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Anti Diabetic Activity of Siddha Herbal Preparation Allipoo Chooranam (*Nymphaea pubescens* flowers) on STZ Induced Diabetic Rats

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ABSTRACT

Background: Siddha medical system is one of the ancient Indian traditional health care systems. A great number of herbs are described in Siddha literature for treatment of Diabetes mellitus. *Nymphaea pubescens* is widely in Siddha system to treat diabetes. **Objective:** To investigate the anti-diabetic activity of Allipoo chooranam (*Nymphaea pubescens* flowers) in streptozotocin (STZ) induced diabetic rats. **Methods:** Acute oral toxicity study was carried out in normal female Wistar rats as per OECD guidelines 423. Wistar rats were rendered diabetic by STZ [50mg/kg b.wt.i.p.]. At a dose of 200 and 400 mg/kg of body weight Allipoo chooranam was administered orally to diabetic rats for 10 days. Glibenclamide was used as a standard drug at a dose of 1.25 mg/kg b.wt. The anti-diabetic activity was determined by estimation of blood glucose level. **Result:** Allipoo chooranam showed significant reduction of blood glucose levels. **Conclusion:** The results exhibited that Allipoo chooranam (*Nymphae pubescens* flowers) possess potent anti-diabetic activity in STZ induced diabetic rats and might be useful in the management of diabetes.

1. INTRODUCTION:

Diabetes mellitus comprises a group of common metabolic disorders that share the phenotype of hyperglycemias. Two broad categories of Diabetics Mellitus are Type I diabetics Mellitus due to B cell destruction, usually leading to absolute insulin deficiency and Type II diabetes mellitus which may range from predominantly insulin resistance with relative insulin deficiency to a predominantly insulin secretary defect with insulin resistance.

The lifestyle changes that have occurred in newly industrialised and developing countries have been followed by a dramatic increase in the incidence and prevalence of Type II diabetics mellitus. The World Health Organisation estimates that more than 150 million people worldwide have diabetics and this number is likely to increase to 300 million by 2025. In India Type II diabetes mellitus approximately affects 90% of all known cases of diabetes.

The World Health Organisation has described traditional medicine as one of the surest means to achieve total health care of the world population. Based on the recommendation made by WHO on diabetes mellitus investigations on hypoglycemic agents from medicinal plants have become more important.

A great number of plants have been described in Siddha literatures for the treatment of diabetes mellitus. One such plant is *Nymphaca pubescens* a large aquatic plant belonging to family Nymphaeceae. The flowers (Alli poo) of this plant are widely used in Siddha medicine as astringent, and coolant. Flowers of *Nymphae pubescens* (Alli poo) are used to treat diabetes, urinary tract ulcers, polyuria, polydypsia and burning sensation of body with the plant having such beneficiary properties and having been traditionally claimed as an anti-diabetic not much work has been conducted in relation to diabetes in the flowers of *Nymphaea pubescens* so far.

Due to lack of sufficient scientific proof on the role *of Nymphae pubescens* flowers in diabetes the current study was done to evaluate the antidiabetic effect of *Nymphaea pubescens* flowers in streptozotocin-induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Plant collection

Nymphaea pubescens (NP) flowers were collected from small ponds in and around Chennai. The plant was authenticated by Dr. Sasikala Ethirajulu, Botanist, Central Research Institute for Siddha, Chennai.

2.2 Preparation of Allipoo Chooranam

The fresh flowers were washed well in running water to remove impurities. Then the flowers were cut into pieces and dried in shade and finely powdered (Figure.1).The powder was moistened with cow's milk. A pot was half filled with milk and water and the mouth of pot was covered and tied with a white cotton cloth. The chooranam was placed above the tied cloth and mouth of pot was closed with another mud pot. The gap between the two mud pots was tied with a wet cloth to avoid evaporation. Then this arrangement was put on fire and boiled until water level gets reduced in the lower pot. Then the powder was taken and stored and stored in airtight container for usage.

Fig. no. 1. Allipoo chooranam.

2.2 Chemicals

Streptazotocine and Glibenclamide (Sigma Aldrich, Missouri, U.S.A) were procured from Ranbaxy Laboratories Ltd. India. All chemicals used were analytical grade quality.

