Andiabetic activity of Nymphaea pubescens Willd-A plant drug of Aquatic flora interest

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ABSTRACT

Nymphaea pubescens Willd (Nymphaeaceae) is a fascinating aquatic plant mentioned in siddha system of medicine, in the treatment of bleeding piles, diabetes and as cardioprotective in the palpitation of the heart. The present study was to evaluate antiidiabetic activity of Nymphaea pubescens in alloxan induced diabetic rats. Albino rats were rendered diabetic by alloxan monohydrate (150mg/kg body weight, intraperitoneally). The ethanolic extract was orally administered to diabetic rats at 200 and 400mg/kg doses daily for 14 consecutive days to determine antiidiabetic activity. The ethanolic extract at 400mg/kg body weight showed significant reduction in blood glucose level up to 99.28mg/dl. Concurrent histopathological examination of pancreas showed comparable regeneration by ethanolic extract which were earlier necrosis by alloxan. Significant result were observed in the estimated parameters, thereby justifying the use of plant in the indigenous system of medicine.

Key words: Alloxan, Antiidiabetic activity, Diabetic rats, Nymphaea pubescens

1. INTRODUCTION

The term diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. The effects of diabetes mellitus include long term damage, dysfunction and failure of various organs. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease. Nymphaea pubescens Willd (Nymphaeaceae) is a perennial aquatic rhizomatic stoloniferous herb. It is commonly known as water lily, which includes about fifty species and widely distributed in tropical and temperate regions, inhabiting stagnant fresh water, ponds, lakes and swamps. The medico ethno-botanical review of the flower of Nymphaea pubescens was used as blood purifier and in the treatment of jaundice. Fruits given with salt for snake bite poisoning followed by blood in urine. Root stock given for cystitis, nephritis, fever, insomnia, jaundice and haemorrhoids. In siddha system of medicine the whole plant was used in the treatment of eydisorder, diabetes and dyspepsia. There are however no scientific studies on antiidiabetic effects of N. pubescens to normal and alloxan induced diabetic rats. The present study was aimed to evaluate antiidiabetic activity of Nymphaea pubescens Willd in alloxan induced diabetic rats.

2. MATERIALS AND METHODS

2.1. Collection and authentication of plant material

The whole plant of Nymphaea pubescens Willd were collected in the ponds of Oomangalam village in Neyveli, Tamilnadu, India, in the month of February 2010 and it was botanically identified and authenticated by Prof.P.Jayaraman, Plant anatomy research centre, Thambaram, Chennai, Tamilnadu, India. A voucher specimen PARC/2007/79 was deposited at the Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai, Tamilnadu, India. The whole plant of N.pubescens was subjected to shade drying and further crushed to coarse powder.

2.2. Drugs and chemicals

Glibenclamide (USV Pharma Ltd, India), Alloxan monohydrate (Lab chemicals, India), one touch glucometer (Johnson & Johnson, India), Ethanol (Qualigens, India) were purchased from respective vendors.

2.3. Preparation of ethanolic extract of Nymphaea pubescens

Shade dried, coarsely powdered whole plant (300g) were extracted with 95% ethyl alcohol (500ml) by soxhlet extractor. After exhaustive extraction, the miscella was filtered, concentrated and dried in vacuo. The extract (yield 4.45%w/w) was suspended in 0.5%w/v carboxy methyl cellulose and used for animal experiments.

2.4. Preliminary phytochemical screening

The preliminary phytochemical analysis of ethanolic extract was carried out for the alkaloid (Mayer’s, Dragendorff’s, Wagner’s, Hager’s test), carboydrates (Molisch’s test), flavonoids (Shinoda test), sterols (Salkowski and Liberman-burchard test), phenolic compounds and tannins, glycosides, saponins and free amino acids.

2.5. Experimental animals and research protocol approval

Wistar albino rats(150-200g) of either sex, maintained in the animals experimental laboratory of College of Pharmacy, Madras Medical College, Chennai, Tamilnadu, India, at room temperature 25°±2°C, relative humidity 75±5% and 12h dark- light cycle. The animals were fed with standard rat pellet and water was available ad libitum. The study was conducted after obtaining ethical committee clearance from the Institutional Animal Ethical Committee of College of Pharmacy, Madras Medical College, Chennai, Tamilnadu, India (vide Proposal No.14/29IAEC/2010) in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA), India.

