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Protective effects of green tea (*Camelia sinensis*) in diet induced hypercholesterolemia and atherosclerosis in wistar albino rats

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Abstract

Green tea (*Camelia sinensis*) supplementation along with high cholesterol diet significantly ($p < 0.05$) reduced the hyperlipidemia by reducing total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and elevation of high density lipoprotein cholesterol (HDL-C) and significantly increased all the cellular antioxidant enzymes and reduced the level of thiobarbituric acid reactive substance (TBARS). Majority of the histopathologically initiated atherosclerotic changes in the aorta and fatty change in the liver of high cholesterol diet group were not observed in the green tea supplemented group. Needs further studies at molecular level in the completely established atherosclerosis in laboratory animal models.

Keywords: Hyperlipidemia, atherosclerosis, hematology, biochemical, histopathology, green tea

Introduction

Hyperlipidemia is accompanied by elevated serum TC, TG, LDL-C and VLDL-C and decreased HDL-C levels [1]. It has been ranked as one of the greatest risk factor in the initiation and progression of atherosclerosis and there by coronary heart disease (CHD) and related heart complications. CHD is the leading causes of mortality in both developed and developing countries, accounting 30% of all worldwide deaths per year [2]. Current reports suggest that by the year 2020, India will have the largest cardio vascular disease (CVD) burden in the world [3].

Lowering serum cholesterol by diet or drugs like statins, fibrates, nicotinic acid and resins slows the rate of progression of atherosclerosis, causes regression of some plaques and reduces the risk of cardiovascular events [4]. Owing to their side effects people are looking for safe alternatives of natural agents [3]. A number of herbal medicines are used for controlling hyperlipidemia and related complications [5]. Tea is one of the most popular beverages consumed worldwide after water. Black tea, green tea and oolong tea, all are derived from the same plant leaves by different levels of oxidization and fermentation, but green tea contains higher concentration of endogenous polyphenols as compared to other teas [6]. The polyphenols found in green tea are commonly known as flavonols or catechins, mainly epicatechin, epicatechin-3-gallate, epigallocatechin and epigallocatechin-3-gallate (EGCG) [7] and these are known to have various health beneficial effects like anti-inflammatory, anti-oxidative, anti-arthritic, anti-angiogenic, anti-metastatic, anti-cancer, anti-obesity, anti-hyperlipidemic, anti-atherosclerotic, neuroprotective, anti-dental caries and antimicrobial (bacterial, viral & fungal) properties studied by various laboratories of the world [8].

Hence the Present study has been carried out to evaluate the anti-hyperlipidemic and anti-atherosclerotic effects of green tea in diet induced hyperlipidemia and atherosclerosis in wistar albino male rats.

Materials and Methods

Procurement of experimental animals: Male wistar albino rats weighing around 200g were procured from Sri Venkateswara Agencies, Bangalore. After acclimatization of one week the rats were grouped and housed in standard poly propylene rat cages (three rats per cage) and maintained at $25 \pm 1^{\circ}\text{C}$ and a 12:12 hour interval light / dark cycle throughout the experimental period of 90 days. The approval of the institutional animal ethical committee was obtained prior to commencement of the experiment.

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Experimental design: A total of 48 healthy wistar albino male rats were divided into 4 groups contains 12 rats in each and were maintained with ad libitum provision of feed and water. Hyperlipidemia and atherosclerosis were experimentally induced by 1% Cholesterol and 15% saturated oil to 1000 g of standard rat chew diet (High cholesterol diet (HCD) and given to group II rats. Group I kept as control and fed with standard rat diet. Group III is green tea control, group IV is HCD along with green tea @ 100mg/kg of rat/day (PO) for 90 days. Six rats from each group were randomly selected for sacrifice at 45 days apart.

Source of cholesterol: Cholesterol extra pure, AR grade with product code No: 97900 was procured from the SRL fine chemicals, Indian Scientific, Tirupati, Andhra Pradesh. Green tea (*Camellia sinensis*) whole leaf extract powder with product code no. P/SVU/CASI-02 was procured from Chemiloids Ltd, Vijayawada, Andhra Pradesh.

Clinical observations: Health condition, behavior, feed and water intake of all the rats was monitored every day and recorded clinical signs and body weight of the animals at 45 days apart.

Hematology: Blood samples were collected in 10% EDTA at each sacrifice from all the sacrificed rats and used for the estimation of TEC, TLC, PCV by micro hematocrit method [9] and Hb by Sahli's method [10].

