International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 6 (5): 738-743, 2010
ISSN 1811-7775

Hypoglycemic and Hypolipidemic Activity of *Pongamia pinnata* (Linn.) Pierre in Streptozotocin-induced Diabetic Rats

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Abstract: The plant *Pongamia pinnata* (Linn.) Pierre of family Leguminosae sub-family Papilioanaceae was evaluated for its hypoglycemic and hypolipidemic activity in streptozotocin induced diabetic rats. A new difuranoflavonone Compound PP (named Pongamiaflavonol), isolated from methanlolic extract of *P. pinnata* pods by column chromatography, was also studied for the activity. It was observed that after 14 days of treatment blood glucose level was reduced by 66.34, 54.82, 63.62 and 67.48 % with Std. Glibenclamiade 3 mg kg^{-1}), *P. pinnata* pods (300 mg kg^{-1}), *P. pinnata* flowers (300 mg kg^{-1}) and PP (100 mg kg^{-1}), respectively. The lipid profile was also studied and was found to be normalized significantly by both the flowers and pods extracts of *P. pinnata* and compound PP.

Key words: *Pongamia pinnata,* Karanja, hypolipidemic, antidiabetic activity, streptozotocin

INTRODUCTION

Diabetes mellitus is one of the common metabolic disorders. In addition to hyperglycemia, hypercholesterolemia and hypertriglyceridemia are the common complications of diabetes mellitus (Riyad *et al*., 1988; Brownlee, 2005; Sharma *et al*., 1996).

Prevalence of diabetes in adults worldwide was estimated to be 4.0% in 1995 and to rise to 5.4% by the year 2025. It is higher in developed than in developing countries. The number of adults with diabetes in the world will rise from 135 million in 1995 to 300 million in the year 2025. The major part of this numerical increase will occur in developing countries. There will be a 42% increase, from 51 to 72 million, in the developed countries and a 170% increase, from 84 to 228 million, in the developing countries. Current estimates are that at least 150 million people worldwide have diabetes, of which two-thirds live in developing countries. The countries with the largest number of people with diabetes are and will be in the year 2025, India, China and the US (King *et al*., 1998). In another study, the total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 (Wild *et al*., 2004).

Insulin and oral hypoglycemic agents like sulphonylureas and biguanides are still the major players in the management of the disease. However, complete cure of the disease has been eluding physicians for centuries and the quest for the development of more effective antidiabetic agents is pursued relentlessly. No single drug is ideal in the treatment due to toxic side effect and sometimes diminution in response after prolonged use (David, 1996). The disadvantage of the presently using antidiabetic drugs are that they have to be given throughout the life and produce side effects (Dixit and Joshi, 1985). The toxicity of oral antidiabetic agents differs widely in clinical manifestations, severity and treatment (Spiller and Sawyer, 2006).

The use of herbal medicine for the treatment of diabetes mellitus has gained importance throughout the world. Many herbal products, including several metals and minerals have been described for the cure of diabetes mellitus in ancient literature. The World Health Organization also recommended and encouraged this practice especially in countries where assess to conventional treatment of diabetes mellitus is not adequate (WHO, 1980). Herbal preparations alone or in combination with oral hypoglycemic agents sometimes produce a good therapeutic response in some resistant cases where modern medicines alone fail (Anturlikar *et al*., 1995). Currently available treatment is far from satisfactory and is expensive.

Pongamia pinnata (Papilionaceae), commonly known as Karanja, is distributed throughout India in tidal and beach forest. It is used medicinally in India, China, Australia and Phillippine Island. In Indian traditional system of medicine, different parts of *P. pinnata* have been used for bronchitis, whooping cough, scabies,

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leprosy, piles, ulcers and rheumatic joints (Meera *et al*., 2003; Wealth of India, 2003). Various investigations have been done on the pharmacological activity of pods and flowers of *P. pinnata*. Significant anti-inflammatory, analgesic, antifilarial and antiulcerogenic activity have been reported in pods (Muruganandan *et al*., 2000; Singh *et al*., 1996; Uddin *et al*., 2003). On the other hand flowers of *P. pinnata* showed significant antihyperglycemic, antilipidperoxidative and antioxidant activity (Pumitha *et al*., 2006; Shirwaikar *et al*., 2003). Juice of the leaves is prescribed to relieve flatulence, dyspepsia, diarrhoea and cough. It is also considered a remedy for leprosy and gonorrhea (Kritikar and Basu, 1995). Flower Pod extract of *P. pinnata* have been reported to show antibacterial as well as antifungal activity (Kumar *et al*., 2010).

