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Anti hyperlipidemic activity of *Adenanthera pavonina* Linn. ethanolic bark extract fractions

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Abstract

Adenanthera pavonina Linn. (Family: Leguminoceae) otherwise called as Bead tree, Red wood (English) and Kanchan Daana, Rakta Kambal (Hindi) is utilized for many remedies traditionally. The bark is used in colonorrhea, ulcers, pharyngopathy, vitiated conditions of vata and gout and rheumatism. Lipid lowering effect of the successive fractions of ethanolic bark extracts of *Adenanthera pavonina* (L.) was evaluated in triton and diet induced hyperlipidemic models of wistar albino rats. The ethyl acetate fraction and n- butanol fraction of ethanolic extract at 400 mg/kg dose levels inhibited the elevation in serum cholesterol and triglyceride levels on Triton WR 1339 administration rats. The extract fractions at the same dose level significantly attenuated the elevated serum total cholesterol and triglycerides in high-fat diet-induced hyperlipidemic rats. The standard dose Atrovastatin studies showed slightly better effects. The lipid lowering activity of the ethyl acetate fraction and n- butanol fraction of ethanolic extracts of *Adenanthera pavonina* Linn. may be attributed to the phytoconstituents present, such as triterpenoids, flavonoids, tannins, and saponins. The findings of the study reveals that ethyl acetate fraction and n- butanol fraction of ethanolic bark can effectively control the blood lipid levels in dyslipidaemic conditions by interfering with the biosynthesis of cholesterol and utilization of lipids. The main reason of the study is to find alternative and safe drugs to treat the hyperglycemia.

Key words: *Adenanthera pavonina* Linn., Antihyperlipidemic activity, Triton WR 1339, High-fat diet, Cholesterol, Triglyceride.

Introduction

Adenanthera pavonina Linn. (Family: Leguminoceae) is a deciduous fast growing, unarmed tree, found naturally in India. Traditionally bark and leaves are used as astringent, vulnerary, anthelmintic and aphrodisiac. The bark is also used in colonorrhea, ulcers, pharyngopathy, vitiated conditions of vata and gout and rheumatism (Vaidyaratnam *et al.*,1994). The seeds are bitter, astringent, sweet, cooling, aphrodisiac, antiemetic and febrifuge. The heart wood is astrigent, aphrodisiac, heamostatic and is useful in dysentery, haemorrhages and vitiated condition of vata. The roots are reported to be emetic in nature (Khare *et al.*,2004).The bark was reported to contain stigmasterol, glucoside, butein, chalcone, dihydrorobenetin, dihydromyricetin, 2,4 dihydrobenzoic acid, robinetin and saponin.

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The present study is an attempt to validate lipid lowering activity of petroleum-ether, diethyl ether, ethyl acetate and n-butanol fractions of ethanolic extract of *Adenanthera pavonina* Linn. bark. From the present study it was revealed that ethyl acetate fraction and n- butanol fraction of ethanolic bark extract of *Adenanthera pavonina* Linn. can effectively control the blood lipid levels in dyslipidaemic conditions by interfering with the biosynthesis of cholesterol and utilization of lipids.

Material and Methods

Plant materials

Adenanthera pavonina Linn. bark were collected from the local area of Salipur, Cuttack, Orissa, during January-February and was authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Center (PARC), Tambaram, Chennai, India, and a voucher specimen holding No.PARC/2007/82, was deposited in the same center. The air dried bark were extracted with petroleum ether (60-80ºC), ethyl acetate, chloroform and ethanol successively by using soxhlet apparatus and the yields were found to be

3.6 w/w, 4.2 w/w, 10.24% w/w 16.78 w/w, respectively, on dried weight (Houghton J.P *et al.*, 1998). The ethanolic extract was further fractionated with petroleum ether, diethyl ether, ethyl acetate and n- butanol successively. Phytochemical screening gave positive test for lipids, steroids, terpenoids, flavonoids, tannins, saponins and sugars in ethanolic extract ; lipids, steroids, terpenoids in petroleum ether fraction; Steroids, terpenoids, flavonoids in diethyl ether fraction; flavonoids, tannins, saponins in ethyl acetate fraction and flavonoids, tannins, saponins for n- butanol fraction of ethanolic bark extract (Harborne, 1998).

Chemicals and reagent

Triton WR 1339 (Sigma USA) which is used to induce hyperlipedimia in rats were brought from commercial sources. Serum Cholesterol and triglycerides were determined by using the kits of Qualigens fine chemicals. Atorvastatin were obtained from DR. Reddy's.Lab, Hyderabad and Sun pharmaceuticals, India respectively.

