

Role of *Boswellia ovalifoliolata* Bal. Henry extract on high fat diet induced hypercholesterolemia

D. Sathis Kumar^{1,2*}, David Banji³, A. Harani⁴, Ch. Pavan Kumar¹ and JN. Ravi Varma⁴

¹Aditya Institute of Pharmaceutical Sciences and Research, Surampalem, Andhra Pradesh, India-533437

²Jawaharlal Nehru Technical University, Hyderabad, Andhra Pradesh, India

³Nalanda College of Pharmacy, Nalgonda, Andhra Pradesh, India-508001

⁴A.U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, India-530003

ABSTRACT

Objective: To evaluate the Antihypercholesterolemic effect of *Boswellia ovalifoliolata* Bal. Henry extract by performing invivo studies and to checkout its effects by evaluating parameters like food consumption, weight gain, fecal fat excretion, serum and liver lipid & biochemical profiles. Even the study includes confirmation of activity by the histopathological studies. **Methods:** Animals were fed with cholesterol rich high fat diet. Food intake, Bodyweight and fecal fat excretion were measured. Serum and liver samples were analyzed for the estimation of lipid profiles and other biochemical parameters by using different kits. Histopathological study on liver, aorta, heart and adipose tissue was done to ensure the activity. **Results:** The animal group administered with methanolic extract of the plant has shown decreased levels of TC, LDL, VLDL, TG, HDL + VLDL, VLDL + LDL, LDL/TC, AI, SGOT, SGPT and elevated levels of HDL, HDL/TC in a dose dependent manner significantly ($p < 0.01$ & $p < 0.05$). The evaluation of liver tissue of animal groups treated with herbal extract and standard had shown increased levels of SOD, GSH and Catalase, whereas levels of SGOT, SGPT, Total glucose, HMG-CoA, lipase, amylase and percentage of monaldehyde were decreased when compared with high fat diet fed rats. Body weight and Food intake in treated groups were significantly lower than that in model control. **Conclusion:** It can be conferred from the present studies that the *Boswellia ovalifoliolata* Bal. Henry extract have strong activity against hypercholesterolemia and obesity suggesting a potential benefit as antihypercholesterolemic agent.

keywords: *Boswellia ovalifoliolata* Bal. Henry, High fat diet, Lipid profile, Histopathological studies

INTRODUCTION

Hypercholesteremia, a known risk factor is considered to be one of the reasons for cardiovascular disease (CAD) and hence as a major cause of premature death globally in many developing and developed countries like India^[1] and the most European countries cardiovascular disease contributes about 40% to all-cause mortality.^[2] It is estimated by World Health Organization that approximately one third of all cardiovascular disease worldwide were caused by high cholesterol, and in the USA, 105 million

people have cholesterol levels to a cardiovascular risk.^[3] Hyperlipidemia is characterized by elevated serum TC, LDL, VLDL and decreased HDL levels. Hyperlipidemia associated lipid disorders are found to be responsible for CAD^[4] of which hypercholesterolemia and hypertriglyceridemia are closely related to ischemic heart disease.^[5,6] The main aim of treatment in patients with hyperlipidemia is to reduce the risk of developing ischemic heart disease or the occurrence of further cardiovascular or cerebrovascular disease.^[7] The treatment of hyperlipidemia involves synthetic hypolipidemic drugs^[8] whose consumption may lead to hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function^[9] which consumption the traditional systems which have immune potential against various diseases. Medicinal plants are used for various research purposes. More than thirteen thousand plants have been studied for various pharmacological properties. Herbal treatment for

*Corresponding author.

D. Sathis Kumar, Associate Professor,
Aditya Institute of Pharmaceutical Sciences and Research,
Surampalem, Andhra Pradesh.
E-mail: satmpdina@yahoo.co.in

DOI: 10.5530/pj.2014.3.16

hypercholesterolemia has been associated with fewer side effects and is relatively cheap, locally available and they are effective in reducing the lipid levels in the system.^[10] Hyperlipidemia is classified as primary or secondary based on complexities associated with disease of which anti-lipidemic drugs are used to treat primary disease, but the secondary type originating from diabetes, renal lipid nephrosis or hypothyroidism requires the treatment of the actual disease condition rather than simple hyperlipidemia based treatment.^[11] Increased LDL formed from VLDL due to high fat consumption that adhere to walls of the blood vessels can block the normal blood flow resulting in the risk which can be prevented by improving the human diet which is highly recommended.^[12] The lipid lowering action of the leaves of medicinal plants which play a major role in hypolipidemic activity is found to be mediated through the inhibition of the hepatic cholesterol biosynthesis and reduction of lipid absorption in the intestine. *Boswellia ovalifoliolata* Bal. Henry (Burseraceae) is distributed throughout hot spot of India. The plant is over exploited for its medicinal uses. The fresh leaf juice used to prevent throat ulcers. The stem is used in stomach ulcers and diabetes. Gum is used in dysentery, inflammations, joint pains, ulcers, arthritis and amoebic dysentery.^[13] Decoction of the stem bark 10 – 25 mL per day reduces rheumatic pains. The gum obtained from the trunk which is highly medicated. Small lumps of fresh light yellow coloured liquid oozes out from the stem and hardens on exposure. Amyrins are the chief constituents of the gum together with resin acids and volatile acids. Shade dried gum is powdered dissolved in water and mixed with curd and given orally to cure amoebic dysentery.^[14] Considering the traditional uses of the plant, the present study was focused on the effect of extract of plant on serum and liver lipid and other biochemical level in high fat diet fed sprague-dawley rats.

MATERIALS AND METHODS

Plant Material

The plant was collected from the surrounding areas of Talagona A.P, India, during November to February. The plant was identified by Botanist, in S.V.University. The plant was identified as *Boswellia ovalifoliolata* Bal. Henry belonging to family – Burseraceae.

