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Hypolipidemic effect of ethanolic extract of leaves of *Bryophyllum pinnatum* in hyperlipidemic rats

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ABSTRACT

This study was undertaken with the objective of studying the hypolipidemic effect of ethanolic extract of leaves of *Bryophyllum pinnatum* in hyperlipidemic rats. Hyperlipidemia was induced in rats by a cocktail diet containing cholesterol, peanut oil, cholic acid and propylthiouracil. Hypolipdemic activity of *Bryophyllum pinnatum* was then evaluated at doses 100mg/kg, 200mg/kg and 400mg/kg and compared with a standard, Atorvastatin. Total cholesterol, triglycerides, HDL, LDL, VLDL and atherogenic index were determined to assess the hypolipidemic effect. Histopathology of the aorta was done to assess the atherogenic changes.The study demonstated dose dependent reduction in serum total cholesterol, triglyceride, LDL, VLDL levels and the atherogenic index at doses 100mg/kg, 200mg/kg and 400mg/kg. The effect was highest with 400mg/kg dose ($p < 0.01$). Though there was increase in the HDL levels after treatment but the increase was not statistically significant. The histopathological analysis showed minimum atherogenic changes in the drug treated groups. From the above results it can be concluded that the ethanolic extract of leaves of *Byophyllum pinnatum* possessed significant hypolipidemic effects and reduced atherogenic changes in cholesterol rich cocktail diet induced hyperlipidemia in rats.

INTRODUCTION

Hyperlipidemia or hyperlipoproteinemia is a metabolic disorder characterized by elevations in any lipoprotein species, also identified as dyslipidemia. High lipid levels speed up a process called atherosclerosis.[1]Atherosclerosis is a major risk factor for cardiovascular diseases. It is well known that cardiovascular diseases (CVDs) are the leading cause of death worldwide, accounting for approximately 17.3 million deaths per year.[2] *Bryophyllum pinnatum* is a perennial herb used in folk medicine[3] which contains a number of active compound groups including alkaloids, triterpenes, flavonoids, steroids, glycosides, phenols and organic acids. [4]

Thus, this study was carried out with the objectives to evaluate the hypolipidemic effect of ethanolic extract of leaves of *Bryophyllum pinnatum* (EEBP) in comparison to standard drug, Atorvastatin in hyperlipidemic rats and its effect in prevention of atherosclerosis.

MATERIALS AND METHODS

Ethical approval

The present study was conducted after getting approval from the Institutional Animal Ethics Committee bearing approval no. MC/05/2015/51.

Plant extract

The shade dried and finely powdered leaves of *Bryophyllum pinnatum* were extracted with 70% ethanol using Soxhlet apparatus. Final yield of the extract was 25.9% (w/w)

Experimental animals

Healthy, pathogen free, colony bred, naïve, male rats of Sprague Dawley variety weighing between 220-250gms were used as experimental animals. They were maintained at 12 hour light and 12 hour dark cycles at a temperature of 24 ± 1 C and humidity of 55 ± 5 % was maintained. They were allowed to acclimatize to the laboratory environment for 2 weeks and were provided water and food ad libitum. Acute toxicity tests as per Organisation for Economic Co- operation and Development

The animals were then divided into six groups each containing six animals. The groups were:

Groups	Day 1 – Day 14	Day 15 – Day 28
Group I *(Normal Control)*	Normal saline 10ml/kg	Normal saline 10ml/kg
Group II *(Hyperlipidemic control)*	Cocktail diet 1ml/100g	Normal saline 10ml/kg
Group III *(Hypolipidemic standard)*	Cocktail diet 1ml/100g	Atorvastatin 20mg/kg
Group IV *Hypolipidemic Test Dose A* *(B. pinnatum 100 mg/kg)*	Cocktail diet 1ml/100g	EEBP 100mg/kg
Group V *Hypolipidemic Test Dose B* *(B. pinnatum 200 mg/kg)*	Cocktail diet 1ml/100g	EEBP 200mg/kg
Group VI *Hypolipidemic Test Dose C* *(B. pinnatum 400 mg/kg)*	Cocktail diet 1ml/100g	EEBP 400mg/kg

(OECD) guidelines 425 was conducted.

