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Research article ***Clinical research***

Evaluation of anti hyperlipedimic activity of hibiscus cannabinus extract in experimental animal model

Gansala Sindhupriya, P.Mary*, Ramya Sri. S

Department of Pharmacology, Samskruti College of Pharmacy, Sangareddy, Telangana, India.
SuraPharma Labs, Dilsukhnagar, Hyderabad, Telangana-500060, India.

Address of Correspondence: P. Mary

ABSTRACT

To investigate the anti Hyperlipidemic activity of methanol extract of *Pterocarpus marsupium s* in male Wistar rats. In this model of Hyperlipidemia, 30 adult male wistar rats (150-200gms) were evenly divided into 5 groups in both groups. Group-1 and Group-2 served as untreated and model controls respectively, while Group-3, 4 and 5 were the treatments groups which were simultaneously treated with standard, 200 and 400 mg/kg extract respectively along with High Fat Diet and Triton x 100. On last day, blood samples for biochemical parameters, were obtained under inhaled diether anaesthesia. In the model of anti diabetic animals were evenly divided into 5 groups. Group-1 and Group-2 served as untreated and model controls respectively, while Group-3, 4 and 5 were the treatments groups which were simultaneously treated with standard, 200 and 400 mg/kg extract respectively after glucose loading. HFD and Triton x 100 treatment caused Hyperlipidemia as evidenced by marked elevation in Cholesterol, Triglycerides, LDL, VLDL and decrease in HDL levels. Co-administration of extract with HFD and Triton x 100 decreased rise Cholesterol, Triglycerides, LDL, VLDL and increase in HDL levels. It was observed that the methanol extract of *Pterocarpus marsupium* conferred Anti- Hyperlipidemia activity by biochemical observation against HFD and Triton-x-100 induced Hyperlipidemia in rats. In the near future could constitute a lead to discovery of a novel drug for treatment of drug induced Hyperlipidemia.

Keywords: *Pterocarpus marsupiums*, Hyperlipidemia, anti diabetic, HFD and Triton-x-100.

INTRODUCTION

Hyperlipidemia is a condition when abnormally high levels of lipids i.e.the fatty substance are found in the blood. This condition is also called hypercholesterolemia/ hyperlipoproteinemia[1]. Human body is complex machinery and for maintaining the homeostasis of various organ and organ system. Any undesirable change will disturb the balance resulting in diseased state. Lipids are fats in the blood stream, commonly divided into cholesterol and triglycerides. Cholesterol circulates in the bloodstream and is involved in the structure and function of cells. Triglycerides(TG) are best viewed as energy that is either used immediately or stored in fat cells.TG are manufactured in the liver from the foods or by being absorbed from the intestine[3]. Virchow in 19thcentury who identified cholesterol crystals in atherosclerotic lesion and stated that endothelial cell injury initiates atherogenesis2. In a modification of this hypothesis it was proposed that the endothelium normally influences the behaviour of arterial smooth muscle cells by providing a barrier to the passage of plasma proteins, and that the major effect of haemodynamic or other factors that injure endothelium is to reduce the effectiveness of the barrier[4].Arteries are normally smooth and unobstructed on the inside, but in case of increased lipid level, a sticky substance called plaque is formed inside the walls of arteries. This leads to reduced blood flow, leading to stiffening and narrowing of the arteries. It has been proved that elevated plasma levels of cholesterol and of LDL are responsible for atherosclerosis in man, and epidemiological data suggests that elevated plasma levels of HDL have a protective effect[5].

Classification of Lipid Concentrations

The cholesterol along with some other types of fats cannot be dissolved in the blood. Moreover, in order to be

transported to and from cells, they have to be specially carried by certain molecules called lipoproteins, which consist of an outer layer of protein with an inner core of cholesterol and triglycerides[6,7]. In addition, the lipoproteins have been found essential for cholesterol to move around the body. The lipids can be classified as TC, triglycerides, LDL, HDL and very low density lipoprotein (VLDL) cholesterol.

Total cholesterol

According to guidelines of National Cholesterol Education Program (NCEP), TC concentrations below 200 mg/dL have been regarded as desirable, whereas, concentrations greater than 240 mg/dL are referred to as hyperlipidemic. However, epidemiological evidence suggests that the risk of cardiac events decreases as TC levels fall approximately to 150 mg/dL. Moreover, TC should be less than 180 mg/dL for children [8,9].