Animals

Male wistar strain albino rats (150-250 gm) were obtained from Central Animal House of Institute of Pharmacy and Technology, Salipur, Cuttack, Orissa India. The animals were housed under standardized environmental conditions (at normal room temp, with a 12 hour light and dark cycle) and fed with standard pellet chow feed and water *ad libitum*. The animal protocol was approved by Institutional Animal Ethical Committee of I. P. T., Salipur, Cuttack, Orissa, India with registration number 1053/ac/07/CPCSEA. All the experiments were performed as per the CPCSEA guidelines.

Triton WR induced hyperlipidemic study

The use of Triton WR 1339 induced hyperlipidemia through accelerated hepatic cholesterol synthesis was suggested as an important approach to screen the action of hypolipidemic drugs (Franz *et al.*,1955). Male wistar rats weighing 200-250 g were divided into 6 groups of 6 animals each. Group-1(Vehicle control) received 0.3% w/v carboxy methyl cellulose (CMC) orally for one week. Group 2-5 received Petroleum-ether fraction, Diethyl ether fraction, Ethyl acetate fraction and n- Butanol fraction of ethanolic extract respectively at the dose of 400mg/kg body weight and Group-6 received Atrovastatin 1mg/kg body weight once daily for one week. On seventh day, 200 mg/kg Triton WR 1339 (isooctyl polyoxyethylene phenol) was injected (ip), to all the six groups of rats immediately after drug administration. Serum total cholesterol and triglycerides were estimated for individual animals in autoanalyser (Microlab 100) on seventh day previous to drug treatment and after 24 hr of Triton administration. Blood was withdrawn from retro-orbital sinus using glass capillary in EDTA coated tubes and serum was separated in cooling centrifuge (Remi, C24) by centrifuging at 2500 rpm for 10 min. The observations made were recorded in Table-1.

Table -1: Effects of the various ethanolic extracts fractions of *Adenanthera pavonina* Linn. bark on lipid profile in triton induced hyperlipidemia.

Groups	Total Cholesterol(mg/dl)		Triglycerides(mg/dl)	
	0 hr	24 hr	0 hr	24 hr
Control	47.0±3.2	182.3±4.2	73.3 ±5.5	356.2 ± 4.7
Pet- ether fraction	52.5±2.7	176.4±3.2	68.5±2.4	312.4 ± 2.6
Diethyl ether fraction	45.3±2.9	174.0±2.6	72.2±3.1	305.5 ± 2.3
Ethyl acetate fraction	45.8±3.7	172.2±2.8	73.4±3.3	298.5 ± 2.3
n- Butanol fraction	45.6±2.3	135.5±5.2*	65.3±5.9	227.3± 2.6*
Atrovastatin	49.5±2.7	118.6±4.3*	68.3±2.4	214.3±2.4*

Values are mean ± SEM of 6 rats in each group*-P<0.001 compared with vehicle (untreated) control

High-fat diet-induced hyperlipidemic study

Hyperlipidemia was induced in male wistar rats weighing 150-180 g by feeding them with a high fat diet, (Table-2) for 4 weeks (Fillionis *et al.*,1956). High-fat diet increased the serum cholesterol and triglycerides to about 75-80% of the normal levels significantly (Table- 3). The rats with significantly higher values of serum cholesterol and triglyceride values compared to that of normal animals were considered to be hyperlipidaemic and six hyperlipidaemic animals were grouped for one treatment. Group 1- received 0.3% w/v CMC and served as vehicle control, Group 2-5 received Pet.ether fraction, Diethyl ether fraction, Ethyl acetate fraction and n- butanol fraction of ethanolic extract

respectively at the dose of 400mg/kg body weight. Hyperlipidaemic animals of sixth group were administered with the standard drug Atrovastatin 1 mg/kg body weight for one week. All the six groups were kept on the same high fat diet throughout drug treatment. Serum total cholesterol, triglyceride of the non-fasted animals was estimated on seventh day after 1hr of dosing.

Table- 2: Constituents of high fat diet

Ingredients	Quantity (g/100g)
Cornflour	25
Milk power	15
Sucrose	15
Casein	5
Egg yolk	3
Lard	1
Salt mixture	1
Cholesterol	1

Statistical analysis

Data are represented as mean ± SEM (Standard error of mean). The group means were compared for significant difference ($p<0.01$) by Student's t test in triton model and paired t-test in diet model.

