



ANTI-DIABETIC ACTIVITY OF METHANOLIC EXTRACT OF *PULICARIA WIGHTIANA* IN ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

The present study was carried out to investigate the anti-diabetic activity of methanolic extract of *Pulicaria wightiana* in alloxan induced diabetic rats for 14 days. Diabetes was induced in rats by administration of alloxan (150 mg/kg, i.p.). After the successful induction of experimental diabetes, the rats were divided into five groups each comprising a minimum of six rats. The methanolic extract at high dose (400 mg/kg) exhibited significant anti-hyperglycemic activity which closes to standard group when compared to control group. However, in groups treated with plant extract the reduction in the blood glucose was slightly less than that achieved with the standard group. The methanolic extract also shows significant improvement in lipid profile in diabetic rats. From this study, it can be concluded that *Pulicaria wightiana* possesses significant anti-diabetic activity in alloxan induced diabetic rats.

Key words: Alloxan, Blood Glucose, Anti-diabetic, *Pulicaria wightiana*

INTRODUCTION

Diabetes mellitus is one of the common metabolic disorders of multiple etiologies that results in significant morbidity and mortality. It is considered as one of the five leading causes of death in the globe. ^[1] The syndrome of diabetes mellitus is characterized by chronic hyperglycemia and an affinity to develop ketoacidosis. In present medicine no satisfactory effective therapy is still available to alleviate diabetes mellitus. ^[2] There is rising require by patients to use natural products with antidiabetic activity due to side effects associated with the use of insulin and oral hypoglycemic agents. ^[3] *Pulicaria wightiana* (Family: Asteraceae) commonly known as sontikli and distributed in various parts of India. Earlier phytochemical studies reveal the presence of Sesquiterpenoids, Diterpenoids and Flavonoids. ^[4] However there is no scientific report available on anti-diabetic activity of *Pulicaria wightiana*. Hence the present study was designed to evaluate anti-diabetic activity of *P. wightiana* in diabetic rats.

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MATERIALS AND METHODS

Chemicals

Alloxan was procured from Hi Media, India. Standard drug Glibenclamide was procured from Cipla Ltd., Diagnostic kits used in this study were procured from Span Diagnostics Ltd., India. All the other chemicals used were of analytical grade.

Plant material

The plant material used in the present investigation was obtained from local area of Kadapa, India. The botanical identity was confirmed at the Department of Botany, SV University, Tirupathi, India by a botanist.

Preparation of the extract

The whole plant product was collected and dried under shadow. The shade-dried whole plant was subjected to pulverization to get coarse powder. The coarsely powder whole plant (1 kg) of *P. wightiana* was used for extraction with methanol in soxhlet apparatus. The extract was evaporated to dryness under vacuum and dried in vacuum desiccator (15.5% w/w).

Animals

Healthy adult male Wistar albino rats between 2 and 3 months of age and weighing about 200–250 g were used for the study. The animals were housed in polypropylene cages, maintained under standard conditions (12 h light: 2 h dark cycle; 25±30°C; 35–60% humidity). They were fed with standard rat pellet diet (Pranav agro Ltd., Bangalore, India) and water *ad libitum*. The Institutional Animal Ethical Committee of

PRRMCP, Kadapa, India (1423/PO/a/11/CPCSEA), approved the study.

Oral glucose tolerance test (OGTT)

The oral glucose tolerance test was performed in overnight fasted (18 h) normal rats. Rats divided into four groups, each consisting of six rats were administered Distilled water, glibenclamide 5 mg/kg, methanolic extract of *P. wightiana* at 200 and 400 mg/kg, respectively. Glucose (3 g/kg) was fed 30 min after the administration of extract. Blood was withdrawn from the retro orbital sinus under ether inhalation at 0, 30, 90 and 150 min of glucose administration and glucose levels were estimated using glucose oxidase-peroxidase method.

Experimental model:

Alloxan monohydrate was weighed individually for each animal according to their body weight and solubilised with saline just prior to injection. Diabetes was induced by injecting it at a dose of 150 mg/kg body weight intraperitoneally. The animals were kept under observation and after 48 hrs blood glucose level was measured by semi-auto analyzer. The diabetic rats (glucose level 200-300 mg/dl) were separated and divided into five different groups for experimental studies, with each group containing six animals. Present study has confirmed that the treatment of methanolic extract of *P. wightiana* for a period of 21 days. 200 & 400 mg/kg of plant extract were screened for anti-diabetic activity against alloxan induced diabetic rats. The anti diabetic activity exhibited by extract was compared with that of standard drug Glibenclamide.

EXPERIMENTAL DESIGN

Group I: Normal (Distilled water).

Group II: Control (Alloxan, 150 mg/kg. ip)

Group III: Alloxan (150 mg/kg. ip) + Glibenclamide (5 mg/kg, p.o).

Group IV: Alloxan (150 mg/kg. ip) + MPW (200 mg/kg, p.o.).

Group V: Alloxan (150 mg/kg. ip) + MPW (400 mg/kg, p.o.).

At the end of the experiment rats were subjected to light ether anaesthesia then blood were collected from the retro - orbital venous plexus using a glass capillary and the blood was centrifuged (2,500 rpm/10min) to get serum. The serum was used for biochemical estimation of blood glucose, cholesterol (TC), triglycerides, HDL and LDL.

Statistical analysis

Data were expressed as the mean \pm SEM; and comparison between the different treatments was carried out using analysis of variance (ANOVA) followed by Tukey multiple comparisons test.

