Short term antidiabetic studies on alcoholic extract of Ipomoea quamoclit linn

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ABSTRACT

Objective: To evaluate the antidiabetic effects of the alcoholic extract of leaves and stem of I. quamoclit employing a short term study.

Materials and Methods: A short term study was carried as a preliminary investigation to evaluate the antidiabetic effect of the alcoholic extract of leaves and stem of I. quamoclit and its normoglycemic and antihyperglycemic activity in streptozotocin (STZ)-nicotinamide induced non insulin-dependent diabetes mellitus rats using Glucose Tolerance Tes t(GTT). Graded doses (250 and 500 mg/kg) of the alcoholic root extract suspended in gum acacia were administered to normal and experimental diabetic rats.

Results: A significant fall in the blood glucose level of extract-treated animals was seen after 1 hour, indicating its hypoglyemic activity. Continuous blood glucose lowering activity was observed up to 4 hours of administration in normoglycemic and diabetic rats. The results were compared with those following the administration of standard oral hypoglycemic agent, glibenclamide.

Conclusion: Administration of the alcoholic extract caused statistically significant decrease in the blood glucose levels of normal and diabetic rats as compared to the normal control and diabetic control groups respectively. Longer duration studies of Ipomoea quamoclit on chronic models may contribute toward the development of a potent antidiabetic drug..

Key words: antidiabetics, rats, Ipomoea quamoclit, streptozotocin-nicotinamide induced model.

INTRODUCTION

In recent times many traditionally used medicinally important plants have been tested in experimental animals for their antidiabetic potential by various investigators. With this in view, the present study was undertaken on an indigenous antidiabetic medicinal plant Ipomoea quamoclit Linn. (Convolvulaceae). This plant is commonly known as Cypress Vine or Indian Pink. Though the plant is often cultivated as an ornamental plant it is used against various disorders in the indigenous system of medicines for fever, vomiting and breast pain[1]. In the Siddha system of medicine the leaves are used in the treatment of diabetes and piles[2]. However, there is no scientific evidence to support these claims. This short term study thus aims to experimentally assess the anitdiabetic effect of the alcoholic extract of leaves and stem of I. quamoclit and its normoglycemic and antihyperglycemic activity in streptozotocin (STZ)-nicotinamide-induced non-insulin-dependent diabetes mellitus rats employing oral glucose tolerance test (OGTT).

MATERIALS AND METHODS

Plant material

The plant Ipomoea quamoclit was collected from Erode, Tamil Nadu, India during the September. The botanical identity of the plant was confirmed by Dr. P. Jayaraman, Botanist, Medicinal Plant Research Unit, Chennai, Tamil Nadu. A voucher specimen (PP 545) has been deposited at the Museum of the Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal.

Preparation of IQ alcoholic extract

The alcoholic extract was prepared by cold

maceration of 500 g of the shade dried plant and dissolving, coarse powder in 1000 ml of ethanol (95%) for five days. The extract was filtered, concentrated, dried *in vacuo* (yield 42g) and the residue stored in a refrigerator at 2–8 °C for use in subsequent experiments.

Phytochemical screening

Preliminary phytochemical screening[3] of the alcoholic extract revealed the presence of phenolic substances, alkaloids, flavonoids and carbohydrates.

Animals

Healthy adult male Wistar Albino rats between two to three months of age and weighing between 150–250 g were used for the study. Housed individually in polypropylene cages, maintained under standard conditions (12 h light and 12 h dark cycle, 25±30 ºC, 35–60% humidity), the animals were fed with standard rat pellet diet (Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee of Kasturba Medical College, Manipal, India as per the guidance of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Acute toxicity

Nulliparous and non-pregnant two-month old female rats were used for the toxicity studies. The animals were marked to permit individual identification and kept in their cages for at least five days prior to dosing. The acute toxicity of IQ alcoholic extract was evaluated by the methodology described in the OECD[4] guidelines for the testing of chemicals. The animals were fasted for four hours prior to dosing. The fasted body weight of each animal was determined and the dose calculated according to its body weight. The alcoholic extract, prepared in drinking water was administered by gavage. The experimental procedure was performed in accordance with the Main Test of the OECD. The animals were observed individually during the first 30 min after dosing, every four hours during the first 12 hours and thereafter daily for 14 days.

