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Hypolipidemic Activity of *Lepidium Sativum* Linn. Seed in Rats

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Abstract: *The hypolipidemic activity of Lepidium sativum (family: Brassicaceae) seed sapogenin and flavonoid extract (LSTS and LSTF) was studied against Triton x-100 and high cholesterol diet (HCD) induced hyperlipidemia on rats. The parameters assessed were serum total protein, total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDLc), low density lipoprotein cholesterol (LDLc), very low density lipoprotein cholesterol (VLDLc), lipoprotein ratio (LDLc/HDLc), risk ratio (TC/HDL), Atherogenic Index (AI) and histopathology of liver. Oral administration of LSTS and LSTF (150, 300 mg/kg body wt./day) in Triton x-100 induced hyperlipidemia exerted significant lipid lowering (P<0.001) effect along with decrease in lipoprotein ration, risk ratio and AI (P<0.05-0.001). LSTF when administered along with HCD feeding simultaneously for 28 days caused significant (P<0.001) lowering of serum lipid parameters, lipoprotein ration, risk ratio and AI at high dose only. Effect of LSTS was non-significant on HCD induced hyperlipidemia. Histopathological findings of rat liver supported the protective role of LSTF in preventing Triton x-100 and HCD induced hepatic steatosis. Flavonoid and sapogenin extract in both the doses showed protection against all parameters of Triton x-100 induced hyperlipidemia where as only flavonoid extract showed good effect against HCD induced hyperlipidemia. The result strongly suggests hypolipidemic activity of L. sativum flavonoids.*

Keyword: *Flavonoid, High cholesterol diet, Hypolipidemic, Lepidium sativum Linn., Triton x-100, Sapogenin, Seeds*

I. Introduction

Cardiovascular disease is a major cause of morbidity and mortality all over the world [1]. Cardiovascular disease is also a leading cause of death in India as well as in western countries [2]. According to World Health Organization (WHO) estimates, in 2003, 16.7 million people around the globe die of cardiovascular disease (CVD) each year. This is over 29 percent of all deaths globally [3].

The underlying primary cause of cardiovascular disease is believed to be atherosclerosis, a progressive multifactorial disease of the arterial wall [4-5]. Central to the pathogenesis of atherosclerosis is deposition of cholesterol in the arterial wall [6]. Increased plasma lipid levels mainly total cholesterol (TC), triglycerides (TG) and low density lipoproteins (LDL) along with decrease in high density lipoproteins (HDL) are known to cause hyperlipidemia which is core in initiation and progression of arteriosclerosis impasse. Hypercholesterolemia refers to elevated serum LDL cholesterol (LDLc) or a combination of high levels of LDL cholesterol and triglycerides and decrease in HDL cholesterol (HDLc). Hypercholesterolemia, a significant cardiovascular risk factor, is one of the major oxidative stresses that generate excess of highly reactive free radicals. This exacerbates the development and progression of atherogenesis induced hypercholesterolemia with a risk of increased LDLc or more accurately LDLc/ HDLc ratio. The atherogenic index can be decreased by reduction in LDLc and increment in HDLc [7, 8]. Hyperlipidemia is characterized by elevated serum TC, LDL and very low density lipoproteins (VLDL), and decreased HDL levels. Hyperlipidemia associated lipid disorders are considered to cause atherosclerotic cardiovascular diseases [9].

Lepidium sativum Linn (Brassicaceae) commonly known as Asaliyo, is an erect, glabrous annual herb cultivated as a salad plant throughout India, Europe and United States. The seeds are used in chronic enlargement of liver and spleen, as carminative adjunct to purgatives, in skin diseases, dysentery, diarrhoea, asthma and in liver complaints. The leaves are mild stimulant and diuretic, serviceable in scorbutic diseases. The root is employed in skin diseases [10-12]. Literature search reveals that the plant has antihypertensive effect [13]. The hypoglycaemic effect of *L. sativum* seed aqueous extract was investigated by Eddouks et al. (2005) in normal and streptozotocin-induced diabetic rats [14]. The seeds are also used in the treatment of bronchial asthma [14]. Patel et al. (2009) reported the presence of flavonoids, coumarins, sulphur glycosides, triterpenes, sterols and various imidazole alkaloids in *L. sativum* seeds [16]. The present investigation was undertaken to screen the antihyperlipidemic effect of *L. sativum* total flavonoid and sapogenin fraction as the potential pharmacological effects of total flavonoid and sapogenin fraction of the plant has not been explored.

II. Materials And Methods

2.1 Plant material

The seeds of *Lipidium sativum* Linn. were purchased in the month of Nov-Dec 2010 from local market of Bhopal (M.P.). The seeds were authenticated by Dr. Zia-ul Hasan, Head, Herbarium and Angiosperm Division (Safia College of Science, Bhopal, M.P.) and a specimen voucher no. 273/bot./safia/2011 was given which was preserved for future reference.

2.2 Preparation of methanolic extract of seed

The trampled *L. sativum* seeds were extracted with soxhlet apparatus using methanol as a solvent. The extract was dried in rotatory evaporator under reduced pressure and stored at -20°C. This extract was used for phytochemical screening.

2.3 Phytochemical screening

Different phytoconstituents like saponins, alkaloids, tannins, anthraquinones, cardiac glycosides, cyanogenetic glycosides, amino acid, protein and flavonoids were tested as per the method described by Kokate et al. (2007) [17].

