

Research article

Tetrahydrocurcumin: Beneficial Effects on HMG-CoA Reductase enzyme and Lipoprotein Lipase Enzymes in High-fat diet-induced Hypercholesterolemia Rabbits

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ABSTRACT: **Introduction:** Obesity is a major factor in causing of atherosclerosis. Tetrahydrocurcumin (THC) may prevent atherosclerosis in high-fat diet-induced hypercholesteremic animals. However, the effect of tetrahydrocurcumin on HMG-CoA reductase and lipoprotein lipase enzymes levels in high-fat diet (HFD)-induced hyperlipidemia rabbits remains unexamined. Therefore, beneficial effect of THC on HMG-CoA reductase and lipoprotein lipase enzymes levels in an animal model with high-fat diet (HFD)-induced hyperlipidemia were examined. In addition, measurement of lipid parameters, anti-oxidant enzyme levels and histopathology studies were also performed. **Methods:** Healthy adult male New Zealand white rabbits (2.0 to 2.5 kg) were used in these studies. A high fatty diet was prepared and fed to the rabbits. Tetrahydroxycurcumin (THC) suspensions (40mg /kg and 80 mg/kg body weight) were given orally to the high-fat diet animals. Simvastatin (20mg/kg body weight) was used as standard drug. The blood was collected and measurement of lipid profiles and antioxidant enzymes levels were performed. In addition, the histopathology studies were performed for all groups. **Results:** Treatment with a high dose of THC (80 mg/kg) showed enhanced hypolipidemic activity compared to a lower dose of THC (40 mg/kg) in high-fat diet (HFD)-induced hyperlipidemia animals. The THC suspensions also possess potent antioxidant activities as demonstrated by their enhanced superoxide dismutase, catalase activity and suppressed lipid peroxidase activity. THC (80 mg/kg) exhibited induction of lipoprotein lipase activity and significantly decreased cholesterogenesis in liver by reducing HMG-CoA reductase activity. **Conclusion:** The results of the present study suggest that THC at a dose 80 mg/kg prevented the development of atherosclerosis in high-fat diet (HFD)-induced hyperlipidemia animals. These protective effects may be associated with the potent antioxidant activities of THC.

KEYWORDS: Tetrahydrocurcumin, HMG-CoA reductase, lipoprotein lipase, atherosclerosis

INTRODUCTION

Hypercholesterolaemia is a main risk factor that contributes to the development of atherosclerosis. Traditionally, factors such as hypercholesterolaemia, obesity, cigarette smoking and sedentary life style have been involved in

the development of cardiovascular disease.^[1,2] Elevation of the blood cholesterol level results in abnormal cholesterol loads into lipoproteins, which in turn results in the deposit of cholesterol on the coronary arteries. Such deposition, eventually leading to atherosclerosis, is a foremost contributory factor in diseases of the coronary arteries.^[3] Lipoprotein lipase (LPL) is a glycoprotein located on luminal surface of capillary endothelial cells. The role of LPL in atherosclerosis is clinically relevant because genetic defects as well as surplus of this enzyme are unpredictably frequent. LPL is known to be expressed by both macrophages and smooth muscle cells; detailed immunocytochemical and *in situ* hybridisation experiments have demonstrated that macrophage-derived foam cells are the primary source of the enzyme within the atherosclerotic

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lesion. In order to investigate the possibility that variations in macrophage LPL synthesis and secretion might constitute a hereditary component of atherosclerosis, LPL gene expression and secretion in macrophages obtained from inbred mouse strains differing in their susceptibility to diet-induced atherosclerosis was studied.^[4] It has been shown that some phyto-compounds in traditional medicinal plants have hypolipidaemic activities due to modulation of gene expression involved in lipid and lipoprotein metabolism.^[5] Many studies have been focused on the phyto-compounds (alkaloids, polyphenols, curcumin and flavonoids) in the prevention and treatment of atherosclerosis. These phytochemicals are widely distributed in plants. The most prominent medicinal plant in Indian medicinal systems is turmeric (*Curcuma longa* L., Family: Zingiberaceae, Rhizomes). Different Indian medicinal system used appropriate proportion of different medicinal plants for the ailment of various diseases. It has been shown to reduce plasma total cholesterol and is also a potent vasorelaxants, as well as reducing the atherogenic properties of cholesterol.^[6] Tetrahydrocurcumin (THC) is an antioxidative substance which is derived from curcumin by hydrogenation. THC and curcumin have identical β diketone structure and phenolic groups, but differ in that THC lacks the double bonds. Tetrahydrocurcumin (Figure 1) also induces a significant reduction in lipid peroxidation (thiobarbituric acid reactive substances and hydroperoxides) and lipids (cholesterol, triglycerides, free fatty acids and phospholipids) in serum and tissues, suggesting its role in protection against lipid peroxidation and its antihyperlipidemic effect. Tetrahydrocurcumin showed antihyperlipidaemic effect and antidiabetic effect in type 2 diabetic rats.^[7,8] These health-promoting effects of turmeric components have mainly attributed to the content of tetrahydrocurcumin (THC). The drug tetrahydrocurcumin was chosen as there was no considerable work was done on its mechanism of action in reducing hypercholesterolemia.

In the present study, as far we know there is no report of tetrahydrocurcumin (THC) effects on HMG-CoA reductase enzyme and lipoprotein lipase enzymes level in hypercholesterolemia in rabbits. Accordingly, the objective of this study was to investigate the effect of tetrahydrocurcumin (THC) on HMG-CoA reductase enzyme and lipoprotein lipase enzymes in high fat diet-induced hypercholesterolemia in rabbits.

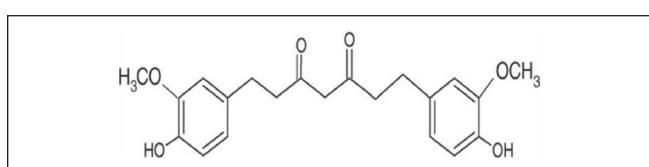


Figure 1: Structure of tetrahydrocurcumin

METHODS

Maintenance of Animals

Healthy adult male New Zealand white rabbits weighing 2.0 to 2.5 kg were obtained from institutional animal breeding house, PSG IMS&R, Peelamedu, Coimbatore, Tamil Nadu. The excremental procedure of the study was approved by "Institutional Animal Ethics Committee" (IAEC) of PSG IMSR of Register no 158/99/CPCSEA

The Animal house was well ventilated and these animals had 12 ± 1 hour day and night schedule with temperature between $11-20 \pm 2$ °C. The animals were housed in large spacious hygienic cages during the course of the experimental period. Rabbits used in antiatherogenic and antihyperlipidaemic studies were fed with prepared high fatty diet and water ad libitum throughout the experimental period.

