

Antihyperlipidemic Activity of *Pongamia pinnata* Leaf Extracts

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The aim of this study was to investigate the possible antihyperlipidemic effect of *Pongamia pinnata* (Leguminosae) leaf extract in triton (400 mg/kg b.w.) induced and atherogenic diet induced hyperlipidemic rats. Petroleum ether, chloroform, ethanol and aqueous extracts of leaves were evaluated for antihyperlipidemic. Antihyperlipidemic drug simvastatin (10mg/kg body wt.) was used as a positive control. The results of the study were expressed as mean± S.E.M. and data was analyzed by using one way analysis of variance test (ANOVA) followed by Dunnett's t-test for multiple comparisons. In diet induced model, chloroform extract showed significant serum lipid lowering parameters like total cholesterol, triglycerides, low density lipoprotein (LDL), very low density lipoprotein (VLDL) and increase in high density lipoprotein (HDL) in hyperlipidemic rats of both models as compared to hyperlipidemic control statistically. In triton induced model, oral administration of (500 mg/kg body wt.) of the chloroform extract and alcoholic extract were able to reduce serum lipid level significantly as compared to hyperlipidemic control. Our results demonstrated that chloroform extract of *P. pinnata* leaves possessed significant antihyperlipidemic activity hence it could be a potential herbal medicine as adjuvant with existing therapy for the treatment of hyperlipidemia.

Key words: Hyperlipidemia, *Pongamia pinnata*, Simvastatin, Triton.

Pongamia pinnata Yaprak Ekstrelerinin Antihiperlipidemik Aktivitesi

Bu çalışmanın amacı *Pongamia pinnata* (Leguminosae) yaprak ekstresinin olası antihiperlipidemik etkisinin triton ile indüklenmiş (400 mg/kg vücut ağırlığı) ve aterojenik diyetle indüklenmiş hiperlipidemik ratlarda incelenmesidir. Antihiperlipidemik ilaç simvastatin (10 mg/kg vücut ağırlığı) pozitif kontrol olarak kullanılmıştır. Çalışmanın sonuçları ortalama±standart hata (S.E.M.) olarak verilmiştir ve tek yönlü varyans analizini (ANOVA) takiben Dunnett's t-testi kullanılarak çoklu karşılaştırmalar yapılmıştır. Diyetle indüklenmiş modelde, kloroformlu ekstre hiperlipidemik kontrol grubu ile istatistiksel olarak karşılaştırıldığında total kolesterol, trigliseritler, düşük dansiteli lipoprotein (LDL), çok düşük dansiteli lipoprotein (VLDL) gibi serum lipid parametrelerini önemli ölçüde düşürmüş ve her iki modelin hiperlipidemik ratlarında yüksek dansiteli lipoprotein (HDL) değerlerini yükseltmiştir. Triton ile indüklenmiş modelde, oral yoldan 500 mg/kg vücut ağırlığı dozunda verilen kloroformlu ekstre ve alkollü ekstre serum lipid düzeylerini hiperlipidemik kontrol grubuna kıyasla önemli düzeyde düşürmüştür. Sonuçlarımız *P. pinnata* yapraklarının kloroformlu ekstresinin önemli antihiperlipidemik aktiviteye sahip olduğunu, bundan dolayı bitkinin hiperlipidemisinin tedavisinde var olan tedaviye adjuvan potansiyel bir bitkisel bir ilaç olabileceğini göstermiştir

Anahtar kelimeler: Hiperlipidemi, *Pongamia pinnata*, Simvastatin, Triton.

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INTRODUCTION

Hyperlipidemia is a secondary metabolic dysregulations associated with increased risk factors for development of diabetes. Beside the cause effect relationship with diabetes, elevated serum level of triglycerides, cholesterol and LDL are major risk factors for the premature development of cardiovascular diseases such as atherosclerosis, hypertension and coronary heart disease (1). Increased plasma lipid levels mainly total cholesterol; triglycerides and LDL along with decrease in HDL are known to cause hyperlipidemia which is the reason for initiation and progression of atherosclerosis impasse (2). Hyperlipidemia with increased concentration of cholesterol, triglycerides carrying lipoproteins is considered to be the cause of arteriosclerosis with its dual sequel of thrombosis and infarction. Hyperlipidemia is caused by over-ingestion of alcohol or foods (1).