Biochemical parameters: At each sacrifice, blood samples from all the groups were collected in to the sterile test tubes. Serum samples were separated without RBC and stored at 4°C. Estimation of TC, LDL-C, VLDL-C, HDL-C, and TG were carried out by using commercially available biochemical kits (Auto Span diagnostics, Bangalore).

Tissue oxidative stress: At each sacrifice, liver and heart tissue pieces were collected and stored at -20°C in the deep freezer until use. Tissue pieces of liver and heart were minced separately and homogenized in 0.05 M ice cold phosphate buffer (pH 7.4) by using a virtis homogenizer to make 10% homogenate. For lipid peroxidation assay, 0.2 ml of the homogenate was used. The remaining part of homogenate was mixed with 10% trichloroacetic acid in the ratio of 1:1, centrifuged at 5000 g for 10 min at 4 °C and supernatant was

used for estimation of reduced glutathione [11]. The remaining part of the homogenate was centrifuged at 15,000 g for 60 min at 4 °C and the supernatant obtained was used for SOD [12], catalase [13] and GPx [14] in liver and aorta of all rats in all groups.

Histopathology – Small tissue pieces of aorta, heart and liver were collected in neutral buffered formalin for routine histoprocessing by paraffin embedding technique and section were stained with Haemotoxylin & Eosin (H&E) [15].

Statistical analysis: The results were analyzed statistically by performing one-way ANOVA [16].

Results and Discussion: The research work was designed with a view to study the beneficial effects of green tea (*Camellia sinensis*) on diet induced hyperlipidemia and atherosclerosis in male wistar albino rats maintained for 90 days. Clinically significant increase in body weight with obesity, sluggishness and poor hair coat was observed in the group II rats compared to control group I [17-21]. It might be due to high cholesterol diet compared to standard rat chew diet of controls. Throughout the experimental period, the green tea ameliorated group was apparently healthy with very shiny hair coat, slim in appearance and physiologically very active and it might be due to its wide spread health benefits of catechins like anti-obesity, anti-hyperlipidemic and anti-oxidative properties [22, 23]. It suggests that long term usage of green tea might be useful for anti-obesity effect.

Clinical pathological parameters like TEC, TLC, PCV and Hb% estimations was carried out and results of TEC, PCV and Hb% of all groups (Group I, II, III & IV) were normal and non-significant ($P < 0.05$) throughout the experimental period. TLC in HCD fed group were non significantly higher when compared to control group I. Mohamed Anwar *et al.* [24] who observed increased WBC and lymphocytes levels in rabbits that were fed with high cholesterol diet. Increased leukocytes count in the present study might be due to increased level of LDL cholesterol, which is responsible for increased viscosity of the blood and thereby resulted into highest TLC [25]. The TLC levels were non significantly reduced in green tea ameliorated group IV, but not to the level of group I. Reduced level of LDL cholesterol might have reduced the level of TLC in the group IV (Table.I)

Table I: Mean values of body weight, serum biochemical and hematological parameters of different experimental groups at 45th and 90th day of experiment

Parameters	Group I		Group II		Group III		Group IV	
	45 th day of study	90 th day of study	45 th day of study	90 th day of study	45 th day of study	90 th day of study	45 th day of study	90 th day of study
Body weight (grams)	240±11.7	309.16±10.2	262.83±13.34	393.33±21.6 ^a	201.6±27.6 ^{abc}	302.5±23.8 ^b	235.8±12.41	309.1±21.3 ^{bc}
Total erythrocyte count (Million/mm ³)	5.78±0.9	6.30±0.2	6.6±0.45	6.83±1.3	6.43±0.98	5.55±0.29	6.8±0.67	6.2±0.9
Total leukocyte count(x ^{mm3} µl)	8.81±1.12	10.22±0.58	12.4±0.76	16.3±0.73	10.5±0.6	11.11±0.55	10.3±1.56	9.57±1.05
Packed cell volume (%)	34.6±5.7	37.83±1.3	39.6±2.6	32.5±2.1	38.6±5.9	36.3±1.6	38.8±5.4	37.8±2.2
Haemoglobin (Hb) (g%)	11.55±1.92	12.61±0.4	13.2±0.8	10.8±0.7	12.89±1.9	11.1±0.5	12.9±1.8	10.9±0.74

Values are Mean ± SD, n= 6. values with different superscripts differ significantly ($P < 0.05$) from the normal control or atherogenic diet or green tea.

