Research Article

Hypolipidemic Effect of *Euphorbia Nivulia* Buch in Experimentally Induced Hypercholesteremic Rats

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ABSTRACT

Hyperlipidemia is associated with abnormally elevated levels of lipids (cholesterol, cholesterol esters, phospholipids and triglycerides) in the blood. Hyperlipidemia is a major modifiable risk factor for cardiovascular diseases. Herbal medicine has proven to be useful in controlling many diseases conditions. *Euphorbia nivulia* tree belonging to the family Euphorbiaceae is one such medicinal tree found in India. Substantial literature is available supporting its medicinal effectiveness. The present investigation was undertaken to study the hypolipidemic effect of *Euphorbia nivulia* Buch in experimentally induced hypercholesteremic rats. Thirty six male albino Wistar rats weighing between 160-175 g were assigned to 6 groups, each groups had 6 rats. Group I served as vehicle control, Group II was fed with hypercholesterol diet (HCD) for 28 days, Group III, IV, V and VI received HCD and atorvastatin 10 mg/kg, hydroalcoholic extract of *Euphorbia nivulia* 100, 200 and 400 mg/kg respectively for 28 days. Results indicated that, animals treated with atorvastatin and *Euphorbia nivulia* 100, 200 and 400 mg/kg b.w showed significant decrease in serum glucose levels, total cholesterol, triglycerides and LDL-C levels ($P<0.001$) and significant increase in HDL-C levels ($P<0.001$). Atherogenic index also decreases significantly ($P<0.001$) in the all the treated groups. No significant change in body weight was reported between the groups. Hence, the hydroalcoholic extract of *Euphorbia nivulia* was found to possess hypolipidemic activity. Comprehensive chemical and pharmacological studies would help to understand the mechanism involved in hypolipidemic effect of the tree.

Keywords: Atorvastatin, *euphorbia nivulia,* hypercholesteremic, hypolipidemic

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INTRODUCTION

It has been well established that nutrition plays an important role in the etiology of hyperlipidemia, atherosclerosis and other coronary heart disease (CHD) complications [1]. Metabolic disorders that involve elevations in any lipoprotein species are termed hyperlipoproteinemia or hyperlipidemia. Hyperlipidemia denotes increased levels of triglycerides [2]. Cholesterol has emerged as an independent risk factor for the development of CHD in the elderly population and adults [3]. Dyslipidemia, including hyperlipidemia and low levels of high density lipoprotein cholesterol (HDL-C) are major causes of increased atherogenic risk.

To reduce the rate of mortality, it is therapeutically recommended to undergo controlled diet or/and drug therapy to lower lipid levels within the normal range. Allopathic hyperlipidemia drugs are available at large scale in the market but the side effects and contraindications of these drugs have marred their popularity [4]. Herbal medication that lowers elevated lipids has gained importance to fill the lacunae created by the allopathic drugs. Plant products are frequently considered to be less toxic and free from side effects than synthetic ones. A number of plants have been found to be useful in hyperlipidemia and been identified as hypolipidemics in Ayurveda [5]. *Euphorbia nivulia* Buch (Fig. 1) belonging to the family Euphorbiaceae has been reported to have various medicinal uses. The root, stem, leaf and

latex are used for abdominal disorders, diabetes, oedema, psychosis, leprosy, anemia and rheumatoid arthritis [6-9]. The present investigation was undertaken to study the hypolipidemic effect of the extract *Euphorbia nivulia* Buch effect in experimentally induced hypercholesteremic rats.



Figure 1: *Euphorbia nivulia* Buch

METHODOLOGY

Collection of plant material

The leaves of *Euphorbia nivulia* Buch were collected from the Ramnagar, Karnataka, identified and authenticated by Dr. MD Rajanna, Botanical garden and Herbarium, UAS, GKVK Campus, Bangalore, Karnataka, India.

Extraction

The leaves of *Euphorbia nivulia* Buch was air dried, powdered and subjected to soxhlet extraction using 70% v/v hydro-alcohol (75-80ºC). Thereafter, the extract was concentrated using rotary flash evaporator (50ºC).

PHARMACOLGICAL SCREENING

Animals

Experimental study was carried out using male albino Wistar rats weighing 160–175g. The animals were procured from Biogen, Bangalore. The animals were housed in polypropylene cages. The cages were maintained clean and hygienic. Animals were acclimatized in light and temperature controlled room with a 12-12h dark-light cycle, temperature 25±2ºC and humidity 50±5%. The mice were fed with commercial pelleted feed and water *ad libitum*. The animal caring and handling were done according to the CPCSEA guidelines. The Institutional Animal Ethics Committee at Visveswarapura Institute of Pharmaceutical Sciences has approved the study.

