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Anti diabetic activity on the flowers of *Nymphaea pubescens* Willd.

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ABSTRACT

To evaluate the antidiabetic activity of flowers of *Nymphaea pubescens* willd. The ethanolic & aqueous extracts of flowers of *Nymphaea pubescens* willd., were screened for antidiabetics. Antidiabetic effect was studied by observing reduction in blood glucose levels in alloxan induced diabetic rats and percentage reductions were calculated. There was statistically significant reduction ($P<0.001$) in blood glucose level in the diabetic rats with the maximum activity at 6h and the percentage reductions were found to be 21.97% and 19.94% at the dose of 400 mg/kg with ethanol and aqueous extracts respectively, when compared with diabetic control groups.

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INTRODUCTION

Diabetes mellitus (DM), a state of chronic hyperglycemia, is a common disease affecting over 124 million individuals worldwide [1,2]. DM is associated with high risk of atherosclerosis and renal, nervous system and ocular damage [3].

The plant is an aquatic perennial herb of the family Nymphaeaceae. It perennate through large, spherical rhizome which produce leaf and flowers seasonally. The rhizome is cooling, sweet, bitter and tonic and is useful in diarrohoea, dysentery, dipsia and general debility. The flowers are astringent and cardiotonic. The seeds are sweet, cooling, constipating, aphrodisiac, stomachic and restorative. They are useful in vitiated conditions of pitta, dipsia, diarrhea and dermatopathy. The root stock and leaves are cooling, bitter, sweet, aromatic, alterant, diuretic and tonic. Rajagopal K & co-workers [4] studied antihyperglycaemi and antihyperlipidaemic effects of *Nymphaea stellata* in alloxan-induced diabetic rats. Larry AW & co-workers [5] isolated 20 antioxidants from the Nymphaea flowers, including two 2S,3S,4S-trihydroxypentanoic acid and myricetin 3-O-(3''-O-acetyl)-alpha-l-rhamnoside.

The present study was undertaken to verify the claim and evaluate the anti-diabetic property of the flowers of *Nymphaea pubescens willd.*

MATERIALS AND METHODS

Plant material

The plant specimens for the proposed study that is *Nymphaea pubescens Willd* were collected from the pond of Kaveripattinam, Dharmapuri, Tamilnadu in the month of July 2008 and authenticated by Dr. P. Jayaraman, Botanist, Plant Anatomy Research Centre (PARC), West Tambaram, Chennai by specimen no. PARC/2008/193.

Chemicals

The drugs and fine chemicals were purchased from Sigma–Aldrich, USA. All other chemicals and solvents were obtained from local firms (India) and were of highest pure and analytical grade.

Plant extracts

The shade dried course powder of flowers (300 gm) was packed well in soxhlet apparatus and was subjected to continuous hot extraction with 90% ethanol/ distilled water for 18 hours. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely. It was dried and kept in a desicator till experimentation. Obtained extract was weighed and % yield was calculated in terms of air-dried powdered crude material.

The aqueous and ethanolic extracts of *Nymphaea pubescence Willd* (12.4 & 14.7 w/w; respectively) were dried under reduced pressure using a rotary flash evaporator and they were kept under refrigeration. Both the extracts were administered to the animals as a suspension in 0.5% (w/v) carboxy methylcellulose sodium (CMC).

Animals

Male Swiss Albino mice weighing between 20 and 25 gm and male Wister rats weighing between 150 and 220 gm were used for study. The animals were obtained from animal house, IRT Perunderai Medical College, Erode, Tamilnadu, India. On arrival, the animals were placed randomly and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24 ± 2^{o} C and relative humidity of 30-70%. A 12:12 light: day cycle was followed. All the animals were allowed to free access to water and feed with standard commercial pelleted rat chaw (M/S Hindustan Lever Ltd., Mumbai). All the experimental protocols used in this study were reviewed by the Institutional Animal Ethics Committee (688/2/C-CPCSEA) and were in accordance with the guidelines of the CPCSEA.

Vehicle

Both plant extracts and Gilbenclamide (3 mg/kg, p.o.) were suspended in 0.5% (w/v) carboxymethylcellulose sodium (CMC) and administered orally to animals. Alloxan monohydrate diluted in normal saline and injected.

