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Anti-diabetic activity of *Ipomoea quamoclit* in Streptozotocin Induced diabetic rats

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Abstract

Present study was undertaken to screen the antidiabetic activity of *Ipomoea quamoclit* whole plant belongs to *Convolvulaceae* family. Extraction and preliminary phytochemical screening were conducted by standard methods. Antidiabetic activity was evaluated by streptozotocin induced diabetic rats where the hydroalcoholic extract of whole plant was administered orally at a dose of 250 and 500 mg/kg for 30 days. Blood glucose levels were estimated at 1st, 10th, 20th and 30th day of study. Lipid profile was studied 30th day of study and body weight of the animals was measured at day 1 and 30. Thirty days administration of hydroalcoholic extract of *Ipomoea quamoclit* whole plant showed a significant (p<0.001) reduction in blood glucose levels at both dose levels under study that is 250 and 500 mg/kg body weight in comparison with diabetic control group. Standard drug also exhibited a significant (p<0.001) reduction in blood glucose levels of the animals in comparison with diabetic control. In biochemical parameters, significant (p<0.05) effect on triglycerides at its lower dose, significant (p<0.05) on total cholesterol levels and HDL, LDL and VLDL levels and also significant (p<0.01) at its high dose that is 500 mg/kg body weight and a good control (p<0.01) over the body weight of the animals in comparison with diabetic control group. Present study gives a scientific evidence for the folklore claim of the plant under study for its use in diabetes.

Keywords: Ipomoea quamoclit, Folklore, Streptozotocin, Diabetes, Lipid profile and Body weight

1. Introduction

Ipomoea quamoclit also called as Quamoclit pinnata belongs to Convolvulaceae family is one of the most important herb in ayurveda and folk medicine. In English it is called as Cypress Vine, Indian Pink and Cupid's Flower^[1]. In Philippines, the leaves are used as poultices for bleeding haemorrhoids. The crushed leaves are used for carbuncles. The seeds are used as a laxative. In India, the powdered roots were given as sternutatory (substance that tends to cause sneezing) where as in Spain, the crushed leaves are used for ulcers and chest pain ^[2]. Plant found its significant use in Siddha Medicine also where the decoction of leaves and stems is used to treat fever, is also used in diabetes ^[3] and in Thailand, is used for snake bites and as snuff, as a laxative and for haemorrhoids and in bloody cough ^[4]. Leaves and stems contain small amounts of alkaloids and cyanogenetic glycosides. Seeds have been reported to contain the resin glycosides, quamoclins I-IV and Jalapin. Pyrrolizidine alkaloids like mono and diesters of platynecine and minalobines like minalobine O and R, ipangulines like ipangualine B_2 and $D_{11}^{[5, 6]}$ and ergoline alkaloids ^[7] and Anthocyanins ^[8] were identified from the plant. Total alkaloid in seeds was found 0.012% ^[9]. The present study deals with pharmacological screening of anti-diabetic activity of Ipomoea quamoclit Linn in a streptozotocin induced diabetic rats with a view to justify its use for diabetes in folk medicine.

2. Materials & Methods

2.1. Collection of Plant Material

For the present study, whole plant of *Ipomoea quamoclit* was collected from the forest area near to the Madanapalli of Chittoor district of Andhra Pradesh, India and the plant was botanically identified and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateshwara University, Tirupati, A.P., India and a voucher specimen (RIPER/ASK/002) was preserved in division of Pharmacognosy, RIPER, Anantapuramu for further reference.

2.2. Extraction

For the present study, 1000 gm of the powdered *Ipomoea quamoclit* was extracted by cold maceration method with ethanol: water (3:2) mixture as solvent. The maceration was

continued for 72 hours with occasionally agitation after which, the contents were filtered and concentrated by rota evaporator ^[10, 11]. A resinous greenish extract was obtained which was calculated for the yield, designated with HAIQ and stored in desiccator till further study.

2.3. Phytochemical Screening

Hydroalcoholic extract of *Ipomoea quamoclit* whole plant was subjected to standard battery of preliminary phytochemical screening ^[10, 11].

2.4 Animals

Adult Swiss albino mice of either sex for acute toxicity studies (20–30 gm) and Wistar rats (150-200 gm), of either sex for pharmacological screening at about 6–8 weeks of age were used in the study. The animals were maintained with free access to food and water and kept at 25 ± 2 ⁰C under a controlled 12 h light/dark cycle. Twelve hours before each experiment animals received only water, in order to avoid food interference with substances absorption ^[12]. The care and maintenance of the animals were carried out as per the approved guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi ^[12, 13]. The research protocols were approved by the Institutional Animal Ethical Committee (IAEC) of Raghavendra Institute of Pharmaceutical Education and Research, Krishnam Reddy Palli cross, Chiyyedu, Anantapuramu-515721, Andhra Pradesh, India.