Composition of high fat diet

The high fatty diet was prepared with following composition of carbohydrates (22%), proteins (47%), Fat (29%).

High Fatty Diet Composition

Bengal Gram	: 30%
Sucrose	: 25%
Whole Milk Powder	: 16%
Yeast	: 1%
Hydrogenated Groundnut Oil	: 10%
Cholesterol	: 5%
Salt Mixture	: 5%
Shark liver oil	: 2%
Egg Yolk Powder	: 7%

The above ingredients were grind along with the drenched Bengal gram and made to a solid consistency, which is much easier for the rabbits to consume. More prominent results could be seen as the composition is contributed by the direct form of cholesterol.

Preparation of tetrahydrocurcumin (THC) solution:

The Powder of tetrahydrocurcumin (THC) was made to suspend in 10% solution of carboxymethyl cellulose (CMC) and final suspension containing drug concentration of 40 mg/kg/day and 80 mg/kg/day body weight were prepared and given to the concern group of the animals. Simvastatin of 20 mg/kg/day body weight was administered as a suspension of carboxymethyl cellulose. The drugs were administered to the animals with the help of oral gauze which was penetrated till the esophagus.

The animals chosen were undergone a post-treatment study which means that animals were fed with the high-fat diet composition for a period of 30 days and then undergone

both the treatment of drug and administration of food for the rest of 60 days.

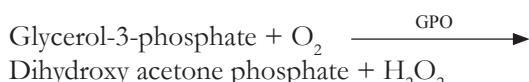
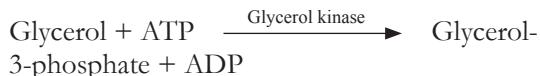
Group 1	Normal	Fed with Normal rabbit pellets
Group 2	HFD control group	Fed with HFD alone, not given any drug
Group 3	SIM 20 mg/kg	Given both HFD and standard drug
Group 4	THC 40 mg/kg	Given both HFD and low dose of test drug
Group 5	THC 80 mg/kg	Given both HFD and high dose of test drug

Body weight analysis: The initial body weight of rabbits was reordered. The body weights of rabbits on 30th, 60th, 90th day were recorder. The weight variation was noted from each group and compared with normal and HFT control groups.

Estimation of Lipid Profile

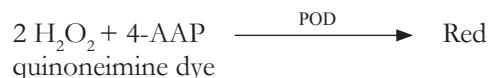
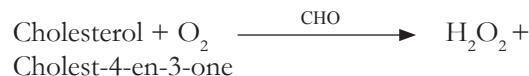
The blood samples were collected periodically from “the marginal ear vein of the rabbits” and these were centrifuged for the isolation of plasma samples which were used for estimation of lipid profiles.

Triglyceride determination: Determination of triglycerides is approached by an enzymatic reaction and formation of red colored compound by GPO-POD method at 510 nm using Merck diagnostic kit. The intensity of the red colour is directly proportional to the concentration of triglyceride in the sample.^[9]



4-AAP: 4-aminoantipyrine, GPO: glycerol-3-phosphate oxidase, POD: peroxidase

Total cholesterol determination: Determination of cholesterol is approached by an enzymatic reaction and formation of red coloured compound by CHOD-PAP method which can be measured at 510nm (Trindlers reaction) using Merck diagnostic kit. The intensity of the red colour is directly proportional to the concentration of total cholesterol in the sample.^[9]

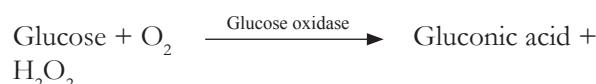


(CHE-Cholesterol esterase, CHO-Cholesterol oxidase, POD-Peroxidase)

HDL cholesterol determination: The chylomicrons, VLDL and LDL of serum are precipitated by phosphotungestic acid and magnesium ions. After centrifugation HDL in the supernatant is measured by an enzymatic method (CHOD-POD) using Beacon diagnostic kit.^[10]

LDL cholesterol determination: It can be determined by using following formula, LDL cholesterol Concentration (mg/dl) = total cholesterol – [HDL cholesterol + triglyceride/5]

Glucose determination: Determination of glucose is approached by an enzymatic reaction and formation of red colored compound was measured at 505nm using Merck diagnostic kit. The intensity of the red color is directly proportional to the concentration of glucose in the sample.^[11]



Antioxidant studies: Blood plasma of rabbits was subjected for estimation of antioxidant enzymes like superoxide dismutase, catalase, reduced glutathione, lipid peroxidase and nitric oxide estimation.

Estimation of superoxide dismutase (SOD): Superoxide dismutase was assayed by taking 0.05 ml of plasma followed by addition of 0.3 ml of sodium pyrophosphate buffer (0.025 M, pH 8.3), 0.025 ml of PMS (186 µM) and 0.075 ml of NBT (300 µM in buffer, pH 8.3). Reaction was started by addition of 0.06ml of NADH. The mixture was incubated at 30 °C for 90 seconds, the reaction stopped by addition of glacial acetic acid (0.025 ml). Then the reaction mixture was stirred vigorously and shaken with 2 ml of n-butanol. The mixture was allowed to stand for 10 minutes and centrifuged. 1.5 ml of n-butanol alone served as blank. The color intensity of the chromogen was read at 560 nm using spectrophotometer. Enzyme activity was expressed as 1 Unit = 50% inhibition/minute/mg of protein.^[12]

Catalase estimation (CAT): The activity of catalase was measured according to the method of Beers and Sizer, 1952.^[13] Catalase measurement was done based on the ability

of Catalase to inhibit oxidation of hydrogen peroxide (H_2O_2). 2.25 ml of potassium buffer (65 mM, pH 7.8) and 100 μ l of the cell lysates or sucrose (90.32 M) were incubated at 25 °C for 30 minutes. H_2O_2 (7.5 mM, 650 μ l) was added to initiate the reaction. The change in absorbance at 240 nm was measured for 2-3 minutes. dy/dx for every minute for each assay was calculated and the results are expressed as CAT units of protein.

$$\text{CAT (U) in } 100 \mu\text{l of sample} = \frac{dy}{dx} \times \frac{0.0003}{38.3956} \times 10^{-6}$$

Lipid peroxidation assay (TBARS): Lipid peroxidation was evaluated by measuring the TBARS content according to the TBA test described by Ohkawa et al., 1997.^[14] with slight modification. 0.2 ml of the cell lysates was taken and to this 0.8 ml saline, 0.5 ml of BHT and 3.5 ml TBA reagent (0.8%) were added and incubated at 60°C in a boiling water bath. After cooling, the solution was centrifuged at 2000 rpm for 10 minutes. The absorbance of the supernatant was determined at 532 nm using spectrophotometer against the black. Standard calibration was plotted using 1,1,3,3-tetra ethoxy propane in the concentration range 0.50-4 μ g. TBARS content were expressed in nanomoles / mg protein.