Preliminary Acute Oral Toxicity Evaluation

Acute oral toxicity studies were performed (Ecobichon, 1997) according to OECD-423 guidelines (acute toxic class method). Swiss mice (n = 3) of either sex selected by random sampling technique were employed in this study. The animals were fasted for 4 h with free access to water only. The extracts (suspended with 0.5% w/v CMC) were administered orally at a dose of 5 mg/kg initially and mortality was observed for 3 days. If mortality was observed in 2/3 or 3/3 animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one mouse out of three animals then the same dose was repeated again to confirm the toxic effect. If mortality was not observed, the procedure was then repeated with higher doses, such as 50, 300 and 2000 mg/kg. The parameters were observed are gross behavioral changes, grooming, alertness, sedation, loss of righting reflex, tremors convulsions in each dose level in initially 24 hours.

Anti-hyperglycemic Activity

Determination of Blood Glucose Level

For glucose determination, blood was obtained by snipping tail with the help of sharp razor. Blood glucose level was monitored by using Hypoguard Advance Blood Glucose Meter,

imported and marketed in India by Nicholas Piramal Ltd. Each time the tail of the mice was sterilized with spirit (Vogal, 2002; Deore et al., 2008).

Hypoglycemic Activity in Normal Fasted Rats

Animals were fasted overnight and were divided in to eight groups of six animal each as follows

Group 1- 0.5 CMC suspension (Control).
Group 2- Gilbenclamide (75mg/ kg, p.o) as Standard group.
Group 3- ethanol extract (100mg/ kg, p.o.).
Group 4- ethanol extract (200mg/ kg, p.o.).
Group 5- ethanol extract (400mg/ kg, p.o.).
Group 6-aqueous extract (100mg/ kg, p.o.).
Group 7-aqueous extract (200mg/ kg, p.o.).
Group 8-aqueous extract (400mg/ kg, p.o.).

After a single dose of drug administration blood sample were collected at 0, 1, 3 and 6 h by tail vain for estimation of glucose level.

Induction of Diabetes

Animals were injected freshly prepared alloxan monohydrate in sterile normal saline at a dose of 150 mg/kg body weight, intraperitoneally (Aruna et al., 1999). To prevent fatal hypoglycemia due to massive pancreatic insulin release, rats were treated with 20% glucose solution intraperitoneally after 6 hr followed by supply of 5% glucose solution bottles in their cages for next 24 hr (Barry et al., 1997). The animals shown blood glucose level >200 mg/dl after 72 hr were considered as diabetic.

Anti-hyperglycemic Activity in Alloxan Induced Rats

In the experiment total of 48 diabetic surviving rats were used. The rats were divided into eight groups of six rats each (Dash et al., 2008).

Group 1: Diabetic Control.
Group 2: Diabetic rats + Gilbenclamide (3 mg/ kg, p.o).
Group 3: Diabetic rats + Ethanol extract (100mg/ kg, p.o.).
Group 4: Diabetic rats + Ethanol extract (200mg/ kg, p.o.).
Group 5: Diabetic rats + Ethanol extract (400mg/ kg, p.o.).
Group 6: Diabetic rats + Aqueous extract (100mg/ kg, p.o.).
Group 7: Diabetic rats + Aqueous extract (200mg/ kg, p.o.).
Group 8: Diabetic rats + Aqueous extract (400mg/ kg, p.o.).

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After a single dose of drug administration blood sample were collected at 0, 1, 3 and 6 h by tail vain for estimation of glucose level.

Statistical analysis

All the results were expressed as mean ± standard error (S.E.M.). Data were analyzed using one-way ANOVA followed by Dunnett' *t*-test. $P < 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Acute oral toxicity studies

The acute oral toxicity of aqueous and ethanolic extracts of flowers of Nymphaea *pubescens Willd* was carried out as per OECD – 423 guidelines. All the doses (5, 50, 300 and 2000 mg/kg, p.o.) of both extracts were found to be non-toxic in acute oral toxicity studies. *Nymphaea pubescence Willd* extracts did not produce any mortality even at the highest dose (2000 mg/kg, p.o.) employed. One tenth of maximum dose of the extract tested for acute toxicity was selected as middle dose i.e. 200 mg/kg and double and half of middle doses were selected for higher and lower dose i.e. 400, 100 mg/kg for further pharmacological studies. The acute oral toxicity studies revealed that LD_{50} >2000 mg/kg for both the extracts.

Hypoglycemic Activity in Normal Fasted Rats (Table 1, Figure 1)

Table 1: Effect of *Nymphaea pubescence Willd.* on blood glucose levels on normal rats.