Chemicals and Reagents

The solvent used for extraction was methanol. Other reagents used were obtained from various commercial sources are of laboratory and analytical grade. All

diagnostic kits purchased from the Reckon diagnostic kit, India. All the parameters were estimated using an automatic analyzer (Robonik Touch, version 2.622A)

Preparation of methanolic extract of *Boswellia ovalifoliolata* Bal. Henry (BOI)

The shade dried plant as a whole was powdered of which 50g was suspended and extracted with 10 volumes of methanol by shaking at room temperature for 15 hours, filtered through filter paper, and the supernatants were pooled. The residue was re-extracted under the same conditions. Pooled extracts were condensed (methanol was removed) by a rotary evaporator. The physical characteristics and percentage yield of methanol extracts were reported. The dried extracts of all solvent were kept in desiccators prior to analysis.^[15]

Experimental Animals

Sprague Dawley rats (5 weeks old) purchased from the Saphthagiri Lab (India) were housed in stainless steel wire-bottomed cages under a 12-h light/12-h dark cycle in a temperature- and humidity-controlled room. They were allowed food and water ad libitum. After a 1 week time period for adaptation to lighting conditions, the healthy animals were used for experimentation. Rat weights at the beginning of the study ranged from 95 to 120 g. The doses of BOI were selected as per literature review^[16] and 1% tween 80 used as a vehicle. Rat chow diet was supplied by Vyas labs (India). Proximate analysis of rat chow diet did show that it contains crude protein 21.3; crude fat 4.10; crude fiber 5.00; carbohydrate 53; total ash 7.4; and minerals and trace elements 5.34% along with other minerals and trace elements like copper 3.20; zinc 35.96; manganese 34.96; and cobalt 0.58mg/100g. Vitamins included A 56; D3 0.169; E 0.404; and B complex 5.073. To the powdered rat chow diet, deoxy cholic acid was added at a ratio of 5 gm for 700gm diet and thoroughly mixed. Simultaneously, cholesterol (5g) was dissolved in 300g warm coconut oil. Oil solution was added slowly into powdered mixture of above to obtain homogeneous soft cake. This cholesterol rich (HFD) preparation was molded in the shape of pellets of about 3g each.^[17] The energy level of the prepared high-fat diet(HFD) was approximately 4996 kcal/kg, whereas rat chow diet was 3030 kcal/kg.

Experimental procedure

The rats were randomly divided in to 5 groups with 6 animals in each. Throughout the experimental period of 35 days, Group I, the normal control (NC) group was fed with normal rat chow diet, Group II (MC) with only

HFD, Group III(STD) was treated with HFD along with standard Atorvastatin (10mg/Kg), Group IV and V were administered with HFD along with BOI 50mg/kg and 100mg/kg respectively. The groups II to V were fed with high fat diet to increase the serum lipid levels before the administration of herbal extract and standard for one week. Dose administration through oral route was started from the 7th day. The animal studies were performed in compliance with protocols and policies approved by the Institutional Animal Ethical Committee of Nalanda College of Pharmacy (NCOP), Nalgonda, India (Voucher no: NCOP/IAEC/Approval/22/2010). Animals were observed daily for any abnormal physical and behavioral changes or signs of toxicity. Food intake (FI), Body Weight (BW), mean food efficiency ratios and fecal fat excretion mass were done for this studies. At the end of the experiment, the rats were fasted for 12 hr and the blood was withdrawn from retro-orbital plexus of rat under the anesthesia which was centrifuged immediately at 3000rpm for 15mins to get the plasma samples and the samples thus obtained were analyzed for the estimation of lipid profiles like TG, TC, HDL & LDL, and other biochemical parameters like glucose, SGOT, SGPT, amylase, lipase, catalase, and TBARS by using diagnostic kits. VLDL, LDL, ratios of HDL/TC, AI and HDL/LDL were calculated to describe serum lipid levels. The liver was immediately removed and stored at deep freeze condition until analyzed. Liver tissues were minced and homogenized (10% w/v) in 0.1M phosphate buffer (pH 7.4). A part of homogenized solution was extracted with chloroform-methanol (2:1, v/v, 2 ml). The residue was analyzed with a TG, TC, HDL, LDL kit. Remaining part of homogenized solution was centrifuged at $5000 \times g$ for 10 min and the resulting supernatant was used for analysis of SGOT, SGPT, Glucose, Lipase and amylase (using diagnostic kit), catalase, SOD, GSH and TBARS activity. For HMG-CoA/mevalonate ratio activity, liver tissue was homogenized in Saline arsenate solution. The remaining liver (after the experiment of hepatic lipid profile), heart, thoracic aorta and adipose tissues were isolated, cleaned and then fixed in a buffer solution of 10% neutral buffered formalin. For the histopathological studies, longitudinal sections of the myocardial tissue, adipose tissue and thoracic aorta were taken at the macroscopic lesions and the liver sections were cut through the macroscopic lesions including capsules. The sections were further cut to $5 \mu\text{m}$ thickness and were stained with H&E.

Statistical analysis

All the data was subjected to ANOVA (Graph pad InStat Demo software version 3.10). The data shown are mean

value and the significance differences was compared by using Dunnett Multiple comparison test at the $P < 0.01$ probability level.