2.4 Extraction of sapogenins and flavonoids

2.4.1 Extraction of total sapogenins from *L. sativum* (LSTS)

Dried and powdered seed, 20 gm was defatted with 60 ml of petroleum ether (40-60°C). The defatted seed powder was extracted with ethanol. The ethanolic extract was dried in rotatory evaporator under reduced pressure and suspended in 100 ml of water. An equal volume of $CHCl_3$: HCl (50 ml: 50 ml) was added to form a suspension**,** refluxed for 30 min and cooled. The chloroform phase was separated and concentrated under reduced pressure and extracted three times with 75 ml of water saturated n-butanol. The n-butanol phase was separated, dried and stored [18].

2.4.2 Extraction of total flavonoids from *L. sativum* (LSTF)

Dried and powdered seed, 100 gm was defatted with 400 ml petroleum ether (40-60°C). The defatted seed was extracted with 400 ml of methanol then volume reduced and concentrated**.** Alkaline hydrolysis of extract was done with 2M NaOH and boiled for 30 min. Hydrolyzed extract was neutralized with 2M HCl drop wise. Aqueous phase was extracted with 50 ml of n-butanol, dried and stored [19].

2.5 Thin layer chromatography (TLC)

Freshly coated plates with silica gel G_{254} was allowed to air dry in room temperature and transferred to oven for activation maintained at 110ºC for 30 min. The flavonoid extracts of *L sativum* seed was dissolved in methanol and filter through Whatman filter paper. Different solvent system were tried to detect maximum possible flavonoid present in methanol soluble fraction. The solvent systems finally optimized as mobile phase is n-butanol: ethanol: water in 4:1:2.2 and 4:1:5 ratio. About 30 µl of sample was applied using thin capillary. The plates were placed into the developing chamber and allowed to run until it reaches a height of about 10 cm from the point of spotting. Spraying agent used for detection of spots was hydrochloric acid. After development the plates were kept in oven maintained at 110ºC to optimal color development. Spots were observed under UV-light. Standard samples of flavonoids i.e, quercetin (JPN Pharma, Mumbai) and ferulic acid (Himedia, Mumbai) was purchased for co-TLC to compare the Rf values.

2.6 Pharmacological screening

2.6.1 Preparation of dose

Extracts of LSTS and LSTF was individually suspended in 1% tween 80 aqueous solutions, a dose of 150, 300 mg/kg was given to the rats once in a day. Suspensions were stored in airtight bottles in a cool place. Standard atorvastatin dose of 10 mg/kg, was prepared by suspending atorvastatin in aqueous 0.3 % methyl cellulose. Standard fenofibrate dose of 65 mg/kg, was prepared in 1% tween 80 aqueous solution.

2.6.2 Animals

Wistar albino rats of either sex weight (120-180 g) were selected for the present study. The animals were acclimatized to the standard laboratory conditions in air conditioned animal house (22±2°C; 55±5% RH) with 12 hour light and 12 hour dark cycles. The animals were fed with standard diet (Golden Feed, India) and water *ad libitum*. Animal study was performed in Radharaman College of Pharmacy, Bhopal with due permission from institutional animal ethical committee (Approval no. IAEC/RCP/Oct.2010/03).

2.6.3 Acute toxicity studies (LD_{50})

LD_{50} was determined according to the guidelines of Organization for Economic Cooperation and Development (OECD) following the fixed dose method (OECD guideline no. 420). Based on the guideline a limit test was performed to categorize the toxicity class (LD_{50}) of the compound. The limit test was performed at 2000 mg/kg (p.o) for LSTS and LSTF. There were no toxic effects or mortality observed up to 14th day. A dose range of 150 mg/kg, 300 mg/kg, oral was selected for screening the pharmacological activity.

2.6.4 High cholesterol diet induced hyperlipidemia

Experimental hyperlipidemic high cholesterol diet (HCD) consists of well pulverized mixture of cholesterol (2%), sodium cholate (1%), coconut oil (25%) and normal laboratory diet (72%). The experimental animals were divided into seven groups, six rats in each group. Group I as normal diet control (0.5 ml saline, p.o), group II as negative control with HCD only, group III as standard drug control along with HCD, Group IV to VII as treated group. In high cholesterol diet model atorvastatin (10 mg/kg, p.o.) was used as standard drug. Group IV and V received HCD and LSTS 150, 300 mg/kg respectively. Group VI and VII received HCD and LSTF at 150, 300 mg/kg respectively. The study period was of 28 days. On 29th day 4 hour after dosing all the animals were sacrificed.

2.6.5 Triton x-100 induced hyperlipidemia

Triton x-100 (100 mg/kg) freshly prepared in physiological saline solution was given by single intraperitoneal injection to overnight fasted experimental rats. In Triton x-100 induced model fenofibrate (65 mg/kg, p.o.) was used as standard drug. Group I as normal control (0.5 ml saline, p.o), group II as negative control received Triton x-100 only, group III as standard drug control along with Triton x-100, Group IV and V received Triton x-100 and LSTS at 150, 300 mg/kg respectively. Group VI and VII received Triton x-100 and LSTF at 150, 300 mg/kg respectively.The study period was of 7 days. On 8th day 4 hour after dosing the animals were sacrificed.

2.6.6 Collection of blood

Blood was collected by cardiac puncture, kept for 30 min in room temperature then centrifuged at 10,000 rpm for 10 min. Serum was separated and stored in refrigerator until biochemical estimations were carried out. Liver were removed, cleaned and weighed. Liver tissue was fixed in 10% formaldehyde and used for histopathological observations.

2.6.7 Biochemical analysis

The serum samples were analyzed in Autoanalyser (Semi auto-analyzer star 21 plus) for estimation of serum total protein, TC, TG, HDLc and LDLc using diagnostic kits procured from Auto Span Diagnostics Pvt. Ltd., Surat, India. VLDLc, lipoprotein ratio (LDLc/HDLc), risk ratio (TC/HDL) and AI were calculated by using formula.

