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Diet-induced hyperlipidemia and hepatic steatosis in wistar albino male rats and its amelioration with whole grain flaxseeds (*Linum usitatissimum*) and green tea (*Camellia sinensis*)

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Abstract
Background: Hyperlipidemia is a disorder of lipid metabolism, characterized by elevated serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoproteins cholesterol (VLDL-C) and decreased high-density lipoprotein cholesterol (HDL-C). Hyperlipidemia induces micro and macrovesicular hepatic steatosis, focal area of hepatic necrosis, portal fibrosis, biliary hyperplasia, and mononuclear cell infiltration. Flaxseeds (*Linum usitatissimum)* lignans (SDG), alpha-linolenic acid (ALA) and omega-3 fatty acid, and green tea (*Camellia sinensis*) polyphenols are known to have various health-beneficial effects including antihyperlipidemic properties. The beneficial effect of flaxseed and green tea was evaluated in experimentally induced hyperlipidemia and hepatic steatosis in wistar albino rats.
Method: Male Wistar albino rats were divided into six groups consisting of 12 rats each. 1% cholesterol along with 15% saturated edible oil to the 1000 g of standard rat chew diet (High cholesterol diet (HCD)) was used for induction of hyperlipidemia and hepatic steatosis. Whole grain flaxseed powder @ 7.5 g/kg/day and green tea @ 100 mg/kg/day were included in the HDC diet and maintained for 90 days along with controls.
Results: High cholesterol diet induced hyperlipidemia and hepatic steatosis. Flaxseed and green tea supplementation significantly reduced hyperlipidemia, increased all the cellular antioxidant enzymes, and reduced the level of thiobarbituric acid reactive substance (TBARS). Hepatic steatosis was greatly reduced in both flaxseed and green tea-supplemented groups. Green tea has shown better amelioration compared to flaxseed.

Keywords: Flaxseeds, green tea, hypercholesterolemia, hyperlipidemia, hepatic steatosis

Introduction
Hyperlipidemia is a disorder of lipid metabolism, characterized by elevated serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoproteins cholesterol (VLDL-C), and decreased high-density lipoprotein cholesterol (HDL-C) (Rahaman *et al.*, 2013) [35]. It is one of the greatest risk factors in the initiation and progression of atherosclerosis and thereby coronary heart disease (CHD) and related heart complications (Eman *et al.*, 2011) [15]. Hyperlipidemia induces micro and macrovesicular hepatic steatosis, focal area of hepatic necrosis, portal fibrosis, biliary hyperplasia, and mononuclear cell infiltration (Curtis *et al.*, 2011 & Basma *et al.*, 2013) [12, 4].
Flaxseed (*Linum usitatissimum)* commonly known as linseed, is a member of the genus Linum in the family Linaceae (Anand *et al.*, 2013) [2]. Flaxseed has a high source of omega-3 fatty acids and alpha-linolenic acid (ALA), soluble and insoluble fibers, phytoestrogen, and the Lignan secoisolariciresinol diglucoside (SDG) (Dupasquier *et al.*, 2007) [14]. The therapeutic activities of flaxseed are well proven, one of them is antiatherosclerotic due to its lipid-lowering activity which is mainly attributed to its Secoisoarciresinol diglucoside (SDG) (Anand *et al.*, 2013) [2].
Green tea (*Camellia sinensis*) was discovered in 3000 BC. 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 concentrations of endogenous polyphenols as compared to other teas (Graham, 1992) [18]. The polyphenols found in green tea are commonly known as flavonols or

catechins, mainly epicatechin, epicatechin -3- gallate, epigallocatechin, and epigallocatechin-3-gallate (EGCG) (Gruenwalked *et al.*, 2000) [19]. Green tea polyphenols 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 in *in vitro* and *in vivo* by various laboratories of the world (Zhao *et al.*,1999) [43].

The present study has been carried out to evaluate the beneficial effects of flaxseeds and green tea in clinical, serum biochemical, tissue antioxidants, and histopathological changes in the liver of experimentally induced hyperlipidemia in Wistar albino male rats.

