Chapter V

Regulatory effect of epigallocatechin gallate on expression of C-reactive protein and other inflammatory markers in an experimental model of atherosclerosis

1. INTRODUCTION

Atherosclerosis is considered to be a chronic inflammatory disease since it develops in response to damage to the vessel wall. It is characterized by the infiltration of mononuclear blood cells into the intima, formation of ‘foam cells’, the proliferation of arterial smooth muscle cells and accumulation of connective tissue components in the inner lining of the arteries (Ross, 1999, Glass and Witztum, 2001). C-reactive protein (CRP) is one of the substances present in the atherosclerotic lesion, more specifically in the vascular intima, where it co-localizes with monocytes, monocyte-derived macrophages and lipoproteins (Torzewski et al., 1998; Zwaka et al., 2001). Two hypotheses have been proposed to explain the role of an elevated serum CRP concentration in the pathogenesis of atherosclerosis. One mechanism may be the ongoing inflammation in the artery, stimulated by Ox-LDL, which leads to the production of cytokines that induce the formation of CRP, an acute phase protein. Alternatively, chronic elevations of acute phase reactants such as CRP, which occur due to smoking, chronic infections, obesity and hypercholesterolemia, may contribute to the development of atherosclerosis. However, these hypotheses are not exclusive and both mechanisms might act together to account for the increase of CRP (Francisco et al., 2006).

CRP is primarily synthesized in hepatocytes, although a growing body of evidence indicates extrahepatic production of CRP, such as in macrophages, arterial tissue, adipose tissue or endothelial cells (Dong and Wright, 1996; Yasojima et al., 2001; Ouchi et al., 2003; Venugopal et al., 2005). The expression of mRNA coding for CRP has also been identified in many different extrahepatic cells, such as islet cells
of the pancreas (Fehsel et al., 1997), neurons (Yasojima et al., 2000), adipocytes (Ouchi et al., 2003) and renal tubular epithelial cells (Jabs et al., 2003). An elevated level of CRP in blood constitutes a stable and reliable marker of systemic inflammation (Gabay and Kushner, 1999; Roberts et al., 2001; Rothkrantz-Kos et al., 2002; Prasad, 2003). The erythrocyte sedimentation rate (ESR) is also frequently used as an indicator of inflammation and as an independent predictor of CVD (Danesh et al., 2000; Godsland et al., 2004).

Feeding a cholesterol-rich diet induces free radical production, followed by oxidative stress and hypercholesterolemia (Bulur et al., 1995) in rats, and also induces signs of inflammation, such as migration of white cells into the endothelium and expression of adhesion molecules in rabbits (Li et al., 1993). Various inflammatory cells, such as macrophages and lymphocytes, are able to generate ROS (Russwurm et al., 1994). Enhanced oxidative stress due to the inflammatory response might thus contribute to the pathogenesis of atherosclerosis. The systemic response to inflammation increases the level of oxidized lipids in serum and enhances the oxidative modification of LDL-cholesterol (Memon et al., 2000). In addition, CRP has a pro-oxidative effect (Kobayashi et al., 2003). Currently both oxidative and inflammatory processes are believed to be involved in the pathogenesis of atherosclerosis (Hansson, 2005), where antioxidant defence systems are also impaired. It would seem logical to neutralise the deleterious effects of ROS by boosting such antioxidant defence systems. In recent years, efforts have been made to evaluate the use of natural antioxidants to alleviate atherosclerosis induced by lipemic oxidative stress. 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. The natural product, EGCG, is the major polyphenolic constituent found in green tea, *Camellia sinensis* L (Demeule et al., 2002; Bettuzzi et al., 2006), and has been characterized as an antioxidant (Xu et al., 2004) with antitumorigenic (Mukhtar and Ahmad, 2000) and antiangiogenic properties (Cao and Cao, 1999).
Administration of EGCG has been reported to ameliorate various pathological states, including cerebral ischemia (Simonyi et al., 2005) and cancer (Lin et al., 1999; Beltz et al., 2006). Interestingly, EGCG has also been found to improve serum lipid profile and antioxidant parameters in erythrocytes, cardiac and hepatic tissue of Wistar rats that have been fed an atherogenic diet (Ramesh et al., 2008; 2009). However, the regulatory effect of EGCG on inflammatory markers, such as CRP and ESR, and leucocyte and platelet counts, has not been described. Hence, the present chapter focuses on an evaluation of the effect of EGCG on expression of CRP and other inflammatory markers in Wistar rats fed an atherogenic-diet.

