



EVALUATION OF ANTIHYPERLIPIDEMIC AND ANTIDIABETIC ACTIVITY OF *PISONIA ACULEATA* IN EXPERIMENTAL RATS

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ABSTRACT

Hyperlipidemia has been associated as a complication with several metabolic diseases, including diabetes mellitus. Results of the diabetic patients are obese and have dyslipidemia and have 2-5 times the risk of cardiovascular diseases. Cardiovascular disease is the primary cause of mortality in the developed and developing nations with the increase in the aging population where there is a surge in the incidence of atherosclerotic cardiovascular disease. One of the most common and lethal manifestations of atherosclerotic is coronary heart disease, accelerating for 50% of the atherosclerotic cardiovascular diseases to men and women younger than 75 years. In this present study is an attempt to investigate its anti hyperlipidemic, anti diabetic activity of alcoholic extract of *Pisonia aculeata* (AEPA) based on the chemical constituents present in the plant. The potential activity was evaluated using albino wistar rats, the hyperlipidemia was induced by Triton X and diabetes was induced by administration of streptozotocin. Two test doses 100 mg/kg; 200 mg/kg for anti hyperlipidemic activity and 300 mg/kg; 600 mg/kg for antidiabetic activity were used based on acute toxicity studies. Anti hyperlipidemic activity mainly due to the modulation of cholesterol levels, LDL, HDL, cholesterol levels, through an improvement of HDL - cholesterol levels and partly to reduce the levels of triglycerides. The extract also has shown good anti diabetic activity.

Keywords: AEPA, Triton X, LDL, HDL, Cholesterol.

INTRODUCTION

Many lipoprotein abnormalities are seen in the untreated, hyperglycemic diabetic patient. The non-insulin-dependent diabetic (NIDDM) patient with mild fasting hyperglycemia commonly has mild hypertriglyceridemia due to over production of TG-rich lipoproteins in the liver, associated with decreased high-density lipoprotein (HDL) cholesterol levels. The more hyperglycaemic untreated NIDDM and insulin-dependent diabetic (IDDM) patient have mild to moderate hypertriglyceridemia due to decreased adipose tissue and muscle lipoprotein lipase, (LPL) activity¹. These patients also have decreased HDL cholesterol levels associated with defective LPL catabolism of TG-rich lipoproteins. Treatment of diabetes with oral sulfonylureas or insulin corrects most of the hypertriglyceridemia and some of the decrease in HDL cholesterol. The abnormality in adipose tissue LPL activity corrects slowly over several months of therapy. The treated NIDDM patient may continue to have mild hypertriglyceridemia, increased intermediate-density lipoprotein levels, small dense low-density lipoproteins (LDL) with increased apoprotein B and decreased HDL cholesterol levels². The central, abdominal distribution of adipose tissue in IDDM is associated with insulin resistance, hypertension and the above lipoprotein abnormalities. Improvement in glucose control, in the absence of weight gain, leads to lower triglyceride and higher HDL cholesterol levels. In addition, the diabetic patient is prone to develop other defects that, in themselves, lead to hyperlipidemia, such as proteinuria, hypothyroidism and hypertension, treated with thiazide diuretics and beta-adrenergic-blocking agents³. When a diabetic patient independently inherits a common familial form of hypertriglyceridemia, he might develop the severe hypertriglyceridemia of the chylomicronemia syndrome. The history of herbal medicine is as that of old human civilization. The documents, many of which are of antiquity, revealed that plants were used medicinally in

China, India, Egypt and Greece long before the beginning of the Christian era. A large portion of the Indian population, even the present time, depends on the Indian system of medicine Ayurveda - Ancient science of life. The well known treatises in Ayurveda are the Charaka Samhita and the Sushruta Samhita. The World Health Organization (WHO) estimates that 75% of the world population use herbal remedies as their primary therapeutic treatment. Every culture has developed the use of simple herbal extracts, used alone or in combination⁴. *Pisonia aculeata* belongs to the family Nyctagenaceae, it grows widely in hill places and it contains various sub species. Literature review shows that it has hepatoprotective and antioxidant activity but no work was done for anti diabetic and hyperlipidemic activity. Therefore the present study was carried out to investigate the anti diabetic and anti hyperlipidemic activity.

MATERIALS AND METHODS

Collection and authentication of crude drug

The whole plant of *Pisonia aculeata* obtained from wild hill regions of Thirupathi of Andra Pradesh, India and authenticated by botanist, freshly collected plant only used for the experiment.