Triglyceride

Triglycerides are another type of fat that is carried in the blood by VLDL. The excess calories, alcohol or sugar in the body get converted into triglycerides and stored in fat cells throughout the body. The triglyceride concentration less than 150 mg/dL is regarded as normal, wheras, concentrations of 200-499 mg/dL are considered as high. Moreover, concentrations of 500 mg/dL or higher are considered dangerous for the development and progression of various CVDs [10].

LDL cholesterol

LDL is commonly known as the bad cholesterol, which is produced by the liver and carry cholesterol and other lipids from the liver to different areas of the body like muscles, tissues, organs and heart. The high levels of LDL indicate much more cholesterol in the blood stream than necessary and hence, increase the risk of heart disease [11]. According to NCEP guidelines, LDL cholesterol concentrations below 100mg/dL are considered optimal, whereas concentrations in the range of 160-189 mg/dL are considered to the higher side.However, increasing evidence supports that normal human LDL cholesterol concentration can be as low as 50 to 70 mg/dL. Moreover, it has been comprehensively seen that the risk of CVDs decreases as LDL cholesterol concentration decreases.

HDL cholesterol

HDL is commonly referred to as the good cholesterol, which is produced by the liver to carry cholesterol and other lipids from tissues back to the liver for degradation [12].High levels of HDL cholesterol have been considered as a good indicator of a healthy heart. The concentrations of 60 mg/dL or higher have been considered as optimal, whereas, HDL concentrations below 40 mg/dL are considered as major risk factor for CVDs. However, HDL is often interpreted in the context of TC and LDL concentrations, and hence may be regarded as less significant when LDL is low.

VLDL Cholesterol

VLDL is similar to LDL cholesterol in the sense that it contains mostly fat and not much protein. VLDL cholesterol is the lipoproteins that carry cholesterol from the liver to organs and tissues in the body. They are formed by a combination of cholesterol and triglycerides. Moreover, VLDLs are heavier than LDL, and are also associated with atherosclerosis and heart disease[13]

MATERIALS AND METHODS

PLAT MATERIAL

The leaves of plant *Pterocarpus marsupium* was collected from hilly region of chittoor district, Tirupathi, A.P, India. The plant was authenticated by Dr. K. Madhav Chetty, Asst. Professor, Dept. of Botany, Sri Venkateshwara University, Tirupathi.

EXPERIMENTAL ANIMALS

Male Wistar rats weighing (180-220g) were provided by animal house of Sigma Institute of Clinical Research and Administration (SICRA Labs), Kukatpally, Hyderabad, India. They were housed in ventilated rooms at a temperature of 24±2ºc with a 12h light/dark cycle and 54±5% relative humidity, maintained on standard pellet and water ad libitum throughout the experimental period. The animals were acclimatized for a period of one week. The experiments were carried out according to the guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA), New Delhi, India and approved by the Institutional Animal Ethical Committee (IAEC) of Sigma Institute of Clinical Research and Administration pvt.ltd. Hyderabad.

DRUGS AND CHEMICALS

Pterocarpus marsupium, all other chemicals and diagnostic kits were provided by Sigma Institute of Clinical Research and Administration.

PREPARATION OF EXTRACT

The collected plant was shade dried for 4 weeks and was ground to course powder using mixer grinder. The powdered plant material leaf (250gm) was extracted with methanol, by Maceration process. Finally extracts were air dried at room temperature. 10.2% and 7.2% w/w extract thus obtained was subjected for evaluation of Hyperlipidemic activity in Triton-X induced Hyperlipidemia and Diet induced Hyperlipidemia induced rats. The test samples of extracts were made in appropriate concentrations using distilled water prior to its use for animal studies.

