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Anti Hyperlipidemic Activity of *Spondias pinnata* Fruit Extracts

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ABSTRACT

Hyperlipidemia is the greatest risk factor of coronary heart disease. Medicinal plants play a major role in hypolipidemic activity. Literature claims that Saponins are able to reduce hyperlipidemia. Based on high saponin content in herbal plants, *Spondias pinnata* (SP) was selected and the present study focus on the anti-hyperlipidemic activity of methanolic extracts of fruits of SP against Triton induced hyperlipidemia in rats. SP was administered at a dose of 100 and 200 mg/kg (*p.o*) to Triton induced hyperlipidemic rats. Fenofibrate was used as reference standard. The statistical analyses were carried out using one way ANOVA followed by Dunnet's multiple comparisons test. The present investigation shows that all triton induced rats displayed hyperlipidemia as shown by their elevated levels of serum and liver cholesterol, triglyceride, PL, VLDL, LDL and the reduction in the HDL level. It can be concluded that SP 100 and 200 mg/kg treatment was effective in cholesterol, PL, TG, VLDL, LDL and HDL in a dose dependant manner.

Keywords: *Spondias pinnata*, Hyperlipidemia, Triglycerides, Lipoproteins.

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INTRODUCTION

Hyperlipidemia is characterized by elevated serum total cholesterol, low density, and very low-density lipoprotein and decreased high-density lipoprotein levels. Hyperlipidemia associated lipid disorders are considered to cause atherosclerotic cardiovascular disease. [1] Among these hypercholesterolemia and hypertriglyceridemia are closely related to ischemic heart disease. [2] The consumption of synthetic drugs leads to hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function. [3] Medicinal plants are used for various research purposes. Medicinal plants play a major role in hypolipidemic activity. The leaves of *Aleurites moluccana*, *Piper betle* suggests that the lipid lowering action is mediated through inhibition of hepatic cholesterol biosynthesis and reduction of lipid absorption in the intestine. [4]

Ethnomedicinal studies are generally cogent in absolute locally important bulb breed abnormally for the analysis of awkward drug. [5] The investigation on plant drugs will be a useful strategy in the discovery of new lead molecules eliciting improved activity by regulating the different mechanisms maintaining the lipid metabolism and thus can be used in treating hyperlipidemia of varied etiology. [6] *Spondias pinnata* (Anacardiaceae) stem heart wood are well known in India as Jangliaam (Hindi), Adavimaamidi, Kondamaamidi (Telugu), Common hog plum, Indian mombin (English). It was found that this plant contains sterols, flavonoids and gums. There are reports which showed that fruits are astringent and antiscorbutic, also used in bilious dyspepsia. Bark astringent and refrigerant, used in diarrhea and dysentery; a paste of it applied in rheumatism. Roots employed for regulating menstruation. Previously isolated compounds are β-Amyrin, oleanolic acid and amino acids (alanine, leucine) from S. *Pinnata.* [7] Essential oil from the pulp yielded carboxylic acids and esters, alcohols, aromatic hydrocarbons. Fruits yield ß-amyrin, oeanolic acid, glycine, cystine, serine, alanine, and leucine. Aerial parts yield lignoceric acid, ß-sitosterol and its glucoside. [7] The fruits are eaten as a vegetable when green and as a fruit when ripe. They are used for flavoring. The flowers are sour and used in curry as a flavoring and also eaten raw as well as the local people make chutney, jam and pickle. Fruits are very nutritious and rich in vitamin A, minerals and iron content. It is astringent, sour, thermogenic, appetizer and aphrodisiac and is good for rheumatism and sore throat. [8] The bark is useful in dysentery and diarrhea and is also prevent vomiting. The root is considered useful in regulating menstruation. The plant is reported to have antitubercular properties. The leaves are aromatic, acidic and astringent. [9] The present study was undertaken to demonstrate the effect of methanolic extract of *Spondias pinnata* on lipid profile of hyperlipidemic rats using standard lipid lowering agent Fenofibrate.

