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RESEARCH ARTICLE

Antihyperlipidemic and Biochemical Effect of Callus and Leaf Extracts of *Pergularia Daemia* (Forssk.) Chiov. on Blood Serum of Streptozotocin-Attenuates White Albino Rats

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ABSTRACT

The aim of the present study was to evaluate the antihyperlipidemic potential and biochemical parameters of various leaf extract of *P. daemia*. Extract was administered orally at a dose of 100, 200 and 300 mg/kg b.w in streptozotocin induced diabetic rats for 21 days. To assess the antihyperlipidemic activities and biochemical effects of this extract, we have also measured the serum levels of total cholesterol, triglycerides, phospholipids, Serum protein, albumin and haemglobin. The various leaf extract of *P. daemia* also resulted a significant recovery in above mentioned biomarkers of lipid profile and biochemical parameters when treated to STZ-induced diabetic rat. Resettlement of all these parameters after the treatment of ethanol, chloroform, aqueous extract of leaf of *P. daemia* is promising which has been reflected here from the comparison with the antidiabetic drug i.e. glibenclamide. The result of this experiment demonstrated that the leaf extracts of *P. daemia* possess a promising antidiabetic efficacy.

KEYWORDS

Antidiabetic, *Pergularia daemia*, Leaf, Streptozotocin, Anti-hyperlipidemic, Biochemical Parameters

INTRODUCTION

Medicinal plants have been in use in traditional health care systems since ancient times and are still the most important health care source for the vast majority of the population around the world.[1,2,3] At present, 80% of the population in developing countries relies largely on plant based drugs for their primary health care needs, and the world market for herbal products based on traditional knowledge was estimated to be worth US $ 60 million.[4] Thirty percent of the drugs sold worldwide contain compounds derived from plant material. Diabetes mellitus (DM) is predominantly characterized by abnormal insulin secretion that leads to elevated glucose. In 2007, the International Diabetes Federation (IDF) estimated that in the South-East Asia region, 54 million people were diabetic and an additional 63 million adults had Impaired Glucose Tolerance (IGT). At the regional level, the number of people with diabetes is expected to increase by 71% between 2007 and 2025.[5]

Pergularia daemia [Forssk.chiov] (Asclepidaceace) commonly known as `Uttamani`, `Velipparuthi` in India, grows in various parts of southern and northern India. The plant is perennial slender foetid-smelling climber. The whole plant is used as an anthelmintic, emmenagogue, antivenom, antiseptic, emetic and expectorant.[6] Extract of this plant is taken orally for gastric ulcers,

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uterine and menstrual complaints.[7] The leaf is useful in treating leprosy and haemorrhoids.[8] The fresh, pulped leaves are applied as a poultice to relieve carbuncles. Leaf juice is used in treating amenorrhea, dysmenorrheal, infantile diarrhea and catarrhal infections.[9,10,11] The present study was undertaken to evaluate the antihyperlipidemic and biochemical properties of *P. daemia* leaf extract in normal and streptozotocin induced diabetic rats.

MATERIALS AND METHOD

Explant Collection

Pergularia daemia was collected from the Southern parts of Pudukkottai district, Tamil Nadu, India, and planted in the J.J. College Botanical Garden. The plants were raised in pots containing mixture of soil and farmyard manure in the ratio of 1:1. Small disease free tender leaf were collected from 5-6 months grown plants, cut into 0.5-1.0 cm segments and used as explants for callus induction.

Media Preparation, Callus Induction and its Proliferation

Murashige and Skoog[12] medium supplemented with auxins viz., 2,4-D, NAA, IAA and cytokinins viz., BAP and Kin alone at different concentrations was used for callus initation. The cultures were incubated under fluorescent lights with 1500-2000 lux for 16 h at 25±1°C and 80±10 relative humidity. Each experiment had 20 replicates and was repeated thrice. The proliferated callus cultures were sub cultured and maintained for 45-50 days on the same medium supplemented with the same growth regulator.

