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Antihyperlipidemic activity of B*auhinia purpurea* extracts in hypercholesterolemic albino rats

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Abstract: *Bauhinia purpurea* is a flowering plant. Several species of this plant are known to possess pharmacological activities. It is a less explored plant, but it possesses phytoconstituents such as flavonoids which are purported to exhibit wide pharmacological activities. In the present study the ethanol extract of unripe pods and leaves of *Bauhinia purpurea* was evaluated for antihyperlipidemic activity in cholesterol high fat diet (CHFD) induced hyperlipidemia. Hyperlipidemia was induced by giving high cholesterol diet in standard rat chow diet for thirty days. The groups of rats selected for the study were treated with atorvastatin, ethanol extract of unripe pods and ethanol extract of leaves daily for the whole period. Changes in body weight and the analysis of serum lipids were carried out at the end of the study. There was a marked decrease in body weight, total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) levels. Also there was a significant increase in high density lipoprotein levels after the treatment with *Bauhinia purpurea* extracts. Ethanol extract of leaves showed a marked effect over body weight reduction and also had a significant effect on the lipoprotein profile. There is a lowered atherogenic index, TC: HDL-c and LDL: HDL-c ratios in the extract treated groups. The present work indicated that *Bauhinia purpurea* extracts significantly suppressed the CHFD induced hyperlipidemia in rats, suggesting the antihyperlipidemic and antiatherogenic potential of the extracts. Further studies are needed to characterize the phytoconstituents responsible for the study.
Key words: Cholesterol diet; leaf extract; unripe pod extract; biochemical parameters.

Introduction

Coronary arterial diseases are responsible for more deaths than all other associated causes combined[1]. Hyperlipidemia is a major cause of atherosclerosis and atherosclerosis-associated conditions, such as Coronary Heart Disease (CHD), ischemic cerebrovascular disease and peripheral vascular disease[2]. Among these hypercholesterolemia and hypertriglyceridemia are closely related to ischemic heart disease[3]. Reduction in serum cholesterol levels reduces the risk for CHD[1]. 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[4]. Currently available hypolipidemic drugs have been associated with a number of side effects[5]. The consumption of synthetic drugs leads to hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver

function[3]. Any herbal treatment for hypercholesterolemia has almost no side effects and is relatively cheap, locally available. They are effective in reducing the lipid levels in the system[6]. Medicinal plants play a major role in antihyperlipidemic activity[3].

Bauhinia purpurea is a species of flowering plant in the family Fabaceae, native to South China (which includes Hong Kong) and southeastern Asia. In the United States of America, the tree grows in Hawaii, coastal California, southern Texas, and southwest Florida. Common names include Hong Kong Orchid Tree, Purple camel's foot, and Hawaiian orchid tree. Several species of this plant are known to possess pharmacological activities. Aqueous extract of leaves have antinociceptive, anti-inflammatory, antipyretic[7], hypoglycaemic[8], nephroprotective[9], antimalarial, antimycobacterial, antifungal and cytotoxic activities[10]. Antioxidant and hepatoprotective activities of *Bauhinia* species have also been reported[11]. Methanol extract obtained from *Bauhinia purpurea* led to the isolation and identification of 6-butyl-3-hydroxy flavone[12].

There is no study reported for treating the hyperlipidemia with ethanol extract of unripe pods and leaves of *Bauhinia purpurea* at the dose of 300 mg kg^{-1} using albino rats fed on a high cholesterol diet. Hence, the present study was undertaken to demonstrate the effect of ethanol extract of unripe pods and leaves of *Bauhinia purpurea* on lipid profile of hyperlipidemic rats using standard lipid lowering agent atorvastatin.

Materials and Methods

Plant material

Bauhinia purpurea leaves and unripe pods were collected in the month of February, from Dhullapally, Rangareddy district, Hyderabad. The plant was authenticated by Dr. Ram Chandra Reddy, Head, department of Botany, Osmania University. A voucher specimen (MRCP-106) is deposited for further reference. Leaves and unripe pods were air dried, powdered to 40 mesh and subjected to Soxhlet extraction with 99% ethanol. The extract was concentrated under reduced pressure. The percentage yield obtained was 23%w/w and 17%w/w with respect to dried leaves and unripe pods respectively. Leaf extract was suspended in 1% Tween-80 due to its sticky constituency and unripe pod extract in 1% gum acacia for oral administration.