2. MATERIALS AND METHODS

2.1. Isolation and purification of EGCG from *Camellia sinensis*

EGCG was isolated from *Camellia sinensis* and purified by methods already 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.

In this phase, the experimental study was performed on rats that had been fed an atherogenic diet (ATH) *ad libitum* for 30 days and then treated for 15 days with EGCG (100 mg/kg body weight).

The rats were divided into three main groups:

Group I (normal) rats received a normal diet for 45 days and daily intraperitoneal administration of saline from days 31-45.
Group II (ATH-fed, saline-treated) rats received ATH for 45 days and daily intraperitoneal administration of saline from days 31-45.

Group III (ATH-fed, EGCG-treated) rats received ATH for 45 days and daily intraperitoneal administration of EGCG (100 mg/kg body weight) from days 31-45.

At the end of the experimental period, all the animals were sacrificed by decapitation. From each animal, blood samples were collected and serum separated; the hepatic tissue was excised. The serum and hepatic samples were stored at -80°C until analysis.

2.3. Determination of inflammatory factors

CRP in each serum sample was assayed by an immunoturbidimetric method using a standard assay kit (Diasys Diagnostics, Holzheim, Germany). ESR was measured by the Westergren method. Total white blood cell (WBC) and platelet counts were performed on whole blood samples using Sysmex KX-24 blood cell counter (Transasia Biomedicals Ltd., Mumbai, India). Blood smears were made from EDTA-treated samples and the differential leucocyte count was done manually.

2.4. Western Blotting

Samples were denatured for 1 min in a boiling water bath with sodium dodecyl sulphate sample buffer, and equal quantities of protein (60 µg) were loaded on a 10% polyacrylamide gel with 4% stacking gel, using the buffer system essentially as described by Laemmli (1970). Following electrophoresis, proteins in the gel were transferred for 60 min at 15V (Bio-Rad semidry blotting apparatus, USA) onto nitrocellulose membranes soaked for at least 30 min in transfer buffer (25 mM Tris-base, 0.193 M glycine, 20% methanol, pH 8.3) (Towbin et al., 1979). Transfer conditions were optimized to ensure complete transfer of proteins in the 118 kDa region of the gel. Membranes were blocked with 5% non-fat dry milk in Tris buffered saline-Tween, TBST [25 mM Tris-base, 150 mM NaCl, pH 7.4; 0.1% (v/v) Tween-20] for 3 hrs, subsequently washed twice for 5 min with Tris buffered-saline, TBS [25 mM
Tris-base, 150 mM NaCl, pH 7.4] and then incubated with monoclonal antibody directed against human CRP (Sigma-Aldrich, St. Louis, MO), 1:400 dilution of antibody in TBS containing 5% (w/v) non-fat dry milk for 2 hrs. The blots were washed 2-3 times with TBST using at least 5 min for each wash and a final 5 min wash with TBS. The blots were then incubated for 1 hr with secondary antibody (goat anti-rabbit IgG-conjugated with alkaline phosphatase, obtained from Genei, Bangalore, India) diluted (1:2000) in TBS containing 5% non-fat dehydrated milk powder. The membranes were then washed 2-3 times with TBST using at least 5 min for each wash and a final 5 min wash with TBS. Membranes were incubated with a color-developing solution containing 5-bromo-4-chloro-3-indolyl phosphate (BCIP). Finally, the developed blots were scanned in a gel documentation system to determine the intensity of the bands (Quantity One Software; Bio-Rad, USA).