Preparation of Extracts

The leaves and calli were dried under shade, coarsely powdered, and extracted with chloroform (60-80°C) followed by alcohol, and then water using Soxhlet apparatus. The extracts so collected were distilled off on a Water bath at atmospheric pressure and the last traces of the solvents were removed in vacuo.[13]

Animals

In the present study healthy, matured male albino rats (wistar strain) were used. Rats weighing 180-230g were obtained from the Periyar College of Pharmaceutical Sciences, Tiruchirapalli, Tamil Nadu, India and kept in plastic animal cages with 12 h light and dark cycle in the institutional animal house. The animals were fed with standard rodent diet and provided water *ad libitum*. After one week of acclimatization the animals were used for the further experiments. Approval from the Institutional Animal Ethical Committee for the usage of animals in the experiments was obtained as per the Indian CPCSEA guidelines.

Chemicals

Streptozotocin (STZ) and glibenclamide were purchased from Sigma Aldrich, St. Louis, MO, USA. All other chemicals and solvents used were of Analytical Grade obtained from E-Merck and Himedia, Mumbai, India.

Acute Toxicity Study

Acute toxicity studies were carried out using Acute Toxic Class Method as per OECD-423 Guideliness.[14] Chloroform leaf extract, ethanol leaf extract, aqueous leaf extract and ethanol leaf callus extract of *P. daema* were administered at a starting dose of 2000 mg/kg b.w of orally to 4 male rats. The animals were observed for mortality and behavioral changes during 48 h.

Experimental Induction of Diabetes

Diabetes was induced in a group of rats after an overnight fast by single intraperitoneal injection of STZ, which was freshly dissolved in 0.1M citrate buffer (pH 4.5). The dose was 40 mg/kg b.w. STZ treated animals were allowed to drink 5% glucose solution overnight to overcome drug induced hypoglycemia. After 48 h of STZ administration, the blood glucose ranges above 200-300 mg/dl was considered as diabetic rats and used for the experiment.

Experimental Design

In the experiment, a total of 162 rats were used, randomly divided into 27 groups of 6 animals each and treatments continued in an aqueous

solution daily using an intragastric tube for 21 days.

Group-I	Normal rats received 3% gum acacia
Group-II, III, IV	Leaf chloroform extract (100, 200, 300 mg/kg b.w.)
Group-V, VI, VII	Leaf ethanol extract (100, 200, 300 mg/kg b.w.)
Group-VIII, IX, X	Leaf aqueous extract (100, 200, 300 mg/kg b.w.)
Group-XI, XII, XIII	Leaf ethanol callus extract (100, 200, 300 mg/kg b.w.)
Group-XIV	Streptozotocin (STZ) 40 mg/kg b.w. (Diabetic control)
Group-XV, XVI, XVII	STZ+Leaf chloroform extract (100, 200, 300 mg/kg b.w.)
Group-XVIII, XIX, XX	STZ+Leaf ethanol extract (100, 200, 300 mg/kg b.w.)
Group-XXI, XXII, XXIII	STZ+Leaf aqueous extract (100, 200, 300 mg/kg b.w.)
Group-XXIV, XXV, XXVI	STZ+Leaf ethanol callus extract (100, 200, 300 mg/kg b.w.)
Group-XXVII	STZ+Glibenclamide (600 µg/kg b.w.)

Biochemical Assays

After the separation of plasma, from blood samples on 21st day of the treated animals the buffy coat was removed and the packed erythrocytes were washed thrice with cold physiological saline. A known volume of the erythrocyte was lysed with cold hypotonic phosphate buffer at pH 7.4. The hemolysate was separated by centrifugation at 2000 rpm for 10 min and the supernatant was used for lipid profile estimations. Activities of antihyperlipidemic marker enzymes like Total Cholesterol[15], Triglycerides[16], Phospholipids[17], Serum protein[18], Haemoglobin[19] and Albumin[20].

Statistical Analysis

Statistical analysis was performed using SPSS Software Package, version 11.5. The values were analyzed by One Way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). All these results were expressed as mean ±SD for six rats in each group, p-values <0.05 were considered as significant.[21]

RESULTS AND DISCUSSION

Callus was initiated from stem and leaf explants of *P. daemia* on basal MS medium supplemented with 2,4-D, α-NAA, BAP, KIN and IBA at different concentrations 1.0 - 3.0 mg/l. The maximum induction rate was observed at 2,4-D 2.0 mg/l combination with 0.5mg/l BAP and 1.0 mg/l α-NAA.

The acute toxicity study revealed the non-toxic nature of the chloroform, ethanol, aqueous and ethanol callus extracts at the tested concentrations. No lethal toxic reactions were observed until the end of the experiment.