Lipid profile: Rats on high cholesterol diet showed a significant ($P<0.05$) increase in serum TC, TG, LDL-C, VLDL-C and significant ($P<0.05$) decrease in HDL-C compared to control rats [1, 26]. It might be due to inclusion of 1% cholesterol and 15% saturated oils to the 1000g of rat diet compared to standard rat chow diet of control group I and it indicated that the diet under trial has established hyperlipidemia in this group of rats.

Low density lipoprotein is a lipoprotein that transports lipids from the liver to the peripheral (extra hepatic) and is often called “bad” cholesterol and constitutes a half to two thirds of cholesterol [27]. High levels of LDLs and its oxidation on the walls of arteries may lead to impaired endothelial relaxation in isolated arterial segments thereby it causes atherosclerosis [28]. High density lipoprotein is often called “good” because it is a lipoprotein that transports lipids from the periphery to the liver. HDL particles enhance the net removal of cholesterol

from a variety of cells, such as smooth muscle cells, fibroblasts and cholesterol laden macrophages [29] and also prevents the oxidation of LDL by virtue of its antioxidant and anti-inflammatory properties [30]. The low levels of HDL in the blood will increase the risk of atherosclerosis and coronary heart disease [31].

Green tea ameliorated group IV significantly ($P<0.05$) reduced the TC, TG, LDL-C and VLDL-C and modestly elevated the HDL-C when compared to group II, but not to the level of group I rats by the end of present experimental period. It might be due to anti-hyperlipidemic, anti-oxidative and anti-atherosclerotic effects of green tea [7, 32, 33]. Green tea catechins influences luminal lipid hydrolysis and intestinal lipid absorption, thereby it modulates the biosynthesis, excretion and intracellular processing of lipids in the body [34] (Table.II).

Table II: Serum biochemical and hematological parameters of different experimental groups at 45th and 90th day of experiment

Parameters	Group I		Group II		Group III		Group IV	
	45 th day of study	90 th day of study	45 th day of study	90 th day of study	45 th day of study	90 th day of study	45 th day of study	90 th day of study
Total cholesterol (mg/dl)	46.03±7.8	50.67±7.04	113.58±11.75 ^a	155.3±10.58 ^a	51.43±4.80 ^b	40.30±3.3 ^b	97.67±4.80 ^{abc}	86.22±6.17 ^{abc}
Triglycerides (mg/dl)	68.70±50.0	67.17±11.08	150.58±33.27 ^a	178.2±39.84 ^a	55.67±5.57 ^b	63.82±15.4 ^b	127.62±23.36 ^{abc}	125.92±67.69 ^{abc}
Low density lipoprotein cholesterol (mg/dl)	17.44±4.7	20.13±2.78	64.88±5.86 ^a	95.9±9.10 ^a	17.95±2.20 ^b	19.88±1.3 ^b	32.03±2.73 ^{abc}	27.58±7.82 ^{abc}
Mean value of VLDL cholesterol (mg/dl)	10.45±1.8	13.43±2.22	21.12±6.65 ^a	31.0±8.78 ^a	9.13±1.11 ^b	10.76±3.1 ^b	18.52±4.67 ^a	15.18±13.54 ^b
High density lipoprotein cholesterol (mg/dl)	28.16±2.8	27.10±5.15	17.58±5.35 ^a	18.4±3.96 ^a	30.35±2.95	29.65±2.7	23.12±5.19	24.45±5.66
Atherogenic index (TC/HDL-C)	2.54±0.33	3.11±0.65	4.2±1.71	5.56±0.89 ^a	2.12±0.13	2.07±0.23 ^{ab}	3.57±0.59	2.54±0.53 ^b

Values are Mean ± SD, n= 6. values with different superscripts differ significantly ($P<0.05$) from the normal control or atherogenic diet or green tea.

Tissue antioxidant enzymes: All the measured liver and heart (Includes aorta) tissue antioxidant enzymes (CAT, SOD, GPx, GR and GST) levels were significantly ($P<0.05$) reduced in group II compared to group I. Prabha *et al.* [21] also reported reduced level of antioxidant enzymes like GST, GPx, GR, GSH and CAT in atherogenic group compared to control group in a study conducted in rats for a period of 90 days. The biological antioxidant defense system is an integrated array of enzymes and antioxidants. GSH, a substrate for GSH-peroxidase, CAT, GST, GPx and GR constitute the first line of cellular antioxidant defense enzymes. Catalase and GPx catalyzes the conversion of hydrogen peroxide to water [35]. GST and GR offers protection against lipid peroxidation by promoting the conjugation of toxic electrophiles with GSH [36]. GSH is required to maintain the normal reduced state and to counteract the deleterious effect of oxidative stress. During the reduction of hydrogen peroxide, GSH is oxidized to GSSG, when GSSG levels increased, the GSH-reductase

activity was activated to convert GSSG in GSH [21].