Preliminary phytochemical screening of *Euphorbia nivulia* Buch extract

Phytochemical analysis was carried out by using the standard procedures. Alkaloids, carbohydrates, flavonoids, glycosides, phytosterols, terpenes, proteins and saponins were qualitatively analysed.

Acute oral toxicity [10]

According to the OECD guideline no. 425, the acute oral toxicity study was performed.

Diet induced hypercholestremia model in rats [11]

Composition of Hyper Cholesterol diet

Normal feed – 92 %, Cholesterol- 2 %, Cholic acid- 1 %, Coconut oil- 5 %

Each group consist of 06 rats

GROUP I : Vehicle control
GROUP II : Hypercholesterol diet (HCD) for 28 days
GROUP III : HCD + Standard (Atorvastatin 10 mg /kg b.w.) for 28 days

GROUP IV : HCD + *Euphorbia nivulia* 100 mg/kg b.w., p.o for 28 days
GROUP V : HCD + *Euphorbia nivulia* 200 mg/kg b.w., p.o for 28 days
GROUP VI : HCD + *Euphorbia nivulia* 400 mg/kg b.w ., p.o for 28 days

All the animals except the vehicle control group were fed with hypercholesterol diet for 28 days. Group II, III, IV, V and VI were treated respectively as mention above. Body weight and food intake was measured everyday. On 28th day animals was fasted for overnight and next day blood was collected and serum was separated and serum cholesterol, HDL, triglycerides and glucose were estimated. LDL and atherogenic index were also calculated.

RESULTS

Preliminary phytochemical screening

Preliminary phytochemical analysis was carried out to detect the secondary metabolites present in hydroalcoholic extract *of Euphorbia nivulia* Buch and showed the presence of alkaloids, flavonoids, saponins, sterols tannins and terpenes.

Acute oral toxicity

Animals administered with single oral dose of *Euphorbia nivulia* extract (2000 mg/kg b.w.) showed neither mortality nor any sign of toxicity in any of the animals. Thus three different doses were selected for the present study – 100, 200 and 400 mg/kg, b.w. respectively.

Diet induced hypercholestremia model in rats

There was no significant gain in body weight of animals fed with HCD when compared with vehicle control. Animals treated with atorvastatin and *Euphorbia nivulia* 100, 200 and 400 mg/kg b.w showed a slight decrease in body weight gain but was not significant when compared with rats fed with HCD (**Table 1**).

Table1: Effect of *Euphorbia Nivulia* on Body Weight Gain in Male Rats

Groups	Final weight (Day 28)	Initial weight (Day 1)	Weight gain
Vehicle control	255.3±3.46	166.5±1.99	88.83±2.91
Hypercholesterol diet (HCD)	263.3±3.81	170.8±1.13	92.50±4.41 ns a
HCD + Atorvastatin 10 mg /kg b.w.	253.2±2.52	169.7±1.30	83.50±3.31 ns b
HCD + *Euphorbia nivulia* 100 mg/kg b.w	257.0±3.41	168.8±1.32	88.17±2.63 ns b
HCD + *Euphorbia nivulia* 200 mg/kg b.w	254.0±3.23	170.7±2.21	83.33±1.89 ns b
HCD + *Euphorbia nivulia* 400 mg/kg b.w	253.3±2.47	169.5±1.23	83.83±3.15 ns b

Values are expressed in terms of SEM ±Mean. Data were analyzed by one way ANOVA followed by Dunnett's *t* test. Number of animals in each group n=6. [a] Comparison made with control group. [b] Comparison made with hyper cholesterol diet (HCD). [ns] non significant.

Animals in HCD group exhibited significant increase in serum glucose levels, total cholesterol, triglycerides and LDL-C levels (P<0.001) and significant decrease in HDL-C levels when compared to vehicle control group. Animals treated with atorvastatin and *Euphorbia nivulia* 100, 200 and 400 mg/kg b.w showed significant decrease in serum glucose levels, total cholesterol, triglycerides and LDL-C levels (P<0.001) and significant increase in HDL-C levels when compared to HCD fed rats (**Table 2**)

Table 2: Effect of *Euphorbia Nivulia* on Serum Glucose, Total Cholesterol, Triglycerides, HDL-C and LDL-C Levels (mg/dL)