2.5. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of mRNA transcripts of CRP

Total RNA was extracted from the freshly isolated liver of the experimental rats using Trizol reagent (Sigma-Aldrich, St. Louis, USA). Briefly, the fresh hepatic tissue (100 mg/ml) was homogenized in Trizol reagent and the homogenate was transferred immediately to a microfuge tube and kept at 4°C for 5 min to permit the complete dissociation of nucleoprotein complexes. Then, 0.2 ml of chloroform was added, vortexed vigorously for 15 seconds and incubated at 4°C for 10 min. After incubation, the mixture was centrifuged at 12,000 ×g for 15 min at 4°C. The upper aqueous phase (600 μl) was carefully transferred to a fresh microfuge tube and an equal volume of isopropanol was added followed by thorough vortexing; the solution was then again incubated at 4°C for 10 min. The incubated mixture was then centrifuged at 12,000 ×g for 10 min; the supernatant was removed and the pellet was washed twice with 75% ethanol by vortexing and subsequently centrifuged at 7,500 ×g for 5 min (4°C). After centrifugation, the ethanol was removed carefully and the pelleted RNA was allowed to dry for 10 min. The isolated RNA was resuspended in 50 μl of RNase-free water; the concentration and purity of total RNA were determined by absorbance at 260/280 nm.
in a UV-spectrophotometer (Sambrook et al., 1989). The purity of RNA obtained was > 1.8.

RT-PCR was performed to quantify the CRP mRNA transcripts against the internal control GAPDH. The primer sequences for CRP and GAPDH are gene-specific; the following primers were used for the generation of cDNAs. CRP (Paul et al., 2004) (amplicon size-280 bp), sense primer: 5'-AGCCTCTCTCATGCTTTTGG-3'; anti-sense primer: 5'-TGTCTCTTTGGGTGAGCATACGA-3'. Rat GAPDH (Nakajjima et al., 2002) (amplicon size-207 bp), sense primer: 5'-TCAAGAAGGTGGTACAGG-3'; anti-sense primer: 5'-GGTCCACCACCTGTGCTGT-3'.

Total RNA was reverse transcribed by Qiagen one-step RT-PCR kit (Qiagen, Germany) according to the manufacturer's instructions. Briefly, 2 μg of RNA template were added with a 'mastermix', containing 10 μl of RT-PCR buffer, 2.0 μl of dNTP mix, 2.0 μl of RT-PCR enzyme and an appropriate volume of 0.6 μM primers and made up to 50 μl with RNase free water. The reverse transcriptase (RT) reaction was performed at 50°C for 30 min followed by initial PCR activation at 95°C for 15 min. The three-step PCR cycles included (i) denaturation at 94°C for 1.5 min, (ii) annealing at 60°C for 1.5 min and (iii) extension at 72°C for 3 min. The PCR amplification was carried out 30 cycles and, to ensure that the products were extended completely, a final extension at 72°C for 10 min was performed. RT-PCR was performed using the thermal cycler (Eppendorf, Germany).

Ten microliters of each PCR product were analyzed by gel electrophoresis on a 2% agarose gel. The molecular size of the amplified products (CRP and GAPDH) was determined by comparison with molecular weight markers (100 bp DNA ladder, Genei, Bangalore, India) run in parallel with the RT-PCR products. Gels were subjected to densitometric scanning and the band intensity of the cDNA fragment of the CRP gene was normalized against the band intensity of the cDNA fragment of GAPDH, using Quantity One Software (Bio-Rad, USA). The experiments were performed in triplicate.
2.6. Statistical analysis

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

3. RESULTS
3.1. Values of inflammatory markers

The mean serum level of CRP was significantly ($P<0.001$) higher in rats fed the atherogenic diet and treated with saline (group II) than that in rats fed a normal diet (group I), while in group III rats (fed the atherogenic diet and treated with EGCG), the mean serum CRP level was significantly lower ($P<0.001$) than that in group II rats (Table 14).

The mean values of inflammatory markers in blood samples of the experimental animals are depicted in Figure 17. The mean values of the ESR, platelet count and total WBC count in blood samples of group II rats were significantly ($P<0.001$) higher than the values in group I rats, while the mean values of these three parameters were significantly ($P<0.001$) lower in the rats fed the atherogenic diet and receiving treatment with EGCG (group III) than those in the rats fed the atherogenic diet and receiving only saline treatment (group II).
Fig. 17. Effect of epigallocatechin gallate (EGCG) on the values of inflammatory markers in blood samples of rats fed an atherogenic diet (ATH)

Group I: Normal; Group II - ATH for 45 days, saline treatment for 15 days; Group III- ATH for 45 days, EGCG treatment for 15 days.