Animals

Albino rats weighing 200-250 g of either sex, 4 months of age were used for this study. The experimental animals were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at 25±3◦ C and 35-60% humidity). Standard pelletized feed and tap water were provided *ad libitum*. The Institutional Animal Ethical Committee of Malla Reddy College of Pharmacy, Hyderabad, with Reg. No. 1217/a/08/CPCSEA, approved the study.

Acute Toxicity studies

The acute toxicity of the ethanol extract of leaves and unripe pods was determined using albino rats of either sex, those maintained under standard conditions. The animals were fasted overnight prior to the experiment. Fixed dose (OECD Guideline No. 423) method of CPCSEA was adopted for the toxicity studies. Mortality was observed at the dose of 1500 mg/kg for the extracts. Hence 1/5th of the LD_{50} dose i.e. 300 mg/kg of the ethanol extracts was selected for the study.

Antihyperlipidemic activity

Animals were divided into 5 groups with 6 animals per group.

Group1: Normal control.

Group2: Hyperlipidemic control (Vehicle 1ml/100gm/day p.o)

Group3: Hyperlipidemic treated with Atorvastatin (5mg/kg, p.o)

Group4: Hyperlipidemic treated with unripe pods extract (300mg/kg, p.o)

Group5: Hyperlipidemic treated with leaf extract (300mg/kg, p.o)

The animals were administered with corresponding treatments for one month.

Induction of Hyperlipidemia:

High Cholesterol diet was prepared by mixing cholesterol 2%, sodium cholate 1% and coconut oil 2%, with powdered standard animal food. The diet which was prepared as pellets was placed in the cage carefully and was administered for 30days[13].

Biochemical assays for lipids:

At the end of treatment period, all the animals were tested for biochemical lipid markers. Blood was collected by cardiac puncture method under ether anesthesia. Serum total cholesterol (TC), triglycerides (TG) was estimated by method of CHOD-PAP and high-density lipoprotein-cholesterol (HDL-c) by the method of GPO-PAP using span diagnostic kits. Serum LDL-c[14], VLDL-c level and atherogenic index was determined by calculation[15].

Statistical Analysis:

All the results were expressed as mean±SEM and subjected to One way analysis of variance followed by

Dunnet's test for comparison between the groups and P<0.05 was considered significant.

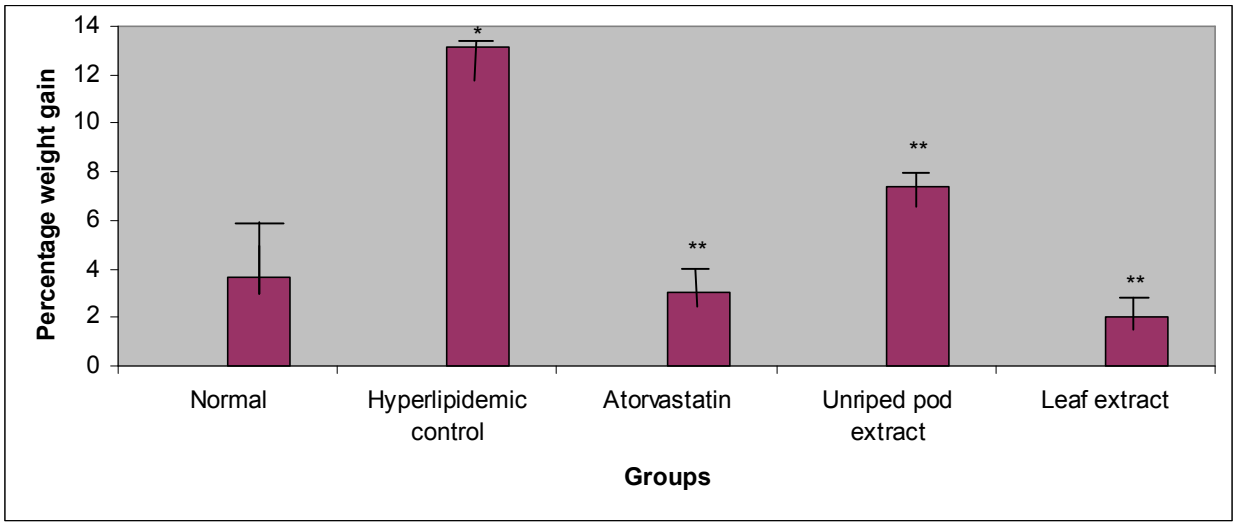

Fig.1: Effect of ethanol extract on the body weight in high cholesterol diet. The values are expressed as mean±SEM, n=6 in each group. *P<0.05 significant as compared to control, **P<0.05, significant as compared to hyperlipidemic control, statistical test employed was ANOVA followed by Dunnet's t test.