Groups	Glucose	Total Cholesterol	Triglycerides	HDL-C	LDL-C
Vehicle control	98.98±2.63	49.68±0.93	52.04±2.56	30.43±0.75	9.77±1.36
Hypercholesterol diet (HCD)	123.0±3.66*** a	82.55±4.09*** a	94.08±1.85*** a	19.54±0.47*** a	44.19±4.19*** a
HCD + Atorvastatin 10 mg /kg b.w.	98.07±3.79*** b	54.27±2.98*** b	67.31±2.26*** b	29.21±0.99*** b	11.60±3.11*** b

HCD + *Euphorbia nivulia* 100 mg/kg b.w	103.0±2.02*** b	67.80±2.97** b	82.41±2.08** b	24.46±1.25** b	31.09±3.17** b
HCD + *Euphorbia nivulia* 200 mg/kg b.w	100.1±3.82*** b	55.80±1.42*** b	69.95±3.49*** b	27.57±1.21*** b	14.24±1.08*** b
HCD + *Euphorbia nivulia* 400 mg/kg b.w	101.0±2.46*** b	49.74±0.89*** b	54.71±1.88*** b	30.54±0.43*** b	7.33±1.36*** b

Values are expressed in terms of SEM ±Mean. Data were analyzed by one way ANOVA followed by Dunnett's *t* test.
Number of animals in each group n=6. [a] Comparison made with control group. [b] Comparison made with hypercholesterol diet group. ***P<0.001 **P<0.01

Animals in HCD group exhibited significant increase in atherogenic index (P<0.001) when compared to vehicle control group. Animals treated with atorvastatin and *Euphorbia nivulia* 100, 200 and 400 mg/kg b.w showed significant decrease in atherogenic index (P<0.001) when compared to HCD fed rats (**Table 3**).

Table 3: Effect of *Euphorbia nivulia* on Atherogenic index

Group	Atherogenic Index
Vehicle control	48.68±0.93
Hyper cholesterol diet (HCD)	81.55±4.09*** a
HCD + Standard (Atorvastatin 10 mg /kg b.w.)	53.27±2.98*** b
HCD + *Euphorbia nivulia* 100 mg/kg b.w	66.80±2.97*** b
HCD + *Euphorbia nivulia* 200 mg/kg b.w	54.80±1.42*** b
HCD + *Euphorbia nivulia* 400 mg/kg b.w	48.74±1.42*** b

Values are expressed in terms of SEM ±Mean. Data were analyzed by one way ANOVA followed by Dunnett's *t* test. Number of animals in each group n=6. [a] Comparison made with control group. [b] Comparison made with hyper cholesterol diet(HCD). ***P<0.001

DISCUSSION

Hyperlipidemia is a major cause of atherosclerosis and also associated with conditions, such as coronary heart disease (CHD), ischemic cerebrovascular disease, and peripheral vascular disease. Hyperlipidemia and high cholesterol diet increase serum TC and LDL-C levels, resulting in an increased risk for the development of atherosclerosis. Thus regulating the serum cholesterol level is an important aspect in atherosclerosis prevention, as it has been shown that atherosclerosis could be suppressed by controlling the level of serum cholesterol. Many allopathic medications which are available in the market lowers the cholesterol but are associated with certain limitations. These results in need of alternative medication which can overcome the drawback associated with allopathic medications. Herbal medications are one among them.

The induction of hyperlipidemia by a high-cholesterol diet in experimental animals has long been used to assess the beneficial effect of hypolipidemic agents on the regulation of cholesterol [12]. Therefore, in this study hypolipidemic effect of hydroalcoholic extract of *Euphorbia nivulia* Buch on a rat model in which hyperlipidemia was induced by high cholesterol diet was evaluated.

Results showed that there was no significant body weight gain in animals fed with HCD when compared with vehicle control. Whereas, rats treated with hydroalcoholic extract of *Euphorbia nivulia* Buch 100, 200, 400 mg/kg, b.w. showed slight decrease in body weight gain but was not significant when compared with HCD.

High glucose levels are seen in HCD fed animals which may be due to insulin resistance and glucose tolerance [13]. Fat cells produce leptin, resistin and adiponectin. Leptin is normally released after a meal and dampens appetite. Resistin and adiponectin both affect cells response to insulin [14]. Fat disrupts the normal balance and functioning of these hormones,

thereby contributing to insulin resistance. High levels of blood circulating triglycerides also interfere with insulin action [15]. Animals treated with hydroalcoholic extract *of Euphorbia nivulia* Buch 100, 200, 400 mg/kg, b.w. showed a significant decrease in blood glucose levels. This may be due to improvement of physiological action of insulin action and prevention of insulin resistance by increasing insulin receptor binding [16].