RESULTS

Food intake and Body weight (BW)

The BW of the rat in the NC group gradually increased during the 5-week period. In contrast, the BW of animals fed with HFD showed rapid increase whereas those fed with the HFD and herbal extracts showed a gradual increase in BW which was significantly less than that of the HFD control in spite of continued and prolonged access to the high-fat diet. Although rats fed with the normal and HF diet continued to show increased BW and FI until the end of the study, % BW reduction was 11.77% for BOI 50; 16.27% for BOI 100 and 38.41% ($p < 0.01$) for STD. Percentage reduction in FI was 5.14%, 6.06% and 5.91% for BOI 50, BOI 100 and STD and % food efficiency ratio was 92.81%, 88.81% and 65.28% ($p < 0.01$) for BOI 50, BOI 100 and STD. The loss of weight in extract treated groups resulted in the massive loss of body lipid and the preservation of proteins, thus increasing the proportion of latter and decreasing that of lipid. The HF diet groups with or without treatment of BOI did not cause diarrhea during the experiment. Food efficiency was increased in the HF group compared with the normal group, but treatment of BOI reduced the food efficiency. BW and food efficiency reduced significantly in atorvastatin treated group than model control group. These results suggest that BOI may prevent an increase of BW induced by a high fat diet; it seemed that low BW in herbal treated groups partially may be due to the loss of appetite. In order to understand change of appetite by ingesting herbal extracts, further research is to be done.

Fecal fat excretion

Fecal dry weight in the MC group was significantly higher than other groups. Fecal excretion of fat in the standard treated group was significantly higher than other groups. It can be considered that Atorvastatin reduce the absorption of cholesterol in the intestine and enhances the excretion of cholesterol. BOI treated groups also increased fecal excretion of fat in a dose dependent manner ($p < 0.05$) and the effect was slightly less than that of standard treated group but more than control groups. Fecal excretion of fat was inversely proportional with serum cholesterol and liver cholesterol.

Serum Analysis

The lipid profile of the serum (TC, LDL, VLDL, TG, HDL+VLDL, VLDL+LDL, LDL/TC, HDL, HDL/TC & AI) and other biochemical parameters like SGOT and SGPT of rats from all groups was summarized in table 1. The MC group showed markedly higher serum TC, LDL, VLDL, TG, HDL+VLDL, VLDL+LDL, LDL/TC & AI levels and lower HDL & HDL/TC levels than the normal control group. Compared with HFD group, in the BOI treated groups TC, LDL, VLDL, TG, HDL+VLDL, VLDL+LDL, LDL/TC & AI were decreased significantly ($p < 0.01$) and HDL & HDL/TC was increased significantly ($p < 0.01$) in a dose dependent manner. These effects may be due to low activity of cholesterol biosynthesis enzymes and low level of lipolysis. The BOI extracts supplementation also results the significant attenuation in the level of serum HDL toward the control level which again strengthens the hypolipidemic effect of the extract. Animals treated with atorvastatin, the standard showed better results than the BOI treated groups in all lipid parameters. The content of HDL in serum implies the activity of LCAT, which plays

a key role in lipoprotein metabolism and may contribute to the regulation of blood lipids. HDL/TC represents the proportion of cholesterol component and may provide valid indices for identifying individuals at risk of peripheral arterial diseases. Constituents present in BOI extracts may decrease the risk of cardiovascular disease by increasing the ratios. The HDL/TC ratio may hasten removal of cholesterol from peripheral tissues to liver for catabolism and excretion. SGOT, SGPT, plasma glucose, % MDA level, amylase and lipase levels were increased and catalase activity was decreased in the MC group. SGOT, SGPT, plasma glucose, amylase, lipase and % MDA (TBARS) were decreased significantly ($p < 0.05$) and catalase activity in plasma was increased in BOI treated groups in a dose dependent manner than MC group after 35th day treatment. Plasma glucose reduction in BOI treated groups indicated that the BOI extract decreases hyperglycemia in obese rats. In the high-fat-diet control, there was little increment in glucose though it is within the range of 77-150mg/dl, where as in the BOI treated animals the determined levels were close to the normal range of glucose level. SGOT and SGPT values were applied to evaluate liver damage in the present study.

Table 1. the data of serum analysis

	TC (mg/dl)	TG (mg/dl)	VLDL (mg/dl)	HDL (mg/ dl)	HDL+VLDL (mg/dl)	LDL (mg/dl)	VLDL + LDL (mg/dl)	AI	HDL/TC (mg/dl)	LDL/TC
Normal control	44.90 ± 6.25**	39.39 ± 6.91**	7.87 ± 1.38**	22.24 ± 2.31	30.12 ± 3.66	14.78 ± 2.71**	22.66 ± 4.07**	1.00 ± 0.10*	0.50 ± 0.02**	0.32 ± 0.02**
model control	167.94 ± 5.24	148.01 ± 6.14	29.60 ± 1.22	31.93 ± 6.33	61.54 ± 7.36	106.39 ± 2.16	136 ± 1.46	4.72 ± 1.17	0.18 ± 0.03	0.63 ± 0.03
BOI 50	123.611 ± 7.225*	103.613 ± 4.124	20.723 ± 0.825	38.424 ± 0.815	59.147 ± 1.557	64.464 ± 6.571**	85.187 ± 7.098**	2.218 ± 0.188	0.313 ± 0.017	0.519 ± 0.023
BOI 100	111.690 ± 4.702**	87.063 ± 5.663*	17.413 ± 1.133*	37.515 ± 1.152	54.928 ± 1.474	56.762 ± 4.035**	74.175 ± 3.558**	1.975 ± 0.036	0.336 ± 0.004	0.507 ± 0.017
STD 10	97.22 ± 7.87**	83.68 ± 1.11*	16.73 ± 0.22*	43.63 ± 1.21	60.37 ± 1.34	36.84 ± 6.53**	53.58 ± 6.67**	1.22 ± 0.12*	0.45 ± 0.02**	0.37 ± 0.03**
	SGOT (IU/L)	SGPT (IU/L)	Total glucose (mg/dl)	Amylase activity (IU/L)	Amylase inhibition (%)	Lipase (IU/L)	Lipase inhibition (%)	Catalase (µmoles/ min/ml)	%MDA	% inhibition (%)
Normal control	9.753 ± 1.352**	12.379 ± 0.65**	47.599 ± 1.909**	69.461 ± 2.989**	7.459 ± 3.982**	185.714 ± 25.754	23.527 ± 10.605	0.014 ± 0.007*	13.826 ± 0.137**	
model control	28.884 ± 0.375	31.509 ± 1.949	110.681 ± 1.056	75.069 ± 0.362**	-0.012 ± 0.482**	242.857 ± 18.898	-0.003 ± 7.782	0.004 ± 0	38.604 ± 1.442	-0.010 ± 3.736
BOI 50	25.508 ± 2.705	28.133 ± 5.664	82.939 ± 6.397*	44.137 ± 7.554*	41.198 ± 10.064*	135.714 ± 7.143	44.116 ± 2.941	0.018 ± 0**	28.063 ± 0.274*	27.298 ± 0.709*
BOI 100	21.756 ± 1.352	21.756 ± 1.352*	79.211 ± 0.313**	42.147 ± 5.393*	43.849 ± 7.185*	128.571 ± 12.372	47.057 ± 5.094	0.022 ± 0.001**	27.105 ± 1.034**	29.781 ± 2.678*
STD 10	13.879 ± 0.375*	15.755 ± 0.65**	64.946 ± 1.075**	19.536 ± 0.829	73.973 ± 1.104	121.429 ± 25.754	49.999 ± 10.605	0.034 ± 0.001**	29.295 ± 4.177*	24.106 ± 10.821*