Preparation of Crude drug for extraction

The whole plant was dried under shade and then coarsely powdered with a mechanical grinder. The powder was passed through the sieve no 40 and stored in an air tight container for further process by continuous hot percolation using ethanol.

Preparation of extracts

The ethanol extraction process was carried, up to 72 h, after completion of extraction, it was filtered and the solvent was removed by evaporation to dryness on a water bath. Brown colour residue was obtained and it was stored in a desiccator⁵.

Animals

Studies were carried out using male albino mice weighing 140-165 g. Animals were supplied by Venkateshwara enterprises, Bangalore, India. The rats were group housed in poly acrylic cages (38 x 23 x 10 cm) with not more than 6 animals per kg and maintained under standard laboratory conditions (temperature 25 ± 2 °C) with a dark and light cycle (14, 10h). They were allowed free access to standard dry pellet diet (Amrut, India) and tap water *ad libitum*. All procedures described were reviewed and approved by IAEC, vide Resolution no: 08/2012-20139(i)/a/CPCSEA/IAEC/SSJ/CHA-SAN/ dt.25.09.2012.

Acute toxicity studies

Albino mice weighing 22-25 g selected by random sampling technique were used in the study. Acute oral toxicity was performed as per OECD- 423 guidelines (acute class method)⁶. The animals were fasted overnight, provided only water after which extract was administered to the groups orally at the dose level of 5 mg/kg body weight by gastric intubation and the groups were observed for 14 days. If mortality was observed in 2 or 3 animals among 6 animals then the dose administered was assigned as a toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose⁷. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2,000 mg/kg body weight. The animals were observed for toxic symptoms such as behavioural changes, locomotion, convulsions and mortality for 72 h.

Oral Glucose Tolerance

Effect of alcoholic extracts of *Pisonia aculeate* (600 mg/kg) and Glibenclamide (0.5 mg/kg) on the glucose tolerance tested at 30 min after glucose administration, the blood glucose concentration increased rapidly from the fasting value and then attains nearly the same value at the end of the study⁸. At 120 min, the extracts treated group blood glucose levels were estimated.

Evaluation of anti hyperlipidemic activity

Triton Induced hyperlipidemia Model

Group- I: Normal control

Group-II: Hyperlipidemic Control Triton - X - 100 (200 mg/kg) was injected subcutaneously in the Rats.

Group-III: Standard group Rats were treated with Lovastatin (5.33 mg/kg p.o) along with Triton treatment.

Group-IV: Test group- I Rats treated with AEPA (100 mg/kg. p.o) along with Triton treatment.

Group-V: Test group- II Rats treated with AEPA (200 mg/kg. p.o.) along with Triton.

Evaluation of antidiabetic activity

Streptozotocin induced diabetic model

Group-I: Normal diet and water *ad libitum* and received vehicle solvent (Normal)

Group-II: Normal diet and water with streptozotocin 50 mg/kg and received vehicle solvent (disease control)

Group-III: Standard treated group Glibenclamide (0.5 mg/kg p.o.)

Group-IV: Normal diet and water with streptozotocin 50 mg/kg and received alcoholic extract (300 mg/kg)

Group-V: Normal diet and water with streptozotocin 50 mg/kg and received alcoholic extract (600 mg/kg)

Analytical procedures

Estimation of blood glucose was carried out by glucose oxidase peroxidase method⁹. The estimation of protein was carried out by the Lowry method^{10,11}. Estimation of serum cholesterol was carried out by Zlatkis method¹². Serum triglycerides were estimated by Foster and Dunn method¹³ and HDL-cholesterol was estimated by Burstein method¹⁴. The serum LDL cholesterol was calculated by Friedwald formula¹⁵. Atherogenic index was calculated by using the formula, $TC-HDL-C/HDL-C$ ¹⁶.

Statistical analysis

The results were expressed as mean \pm S.D. The statistical analysis of results was carried out using Student t-test and one-way ANOVA followed by Dennett's multiple comparison tests.

Table 1: Effect of *Pisonia aculeata* on Oral GTT

Treatment	Changes in blood glucose levels (mg/dl)			
	0 min.	30 min.	60 min.	120 min.
control group	96.60 \pm 2.8	97.42 \pm 2.8	97.22 \pm 2.6	96.82 \pm 2.4
Standard group	97.27 \pm 4.62	99.20 \pm 6.6*	99.43 \pm 4.6*	98.66 \pm 3.9*
Alcohol extract (100 mg/kg)	99.21 \pm 5.6	100.14 \pm 5.5*	99.41 \pm 3.8**	98.12 \pm 5.9**
Alcohol extract (200 mg/kg)	100.38 \pm 5.8	104.24 \pm 5.2**	101.2 \pm 4.4**	99.42 \pm 6.4**

Values are expressed as mean \pm SEM, *P<0.001** P<0.01, when compared with control. n=6.