The serum cholesterol level too increases in diabetic animals. It was 215.42 mg/dl on the 21st day for diabetic animals for normal animals it was 97.79 mg/dl on the 21st day (Table 1). The levels of cholesterol were found lower (142.4 mg/dl) in animals treated with 300 mg/kg of chloroform leaf extract compared with untreated ones. The same dose required serum cholesterol on the 21st day such as 121.80 mg/dl to ethanol extract and 131.11 mg/dl to aqueous extract. Groups treated with 300 mg/kg of ethanol callus extract showed 113.02 mg/dl of serum cholesterol, whereas 600 µg/kg of glibenclamide produced 146.08 mg/dl on the 21st day.

The concentration of triglycerides was found significantly higher in diabetic animals. It was 147.82 mg/dl more or less double the normal of 69.35 mg/dl. But the triglycerides levels were lower in diabetic animals treated with 300 mg/kg of chloroform extract (121.30 mg/dl),

Table 1: Changes in levels of total cholesterol, triglycerides, and phospholipids in serum of normal and experimental animals

Control & Treatment		Groups	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	Phospholipids (mg/dl)
Normal		I	97.79 ±1.82	69.35±1.92	72.16±1.46
Chloroform	100 mg/kg	II	107.32±1.54	73.27±8.91	80.93±4.05
	200 mg/kg	III	108.20±1.56	75.48±3.55	73.61±1.63
	300 mg/kg	1V	112.27±1.02	79.20±1.75	77.85±1.8
Ethanol	100 mg/kg	V	109.45±2.55	79.46±2.05	80.40±2.81
	200 mg/kg	VI	109.23±2.61	77.02±5.12	73.12±1.92
	300 mg/kg	VII	111.83±4.71	78.63±5.29	72.97±1.65
Aqueous	100 mg/kg	VIII	101.75±1.21	71.58±1.36	77.65±2.70
	200 mg/kg	IX	109.36±1.05	68.29±1.33	80.31±1.77
	300 mg/kg	X	110.22±1.55	72.85±1.38	80.90±1.73
Ethanol callus	100 mg/kg	XI	99.70±2.76	75.60±2.66	72.70±1.56
	200 mg/kg	XII	104.4±2.61	74.70±1.55	73.00±2.67
	300 mg/kg	XIII	105.5±2.57	72.6±01.58	73.40±2.58
Diabetic (STZ)	40 mg/kg	XIV	215.42±2.67*	147.82±2.92*	146.71±2.34*
STZ + Chloroform	100 mg/kg	XV	145.8±0.82#	135.51±1.63#	131.40±1.82#
	200 mg/kg	XVI	144.82±1.43#	128.82±1.12#	121.9±1.19#
	300 mg/kg	XVII	142.4±2.81#	121.3±1.27#	119±1.47#
STZ + Ethanol	100 mg/kg	XVIII	128.25±1.32#	91.16±1.82#	102.5±3.35#
	200 mg/kg	XIX	125.14±1.24#	85.26±1.64#	94.43±1.62#
	300 mg/kg	XX	121.80±1.27#	84.30±1.16#	90.81±1.25#
STZ + Aqueous	100 mg/kg	XXI	137.82 ±1.08#	94.12±1.97#	109.3±1.18#
	200 mg/kg	XXII	135.56±1.84#	93.16±0.08#	107.8±1.24#
	300 mg/kg	XXIII	131.11±1.24#	92.6±2.47#	106.1±1.43#
STZ + Ethanol callus	100 mg/kg	XXIV	119.4±1.23#	83.44±2.40#	87.13±1.32#
	200 mg/kg	XXV	114.51±1.65#	82.41±2.42#	85.25±1.91#
	300 mg/kg	XXVI	113.02±2.62#	82.02±1.32#	84.01±1.32#
STZ + Glibenclamide	600 µg/kg	XXVII	146.8±4.08#	99.16±5.08#	140.6±6.33#

Values are expressed as mean ± SD (n=6).

in the ethanol extract treated groups it was (84.30 mg/dl), in the aqueous extract treated groups it was (92.60 mg/dl) and in the ethanol callus extract treated groups 82.02 mg/dl. It was 99.16 mg/dl in the groups treated with glibenclamide at a dose of 600 µg/kg (Table 1).