Values are in mean ± SEM; Number of animals in each group =6; ** $P < 0.01$ & * $P < 0.05$ Vs Normal Control; BOI 50 = treated with 50mg/kg dose of methanolic extract of *Boswellia ovalifoliolata* Bal. Henry; BOI 100 = treated with 100mg/kg dose of methanolic extract of *Boswellia ovalifoliolata* Bal. Henry; STD 10 = treated with Atorvastatin (10mg/Kg).

Liver profile

In liver tissue, HDL, HDL/TC, SOD, GSH and Catalase were decreased and TC, TG, VLDL, HDL/VLDL, SGOT, SGPT, Total glucose, lipase, amylase, HMG-CoA/mevalonate ratio and % of monaldehyde were increased in high fat diet rats as compared to normal rats. In liver, HDL ($p < 0.05$), HDL/TC, SOD, GSH, HMG-CoA/mevalonate ratio and Catalase were increased with supplementation of BOI extracts and standards, whereas TC ($p < 0.05$), TG, VLDL, HDL/VLDL, SGOT, SGPT, Total glucose ($p < 0.01$), lipase, amylase, and % of MDA were decreased as compared to model control (high fat diet rats) in dose dependent manner. Biochemical profile in liver of rats from all groups was summarized in table 2. In the groups treated with BOI extracts and standard, the BW and liver triacylglycerol level were reduced significantly when compared with high-fat diet-fed group. The increase in the intracellular deposition of TG in liver is well documented and demonstrated to attenuate glucose metabolism by interfering with insulin signaling and insulin secretion. Theoretically, Lipase inhibitors should inhibit fat accumulation in adipose tissue. On the other

hand, inhibition of lipase in muscle may slow down clearance of circulating triacylglycerols. BOI extracts increased the fecal lipid content, possibly by inhibiting PL and other gastro-intestinal lipases, decreasing the digestibility of dietary fat. The accumulation of triglycerides in the liver, induced by a high fat diet was reduced by the consumption of herbal extracts, possibly because the inhibition of gastric lipases and the subsequent reduction of the intestinal fat absorption and the reduction of the lipolysis. Such effects should, in turn, result in the suppression of hydrolysis and absorption of triglycerides. Since herbal extracts inhibited both lipase and amylase, reduced triglycerides in liver and lipolysis, the compounds in herbal extracts would have potential to reduce the body fat in animals. Though serum and liver TC and TG were increased in MC, the BOI extract treatment decreased ($p < 0.05$) those values. Conversely, higher ($p < 0.05$) fecal cholesterol outputs were measured in animals treated with herbal extracts as compared to MC. The liver Monaldehyde content was used to represent the liver peroxidation status, while liver GSH, CAT and SOD were used to evaluate the liver antioxidant capacity in the present study. Lower ($p < 0.05$) liver MDA and higher ($p < 0.05$)