Elevated lipid levels result from increased absorption through the gut or enhanced endogenous synthesis therefore two ways are feasible to reduce hyperlipidemia; to block endogenous synthesis or to decrease absorption. Both factors can be evaluated in normal animals without artificial diets.

Pongamia pinnata (Leguminosae) is a glabrous, semi-evergreen tree, growing up to 18 m or higher, with a short bole, spreading crown with grayish green or brown bark. Leaves are imparipinnate, alternate, and leaflets are 5-7 in number, ovate in shape and opposite in arrangement. This tree is popularly known as *Karanja* in Hindi, Indian Beech in English or *Derris indica* (synonym), and *Hongae* in Kannada. *P. pinnata* occurs all over India in the bank of rivers streams and planted as an avenue tree in gardens. The leaves of *P. pinnata* have been used in Ayurvedic medicine as digestive, laxative, anthelmintic, to cure piles, wound healing, relieving rheumatic pains, for cleaning ulcers in gonorrhoea and scrofulous enlargement. Previous studies have demonstrated that *P. pinnata* is rich in flavonoids and related compounds. Seeds and seed oil, flowers and stem bark contain karanjin, pongapin, pongaglabrone, kanugin, desmethoxykanugin and pinnatin (3). Furanoflavonoid glucosides

(pongamosides A-C) and flavonol glucoside (pongamoside D) have also been reported (4). The leaves and stem of the plant consist of several flavone and chalcone derivatives such as pongone, galbone, pongalabol, pongagallone A and B (5).

Anticonvulsant effect of the leaf extract was established by using pentylene tetrazole induced convulsion (PTZ) model in rats (6). Leaf extract showed significant activity on the levels blood ammonia, and serum lipid profiles (cholesterol, triglycerides, phospholipids, free fatty acids) for its protective effect during ammonium chloride induced hyperammonemia in Wistar rats (7). Studies on stem bark (8), fruits (9), and flowers (10) of *P. pinnata* showed significant antihyperglycemic activity with additional of flowers whereas studies on seed oil (11) proved to have fluorescent pyranoflavonoid.

Antihyperammonemic efficacy of the leaf extract was investigated on blood ammonia, plasma urea, uric acid, non-protein nitrogen and serum creatinine in control and ammonium chloride induced hyperammonemic rats (12). Ethanolic extract of leaves possessed significant anti-inflammatory activity in acute, subacute and chronic models of inflammation without ulcerogenic activity suggesting its potential as an anti-inflammatory agent for the use in treatment of various inflammatory diseases (13).

The search for new drug with the ability of reduce or regulate serum cholesterol and triglyceride concentrations has gained momentum over the years, resulting in a plethora of publications reporting significant activity of a variety of natural and synthetic agents. Molecular modification of naturally occurring compounds has also given rise to potent agents like pravastatin and simvastatin; the former prepared by replacement of the methyl group of naturally occurring lovastatin by a hydroxyl group and the latter a methylated derivative of compaction. In continuation of our search for plant derived antihypercholesterolemic and hypolipidemic agents, we directed our attention to some Indian medicinal plants for which antihyperlipidemic activity has not been scientifically validated.

EXPERIMENTAL

Plant material

Leaves of *Pongamia pinnata* were collected in and around local forest area of Sirsi in Western Ghats, Karnataka and authenticated by the Botanist Prof. G. S. Naik, Department of Botany, G. C. Science and Art College, Ankola. A voucher herbarium specimen number GCSAC/PP/01 was also preserved in the same college. The collected leaves were dried and powdered to coarse consistency in cutter mill. The powder was passed through 40 # mesh particle size and stored in an airtight container at room temperature.

Atherogenic diet and chemicals

Experimental hyperlipidemic diet: Experimental diet consists of well pulverized mixture of cholesterol (2%), cholic acid (1%), peanut oil (10%), sucrose (40%) and normal laboratory diet (47%).

Experimental hyperlipidemic agent: A suspension of triton –WR 1339 (S D Fine chemicals) in 0.15 M NaCl was used for inducing hyperlipidemia in experimental rats.

Simvastatin (Dr. Reddy's Laboratories, Hyderabad), Diagnostic kits for estimation were purchased from Merck Diagnostics India Ltd. anesthetic ether (Ozone International, Mumbai), and all other chemicals were of analytical grade.