2.7 Statistical Analysis

All the experiments were conducted using six animals in each group and the results are reported as Mean ± SEM. The one way ANOVA (non parametric) was performed to assess the statistical significance, followed by Dunnett t-test. P value less than 0.05 was considered significant.

III. Results

The Phytochemical tests of methanolic extract (8.59%) of *L. sativum* seed indicated the presence of alkaloid, carbohydrate, flavonoids, saponins, glycosides, tannins and phenolic compounds. Total sapogenin and total flavonoids extract of *L. sativum* showed 2.l6 and 2.72% yield respectively. Shinoda test, alkaline reagent test, zinc hydrochloride test and lead acetate test was positive in flavonoid extracted of *L. sativum*. Froth formation test was negative and haemolysis test was positive for extracted sapogenin, where as it showed negative results for alkaloid, carbohydrate, phenolic compounds tests.

TLC of extracted flavonoid

Flavonoid extract, LSTF was obtained as a yellow powder (m.p. 329°C) and its UV spectrum was consistent with that of flavonoid with maxima at 252, 265 and 342 nm. The LSTF and standard samples of flavonoids were subjected to TLC analysis on silica gel 60 F_{254} plates using n-butanol: ethanol: water (4:1:2.2) and n-butanol: acetic acid: water (4:1:5). Flavonoid extract showed the two spots having R_f values 0.64 and 0.88 in n-butanol: ethanol: water (4:1:5) and 0.60, 0.64 and 0.89 in n-butanol: acetic acid: water (4:1:2.2). The R_f of standard quercetin and ferulic acid was found to be identical with Rf value respectively 0.64-0.65 and 0.88-0.89

the LSTF (Table 1, Fig. 1 and Fig. 2). LSTF showed presence of two components quercetin and ferulic acid with matching Rf.

Acute toxicity study

LSTS and LSTF extract did not show any sign and symptom of toxicity and mortality up to 2000 mg/kg, p.o. when tested as per OECD guideline 425.

Effect on body weight and liver weight of high cholesterol diet fed rat

Vehicle control group showed 12.74% increase in body weight after 28th day, where as HCD treated group showed a drastic increment of 17.20% in body weight followed by increased food consumption. Standard drug atrovastatin (13.17%), LSTS (12.89%) and LSTF (12.96%) showed dose dependent decrement in body weight in comparison to the HCD treated animals. Administration of HCD extreme significantly ($P<0.001$) increased the relative liver weight compared to vehicle control group where as LSTS and LSTF along with HCD for 28 days resulted in non-significant change in relative liver weight of animal compared to HCD group (Table 2).

Effect on serum biochemical parameters of high cholesterol diet fed rat

Results of the biochemical parameters revealed that the levels of TC, TG, LDLc, VLDLc and total protein in serum was severely increased whereas a mark decrease in level of HDLc was observed in comparison to vehicle control group. LSTS treatment also has non-significant effect towards normalization of TG, HDLc and LDLc whereas, the levels of VLDLc has decreased extreme significantly ($P<0.001$). LSTS treatment also has non-significant effect on elevated LDLc/HDLc ratio, risk ratio (TC/HDL) and AI of rat. Treatment of LSTF (150 and 300 mg/kg) extreme significantly ($P<0.01$-0.001) reduced the level of TC, TG and VLDLc whereas, LDL and HDLc was normalized at LSTF 300 mg/kg only compared to HCD treated group. Results revealed that LSTF at 300 mg/kg has significantly decreased the LDLc/HDLc and risk ratio as well as AI in HCD treated animals. Atorvastatin treatment decreased serum TC, TG, LDLc and VLDLc and LDLc/HDLc and TC/HDL ratio and AI extreme significantly ($P<0.001$) as presented in Table 3.

Effect on body weight and liver weight of Triton x-100 induced hyperlipidemic rat

Administration of Triton x-100 has resulted in increase of body weight gain (11.11%) compared to vehicle control group (6.66%). LSTS and LSTF at 150 and 300 mg/kg dose showed dose dependent decease in body weight by 5.81, 4.41, and 4.92 and 3.56% respectively (Table 4). Administration of Triton x-100 showed significant ($P<0.01$) increase in relative liver weight compared with the normal control group. Treatment with fenofibrate, LSTS and LSTF (300 mg/kg) resulted in significant ($P<0.05$) decrease in liver weight compared to Triton x-100 group.

Effect of on serum biochemical parameters of Triton x-100 induced hyperlipidemic rat

The levels of TC, TG, LDLc and VLDLc in serum was increased significantly ($P<0.001$) in Triton x-100 intoxicated animals. LSTS and LSTF (150 and 300 mg/kg) groups showed extreme significant decrease ($P<0.001$) in serum TC, TG, LDLc and VLDLc level in comparison to Triton x-100 treatment. HDLc level was increased significantly ($P<0.001$) by both LSTS and LSTF treatment. LSTS and LSTF in both the tested doses resulted in significant ($P<0.001$) decrease in LDLc/HDLc, risk ratio and AI ($P<0.05$-0.001) compared to Triton x-100 treated animals (Table 5).