Experimental design for evaluation of hypolipidemic action:

Hyperlipidemic cocktail diet:

Hyperlipidemic cocktail solution was prepared dissolving 100g of cholesterol, 30g of propylthiouracil and 100g of cholic acid in 1litre of peanut oil.[5,6]

Test extract and Atorvastatin were suspended in 1% carboxy methycellulose and administered orally.

Method:

1. Day zero: The baseline serum lipid levels of all the animals were estimated.

2. Day one: Group I received normal saline at the dose of 10ml/kg while animals from group II to group VI received hyperlipidemic cocktail at the dose of 1ml/100g per orally

3. Day two to Day 14: The normal saline and cocktail were administered in the same manner as Day one.

4. Day 15: i) Blood was collected from all the groups from the tail vein for estimation of lipid profile.

ii) Group I and group II received normal saline at the dose of 10ml/kg.

iii) Ethanolic extract of *Bryophyllum pinnatum* was given to all the animals of group IV, group V and group VI at the dose of 100mg/kg, 200mg/kg and 400mg/kg respectively per orally.

iv) Group III received Atorvastatin at the dose of 20mg/kg per orally.

5. Day 16 to Day 28: The drugs were administered in the same manner as Day 15.

6. Day 29: i) Blood was collected from all the groups by cardiac puncture for estimation of lipid profile.

ii) The animals from all the groups were anaesthesized with ketamine (50mg/kg i.p.) and sacrificed by cervical dislocation. The thoracic aorta was identified and then excised, cleaned and stored in 10% formalin solution. The specimens were then prepared for histopathological analysis.

Duration of the experiment: 28 days.

Estimation of serum lipid levels and atherogenic index:

1. The serum lipid levels were estimated using the commercial biochemical assay kits. They were analysed in the Rayoto semiauto chemistry analyzer (RT 9600). Total cholesterol (TC), triglycerides (TG) and high density lipoprotein (HDL) were analysed by the assay kits while low density lipoprotein (LDL)

and very low density lipoprotein (VLDL) were calculated by mathematical formulae (Friedewald formulae):

LDL = Total cholesterol (HDL - VLDL)

VLDL = Triglyceride/5

2. The atherogenic index was then calculated for each group by the following formula:

Atherogenic index (AI) = (Total cholesterol HDL)/HDL[7]

Statistical analysis

The statistical analysis was carried out using graph pad prism 5.01 software. Data were expressed as mean ± SEM. Results were analyzed by one way analysis of variance (ANOVA), followed by Bonferroni's multiple comparison test. p value < 0.05 was considered as statistically significant.

RESULTS

Table 1 shows the values of serum total cholesterol, triglycerides, HDL, LDL and VLDL of day 29 expressed in mg/dl. Table 2 shows the atherogenic index recorded on day 29. The results are presented as mean ± standard error of mean (SEM) of six animals in each group.On day zero, the baseline serum lipid levels in Group I-VI estimated were comparable (p > 0.005). On day 15, after receiving cocktail diet for 14 days the serum lipid levels were measured to assess the induction of hyperlipidemia. The the serum lipid levels of group II to group VI were significantly higher except HDL levels when compared to baseline (Day 0) and normal control but was not statistically significant when compared to the disease control group (Group II).On day 29, after treatment for 14 days in Group III to Group VI the serum lipid levels were measured again. There was significant decrease (p value < 0.05) in the serum total cholesterol, triglycerides, LDL, VLDL and atherogenic index at all doses of the extract when compared to the disease control group (Group II). Group VI (EEBP 400mg/kg) showed the most significant effect though the effect was not significantly higher than the Atorvastatin treated group (Group III).The study demonstrated

Table 1 : Effect of ethanolic extract of leaves of Bryophyllum pinnatum on serum total cholesterol, triglycerides, HDL, LDL and VLDL in hyperlipidemic cocktail induced hyperlipidemia in rats.