Materials and Methods

Procurement of experimental animals

Male Wistar albino rats weighing around 200 g were procured from Sri Venkateswara Agencies, Bangalore. Rats were acclimatized to the experimental conditions for one week and were grouped and housed in standard polypropylene rat cages (three rats per cage) during the experiment. They were maintained at 25±10c and a 12:12 hour interval light / dark cycle and provided standard laboratory animal feed and ad libitum water throughout the experimental period of 90 days. The approval of the institutional animal ethical committee was obtained before the commencement of the experiment.

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. Dietary grade flaxseed in the ground farm was procured from the local market.

Experimental design

A total of 72 healthy Wistar albino male rats were divided into 6 groups of 12 rats in each and maintained for 90 days. Group I: basal diet, Group II (High cholesterol diet (HCD): 1% cholesterol along with 15% hydrogenated oil in 1000 g standard rat chew diet for production of hyperlipidemia and hepatic steatosis. Group III (Flaxseed control) flaxseed @ 7.5 g/kg b.w of rats/day along with standard basal rat chew diet. Group IV (Green Tea control): standard rat chow diet and green tea @ 100 mg/kg/day. Group V: ground flaxseed @ 7.5 g/kg b.w of rats/day along with HCD. Group VI: green tea @ 100 mg/kg/day along with HCD. Six rats from each group were randomly sacrificed 45 days apart.

Clinical observations

The health condition, behavior, feed, and water intake of all the rats were monitored throughout the experimental period. The body weights of the animals were recorded on the 45th and 90th days of the experiment.

Hematology

Blood samples were collected in 10% EDTA at each sacrifice from all the sacrificed rats and used for the estimation of TEC, TLC, and PCV by microhematocrit method (Jain, 1986) [23] and Hb by Sahli's method.

Biochemical parameters

At each sacrifice, blood samples from all the groups were collected into sterile test tubes. After blood clots, clear serum samples were separated without RBC and stored at 4 °C. Estimation of TC, LDL-C, VLDL-C, HDL-C, and TG was carried out by using commercially available biochemical kits (Auto Span diagnostics, Bangalore).

Tissue oxidative stress

Liver and heart tissue pieces were collected and stored at –200C 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 the lipid peroxidation assay, 0.2 ml of the homogenate was used. The remaining part of the homogenate was mixed with 10% trichloroacetic acid in the ratio of 1:1, centrifuged at 5000 g for 10 min at 40 C, and the supernatant was used for the estimation of reduced glutathione (GR). The remaining part of the homogenate was centrifuged at 15,000 g for 60 min at 40 C and the supernatant obtained was used for superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) in liver and aorta of all rats in all groups.

Histopathology: Small tissue pieces of liver were collected in neutral buffered formalin for routine histoprocessing by paraffin embedding technique and sections were stained with Haemotoxylin & Eosin (H&E). The results were analyzed statistically by one-way ANOVA.

Results and Discussion

During the experimental study, no abnormal clinical symptoms were observed in control group I, group III, and group IV rats throughout the study period. Whereas obesity, sluggishness poor hair coat was observed in the high cholesterol diet-fed group II rats and is in concurrence with Prabha *et al*., (2013) [32]. Moderate obesity was observed in the cholesterol diet supplemented with flaxseed group V. No such obesity was observed in the green tea-supplemented group VI (Fig 1&2). Farjad and Hasan (2012) [17] observed a significant reduction in the body weight of Wistar male rats fed on a fat-enriched diet along with green tea @ 100mg/kg/day. Green tea catechins, especially EGCG appear to have anti-obesity and anti-diabetic effects (Sabu *et al*., 2010) [38]. Throughout the experimental period, the green tea ameliorated group was healthy with a very shiny hair coat, slim in appearance, and physiologically very active and it might be due to widespread health benefits like ant obesity, anti-hyperlipidemic, and anti-oxidative properties of green tea (Yasuo *et al*., (2012) [42].