Significant ($p<0.05$) increase in TBARS levels in the tissues of liver and heart (includes aorta) were observed in group II compared to group I rats; it indicates an increased amount of oxidative stress in the HCD fed rats [18]. Unsaturated fatty acids of cell membrane is the target for free radicals for the generation of MDA or TBARS [37].

Green tea ameliorated group showed significant ($P<0.05$) increase in all the antioxidant enzymes measured and reduced the TBARS levels compared to group II rats [38, 39, 40]. Green tea polyphenols have been demonstrated as powerful antioxidants, as it binds and neutralizes free radicals by its structural hydroxyl groups and also chelates metallic ions that generates radical oxygen species [7, 41]. Green tea control group III also showed significant ($P<0.05$) increase in all the antioxidant enzymes, which indicates its action against the oxidative stress. (Table. III).

Table III: Effect of green tea (*Camellia sinensis*) on tissue antioxidants of rats at 45th and 90th day of experiment

Parameters	Group I		Group II		Group III		Group IV	
	45 th day of study	90 th day of study	45 th day of study	90 th day of study	45 th day of study	90 th day of study	45 th day of study	90 th day of study
TBARS (nmol TBARS/ g tissue) in the liver	1.38±0.42	1.36±0.27	1.8±0.32	2.02±0.23	1.28±0.25	1.32±0.17	1.56±0.17	1.62±0.16
TBARS (nmol TBARS/ g tissue) in the heart	1.98±0.26	1.84±0.32	3.4±0.23	4.1±0.17	2.1±0.33 ^b	1.98±0.24 ^b	2.58±0.23 ^{abcd}	2.8±0.31 ^{abcd}
Catalase activity (nM of H ₂ O ₂ decomposed /min/mg of protein) in the liver	0.25±0.020	0.28±0.020	0.15±0.03	0.14±0.02	0.26±0.04	0.27±0.04	0.18±0.032	0.20±0.025
Catalase activity (nM of H ₂ O ₂ decomposed /min/mg of protein) in the heart	0.3±0.032	0.35±0.024	0.19±0.02	0.14±0.012	0.32±0.014	0.29±0.018	0.27±0.018 ^{abc}	0.28±0.011 ^{abc}
SOD activity (U/min/mg of protein) in the liver	18±1.1	16±1.8	14±1.02	12±2.2	18±1.3	19±2.1	16±0.8	17±1.7
SOD activity (U/min/mg of protein) in the heart	15±1.2	14±1.7	10±1.52	9±1.8	14±1.44 ^b	15±1.65 ^b	11±0.76 ^{ac}	12±1.67 ^{bc}
GPx activity (U/min/mg of protein) in the liver	28±1.1	26±1.6	22±0.9	20±1.2	28±0.9	29±1.1	26±1.3	27±1.5
GPx activity (U/min/mg of protein) in the heart	24±1.3	23±0.8	19±1.4	15±1.4	25±0.99 ^b	24±0.98 ^b	23±1.32 ^b	20±0.97 ^b
Glutathione reductase (nmol of GSSG utilized/min/mg protein) in the liver	7.54±0.23	6.9±0.32	4.5±0.34	3.5±0.19	8.1±0.43 ^{ab}	7.9±0.53 ^b	5.9±0.29 ^{abc}	4.8±0.38 ^{bc}
Glutathione reductase (nmol of GSSG utilized/min/mg protein) in the liver	9.77±0.34	8.8±0.24	5.5±0.28	4.8±0.21	8.9±0.21 ^{abc}	8.2±0.33 ^b	6.6±0.38 ^{abc}	7.5±0.22 ^{abc}

Mean values with different superscripts differ significantly (*P*<0.05)

Liver: Gross changes in different organs are not conspicuous except in liver. HCD fed rat liver were enlarged, soft and pale yellow in colour and degree of changes were higher in 90th day slaughter compared to 45th day (Fig.1)^[42]. Microscopically, very conspicuous micro and macro vesicular hepatic steatosis was observed (Fig.2)^[18,43]. Enlargement of liver is primarily due to the accumulation of lipids in the cytoplasm of the hepatocytes and it might be due to inclusion of 1% cholesterol and 15% saturated fat to the rat diet. Severity of enlargement and paleness was less in green tea ameliorated group and it was completely ameliorated the fatty vacuoles in the hepatocytes microscopically by the end of the study period compared to presence of few fatty vacuoles by 45th day of study (Fig.3), indicates long-term usage of green tea might be useful against the hepatic steatosis and it was evidenced biochemically by reduced level of serum TC, LDL, TG, VLDL and increased HDL values^[32, 33, 44, 45]. No such fatty change was observed both gross and microscopically in control groups I & III.