2.6. Acute toxicity study

Acute toxicity study was performed according to OECD guidelines 423. The methods uses defined doses at 5, 50, 300, 2000mg/kg body weight. The starting dose of ethanolic extract 2000mg/kg body weight was administered. They were continuously observed for 3 days to detect changes in the skin, fur, eyes, mucous membrane, respiratory, circulatory, autonomic central nervous system, somoto motor activity and behavior pattern. A group of animals treated with the vehicle 0.1ml of 0.5%w/v carboxy methyl celullose served as control. Based on the results of preliminary toxicity testing, the dose of 200mg and 400mg/kg body weight of N.pubescens were chosen for further experiments.

2.7. Induction of experimental diabetes

Diabetes was induced in overnight fasted adult wistar strain albino rats were fasted overnight and administered with alloxan 150mg/kg i.p. route. Fasting blood sugar levels were determined on fifth day after administering alloxan to confirm stable hyperglycemia. The diabetic rats after confirmation of stable hyperglycemia were divided into five groups of six rats each. Day five of induction was designated as day zero for extract administration. Drugs and doses were administered as mentioned below.

2.8. Experimental design

The rats were divided into following groups of six rats each

Group I: Normal control (1ml of 0.5%w/v Carboxy methyl cellulose) Group II: Diabetic control rats (Alloxan Monohydrate 150mg/kg b.w) Group III: Glibenclamide (0.25mg/kg b.w) treated diabetic rats

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Group IV: Ethanolic extract (200mg/kg b.w suspended in 0.5% CMC)

Group V: Ethanolic extract (400mg/kg b.w suspended in 0.5% CMC)

Treatment was continued for 14 consecutive days. Fasting blood sugar was estimated on days 0, 7 and 14 of extract administration, collected by tail tipping method using a glucometer. Initial and final changes in body weight were observed.

2.9. Histopathological study

On fourteenth day the animals were sacrificed by mild chloroform anaesthesia, the pancreas was excised and stored in 10% formalin after washing with normal saline. The tissues were washed, dehydrated with alcohol, cleared with xylene and paraffin blocks were made. Serial section of 5µm thickness were cut using a rotary microtome. The sections were deparaffinised with xylene and hydrated in descending grades of alcohol. The slides were then transferred to haemotoxylin for 10min, followed by rinsing with water, dehydrated with ascending grades of alcohol, cleared with xylene and mounted.

2.10. Statistical analysis

Results were expressed as mean ± standard error. Statistical analysis was done by using repeated measure one way anova followed by Dunnett’s multiple comparison test[8].

3. RESULTS AND DISCUSSION

3.1. Preliminary phytochemical screening

Preliminary phytochemical analysis of ethanolic extract of N.pubescens revealed the presence of alkaloids, carbohydrates, glycosides, sterols, phenolic compounds and tannins, amino acids, proteins and flavonoids. The results are shown in table 1.

3.2. Acute toxicity study

Acute toxicity studies revealed the non toxic nature of the ethanolic extract of N.pubescens. There was no lethality or any toxic reactions found at any of the doses selected until the end of study period.

3.3. Blood glucose and body weight

Alloxan monohydrate injection caused diabetes mellitus, which may be due to destruction of B cells of the islet of langerhans of the pancreas. Induction of diabetes in the experimental rats was confirmed by the presence of a high fasting blood sugar level. The effect of ethanolic extract of whole plant N.pubescens on normal and alloxan induced diabetic animals are presented in table 2. The difference between the experimental and control rats in lowering the fasting blood sugar levels was statistically significant (P<0.005) in diabetic rats administered with ethanolic extract at 400mg/kg b.w.

Induction of diabetes with alloxan is associated with the characteristic loss of body weight, which is due to increased muscle wasting and due to loss of tissue protein[9]. Diabetic rats treated with the aqueous extract showed an increase in body weight as compared to the diabetic control which may be due to its protective effect in controlling muscle wasting i.e., reversal of gluconeogenesis.

The ethanolic extract did not produce any significant effects on normal animals, which further confirms the antidiabetogenic action of the extract.
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3.4. Histopathological studies
Histopathology studies also supported our findings. Alloxan was suspected to destroy pancreatic ß cells. Diabetic rats showed reduced islet cells, which were restored to near normal upon treatment with the extract (Fig.1). No such changes are found in the normal rats. The mode of action of N.pubescens may be due to membrane stabilization and cell generation.

4. CONCLUSION
Studies are in progress in our laboratory to elucidate the molecular and cellular mechanism of the extract and its principle constituent. Longer duration studies on chronic models may contribute towards the development of potent antidiabetic drug.

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