2.5 Gross behavioural and toxicity studies

The hydro alcoholic extract of whole plant of *Ipomoea quamoclit* was screened for the gross behavioural and toxicity studies in selected Swiss albino mice. Groups of mice comprising six animals each were treated with 100, 200, 400,800, 1000, 2000 and 3000 mg/kg of the extract suspended in 0.5% w/v sodium carboxy methyl cellulose were administered orally, via a gastric catheter. The animals were then observed continuously for first four hours for any behavioural changes and for mortality if any at the end of 72 h. However, no mortality was observed in the animals ^[12, 13]. Hence HAIQ was selected to screen for its anti-diabetic activity in streptozotocin induced diabetic rats at dose level of 250 mg/kg and 500 mg/kg body weight.

2.6 Induction of diabetes

Rats were made diabetic by single intraperitoneal administration of streptozotocin at a dose of 55 mg/kg dissolved in 0.1 M citrate buffer, pH 4.5. Forty-eight hours later, blood samples were collected and glucose levels were determined to confirm the development of diabetes. Only those animals which showed hyperglycemia (blood glucose levels >180 mg/dl) were used in the experiment ^[14].

2.7 Experimental design: Studies on Streptozotocininduced diabetic rats

The rats were divided into six groups comprising of six animals each (n=6).

Group 1: Normal control; normal rats received 1% w/v CMC, orally for 30 days,

Group 2: Diabetic control; diabetic rats received 1% w/v CMC, orally for 30 days,

Group 3: Standard; diabetic rats treated with Glibenclamide (10 mg/kg, orally) in aqueous solution for 30 days,

Group 4: Test I; diabetic rats treated with HAIQ (250 mg/kg, orally) suspended in 1% w/v CMC solution for 30 days. Group 4: Test II; diabetic rats treated with HAIQ (500 mg/kg, orally) suspended in 1% w/v CMC solution for 30 days.

2.8 Collection of blood samples

Blood samples (1.5 ml) were collected on 1st, 10th, 20th and 30th day of study by retro orbital plexus under mild anaesthesia and allowed to clot and were centrifuged at 3000 rpm for 15 min. The serum was separated and used for the estimation of blood glucose levels by glucometer ^[15, 16].

2.9 Biochemical estimations and body weight

The lipid profile in STZ induced diabetic model were evaluated on 30th day after withdrawal of blood for estimation of blood glucose levels. Serum lipid profile, including total cholesterol (CHOD/PAP method), triglycerides (GPO/PAP method), HDL cholesterol (PEG Precipitation method), LDL cholesterol and VLDL cholesterol were estimated. A change in body weight was determined by weighing the animals on day 1 and 30 ^[17, 18].

2.10 Statistical significance

All the results are expressed as the mean \pm S.E.M. The data were analyzed for statistical significance by one-way analysis of variance (ANOVA) followed by Bonferroni's test using computerized Graph Pad Prism, version 4.5 software (Graph Pad Software Inc). Statistical significance was set accordingly.

3. Results

3.1 Extraction

A thick green viscous matter about 36.4 gm was obtained from 1000 gm of *Ipomoea quamoclit* whole plant by maceration and the percentage was found to be 3.64% w/w.

3.2 Preliminary phytochemical analysis

Preliminary phytochemical analysis of whole plant of *Ipomoea quamoclit* was carried out and it showed the presence of alkaloids, carbohydrates, saponins, phytosterols, phenolic compounds, tannins, flavonoids, proteins, amino acids, terpenoids, gums and mucilages.

3.3 Antidiabetic activity

3.3.1 Effect on blood glucose levels

The initial administration of streptozotocin at 55 mg/kg caused marked elevation in blood glucose levels of the animals under study. Thirty days administration of hydroalcoholic extract of *Ipomoea quamoclit* whole plant showed a significant (p<0.001) reduction in blood glucose levels at both dose levels under study that is 250 and 500 mg/kg body weight in comparison with diabetic control group (figure 1). Standard drug also exhibited a significant (p<0.001) reduction in blood glucose levels of the animals in comparison with diabetic control. The results were shown in table number 1.

3.3.2 Effect on lipid profile

In biochemical parameters, thirty days administration of hydroalcoholic extract of *Ipomoea quamoclit* whole plant showed a significant (p<0.05) effect on triglycerides at its lower dose, significant (p<0.05) on total cholesterol levels and HDL, LDL and VLDL levels and also significant (p<0.01) and p<0.001) at its high dose that is 500 mg/kg body weight (figure 2). The results were shown in table number 2.

3.3.3 Body weight

Administration of hydroalcoholic extract of *Ipomoea* quamoclit whole plant exhibited a good control (p<0.01) over

the body weight of the animals in comparison with diabetic control group (figure 3). The results were shown in table number 3.

