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ANTI-DIABETIC ACTIVITY OF *PISONIA ACULEATA* LEAF IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

Aim: *The aim of the present study was to evaluate the antihyperglycemic effect of leaves of Pisonia aculeata (Family: Nyctaginaceae) on alloxan-induced diabetes in rats.* ***Methods:*** *Ethylacetate and ethanolic extract of the leaves of Pisonia aculeata were administered orally at doses of 100mg/kg and 200 mg/kg in normal and alloxan-induced diabetic rats. Body weight and blood glucose levels were estimated on 4th, 7th, and 10th day of the treatment. Blood samples were collected on the 10th day for estimation of biochemical parameters (serum urea, serum creatinine, serum cholesterol, serum protein).* ***Results:*** *Both extracts were able to produce a significant hypoglycemic effect as well as reduction in serum urea, serum creatinine and serum cholesterol.* ***Conclusion:*** *The present study suggests that ethanolic extract of Pisonia aculeata leaves possesses potent antidiabetic property. Further studies on Pisonea aculeata leaves and its isolated compounds are necessary to elucidate the exact mechanism of action and to develop it as a potent antidiabetic drug.*

KEY WORDS

Pisonia aculeata, Alloxan, Diabetes mellitus, Blood glucose.

INTRODUCTION

Diabetes is the most common disease associated with carbohydrate metabolism and is a major cause of disability and hospitalization [1]. Type II diabetes is by far the commonest form of the disease globally, with rapidly developing countries being at the forefront of this epidemic affecting at least 15 million people having complications which include hypertension, atherosclerosis and microcirculatory disorders [2]. India has today become the diabetic capital of the world with over 20 million diabetes and this number is set to increase to 57 million by 2025[3]. Diabetes mellitus is ranked seventh among the leading causes of death and is considered third when its fatal complications are taken into account [4].Diabetes mellitus is a multi-factorial disease which is characterized by hyperglycemia[5], lipoprotein abnormalities [6],raised basal metabolic rate[7],defect in reactive oxygen species scavenging enzymes[8]

A wide number of traditional medicinal plants are still being used to treat diabetes mellitus. Several beneficial role such as correcting alternated carbohydrate metabolism, maintaining integrity and function of β-cells, insulin secreting activity, enhancing glucose up take and utilization and antioxidant properties present in traditional medicinal plant and their constituents offer exciting opportunity to develop them in to novel therapeutics[9] In the present study we selected a plant namely *Pisonia aculeata* . It is a monoecious evergreen medium sized tree used traditionally for its curative property in treating diabetes, rheumatism and ulcer disorders [10]. From the literature survey, *Pisonia aculeata* has been reported to contain several secondary metabolites like flavonoids, coumarins, flavones, steroids,

triterpenoids [11]. Thus the present study was initiated to evaluate anti diabetic activity of various extract of leaf of *Pisonia aculeata*.

MATERIAL AND METHODS

The plant of *Pisonia aculeata* collected from Chittoor district was authenticated by Dr. K. Madhava Chetty, Ph.D., Department of Botany, S.V University, Tirupathi with Voucher Specimen No: 0603. The leaves of *Pisonia aculeata* were shade dried after collection of 15 days and was coarsely powdered. The powdered leaf was defatted with petroleum ether and then subjected to continuous hot extraction in Soxhlet apparatus with ethylacetate and ethanol. The extract was filtered through a cotton plug, followed by Whatmann filter paper (No.1). The extract was evaporated under reduced pressure using Rotovac evaporator at a low temperature (40-60ºC)[12].

Phytochemical studies:

The various extract of *Pisonia aculeata* were subjected to preliminary phytochemical screening [13] and it revealed the presence of alkaloids, flavonoids, terpenoids, steroids, tannins and carbohydrates.

Animals

Wistar Albino rats (**150-200 grams**) of either sex were used in this investigation. They were maintained at standard housing condition and fed with commercial diet (Hindustan Lever Ltd., Bangalore) and provided with water *ad libitum* during the experiments. Permission was obtained from the Institutional Animal Ethical Committee prior to the study.

Acute toxicity studies

Acute toxicity study was performed for various extracts of *Pisonia aculeata* according to the acute toxic classic methods as per OECD guidelines [14]. The animals were kept fasting overnight providing only water, after which the various extracts were administered orally at the dose of 2000mg/kg which was prepared by dissolving the extract in distilled water and the concentration was adjusted in such a way that it did not exceed 1ml/100g body weight of the rat. The extract was then administered (p.o) and animals were observed for any behavioral changes, toxicity and mortality up to 48 hrs.

Induction of diabetes

Diabetes mellitus was induced by single I.P injection of freshly prepared solution of alloxan monohydrate (120mg/kg body weight) dissolved in physiological saline in overnight fasted Wistar rats [15]. Diabetes was assessed in alloxan injected rats by determining the blood glucose concentration. Rats with blood glucose level above 250mg/dl were selected for the experimental study.