High cholesterol diet-fed group II rats showed a non-significant increase in body weight in comparison with control group I which received a normal rat diet by the 45th day and a significant increase by the end of the experimental period. It is under the reports of Farjad and Hasan (2012) [17], Olubukola *et al*., (2012) [28], Pande *et al*., (2012) [29], and Faheemuddin *et al*., (2013) [16] and it might be due to high-calorie fat (1% cholesterol and 15% saturated oils) present in the high cholesterol control compared to standard normal rat chew control. A non-significant reduction in body weight was observed in the flaxseed ameliorated group throughout the

experiment period when compared to high cholesterol diet-fed group II. It is contrary to Parameshwari and Nazni (2012) [30] who reported a moderate reduction in body weight when given roasted flaxseed along with chapattis to hyperlipidemic patients. However, ameliorated with green tea group VI significantly (p<0.05) reduced the body weight by the 90th day of the experiment compared to a non-significant reduction by the 45th day experiment. It might be due to the anti-obesity action of green tea, as it suppresses adiposity (Tariq and Riyaz 2013) [40]. Green tea affects the expression of lipid metabolism genes, especially hepatic expression of the lipid catabolism genes acyl-coenzyme A oxidase 1, palmitoyl (ACOX1), acyl-coenzyme A dehydrogenase, c-4 to c-12 straight chain (ACADM), and peroxisome proliferator-activated receptor alpha (PPAR-α) (Bornhoeft *et al.* 2012 and Ryou *et al.* 2012) [6, 37]. Tea catechins, especially EGCG, appear to have anti-obesity effects (Sabu *et al.*, 2010) [38]. It suggests that long-term usage of green tea might be useful for the anti-obesity effect.

Clinical parameters like total erythrocyte count (TEC), total leukocyte count (TLC), packed cell volume (PCV), and hemoglobin percentage of all groups (Group I, II, III, IV, V & VI) were normal and non-significant (p<0.05) throughout the experimental period. Total leukocyte count in high cholesterol diet fed group II was non significantly higher when compared to control group I. Similar reports were made by Mohamed *et al*., (2008) [25] who observed increased leukocyte levels in rabbits that were fed with high cholesterol diet. Increased leukocytes count in the present study might be due to increased levels of LDL cholesterol which is responsible for increased viscosity of the blood and thereby resulted in the highest TLC (Huang *et al*., 2001) [21]. The TLC levels were non significantly reduced in ameliorated groups V and VI but not to the level of group I. Reduced level of LDL cholesterol might have reduced the level of TLC in the flaxseed and green tea ameliorated groups (Table I).

Rats in high cholesterol diet group II showed a significant (p<0.05) increase in serum TC, TG, LDL-C, and VLDL-C and a significant decrease in HDL-C compared with control group I rats that received standard basal diet. These observations are inconsistent with the reports of Farzad *et al*., (2012) [17], Abbass *et al*., (2012) [1], and Rahaman *et al*., (2013) [35]. Increased serum lipid parameters in the present study might be due to the inclusion of 1% cholesterol and 15% saturated oils in the 1000g of rat diet compared to the standard rat chew diet of the control group and it indicated that the diet under trial has established hyperlipidemia in the group II rats. The rise in serum cholesterol can be attributed to the reduced catabolic rate of serum TC or reduced activity of hepatic cholesterol -7-alpha-hydroxylase, the rate-limiting enzyme in bile acid synthesis from cholesterol (Zulet *et al*., 1999) [44].

Low-density lipoprotein is a lipoprotein that transports lipids from the liver to the peripheral (extrahepatic) and is often called “bad” cholesterol and constitutes half to two-thirds of cholesterol (Murry *et al*., 1996). High-density lipoprotein (HDL) cholesterol 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 (Wolfgang and Antonio 1995) [41]. HDLs also prevent the oxidation of LDL through their antioxidant and anti-inflammatory properties (Chiozie and Chidinma 2009) [9]. The low levels of HDL in the blood will increase the risk of atherosclerosis, coronary heart disease, and severe fatty change in the liver (Moeliandari and Wijaya 2002) [24].

