepatic tissue correlated with the abnormal biochemical parameters observed in the present study. However, treatment with EGCG appeared to ameliorate the adverse effects on the hepatic tissue, as suggested by the presence of only minimal or partial fatty changes. Sudhahar et al. (2007) reported that the administration of lupeol and lupeol linoleate to hypercholesterolemic rats resulted in reduction of fatty changes in hepatic tissue. EGCG, the major catechin of green tea, appears to prevent hepatic lipidemic-oxidative injury by circumventing lipid and oxidative abnormalities.

5. CONCLUSION

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 mechanism: 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 excretion of cholesterol and bile acids.