(Mean serum levels expressed in mg/dl ± SEM)

GROUPS n=6	TCH	TGL	HDL	LDL	VLDL
Normal control	86.333 = 0.769	84.333 ± 2.329	30.833 ± 4.838	36.233 ± 3.905	17.266 ± 0.154
Disease control	179.333 ± 2.704 [a]	202.666 ± 3.347[a]	31.166 ± 1.234	135.633 ± 1.923 [a]	35.866 ± 0.541[a]
Hypolipidemic Standard	92.333 = 2.388 [b]	102.333 ± 2.117 [a, b]	47.833 ± 2.763 [a, b]	36.033 ± 4.296 [b]	18.466 ± 0.477
Test drug 100mg/kg	157.8333 ± 2.019 [a, b]	174.666 ± 1.805 [a, b]	33.833± 3.832[a]	109.266 ± 3.911 [a, b]	31.566 ± 0.403 [a]
Test drug 200mg/kg	124.333 ± 2.0367 [a, b]	147.666 ± 1.284 [a, b]	34.833 ± 3.585 [a]	87.966 ± 3.856 [a, b]	24.866 ± 0.407 [a, b]
Test drug 400mg/kg	104.333 ± 2.490 [a, b]	117.5 ± 2.22 [a, b]	36.166± 4.406 [a]	60.466 ± 3.752 [a, b]	20.866 ± 0.498 [a, b]

a=p < 0.05 when compared to the Normal control group, Group I

b=p < 0.05 when compared to the Hyperlipidemic control group, Group II

dose dependent reduction in serum total cholesterol, triglyceride, LDL, VLDL levels and the atherogenic index at doses 100mg/kg, 200mg/kg and 400mg/kg. Though there was increase in the HDL levels after treatment but the increase was not statistically significant. There was however no significant difference in the serum lipid levels in the normal control group (Group I) throughout the duration of the study.

Histopathology:

Fig 1 shows the histological study of all the groups. A considerable difference was observed in the hyperlipidemic control group (II) and the test drug treated groups(IV-VI) when compared with normal control group(I). The aorta of the normal control group showed a normal architecture with intact endothelial lining and compact vessel wall with no infiltration

Fig. 1 : Histopathological study of the effect of ethanolic extract of leaves of *Bryophyllum pinnatum* on the aorta in hyperlipidemic cocktail induced hyperlipidemia in rats.