Co-treatment with the flaxseed along with high cholesterol diet significantly (p<0.05) reduced the TC, TG, LDL-C, and VLDL-C and mildly elevated the HDL-C when compared to group II, but not to the level of group I by the end of the experimental period. Present observations are consistent with the earlier reports of Prasad (1997 & 2005), Patade *et al*., 2008, Delfin *et al*., 2010 and Anand *et al*., 2013) [49, 31, 13, 2]. It might be due to the hypolipidemic effects of flaxseeds. Flaxseed Omega 3 fatty acids play a role in reducing the risk of cardiovascular diseases by reducing hypertension, cholesterol, triglycerides, and free radicals (Morris 2003) [26]. Flaxseed fibers also reduce blood cholesterol levels by delaying and reducing their absorption from the intestines. Flavonoids and phenolics of flax lignan complex are potent antioxidants and exhibit hypolipidemic and anti-atherogenic effects and it is synergistic with the action of flax SDG (Anand *et al*., 2013) [2]

Green tea ameliorated group VI significantly (p<0.05) reduced the TC, TG, LDL-C, and VLDL-C and modestly elevated the HDL-C when compared to high cholesterol diet fed group II, not to the level of control group I rats by the end of the present experimental period. It might be due to the antihyperlipidemic, anti-oxidative, and anti-atherosclerotic responses of green tea (Zhao *et al*., (1999) [43]. These results are under Farzad *et al*., (2012) [17], Hussein *et al*., (2012) [22], and Abbass *et al*., (2012) [1]. Green tea catechins affect lipid metabolism by various mechanisms and prevent atherosclerosis and liver fatty change in various models of hyperlipidemia. In addition, catechins influence luminal lipid hydrolysis and intestinal lipid absorption and they also up-regulate hepatic LDL receptor expression, thereby modulating the biosynthesis, execration, and intracellular processing of lipids (Bursill *et al*., 2007) [7]. Catechins also have a direct inhibitory effect on cholesterol synthesis due to potent and selective inhibitors of squalene epoxidase, a likely rate-limiting enzyme of cholesterol biosynthesis (Abbass *et al*., 2012) [1]. In the present study, green tea ameliorated all hyperlipidemic effects better compared to flaxseeds and it might be due to its widespread health-beneficial effects of polyphenols especially catechins (Guo *et al*., 1996) [20] compared to mild hyperlipidemic action of flaxseeds (Patade *et al*., 2008) [31]

High cholesterol diet group II rats showed significantly (p<0.05) increased levels of both CRI-I and CRI-II by the end of the experiment compared to control rats fed on the standard diet. It is following the earlier reports of Kanthilal *et al*., (2012) [46], Rahman *et al*., (2013) [35]. It indicates that HDL-C is decreased in proportion to increased TC, and LDL-C and it is significantly (p<0.05) evidenced by all serum biochemical parameters. Atherogenic index (AI) indicates the deposition of foam cells or plaque or fatty infiltration or lipids in the liver. The higher the AI, the higher the risk of the above organs (Renuka *et al*., 2014) [36]. Both flaxseed and green tea ameliorated groups significantly (p<0.05) reduced both CRI-I and CRI-II of the atherogenic index by the 90th day of the

experiment compared to a non-significant decrease on the 45th day of the experiment. Kanthilal *et al*., (2012) [46] stated that reduced AI indicates protection against cardiovascular disease.

The ability of flaxseed and green tea to protect against liver anti-oxidant enzyme depletion was investigated. Results of the present study revealed a significant (p<0.05) increase in TBARS levels in the liver of HCD group II compared to group I rats on a standard diet, which may indeed indicate an increased amount of oxidative stress in the HCD-fed rats (Olubukola *et al*., (2012) [28]. Hypercholesterolemia induces oxidative stress by causing a reduction in the tissue defense antioxidant enzymes, leading to the acceleration of lipid peroxidation, cellular injury, atherosclerosis, and heart disease (Shah *et al*., 2007) [39]. Cell membranes contain unsaturated fatty acids, which are a target for free radicals that resulted in the peroxidation of lipid membranes and the generation of MDA or TBARS (Basma *et al*., 2013) [4]. HCD diet in the present study induced hypercholesterolemia indicated by an increased serum lipid profile and which might be increased the thiobarbituric reacting substances (TBARS) and oxygen radicals in the liver. All other antioxidant enzymes (CAT, SOD, GPx, Reduced glutathione, and Glutathione S transferase) activity was reduced in HCD-fed group II compared to control I rats fed on the standard diet. Prabha *et al*., (2013) [32] also reported a reduced level of antioxidant enzymes like GST, GPx, GR, GSH, and CAT in the high cholesterol group compared to the control group in a study conducted in rats for 90 days. Beshbishy, (2005) [5] stated that the activities of some intracellular antioxidant enzymes decreased with the increase of lipid peroxidation levels and it might be the reason for the reduced activity of all the antioxidant enzymes, except TBARS of HCD-fed group II in the present study. The biological antioxidant defense system is an integrated array of enzymes and antioxidants. GSH, a substrate for GSH-peroxidase, CAT, GST, GPx, and GR constitutes the first line of cellular antioxidant defense enzymes. Catalase and GPx catalyze the conversion of hydrogen peroxide to water (Rocha *et al*., 2009) [46]. GST and GR offer protection against lipid peroxidation by promoting the conjugation of toxic electrophiles with GSH (Jakoby 1998). 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 into GSH (Prabha *et al*., 2013) [32] (Table II).