Values are expressed as median of nine rats in each group.

Statistical analyses (* represent P<0.001)

*Group I vs. group II values

bGroup II vs. group III values
Table 15 shows the differential leucocyte counts (percentages) in blood samples of the experimental animals. The mean percentages of neutrophils and monocytes were found to be significantly ($P<0.001$ and $P<0.01$, respectively) higher in rats fed the atherogenic diet and treated with saline (group II) than those in rats fed the normal diet (group I), whereas the mean percentages of neutrophils and monocytes in blood samples of group III rats (fed the atherogenic diet and treated with EGCG for 15 days) were significantly ($P<0.001$ and $P<0.01$ respectively) lower than those noted in group II rats. A significantly ($P<0.001$) lower mean lymphocyte percentage was noted in blood of group II rats than that in group I rats, whereas the lymphocyte percentage in blood of group III rats was significantly ($P<0.001$) higher than that in group II rats. No significant differences were noted between the mean eosinophil percentages in blood samples of the three groups of rats, while basophils were absent in blood samples of all the groups.

3.2. Expression of C-reactive protein

The level of CRP in the serum samples of different groups of rats was also compared by immunoblotting (Figs. 18a & 18b). The intensity of the band corresponding to CRP was significantly ($P<0.001$) lower in group III rats (fed the atherogenic diet and treated with EGCG) than that in group II rats, and was also significantly ($P<0.001$) lower in normal (group I) rats than that in group II rats (the protein loading control was confirmed by the antibody, $\beta$-actin). Figures 18c & 18d demonstrate that there was a significant ($P<0.01$) positive correlation ($r=0.881$) between the band intensity levels of CRP and quantitative levels of CRP.

3.3. Transcript levels of CRP mRNA

CRP mRNA was analyzed by RT-PCR and the transcript levels were compared between the experimental groups (Figs. 19a & 19b). In group III (atherogenic diet-fed, EGCG-treated) rats, the level of the transcript of the CRP gene was found to be significantly ($P<0.001$) lower than that in group II (atherogenic diet-fed, saline-treated)
Table 14. Effect of epigallocatechin gallate (EGCG) on the level of C-reactive protein (CRP) in serum samples of rats fed an atherogenic diet (ATH)

<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameter studied</th>
<th>Group I rats (Normal)</th>
<th>Group II rats (ATH for 45 days, saline treatment for 15 days)</th>
<th>Group III rats (ATH for 45 days, EGCG treatment for 15 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CRP (mg/l)</td>
<td>1.16 ± 0.35</td>
<td>6.28 ± 1.61&lt;sup&gt;a*&lt;/sup&gt;</td>
<td>3.02 ± 0.57&lt;sup&gt;b*&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of nine rats in each group
Statistical analyses (* represents P <0.001)
<sup>a</sup>Group I vs. group II values
<sup>b</sup>Group II vs. group III values

Table 15. Effect of epigallocatechin gallate (EGCG) on differential leucocyte percentages in blood samples of rats fed an atherogenic diet (ATH)

<table>
<thead>
<tr>
<th>Parameters studied</th>
<th>Group I rats (Normal)</th>
<th>Group II rats (ATH for 45 days, saline treatment for 15 days)</th>
<th>Group III rats (ATH for 45 days, EGCG treatment for 15 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophil (%)</td>
<td>10 ± 1</td>
<td>22 ± 3&lt;sup&gt;**&lt;/sup&gt;</td>
<td>12 ± 2&lt;sup&gt;b*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>87 ± 6</td>
<td>73 ± 5&lt;sup&gt;**&lt;/sup&gt;</td>
<td>84 ± 3&lt;sup&gt;b*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Monocyte (%)</td>
<td>2 ± 1</td>
<td>4 ± 1&lt;sup&gt;†&lt;/sup&gt;</td>
<td>3 ± 1&lt;sup&gt;b‡&lt;/sup&gt;</td>
</tr>
<tr>
<td>Eosinophil (%)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Basophil (%)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of nine rats in each group
Statistical analyses (*, † and ‡ represent P <0.001, P <0.01 and P <0.05, respectively)
<sup>**</sup>Group I vs. group II values
<sup>b</sup>Group II vs. group III values

Abbreviation
ND- Not detected
Fig. 18. (a). Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) pattern of **C-reactive protein (CRP)** protein in serum samples of experimental groups M-Protein marker, L1-Group I; L2-Group II; L3-Group III

(b). Immunoblot analysis of CRP with β-actin as loading control in serum samples of experimental groups

(c). Box-plot showing the band intensity levels of CRP (* Significance at P<0.001; a-Group I vs. group II values; b-Group II vs. group III values)

(d) Positive correlation noted between band intensity and quantitative levels of CRP.