MATERIALS AND METHODS

Plant Material Collection

Spondias pinnata (Anacardiaceae) fruits were collected from the Salur, Vizianagaram district area, Andhra Pradesh, India in the month of October 2016 and authenticated by the taxonomist, Department of Botany, Andhra University and the specimen voucher no AUCP/BGR/2015/S22 was preserved in the Department.

Acute toxicity studies

Acute toxicity studies were performed for extracts of selected plant according to the toxic classic method as per guidelines. None of these extracts showed mortality even at a dose of 1000mg/kg and therefore considered safe. Toxicological studies were conducted in mice (N=6) for all the extracts as per the Irvin's method [10] at the doses of 100, 300 and 1000 mg/kg, no mortality was observed.

Experimental Animals

Wistar albino adult male rats weighing 200-250 g were obtained from the Mahaveer enterprises, Hyderabad, India. The animals were housed under standard environmental conditions (temperature of 22 ± 1°C with an alternating 12 h light–dark cycle and relative humidity of 60 ± 5%), one week before the start and also during the experiment as per the rules and regulations of the Institutional Animal Ethics committee and by the Regulatory body of the government (Regd no. 516/01/a/CPCSEA). They were fed with standard laboratory diet supplied by M/s. Rayans biotechnologies Pvt. Ltd., Hyderabad, Andhra Pradesh, India. Food and water was allowed *ad libitum* during the experiment.

Antihyperlipidemic studies

The animals were divided into five groups of five rats each. The first group was given standard pellet diet, water and orally administered with 5% CMC. The second group was given a single dose of triton administered at a dose of 400 mg/kg, *p.o.* After 72 hours of triton injection, this group received a daily dose of 5% CMC (*p.o*) for 7 days. The third and fourth group was administered a daily dose of SE aqueous extract 100 mg/kg and 200 mg/kg suspended in 5% CMC *p.o.*, for 7 days, after inducing hyperlipidemia. Fifth group was administered with the standard Fenofibrate 65 mg/kg *p.o.* for 7 days. [11]

Collection of blood

On the 8th day, blood was collected by retero orbital sinus puncture, under mild ether anaesthesia. The collected samples were centrifuged for 10 minutes. Then serum samples were collected and used for various biochemical experiments. The animals were then sacrificed and the liver collected. [12]

Liver lipid extraction

The liver was homogenized in cold 0.15M KCl and extracted with $CHCl_3$ and CH_3OH (2% v/v). This lipid extract was used for the estimation of lipid parameters. [13]

Biochemical Analysis

The serum and liver extract were assayed for total cholesterol, triglycerides, phospholipids, high-density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL) using standard protocol methods. [14]

RESULTS AND DISCUSSION

The methanol extract of *Spondias pinnata* was found to be non–toxic up to the dose of 2 g/kg and did not cause any death of the tested animals. The Phytochemical tests with the methanol extract of *Spondias pinnata* indicated the presence of carbohydrates, flavanoids, glycosides, terpenes, saponins and gums and mucilage. Hyperlipidemia is associated with heart disease, which is the leading cause of death in the world. The lowering of the levels of harmful lipids to satisfactory values has

been confirmed by several experimental animal and interventional studies indicating lowered morbidity and mortality in coronary heart diseases. The results are discussed under the lipid profile in serum and the lipid profile in liver. Lipid profile in serum and liver indicates that increased phospholipids (PL), triglyceride (TG) and cholesterol levels were significantly reduced by treatment of 100 and 200 mg/kg of *Spondias pinnata*. LDL and VLDL levels were significantly increased in triton-injected animals to control rats. The results are shown in Tables 1, 2. The SP markedly lowers the levels of serum cholesterol and VLDL. The decrease in cholesterol may indicate increased oxidation of mobilized fatty acids of inhibition or lipolysis. The present investigation shows that all triton induced rats displayed hyperlipidemia as shown by their elevated levels of serum and liver cholesterol, triglyceride, PL, VLDL, LDL and the reduction in the HDL level. It can be concluded that SP 100 and 200 mg/kg treatment was effective in cholesterol, PL, TG, VLDL, LDL and HDL in a dose dependant manner.