Histopathology of liver in high cholesterol diet treated rat

Liver section of normal rat liver showed absence of cellular degeneration or sign of necrosis (Fig. 3A). Liver section of HCD treated rats showed marked vascular congestion, fatty deposition, foamy degeneration of hepatocytes and steatosis (Fig. 3B). Atorvastatin treated rat liver showed mild fatty degeneration and inflammatory changes in the hepatocytes with some degree of swelling (Fig. 3C). Liver section of LSTS (150 mg/kg) treated rats showed cellular degeneration, foamy degeneration, steatosis and necrosis (Fig. 3D). Liver section of LSTS (300 mg/kg) treated rats showed mild fatty deposition, inflammatory changes and focal nacrosis with few recovered normal hepatocytes (Fig. 3E). Liver section of LSTF (150 mg/kg) treated rats showed marked microvesicular fatty changes, hepatocyte ballooning and degenerative changes (Fig. 3F). Liver section of LSTF (300mg/kg) treated rats showed very less fatty deposition and foamy degeneration of hepatocytes with recovered normal hepatocytes **(**Fig. 3G).

Histopathology of liver in Triton x-100 treated rat

Liver section of vehicle control rats showed normal hepatic architecture (Fig. 4A) where as Triton x-100 treatment induced severe necrosis, cellular degeneration and sinusoidal dilation (Fig. 4B). Fenofibrate

treated rat showed moderate hydropic cell degeneration, necrosis and inflammation (Fig. 3C). LSTS (150 mg/kg) treated rat liver sections showed marked hydrophobic and fatty changes with diffused necrosis (Fig. 4D). LSTS (300 mg/kg) treatment showed cellular degeneration, diffused necrosis with lymphocyte infiltration (Fig. 4E). LSTF at 150 mg/kg showed hydrophobic cellular degeneration and necrosis but at 300 mg/kg showed only moderate cellular degeneration and necrosis with inflammatory changes (Fig. 4G).

IV. Discussion

L. sativum has wide range of traditional uses in treating different ailments from ancient time. Very little pharmacological work has been done on medicinal application of the isolated and extracted compounds of *L. sativum* making it imperative that more pharmacological and clinical studies should be conducted to investigate unexploited potential of this plant. Very less information is available regarding the chemical constituents like flavonoid and sapogenin of this plant due to lack of phyto-pharmacological studies. The flavonoids and sapogenins are the class of phyto-compounds which are attributed to have many pharmacologic activities.

The hypoglycaemic effect of *L. sativum* seed aqueous extract was reported in normal and streptozotocin-induced diabetic rats [14]. Maghrani et al. (2005) studied antihypertensive and diuretic effects of the aqueous extract in normotensive and spontaneously hypertensive rats. Daily oral administration of the aqueous *L. sativum* extract (20 mg/kg for 3 weeks) exhibited a significant decrease in blood pressure in spontaneously hypertensive rats [13]. Radwan et al. (2007) reported hepatoprotective activity of petroleum ether and 80% alcoholic extract of *L. sativum* using hepatocyte monolayer culture from rat. Both the petroleum ether and 80% ethanolic extracts showed hepatoprotective effect on the hepatocyte against CCl_4 cytotoxicity at the concentration of 50 µg/ ml where, the LC_{50} were 150 µg/ ml and 200 µg/ ml respectively [20]. Afaf et al. (2008) investigated hepatoprotective effect of *L. sativum* seed against CCl_4 induced hepatic damage in rats. The methanolic seed extract at 200 and 400 mg/kg dose reduced serum glutamic oxaloacetic transaminase, glutamic pyruvate transaminase, alkaline phosphatase and bilirubin level [21]. Shinde et al. (2010) reported the protective effect of *L. sativum* aqueous extract against doxorubicin-induced nephrotoxicity in rats. The level of superoxide dismutase, catalase, reduced glutathione were elevated and tissue malondialdehyde were declined significantly in the *L. sativum* (200 and 400 mg/kg) treated groups. Histopathological examination showed that *L. sativum* markedly ameliorated doxorubicin-induced renal tubular necrosis [22].

Increased serum lipid levels mainly TC, TG and LDLc along with decrease in HDLc are known to cause hyperlipidemia with progression to arteriosclerosis. Therefore, prime consideration in therapy for hyperlipidemia and arteriosclerosis is to enervate the elevated serum levels of TC, TG and LDL along with increase in HDL lipids. All these previous studies indicate that *L. sativum* may have effect on disorders related to liver and metabolic abnormalities with possible presence of antioxidant activity. In the present work our aim was to extract flavonoid and sapogenin from the *L. sativum* seed and screen its anti-hyperlipidemic property against HCD and Triton x-100. The extraction and identification was carried out using standard method. Flavonoid fraction of *L. sativum* seed showed presence of quercetin and ferulic acid.

HCD fed group showed maximum percentage increased in body weight on 28^{th} day. The observation was in accordance with Kumar et al., (2010) stating that HCD had a significant increase in body weight. The body weights of Triton x-100 induced hyperlipidemic group were also higher from normal animals [23]. Sodipo et al. (2011) reported increase body weight gain by Triton x-100 treatment. HCD as well as Triton x-100 treatment induced hyperlipidemia evident by increase in the mean relative weights of liver might be due to excessive accumulation of lipids [24, 1]. Flavonoid content of *L. sativum* had significant effect in lowering relative liver weight against Triton induced fatty liver.

A number of clinical studies suggested that the increased risk of coronary heart disease is associated with a high serum concentration of TC, LDLc and triglyceride. On the other hand, low serum concentration of HDLc is also responsible for coronary heart disease. Preclinical observations demonstrate that hyperlipidemia promotes accumulation of oxidatively modified LDL in the arterial wall, promoting endothelial dysfunction and development of atherosclerosis and congestive heart diseases [25]. HCD feeding has significantly increased serum levels of TC, TG and LDLc along with decrease in HDLc. Dietary cholesterol administration significantly increases the concentrations of the hepatic TC and TG in rats indicating disturbed hepatic lipid metabolism. Hepatic TG level is controlled mainly by TG synthesis, beta-oxidation and secretion in the form of lipoprotein [26]. Hepatic TG accumulation by high dietary cholesterol is involved in the stimulation of fatty acid and TG synthesis in rats. The hepatic TG accumulation caused by dietary cholesterol is attributed to the reduction of fatty acid beta-oxidation, afflux and secretion of LDL [27].