Table 2. the data of liver profile

	TC ($\mu\text{mol/gm}$)	TG ($\mu\text{mol/gm}$)	VLDL ($\mu\text{mol/gm}$)	HDL ($\mu\text{mol/gm}$)	HDL+VLDL ($\mu\text{mol/gm}$)	HDL/TC ($\mu\text{mol/gm}$)	SGOT (IU/L)	SGPT (IU/L)	SOD (U/mg of protein)	GSH (mg/g of tissue)
Normal control	7.40 \pm 1.5**	18.36 \pm 1.69**	3.67 \pm 0.34**	0.94 \pm 0.109**	8.96 \pm 0.82**	0.14 \pm 0.04	100.90 \pm 14.53**	121.91 \pm 6.31**	3.48 \pm 0.02	4.21 \pm 0.02
model control	20.32 \pm 1.13	45.70 \pm 1.3	9.14 \pm 0.27	1.81 \pm 0.12	21.79 \pm 0.71	0.09 \pm 0.01	302.34 \pm 20.72	310.59 \pm 20.47	1.59 \pm 0.013	1.7 \pm 0.078
BOI 50	18.438 \pm 0.883	41.199 \pm 1.187	8.24 \pm 0.238	1.818 \pm 0.226	19.821 \pm 0.362	0.098 \pm 0.007	242.322 \pm 35.925	281.708 \pm 45.230	1.94 \pm 0.008	2.033 \pm 0.225
BOI 100	17.712 \pm 0.768	39.757 \pm 0.639*	7.951 \pm 0.128*	2.006 \pm 0.063	19.371 \pm 0.306*	0.113 \pm 0.003	226.567 \pm 12.704	232.944 \pm 13.690	2.107 \pm 0.002	2.067 \pm 0.235
STD 10	10.01 \pm 0.50**	39.10 \pm 0.72**	7.82 \pm 0.14**	2.57 \pm 0.27**	19.63 \pm 0.58	0.25 \pm 0.03**	147.04 \pm 13.16**	156.79 \pm 7.5**	3 \pm 0.079	3.89 \pm 0.021
	Total glucose ($\mu\text{mol/gm}$)	Amylase (IU/gm)	% inhibition	Lipase (IU/gm)	lipase inhibition	Catalase ($\mu\text{mol/gm}$)	% catalase inhibition	index of HMGR	% of MDA	% inhibition
Normal control	108.68 \pm 4.58**	0.25 \pm 0.06**	7.84 \pm 25.11**	2 \pm 0.38	0 \pm 19.24	1.91 \pm 0.29**	-0.23 \pm 15.03**	1.80 \pm 0.11**	20.39 \pm 1.121**	
model control	248.90 \pm 3.95	0.27 \pm 0.007	-0.05 \pm 2.63	2 \pm 0.38	0 \pm 19.24	0.18 \pm 0.02	87.48 \pm 1.02	2.03 \pm 0.04	53.11 \pm 3.85	-0.008 \pm 7.25
BOI 50	187.457 \pm 6.8**	0.094 \pm 0.019	65.771 \pm 6.966	1.556 \pm 0.222	22.222 \pm 11.111	1.018 \pm 0.028*	45.263 \pm 1.404*	2.804 \pm 0.112	44.216 \pm 2.926	16.746 \pm 5.510
BOI 100	179.162 \pm 1.333**	0.087 \pm 0.013	68.404 \pm 4.561	1.333 \pm 0.385	33.333 \pm 19.245	1.145 \pm 0.023**	38.83 \pm 1.187**	3.138 \pm 0.166	42.574 \pm 1.306	19.839 \pm 2.459
STD 10	139.53 \pm 3.66**	0.02 \pm 0.00**	89.46 \pm 2.63**	0.66 \pm 0.38	66.66 \pm 19.24	1.38 \pm 0.03**	26.55 \pm 1.77**	3.54 \pm 0.09	36.68 \pm 5.46**	30.92 \pm 10.28**

Values are in mean \pm SEM; Number of animals in each group =6; ** $P < 0.01$ & * $P < 0.05$ Vs Normal Control; BOI 50 = treated with 50mg/kg dose of methanolic extract of *Boswellia ovalifoliolata* Bal. Henry; BOI 100 = treated with 100mg/kg dose of methanolic extract of *Boswellia ovalifoliolata* Bal. Henry; STD 10 = treated with Atorvastatin (10mg/Kg).

GSH, CAT and SOD were observed in the NC and BOI extract treated groups than the others. In high fat diet fed groups, cholesterol synthesis was increased and the index of HMG-CoA/mevalonate ratio was decreased. In BOI and standard treated groups, cholesterol biosynthesis in liver was decreased while the index of HMG-CoA/mevalonate ratio was increased as compared to the MC group.

Histopathological studies for adipose tissue

Adipose tissues used were collected from subcutaneous region of the abdomen of rat. The histological appearance of epididymal adipocyte was irregular (exhibited heterogeneous sizes) in MC group compared to NC group. However, this morphological change mildly appears in BOI treated groups. The size of epididymal adipocytes was significantly bigger in MC group compared to other groups and the herbal treated groups showed moderate adipocyte size to that of NC group. These results suggest that BOI treated extract supplementation can inhibit lipid accumulation in epididymal adipocyte tissue. No significant change in number of nuclei was observed when compared with Model control. Figure 1, expressed the histopathological studies on adipose tissue.

Histopathological studies for Liver

Fatty liver disease is a new clinic-pathological entity of emerging importance, now recognized as the most common cause of abnormal liver. It is characterized by a wide spectrum of liver damage, i.e. simple steatosis may progress to advanced fibrosis and to cryptogenic cirrhosis via steatohepatitis, and ultimately to hepatocellular carcinoma. Normal control and standard treated group liver tissues are of normal size. Observation of tissues in MC rats also showed large vacuoles; fat degeneration; cumulative fatty cyst; vascular congestion moderate; dilatation marked; increase hepatocyte size; distinct enlargement of sinusoids; and sinusoidal dilatation with congestion. The livers of model control were clearly steatotic. In BOI treated group liver, few small fatty droplets, periportal inflammation, mild congestion; increased size of nucleus with prominent nucleolus; fibrosis were observed. Standard showed very few lipid droplets and the architecture of hepatocytes are found to be very similar to that of Normal control group. All treated livers have significantly reduced fat liver depots than the model control, as evaluated by H&E staining. The liver of treated animals had shown decreased lipid droplets, but there is a slight change