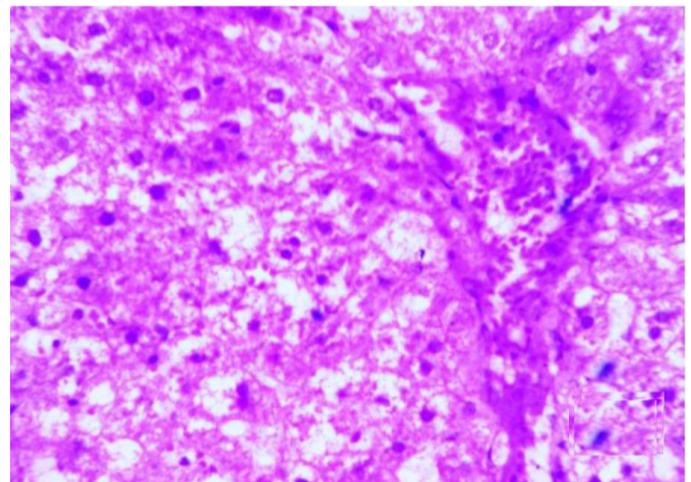


Fig 2: Liver: Group II: Section of liver showing mild to moderate micro and macro vascular fat vacuoles in the hepatocytes. H&E X400.

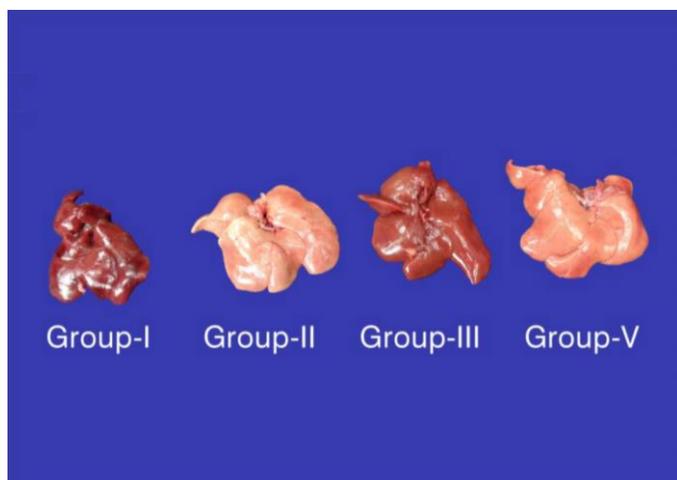


Fig 1: Group IV: Note modestly reduced size and paleness by 90th day of study

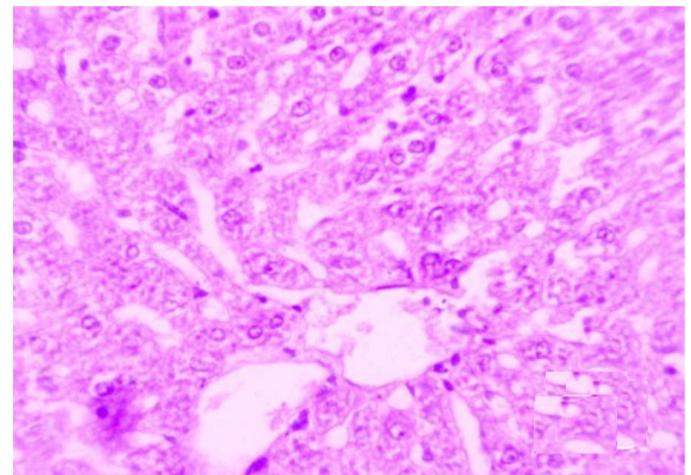


Fig 3: Liver: Group IV: Section showing normal hepatic architecture with prominent cytoplasmic staining. H&EX400

