

Antihyperglycaemic and antihyperlipidaemic effects of *Nymphaea stellata* in alloxan-induced diabetic rats

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ABSTRACT

Introduction: This study aims to investigate *Nymphaea stellata* (*N. stellata*) flower extract for antihyperglycaemic and antihyperlipidaemic effects in diabetic rats induced by alloxan. Its effect was compared with that of glibenclamide, a reference antidiabetic drug.

Methods: Diabetic animals were randomly divided into five groups and treated orally with different doses (200, 300 and 400 mg/kg body weight) of flower extract once a day for 30 days. The body weight of each animal was determined, to assess any possible weight gain or loss in experimental animals compared with control groups. On the 31st day, those administered 300 mg/kg of *N. stellata* flower showed more promising results with regard to fasting blood glucose (FBG), plasma insulin levels, haemoglobin counts, urine sugar levels, food intake, water intake, urea and protein when compared to those treated with other doses. Therefore, 300 mg/kg dose was used for further biochemical studies. Total lipids (TL), total cholesterol (TC), triglycerides (TG), phospholipids, free fatty acids (FFA), low density lipoproteins (LDL), very low density lipoproteins (VLDL), atherogenic index (AI) and high density lipoproteins (HDL) levels, on normal and diabetic rats treated with the dose of 300 mg/kg, were evaluated.

Results: The flower extract shows a significant (p-value is less than 0.001) reduction in levels of FBG, water intake, food intake, urine sugar, blood urea, TL, TC, TG, FFA, phospholipids, LDL, VLDL and AI. It also shows a significant increase in body weight, plasma insulin, protein, haemoglobin and HDL levels.

Conclusion: Our results suggest that *N. stellata* flower extract exhibit antihyperglycaemic as well as antihyperlipidaemic effects on alloxan-induced diabetic rats.

Keywords: alloxan, antihyperglycaemic, diabetes mellitus, *Nymphaea stellata* flower extract

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INTRODUCTION

Non-insulin dependent diabetes mellitus (NIDDM) is a multifactorial disease, which is characterised by hyperglycaemia and lipoprotein abnormalities.⁽¹⁾ These traits are hypothesised to damage cell membranes, which results in excess generation of reactive oxygen species. NIDDM has also been associated with an increased risk for developing premature atherosclerosis due to an increase in triglycerides (TG) and low-density lipoproteins (LDL), and decrease in high density lipoprotein levels (HDL).⁽²⁾ An ideal oral treatment for diabetes mellitus would be a drug that not only controls the glycaemic level, but also prevents the development of atherosclerosis and other complications associated with diabetes mellitus. The role of traditional medicines in the solution of health problems is invaluable on a global level. This is all the more striking when we consider the fact that approximately 80% of the people living in less developed countries rely exclusively on traditional medicines for their healthcare needs.

Nymphaea stellata (*N. stellata*) wild, belonging to the family of Nymphaeaceae, commonly known as Indian water lily is a perennial aquatic herb. The flowers are used for the treatment of liver disorders in traditional medicine⁽³⁾ and has proven hepatoprotective activity.⁽⁴⁾ *N. stellata* seeds are prescribed for those with diabetes mellitus in the Ayurvedic system of medicine.⁽⁵⁾ The flowers were used in the treatment of diabetes mellitus (Madhumeha) in ancient times in the siddha system of medicine, which is popular in peninsular India. No scientific reports for its antihyperglycaemic and antihyperlipidaemic activities have been made so far. The present paper deals with the antihyperglycaemic and antihyperlipidaemic effects of *N. stellata* flower extracts on alloxan-induced diabetic rats.

METHODS

Alloxan monohydrate was purchased from Sigma Chemical Co, St Louis, MO, USA. Glibenclamide was received as gift sample from Micro Labs, Hosur, Tamil Nadu, India. One touch glucometer (Accu-chek sensor) of Roche Diagnostics, Germany, total cholesterol (TC), TG,

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and HDL kits were purchased from Roche Diagnostics GmbH, Mannheim, Germany. All other chemicals and reagents were of analytical grade.