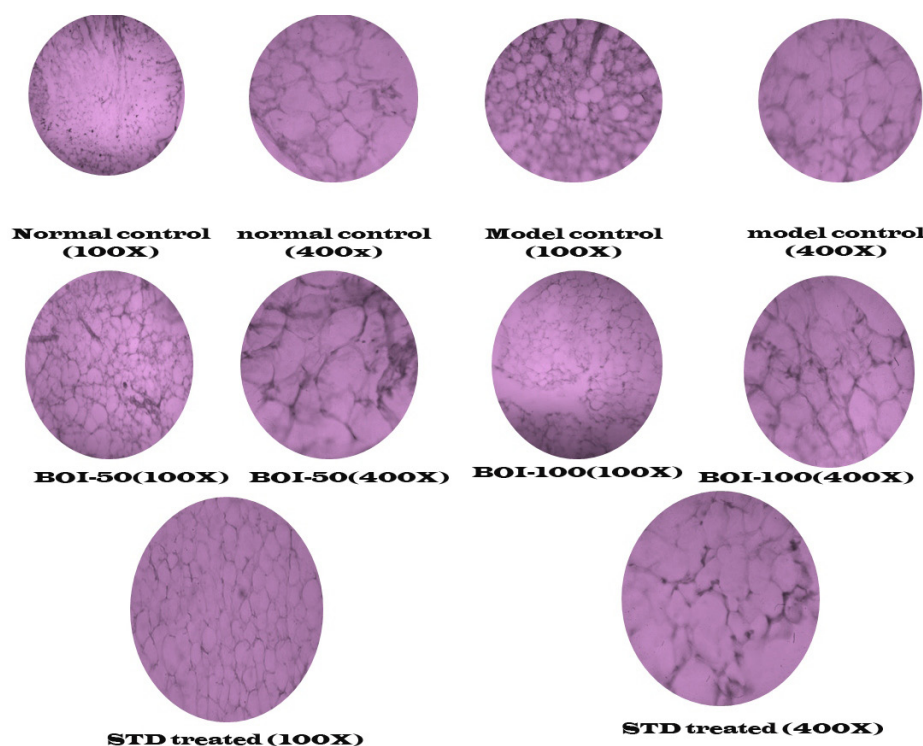


Figure 1. histopathological studies for adipose tissues (100X = hematoxylin–eosin stains with 100 magnification; 400X = hematoxylin–eosin stains with 400 magnification; BOI 50 = treated with 50mg/kg dose of methanolic extract of *Boswellia ovalifoliolata* Bal. Henry; BOI 100 = treated with 100mg/kg dose of methanolic extract of *Boswellia ovalifoliolata* Bal. Henry; STD treated = treated with Atorvastatin (10mg/Kg)).

in the morphology of hepatocytes. Figure 2 expressed the histopathological studies on liver.

Histopathological studies for Aorta

Normal control group and standard treated aortas were normal but in model control, atherosclerotic plaque was observed on aorta wall. Histopathology of aorta of MC rats indicated lesion with abnormal overlaying endothelium. Observation of tissues in MC rats also showed cholesterol deposits and fatty infiltration. BOI treated group aortas were similar to Normal control except with few inflammatory cells in the vessel wall and congestion. Figure 3 expressed the histopathological studies on aorta.

Histopathological studies for Heart muscle

Normal control group and standard treated groups were normal but in model control, few lipid droplets were present on wall. Histopathology of heart of MC rats indicated hyaline degeneration in muscles. BOI treated group aortas were similar to Normal control. Observation of tissues in herbal treated rats also showed mild hyaline degeneration in muscles. Figure 4 expressed the histopathological studies on heart.

Discussion

The oxidative modification of LDL and its accumulation in serum is a primary event in the proceeding of atherosclerosis. It is generally believed that antioxidants, which increase LDL oxidation resistance of the body, could inhibit atherosclerosis, though there is no direct evidence yet. The major advantages found in these pathological disorders have been in relation to fat deposition and serum triglycerides. In this way, a statistically significant decrease ($P < 0.05$) has been found in the serum triglyceride levels of animals treated with BOI extracts as compared with MC. Some authors have associated this outcome with alterations in fat assimilation in the digestive tract and changes in triglyceride storage and mobilization in adipose tissue. Moreover, it has been reported that β -3-adrenergic agonists produce an increase in the rate of lipolysis and a decrease in lipid synthesis in adipose tissue. The reduction in lipase activity in serum and liver of BOI treated groups may be explained by these findings. The results suggested that BOI extracts reduced the extent of hypercholesteremia. It may be due to the inhibition of intestinal absorption of cholesterol and the acceleration of catabolism of cholesterol to bile acid. Also