Histopathological studies: At the end of the 90th day of study the experimental animals were euthanized by using ketamine 35mg/kg IM. The aortas were isolated for histopathological study. The aortas were excised quickly and fixed in 10% buffered neutral formalin. Paraffin sections 5-10 μ were prepared, stained with haematoxylin and eosin and mounted in neutral DPX medium.

Plasma lipoprotein lipase (LPL) Assay: Lipoprotein lipase was assayed in plasma and tissues by the method of Korn, 1962.^[15] Albumin was added to the incubation tube to bind the unesterified fatty acids which otherwise will inhibit the reaction. Ammonium ions were also added to provide the necessary activating cation. The amount of glycerol liberated was determined calorimetrically.

a. Preparation of sample: A known amount of the sample (plasma or tissue) was homogenized in 20 ml of acetone. This was filtered and the residue in the filter paper was scrapped off, weighed and suspended in 1.0 ml of 0.025 M ammonia for 30 min at 0°C for complete extraction of enzyme. This extract was used as the enzyme source.

b. Incubation: The incubation mixture consisted of 0.4 ml albumin, 0.1 ml ammonium sulphate, 0.1 ml substrate, 0.1 ml enzyme and sufficient water to make up a final volume of 1.0 ml. The reagents were kept cold while mixing and the tubes were incubated at 37 °C for 1 hour removing aliquots of 0.2 ml at 15 or 30 min intervals. The first sample was removed before placing the tube in the water bath. The samples were

transferred directly into a 10 ml conical tip centrifuge tube containing 0.1 ml of 1N H_2SO_4 .

c. Glycerol determination: Periodate (0.1 ml) was added to the centrifuge tube, mixed well and allowed to stand at room temperature for 5 minutes. Then 0.1 ml sodium arsenite reagent was added, mixed well and again allowed to stand at room temperature for 10 min. This was followed by the addition of 9.0 ml chromotropic acid, mixed by inversion (covering the top of the tube by paraffin) and placed in a boiling water bath for 30 min, cooled and the volume adjusted to 10 ml with water. The optical density was read at 570 nm. The assay was standardized against a glycerol solution of known molarity. The activity of lipoprotein lipase is expressed as μ moles of glycerol liberated/h/ml plasma or μ moles of glycerol liberated/hr/mg protein.

Tissue Hydroxy-methyl glutaryl coenzyme A reductase (HMG CoA reductase) Assay: The ratio between HMG CoA and mevalonate in tissues was taken as an index of the activity of HMG-CoA reductase as described by Rao et al., 1975.^[16] Equal volumes of fresh 10% tissue homogenate and dilute perchloric acid were mixed, kept for 5 minutes and centrifuged at 2000Xg for 10 min. To 1.0 ml of filtrate, 0.5 ml of freshly prepared hydroxylamine reagent (alkaline hydroxylamine reagent in the case of HMG-CoA) was added, mixed and after 5 min 1.5 ml of $FeCl_3$ was added and shaken well. Readings were taken after 10 min at 540 nm against a similarly treated saline-arsenate blank. The ratio of HMG-CoA to mevalonate was calculated. Lower ratio indicates higher enzyme activity and vice versa.

Statistical analysis: All data analyses were performed using Graph Pad Instat DTCG through one way ANOVA and Bonferroni multiple comparisons test was used to compare all treatment groups with their control respective day's treatment and Dunnett comparison test with control. The significance was expressed as *P < 0.05, **P < 0.01, ***P < 0.001.

RESULTS

Body weight and plasma glucose analysis

In the present study, a significant increase in the body weight was observed in the HFD group after 30 days of dietary treatment, and it was maintained alike till the successive 60th and 90th day of the study when compare with the 0 day. The body weights of the simvastatin (SIM) treated group of the animals were increased slightly before the treatment was started and it decreased significantly ($p < 0.05$) on the 60th day of the study and maintained as such till termination of the study. However, there was no much more difference in the body weights of the test drug

treated animals when compare with the simvastatin (SIM) treated animals (Figure 2).

Both the doses of THC (40 mg/kg, 80 mg/kg) had not interrupted the food intake of the respective group of rabbits. There was a noticeable increase in the blood glucose level of the HFD control group of rabbits and it was almost a pre-diabetic condition. In SIM treated group of rabbits, there was an increase in the blood glucose level at the 30th day and this was maintained till termination of the study. The THC treated animals also shown the same report

regarding the blood glucose level, an initial increment on the 30th day of study and being sustained on the 60th, 90th day of the study (Figure 3).

Plasma lipid profile analysis

In the blood plasma of rabbit fed with high fat diet, it was observed that the total lipids such as total cholesterol (Figure 4), triglycerides (Figure 5), and LDL (Figure 6), cholesterol were elevated significantly after 30 days when compared with the zero days. Because till this period only the high fat diet was given to the animals and the