RESULTS

Oral glucose tolerance test (OGTT)

The blood glucose levels in the control group increased to peak level 30 minutes after glucose load. The methanolic extract showed a significant reduction in blood glucose levels from 90 min onwards in oral glucose tolerance test (table 1).

Anti-diabetic study

The animals treated with alloxan showed significant increase in the serum glucose levels was observed when compared to the normal animals. This indicates that alloxan induces persistent diabetes mellitus. On administration of MPW (200 and 400 mg/kg, p.o.) showed a significant decrease in the serum glucose levels when compared to diabetic control group. The MPW was significantly reduced cholesterol, triglycerides and LDL as well as significantly raises HDL level. (table 2 & table 3).

DISCUSSION

Management of diabetes with the agents devoid of any side effects is still a challenge in the medicinal system. This concern has led to an increased demand for natural products as anti diabetic agents. Alloxan induces diabetes with a single dose of administration by selective necrotic action on the beta cells of pancreas leading to the insulin deficiency. [5] In the present study, the anti-hyperglycemic activity of MPW was evaluated in alloxan-induced diabetic rats. Both doses of MPW Showed dose dependent and significant reduction in blood glucose concentration in diabetic rats. Maximum reduction of serum glucose level occurred at 7th day. Oral administration of Glibenclamide (5 mg/kg, p.o) showed the maximum reduction in glucose levels. Treatment with MPW leads to regeneration of the β -cells of the pancreas and potentiation of insulin secretion from surviving β -cells; the increase in insulin secretion and the consequent decrease in glucose levels may lead to inhibition of lipid peroxidation and control of lipolytic hormones. In this context, a number of other plants have also been reported to have antihyperglycemic, antihyperlipidemic and insulin stimulatory effects. [6] Excess of fatty acid in plasma produced by the alloxan-induced diabetes promotes the liver conversion of some fatty acids into phospholipids and cholesterol. [7] The higher lipid levels seen in diabetic rats were due to increased mobilization of free fatty acids from peripheral depots and also due to lipolysis caused by hormones. [8] MPW produced significant beneficial effects in the lipid profile in diabetic rats, reducing TC, TG, and LDL, significantly. Earlier phytochemical studies reveal the presence of Sesquiterpenoids, Diterpenoids and Flavonoids. [4] These components may exert its anti diabetic activity by regeneration of the β -cells of the pancreas and potentiation of insulin secretion from surviving β -cells.

Table 1: Effect of MPW on OGTT

Groups	Serum glucose (mg/dl) (mean ± SEM)			
	Time (min) after glucose administration			
	0	30	90	150
I	54.10 ± 3.067	109.76 ± 11.21	91.97 ± 4.445	71.26 ± 3.618
II	55.60 ± 4.794	111.13 ± 14.04	62.87 ± 4.209***	51.57 ± 2.631***
III	55.32 ± 5.178	109.84 ± 12.39	71.38 ± 3.739**	62.3 ± 2.097*
IV	54.36 ± 3.407	111.14 ± 11.91	66.95 ± 3.397***	56.19 ± 2.002**

* indicate p<0.05, ** indicate p<0.01, *** indicate p<0.001 when compared to control group

Table 2: Effect of MPW on blood glucose level in Alloxan induced diabetic rats

Groups	Serum glucose (mg/dL) (Mean ± SEM) on			
	Initial day	7 th day	14 th day	21 st day
I	66.30 ± 3.361	68.83 ± 4.474	66.49 ± 5.586	65.96 ± 3.384
II	177.93 ± 15.59	186.66 ± 9.744###	210.44 ± 12.24###	241.35 ± 10.03###
III	240.47 ± 15.54	104.01 ± 11.45***	90.83 ± 4.447***	65.79 ± 7.741***
IV	408.72 ± 21.11	391.94 ± 20.92***	103.5 ± 10.927***	73.74 ± 11.47***
V	224.11 ± 17.71	125.44 ± 4.369***	71.2 ± 7.907***	59.79 ± 3.056***

All values are shown as mean ± SEM and n=6.

Indicate p<0.001 when compared to normal group (G-I).

** Indicate p<0.01, *** Indicate p<0.001 when compared to control group (G-II)

Table 2: Effect of MPW on lipid profile in Alloxan induced diabetic rats

Groups	Serum glucose (mg/dL) (Mean ± SEM) on			
	Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
I	50.43 ± 2.70	95.73 ± 2.50	16.92 ± 1.98	35.19 ± 2.01
II	77.62 ± 1.22###	154.27 ± 4.31###	10.22 ± 1.89###	58.10 ± 1.12###
III	52.95 ± 2.80***	96.19 ± 3.68***	13.01 ± 0.99***	38.63 ± 1.98***
IV	57.85 ± 2.10***	101.88 ± 3.44**	14.28 ± 0.98***	42.32 ± 3.62***
V	54.40 ± 1.32***	98.30 ± 3.56***	13.36 ± 1.00***	40.66 ± 3.50***

All values are shown as mean ± SEM and n=6.

indicate p<0.001 when compared to normal group (G-I).

* indicate p<0.05, ** indicate p<0.01, *** indicate p<0.001 when compared to control group (G-II)

CONCLUSION

On the basis of the aforementioned results, we concluded that *Pulicaria wightiana* shows a

significant anti-hyperglycemic effect in diabetic rats and when its effect is comparable to that of standard. Therefore, this medicinal plant is

considered to be effective and alternative treatment for diabetes.

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