Decoctions of flowers of *P. pinnata* have been reported to be used to quench the excess thirst by diabetes patient traditionally (Kritikar and Basu, 1995).

As the plant flowers for a short period, the pods may be used as an alternate in effect. The antihyperglycemic activity of *P. pinnata* flowers have been already reported in steptozotocin induced diabetic rats but the activity has not been reported in pods so far. Therefore, a comparative hypoglycemic and hypolipidemic study of pods, flowers and a new isolated compound PP from *P. pinnata* pods was investigated in streptozotocin induced diabetic rats in the present study.

MATERIALS AND METHODS

Plant material: The pods and flowers of *P. pinnata* were collected from Jamia Hamdard campus in October 2006 and were identified by Dr. Javed Ahmad, Department of Botany, Jamia Hamdard. (Voucher No. PRL-001-06). The voucher specimens are kept for the record in Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi. Flowers of plant were dried in shade in for 7 days in room temperature and pods were dried at 60°C for 6 h. Both flowers and pods were powdered and extracted with water by soxhlet extraction. The extracts were vaccum dried in rotator vacuum film evaporator (Perfit Model No. 5600 Buchi type). Extractive yield of aqueous extract pods and flowers were 12.5 and 15.8 % of dry plant, respectively.

Methanolic extract of *P. pinnata* pods was fractionated by column chromatography. A new difuranoflavonone named Pongamiaflavonol $C_{20}H_{12}O_6$ (PP) was isolated from methanlolic extract of *P. pinnata* pods by column chromatography.

Animal: Colony bred, healthy Wistar Albino rats were obtained from the animal house of Jamia Hamdard, New Delhi, after obtaining approval from institutional ethical committee. All the animals were weighted (200-250 g) and marked separately. Animals having similar weight and sex were kept in same group. The animals were housed in standard cages (48×35×22 cm) at room temperature (25± 2°C), with artificial light from 7.00 a.m. to 7.00 p.m. and provided with pelleted food and water *ad libitum*.

Experimental method

Acute toxicity study: Acute toxicity was performed for aqueous extract according to the acute toxic classic method as per guidelines of Organization for Economic Cooperation and Development (OECD, 1996). Albino wistar rats were used and animals were kept fasting for overnight providing only water, after which the extract was administered orally at the dose of 300 mg kg^{-1} and observed for 24 h. If the mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If the mortality was observed in one animal, then the same dose was repeated to confirm the toxic dose. If mortality was not observed, the procedure repeated for further higher dose, i.e., 2000 mg kg^{-1}. One tenth of maximum dose of the extract tested for acute toxicity was selected as dose, i.e., 300 mg kg^{-1}.

Antidiabetic activity: Albino Wistar rats (200-250 g) were randomly divided into six groups with six animals in each group. Except the group I (normal control), in animals of all other groups, diabetes was induced by injecting Streptozotocin (50 mg kg^{-1} i.p. for 4 consecutive days) which was freshly prepared in citrate buffer (pH 4.5). After 4 days these hyperglycemic rats were used for the study.

Group I: (normal control) rats received only buffer (orally).

Group II: (diabetic control) these rats were kept without any treatment to study the diabetic nature of rat.

Group III: (standard) received the reference standard drug glibenclamide (3 mg kg) administered orally.

Groups IV: *P. pinnata* pods extract *P. pinnata* pods extract (300 mg kg^{-1}) in 1% Carbo xy methyl cellulose (CMC) through oral route.

Groups V: (*P. pinnata* flowers extracts): *P. pinnata* pods extract (300 mg kg^{-1}) in 1% CMC through oral route.

Groups VI: (pure compound PP-2) received new isolated difurano flavonoids (100 mg kg^{-1}) in 1% CMC through oral route.

After 14 days of treatment, the blood glucose level was estimated with One Touch Basic plus Glucometer using strips (Lifescan Inc. USA).