PHYTOCHEMICAL SCREENING

Preliminary phytochemical investigation was carried out on Methanol extract of *Pterocarpus marsupium* leaf for detection of various phytochemical by standard methods

DETERMINATION OF ACUTE ORAL TOXICITY

Acute toxicity studies were performed according to OECD-423 guidelines category IV substance (acute toxic class method). Albino rats (n=3) of either sex selected by random sampling technique were employed in this study. The animals were fasted for 4 hrs with free access to water only. The plant extracts of *Pterocarpus marsupium* were administered orally with maximum dose of 2000 mg/kg body weight. The mortality was observed for three days. If mortality was observed in 2/3 or 3/3 of animals, then the dose administered

was considered as a toxic dose. However, if the mortality was observed only one rat out of three animals then the same dose was repeated again to confirm the toxic effect. If mortality was not observed, the procedure was then repeated with higher dose (Organization for economic Co-operation and development, 2001).

Chemicals

Triton X-100(a non-ionic detergent, iso octyl polyoxy ethylene phenol, formaldehyde polymer) was obtained from Technicho lab chemicals, Coimbatore. Atorvastatin was obtained from Moral labs, Chennai. All other chemicals were of analytical grade and obtained locally.

Table 1: High Fat Diet Composition

Composition	Normal diet (%)	High Fat diet (%)
Protein (Milk powder)	12	10
Carbohydrates(Wheat flour)	71	61
Sugar	05	05
Fat (Butter)	05	16
Salts	04	04
Vitamins	01	02
Fibers	02	01
Cholesterol	--	01
Total Weight	100g	100 g

Experimental Animals

Wistar albino adult male rats weighing 200-250g were obtained from the animal house. The animal were grouped and housed in polyacrylic cages (38x 23x 10 cm) with not more than five animals per cage and maintained under standard laboratory under standard laboratory conditions (temperature 25+2oC) with dark and light cycle (14/10 hour). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water ad libitum. The mice were acclimatized to laboratory condition for 10 days before commencement of experiment. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) constituted under CPCSEA.

Induction of Hyperlipidemia by Triton-x-100

Hyperlipidemia was induced in Wistar albino rats by single intraperitoneal injection of freshly prepared solution of Triton-X-100 (100 mg/kg) in physiological saline solution after overnight fasting for 18 h.

The animals were divided into five groups of six rats each.

I. The first group was given standard pellet diet, water and orally administered with 2% Tween 80.
II. The second group was given a single dose of triton administered at a dose of 100mg/kg, i.p. After 72 hours of triton injection, this group received a daily dose of 2% Tween 80 (p.o) for 7 days.
III. The third group was administered a daily dose of Atorvastatin 10 mg/day
IV. Fourth group *Pterocarpus marsupium* 200mg/kg suspended in 2% Tween 80, p.o., for 7 days, after inducing hyperlipidemia.
V. Fifth group was administered with the *Pterocarpus marsupium* 400 mg/kg, p.o. for 7 days.

STATISTICAL ANALYSIS

All data were expressed as the mean ± SEM. For statistical analysis of the data, group means were compared by one-way analysis of variance (ANOVA) followed by Dunnett's test, $P<0.05$ was considered significant.

RESULT AND DISCUSSION

Determination of Acute Oral Toxicity of EBG

The plant leaf extract of Pterocarpus marsupium didn't show any mortality and toxicity even at highest dose of 2000 mg/kg body weight employed. The present research study was carried out using dose (400mg/kg body weight) for Hyperlipidemic activity.

Table 2: Toxicity record sheet: The toxicity record sheet is as follows

		Toxicity		Time Of Death	Observation										
		Onset	Stop		Skin colour	Eyes	Resp	CNS	Tre	Con	Sali	Diah	Sleep	Leth	
1.	MBG	x	X	x	x	X	x	X	x	x	x	x	X	x	

(TRE-Tremor, CON-Convulsions, SALI- Salivation, Diah - Diarrhea, LET-Lethargy) × = Negetive Ø = Positive

Evaluation of Anti Hyperlipidaemic activity of Pterocarpus marsupium In Rats

MEAN AND S.E.M OF PARAMETERS OF THE ANIMALS

Table 3: Triton-X-100 Induced Model

TEST	NORMAL	CONTROL	STANDARD	T1	T2
ALP	73.12±5.219	160.29±2.01***	123.61±2.19***	124.14±1.46***	79.1±0.249
GPT	36.91±2.109	63.18±2.159***	42.81±1.519	40.84±3.591***	34.28±2.642**