(•) Values in group I; (▲) Values in group II; (■) Values in group III
Group I- Normal; Group II-ATH for 45 days, saline treatment for 15 days; Group III-ATH for 45 days, EGCG treatment for 15 days.
Fig. 19. (a). Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of C-reactive protein (CRP) mRNA transcript levels in hepatic tissue visualized on an ethidium bromide-stained agarose gel. M-100 bp DNA ladder; L1- Group I; L2- Group II; L3- Group III

(b). The results depicted are normalized to levels of GAPDH gene. Data are expressed as mean ± SD Value of ratios of intensity for CRP gene divided by that for GAPDH (tested in triplicate)

*Significance at P<0.001; a-Group I vs. group II values; b-Group II vs. group III values

Group I- Normal; Group II-ATH for 45 days, saline treatment for 15 days; Group III-ATH for 45 days, EGCG treatment for 15 days.
rats. The level of the CRP gene transcript was found to be significantly ($P<0.001$) higher in group II rats than that in normal rats (group I).

4. DISCUSSION

Atherosclerosis is currently accepted to be an inflammatory disease. Inflammatory mechanisms play a central role in all phases of atherosclerosis, from the initial recruitment of circulating leukocytes to the arterial wall upto the rupture of unstable plaques, which results in the clinical manifestations of the disease. Major risk factors, including hypercholesterolemia (especially of Ox-LDL particles), participate in the initiation and progression of the inflammatory process. C-reactive protein (CRP), formerly considered solely as a biomarker of inflammation, is now viewed as a prominent participant in endothelial dysfunction and atherosclerosis (Szmitko *et al.*, 2003; Verma and Yeh, 2003). Elevated levels of CRP in plasma / serum have been reported in numerous disease states (Prasad, 2003). Much of the clinical interest in CRP is focused on the association of chronically elevated CRP levels with increased risk of atherosclerosis and CVD (Ridker *et al.*, 1997; 1998; 2000; Morrow and Ridker, 2000). In addition to being a serum biomarker, CRP is involved in the pathogenesis of atherosclerosis by enhancing uptake of LDL-cholesterol into macrophages to form foam cells (Zwaka *et al.*, 2001), facilitating leukocyte transmigration (Pasceri *et al.*, 2001) and platelet-activating factor (Rouis *et al.*, 1998), stimulating monocytes to produce tissue factor and proinflammatory cytokines through the upregulation of nuclear factor κB (Morrow and Ridker, 2000), and also stimulating vascular smooth muscle migration, proliferation, neointimal formation and production of ROS (Wang *et al.*, 2003). Oxidative stress, defined as a disruption of the delicate balance between oxidative and antioxidative processes, is believed to play an important role in the pathogenesis of hypercholesterolemic atherogenesis (Steinberg *et al.*, 1989). ROS are reported to be generated during hypercholesterolemic atherogenesis (Prasad and Kalra, 1993; Prasad *et al.*, 1994). Therefore, much research has been oriented towards supplementation of the diet with natural antioxidant compounds in order to attenuate oxidative stress-induced pathogenesis of diseases.
In the present study, the inflammatory marker CRP was investigated. The mean serum level of CRP was significantly higher in atherogenic diet-fed, saline-treated rats than in normal rats (Table 14), which corroborates the earlier findings of elevated serum levels of CRP in CVD (Ridker et al., 1997; 1998; 2000) and in acute coronary syndrome (Sivaraman et al., 2004). Interestingly, the mean serum level of CRP was significantly lower in atherogenic diet-fed, EGCG-treated rats than in atherogenic diet-fed, saline-treated rats. Earlier studies have shown that pravastatin reduces the level of plasma/serum CRP in patients with hypercholesterolemia (Ridker et al., 1999), coronary artery disease (Nissen et al., 2005), dyslipidemia (Kent et al., 2003) and stable ischemic heart disease (Takeda et al., 2003). The data presented herein show that green tea catechins, including EGCG, lower the serum levels of CRP.