Results and Discussion

The systemic administration of the surfactant Triton to rats resulted in an enormous elevation of serum cholesterol and triglycerides at 24 hr (Table -3). The petroleum-ether fraction, diethyl ether fraction, ethyl acetate fraction and n-butanol fraction of ethanolic extracts of *Adenanthera pavonina* Linn. inhibited the highly significant elevation in cholesterol at 400 mg/kg dose levels, respectively as compared to that of untreated vehicle control group. Similarly the above successive ethanolic extract fractions of *Adenanthera pavonina Linn* lowers the Triglycerides levels in comparison to that vehicle control rats (Table- 2). Atrovastatin, the lipid controlling mechanism of which is inhibition of synthesis of cholesterol in the liver, was employed as the standard drug in Triton induced model. The treatment with Atrovastatin resulted in a slightly better effect than *Adenanthera pavonina* Linn. These results indicate that the various fractions of ethanolic extract of *Adenanthera pavonina* Linn. may interfere with cholesterol biosynthesis as Triton accelerates the hepatic synthesis of cholesterol (Gerhard, 1997).

Table -3: Effects of the various ethanolic extracts fractions of *Adenanthera pavonina* Linn. bark on lipid profile of hyperlipidemic wistar rats in diet-induced hyperlipidemia

Groups	Total cholesterol (mg/dl)			Triglycerides(mg/dl)		
	Normal value	On induction of hyperlipemia		Normal value	On induction of hyperlipemia	
		0^{th} day	7^{th} day		0^{th} day	7^{th} day
Control	50.6±2.5	71.2±3.2	77.5±2.8	68.7±2.8	131.5±2.1	172.5±4.2
Petroleum-ether fraction	48.8±2.2	74.5±1.7	71.7±4.2	70.6±2.2	133.7±2.5	164.2±3.1
Diethyl ether fraction	48.3±2.2	75.2±1.4	69.1±1.1	67.7±3.6	135.1±2.2	160.1±2.1
Ethyl acetate fraction	47.3±2.3	74.1±2.6	51.5±1.5*	65.2±2.7	134.3±2.8	95.7±2.6*
n-butanol fraction	47.3±2.1	72.1±2.5	50.5±1.3*	62.2±3.1	129.3±2.6	89.7±2.6*
Atrovastatin	48.3±3.2	74.5±2.5	48.5±1.7*	64.2±3.5	132.7±2.5	87.6±2.7*

Values are mean ±SEM of 6 rats in each group*- Represent values significantly different in paired t-test as compared to 0th day values ($P<0.01$)

Triton induced hypercholesterolemia, though simple and rapid for evaluating hyperlipidemic compounds, is rather artificial. Hence the lipid controlling potential of different fraction of *Adenanthera pavonina* Linn. bark was further validated in diet-induced hyprlipidemic rat model. When male wistar albino rats were kept on high-fat diet supplemented with 1% cholesterol for 4 weeks, there was elevated serum cholesterol levels and triglyceride levels were almost doubled (Table 3). Elevated circulating lipid levels may be the outcome of inhibitory effect of high dietary fat intake on lipogenesis (Rothwell *et al.*,1983). The treatment of hyperlipidemic rats with Ethyl acetate fraction and n-butanol fraction of ethanolic extract of *Adenanthera pavonina* Linn. bark for one week significantly brought down the elevated serum total cholesterol and triglycerides (Table-3). Similar to Gemfirozil (50 mg/kg) the standard fibrate drug used, the Ethyl acetate fraction and n- Butanol fraction of ethanolic bark extract may

have enhanced the breakdown of lipids, thus modifying the altered lipid metabolism induced by high fat-diet. A significant reduction of cholesterol and triglyceride by ethyl acetate fraction and n-butanol fraction of ethanolic bark extracts of *Adenanthera pavonina* Linn. treatment demonstrates the protective efficacy of these fractions against atherogenesis. The lipid lowering activity of the Ethyl acetate fraction and n- Butanol farction of ethanolic extracts of *Adenanthera pavonina* Linn. may be attributed to the phytoconstituents present, such as triterpenoids, flavonoids, tannins, and saponins in it, as reported for other plant extracts (Pengelly *et al.*,2004; Regisusan *et al.*,1998 and Marudamuthu *et al.*,2008).

Elevated serum concentrations of total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) appear to increase the risk of individual in developing coronary heart disease (CHD). The investigation of lipid lowering activity on herbs will be useful strategy in the discovery of new lead molecules eliciting improved activity by regulating through different mechanism of action. The plant extracts maintaining the lipid metabolism and thus can be used in treating hyperlipidemia of varied etiology. Saponin derived from *Medicago sativa* were reported to reduce blood cholesterol by competing with cholesterol at binding sites or interfering with cholesterol biosynthesis in the liver (Lanksy *et al.*, 1993). Phenolic active principle present in *Anethum graveolens* were observed to be responsible for lowering TC and LDL-C and elevating HDL-C in hypercholesterolemia rats (Yazdanparast *et al.*, 2008). The findings of the study reveals that Ethyl acetate fraction and n- Butanol fraction of ethanolic bark extract of *Adenanthera pavonina* Linn. can effectively control the blood lipid levels in dyslipidaemic conditions by interfering with the biosynthesis of cholesterol and utilization of lipids.

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