Estimation of blood glucose and lipid profiles: Blood was taken from the orbital sinus of each rat with the help of a capillary tube for the estimation of blood sugar (Semalty and Semalty, 2008). The Institutional ethics committee approved all experimental protocols. One drop of the blood is spread on the slide of Glucometer to estimate the blood glucose level of experimental animals. The blood glucose level, displayed on the screen of glucometer was recorded. The decrease in percentage of glucose level experimental animal was given was calculated, the percent decrease in blood glucose level can be calculated using by formula:

$$\text{Percent decrease in blood glucose level} = \frac{(\text{Before-After treatment}) \times 100}{\text{Before treatment}}$$

For determination of lipid profiles, serum was separated by immediate centrifugation of blood samples using REMI ultra cooling centrifuge at 3000 rpm for 5 min at room temperature and was directly used for estimating triglyceride (TG), total cholesterol, HDL-cholesterol by using respective span diagnostic kits (Anreddy *et al*., 2010). VLDL (very low density lipoproteins) cholesterol was calculated as: TG/5; on the other hand LDL (low density lipoproteins) cholesterol was calculated using the following formula:

LDL cholesterol = Total cholesterol-(HDL+VLDL)

Statistical analysis: The data are represented as Mean±SEM and statistical significance between treated, untreated and control group was analyzed by ANOVA followed Dunnett's multiple comparison *t*-tests. Student's t-test. $p<0.05$ implies significance.

RESULTS

Extractive yield of aqueous extract pods and flowers were 12.5 and 15.8% of dry plant respectively. A new difuranoflavonone named Pongamiaflavonol $C_{20}H_{12}O_6$ (PP) was isolated from methanlolic extract of *P. pinnata* pods by column chromatography.

The dose of 300 mg kg^{-1} body weight per oral did not produce any toxic effect. Antidiabetic activity of aquous extract of *P. pinnata* was significantly observed after 14 days of treatment compared to diabetic control (Table 1). Administration of Streptozotocin (50 mg kg^{-1}) led to elevation of blood glucose level. A comparative chronic antidiabetic study was carried out between aqueous extract of *P. pinnata* pods and *P. pinnata* flowers. It was observed that after 14 days of treatment, blood glucose level was reduced by 66.34, 54.82, 63.62 and 67.48% with Std. Glibenclamiade 3 mg kg^{-1}), *P. pinnata* pods (300 mg kg^{-1}), *P. pinnata* flowers (300 mg kg^{-1}) and PP (100 mg kg^{-1}), respectively. Therefore it can be concluded that both flowers and pods extract of *P. pinnata* showed significant antidiabetic activity. Similarly Compound PP showed significant results as compared to standard drug.

The lipid profiles was also studied and were found to be normalized significantly by both the extracts of *P. pinnata* and compound PP (Table 2). Table 2 depicts the levels of total cholesterol, LDL, HDL, VLDL and triglyceride in control and streptozotocin-induced diabetic rats. The levels of cholesterol and triglyceride increased

Table 1: Antidiabetic activity of *P. pinnata*

		Blood glucose level (mg/100 mL)		
Group	Treatment	Initial	After STZ	After 14 days
I	Normal	64.33±2.69	---	61.66±2.47
II	Diabetic control	68.33±1.87	284.47±8.14	296±6.02
II	Std. Glibenclamiade 3 mg kg^{-1})	74.53±2.34	289.71±8.32	97.50±2.17**
IV	*P .pinnata* pods (300 mg kg^{-1})	71.66±3.39	277±8.39	125.16±9.20**
V	*P .pinnata* flowers (300 mg kg^{-1})	83.66±4.86	288±66±6.46	105±7.12**
VI	PP-2 (100 mg kg^{-1})	76.50±8.19	288.50±7.98	93.83±3.39**

Values are Mean±SEM (n=6), **p<0.01

Table 2: Effect of 14 days treatment of extracts and compound of *P. pinnata* on serum lipid profile

	Body weight (g)						
Treatment	Initial	Final	Cholesterol	LDL	HDL	VLDL	Triglycerides
			(mg dL^{-1})				
Normal control	210	222	71.76±2.21	43.28±1.17	28.37±0.86	14.92±1.20	67.66±1.01
Diabetic control	215	195	152.37±3.45	51.85±0.50	14.04±0.56	37.80±0.48	189.04±2.44
Standard	202	216	98.24±2.14	45.74±1.47	31.37±1.52	14.36±0.22	71.85±1.12
Pods	216	225	91.36±1.50	53.05±1.27	36.96±1.10	16.12±0.31	80.13±1.72
Flowers	232	240	86.72±0.95	47.97±0.76	33.34±0.82	14.62±0.19	73.16±0.97
Compound PP-2	219	235	83.44±0.79	43.77±1.87	29.55±2.02	14.22±0.42	71.13±2.14

Values are Mean±SEM compared with diabetic control group

from 71.76 and 67.66 mg dL^{-1} in normal control to 152.37 and 189.04 mg dL^{-1} in diabetic animals, respectively. After treatment with compound PP, the higher levels of cholesterol and triglyceride, decreased to 83.44 and 71.13 mg dL^{-1}, respectively which are almost near the levels of normal control. The level of HDL in serum of diabetic animals decreased to 14.04 mg dL^{-1} from 28.37 mg dL^{-1} in normal control. These lowered levels of HDL were restored significantly in pod extract (36.96 mg dL^{-1}), flower extract (33.34 mg dL^{-1}) and Compound PP (29.55 mg dL^{-1}) treated diabetic rats. Moreover, Pod extract was found to be the most effective in improving the HDL levels. The restoration of HDL was better even with flower extract and compound PP also in comparison with that of standard (31.37 mg dL^{-1}).