2.3 Experimental Animals

Wistar rats of either sex weighing 160-200 gm were used for the study. The animals were kept in polypropylene eagers at 25 ± 2°C with relative humidity 45-55% under 12h light and 12h dark cycles. They were fed with standard laboratory animal feed (Poultry Research Station, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India) and water ad labium. The experimental protocol was approved by the IAEC (Ref. No. 23/16 – CLBMCP dated 14.12.2006).

2.4 Acute toxicity study

Female Wistar rats weighing 150-200 gm were used for the study. The starting dose level of the Allipoo Chooranam was 2000 mg/kg body weight (per oral). As most of the crude extracts posses LD50 value more than 2000 mg/kg p.o., the starting dose used was 2000 mg/kg p.o. Dose volume was administered 0.1 ml/10 gm body weight to the rat which were fasted overnight with water ad libitum. The food was withheld for a further 3 to 4 hours after administration and observed for signs of toxicity. Body Weight of the rats before and after termination were noted and any changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous systems and somatomotor activity and behaviour pattern were observed, and also signs of tremors, convulsion, salivation, diarrhoea, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity were also noted.

2.5 Induction of diabetes mellitus:

Wistar rats of either sex weighing 160-200 gm were used. The experimental protocol was approved by the IAEC (Ref. No 23/16 - CLBMCP dated 14.12.2006).After fasting for 18 hours, 25 rats were injected intraperitoneally with a single dose of 50 mg/kg streptozotocin (STZ) after dissolving it in freshly prepared ice-cold citrate buffer (pH 4.5). After the injection, they had free access to feed and water and were given 5% glucose solution to drink overnight to counter the hypoglycemic shock.

Development of diabetes was confirmed after 48 hours of the STZ injection. The animals having blood glucose level more than 200 mg/dl were selected for the experimentation. The blood glucose was estimated by using One Touch blood glucometer. (Lifescan, U.S.A). The blood was collected from the tail vein of the rats. From out of 25 animals, 5 animals died before grouping and 2 animals were omitted from the study because of mild hyperglycemia (below 180 mg/dl). The 18 diabetic animals were divided into 3 groups, each having 6 animals.

Experimental Protocol:

Group I - Animals received Allipoo Chooranam 200 mg/kg (p.o)
Suspended in 1% SCMC, daily for 10 days.
Group II - Animals received Allipoo Chooranam 400 mg/kg (p.o)
suspended in 1% SCMC, daily for 10 days.
Group III - Animals received standard glibenclamide 1.25 mg/kg (p.o)
suspended in 1% SCMC, daily for 10 days.
Blood samples were obtained by a tail puncture at 3, 5, 7 and 10 days of treatment. The blood glucose level was determined using an electronic glucometer.

2.6 Statistical Analysis:

Results were expressed in ± standard deviation. The statistical analysis was carried out using one-way ANOVA (Analysis of Variance) followed by Dunnett's't' test, P values < 0.05 were considered as significant.

3. RESULTS

3.1Acute Oral Toxicity Study

Nymphaea pubescens flowers (Allipoo Chooranam) did not exhibit any significant toxicity at a dose of 2000 mg/kg/b. wt. So the drug is safe for administration.

3.2 Anti-diabetic activity of Allipoo chooranam against STZ induced diabetic rats.

S. No	Group	Blood glucose level (mg/dl)				
		48hrs after STZ	After drug Administration			
			3rd day	5th day	7th day	10th day
1.	Group I	268.42 ± 39.76	262.42 ±42.86^{NS}	225.62 ± 22.06*	197.45 ± 36.72**	154.83 ± 30.25***
2.	Group II	266.67 ± 31.91	260.67 ± 31.05^{NS}	215 ± 18.85**	172.32 ± 41.36**	116.66 ± 13.01***
3.	Group III	289.17 ± 79.62	253.67 ± 58.59^{NS}	186.83 ± 40.58**	162.54 ± 41.24***	104.5 ± 27.42***

Values are mean ± S.D of six animals in each group.