Plant extract

2.5 kg of the fresh air-dried, powdered crude drug of *P. pinnata* leaves were extracted with petroleum ether (60-80 °C), chloroform, 95% ethanol and chloroform water by adopting simple maceration procedure at room temperature for 48 h in conical flask with occasional shaking and stirring. The extract was filtered and concentrated to dryness at room temperature to avoid the decomposition of the natural metabolites (14). All the extracts were preserved in a refrigerator till further use. Standardization of crude drug was carried out for morphology; microscopy and physicochemical parameters e.g. total ash, acid insoluble ash, moisture content and foreign organic matter. Preliminary phytochemical analysis was carried out in all 4 extracts by different methods of

phytochemical analysis (15). An extract volume was suspended in distilled water and was orally administered to the animals by gastric intubation using a force feeding needle during the experimental period.

Animals

Adult albino rats of wistar strain (150-200 g) of either sex were procured and housed in the animal house of K L E S College of Pharmacy, Ankola with 12 h light and 12 h dark cycles. Standard pellets obtained from Goldmohar rat feed, Mumbai India, were used as a basal diet during the experimental period. The control and experimental animals were provided food and drinking water ad libitum. All the animal experiments were conducted according to the ethical norms approved by CPCSEA, Ministry of social justice and empowerment, Government of India and ethical clearance was granted by institutional ethical committee in resolution no. 1/18/2007 held on 23rd November 2007 at J N Medical college, Belgaum (Ethical committee IAEC reg. no.: 627/02/a/CPCSEA).

Preparation of dose for dried extracts

The petroleum ether (60-80 °C), chloroform, alcoholic and aqueous extracts (500 mg/kg) of the plant were formulated as suspension in distilled water using Tween-80 as suspending agent. The strength of the suspension was according to the dose administered and was expressed as weight of dried extract (16).

Preparation of standard drugs

Simvastatin 10 mg/kg was used as the reference standard drug for evaluating the antihyperlipidemic activity which was made into suspension in distilled water using Tween-80 as a suspending agent.

Acute oral toxicity studies

The acute oral toxicity studies of extracts were carried out as per the OECD guidelines, draft guidelines 423 adopted on 17th December 2001 received from CPCSEA, Ministry of social justice and empowerment, Govt. of India. Administration of the stepwise doses of all 4 extracts of *P. pinnata* from 50 mg/kg up to the dose 5000 mg/kg caused no considerable signs of toxicity in the tested animals. One tenth of upper limit dose were

selected as the levels for examination of antihyperlipidemic activity (17).

Diet-induced hyperlipidemic model

The animals were selected, weighed then marked for individual identification. In this model rats were made hyperlipidemic by the oral administration of atherogenic diet for 20 days by mixing with regular pellet diet. Rats were given free access to the feed ad libitum. The rats were then given plant extracts suspended in 0.2% tween 80 at the dose of 500 mg/kg b.w. once daily in the morning through gastric intubation for 14 consecutive days. During these days, all the groups also received atherogenic diet in the same dose as given earlier. The control animals received the hyperlipidemic diet and the vehicle. At the end of treatment period, the animals were used for various biochemical parameters. Blood was collected by orbital plexus of rat under ether anesthesia and centrifuged by using centrifuge at 2000 rpm for 30 minute to get serum (18).

Triton-induced hyperlipidemic model

Animals kept for fasting for 24 h, were injected a saline solution of triton at the dose of 400 mg/kg intra-peritoneally. The plant extracts, at the dose of 500 mg/kg, were administered orally through gastric intubation, the first dose being given immediately after triton injection and second dose 20 h later. After 4 h of second dose the animals were used for various biochemical parameters. Blood was collected by orbital plexus of rat under ether anesthesia and centrifuged by using centrifuge at 2000 rpm for 30 minute to get serum (1).

Experimental design

Animals were divided into seven different groups with six animals in each group. Group I served as normal control and this group did not receive atherogenic diet and triton except regular standard pellet diet). Group II was positive control which was given standard antihyperlipidemic drug simvastatin (10 mg/kg/day p.o.). Group III was hyperlipidemic control and this group did not receive any treatment except atherogenic diet in case of diet induced and triton in case of triton induced hyperlipidemia Group IV, V,

VI and VII received different extracts of *P. pinnata* leaves (500 mg/kg/day, p.o.). Treatment period for all these groups was 14 days in atherogenic diet induced hyperlipidemia and 48 hours in case of triton induced hyperlipidemia.