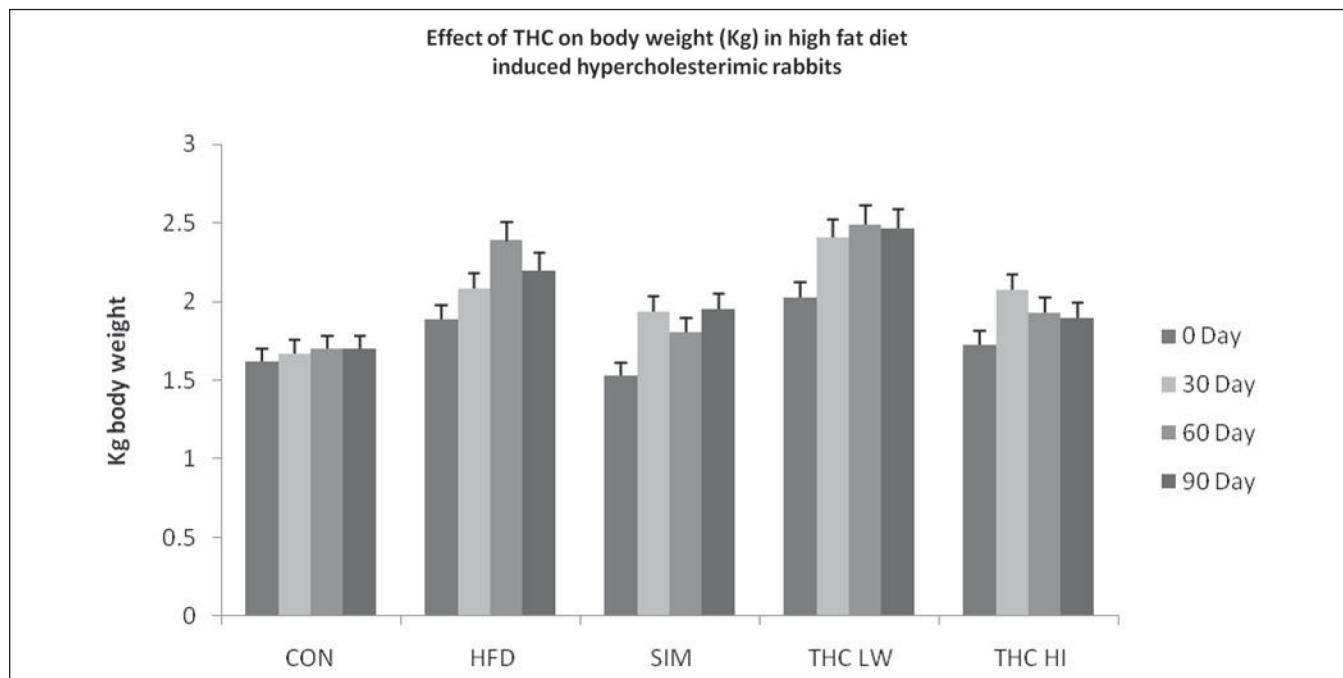


Figure 2: CON-Control; HFD-High fat diet; SIM-Simvastatin (20 mg/kg bw); THC LW-Tetrahydrocurcumin low dose (40 mg/kg bw); THC HI-Tetrahydrocurcumin high dose (80 mg/kg bw). Values are expressed in terms of mean \pm S.E.M. *p < 0.05 – considered as significant.

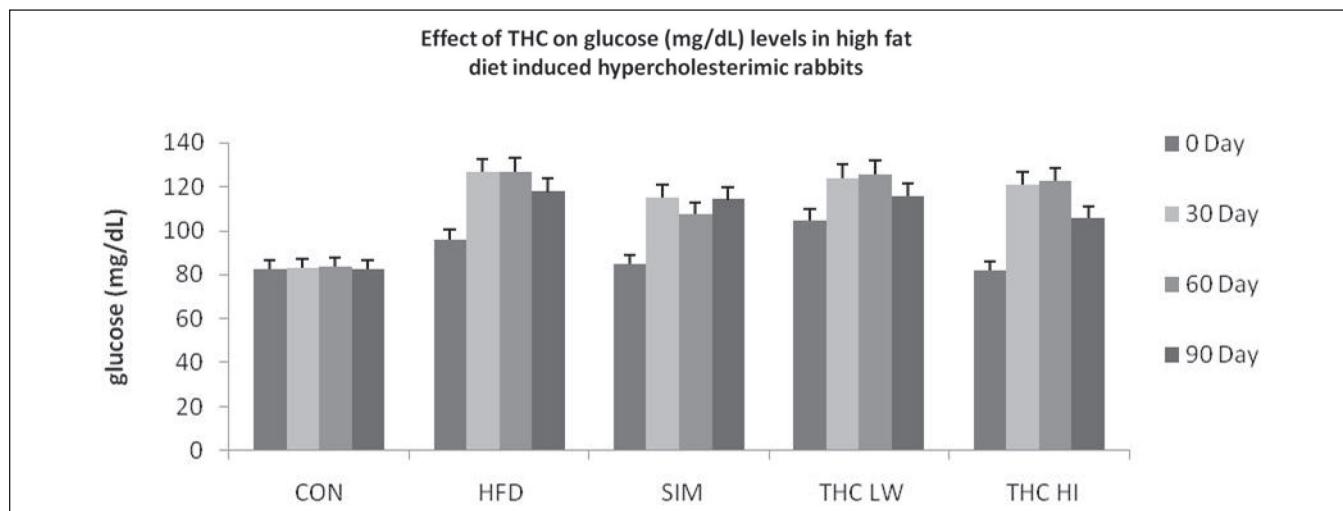


Figure 3: CON-Control; HFD-High fat diet; SIM-Simvastatin (20 mg/kg bw); THC LW Tetrahydrocurcumin low dose (40 mg/kg bw); THC HI-Tetrahydrocurcumin high dose (80 mg/kg bw). Values are expressed in terms of mean \pm S.E.M.

drug treatment was not started. However, the HDL cholesterol level was reduced after the 30 days of study as compared with the zero days in all groups of animals (Figure 7).

In this study, treatment with two different doses of tetrahydrocurcumin (40 & 80 mg/kg body weight) showed hypolipidaemic effect after 30 days on rabbits. In the 60th day of study, TC level in the HFD control group increased

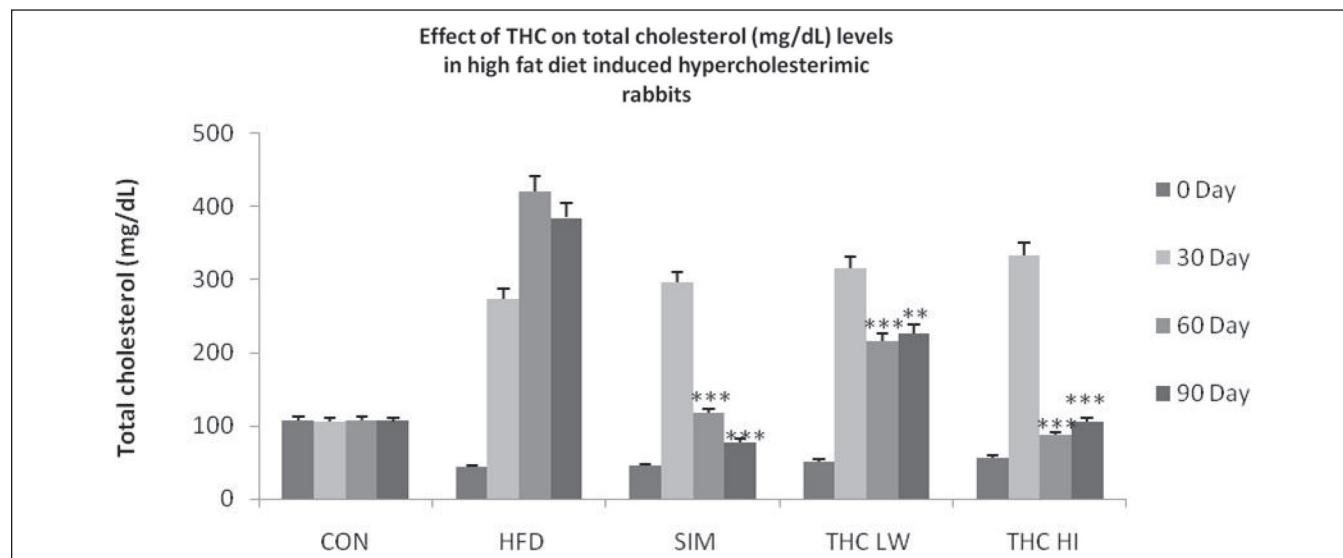


Figure 4: CON-Control; HFD-High fat diet; SIM-Simvastatin (20 mg/kg bw); THC LW-Tetrahydrocurcumin low dose (40 mg/kg bw); THC HI-Tetrahydrocurcumin high dose (80 mg/kg bw). Values are expressed in terms of mean \pm S.E.M. ***p < 0.001, **p < 0.01-considered as significant.