The addition of flaxseeds to the HCD in group V rats improved all the anti-oxidant enzymes and modestly reduced the levels of TBARS. It might be due to the antioxidant action of flaxseeds. Anand *et al., (*2013) [2] reported elevation of reduced glutathione (GSH) and Superoxide dismutase (SOD) in doxorubicin-induced cardiotoxic rats along with administration of flax lignin concentrate (FLC) @ 500mg/kg and Omega 3 fatty acid @ 1ml/kg. Prasad (2005) [49] reported a modestly reduced level of TBARS in the flaxseed-fed group in a study conducted on rats. Eicosanoids derived from omega-3 fatty acids of flaxseeds improve heart & liver functions by reducing oxygen free radicals (Jenkins *et al*., 1999, Lucas *et al*., 2008) [47, 31].

Green tea ameliorated group VI of the present study, showed a significant increase in all the antioxidant enzymes, and reduced the TBARS levels compared to group II rats fed on HCD. These results are consistent with Beshbishy (2005) [5], and Awoniyi *et al*., (2011) [3]. Green tea polyphenols have been demonstrated as powerful antioxidants (Guo *et al*., 1996 and Zhao *et al*., 1999) [20, 43] and it is a result of the binding and neutralization of free radicals by its structural hydroxyl groups and also by chelating metallic iron, as that can generate radical oxygen species (Tariq and Riyaz 2013) [40]. Green tea alone fed group also showed a significant increase in all the antioxidant enzymes, which indicates its powerful action against oxidative stress.

Gross changes in different organs are not conspicuous except in the liver. The liver was enlarged, soft and pale yellow in HCD-fed group II and the degree of changes was higher in 90th day slaughtered rats compared to 45th day slaughter rats of the same group. Similar changes were observed by Burt *et al*., (1998). The severity of enlargement and paleness were less in flaxseed and green tea treatment group V & VI and it might be due to the correction of altered lipid metabolism evidenced by biochemical parameters in the present study. No specific gross changes were observed in the standard diet, flaxseed, and green tea control group I, III & IV (Fig 3&4).

Microscopically, in the HCD group rats microvesicular hepatic steatosis was observed predominantly and macrovesicular fatty change in a few rats (Fig 5 & 6). These changes were very mild and less conspicuous in the ameliorated group of flaxseed group V and green tea group VI by the 45th day and the complete absence of steatosis by the 90th day of the study (Fig 7 & 8). Liver steatosis was absent in control, green tea and flaxseed alone fed group I, III, and IV. Lesions in group II are consistent with the reports of Curtis Green *et al*., (2011) [12] and Olubukola *et al*., (2012) [28]. Enlargement of the liver is primarily due to the accumulation of lipids in the cytoplasm of the hepatocytes and it is evidenced grossly by enlarged fatty liver and fatty change microscopically. These changes in group II might be due to the inclusion of 1% cholesterol and 15% saturated fat in the rat diet. Macrovesicular steatosis is generally reversible in most cases while microvesicular steatosis is generally a more severe disease than the macrovesicular and is observed in a variety of conditions in which there is either an inherited or an acquired defect in beta-oxidation of fatty acids (Burt *et al*., (1998). Complete absence of steatosis was observed in the green tea ameliorated group by the 90th day of study, contrary to the persistency of a few vacuoles in flaxseed-supplemented group V on the same day. Flaxseed supplementation did not completely reduce the hepatic vacuoles even by the end of the study period and it might be due to the partial ameliorating effect of flaxseeds on serum lipid parameters (Prasad *et al*., (1998) [48], Patade *et al*., (2008) [31] and Prasad *et al*., (2009) [34]. On the other hand, green tea completely ameliorated the fatty vacuoles in the hepatocytes by the end of the study period compared to the presence of few fatty vacuoles by the 45th day of the study, indicating long-term usage of green tea might be useful against the hepatic steatosis and it was evidenced biochemically by the reduced level of serum TC, LDL, TG, VLDL, and increased HDL levels and it is in line with the reports of Farjad and Hassan (2012) [17], Hussein Abdel Maksoud *et al*. (2012) [22] and Abbass *et al*., (2012) [1].