Aorta: Aorta from HCD fed group revealed moderate initiation of atherosclerotic lesions with degeneration of endothelial cells, sub intimal lipid laden macrophages (foam cells), slight to moderate thickening of the tunica intima with proliferation of few smooth muscle cells (SMC) (Fig.4) [1, 18, 20, 33]. It might be due to oxidation of high level of serum LDL-C with reduced levels of tissue antioxidants. Van Boven *et al.* [46] stated that low density lipoproteins leads to altered gene expression of endothelial cells and transformation of SMCs into pro inflammatory intimal layer, thereby initiation atherosclerotic lesions. Except erythrocytes adherence to the wall of the endothelium and presence of few fat cells under the sub endothelium, all other initiated atherosclerotic lesions in the aorta were minimized by 90th day of green tea ameliorated group (Fig.5). It might be due to the anti hyperlipidemic and anti-atherosclerotic action of green tea catechins, which reduced the elevated serum TC, TG, LDL-C and VLDL-C and elevated the level of HDL-C that were observed biochemically [32, 33, 34].

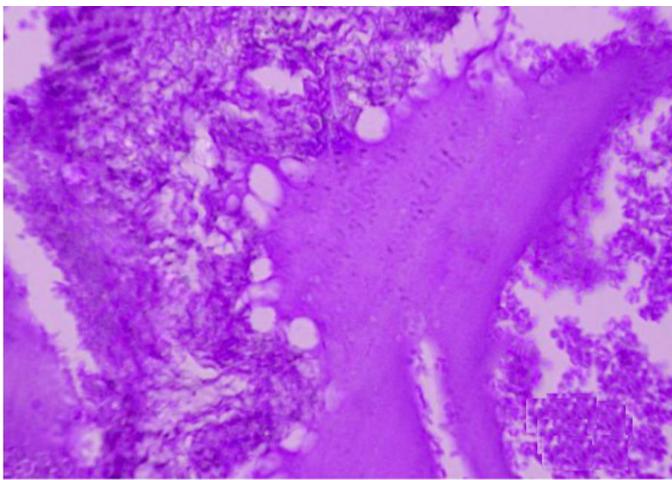


Fig 4: Aorta: Group II: Section showing vascular degenerative changes with sub intimal fat vacuolation and thrombus in the lumen of aorta. H&E X400.

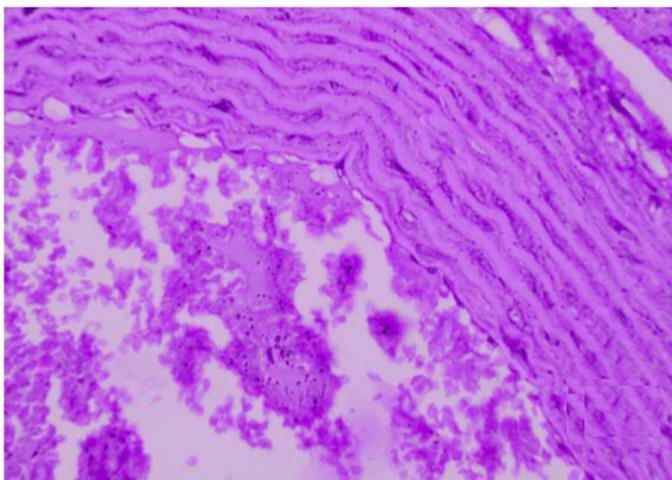


Fig 5: Aorta: Group VI: Section showing mild endothelial degeneration, few fat cells under the sub endothelium and attachment of very small micro thrombi to the wall of endothelium with normal wavy appearance of vascular elastic fibers. H&E X400

Conclusion: High cholesterol diet of present study was established the hyperlipidemia evidenced by elevated levels of TC, TG, LDL-C, VLDL-C, Tissue TBARS and decreased level of HDL-C and all the measured tissue anti oxidant

enzymes (GST, GPx, GR, GSH and CAT) there by it might be initiated the atherosclerotic lesion on the endothelium of aorta, but failed to form the complete atherosclerosis and it may be due to inclusion of low level of cholesterol (1% cholesterol) and also may be due to short span of study period. On the other hand, green tea supplementation effectively controlled the dyslipidemia and enhanced all the tissue anti-oxidant enzymes, thereby it might have prevented the oxidation of LDL-C and atherosclerotic changes in the aorta. Present study results strongly indicate that, the green tea is having the anti-obesity, anti-hepatic steatosis, anti-inflammatory and anti-atherosclerotic properties and ameliorated the hyperlipidemia associated atherosclerotic changes to the maximum extent. Molecular evaluation of green tea needs to be studied in completely established atherosclerosis in laboratory animal models as it is one of the leading cause of morbidity and mortality in human beings now a days.

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