An increase in TC and TG levels in HCD fed animals can be attributed to increase in both *de novo* synthesis and intestinal absorption of cholesterol [17]. Cholesterol feeding alone however does not affect the serum TG level. It is assumed that a high level of saturated fat in addition to cholesterol is required to significantly elevate serum TG level in rat model [18]. When the rats are fed with HCD, there is a significant increase in triglyceride levels when compared to normal control. This may be due to absorption from the intake food; fat and liver cells synthesize and stored triglycerides. Cholic acid also aids in the absorption of cholesterol.

Diet containing saturated fatty acids increases the activity of HMGCoA reductase (the rate–determining enzyme in cholesterol biosynthesis); this may be due to higher availability of acetyl CoA, which stimulated the cholesterogenesis rate [19]. Moreover, this could be associated with a down regulation in LDL receptors by the cholesterol and saturated fatty acids in the diet, which could also explain the elevation of serum LDL-C levels either by changing hepatic LDLR (LDL-receptor) activity, the LDL-C production rate or both [20]. The activity of cholesteryl ester transfer protein (CETP), a key enzyme in reverse cholesterol transport and HDL metabolism increase in HCD and mediates the transfer of cholesteryl esters from HDL-C to triglyceride-rich particles in exchange for triglycerides. This leads to increased plasma concentrations of TG & decreased concentrations of HDL-C [21]. Lecithin Cholesterol O-acyltransferase (LCAT) enzyme is involved in the trans-esterification of cholesterol, the maturation of HDL and the flux of cholesterol from cell membranes into HDL [22]. The activity of the enzyme tends to decrease in diet-induced hypercholesterolemia. The increase in the concentrations of LDL and VLDL observed are mainly due to the dietary carbohydrates and cholesterol [23]. Most of the cholesterol in the mature lesion originates from circulating LDL particles. These particles cross the endothelium into the intimal of blood vessels. In their native form they are unfavorable for uptake into intimal macrophages and most return to the circulation. However, some particles may be oxidized by local cells possibly facilitated by the presences of transition metal ions and binding to proteoglycans. After oxidative modification the LDL particles are rapidly taken up into macrophages via the scavenger receptor. Subsequent loading with cholesteryl esters forms so called foam cells, which could be responsible for the initiation of atherosclerosis [24].

Animals are treated with hydroalcoholic extract *of Euphorbia nivulia* Buch (100, 200, 400 mg/kg, b.w.) showed a significant decrease in the levels of triglycerides when compare to the HCD fed hyperlipidemic rats. This may be due to increased triglyceride-rich lipoprotein (TRL) lipolysis, induction of hepatic fatty acid (FA) uptake and reduction of hepatic triglyceride production or block the release of free fatty acids from adipose tissue [25-26].

Animals are treated with hydroalcoholic extract *of Euphorbia nivulia* Buch (100, 200, 400 mg/kg, b.w.) showed a significant decrease in the levels of cholesterol and LDL-C when compared to the HCD fed hyperlipidemic rats. This may be due to increased stimulation of bile acid synthesis leading to an increased utilization of cellular free cholesterol and thus help in reduction of cholesterol, increased removal of LDL particles [27], reduction in neutral lipid (cholesteryl ester and triglyceride) exchange between VLDL and HDL [28], increase in HDL production and stimulation of reverse cholesterol transport [29], inhibition or down regulation of HMG-CoA reductase activity inhibition of oxidative stress, increase in the level of serum HDL, increase in the activity of lipoprotein lipase and plasma LCAT, which are known to involve in transport of tissue cholesterol to liver for its excretion.

Animals are treated with hydroalcoholic extract *of Euphorbia nivulia* Buch (100, 200, 400 mg/kg, b.w.) showed a significant increase in the levels of HDL-C levels when compare to the HCD fed hyperlipidemic rats. This may be due to the mobilization of cholesterol from peripheral cells to the liver by the action of LCAT [30]. The increased HDL-C facilitates the transport of TG or cholesterol from serum to liver by a pathway termed 'reverse cholesterol transport' where it is catabolized and excreted out of the body [31]. Inhibition of the action of TG-lipase which may contribute to the rapid catabolism of blood lipids through extra hepatic tissues, increased conversion of cholesterol to bile acids and salts, prevention of accumulation of lipids in liver, compete with cholesterol binding sites or interfere with cholesterol bio synthesis in liver, thereby reducing blood cholesterol level [32-34].

CONCLUSION

Hence, the hypocholesterolemic effect of the extract *Euphorbia nivulia* Buch seems to be mediated through any one or more mechanisms mentioned above. The exact mechanism has to be further explored.

Comprehensive chemical and pharmacological studies would help discover the exact mechanism of hypolipidemic effect of this indigenous tree and pave the way for its large scale commercial use as a cardio protective/curative drug.

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