Treatment with flavonoid and sapogenin extract, at two different doses (150, 300 mg/kg) significantly decreased the levels of increased serum lipid parameters in comparison to the control group. The sapogenin and flavonoid extract showed protective action at 300 mg/kg dose and demonstrated a significant decrease in the raised Triton x-100 induced levels of serum TC, LDLc and triglycerides. Flavonoid extract showed potential reduction of

serum lipid against HCD induced hyperlipidemia. It is widely accepted that reduction in serum HDL is a risk factor for developing atherosclerosis. HDL facilitates the translocation of cholesterol from the peripheral tissue, such as arterial walls to liver for catabolism. The increase in HDL may slow down the atherosclerotic process. Increased levels of HDL (cardio protective lipid) after administration of flavonoid extracts of *L. sativum* concluded that the extract is a potent cardio protective agent and this effect may be due to the increase in the activity of lecithin: cholesterol acyl transferase (LCAT), which play a key role in incorporating the free cholesterol into HDL which is taken back by the liver cells. Several studies showed that an increase in HDLc is associated with a decrease in coronary risk high levels of TC and LDLc are major coronary risk factors. Treatment of flavonoid and sapogenin extract of *L. sativum* lowered both TC and LDLc in Triton induced hyperlipidemic rats. Flavonoid showed better efficiency towards increasing the level of HDLc in Triton treated groups.

The higher the AI, the higher is the risk of the cardiovascular disease [28]. AI indicates the deposition of foam cells or plaque or fatty infiltration of lipids in heart, coronaries, aorta, liver and kidneys. The rise in AI in hyperlipidemic rats enhances the probability of cardiovascular pathogenesis and endothelial dysfunction. AI in HCD treated animals were increased moderately where as Triton induced very high rise in AI value. Significant decrease in AI value was observed in *L. sativum* flavonoid supplemented animals, suggesting the atheroprotective/cardio protective potential of flavonoid content of *L. sativum* [29].

These results indicate the cholesterol lowering activity of the *L. sativum* flavonoid against both HCD and Triton which can be a result of rapid catabolism of LDLc through its hepatic receptors for final elimination in the form of bile acids as demonstrated [30]. Flavonoids and anthocyanins, a heterogenous group of ubiquitous plant polyphenols, have exhibited a variety of pharmacological activities, including the anti-atherogenesis and antioxidant effect [31]. Report of Hicham et al. (2008) strongly suggests that the hypolipidemic activity of *Ocimum basilicum* is attributable to the presence of valuable polyphenolic compounds [32].

Triton has been widely used to block clearance of triglyceride-rich lipoproteins to induce acute hyperlipidemia in several animals [33]. The severe increase in plasma cholesterol and triglycerides due to Triton injection results mostly from an increase of VLDL secretion by the liver accompanied by a strong reduction of VLDL and LDL catabolism [34]. The reduction of TC by the *L. sativum* sapogenin and flavonoid both may be associated with a decrease of its LDL fraction, which is the target of several hypolipidemic drugs. The increased level of HDLc and decreased TC and LDL which is evident from the results could be due to an increased cholesterol excretion and decreased cholesterol absorption through gastro intestinal tract. It is found that saponins increase the permeability of intestinal mucosal cells in vitro, inhibit active mucosal transport and facilitate uptake of substances that are normally not absorbed [35]. Saponins also lower transmural potential difference across the small intestine of the rat [36]. Saponins have long been known to have a lytic action on erythrocyte membranes and this property has been used for their detection. The haemolytic action of saponins is believed to be the result of the affinity of the aglycone moiety for membrane sterols, particularly cholesterol, with which they form insoluble complexes [37]. Reshma et al. (2002) have reported hypolipidemic activity of the saponins from *Acorus calamus* [38]. The high amount of saponins and phytosterol present in *Eclipta prostrate* is responsible for the hypolipidemic effect as reported by Dhandapani (2007) [39]. Flavonoids of the citrus peel extract are a group of polyphenolic antioxidants that exhibit a wide range of biological effects including hypocholesterolemic action. Kurowska et al. (2000) reported reduced serum LDLc in rabbits given orange juice containing naringin and hesperitin as citrus flavonoids [40]. A high cholesterol diet supplemented with a citrus flavonoid lowered plasma and hepatic cholesterol level in rats [41].

Flavonoid and sapogenin extracts of *L. sativum* has reveled hepatoprotective activity against the hyperlipidemic effect of HCD and Triton x-100, which was also supported by histological observations. *L. sativum* significantly reverse HCD and Triton x-100 induced steatosis indicating protective and curative effect against fatty liver. Histopathological observations confirm the curative efficacy of *L. sativum* against HCD and Triton x-100 induced liver damage as shown by decrease in severity of hyperlipidemic effects in 300 mg/kg treated dose comparable to standard drug. Microvesicular fatty changes, hepatocyte ballooning and steatosis was markedly corrected by flavonoid extract. However, the degree of hepatic fat accumulation was substantially alleviated by saponin treatment and the number of infiltrating macrophages was also reduced. In the present study *L. sativum* flavonoid was found to contain quercetin and ferulic acid, which reduced the serum TG and TC level very effectively in HCD feed animals. The result strongly suggests that the hypolipidemic activity of *L. sativum* can be attributed to the presence of the saponins and flavonoids.