Anti-diabetic activity

Fasting blood glucose was determined after depriving food for 16 hrs with free access of drinking water. Hyperglycemia was induced by a single intra peritoneal injection of 120 mg/kg of alloxan monohydrate in sterile saline. After 5 days of alloxan injection, the hyperglycemic rats (glucose level>250 mg/dl) were separated and divided into different groups comprising of 6 rats each for the anti-diabetic study. The treatment (p.o) was started from the same day except normal control and diabetic groups for a period of 10 days. During this period, animals in all groups had free access to standard diet and water. Body weight and blood glucose levels were estimated on 4th, 7th and 10th day of the treatment. On the 10th day, blood samples were collected from overnight fasted rats by cardiac puncture under mild ether anesthesia for biochemical estimation [16].

Grouping of animals:

Rats are divided into 7 groups, each group containing 6 rats, the treatment schedules of animals belonging to different groups are shown below.

Group A: Served as normal control (food and distilled water).

Group B: Served as diabetic control - 2% alloxan monohydrate, 120 mg/kg body wt. This was followed by a daily administration of distilled water (10 ml/kg body wt per day). This group served as control for group D, E, F and G

Group C: Alloxan + Glibenclamide (10 mg/kg, p.o.) served as standard.

Group D: Alloxan + Ethanol extract of *Pisonia aculeata* (100 mg/kg, p.o.)

Group E: Alloxan + Ethanol extract of *Pisonia aculeata* (200 mg/kg, p.o.)

Group F: Alloxan + Ethyl acetate extract of *Pisonia aculeata* (100 mg/kg, p.o.)

Group G: Alloxan + Ethyl acetate extract of *Pisonia aculeata (*200 mg/kg, p.o.)

RESULTS

Effects of *Pisonia aculeata* leaf extract on fasting blood glucose level in diabetic rats.

A marked rise in fasting blood glucose level observed in diabetic control compare to normal control rats (**Table.1**). Ethanolic and ethyl acetate extract of *Pisonia aculeata* (at 100&200 mg/kg) exhibited a dose dependent significant anti hyperglycemic activity on 4th, 7th & 10th day post treatment. The ethylacetate (100mg/200mg) extract dose also caused reduction in blood glucose level but the results were found statistically insignificant. The antihyperglycemic effect of ethanolic extract (100mg/200mg/kg) was found less effective than the reference standard glibenclamide produced a significant reduction in blood glucose compared to diabetic control.

Table 1: Effect of *Pisonia aculeata* leaf extracts on fasting blood glucose level in alloxan induced diabetic rats

Group	Treatment	Fasting Blood Glucose Level (mg/dl)			
		Basal Value	4th Day	7th Day	10th Day
1.	Normal Control	91.43 ± 2.63	91.62 ± 2.92	92.32 ± 1.63	87.34 ± 3.24
2.	Diabetic Control	294.64 ± 6.30	295.6 ± 4.30	297.3 ± 5.46	298.6 ± 8.36
3.	Alloxan + Glibenclamide (10 mg/kg)	287.46 ± 3.20	208.36 ± 6.00***	186.4 ± 6.22***	178.34 ± 6.32***
4.	Alloxan + Ethanolic Acid (100 mg/kg)	290.43 ± 5.43	262.48 ± 3.64	235.3 ± 8.12*	212.36 ± 7.34**
5.	Alloxan + Ethanolic Acid (200 mg/kg)	286.34 ± 5.43	241.34 ± 3.12	206.4 ± 9.87*	194.07 ± 9.78**
6.	Alloxan + Ethylacetate Extract (100 mg/kg)	290.34 ± 3.24	268.82 ± 4.62	247.6 ± 7.30	238.32 ± 4.32**
7.	Alloxan + Ethylacetate Extract (200 mg/kg)	284.36 ± 4.32	250.32 ± 4.17	228.43 ± 6.34*	223.07 ± 8.32**

(Values are Mean ± SEM; n=6, *P<0.05, **P<0.01, ***P<0.0001 vs Diabetic Control)

Table 2: Effect of *Pisonia aculeata* leaf extracts on biochemical parameters in alloxan induced diabetic rats

(Values are Mean ± SEM; n=6, **P<0.01, ***P<0.001 vs Diabetic Control)