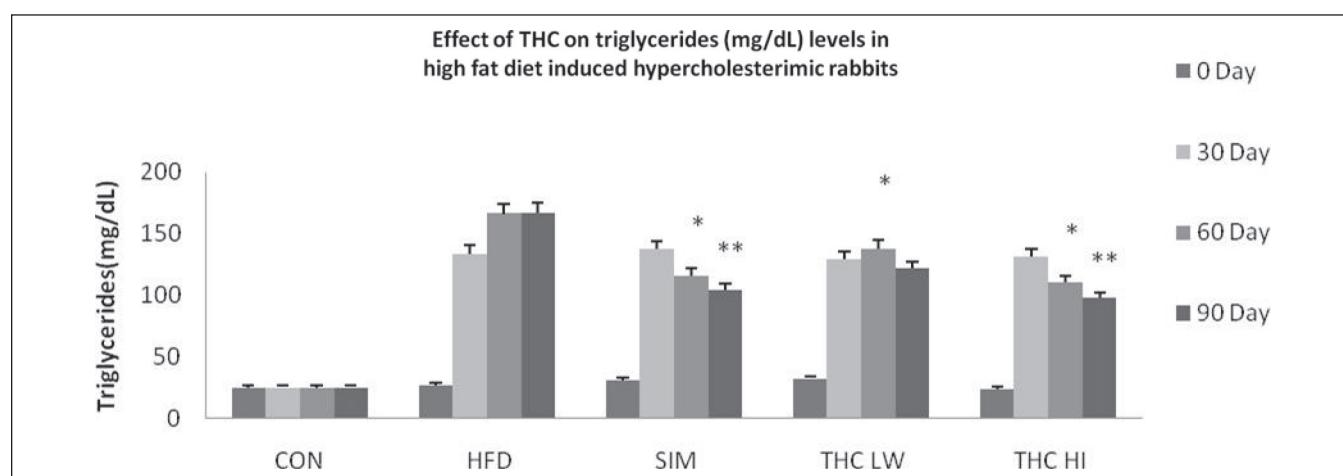


Figure 5: CON-Control; HFD-High fat diet; SIM-Simvastatin (20 mg/kg bw); THC LW-Tetrahydrocurcumin low dose (40 mg/kg bw); THC HI-Tetrahydrocurcumin high dose (80 mg/kg bw). Values are expressed in terms of mean \pm S.E.M. *p < 0.05, **p < 0.01-considered as significant.

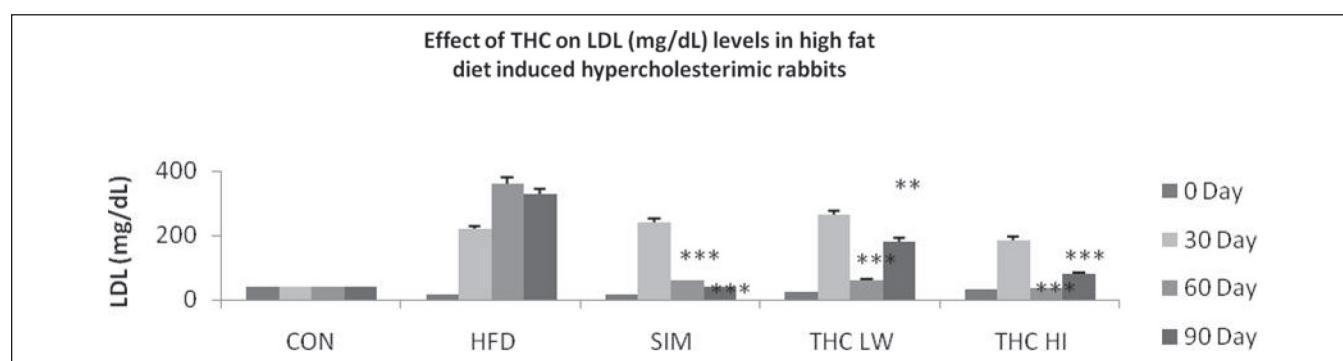


Figure 6: CON-Control; HFD-High fat diet; SIM-Simvastatin (20 mg/kg bw); THC LW-Tetrahydrocurcumin low dose (40 mg/kg bw); THC HI-Tetrahydrocurcumin high dose (80 mg/kg bw). Values are expressed in terms of mean \pm S.E.M. ***p < 0.001, **p < 0.01-considered as significant.

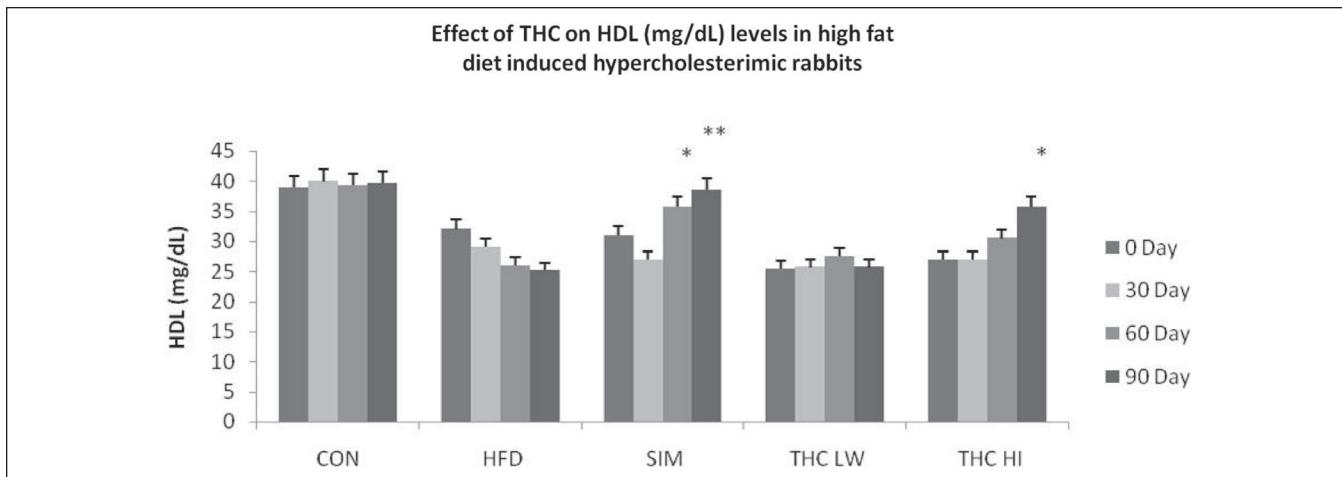


Figure 7: CON-Control; HFD-High fat diet; SIM-Simvastatin (20 mg/kg bw); THC LW-Tetrahydrocurcumin low dose (40 mg/kg bw); THC HI-Tetrahydrocurcumin high dose (80 mg/kg bw). Values are expressed in terms of mean \pm S.E.M. * $p < 0.05$, ** $p < 0.01$ -considered as significant.