Table 1: Effect of *Bauhinia purpurea* on serum lipid level in CHFD induced hyperlipidemia

Groups	Dose (mg/kg)	TC	TG	HDL	LDL	VLDL
Normal	--	120.81±1.1	107.13±2.9	51.45±2.7	42.14±5.0	23.91±3.9
Hyperlipidemic Control	Tween-80 1%	300.31±2.4*	286.47±3.0*	30.41±2.9*	200.62±2.5*	60.44±1.2*
Atorvastatin	5	138.44±2.8**	128.41±2.1**	75.42±1.8**	37.33±2.1**	5.60±4.0**
Unripe pod extract	300	212.43±2.9*	206.26±1.6*	68.42±3.0**	103.67±6.5*	41.25±2.9*
Leaf extract	300	181.37±3.6**	166.43±1.8**	70.55±2.4**	54.90±3.8**	35.41±2.0**

The values are expressed as mean±SEM, n=6 in each group. *P<0.05 significant as compared to control, **P<0.05, significant as compared to hyperlipidemic control, statistical test employed is ANOVA followed by dunnet's t test.

Table 2: Effect of *Bauhinia purpurea* on serum lipoprotein ratio

Groups	Dose (mg/kg)	Atherogenic Index	TC: HDL-c	LDL: HDL-c
Normal	--	1.2	2.3	0.8
Hyperlipidemic control	Tween-80 1%	8.5	9.8	6.5
Atorvastatin	5	0.83	1.8	0.5
Unripe pod extract	300	2.1	3.1	1.5
Leaf extract	300	1.3	2.5	0.7

Results and Discussion

Many phytochemical analysis reports revealed the presence of flavonoids, carbohydrates, glycosides, tannins, volatile oils, anthocyanidins, lactones and terpenoids as reported by Bhartiya & Gupta[16]. Chemical tests were carried out on the *Bauhinia purpurea* extracts using the standard procedures available in text books which revealed the presence of carbohydrates, proteins, alkaloids, flavonoids, triterpenes, glycosides and steroids.

Rats fed with CHFD, for one month displayed an increase in body weight as compared to normal rats. Treatment with ethanol extract of unripe pods (300mg/kg/day) and leaves (300mg/kg/day) showed only slight increase in body weight to 7.4% and 2.0%, respectively, as compared to hyperlipidemic group (13.11%). The hyperlipidemic animals when treated with leaf extract shows only slight increase in body weight (2.0%), which was comparable to atorvastatin treatment (3.06%). The results suggest the potential of *B. purpurea* extracts against obesity (Fig.1).

There was significant increase in the levels of serum TC, TG, LDL-c and VLDL-c in CHFD induced rats and also there was a significant reduction in HDL-c levels in these animals. Treatment with unripe pods extract and leaf extract showed a marked reduction in TC, TG and LDL-c levels. But there was a significant rise in HDL-c levels in all the groups. Atorvastatin also produced significant reduction in serum TC, TG, LDL-c levels and a rise in HDL-c levels (Table1).

There was a marked reduction in TC: HDL-c ratio, LDL: HDL-c ratio and the atherogenic index after the treatment of rats with 300mg/kg dose of ethanol extract of unripe pods and leaves of *Bauhinia purpurea* (Table 2). TC: HDL-c ratio, LDL: HDL-c ratio is an effective predictor of coronary risk[17]. Atherogenic index is an important indicator of CHD risks at both high and low serum cholesterol level[18]. The cholesterol lowering effect of the extracts might be due to inhibition of dietary cholesterol absorption and/or esterification. Since two enzymes are involved in these two processes pancreatic cholesterol esterase[19] and intestinal acyl Co-A-Cholesterol acyl transferase enzyme (ACAT)[20], thus it could be suggested that the extracts inhibits one or both enzymes activity. In the present study, the activity of the extracts may be due to direct inhibition of cholesterol absorption or due to increased biliary excretion of sterol and /or bile acids and the block of cholesterol movement from the liver to the blood; as cholic acid was one of the ingredients of cholesterol high fat diet.

Conclusion

The activity may be due to the presence of polyphenolic compounds flavonoids, tannins and proanthocyanidines in the ethanol extracts, which reduce oxidation of LDL-c[21]. This needs to be studied further by assay of oxidized LDL. The ethanol extract of leaves has significant weight reduction property than unripe pods extract which was comparable to that of Atorvastatin. Ethanol extract of leaves also had a marked effect on antihyperlipidemic activity.

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