GOT	42.28±2.161	54.39±1.214*	40.11±0.210	41.98±1.224	45.83±3.051
TP	40.06±1.389	33.86±2.506***	20.43±4.589***	17.02±3.259***	20.17±2.381***
HDL	52.67±0.310***	28.52±0.291	43.18±6.159**	50.14±3.019	55.21±3.150
TG	50.26±3.216	82.51±2.510***	80.43±2.358***	80.0±1.426***	60.32±2.259***
TC	67.20±3.152	150.13±3.256***	67.01±2.186	97.15±2.501***	93.06±2.630***
VLDL	10.01±0.233	15.59±0.627***	13.85±0.352***	15.45±0.356***	13.30±1.464***
LDL	11.56±2.692	101.63±5.069***	10.63±3.114	33.83±4.159	21.98±2.700
AI	0.41±0.239	3.29±0.358	0.42±0.189	0.96±0.328	0.51±0.496
CRR	3.12±0.521	7.63±0.491	2.68±0.122	2.23±0.214	2.16±0.161

Table 4: Body Weight

TRITON X100	NORMAL	CONTROL	STANDARD	T1	T2
Before treatment	171.1±0.21	173.96±0.89	176.0±2.22	174.28±0.13	171.0±0.85
After treatment	185.0±1.65	241.0±0.90***	197.33±0.71***	225.18±0.17***	244.83±1.14***

N = 6; Significance: * *P*<0.001, ** *P*<0.01, * *P*<0.05 from control**

DISCUSSION

The result of this study showed that oral administration Methanol extract of *Pterocarpus marsupium* leaf. From the results of clinical studies, Phytochemical screening of methanol extract of the leaf and part of *Pterocarpus marsupium* revealed the presence of flavonoids, alkaloids, tannins, Flavonoids have been shown to exert their antioxidant activity by various mechanisms by scavenging or quenching free radicals or by inhibiting enzymatic systems responsible for free radical generation.

Previous studies have reported some of these phytocomponents to elicit a wide range of biological activities which include hypolipidemia among others. Specifically, saponin is known to elicit serum cholesterol lowering activity by causing resin-like action, thereby reducing the enterohepatic circulation of bile acids. In the process, the conversion of cholesterol to bile acid is enhanced in the liver resulting in concomitant hypocholesterolemia.

Literature has reported the hypolipidemic effects of flavonoids, alkaloids and tannins. The presence of these phytocomponentsin the extract in high concentrations could account for these observed biological effects, particularly hypolipidemic effects. The mechanism by which the extract exert the hypoglycemic effect may appear to be related to presence of flavonoid among other secondary metabolites or bioactive chemical constituents found in the plant extract which may be an active constituents in a group or an individual responsible for the Hyperlipidemic activity of the plant extract.

The plant extract of *Pterocarpus marsupium* didn't show any mortality and toxicity even at highest dose of 2000mg/kg body weight employed. Hence, present research study was carried out using dose 400mg/kg body weight.

The presence of the steroids reduces the absorption of cholesterol and decreases the cholesterol concentration. Secondary metabolite like the flavonoids, saponins, reduces the cholesterol levels. Saponins will act as anti hyperlipidaemics by binding with the cholesterol and is readily absorbed by the bile acids causing the reduction in extra hepatic circulation and increases the metabolism of cholesterol to sterols through the fecal excretion. Saponins will as reported to increase the lipoprotein lipase activity and helps in the faster removal of free fatty acids from circulation causes decrease in fatal cholesterol.

Elevated cholesterol levels will promotes the atherosclerosis. High cholesterol levels are associated with the increased incidence of coronary heart diseases. Reduction in the cholesterol and the HDL concentration significantly reduces the cholesterol levels.

Atorvastatin is a member of the drug class of statins, it is the first specific inhibitor used for lowering cholesterol (hypo-lipidemic agent) in those with hyper-cholesterolemia and so preventing cardio vascular disease. It is a naturally occuring drug found in food such as oyster mushrooms and red yeast rice.It reduces the levels of "bad" cholesterol (LDL) and Triglycerides in the blood, while increasing levels of "good" cholesterol (HDL). It is an inhibitor of 3-hydroxy-3 methyl glutaryl-CoA reductase (HMG-CoA reductase), an enzyme that catalyses the conversion of HMG-CoA to mevalonate.Mevalonate is a required building block for cholesterol biosynthesis and Atorvastatin interferes with its production by acting as a reversible competitive inhibitor foe HMG-CoA,which binds to the HMG-CoA reductase. It works by slowing the production of cholesterol in the body. Buildup of cholesterol and fats along the walls of the blood vessels (A process known as Atherosclerosis) decreases blood flow and therefore, the oxygen supply to the heart, brain and other parts of the body. Lowering blood levels of cholesterol and fats may help to decrease the risk of heart disease, Angina (chest pain), strokes and Heart attacks. In addition to taking a cholesterol-lowering medication, making certain changes in our daily habits can also lower the blood cholesterol levels.