Table 2: Lipid Profiles in Triton Induced Hyperlipidemia

Groups	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	Anti atherogenic index (%)
Control	90.88 \pm 2.38	75.35 \pm 1.62	36.9 \pm 1.77	30.62 \pm 1.32	95.96
Triton(200mg/kg)	135.9 \pm 1.8*	121.6 \pm 81*	31.87 \pm 1.65*	51.95 \pm 1.75*	35.91
Triton+ aqueous extract (100 mg/kg)	99.12 \pm 2.9**	88.91 \pm 1.23**	32.02 \pm 1.63**	38.68 \pm 1.85**	56.28
Triton+ alcohol extract (200 mg/kg)	95.3 \pm 1.89***	80.52 \pm 2.06***	33.13 \pm 1.71***	35.27 \pm 1.76***	69.6
Triton+ Lovastatin(5.33mg/kg)	92.25 \pm 1.59***	76.05 \pm 1.79***	36.03 \pm 1.86***	32.58 \pm 1.85***	76.02

Values are expressed as mean \pm SEM, * P<0.05, ** P<0.01. *** P<0.001 vs control group

Table 3: Effect of Extract on Blood Glucose of Diabetic Treated Group (mg/dl)

Group	Day 0	Day 5	Day 10	Day 15
Control	76.85 \pm 2.1	75.32 \pm 4.2	74.92 \pm 3.6	74.72 \pm 2.9
Disease control	278.54 \pm 9.2*	280.95 \pm 10.5*	285.73 \pm 11.4*	292.72 \pm 9.8*
Extract 300 mg/kg	261.36 \pm 11.9	110.65 \pm 7.8**	91.45 \pm 1.6**	87.73 \pm 1.8**
Extract 600 mg/kg	250.76 \pm 16.5	90.59 \pm 4.4**	83.58 \pm 3.2**	75.81 \pm 2.9**
Standard 0.5 mg/kg	239.53 \pm 13.3	95.45 \pm 2.8**	81.69 \pm 1.5**	72.91 \pm 2.4**

Values are expressed as mean \pm SEM, *p<0.001 compared with normal group, **p<0.001 compared with STZ control group

RESULTS**Phytochemical tests**

The Phytochemical tests with the methanol extract indicated the presence of carbohydrates, flavanoids, glycosides, terpenes, saponins.

Oral Glucose Tolerance

Effect of ethanolic extracts of *Pisonia aculeata* (600 mg/kg) and Glibenclamide (0.5 mg/kg) on the glucose tolerance has been shown in the Table 1. At 30 min after glucose administration, the blood glucose concentration increased rapidly from the fasting value and then attains nearly the same value at the end of the study. At 120 min, the extracts treated group blood glucose level was significantly decreased from 99.21 ± 5 to 98.12 ± 5.96 and 100.30 ± 5.8 to 99.42 ± 6.4 mg/dl respectively.

Evaluation of anti hyperlipidemic effect of *Pisonia aculeata*

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 triglyceride (TG) and cholesterol levels were significantly reduced by treatment of 100 and 200 mg/kg of AEPA. LDL levels were significantly increased in triton-injected animals to control rats. The results are shown in Table 2. The AEPA markedly lowers the levels of serum cholesterol and LDL. 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, LDL and the reduction in the HDL level. It can be concluded that SE 100 and 200 mg/kg treatment was effective in cholesterol, TG, LDL and HDL in a dose dependant manner.

Evaluation of anti diabetic effect of *Pisonia aculeata*

The effect of single oral administration of alcoholic extracts of *Pisonia aculeata* is shown in Table 3. Experimental studies reveals that the alcohol extracts from *Pisonia aculeata* (100 and 200 mg/kg) orally administered produced a significant decrease in the blood glucose level in the model of streptozotocin induced acute diabetes in rats. Maximum reduction in blood glucose level was seen at dose of 200 mg/kg of extracts of plant.

DISCUSSION

All these beneficial effects of *Pisonia aculeata* are especially hopeful in preventing hyperglycemia, cardiovascular, hyperlipidemia diseases. In conclusion, this study has undoubtedly provided scientific confirmation and evidence for the safe use of the whole plant of *Pisonia aculeata* by traditional healers in the treatment of hyperlipidemia and diabetes. However the nature of the active principle(s)

responsible for all these positive effects requires further investigation.

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