V. Conclusion

In conclusion, the flavonoid and sapogenin extracts of *L. sativum* Linn. seed significantly reduced the Triton x-100 induced hyperlipidemia in rats and flavonoid extract showed protection against all parameters (TC, TG, LDLc and VLDLc) of HCD diet induced hyperlipidemia indicating promising anti-hyperlipidemic effect.

This effect needs further investigation on various models of hyperlipidemia to elucidate mechanism of action of *L. sativum* as hypolipidemic. The flavonoid and sapogenin extracts of *L. sativum* should be further subjected to isolation and structural elucidation of the compounds.

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Table 1: TLC of flavonoid extract of *L. sativum* seed

Solvent system	Ratio	No. of spots	Rf value (sample)	Rf value (standard)	
				Quercetin	Ferulic acid
N-butanol: ethanol: water	4:1:5	2	0.64, 0.88	0.64	0.88
N-butanol:acetic acid: water	4:1:2.2	3	0.60, 0.64, 0.89	0.65	0.89

Table 2: Effect of sapogenin and flavonoid extract of *L. sativum* seed on body weight and liver weight of high cholesterol diet fed rat

Treatment (mg/kg, p.o)	% Increase in Body Weight				Liver wt./100 gm body wt. (M ± SEM)
	7th Day	14th Day	21st Day	28th Day	
Normal diet control	3.11	5.29	8.49	12.74	3.76 ± 0.23
Negative control (HCD)	7.20	9.59	13.26	17.20	5.52 ± 0.31^{c}
Atorvastatin (10)	4.30	7.35	9.05	13.17	3.96 ± 0.12^{ns}
LSTS (150)	6.06	8.03	12.12	16.26	4.70 ± 0.80^{ns}
LSTS (300)	5.32	6.13	10.19	12.89	4.58 ± 0.27^{ns}
LSTF (150)	4.90	6.90	10.50	13.06	4.61 ± 0.67^{ns}
LSTF (300)	4.17	6.50	9.11	12.96	4.44 ± 0.98^{ns}

Data expressed as M ± SEM and n = 6 in each group. $^{c}P<0.001$ compared to vehicle control. All the values are non-significant (ns) when compared to high cholesterol diet (HCD) group.

Table 3: Effect of sapogenin and flavonoid extract of *L. sativum* seed on serum biochemical parameters of high cholesterol diet fed rats

Treatment (mg/kg, p.o)	Serum biochemical parameters								
	TC (mg/dl)	TG (mg/dl)	HDLc (mg/dl)	LDLc (mg/dl)	VLDLc (mg/dl)	Total Protein (gm/dl)	LDLc /HDLc Ratio	Risk ratio TC/HDL	Athero-genic Index (AI)
Normal diet control	130.82 ± 8.40	78.45 ± 4.28	47.05 ± 2.31	107.15 ± 7.09	20.69 ± 1.30	6.25 ± 1.11	2.27 ± 0.08	2.78 ± 1.04	2.71 ± 0.82
Negative control (HCD)	249.53 ± 12.43^{c}	167.75 ± 11.92^{c}	35.25 ± 1.09^{c}	170.73 ± 10.09^{c}	83.55 ± 3.67^{c}	6.32 ± 0.97^{ns}	4.84 ± 0.21^{c}	7.07 ± 0.30^{c}	7.21 ± 0.75^{c}
Atorvastatin (10)	$144.73 \pm 9.03^{***}$	$60.14 \pm 4.08^{***}$	$52.36 \pm 3.51^{***}$	$82.41 \pm 4.98^{***}$	$22.02 \pm 1.15^{***}$	6.87 ± 0.78^{ns}	$1.57 \pm 0.68^{***}$	$2.76 \pm 0.04^{***}$	$1.99 \pm 0.07^{***}$
LSTS (150)	236.86 ± 12.52^{ns}	$101.45 \pm 6.09^{***}$	34.48 ± 1.56^{ns}	168.17 ± 6.67^{ns}	$44.29 \pm 2.11^{***}$	6.62 ± 0.76^{ns}	4.87 ± 0.26^{ns}	6.86 ± 0.06^{ns}	6.16 ± 0.71^{ns}
LSTS (300)	228.34 ± 14.96^{ns}	$89.66 \pm 5.98^{***}$	38.42 ± 1.77^{ns}	141.97 ± 6.98^{ns}	$40.93 \pm 2.98^{***}$	5.86 ± 0.45^{ns}	3.69 ± 0.05^{ns}	5.94 ± 0.89^{ns}	4.76 ± 0.93^{ns}
LSTF (150)	$180.35 \pm 11.35^{**}$	$66.33 \pm 4.12^{***}$	37.66 ± 1.85^{ns}	$135.05 \pm 7.48^{*}$	$33.26 \pm 1.01^{***}$	6.36 ± 0.58^{ns}	3.58 ± 0.34^{ns}	4.78 ± 0.03^{ns}	$4.46 \pm 0.26^{*}$
LSTF (300)	$167.03 \pm 10.03^{***}$	$57.70 \pm 3.48^{***}$	$46.93 \pm 2.16^{*}$	$132.66 \pm 8.01^{*}$	$31.54 \pm 1.96^{***}$	6.13 ± 0.92^{ns}	$2.82 \pm 0.18^{**}$	$3.55 \pm 0.16^{***}$	$3.49 \pm 0.61^{**}$

Data expressed as M ± SEM and n = 6 in each group. $^{c}P<0.001$ compared to vehicle control, *P<0.05, **P<0.01, ***P< 0.001 and ns= non significant when compared with high cholesterol diet group.