Effect of different extracts of Pterocarpus marsupium on serum lipid profile and Atherogenic Index, % protection

The serum level of triglycerides and cholesterol and it can be seen that the HFD group and Triton-x-100 show significant hyperlipidemia when compared with the normal control group. The extract treated groups and the standard treated group significantly decreased the serum levels of cholesterol and triglycerides when compared with the HFD control group and Triton-x-100 ($p<0.05$). The effect of ethanol extract on serum lipid levels was as better that of the standard treated group, showing the hypolipidemic potential of the plant. An increase of HDL-cholesterol level was also observed.

Decrease in glucose levels are observed in methanolic extract compared to HFD control group ($p<0.001$). Both 200 and 400 mg/kg body wt. 0.obtusata treated animals and 10 mg/kg body wt of Atorvastain treated animals in both models showed decrease in the atherogenic index and increased percentage of protection.

Effect of different extracts of Pterocarpus marsupium on Total protein profile

The serum level of total protein and it can be seen that the Triton-x-100 group shows significant decrease in total protein levels when compared with the normal control group. The extract treated groups and the standard treated group significantly increased the serum levels of total protein when compared with the Triton-x-100 control group ($p<0.001$). The effect of methanol extract on levels was better as that of the standard treated group, showing the hypolipidemic potential of the plant.

Effect of different extracts of Pterocarpus marsupium on SGOT, SGPT and ALP levels

AST, ALT, SGOT, SGPT, and GGT and Alkaline Phosphates are abbreviations for proteins called enzymes which help all the chemical activities within cells to take place. Injury to cells releases these enzymes into the blood. They are found in muscles, the liver and heart. Damage from alcohol and a number of diseases are reflected in high values. AST/SGOT, ALT/ SGPT are also liver and muscle enzymes. They may be elevated from liver problems, hepatitis, excess alcohol ingestion, muscle injury and recent heart attack. An atherogenic diet has been reported to induce glomerulosclerosis/nephropathy and mild tubular and hepatic damage experimental rats In case of the effect of methanol extract on enzymes (SGOT, SGPT and ALP), the extract shows significantly lower levels of SGOT, SGPT and ALP in comparison to Triton-x-100 control group ($p<0.05$). Here the maximum reduction was observed for standard followed by methanolic extract. Therefore, it can be confirmed that, in present investigation significant Hyperlipidemic potential of *Pterocarpus marsupium* herb may be due to flavonoids, alkaloids, tannins content, which were confirmed by preliminary phytochemical screening.

CONCLUSION

Phytochemical screening of the extract shows the presence of chemical constituents like Alkaloids, steroids, fixed oils, cardiotonic aglycones, flavonoids, saponins, carbohydrates, proteins, resins. Acute toxicity tests were performed according to the OECD guide line no.423, LD50 value was found to be 200mg/kg and 400mg/kg. Anti Hyperlipidaemic activity was performed by using the high fat diet and Triton-x-100 induced method. In the present study an increase in plasma HDL-cholesterol with a concomitant percentage decrease from other lipid was observed. It can be concluded from the present data that the levels of total serum cholesterol, triglyceride and MDA which are actually raised in high fat diet, can be lowered significantly with Pterocarpus marsupium And total proteins which is actually lowered in Triton-x-100 can be raised significantly with Pterocarpus marsupium. Atherogenic index which actually raised in atherogenic diet and Triton-x-100, can be lowered significantly with Hibiscus cannabinus and a very good % protection was seen with *Pterocarpus marsupium s* and standard drug. In nutshell the extract of *Pterocarpus marsupium* possesses significant\anti Hyperlipidaemic activity, which is the first claim in this respect.

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