N. stellata flowers were collected from the Vadakara district, Kerala, India. They were carefully identified and authenticated by Dr P Daniel, Professor of Botany, Botanical Survey of India, Tamil Nadu Agricultural University, Coimbatore, India. A voucher specimen (BSI-960) of this plant was deposited in the herbarium of the university. Shade-dried *N. stellata* flowers were pulverized using a mixer grinder. About 1 kg of coarse powder was chopped in ethanol (1:1, w/v) and cold macerated for three days with occasional stirring. The suspension was filtered through a fine muslin cloth and the extract was concentrated on rotavapour under reduced pressure and then lyophilised (yield: 6.8% w/w, dry weight).

Male Wistar strains of rats, each weighing 150–200 g, obtained from the Animal Breeding Centre of Kerala Agricultural University, Mannuthy, Trichur, Kerala, India, were used for the study. They were housed in polypropylene cages lined with husk, renewed every 24 h under 12/12 h light/dark cycles at 25–30°C and at 45%–55% relative humidity. The animals were fed with a standard rat pellet diet and tap water was supplied *ad libitum*. A freshly prepared solution of alloxan monohydrate (120 mg/kg body weight), in sterile normal saline solution, was injected intraperitoneally to overnight fasted rats.⁽⁶⁾ Blood glucose was measured after 72 hours of alloxanisation by one-touch glucometer, and it was confirmed by testing for glucosuria using glucose indicator sticks.^(7,8) Rats showing fasting blood glucose (FBG) levels > 250 mg/dL were selected for the study.

The rats were divided into six groups with six rats in each group as follows; Group I: Normal control rats; Group II: Diabetic control rats; Group III: Diabetic rats that received *N. stellata* flower extract (200 mg/kg body weight); Group IV: Diabetic rats that received *N. stellata* flower extract (300 mg/kg body weight); Group V: Diabetic rats that received *N. stellata* flower extract (400 mg/kg body weight); Group VI: Diabetic rats that received glibenclamide (2 g/kg body weight) for a period of 30 days orally.

After the experimental regimen, the animals were fasted overnight and sacrificed by cervical dislocation under mild anaesthesia. Blood was collected on decapitation in two different tubes, one with anticoagulant for plasma and another without anticoagulant for serum separation. Serum and plasma was separated by centrifugation at 2,500 rpm for 15 min, and utilised for biochemical studies.

Blood glucose and urea levels were estimated by an enzymatic glucose oxidase peroxidase (GOD-POD) method⁽⁹⁾ using a commercial kit (Span Diagnostics, Surat India). Plasma insulin was assayed by AxSYM

autoanalyser (Abbott Laboratory, Abbott Park, IL, USA). TC, TG and HDL were analysed by kits (Roche Diagnostics GmbH, Mannheim, Germany) on Hitachi autoanalyser. LDL, very low density lipoproteins (VLDL) and atherogenic index (AI) levels were calculated using the formula of Friedewald et al,⁽¹⁰⁾ phospholipids, free fatty acids (FFA) and total lipids (TL) were analysed by the method of Zilversmit and Davis,⁽¹¹⁾ Falholt et al⁽¹²⁾ and Folch et al,⁽¹³⁾ respectively. Haemoglobin was estimated by the method of Drabkin and Austin⁽¹⁴⁾ and protein was determined by the method of Lowry et al.⁽¹⁵⁾ Values reported are mean of six experiments \pm standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA). Tukey's test was used for multiple comparisons. The values were considered to be significantly different when $p < 0.05$.