Oral glucose tolerance test (OGTT)

The oral glucose tolerance test[5] was performed in overnight fasted (18 hr) normal animals. The rats divided into four groups (n=6) were administered 2% gum acacia solution, alcoholic extract (250 mg/kg), alcoholic extract (500 mg/kg) and glibenclamide (0.25 mg/kg) respectively. Glucose (3 g/kg) was fed 30min after the administration of extracts. Blood was withdrawn from the retro orbital sinus under ether inhalation (to minimize the distress) at 0, 60, 90, 120 and 180 min of extract administration. The fasting blood glucose levels were estimated by glucose oxidase–peroxidase reactive strips (Accu-chek, Roche Diagnostics, USA). The percentage decrease in the glucose concentrations were calculated with the formula = [(Gh-Gf)/Gf] X 100. Where Gh = the highest blood glucose concentration during the study; Gf- fasting blood glucose concentration.

Normoglycemic study

For normoglycemic study, the rats were divided into four groups (n=6) and were administered 2% gum acacia solution, alcoholic extract (250 mg/kg), alcoholic extract (500 mg/kg) and glibenclamide (0.25 mg/kg) respectively. Blood samples were withdrawn from the retro orbital sinus under ether inhalation at 0, 1, 2, 3 and 4 hr of extract administration[6]. The fasting blood glucose levels were estimated by glucose oxidase–peroxidase reactive strips.

Antihyperglycemic activity

Type II diabetes was induced[7] in overnight fasted animals by a single intraperitoneal injection of 60mg/kg Streptozotocin (Sigma Aldrich, Germany), 15 min after the i.p. administration of 120mg/kg nicotinamide (Qualigens Fine Chemicals, division of Glaxo, Mumbai, India). Hyperglycemia was confirmed by the elevated glucose level in the blood, determined at 72hr and then on Day 7 after injection. The

animals exhibiting fasting blood glucose level of 200-350 mg/dl were used for the antidiabetic study.

The diabetic rats were divided into four groups (n=6) and were administered 2% gum acacia solution, alcoholic extract (250 mg/kg), alcoholic extract (500mg/kg) and glibenclamide (0.25 mg/kg) respectively. Blood samples were withdrawn from the retro-orbital sinus under ether inhalation at 0, 1, 2, 3 and 4 hr of extract administration. The fasting blood glucose levels were estimated by using glucose oxidase-peroxidase reactive strips.

Statistical analysis

Data was expressed as mean ± S.E.M. The significance of the difference between the means of the test groups and control group was established by One Way ANOVA followed by *post hoc* Levene's test for variance using SPSS, version 10. The values were considered significant when $p < 0.05$.

RESULTS

Acute toxicity studies revealed the non-toxic nature of the alcoholic extract of IQ up to a dose level of 2000 mg/kg body weight in rats. There was no lethality or toxic reaction found at any of the doses selected until the end of the study. Induction of diabetes in the experimental rats was confirmed by the presence of high blood glucose levels. The difference between experimental and control rats was statistically significant ($p<0.05$). In OGTT, the alcoholic extract of IQ caused significant decrease in blood glucose level at both dose levels at 60 and 90 min as compared to the control (Table 1). The alcoholic extract caused significant decrease in the hyperglycemic peak (74.4% & 78.1%) at the dose levels of 250 and 500 mg/kg body weight respectively in relation to the control group and glibenclamide (43.5%). In normoglycemic studies the mean blood glucose level decreased, 4 hr after administration of the alcoholic extract at the dose level of 250 and 500 mg/kg body weight from 82.0 and 84.3 mg/dL to 51.1 and 48.8 mg/dL respectively (Table 2). The fasting blood glucose level of diabetic rats significantly ($p<0.05$) reduced from 296.1 and 305.0 mg/dL to 248.4 and 255.0 mg/dL, four hours after administration of the alcoholic extract at the dose level of 250 and 500 mg/kg body weight respectively, which is comparable to the effect of glibenclamide (0.25 mg/kg) (Table 3).

Table 1. Effect of alcoholic root extract of P. viscida on the glucose tolerance test Values are mean± S.E of 6 animals in each group; [a]p<0.01 vs control; [b]p<0.05 vs control; [a*]p<0.01 vs Glibenclamide and [b*]p<0.05 vs Glibenclamide.

Group (n=6)	Treatment	Fasting blood glucose levels (mg/dl) (Mean± S.E.M)				
		0 min	60 min	90 min	120 min	180 min
I	Control	82.6 ± 3.1	126.0 ± 5.2	110. 0 ± 4.5	102.3 ± 2.4	89.6 ± 3.1
II	Extract (250 mg/kg)	88.1 ± 3.3	100.0 ± 2.9[b]	92.8 ± 3.1[a]	81.0 ± 2.2[b]	77.6 ± 2.1[b]
III	Extract (500 mg/kg)	74.1 ± 1.5	82.66 ± 2.0[a]	75.5 ± 3.3[a]	69.3 ± 2.1[a]	65.5 ± 2.0[a]
IV	Glibenclamide (0.25 mg/kg)	78.6 ±1.5	99.1 ± 1.3[b]	83.8 ± 1.6[b]	72.8 ±1.5[a]	67.1 ± 1.4[a]