The result strongly suggests that the hypolipidemic activity of this medicinal plant could be attributed to the presence of the valuable saponins and flavonoids in the extract. This extract supplementation also resulted in significant attenuation in the level of LDL and HDL in serum towards the control level, which again strengthens the hypolipidemic effect of this extract. The antihyperlipidemic activity of *Spondias pinnata* (100 and 200 mg/kg) against Triton Wr-1339 showed significant activity when compared to fenofibrate treated groups in a dose dependant manner. This result suggests that cholesterol-lowering activity of the herb extract can be result from the rapid catabolism of LDL cholesterol through its hepatic receptors for final elimination in the form of bile acids as demonstrated. [15] It is well known that HDL-Cholesterol levels have a protective role in Coronary artery disease. [16] Similarly increased level of serum LDL-cholesterol results in increased risk for the development of atherosclerosis. [17] Triton WR-1339 acts as a surfactant and suppresses the action of lipases to block the uptake of lipoproteins from circulation by extra hepatic tissues, resulting into increased blood lipid concentration. [18] Triton induces hyperlipidemia by increasing the hepatic synthesis of cholesterol and triglycerides. [19-20]

Table 1: Effect of Methanolic extract of *Spondias pinnata* on HDL, LDL, VLDL in serum of control and Experimental Rats

Groups	Parameters		
	HDL	LDL	VLDL
Group-I Control	23.82 ± 2.11	22.49 ± 1.78	13.76 ± 4.25
Group-II Trition treated	18.20 ± 5.12*	15.48 ± 4.12*	22.68 ± 3.63*
Group-III Trition + SP (100 mg/kg)	20.42 ± 4.26**	35.56 ± 2.24**	18.32 ± 2.54**
Group-III Trition + SP (200 mg/kg)	24.80 ± 1.21**	29.89 ± 3.56**	16.30 ± 2.15**
Group-III Trition + fenofibrate	25.02 ± 2.10**	26.32 ± 3.30**	15.45 ± 3.10**

Values are in mean + SE; Number of animals in each group = 5; *p< 0.05 Vs Group I; **p<0.05 Vs Group II.

Table 2: Effect of Methanolic extract of *Spondias pinnata* on Cholesterol, Triglycerides, Phospholipids in serum of control and Experimental Rats

Groups	Parameters		
	HYPERLIPIDEMIC	TRIGLYCERIDES	PHOSPHOLIPIDS
Group-I Control	61.49 ± 4.09	72.50 ± 2.27	145.42 ± 6.28
Group-II Trition treated	182.31 ± 8.25*	112.20 ± 5.31*	197.28 ± 8.35*
Group-III Trition + SP (100 mg/kg)	77.45 ± 10.21**	84.16 ± 4.15**	181.56 ± 7.62**
Group-III Trition + SP (200 mg/kg)	69.83 ± 4.36**	75.28 ± 5.38**	165.32 ± 6.25**
Group-III Trition + fenofibrate	66.33 ± 3.52**	72.30 ± 2.46**	68.28 ±7.52**

Values are in mean + SE; Number of animals in each group = 5; *p< 0.05 Vs Group I; **p<0.05 Vs Group II.

Triton Wr-1339 has been widely used to block clearance of triglyceride-rich lipoproteins to induce acute hyperlipidemia in several animals. This model is widely used for a number of different aims particularly, in rats it has been used for screening natural or chemical hypolipidemic drugs. Interestingly, the results of the present study show that extract of *Spondias pinnata* produced a significant reduction in cholesterol level and also it reversed Triton induced hyperlipidemia in rats. The results obtained from the pharmacological screening have led to the conclusions that, methanolic extract of *Spondias pinnata* fruits have significant antihyperlipidemic activity. Hence it can be exploited as antihyperlipidemic therapeutic agent or adjuvant in existing therapy for the treatment of hyperlipidemia.

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