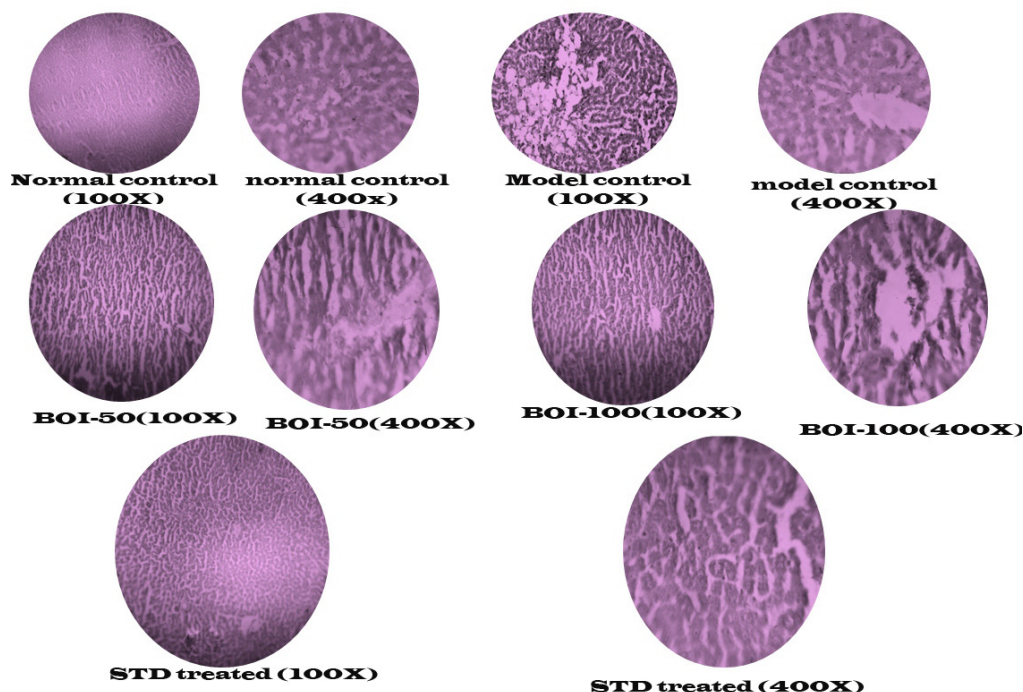


Figure 2. histopathological studies for liver (100X = hematoxylin–eosin stains with 100 magnification; 400X = hematoxylin–eosin stains with 400 magnification; BOI 50 = treated with 50mg/kg dose of methanolic extract of *Boswellia ovalifoliolata* Bal. Henry; BOI 100 = treated with 100mg/kg dose of methanolic extract of *Boswellia ovalifoliolata* Bal. Henry; STD treated = treated with Atorvastatin (10mg/Kg)).

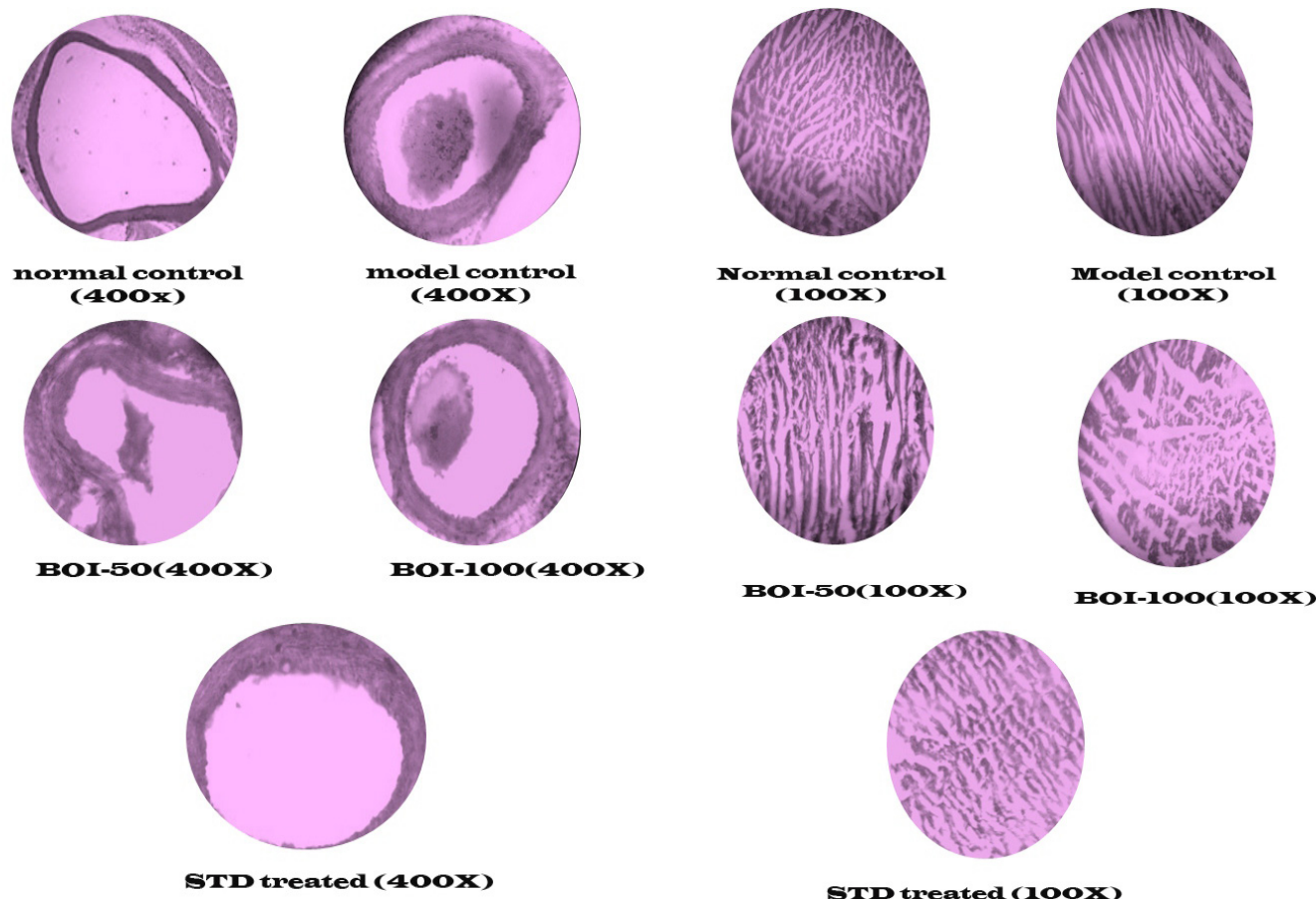


Figure 3. histopathological studies for aorta (100X = hematoxylin–eosin stains with 100 magnification; 400X = hematoxylin–eosin stains with 400 magnification; BOI 50 = treated with 50mg/kg dose of methanolic extract of *Boswellia ovalifoliolata* Bal. Henry; BOI 100 = treated with 100mg/kg dose of methanolic extract of *Boswellia ovalifoliolata* Bal. Henry; STD treated = treated with Atorvastatin (10mg/Kg)).