Table 2. Hypoglycaemic effect of alcoholic root extract of *P. viscida* in normal rats

Group (n=6)	Treatment	Fasting blood glucose levels (mg/dl) (Mean± S.E.M)				
		0 hr	1 hr	2 hr	3 hr	4 hr
I	Control	87.8 ±3.1	87.5 ± 3.0	86.6 ± 3.2	86.5 ±3.2	86.1 ±2.8
II	Extract (250 mg/kg)	82.0 ±2.8	72.0 ±2.5	68.1 ±2.5[b]	57.1 ±2.78[a]	51.1 ±2.8[a]
III	Extract (500 mg/kg)	84.3 ±2.8	71.0 ±2.5[b]	65.8 ±2.9[b]	55.3 ±3.3[a]	48.8 ±3.1[a]
IV	Glibenclamide (0.25 mg/kg)	82.1 ± 2.4	72.8 ± 1.8	68.1 ± 1.9[b]	60.1 ±2.3[a]	54.8 ±2.2[a]

Values are mean± S.E of 6 animals in each group; [a]p<0.01 vs control; [b]p<0.05 vs control; [a*]p<0.01 vs Glibenclamide and [b*]p<0.05 vs Glibenclamide.

Table 3. Antihyperglycaemic effect of alcoholic root extract of *P. viscida* in diabetic rats

Group (n=6)	Treatment	Fasting blood glucose levels (mg/dl) (Mean± S.E.M)				
		0 hr	1 hr	2 hr	3 hr	4 hr
I	Control	292.6 ± 9.1	291.3 ± 8.6	292.0 ± 8.02	291.6 ± 8.3	290.5 ± 8.0
II	Extract (250 mg/kg)	296.1 ± 9.6	262.1 ±12.9[b]	258.1 ± 12.7[b]	254.1 ±12.7[a]	248.5 ± 12.5[a]
III	Extract (500 mg/kg)	305.0 ±13.9	270.5 ±14.5[b]	263.5 ±14.1[a]	258.6 ± 14.4[a]	255.0 ± 14.7[a]
IV	Glibenclamide (0.25 mg/kg)	292.16 ±7.85	275.0 ±8.5[c]	262.1 ± 5.7[b]	256.8 ±5.2[a]	253.1 ±4.7[a]

Values are mean± S.E of 6 animals in each group; [a]p<0.01 vs control; [b]p<0.05 vs control; [a*]p<0.01 vs Glibenclamide and [b*]p<0.05 vs Glibenclamide.

DISCUSSION

The present paper studies the antidiabetic effect of the alcoholic root extract of *Pseudarthria viscida* on glucose loaded, normal and streptozotocin-nicotinamide induced type II diabetic rats. Over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues is the fundamental base of hyperglycemia in diabetes mellitus (Latner, 1958). In our study the difference observed between the initial and final fasting blood glucose levels of different groups under investigation revealed a significant elevation in blood glucose in the diabetic control group as compared to normal animals. Administration of the alcoholic extract of PV caused statistically significant decrease in the blood glucose levels of normal and diabetic rats as compared to the normal control and diabetic control groups respectively. Insulin primarily controls the glycolytic pathway by regulating the cell entry of glucose and its phosphorylation for further metabolism[8].A number of plants have been observed to exert hypoglycemic activity through insulin-release stimulatory effects[9].

In our studies, preliminary phytochemical investigation revealed the presence of phenolic substances, flavonoids and alkaloids. Cherian et al.[10] isolated the leucopelargonidin derivatives from the bark of Ficus bengalensis and demonstrated its significant in vitro insulin secretion from β-cells. Epicatechin, tannin isolated from the ethanol extract of Pterocarpus marsupium bark has also been shown to possess a significant

anti-diabetic effect, by enhancing insulin release and by the conversion of proinsulin to insulin *in vitro*[11].Phenolic constituents such as marsupin and pterostilbene lowered the blood glucose level significantly in STZ diabetic rats, and the effect was comparable to that with metformin. The flavonoid fraction of *P. marsupium* has been shown to cause pancreatic β cell degranulation, which may explain the anti-diabetic mechanism of the plant[12].

CONCLUSION

In our study, the administration of the alcoholic extract of IQ caused significant decrease in the blood glucose levels of normal and diabetic rats. Hence, the significant antihyperglycemic activity exerted by the alcoholic root extract of Ipomoea quamoclit may be attributed to the presence of phenolic compounds, flavonoids or alkaloids. Studies are in progress to elucidate the molecular and cellular mechanism of the extract. Longer duration studies of *Ipomoea quamoclit* on chronic models may contribute toward the development of a potent antidiabetic drug.

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