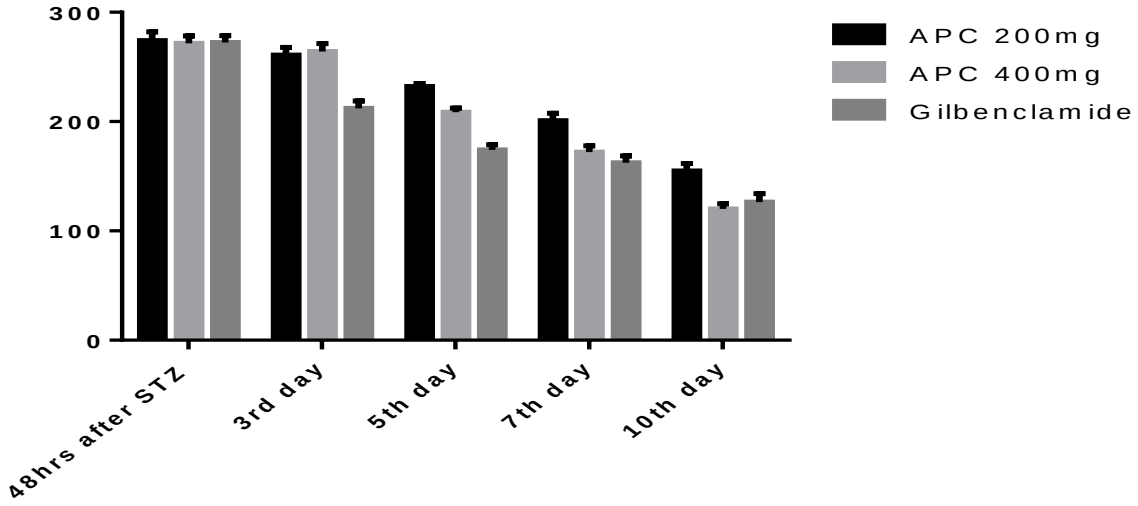
NS - Non significant

* - P<0.05

** - P<0.0l

*** - P < 0.001

Comparison: Respective diabetic level Vs drug treatment 3rd, 5th, 7th and 10th day.



Allipoo chooranam showed significant reduction in blood sugar levels from the fifth day of study. The p value was < 0.05 on 5th day at a dosage of 200 mg per kg b.w. and p < .01 at a dosage of 400 mg per kg. b. W. p < 0.01 on 7th day and on 10th day p < 0.001 was observed with a dose of 200 mg and 400 mg per kg. b.w.

DISCUSSION

Diabetes mellitus, a lifestyle disorder is becoming a great challenge to healthy living of human. According to the data from WHO, the highest increases in diabetes prevalence are amongst low and middle-income countries, predominantly within the 40-59 years age group, although a tendency is seen for onset at a younger age. Thus treatment and management of diabetes impose significant socio-economic burdens from cost productivity and slow economic growth. The world Health Organization Expert Committee on diabetes recommended that traditional medicinal herbs be further investigated as they are frequently considered to be less toxic and from side effects (Halbertein 2005). To assure the medical field regarding the usage of medicinal plants, the present study on *Nymphacea pubescens* flowers (Allipoo Chooranam) was undertaken.

The Sidha literature Agaithiyar Gunavagadam gives evidence that Allipoo (*Nymphaea pubercens*) is widely used to treat polyuria, urinary treat ulcers, polydypsie, fatigue, burning sensation and minimizes Azhal humour. Moreover these flowers possess coolant and emollient action. *Nymphaea pubescens* flowers are astringent in taste. As per Siddha literature, astringent taste is due to earth and air boothams and astringent taste pacifies the dearranged Azhal humors which is the primary cause of Diabetes and regularizes three body

vital humors'. It also strengthens the seven physical constituents. Allipoo chooranam provided significant relief in diabetes mellitus. On comparison, both the groups were equally significant, in which group 1 showed better effect than other groups.

Nymphaea pubescens flowers (Allipoo Chooranam) produced significant hypoglycemic activity on streptozotocin induced diabetic rats. STZ causes damage to pancreatic cells, hepatocytes, nephrons and cardiomyocytes. The phytochemical study showed presence of flavonoids, tanninsand phytosteroids. Flavonoids are effective antioxidants. Flavonoids bring about regeneration of pancreatic islets and probably increases insulin release in STZ induced diabetic. The anti-diabetic activity of Allipoo chooranam may be due to the ppresence of flavonoids.

CONCLUSION

The pharmacological study showed that Allipoo chooranam possesses potent anti-diabetic activity in STZ induced diabetic rats.The findings of the experimental animal study provides scientific support to the traditional Siddha medical use of *Nymphaea pubescens* flowers (Allipoo chooranam) in the management of diabetes mellitus. However further study is needed to ascertain mechanism of action to develop as a potent anti-diabetic agent.

Conflict of interest:

Authors declare no conflict of interest.

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