Apart from CRP, other inflammatory markers such as the ESR, total WBC count and platelet count, have been shown to be significantly and positively correlated with CVD (Thaulow et al., 1991; Huang et al., 2001) and with acute coronary syndrome (Sivaraman et al., 2004). The ESR appears to be a strong predictor of coronary heart disease (Erikssen et al., 2000). In the present study, the mean values of the ESR, WBC and platelet counts were significantly higher in atherogenic diet-fed, saline treated rats than in normal (group I) rats. However, the mean levels/counts of ESR, WBC and platelet counts were significantly lower in atherogenic diet-fed, EGCG-treated (group III) rats than in atherogenic diet-fed, saline-treated (group II) rats (Fig. 17). Platelet counts have been shown to improve following the administration of aspirin in coronary artery disease (Markuszewski et al., 2006; Faraday et al., 2009) and acute coronary syndrome (Modica et al., 2007). Prentice et al. (1982) reported that neutrophil, eosinophil, and monocyte counts are predictive of coronary artery disease. However, there appears to be an inverse relation between lymphocyte count and cardiovascular risk (Horne et al., 2005; Dragu et al., 2008). In the present investigation, administration of EGCG for 15 days to rats that had been fed an atherogenic diet brought about a decrease in the mean percentages of neutrophils and monocytes and an
increase in the mean percentage of lymphocytes, when compared to the values occurring in rats that had been fed an atherogenic diet and treated with saline. Interestingly, no such significant difference was observed between the mean percentages of eosinophils in the different experimental groups; these findings are consistent with those of an earlier study that reported that the eosinophil count was virtually unrelated to coronary atherosclerosis (Kawaguchi et al., 1996). Similarly, Tani et al. (2008) have reported that treatment with pravastatin, a potent lipid-lowering drug, can reduce the leukocyte count in patients with CAD. Thus, the present study demonstrates that the administration of EGCG results in near normal values of ESR, platelet and total WBC counts in rats fed an atherogenic-diet.

In the present study, immunoblotting of serum CRP was performed to assess the level of its expression in different groups of experimental rats. The expression of CRP was found to be significantly higher in the serum samples of atherogenic diet-fed, saline-treated (group II) rats than that in serum samples of normal (group I) rats and of atherogenic diet-fed, EGCG-treated (group III) rats. Interestingly, these findings are consistent with the observations made on CRP levels in the quantitative studies. Again, these original findings suggest that EGCG treatment is able to modulate the CRP level to near normal levels. Kaur et al. (2007) have reported that treatment with the powerful antioxidants, resveratrol and quercetin, decreases cytokine-induced CRP expression in Hep3B cells.

To further underline the relevance of the present investigation, the expression of the CRP gene in the hepatic tissue of the experimental rats was analysed by RT-PCR. The level of the CRP gene transcript was higher in hepatic tissue of atherogenic diet-fed, saline-treated (group II) rats than that in atherogenic diet-fed, EGCG-treated (group III) rats and that in normal (group I) rats. These findings suggest that EGCG treatment is able to modulate CRP expression at both mRNA and protein levels, therein reducing the risk of atherosclerosis. Interestingly, a recent study reported a decrease of mRNA expression of CRP and of the level of inflammatory mediators following the
administration of the phenolic flavonoid, procyanidin, from grape-seed to rats that had been fed a high-fat diet (Terra et al., 2009).

5. CONCLUSION

The results of the present study indicate that green tea catechins, and particularly EGCG, the major component of green tea, can reduce inflammatory markers in rats fed an atherogenic diet. The expression of CRP protein and levels of its mRNA transcript are decreased in atherogenic diet-fed, EGCG-treated rats, when compared to atherogenic diet-fed, saline-treated rats. The findings strongly suggest that EGCG possesses anti-inflammatory properties, and these characteristics may play a role in its putative anti-atherogenic effect in Wistar rats fed an atherogenic diet.