Collection of blood

Blood was collected by retro-orbital sinus puncture, under mild ether anesthesia. The collected samples were centrifuged for 10 minutes.

Biochemical analysis

The serum was assayed for total cholesterol, triglycerides, phospholipids, high-density lipoprotein (HDL), low density lipoprotein (LDL), and very low density lipoprotein (VLDL) using standard protocol method. Serum total cholesterol, triglyceride was estimated by the method of CHOD-PAP and high density lipoprotein by the method of GPO-PAP. Low density and very low density cholesterol were calculated by using Friedwald formula and VLDL: TG/5 respectively (19, 20).

Statistical Analysis

The results of the study were expressed as mean \pm S.E.M. Data was analyzed by using one way analysis of variance test (ANOVA) followed by Dunnett's t-test for multiple comparisons. Values with $P < 0.05$ were considered significant (21).

RESULTS

Standardization parameters for *P. pinnata* leaves crude drug and extract were determined and all the parameters were found to be within Indian herbal pharmacopoeia and Ayurveda pharmacopoeia standards limit. Crude powder taken for extraction was of green color with slight bitter taste. Losses on drying, total ash, acid insoluble ash and water soluble ash were 3.67%, 6.35%, 3.54% and 1.05% w/w respectively.

Thin layer chromatography of *P. pinnata* leaves revealed yellow/orange spots/florescence with R_f values 0.56, 0.72, 0.43, 0.25, and 0.86. Antimony (III) chloride (10%) reagent was used as spraying agent for

detection of flavonoids. Phytochemical screening of all the extracts of *P. pinnata* showed the presence of various phytochemical constituents like flavonoids, triterpenoids, carbohydrates, tannins, phytosterols and traces of alkaloids.

Acute toxicity study

As per (OECD) draft guidelines 423 adopted on 17th December 2001 received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), young female albino rats were given 50-5000 mg/kg b.w. of *P. pinnata* extract for the purpose of toxicity study. Animals were observed at regular time intervals at least once during the first 30 minutes of initial dosing during the first 24 hrs. In all the cases no death was observed within first 24 hrs. Additional observations like behavioral changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous systems and somato motor activity and behavior pattern were also found to be normal. Attention was also given to observation of tremors and convulsions. Overall results suggested the LD₅₀ value as 5000 mg/kg. Hence therapeutic dose was calculated as 1/10th (500 mg/kg of the lethal dose for the purpose of antihyperlipidemic investigations.

The effect of various extracts, obtained from simple maceration of leaves of *P. pinnata* were studied on serum lipids and lipoproteins level of triton (400 mg/kg induced hyperlipidemic rats and results are expressed as change in serum lipid and lipoprotein levels.

Triton-induced hyperlipidemic model

As expected, administration of triton WR1339 led to elevation of serum lipid and lipoprotein levels, which were maintained over a period of study in hyperlipidemic control group and these rats, were given treatment with aqueous, alcoholic, chloroform and petroleum ether extracts of *P. pinnata* leaves. The results were comparable with reference standard simvastatin. There was a significant elevation in serum lipids and lipoproteins in triton induced hyperlipidemic control rats when compared with normal control. At this time an increased level of

HDL-Cholesterol was also observed. *P. pinnata* leaves chloroform extract reduced serum lipids significantly ($p < 0.001$) as compared to hyperlipidemic control statistically (Tables 1 and 2).

Diet-induced hyperlipidemic model

In diet induced model, chloroform extract showed significant serum lipid lowering effects in hyperlipidemic rats which brought down total cholesterol 75.16 ± 2.30 , triglycerides 70.83 ± 1.86 , phospholipids 81.83 ± 2.21 , LDL 48.33 ± 2.92 , VLDL 26 ± 1.48 and increased level of HDL 27 ± 1.50 in comparison of diet induced hyperlipidemic control total cholesterol 101.16 ± 2.61 , triglycerides 86 ± 2.28 , phospholipids 107.66 ± 2.64 , LDL 81 ± 2.55 , VLDL 35 ± 1.14 and HDL 21.08 ± 1.17 at 14th day.