DISCUSSION

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia associated with several other factors including dyslipidemia, which are involved in the development of micro- and macrovascular complications. The currently available drug regimens for management of diabetes mellitus have certain drawbacks and therefore, there is a need to find safer and more effective antidiabetic drugs (Grover *et al*., 2002; Daisy *et al*., 2009; Rajagopal and Sasikala, 2008). Chronic Diabetes mellitus is associated with several complications such as atherosclerosis, myocardial infarction, nephropathy etc. These complications have long been assumed to be related to chronically elevated glucose level in blood (Alarcon-Aguilara *et al*., 2000).

Streptozotocin (STZ) is an alkylating agent antibiotic that experimentally produces diabetes due to β-cell death by the mechanism of DNA damage in rodent islets (Yang and Wright, 2002). During STZ metabolism, various toxic intermediates are produced, including methyl cations, methyl radicals, reactive oxygen species (ROS) and nitric oxide (NO). Beta cells are very susceptible to oxidative changes since they possess a low antioxidative capacity (El-Sayed *et al*., 2009).

In the present study, both flowers and pods extract of *P. pinnata* showed significant hypoglycemic activity in the STZ induced diabetic rats. After 14 days of treatment, antidiabetic activity was found to be in the decreasing order of compound PP (100 mg kg^{-1})> Std. (Glibenclamide 3 mg kg^{-1})> *P. pinnata* flowers' extract (300 mg kg^{-1})> *P. pinnata* pods' extract (300 mg kg^{-1}). The orally administered PpFAet (300 mg $orally^{-1}$) to diabetic rats showed a significant antihyperglycemic activity. Compound PP (A new difuranoflavonone named Pongamiaflavonol) was isolated from methanlolic extract of *P. pinnata* pods. The compound PP showed the best hypoglycemic activity and this indicates that pod extract is richer in hypoglycemic content than that of flower. In previous investigations on *P. pinnata,* the hypoglycemic activities have been reported in flower and bark also (Punitha *et al*., 2006; Badole and Bodhankar, 2009a, b). Our study supported the presence of antihyperglycemic activity of the plant with a focus on activity of flower and flower pod extract. The activity has been explored and reported in the pod very first time.

Insulin deficiency or insulin resistance is associated with hypercholesterolemia and hypertriglyceridimia (Sharma *et al*., 1996). The abnormally high concentration of serum lipids in diabetes mellitus is mainly due to an increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. The marked hyperlipidaemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots (Al-Shamaony *et al*., 1994). HDL removes cholesterol from non-hepatic tissues to liver through the process known as reverse cholesterol transport. Several studies have documented reduction in plasma HDL cholesterol in diabetic rats and diabetic patients due to defect in reverse cholesterol transport. Our results support these observations. Liver plays an important role in the catabolism and excretion of cholesterol. Profound increases in plasma and tissue lipids (cholesterol, phospholipids, triglycerides and free fatty acids) were reported in diabetic animals. Triglycerides accumulation in the liver of diabetic rats is due to enhanced synthesis or decreased output from liver as VLDL or combination of both. Oral administration of flower extract, pod extract and compound PP of *P. pinnata* restored the levels of lipids and lipoproteins in diabetic rats. Administration of flower extract, pod extract and compound PP to diabetic rats brought down serum cholesterol, LDL, VLDL, triglycerides and increased HDL– cholesterol level.

CONCLUSION

In conclusion this study established the antidiabetic potential of both the flowers and pods extracts. It also suggested that pods to be good substitute for flowers in effect. It is also noteworthy that our study tried to correlate the antidiabetic effect of pod extract to one of its compound PP (a difuranoflavonone) which showed significant antidiabetic activity. The flower and pod extract (and also compound PP) showed significant hypolipidemic activity. Therefore, the aquous extract of

flowers and pods of *P. pinnata* possesses antidiabetic activity and it may be used in diabetic conditions with or without cardiovascular complications.

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