Drug Treatment	Group	Dose (mg/kg)	Blood Glucose level mg/dl			
			Initial	1h	3h	6h
Control	Group 1	--	85.67 ± 2.60	84.83 ± 1.49	83.83 ± 1.42	83.17 ± 1.45
Gilbenclamide	Group 2	3	84.33 ± 2.95	55.17 ± 2.49 ***	50.33 ± 2.16 ***	53.33 ± 2.43 ***
Ethanol extract	Group 3	100	81.17 ± 1..49	77.33 ± 1.45	75.67 ± 1.33	74.50 ± 1.09
Ethanol extract	Group 4	200	83.33 ± 2.32	72.50 ± 1.89 **	66.17 ± 1.49***	67.33 ± 2.59 ***
Ethanol extract	Group 5	400	81.83 ± 2.32	68.50 ± 1.88 ***	59.67 ± 2.22 ***	61.17 ± 2.07 ***
Aqueous extract	Group 6	100	82.17 ±2.21	78.67 ± 1.52	76.17 ± 1.05	75.17 ± 1.42
Aqueous extract	Group 7	200	81.67 ± 2.23	75.17 ± 2.49 *	69.17 ± 2.28 *	69.33 ± 1.38 ***
Aqueous extract	Group 8	400	83.67 ± 2.80	71.83 ± 2.09 ***	63.50 ± 1.75 ***	65.83 ± 2.52 ***

Values are Mean ± SEM; n= 6 animals in each group; *p < 0.05, **p < 0.01, ***p < 0.001 as compared with control

The ethanol and aqueous extracts of *Nymphaea pubescence Willd* were showed a significant dose dependent hypoglycemic effect at 1h, 3h and 6h in normal fasted rats after a single dose of extracts when compared to control group of animals. The lower dose (100 mg/kg, p.o.) of both extracts did not show any significant changes up to 6h in the blood glucose level as compared with control groups. The maximum hypoglycemic effect was created by 200 mg/kg of

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ethanol and aqueous extracts on after 3h of drug administration and the percentage reduction in blood glucose levels were 21.07% and 17.49% respectively.

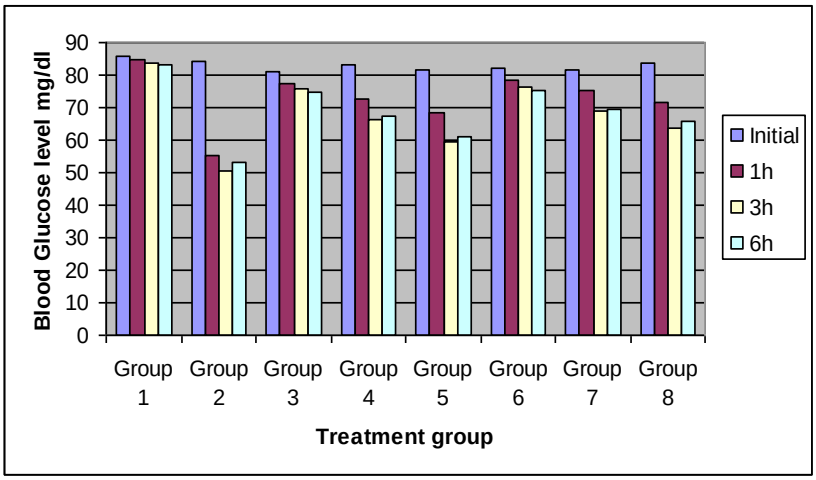

Figure 1. Graphical Representation of Effect of *Nymphaea Pubescence Willd* on Blood Glucose Levels on Normal Rats

The highest dose of extracts 400 mg/kg by oral showed the significant decline (P<0.001) in normal glucose levels at 1h, 3h and 6h and the maximum percentage reduction at 3h as 28.82% and 24.25% when compared with control. The highest reduction were observed like 39.96% in Gilbenclamide (3 mg/ kg, p.o), used as a standard drug on 3h as expected.

Anti-hyperglycemic Activity in Alloxan Induced Rats (Table 2, Figure 2)

Table 2: Effect of *Nymphaea pubescence Willd.* on blood glucose levels on alloxan diabetic rats.