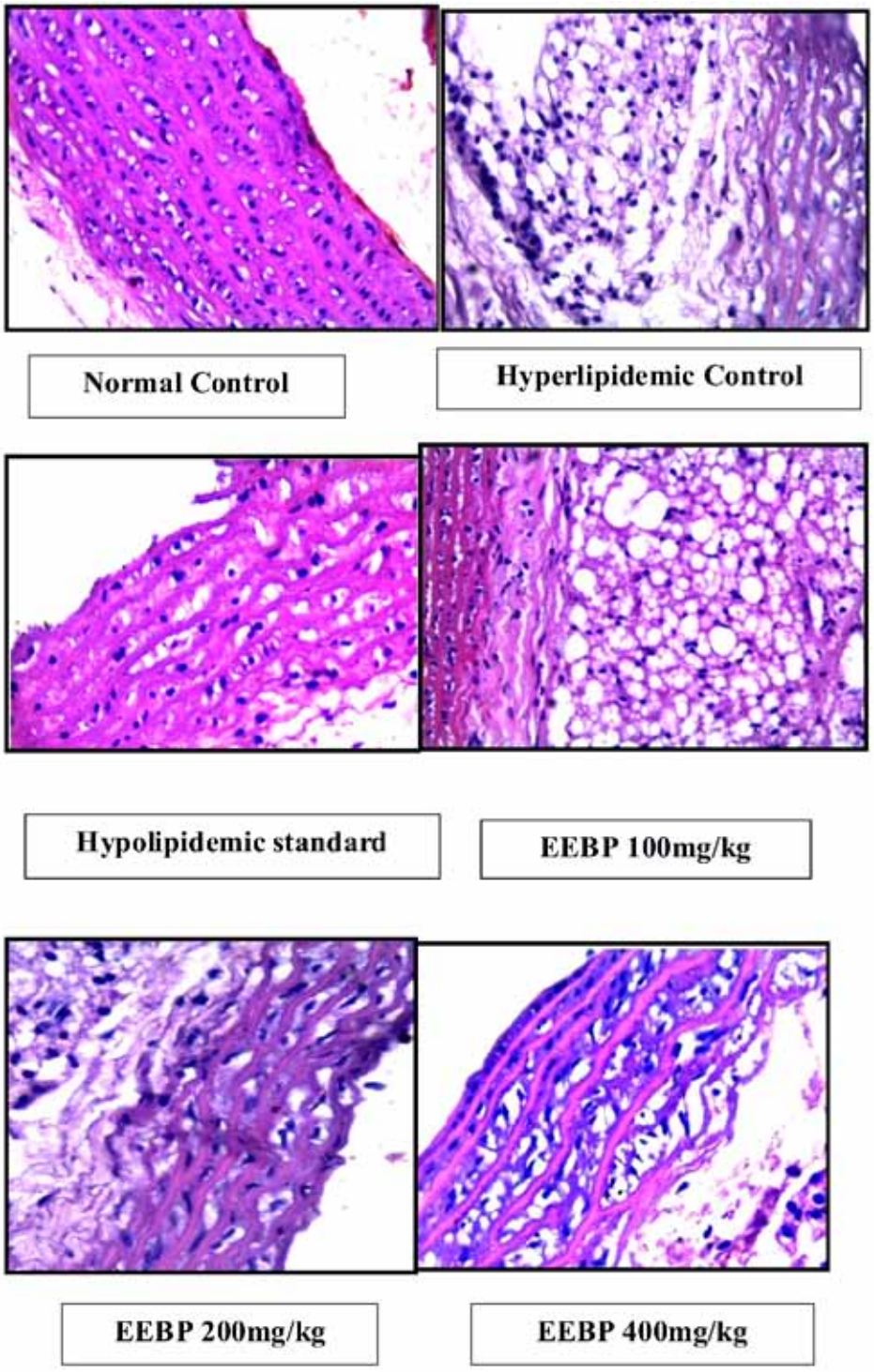

Table 2 : Effect of ethanolic extract of leaves of *Bryophyllum pinnatum* on atherogenic index in hyperlipidemic cocktail induced hyperlipidemia in rats.

(Mean atherogenic index ± SEM)

GROUPS	ATHEROGENIC INDEX
n=6	On Day 29
Normal control	1.976 ± 0.38
Disease control	5.542 ± 0.177
Hypolipidemic Standard	1.203 ± 0.188 [b]
Test drug 100mg/kg	4.688 ± 0.785 [a, b]
Test drug 200mg/kg	3.522 ± 0.463 [a, b]
Test drug 400mg/kg	2.492 ± 0.342 [a, b]

a=p < 0.05 when compared to the Normal control group, Group I

b=p < 0.05 when compared to the Hyperlipidemic control group, Group II

between the layers of the wall. There was no deposition of fat cells or foam cells in this group. Deposition of abundant fat cells and foam cells with disruption of the endothelial lining were observed in the hyperlipidemic control group. There was widening of the arterial wall due to deposition of fat cells and inflammatory cells between the layers of the wall. These changes were least in the Atorvastatin treated group. In Group IV (100mg/kg EEBP) there was abundant deposition of fat cells and inflammatory cells but was lesser than the hyperlipidemic control group. Group VI showed very little changes with only few fat cells and inflammatory cells in the arterial wall.