Group	Treatment	Serum Urea (mg/dl)	Serum Creatine (mg/dl)	Serum Cholesterol (mg/dl)	Serum Protein (mg/dl)
1.	Normal Control	30.48 ± 3.26	0.52 ± 0.06	104.32 ± 1.89	6.18 ± 0.56
2.	Diabetic Control	62.84 ± 2.14	1.21 ± 0.03	168.36 ± 1.32	4.63 ± 0.36
3.	Alloxan + Glibenclamide (10 mg/kg)	34.32 ± 1.34***	0.60 ± 0.04***	120.42 ± 2.36***	6.12 ± 0.06***
4.	Alloxan + Ethanolic Acid (100 mg/kg)	49.38 ± 1.48**	1.10 ± 0.08**	158.38 ± 3.12**	5.46 ± 0.13**
5.	Alloxan + Ethanolic Acid (200 mg/kg)	43.68 ± 4.32***	0.83 ± 0.04***	148.36 ± 3.23***	5.91 ± 0.32***
6.	Alloxan + Ethylacetate Extract (100 mg/kg)	43.68 ± 1.46**	1.30 ± 0.05**	162.23 ± 1.46**	4.98 ± 0.12**
7.	Alloxan + Ethylacetate Extract (200 mg/kg)	59.36 ± 1.58**	1.18 ± 0.04**	154.32 ± 2.64**	5.28 ± 0.16**

Table 3: Effect of *Pisonia aculeata* leaf extracts on Body weight in alloxan induced diabetic rats (Values are Mean ± SEM; n=6, *P<0.001 vs Diabetic Control)

Group	Treatment	Body Weight (gms)			
		Basal Value	4th Day	7th Day	10th Day
1.	Normal Control	202.56 ± 2.32	203.61 ± 2.16	204.32 ± 2.62	206.12 ± 2.82
2.	Diabetic Control	203.64 ± 2.76	182.32 ± 2.28	166.32 ± 2.24	152.00 ± 1.64*
3.	Alloxan + Glibenclamide (10 mg/kg)	206.32 ± 2.82	204.24 ± 2.12*	199.24 ± 2.32*	194.32 ± 1.68*
4.	Alloxan + Ethanolic Acid (100 mg/kg)	205.84 ± 2.16	196.24 ± 3.21*	191.28 ± 2.32*	187.24 ± 2.16*
5.	Alloxan + Ethanolic Acid (200 mg/kg)	205.32 ± 1.18	194.32 ± 2.18*	189.36 ± 2.82*	183.26 ± 1.38*
6.	Alloxan+Ethylacetate Extract (100 mg/kg)	206.24 ± 2.24	197.32 ± 1.16*	193.16 ± 1.34*	189.16 ± 1.32*
7.	Alloxan+Ethyacetate Extract (200 mg/kg)	206.12 ± 1.19	196.12 ± 1.36*	192.12 ± 4.32*	186.89 ± 1.34*

Effect of *Pisonia aculeata* leaf extract on biochemical parameters;

Serum urea, serum creatinine &serum cholesterol levels were decreased significantly in a dose related manner by ethanolic extract of *Pisonia aculeata* at 100/200mg/kg due to 10 days of treatment, where as protein level was increased significantly when compare to diabetic control group. However, the ethylacetate extract at dose (100mg/kg) failed to reverse the altered biochemical parameters. (**Table 2**)

Effect of *Pisonia aculeata* leaf extract on body weight in diabetic rat;

Normal control animals were found to be stable in their body weight but diabetic rats showed significant reduction in body weight during drug treatment .Alloxan mediated body weight reduction was significantly reversed by ethanolic extract in dose dependent manner (100mg/200mg/kg).the effect of ethyl acetate extract 100mg/kg on body weight of the animals was found statistically not significant when compared to the standard glibenclamide.

DISCUSSION

The Pancreas is the primary organ involved in sensing the organisms dietary and energetic states via glucose concentrations in the blood and in response to elevated blood glucose, insulin in secreted [17]. Alloxan is one of the usual substances used for the induction of diabetes mellitus apart from streptozotocin. Alloxan has a destructive effect on the beta cells of the pancreas [18].Alloxan causes a massive reduction in insulin release by the destruction of β cells of the Islets of Langerhans, there by inducing hyperglycemia [19].

The results in present study indicate that *Pisonia aculeata* leaf extract was found to reduce the glucose level in animals made diabetic with alloxan. Alloxan has been shown to induce free radical production and cause tissue injury. In the present investigation ethanolic extract of *Pisonia aculeata* leaf demonstrated significant antidiabetic activity. The results from the present study also indicate that *Pisonia aculeata* leaf extract can reduce the levels of serum urea, serum creatinine, serum cholesterol, and increase the serum protein and confirms the possibility that the major function of the extract is on the regeneration of vital tissues of pancreas, thereby reducing the causation of diabetes in the experimental animals. Overall results demonstrate the antidiabetic activity of *Pisonia aculeata* leaves, but the activity may be due to presence of chemical constituents like alkaloids, flavonoids and terpenoids in leaves.

CONCLUSION

From this study, we can state that the ethanolic extract of *Pisonia aculeata* has beneficial effects on blood glucose level as well as improving hyperlipidemia and other metabolic aberrations. It has the potential to impart therapeutic effects in diabetes and needs long term studies on its extracts and its isolated compounds.

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