Drug Treatment	Group	Dose (mg/kg)	Blood Glucose level mg/dl			
			Initial	1h	3h	6h
Diabetic Control	Group 1	--	222.17 ± 4.22	221.17 ± 4.10	220.17 ± 3.83	221.50 ± 3.73
Alloxan + Gilbenclamide	Group 2	3	221.83 ± 3.61	172.50 ± 2.79***	147.33 ± 2.50***	144.33 ± 2.87***
Alloxan + Ethanol extract	Group 3	100	225.83 ± 3.38	217.17 ± 3.18	214.50 ± 2.81	213.50 ± 2.17
Alloxan + Ethanol extract	Group 4	200	218.17 ± 4.70	203.33 ± 2.49**	195.17 ± 2.27***	188.83 ± 2.87***
Alloxan + Ethanol extract	Group 5	400	224.17 ± 4.70	184.33 ± 2.20***	176.67 ± 2.45***	172.83 ± 2.10***
Alloxan + Aqueous extract	Group 6	100	222.00 ± 3.99	218.50 ± 4.04	215.83 ± 4.22	216.33 ± 4.01
Alloxan + Aqueous extract	Group 7	200	217.33 ± 4.80	207.33 ± 4.15*	197.50 ± 3.13***	192.17 ± 1.40***
Alloxan + Aqueous extract	Group 8	400	220.67 ± 4.55	189.83 ± 2.57***	183.83 ± 3.27***	177.33 ± 1.94***

Values are Mean ± SEM; n= 6 animals in each group;*p < 0.05, **p < 0.01, ***p < 0.001 as compared with Diabetic control

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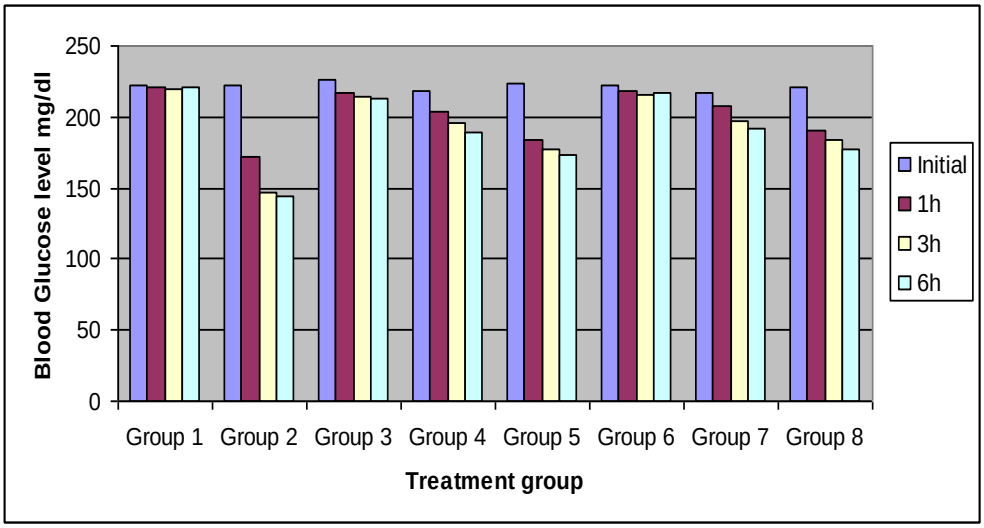

Figure 2. Graphical representation of effect of *Nymphaea pubescence Willd* on blood glucose levels on alloxan diabetic rats.

The dose dependent reduction in blood glucose levels were also observed in alloxan induced diabetic rats at 1h, 3h and 6h after treated with a single dose of *Nymphaea pubescence Willd* extracts. There was statistically significant reduction ($P<0.001$) in blood glucose level in the diabetic rats with the maximum activity at 6h and the percentage reductions were found to be 21.97% and 19.94% at the dose of 400 mg/kg with ethanol and aqueous extracts respectively, when compared with diabetic control groups. Similarly, 200 mg/kg of *Nymphaea pubescence* extracts treated by oral showed the maximum significant reduction ($P<0.001$) in blood glucose levels in 6h and the percentage reductions like 14.75% and 13.24% respectively with ethanol and aqueous extracts as compared with diabetic control animals. The lower dose (100 mg/kg, p.o.) of both extracts did not show any considerable changes up to 6h in the diabetic blood glucose level in diabetic induced rats. Moreover, the standard drug recorded the significant reduction ($P<0.001$) in diabetic induced glucose level at 1h, 3h and 6h after treatment and the maximum percentage reduction was 34.84%.

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