Table 1: 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		Group V		Group VI	
	45th day of study	90th day of study	45th day of study	90th day of study	45th day of study	90th day of study	45th day of study	90th day of study	45th day of study	90th day of study	45th day of study	90th day of study
Body weight (grams)	240±11.7	309.16±10.2	262.83±13.34	393.33±21.6^{a}	242.33±9.47	325±13.4^{b}	201.6±27.6^{abc}	302.5±23.8^{b}	245±17.8^{d}	383.3±43.7^{acd}	235.8±12.41^{d}	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	6.41±0.30	6.7±0.5	6.43±0.98	5.55±0.29	7±0.60	7.46±1.02	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	9.16±3.7	10.9±0.62	10.5±0.6	11.11±0.55	10.9±0.4	10.5±1.05	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	38.5±1.8	37.83±1.7	38.6±5.9	41.3±1.2	40.5±3.4	41.3±1.2	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	12.83±0.6	12.61±0.5	12.89±1.9	11.1±0.5	13.5±1.15	13.7±0.4	12.9±1.8	10.9±0.74
Total cholesterol (mg/dl)	46.03±7.8	50.67±7.04	113.58±11.75^{a}	155.3±10.58^{a}	48.75±2.75^{b}	46.06±4.27^{b}	51.43±4.80^{b}	40.30±3.3^{b}	99.0±6.9^{abcd}	83.8±4.1^{abcd}	97.67±4.80^{abcd}	86.22±6.17^{abcd}
Triglycerides (mg/dl)	68.70±50.0	67.17±11.08	150.58±33.27^{a}	178.2±39.84^{a}	57.43±9.14^{b}	67.83±34.93^{b}	55.67±5.57^{b}	63.82±15.4^{b}	122.1±17.6^{abcd}	115.8±21.0^{abcd}	127.62±23.36^{abcd}	125.92±67.69^{abcd}
Low density lipoprotein cholelsterol (mg/dl)	17.44±4.7	20.13±2.78	64.88±5.86^{a}	95.9±9.10^{a}	16.31±2.85^{b}	17.45±4.69^{b}	17.95±2.20^{b}	19.88±1.3^{b}	30.4±2.9^{abcd}	28.9±2.4^{abcd}	32.03±2.73^{abcd}	27.58±7.82^{abcd}
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}	11.49±1.83^{b}	11.65±5.32^{b}	9.13±1.11^{b}	10.76±3.1^{b}	17.4±3.5^{a}	14.2±4.2^{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}	29.95±2.93	28.96±3.02	30.35±2.95	29.65±2.7	22.2±2.4	21.7±2.6	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}	2.36±0.32	2.78±0.52^{b}	2.12±0.13	2.07±0.23^{ab}	3.2±0.16	2.63±0.264^{b}	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 high cholesterol diet or flaxseed control.