Table 1: Effect of Ipomoea quamoclit whole plant on blood glucose levels of STZ induced diabetic rats

Group	Mean Blood glucose conc. (mg/dl.)				
	1 st Day	10 th Day	20 th Day	30 st Day	
Normal control	89.16 <u>+</u> 0.08	88.48 <u>+</u> 0.22	89.1 <u>+</u> 0.11	88.4 <u>+</u> 0.12	
Diabetic control	195.24 <u>+</u> 0.29	203.5 <u>+</u> 0.15	216.5 <u>+</u> 0.08	239.5 <u>+</u> 0.16	
Glibenclamide 10mg/kg	189.7 <u>+</u> 0.39 ^a	154.2 <u>+</u> 0.23 ^b	141.3 <u>+</u> 0.14 ^b	130.1 <u>+</u> 0.24 ^c	
HAIQ 250 mg/kg	185.97 <u>+</u> 1.03 ^a	181.07 <u>+</u> 0.34 ^b	170.70 <u>+</u> 0.32 ^b	162.20 <u>+</u> 1.28 ^c	
HAIQ 500 mg/kg	191.47 <u>+</u> 0.66 ^b	173.47 <u>+</u> 0.20 ^b	165.46 <u>+</u> 0.46 ^c	155.58 <u>+</u> 0.81°	
All values were expressed as Mean SEM one way ANOVA followed by Penferreni's test as n < 0.05, b; n < 0.01 and as n < 0.001 when compared					

All values were expresses as Mean \pm SEM, one way ANOVA followed by Bonferroni's test, a: p<0.05, b: p<0.01 and c: p<0.001 when compared to diabetic control group; HAIQ- Hydroalcoholic extract of *Ipomoea quamoclit* whole plant

Table 2: Effect of Ipomoea quamoclit whole plant on lipid profile of STZ induced diabetic rats

Crown	Mean Lipid profile (mg/dL)					
Group	Triglycerides	Total cholesterol	HDL	LDL	VLDL	
Normal control	67.58 <u>+</u> 0.49	74.25 <u>+</u> 0.32	32.08 <u>+</u> 0.18	38.50 <u>+</u> 0.20	12.21 ± 0.11	
Diabetic control	157.32 <u>+</u> 1.38	143.16 <u>+</u> 0.39	14.37 <u>+</u> 0.12	93.70 <u>+</u> 0.27	43.58 <u>+</u> 0.19	
Glibenclamide 10mg/kg	68.00 <u>+</u> 0.06 ^a	78.96 <u>+</u> 0.32 ^a	31.16 <u>+</u> 0.13 ^b	38.62 <u>+</u> 0.23 ^b	14.98 <u>+</u> 0.12 ^c	
HAIQ 250 mg/kg	63.44 <u>+</u> 0.23 ^a	84.44 <u>+</u> 0.24	25.20 <u>+</u> 0.12 ^a	42.02 <u>+</u> 0.54 ^b	14.33 <u>+</u> 0.22 ^a	
HAIQ 500 mg/kg	65.40 <u>+</u> 0.24	72.15+0.31ª	30.82 <u>+</u> 0.12 ^b	41.23 <u>+</u> 0.30	14.58 <u>+</u> 0.17 ^c	

All values were expresses as Mean \pm SEM, one way ANOVA followed by Bonferroni's test, a: p<0.05, b: p<0.01 and c: p<0.001 when compared to diabetic control group; HAIQ- Hydroalcoholic extract of *Ipomoea quamoclit* whole plant

Table 3: Effect of Ipomoea quamoclit whole plant on body	weight of
STZ induced diabetic rats	

Creare	Mean Body weight (gm)		
Group	1 st day	30 th day	
Normal control	165.39 <u>+</u> 0.39	179.79 <u>+</u> 0.76	
Diabetic control	167.5 <u>+</u> 0.42	106.85 <u>+</u> 0.62	
Glibenclamide 10mg/kg	168.66 <u>+</u> 0.62 ^a	188.34 <u>+</u> 0.38 ^a	
HAIQ 250 mg/kg	174.37 <u>+</u> 0.30	178.76 <u>+</u> 0.82	
HAIQ 500 mg/kg	176.71 <u>+</u> 0.34 ^a	180.92 <u>+</u> 0.22 ^a	

All values were expresses as $Mean\pm$ SEM, one way ANOVA followed by Bonferroni's test, a: p<0.01 when compared to diabetic control group; HAIQ- Hydroalcoholic extract of *Ipomoea quamoclit* whole plant



Fig 1: Effect of *Ipomoea quamoclit* whole plant on blood glucose levels of STZ induced diabetic rats



Fig 2: Effect of *Ipomoea quamoclit* whole plant on lipid profile of STZ induced diabetic rats



Fig 3: Effect of *Ipomoea quamoclit* whole plant on body weight of STZ induced diabetic rats

4. Conclusion

From the above findings, it can be concluded that thirty days administration of hydroalcoholic extract of *Ipomoea quamoclit* whole plant exhibited a significant reduction in the blood glucose levels of the streptozotocin induced diabetic rats. This may be probably due to the presence of different phytoconstituents like alkaloids, saponins, phytosterols, phenolic compounds, flavonoids ^[16]. Further studies are under progress to isolate and identify the pure phytoconstituents that are responsible for the anti-diabetic activity. Present study supports the folklore claim of the whole plant of *Ipomoea quamoclit* in the treatment of diabetes.

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