The phospholipids content was higher in diabetic rats (146.71 mg/dl) almost double the normal values of 72.16 mg/dl on the 21st day. After treatment with 300 mg/kg chloroform leaf extract it was 119 mg/dl; with the diabetic animals treated with 300 mg/kg of ethanol extract it was 90.81 mg/dl; with doses of 300 mg/kg of aqueous extract 106.1 mg/dl. Diabetic animal treated with 300 mg/kg of ethanol extract of callus showed 84.01 mg/dl of phospholipids on the 21st day. Diabetic animal treated with 600 µg/kg of glibenclamide showed 140.6 mg/dl of phospholipids (Table 1).

Diabetic control is compared with normal; *Values are statistically significant at P^{*} <0.05 compared to normal.

Treated groups are compared with diabetic control; # Values are statistically significant at $P^{\#}$ <0.05 compared to diabetic control.

The serum protein concentration decreased in diabetic animals. It was 3.98 g/dl. With the normal group it was 8.11 g/dl on 21st day (Table 2). The level of protein increased in leaf extract treated animals 4.63 g/dl protein was recorded in the 300 mg/kg of chloroform leaf extract treated animals. In the ethanol extract treated specimen it was 5.62 g/dl; in the aqueous extract treated group it was 4.88 g/dl and in the ethanol callus extract treated group it was 8.84 g/dl. In the 600 µg/kg of glibenclamide treated group, the scores were 5.10 g/dl.

The albumin level decreased in diabetic animals. It was 2.15 g/dl. In normal animal it was 6.98 g/dl on 21st day (Table 2). The levels of albumin increased in animals treated with all the three extracts. When the extracts were administrated at the rate of 300 mg/kg, the groups recorded the albumin levels varying from 5.45 to 3.65g/dl. The minimum level was recorded in the group treated with chloroform leaf extract 3.65g/dl. The ethanol extract treated group recorded 4.95 g/dl. The group treated with aqueous extract recorded 4.63 g/dl and the group treated with ethanol callus extract had the high value (5.45 g/dl). In the group treated with glibenclamide at a dose of 600 µg/kg the albumin level 5.19 g/dl on the 21st day.

The concentration of hemoglobin content decreased in diabetic animals. It was 8.81 g/dl. In normal animals it was 14.31 g/dl on 21st day (Table 2). The level of haemoglobin increased in leaf extracts treated animals. It was 9.89 g/dl in the chloroform leaf extract treated animals. The ethanol extract treated sample registered 12.60 g/dl. In the case of the aqueous extract treated animals it was 11.59 g/dl; while the ethanol callus extract treated groups showed 13.55 g/dl of hemoglobin. In the glibenclamide (600 µg/kg) treated groups it was 12.81 g/dl.

Diabetic control is compared with normal; *Values are statistically significant at P^{*} <0.05 compared to normal.

Treated groups are compared with diabetic control; # Values are statistically significant at $P^{\#}$ <0.05 compared to diabetic control.

Increased levels of serum triglycerides and cholesterol observed in streptozotocin-induced diabetic rats were in more or less agreement with other studies.[22,23] The abnormally high concentration of serum lipids in diabetic animals is due mainly to an increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone-sensitive lipase.[24] Excess fatty acid in the serum of diabetic rats is converted into phospholipids and cholesterol in the liver. These two substances along with the excess triglycerides formed at the same time in the liver may be discharged into the blood in the form of lipoproteins.[25]

Hypertriglyceridaemia is common among diabetic patients and is responsible for vascular complications.[26] In the study of Bruan and Severson[27] it was concluded that deficiency of lipoprotein lipase activity may contribute significantly to the elevation of triglycerides in diabetes. Lopes Virella *et al.* [28]

Table 2: Changes in the level of serum protein, albumin and haemoglobin in normal and experimental animals