In diet induced model, standard antihyperlipidemic agent simvastatin 10 mg/kg body weight also able to reduce the elevated serum lipid level towards the normal. It brought down total cholesterol 68 ± 2.86 , triglycerides 65.33 ± 1.80 , phospholipids 75 ± 1.52 , LDL 43.83 ± 2.18 , VLDL 24 ± 1.46 and increased level of HDL 28 ± 1.57 when compared to diet induced hyperlipidemic control total cholesterol 101.16 ± 2.61 , triglycerides 86 ± 2.28 , phospholipids 107.66 ± 2.64 , LDL 81 ± 2.55 , VLDL 35 ± 1.14 and HDL 21.08 ± 1.17 at 14th day.

DISCUSSION

Treatment with *P. pinnata* leaves extract (500 mg/kg) significantly decreased the level of cholesterol, triglycerides, phospholipids, VLDL and LDL as compared to hyperlipidemic control. There was significant increase in the HDL as compared to control. This effect may be due to the increased activity of lecithin: cholesterol acetyl transferase which incorporates free cholesterol, free LDL into HDL and transferred back to VLDL and intermediate density lipoprotein.

Table 1. Effect of *Pongamia pinnata* leaves extracts on serum total cholesterol, triglycerides and phospholipids level in triton induced hyperlipidemic rats.

Group Name ^a (Dose)	Values are expressed as mg/dL, Mean±S.E.M.											
	Cholesterol				Triglycerides				Phospholipids			
	6 hr	24 hr	48 hr		6 hr	24 hr	48 hr		6 hr	24 hr	48 hr	
Normal control (vehicle only)	60.83±1.32	68.17 ±1.70	64.66±1.54		65.33±2.14	67.16±2.02	64.83±1.70		75.50±3.30	78.33±0.91	74.00±1.36	
Hyperlipidemic control	106.50±4.08	260.33±5.92	178.50±3.64		101 ±3.00	208.66±5.46	107.83±3.20		106.50±2.32	185.83±2.60	100.83±2.48	
Simvastatin (10mg/kg)	83.67±1.83**	176.17±7.56**	73.83±2.82**		81.50±1.74**	172.50±3.63**	79.66±3.98**		91.66±1.72**	139.66±1.33**	80.33±1.40**	
Chloroform extract (500mg/kg)	86.00±2.67**	190.00±4.32**	84.00±2.54**		83.33±1.68**	187.66±4.91**	82.00±2.62**		92.50±3.64	146.66±2.18	86.00±2.62**	
Alcoholic extract (500 mg/kg)	88.83±1.51**	194.16±5.02**	90.16±1.99**		85.16±3.53**	175.00±3.08**	84.33±4.36**		95.00±2.70	152.83±2.38	88.00±2.46*	
Aqueous extract (500 mg/kg)	94.16±3.65*	220.83±7.93	136.00±2.74*		90.66±2.12*	178.33±3.84**	89.16±3.07**		99.33±3.78	160.16±3.67	93.33±1.43ns	
Petroleum ether extract (500 mg/kg)	102.00±2.70ns	260.00±5.132ns	172.33±2.27ns		98.83±2.81ns	194.66±3.87ns	97.50±4.08ns		101.16±2.67	167.00±4.02	98.00±3.59 ^{ns}	

^a mg/kg/day for 48 hours. Values are expressed as mean±S.E.M.; N=6. Values are statistically significant at * p < 0.05 and more significant at ** p < 0.01.

** p < 0.01vs Hyperlipidemic control (ANOVA).

Table 2. Effect of *Pongamia pinnata* leaves extracts on LDL, VLDL and HDL level in triton induced hyperlipidemic rats.