linearly in comparison to the other groups, while triglyceride levels significantly ($p < 0.05$) decreased in the high dose of THC treated animal groups as compared with HFD control animals. However, low dose of THC treated animals not shown any significant reduction of triglycerides level. The total cholesterol levels in high as well as low doses of THC drug treated animals shown significant decrease when compared with the HFD control animals. The reduction in the total cholesterol level of the two doses of THC ($p < 0.001$) were nearly as that much of the effect of SIM treated animals ($p < 0.001$) as evidenced by comparison with the HFD control group. The LDL cholesterol level in the HFD control group increased drastically when compared to the drug treated animal groups, while in the high dose of THC treated groups the plasma LDL cholesterol levels significantly decreased as that of animal treated with simvastatin. Whereas, low dose of THC treated animals also shown significant reduction of LDL cholesterol which is near to the SIM treated groups. No significant differences were observed in plasma HDL cholesterol levels between the HFD control and THC treated groups at the 60th day of observation. Though, the animals treated with the SIM ($p < 0.05$) had shown significant increase of HDL cholesterol when compare with HFD control group animals. In the 90th days of study, TC level in the HFD control group reached plateau, while triglyceride levels significantly decreased in the high dose of THC and SIM treated animal groups ($p < 0.01$) as compared with HFD control animals. However, low dose of THC treated animals had shown significant reduction of triglycerides level ($p < 0.05$). The HFD control group animals total cholesterol levels also attained plateau as that of triglycerides level. The high dose of THC drug treated animals had shown significant decreases of total cholesterol when compared with the HFD control animals. The

reduction in the total cholesterol level of the high doses of THC ($p < 0.001$) were nearly as that much of the effect of SIM treated animals ($p < 0.001$) as evidenced by comparison with the HFD control group. Whereas, low dose of THC drug treated animals had shown significant decreases of total cholesterol when compared with the HFD control animals, such effect is not equal to that of reference drug treatment. The LDL cholesterol level in the HFD control group become sustained at the 90th day , while in the high dose of THC treated groups of plasma LDL cholesterol levels significantly decreased as that of animal treated with simvastatin. Whereas, low dose of THC treated animals also shown significant reduction of LDL cholesterol which is not near to the SIM treated groups. Significant differences were observed in plasma HDL cholesterol levels between the HFD control and high dose of THC ($p < 0.01$) treated groups. Though, the animals treated with the SIM ($p < 0.001$) had shown highly significant increase of HDL cholesterol level when compare with high dose of THC control group animals. On the other hand, the low dose of THC treated animals neither increased nor decreased the HDL cholesterol throughout the study.

Liver HMG-CoA reductase and lipoprotein lipase activity analysis

The liver HMG-CoA reductase enzyme activity of the HFD control rabbits were shown in Figure 8, which indicates the ratio of HMG-CoA to mevalonate. The ratio (absorbance of HMG-CoA/absorbance of mevalonate) is taken as an index of the activity of HMG-CoA reductase required to convert HMG-CoA to mevalonate. Lower ratio indicates higher enzyme activity and vice-versa. The increased ratio was found for the all drug treated animals as compared with the HFD control group. SIM and high dose of THC groups had shown

significant ratio ($p < 0.01$) which indicates decrease in the enzyme activity and cholesterologenesis as well. On the other hand the low dose of THC treated animals had shown a less significant ratio ($p < 0.05$) when compared to the other two groups of drug treatment. The liver lipoprotein lipase activity considerably decreases with the high fat diet intake in the animals. SIM and high dose of THC groups had shown significant increase ($p < 0.01$) in enzyme activity in comparison with HFD control group. Furthermore, this was evidenced by a significant reduction of total cholesterol, triglycerides and LDL cholesterol. On the other hand the low dose of THC treated animals had not shown any significant increase in the enzyme activity was observed.

Antioxidant analysis

The high fat diet increased free radicals level as indicated by increased catalase, SOD and lipid peroxidase levels (Table 1) in comparison to the animals before the feeding of high fat diet. The both high and low dose of THC significantly increased the catalase ($p < 0.01$) and SOD ($p < 0.01$) activity level in comparison to the HFD control. Whereas, the lipid peroxidase levels were decreased significantly ($p < 0.01$) in the higher dose of THC treated animals when compared with low THC.

Histopathological studies: Aorta rabbits samples

HFD control rabbits sections studies showed cross sections of aorta. The aorta showed a thin tunica intima, lined by endothelial cells, a large tunica media composed of smooth muscle cells and elastic fibers, tunica adventitia. The tunica intima in some foci appears elevated with subintimal collections of macrophage derived foam cells (lipid containing macrophages). Thin collagen bundles are also seen. However there are no smooth muscle cells or lymphocytes or a necrotic core. These findings are consistent with that of fatty streaks. This lesion is found in 3 of the 5 cross sections of the aorta (Figure 9 (a)). Reference drug treated rabbits fed with high fat diet sections studies showed cross sections of aorta. The intima looks completely normal. There was no evidence of fatty streak lesion. The tunica media and adventitia were also normal (Figure 9 (b)). THC low dose treated rabbits fed with high fat diet section studies showed cross sections of aorta. The tunica intima in some areas showed persistence of the fatty streak lesion (Figure 9 (c)). However this has been seen in only 1 of out of the 5 cross sections of the aorta. The tunica media and adventitia are normal. Treatment with high dose THC rabbits fed with high fat diet section studies exhibited cross sections of aorta. The tunica intima, media and adventitia are normal. No fatty streak lesions are found (Figure 9 (d)).