Table 2: Effect of flaxseed (*Linum usitatissimum)* and 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		Group V		Group VI	
	45th day of study	90th day of study	45th day of study	90th day of study	45th day of study	90th day of study	45th day of study	90th day of study	45th day of study	90th day of study	45th day of study	90th 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	1.34±0.19	1.03±0.24	1.28±0.25	1.32±0.17	1.48±0.32	1.52±0.26	1.56±0.17	1.62±0.16
Catalase activity (nM of H_2O_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	0.24±0.03	0.25±0.02	0.26±0.04	0.27±0.04	0.16±0.05	0.17±0.06	0.18±0.032	0.20±0.025
SOD activity (U/min/mg of protein) in the liver	18±1.1	16±1.8	14±1.02	12±2.2	17±0.9	18±1.8	18±1.3	19±2.1	15±0.9	14±2.3	16±0.8	17±1.7
GPx activity (U/min/mg of protein) in the liver	28±1.1	26±1.6	22±0.9	20±1.2	27±1.3	28±0.8	28±0.9	29±1.1	25±1.6	24±1.2	26±1.3	27±1.5
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	7.8±0.35^{b}	7.1±0.43^{b}	8.1±0.43^{ab}	7.9±0.53^{b}	5.4±0.13^{abcd}	4.5±0.33^{bcd}	5.9±0.29^{abcd}	4.8±0.38^{bcd}
Glutathione S transferase (mmol CDNB - GSH conjugate formed/min/mg protein)	24±0.42	23±0.45	16±0.53^{a}	12±0.33^{a}	25±0.49^{ab}	24±0.29^{ab}	26±0.63^{abc}	25±0.53^{abc}	19±0.62^{abcd}	19±0.35^{abcd}	18±0.54^{abcde}	20±0.48^{abcde}

Mean values with different superscripts differ significantly (p<0.05)

Fig 1: Group II (HCD group) rat showing obesity with poor hair coat compared control group I

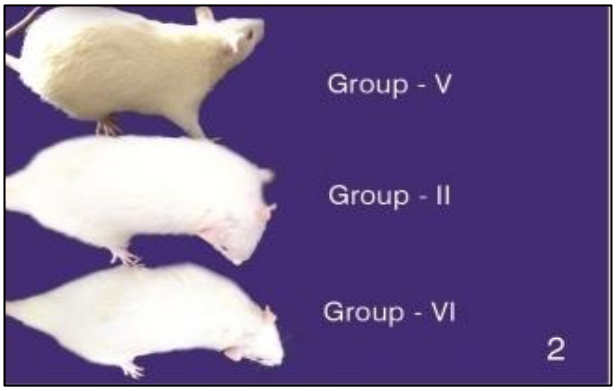

Fig 2: Note modest obesity in group V and normal healthy appearance in group VI (compared to group II rats.

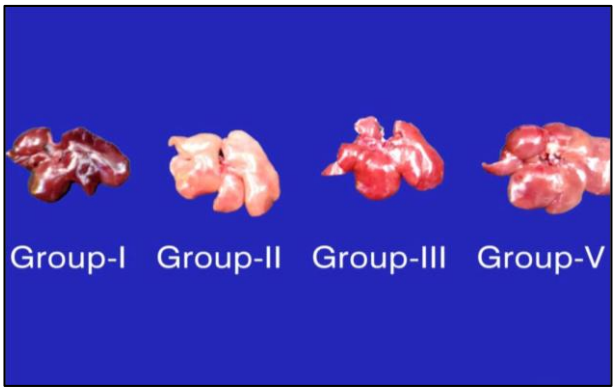

Fig 3: Liver: Group II: Note the degree of enlargement and paleness by 90th day of study compared to control and amelioration of flaxseed group V

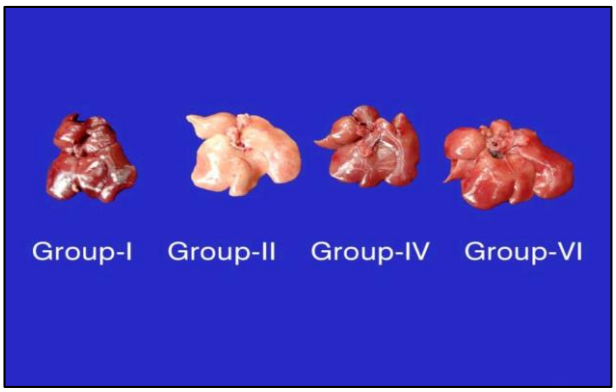

Fig 4: Liver: Note the degree of enlargement and paleness in group II compared to control and amelioration of green tea in group VI