Table 4: Effect of sapogenin and flavonoid extract of *L. sativum* seed on body weight and liver weight of Triton x-100 induced hyperlipidemic rat

Treatment (mg/kg, p.o)	% Increase in body weight on 7th day	Liver wt./100gm body wt
Vehicle control	6.66	3.03 ± 0.22
Triton x-100 (100)	11.11	4.82 ± 0.16^{b}
Fenofibrate (65)	5.03	$3.06 \pm 0.21^{**}$
LSTS (150)	5.81	3.83 ± 0.32^{ns}
LSTS (300)	4.41	$3.46 \pm 0.29^{*}$
LSTF (150)	4.92	3.68 ± 0.28^{ns}
LSTF (300)	3.56	$3.53 \pm 0.39^{*}$

Data expressed as M ± SEM, n = 6 in each group. $^{b}P<0.01$ compared to vehicle control, $^{*}P<0.05$, **P<0.01 and ns= non significant when compared to triton treated group.

Table 5: Effect of sapogenin and flavonoid extract of *L. sativum* (Linn.) seed in serum biochemical parameters on Triton x-100 induced hyperlipidemia using rats

Treatment (mg/kg, p.o)	Serum biochemical parameters								
	TC (mg/dl)	TG (mg/dl)	HDLc (mg/dl)	LDLc (mg/dl)	VLDLc (mg/dl)	Total Protein (gm/dl)	LDLc /HDLc Ratio	Risk ratio TC/HDL	Athero-genic Index (AI)
Vehicle control	134.64 ± 10.56	103.67 ± 8.23	43.24 ± 2.87	112.67 ± 10.98	20.73 ± 1.24	7.48 ± 0.92	2.60 ± 0.02	3.11 ± 0.98	3.08 ± 0.23
Triton x-100 (100)	263.10 ± 12.34^{c}	300 ± 11.26^{c}	20.72 ± 2.80^{c}	162.38 ± 11.89^{c}	90.0 ± 4.35^{c}	6.60 ± 1.02^{ns}	7.83 ± 1.04^{c}	12.69 ± 2.67^{c}	12.18 ± 2.87^{c}
Fenofi-brate (65)	71.20 ± 4.17***	120.50 ± 9.12***	48.17 ± 3.02***	92.93 ± 4.23***	40.10 ± 2.88***	9.30 ± 1.08^{ns}	1.92 ± 0.07***	1.47 ± 0.63***	2.76 ± 0.43***
LSTS (150)	162.3 ± 14.19***	167.76 ± 11.23***	37.04 ± 2.23***	124.51 ± 10.02***	52.75 ± 3.07***	8.67 ± 1.05^{ns}	3.36 ± 0.01***	4.38 ± 0.89**	4.78 ± 0.42**
LSTS (300)	157.61 ± 11.56***	157.34 ± 11.70***	42.10 ± 2.98***	112.05 ± 10.09***	41.46 ± 2.01***	8.41 ± 1.13^{ns}	2.66 ± 0.01***	3.74 ± 0.72***	3.64 ± 0.47**
LSTF (150)	182.44 ± 13.92***	217.00 ± 11.13***	34.51 ± 2.08***	116.53 ± 9.12***	63.40 ± 3.28***	8.26 ± 1.02^{ns}	3.37 ± 0.73***	5.28 ± 1.21**	5.21 ± 1.78*
LSTF (300)	163.56 ± 13.86***	188.99 ± 14.02***	47.80 ± 3.81***	102.97 ± 7.12***	47.79 ± 2.67***	8.50 ± 1.70^{ns}	2.15 ± 0.56***	3.42 ± 0.56***	3.15 ± 0.88***

Data expressed as Mean ± SEM and n = 6 in each group. ^{c}P<0.001 compared to vehicle control, *P<0.05, **P<0.01, ***P<0.001 and ns= non significant, compared with triton x-100 treated group.

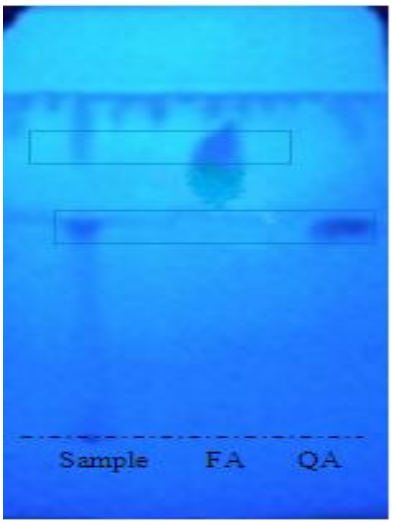

Figure 1: TLC of flavonoid extract of *L. sativum* seed sample in n-butanol: acetic acid: water (4:1:5).
Standard Quercetin showed Rf 0.64 and Ferulic acid Rf 0.88. Sample showed presence of two components with matching Rf 0.64 (Quercetin) and 0.88 (Ferulic acid).
Sample: flavonoid extract of *L. sativum* seed; QA: Quercetin and FA: Ferulic acid.

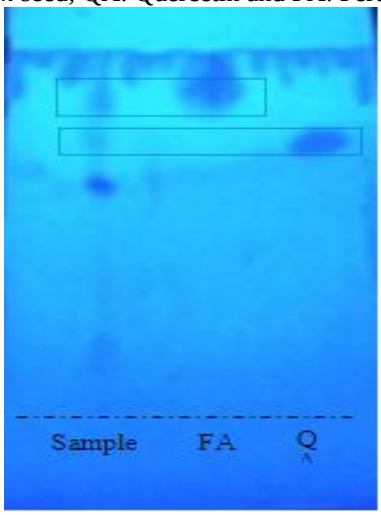

Figure 2: TLC of flavonoid extract of *L. sativum* seed sample in n-butanol: ethanol: water (4:1:2.2).