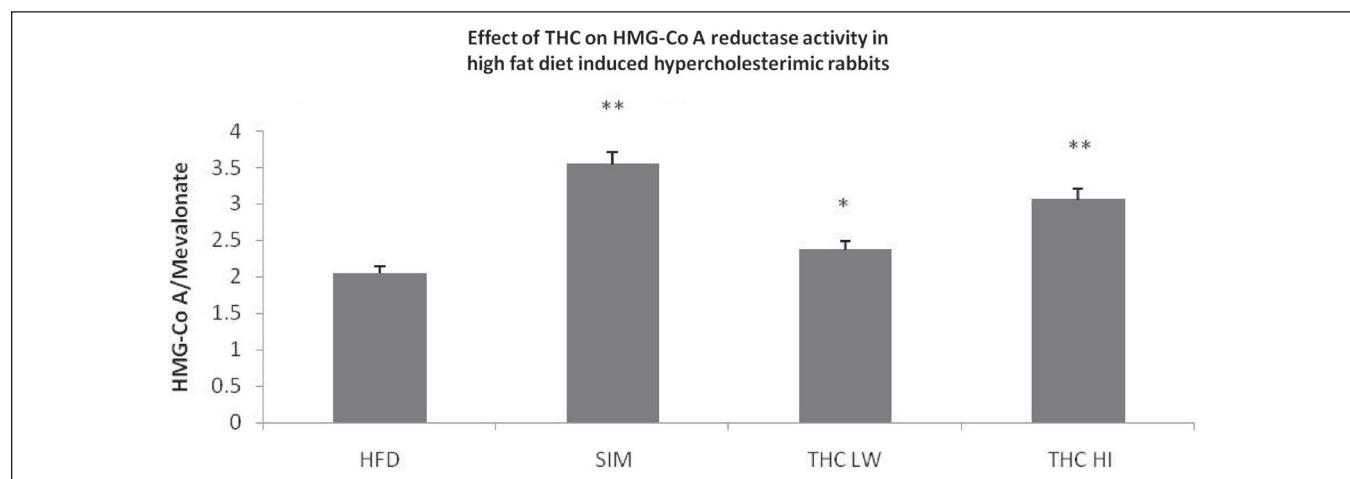


Figure 8: HFD-High fat diet; SIM-Simvastatin (20 mg/kg bw); THC LW-Tetrahydrocurcumin low dose (40 mg/kg bw); THC HI-Tetrahydrocurcumin high dose (80 mg/kg bw). Values are expressed in terms of mean \pm S.E.M. ** $p < 0.01$, * $p < 0.05$ -considered as significant.

Table 1: Effect of THC on plasma SOD, CAT and LPO levels in high fat diet induced hypercholesterolemic rabbits (n=4)

Groups	Units/mmoles of plasma		Nanomoles/ml of plasma
	SOD	CAT	
CON	55.84 \pm 3.84	51.84 \pm 1.75	345.7 \pm 40.6
HFD	29.84 \pm 1.44	34.73 \pm 0.66	852.3 \pm 48.2
Simvastatin	54.34 \pm 4.77*	46.49 \pm 3.03	585.1 \pm 100*
THC 40 mg/kg	56.53 \pm 5.57**	56.12 \pm 5.47**	580.6 \pm 42.6*
THC 80 mg/kg	68.37 \pm 7.42**	58.74 \pm 3.47**	517.9 \pm 19.1**

CON-Control; HFD-High fat diet; THC-Tetrahydrocurcumin. Values are expressed in terms of mean \pm S.E.M. statistical analysis carried by Graph Pad InStat DTCG through one way ANOVA . **, $p < 0.01$, * $p < 0.05$

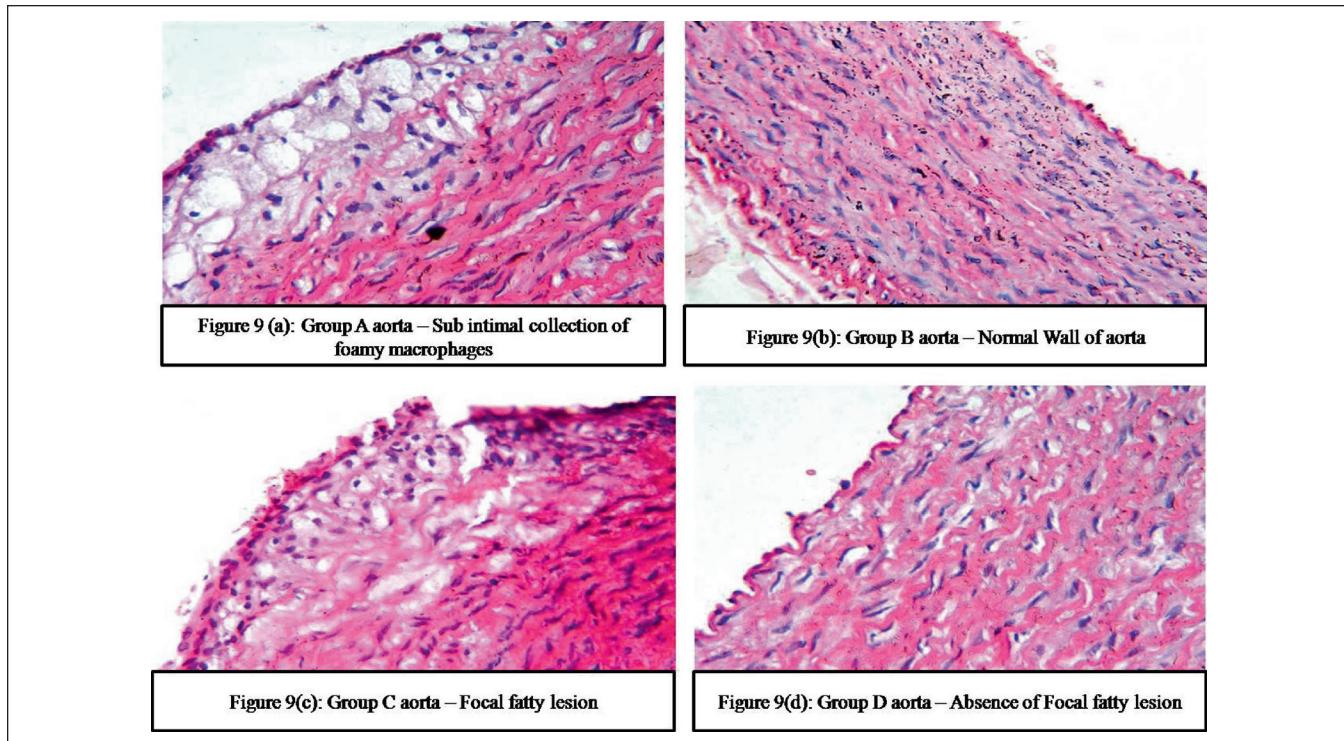


Figure 9: Histopathological studies, the aortas were excised quickly and fixed in 10% buffered neutral formalin. Paraffin sections 5-10 μ were prepared, stained with haematoxylin and eosin and mounted in neutral DPX m. Group A-HFD control group; Group B-SIM (20 mg/kg) treated group; Group C-THC (40 mg/kg) group; Group D-THC (80 mg/kg) group.