Figure 4. histopathological studies for heart (100X = hematoxylin–eosin stains with 100 magnification; 400X = hematoxylin–eosin stains with 400 magnification; BOI 50 = treated with 50mg/kg dose of methanolic extract of *Boswellia ovalifoliolata* Bal. Henry; BOI 100 = treated with 100mg/kg dose of methanolic extract of *Boswellia ovalifoliolata* Bal. Henry; STD treated = treated with Atorvastatin (10mg/Kg)).

many phenolic compounds have been shown to possess hypolipidemic and antihypercholesterolemic activity by increasing the fecal cholesterol excretions and LDL receptor activity.^[18,19] Atorvastatin which was used as standard drug in this study is a HMGR inhibitor which catalyzes the committed step in cholesterol biosynthesis. Statins are HMGR inhibitors that effectively lower serum cholesterol levels and are widely prescribed in the treatment of hypercholesterolemia. Rats treated with Atorvastatin showed marked reduction in all serum lipoproteins and increase in HDL level as compared with HFD group.^[20] The inferred results were correlated with our previous reports that the phenolic compounds were the major contributors for antihypercholesterolemic activity of the extract. The present study suggests that both doses of BOI extracts are capable of exerting antihypercholesterolemic effects in high fat diet induced rats.

CONCLUSION

It can be concluded that the present study supports the folk information regarding the anti hypercholesterimic activity of *Boswellia ovalifoliolata* Bal. Henry by administering the macerated methanol extract of the plant to the rats which reduced hyperlipidemia. Further studies are required to isolate active principles from the extract to specify the extent of activity of each one to assess its anti-hypercholesterolemic effect.

ACKNOWLEDGEMENT

Authors acknowledge the management, Aditya institute of Pharmaceutical Sciences and Research and Nalanda College of Pharmacy for their technical support.

Authors acknowledge Mr. Madhavachetty, Botanist, S.V. University, Thirupati, Andhra Pradesh who was identified and authenticated the plant.

CONFLICTS OF INTEREST

We didn't receive any type of funds from government institution or any other funding agency or our college management. This work has done by our own expenditure.

REFERENCES

1. Verlecar XN, Jena KB, Chainy GBN. Biochemical markers of oxidative stress in *Perna viridis* exposed to mercury and temperature. *Chemico-Biological Interactions*. 2007;167: 219–26.
2. Kromhout D. Epidemiology of cardiovascular diseases in Europe. *Public Health Nutr*. 2001; 4(2B): 441–57.
3. George AM, Demosthenes BP, Christos P, Dimitra X, George P, Christodoulos S. Prevalence of self-reported hypercholesterolemia and its relation to dietary habits, in Greek adults; a national nutrition and health survey. *Lipids in Health and Disease*. 2006; 5: 361–64.
4. Saravanan R, Rajendra Prasad N, Pugalandi KV. Effect of Piper betle leaf extract on alcoholic toxicity in the rat brain. *J. Med. Food*. 2003; 6: 261–65.
5. Kaesancini AY, Krauss RM. Cardiovascular disease and hyperlipidemia. *Cur. Opinion in lipidology*. 1994; 5: 249–51.
6. Davey Smith G. Cholesterol lowering and mortality: the importance of considering initial level of risk. *Int. Med. J*. 1993; 306: 1367–73.
7. Davey Smith G, Pekkanen J. Should there be a moratorium on the use of cholesterol lowering drugs? *Br. Med J*. 1992; 304: 43–40.
8. Brown SL. Lowered serum cholesterol and low mood. *Br. Med. J*. 1996; 313: 637–38.
9. Speight TM. *Drug treatment Principles and Practice of clinical Pharmacology and therapeutics*. 3rd ed, Auckland: ADIS press Ltd. 1987; pp. 599.
10. Berliner JA, Heinecke JW. The role of oxidized lipoproteins in atherogenesis. *Free. Radic. Biol. Med*. 1996; 20: 707–27.
11. Suzuki T, Suzuki Y. Current topics of lipid dynamics and pathobiology in membrane lipid rafts. *Biol. Pharm. Bull*. 2006; 29(8): 1538–41.
12. Ryan D H. Diet and exercise in the prevention of diabetes. *Int. J. Clin. Pract*. 2003; 134: 28–35.
13. Sathis Kumar D, Srisutherson N, Pradeep Kumar Reddy B, Vinitha S, Yadhagiri Rao T, David Banji. Pharmacognostical study on *Boswellia ovalifoliolata* Bal. Henry, *Journal of pharmacy research*. 2011; 4(5): 1374–75.
14. Mitchell RN, Cotran RS. In: *Robinsons Basic Pathology*. Edtn 7. Harcourt Pvt. Ltd, New Delhi, India. 2000; 33–42.
15. Singleton VL, Orthofer R, Lamuela Raventos RM. Analysis of total phenols & other oxidation substrates & antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol*. 1999; 299: 152–78.
16. Sakuntala Devi PR, Adilaxamma K, Srinivasa Rao G, Srilatha Ch, Alpha Raj M, Safety evaluation of alcoholic extract of *Boswellia ovalifoliolata* stem-bark in rats, *Toxicological international*. 2012; 19(2): 115–20.
17. Vishnu Kumar, Pradyumn Singh, Ramesh Chander, Farzana Mahdi, Sushma Singh, Ranjana Singh, Ashok kumar Khanna et al. Hypolipidemic activity of *Hibiscus rosa sinensis* root in rats. *Indian journal of biochemistry and biophysics*. 2009; 46: 507–10.
18. Chan PT, Fong WP, Cheng YL, Huang Y, Chen ZY. Jasmine green tea epicatechins are hypolipidemic in hasters fed a high fat diet. *Journal of Nutrition*. 1999; 129: 1094–101.
19. Tikkanen MJ, Wahaink OS, Vimha V, Adcercrentz H. Effect of Soyabean phytoestrogen intake on LDL oxidation resistance, *Proc. Natl. Acad. Sci*. 1998; 95: 3106–110.
20. Varsha D, Shubhangi S, Mangesh P, Naikwade NS. Antihyperlipidemic Activity of *Cinnamomum tamala* Nees on High Cholesterol Diet Induced Hyperlipidemia. *International Journal of Pharm Tech Research*, 2010; 2(4): 2517–21.