Standard Quercetin showed Rf 0.65 and Ferulic acid Rf 0.89. Sample showed presence of three components as Rf 0.60 (unknown) and with matching Rf of 0.64 (Quercetin) and 0.89 (Ferulic acid).
Sample: flavonoid extract of *L. sativum* seed; QA: Quercetin and FA: Ferulic acid.

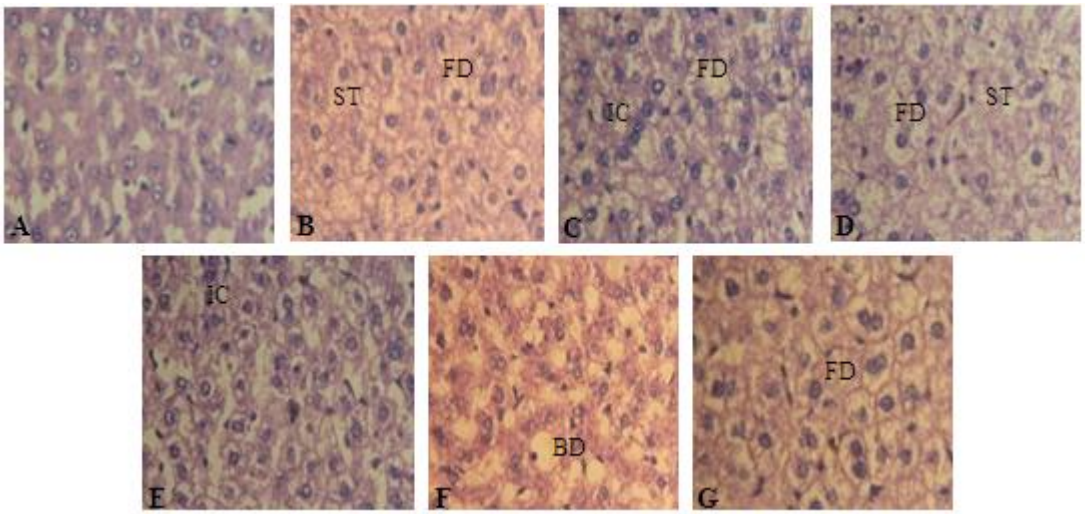

Figure 3: Photomicrograph of rat liver sections of high cholesterol diet induced hyperlipidemic rat treated with sapogenin and flavonoid extract of *L. sativum* (Linn.) seed (40×).

A: Vehicle control; B: High cholesterol diet treated (HCD); C: HCD + atorvastatin; D: HCD + *L. sativum* total sapogenin (LSTS) 150 mg/kg; E: HCD + LSTS 300 mg/kg; F: HCD + *L. sativum* total flavonoid (LSTF) 150 mg/kg; G: HCD + LSTF 300 mg/kg treated rat. Balloning degeneration (BD), Steatosis (ST), Fatty degeneration (FD) and Inflammatory changes (FC).

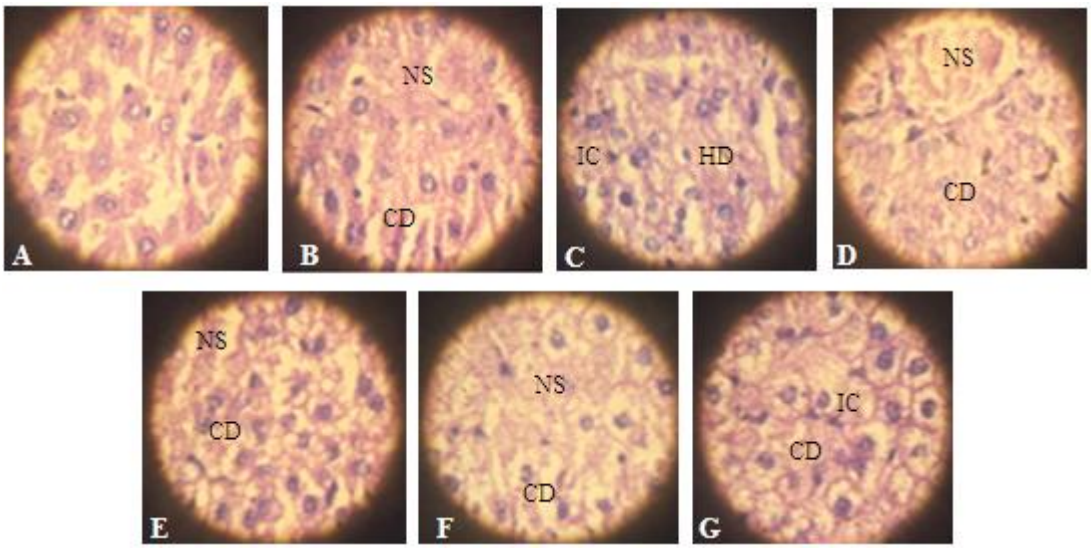

Figure 4: Photomicrograph of rat liver sections of triton x-100 induced hyperlipidemic rat treated with sapogenin and flavonoid extract of *L. sativum* (Linn.) seed (50×).

A: Vehicle control; B: Triton x-100; C: Triton x-100 + fenofibrate; D: Triton x-100 + *L. sativum* total sapogenin (LSTS) 150 mg/kg; E: Triton x-100 + LSTS 300 mg/kg; F: Triton x-100 + *L. sativum* total flavonoid (LSTF) 150 mg/kg; G: Triton x-100 + LSTF 300 mg/kg treated rat. Inflammatory changes (FC), Severe necrosis (NS) and cellular degeneration (CD).