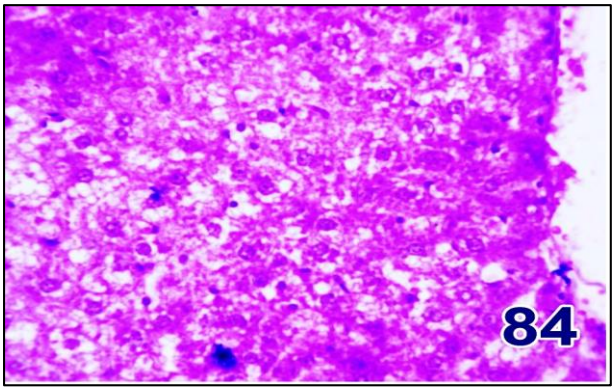

Fig 5: Liver: Group II: Section of liver showing mild to moderate micro and macro vascular fat vacuoles in the hepatocytes. H&E X400

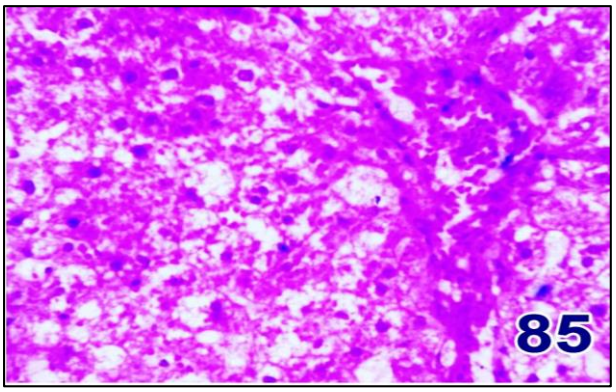

Fig 6: Liver: Group II: Note fatty vacuolation in the hepatocytes and venous congestion. H&E X400

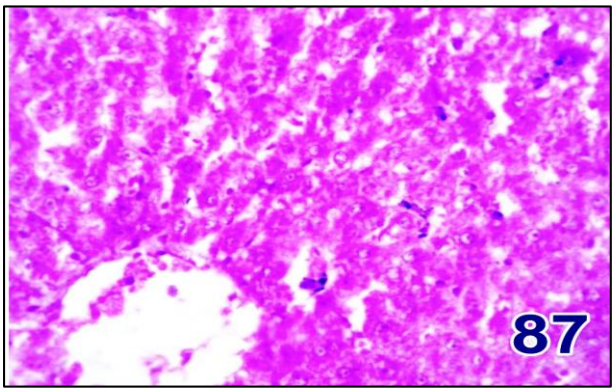

Fig 7: Liver: Group V: Note very mild micro vesicular fatty changes in the hepatocytes H&E X400

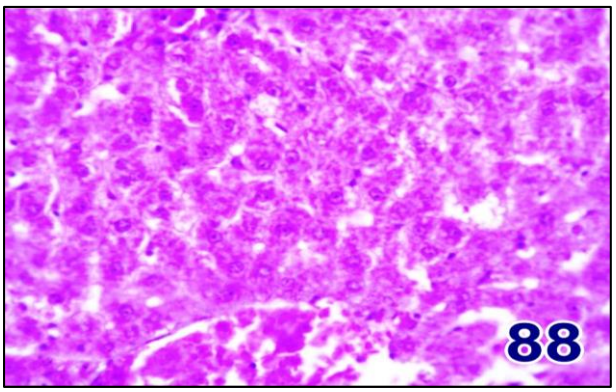

Fig 8: Liver: Group VI: Note complete absence of fat vacuoles in the hepatocytes. H&E X400

Conclusion

High cholesterol diet (HCD) of the present study was established the hyperlipidemia and hepatic steatosis evidenced by increased TC, TG, LDL-C, VLDL-C and low HDL-C and elevated the total leukocyte count and tissue TBARS levels along with decrease in other cellular anti-oxidant enzymes and fatty vacuoles in the hepatocytes. Flaxseed and Green tea supplementation effectively controlled the dyslipidemia and enhanced all the tissue anti-oxidant enzymes and completely ameliorated the fatty change of liver. Green tea has shown more significant in ameliorating the hyperlipidemia and hepatic fatty change than flaxseed. Present study results strongly indicate that, the green tea is having the anti-obesity, anti-hepatic steatosis, anti-inflammatory properties. Bothe flaxseed and green tea needs further molecular evaluation for their potential utilization as a food substance and beverage.

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