Group Name ^a (Dose)	Values are expressed as mg/dl, Mean±S.E.M.											
	LDL				VLDL				HDL			
	6 hr	24 hr	48 hr	6 hr	24 hr	48 hr	6 hr	24 hr	48 hr	6 hr	24 hr	48 hr
Normal control (vehicle only)	55.50±1.23	57.33 ±0.55	60.16±1.70	21.50±0.428	18.50±0.56	15.16±0.74	38.83±1.83	39.66±1.47	44.50±1.94			
Hyperlipidemic control	105.66±2.31	194.66±3.11	97.66±2.97	29.16±1.13	34.83±2.05	32.83±2.13	18.66±1.68	16.33±2.24	20.33±1.52			
Simvastatin (10mg/kg)	64.83±2.27	140.66±3.31	66.00±2.11**	24.33±1.05	27.83±1.4	19.00±0.73	31.66±3.50**	25.00±2.03**	42.00±2.80**			
Chloroform extract (500 mg/kg)	84.00±2.74**	168.00±4.16**	81.33±3.46**	24.16±1.75	28.16±1.81*	23.16±1.16**	29.16±1.62**	24.66±1.89**	33.50±1.14**			
Ethanollic extract (500 mg/kg)	90.16±3.01**	172.00±1.06**	83.16±2.63**	26.66±1.99	31.66±1.30	27.50±1.74*	27.00±2.03*	22.00±1.36**	30.16±2.00**			
Aqueous extract (500 mg/kg)	97.33±2.02ns	182.16±3.85*	93.00±3.24ns	28.00±2.01	33.00±2.21	29.33±2.01ns	22.33±1.33ns	20.50±1.11ns	26.33±1.60ns			
Petroleum ether extract (500 mg/kg)	102.00±2.00ns	190.16±3.40ns	97.66±2.61ns	29.00±1.65	34.16±1.24	30.33±2.10 ^{ns}	19.16±1.90ns	18.16±1.83	21.00±1.57ns			

^a mg/kg/day for 48 hrs. Values are means±S.E.M. N=6. Values are statistically significant at * p < 0.05 and more significant at ** p < 0.01.
** p < 0.01 vs Hyperlipidemic control (ANOVA).

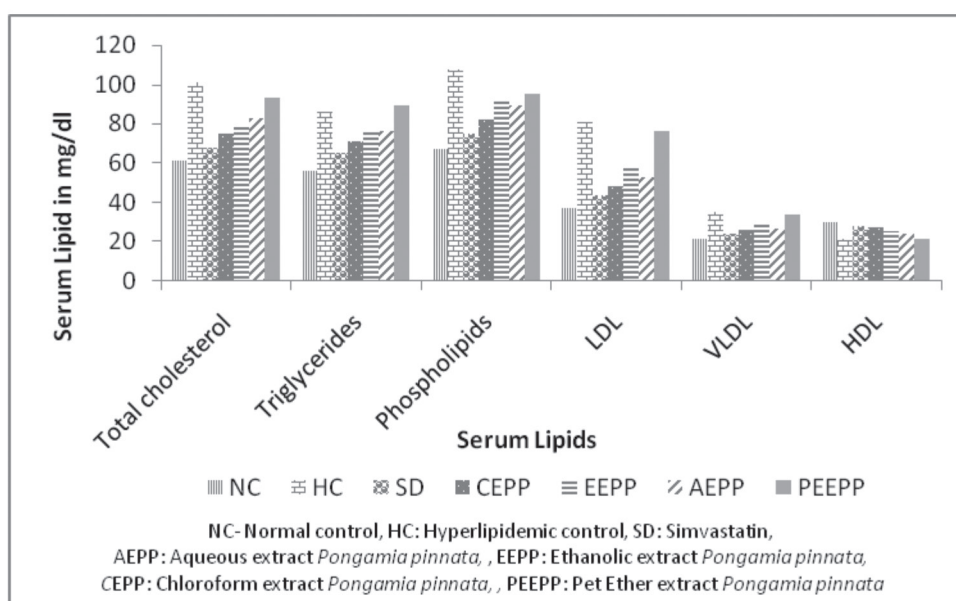


Figure 1. Effect of *Pongamia pinnata* leaves extract on serum lipid parameters in diet induced hyperlipidemic rats

Decrease in the triglyceride level may be due to the increase in activity of the endothelium bound lipoprotein lipase which hydrolyses the triglyceride into fatty acid or due to inhibition of lipolysis so that fatty acids do not get converted to triglyceride.

Hepatic cholesterol synthesis is accelerated by triton WR 1339. Moreover, triton physically alters very low density lipoproteins rendering them refractive to the action of lipolytic enzymes of blood and tissues, preventing or delaying their removal from blood (22). Hence the hypolipidemic effect of extracts could be due to an increased catabolism of cholesterol into bile acids.

CONCLUSION

The results obtained from the pharmacological screening have led to the conclusions that, chloroform extract of *P. pinnata* leaves have significant antihyperlipidemic activity. Hence it can be exploited as antihyperlipidemic therapeutic agent or adjuvant in existing therapy for the treatment of hyperlipidemia. Further study by measurement of heparin-releasable plasma LpL activity and LCAT activity is significant can be undertaken.

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