DISCUSSION

The current study has demonstrated the effect of tetrahydrocurcumin on HMG-CoA reductase enzyme and lipoprotein lipase enzymes activity for cholesterologenesis and plasma lipids regulation respectively. Lipoprotein lipase (LPL) expressed by the parenchymal cells of several extrahepatic and concerned with the uptake of circulating triglyceride rich lipoproteins (Chylomicrons, VLDL and LDL) by the extrahepatic tissues thereby providing non-esterified fatty acids and 2-monoacylglycerols for tissue utilization.¹⁷ The lower activity of this enzyme indicates decreased uptake of circulating triglyceride rich lipoproteins. In our study, there was an elevation in the triglycerides and LDL in the high fat diet fed animals and also decreased hepatic lipoprotein lipase activity was noticed. On the other hand there was a significant reduction of LDL lipoprotein and increased hepatic lipoprotein lipase activity upon the drug treatment in the dose range of 80 mg/kg THC.

Several study revealed that several LPL-deficient patients develop relatively advanced atherosclerosis. Additionally, individuals who are heterozygous for LPL mutations that reduce enzymatic activity have been reported to be predisposed to premature atherosclerosis.¹⁸ It was described that hypertriglyceridaemia that is associated with insulin resistance and type II diabetes is implicated in the acceleration of atherosclerosis and is mediated largely via

the suppression of adipose tissue LPL expression by cytokines such as tumour necrosis factor- α (TNF- α) and interleukin (IL)-6. In this present study, glucose estimation along with the lipid parameters was carried out and our study showed the hypertriglyceridemia, a pre-diabetic condition and an atherogenic lesion in the high fat diet fed animals (Figure 8 (a)). However, it was not the case with high dose of THC and SIM treated group of animals as evidenced by the histopathological study (Figure 8(d)). Efficient lipolysis of triglyceride-rich lipoproteins in adipose tissue, heart and skeletal muscle generally drives the profile of circulating lipoproteins in a non-atherogenic direction. For instance, over expression of LPL is highly effective in normalizing the atherosclerotic lipoprotein profiles of both apoE-deficient and LDL receptor-deficient mice and protects wild-type mice against diet-induced hyperlipidemia. Additionally, administration of higher dose of THC increased the tissue LPL activity and showed protection against atherosclerosis due to elevation of HDL. The HDL has a dual anti-atherogenic effect: it functions as a removal vehicle for cholesterol, transporting it away from areas of production to the liver where it can be excreted as bile salts and, in addition, it has been shown to prevent the oxidation of LDL by metal ions. The liver occupies a key position in cholesterol metabolism. Hepatocytes derive cholesterol from circulating lipoproteins or by *de novo* synthesis and use it for membrane synthesis, bile acid synthesis and secretion, secretion of free sterol into the

bile, lipoprotein formation, and storage of excess sterol as cholesterol ester. The enzyme HMG-CoA catalysis the rate limiting step in cholesterol biosynthesis in the tissues and its activity closely correlates with cholesterogenesis in the tissues. Regulation appears to involve changes in the rate of reductase synthesis, modulation of catalytic activity and the action of hormones. Defective regulation of HMG-CoA reductase has been demonstrated in hepatic tumor cells and has been implicated in familial hypercholesterolemia. The increased activity of the enzyme in the liver of rabbits fed with high fat diet corresponds with increased cholesterogenesis, as indicated by the higher incorporation of blood plasma total cholesterol and LDL cholesterol was observed in our current study. The animals treated with THC (80 mg/kg) and SIM (20 mg/kg) had shown decreased activity of enzyme as evidenced by the reduction in plasma total cholesterol and LDL cholesterol. By inhibiting HMG-CoA reductase, statins reduce the hepatocyte cholesterol content and increase expression of low-density lipoprotein (LDL) receptors, responsible for LDL cholesterol uptake via receptor-mediated endocytosis.¹⁹ Additionally, a second mechanism of LDL reduction may relate to LDL and very-low-density lipoprotein (VLDL) interactions. However, increases in HMG-CoA reductase synthesis shortly after statin therapy restore cellular VLDL levels, and the ultimate effect of reductase inhibition is enhanced LDL receptor expression and lower plasma LDL in the setting of normal cellular cholesterol content.²⁰ The same inference could be attained in our study in the animal treated with the high dose of THC. The depletion of free radical scavenger system due to increased oxidative stress in the cell in high fat diet induced hyperlipidemia in animals. Hyperlipidemia associated with changes in lipid peroxidation in plasma shows decreased activities of key antioxidants SOD, CAT and LPO which play an important role in scavenging the toxic intermediate of incomplete oxidation. SOD and CAT are the two major scavenging enzymes that remove toxic free radicals *in vivo*. Previous studies have reported that the activity of SOD is low in hyperlipidemic condition. The higher concentrations of lipidperoxides are found in patients with ischaemic heart disease and with peripheral arterial disease.²¹ A decrease in the activity of these antioxidants can lead to an excess availability of superoxide anion and hydrogen peroxide in biological systems, which in turn generate hydroxyl radicals resulting in initiation and propagation of lipid peroxidation.²² The result of increased activities of SOD and CAT suggest that THC contains a free radical scavenging activity, which could exert beneficial effect against pathological alteration caused by the presence of oxygen and hydroxyl free radicals. The increased activity of SOD accelerates dismutation of oxygen free radical to hydrogen peroxide that is removed by CAT.²³ This action could involve in the mechanisms related to scavenging activity of THC was observed in our antioxidant estimations study.

In the present study, the results suggested that the influence of severe hyperlipidemia induced in this study may be too strong to be overcome by THC, although some parameters had a tendency to be improved by the administration of THC to high fat diet fed rabbits. The dose 80 mg/kg of THC was presumed to be high enough for the lowering of lipid parameters, increased lipoprotein lipase enzyme activity and antioxidant effects has shown by its anti-atherosclerotic effects. However, lower doses may reduce free radical and lipid parameters level in blood plasma which may retard the development of atherosclerosis.

CONCLUSION

High dose of THC (80 mg/kg) showed appreciable hypolipidemic activity rather than low dose of THC (40 mg/kg) in high fat diet-fed rabbits. It is also considered to possess potent antioxidant activities due to significantly enhanced superoxide dismutase, catalase activity and suppressed lipid peroxidase activity. THC has been shown to induce lipoprotein lipase activity and significantly decreased cholesterogenesis in liver by reducing HMG-CoA reductase activity. Therefore, the present study indicated that tetrahydrocurcumin (THC-80 mg/kg body weight) could prevent atherosclerosis in high fat diet-fed rabbits.

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