Control & Treatment		Groups	Serum protein (g/dl)	Albumin (g/dl)	Haemoglobin (g/dl)
Normal		I	8.11±0.87	6.98±0.85	14.31±5.78
Chloroform	100 mg/kg	II	8.85±0.55	7.45±0.89	14.55±3.81
	200 mg/kg	III	8.90±0.59	7.62±0.67	14.15±0.63
	300 mg/kg	1V	8.52±0.44	7.86±0.31	14.88±0.35
Ethanol	100 mg/kg	V	8.72±0.49	7.77±0.72	13.85±4.13
	200 mg/kg	VI	8.56±0.78	8.53±0.14	14.75±0.54
	300 mg/kg	VII	8.53±0.26	7.21±0.14	13.87±0.36
Aqueous	100 mg/kg	VIII	8.91±0.45	6.55±0.61	14.81±4.07
	200 mg/kg	IX	8.74±0.62	7.96±0.22	14.30±0.87
	300 mg/kg	X	8.78±0.36	8.53±0.23	14.92±0.95
Ethanol callus	100 mg/kg	XI	8.61±0.54	7.62±0.08	13.33±0.17
	200 mg/kg	XII	8.43±0.72	7.92±0.31	13.54±0.58
	300 mg/kg	XIII	8.17±0.54	7.73±0.39	14.13±0.21
Diabetic (STZ)	40 mg/kg	XIV	3.98±0.61*	2.15±0.46*	8.81±3.71*
STZ + Chloroform	100 mg/kg	XV	4.02±1.08#	3.02±0.25#	9.02±2.18#
	200 mg/kg	XVI	4.58±0.91#	3.13±0.61#	9.74±1.27#
	300 mg/kg	XVII	4.63±0.22#	3.65±0.37#	9.89±1.35#
STZ + Ethanol	100 mg/kg	XVIII	4.95±1.58#	4.77±0.22#	11.89±1.07#
	200 mg/kg	XIX	5.09±0.13#	4.81±0.52#	12.05±1.18#
	300 mg/kg	XX	5.62±0.25#	4.95±0.83#	12.60±1.23#
STZ + Aqueous	100 mg/kg	XXI	4.64±0.41#	4.22±0.43#	10.65±1.29#
	200 mg/kg	XXII	4.78±0.85#	4.61±0.45#	10.66±1.79#
	300 mg/kg	XXIII	4.88±0.36#	4.63±0.86#	11.59±2.23#
STZ + Ethanol callus	100 mg/kg	XXIV	7.10±0.35#	4.97±0.32#	13.12±3.58#
	200 mg/kg	XXV	8.18±0.06#	5.02±0.62#	13.45±1.36#
	300 mg/kg	XXVI	8.84±0.14#	5.45±0.72#	13.55±1.19#
STZ + Glibenclamide	600 µg/kg	XXVII	5.10±0.24#	5.19±0.88#	12.81±1.48#

Values are expressed as mean±SD (n=6).

reported that treatment of diabetes with insulin served to lower plasma triglycerides levels by returning lipoprotein lipase levels to normal. In the present study the decreasing levels of plasma triglycerides and cholesterol levels following the treatment with *P. daemia* leaf extract and glibenclamide may be due to their stimulating effect on insulin secretion.

A decrease in serum protein content with concomitant increase in protein level of diabetic rats was observed in the present study. Advanced oxidative protein products (AOPP), reactive oxygen species (ROS) and free radicals produce protein carbonyl products **(**PCO) and are considered as markers of oxygen-mediated protein damage. They also indicate changes in glomerular filtration barrier that result in the increased permeability of the membrane.[29,30]

Serum protein, albumin and haemoglobin levels were reduced in diabetic rats as reported by Prakasam *et al*.[31] from their herbal drug antidiabetic study. Serum protein, albumin and haemoglobin level reduction may be due to increased protein catabolism caused by streptozotocin.[32] The results of the present study demonstrated that the treatment of diabetic rats with the leaf extract of *P. daemia* caused a noticeable elevation in the plasma total protein and albumin levels as compared with their normal levels. Such improvement of serum protein and albumin was previously observed after the oral administration of *Balanites aegyptiaca* (*B. aegyptiaca*) to experimentally diabetic rats.[33] It has been established that insulin stimulates the incorporation of amino acids into proteins.

CONCLUSION

The different solvents viz., chloroform, ethanol and aqueous leaf extracts and ethanol leaf derived callus extract were tested for the Anti-hyperlipidemic and Biochemical activity. Among the three solvents tested, ethanol extract has high moderately effect followed by aqueous and chloroform extract. Among the tested concentration 300 mg/kg were highly susceptible for leaf and leaf derived callus extracts. The statistics inhibitory activity was observed in ethanol extract of both leaf and leaf derived callus extracts. In the current study therefore, the main emphasis was on the potentialities of the use of *in vitro* derived callus of *P. daemia* and field grown leaf extracts of *P. daemia* for Antihyperlipidemic effect and Biochemical parameters has been demonstrated for the first time.

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