DISCUSSION

The hypolipidemic action of *Bryophyllum pinnatum* can be attributed to its phytochemical constituents. The phytochemical screening of *Bryophyllum pinnatum* by Prasad et al revealed the presence of steroids, terpenoids, flavonoids, phenolics, tannins, alkaloids, glycosides, carbohydrates & proteins. Ethanolic extract of the leaves showed positive tests for flavonoids, steroids, terpenoids, phenolics, tannins, alkaloids and glycosides. [8]

Ogbonnia et al in their study suggested that the synergistic interaction of polyphenols, steroids and tannins contents in the extract may impart hypolipidemic property to the extract. [9]

Many studies have shown that flavonoids possess hypolipidemic activity.

De Whalley et al in their cell culture studies showed that flavonoids inhibit the oxidative modification of low density lipoproteins by macrophages. [10]

Hodek et al reported that flavonoids activate multi enzyme systems, such as cytocrome P450 and b5. Due to this effect, flavonoids act on body lipid constituents like steroids and bile acids, and influence lipid metabolism. They increase bile acid excretion because cytochrome P450 enzymes bind some compounds to the bile acids and therefore reduce cholesterol level in the body. [11]

Gomes et al reported the triglyceride-lowering effect of flavonoids, while G. V. Gnoni et al showed quercetin induced decrease in both de novo fatty acid and triglyceride synthesis, with a consequent reduction in VLDL-TG formation. [12,13]

Tania et al in their study reported that, flavonoids lower triglyceride levels, probably through activation of cAMP synthesis. cAMP activates protein kinase which in turn increases triglyceride hydrolysis, and hence reduces its levels in blood and liver. [14]

Kirk et al demonstrated that dietary isoflavones reduce plasma cholesterol and atherosclerosis in C57BL/6 mice. These findings suggested that isoflavones might lower cholesterol levels by increasing LDL receptor activity.[15]

Jean Bruneton in his book "Pharmacognosy, Phytochemistry, Medicinal Plants" reported that steroid containing drugs have been found to lower blood cholesterol and lipids levels in animals and increases HDL-cholesterol/total cholesterol ratio.[16] Furthermore, reports by Ikeda et al and Plosch et al have suggested that several plant sterols reduce serum cholesterol by the inhibition of intestinal cholesterol absorption.[17,18]

Niroumand et al. demonstrated that atherogenic index has as predictive value for atherosclerosis and can be used as an available index of highest sensitivity for assessing cardiovascular risk factors, and for predicting the acute coronary events.[19]

Sindhu et al showed that various solvent extracts from *Bryophyllum pinnatum* leaves showed varying degrees of antioxidant activity in different test systems in a dose-dependent manner.They stated that the many pharmacological effects of phenolic compounds flavonoids are linked to their ability to act as

strong antioxidants and free radical scavengers. These support the role of *Bryophyllum pinnatum* in preventing atherosclerosis as antioxidants are strong antiatherosclerotic agents.[20]

Thus, the hypolipidemic effect of *Bryophyllum pinnatum* can therefore be attributed to its phytochemical constituents. The flavonoids, steroids, alkaloids, glycosides, tanins etc present in *Bryophyllum pinnatum* can be considered to be the active constituents responsible for the hypolipidemic activity demonstrated in the present study. The role and different mechanisms of flavonoids and sterols in lowering serum lipid levels have been demonstrated in other studies and these might be the mechanism of *Bryophyllum pinnatum* in causing hypolipidemia. Moreover, *Bryophyllum pinnatum* also prevents the development of atherosclerosis by decreasing the hyperlipidemic changes as well as by its antioxidant property. However, further investigations can provide more accurate details about the mechanisms by which *Bryophyllum pinnatum* acts as a hypolipidemic.

CONCLUSION

From the above results it can be concluded that the ethanolic extract of leaves of *Byophyllum pinnatum* possessed significant hypolipidemic effects and reduced atherogenic changes in cholesterol rich cocktail diet induced hyperlipidemia in rats but further investigations have to be done to understand the exact mechanism of the effects.

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