RESULTS

Table I illustrates the variations in body weight, food and water intakes of normal control, diabetic control and diabetic treatment groups after 30 days. Alloxan significantly reduced the body weight compared with the controls ($p < 0.001$), which gained significant weight. Although the extract at 200 and 400 mg/kg body weight ameliorated this weight loss, the extract at 300 mg/kg demonstrated a significant beneficial effect when compared with the reference drug glibenclamide. Diabetic control rats showed significantly higher intake of food and water when compared with normal control groups ($p < 0.001$). The food intake was significantly decreased in diabetic rats treated with *N. stellata* flower extract (200, 300 and 400 mg/kg) and glibenclamide. The effect of *N. stellata* at a dose of 300 mg/kg body weight was more significant than at 200 and 400 mg/kg.

Table II shows the effect of *N. stellata* flower extract on FBG in control and diabetic animals. Alloxan caused a significant increase in the FBG of experimental animals compared with control ($p < 0.001$). The FBG was significantly reduced after 30 days of treatment in all animals except non-diabetic control animals. The effect of the flower extract at a dose of 300 mg/kg body weight was more highly significant than 200 mg/kg, 400 mg/kg and glibenclamide 2 g/kg body weight. Therefore the dose of 300 mg/kg was used for further biochemical studies. There was significant decrease in total haemoglobin, plasma insulin and total protein levels in alloxan-induced diabetic rats, when compared to normal control rats. Administration of the flower extract at various doses and glibenclamide tends to significantly bring the level to normal. The blood urea level was significantly increased in diabetic induced rats. This was significantly reduced by the administration of the flower extract. The effect of standard drug glibenclamide on urea in diabetic rats was comparable to that of the herbal extract.

Table I. The dose response effects of *Nymphaea stellata* on body weight, food and water intakes in normal and alloxan-diabetic rats.

Groups	Body weight (g)		Change (%)	Food intake (g/day)	Water intake (ml/day)
	Initial	Final			
Normal control	186 ± 8.40	199 ± 09.85	6.98↑	55.42 ± 5.28	210.65 ± 18.20
Diabetic control	185 ± 12.70	143 ± 12.72**	22.71↓	69.28 ± 4.27**	352.42 ± 20.25**
Diabetic + <i>N. stellata</i> (200 mg/kg)	191 ± 17.72	194 ± 10.32†	1.57↑	63.25 ± 5.87†	335.65 ± 19.56†
Diabetic + <i>N. stellata</i> (300 mg/kg)	183 ± 18.50	201 ± 15.80†	9.83↑	61.32 ± 4.53†	310.45 ± 26.72†
Diabetic + <i>N. stellata</i> (400 mg/kg)	195 ± 11.50	201 ± 12.20†	3.07↑	63.27 ± 4.29†	324.87 ± 21.84†
Diabetic + Glibenclamide (2 g/kg)	185 ± 11.80	198 ± 13.50†	7.02↑	62.05 ± 3.27†	318.38 ± 17.49†

Data is expressed as mean ± SEM (n = 6).

† p < 0.001 when compared to diabetic control rats; ** p < 0.001 when compared to normal control rats.

Table II. The dose response effects of *Nymphaea stellata* on FBG, haemoglobin, plasma insulin, total protein, urea and urine sugar levels in normal and alloxan-diabetic rats.

Groups	Glucose (mg/dl)	Haemoglobin (g/dl)	Plasma insulin (µu/ml)	Total protein (g/dl)	Urea (mg/dl)	Urine sugar
Normal control	80.08 ± 0.23	2.38 ± 5.82	25.58 ± 0.32	10.20 ± 1.02	35.58 ± 1.51	Nil
Diabetic control	275.68 ± 0.35**	05.96 ± 3.62**	16.00 ± 0.41**	5.82 ± 0.83**	53.52 ± 0.86**	+ + +
Diabetic + <i>N. stellata</i> (200 mg/kg)	32.66 ± 15.4†	06.67 ± 3.58*	17.28 ± 2.50*	7.95 ± 1.24†	49.23 ± 5.45†	+ +
Diabetic + <i>N. stellata</i> (300 mg/kg)	89.88 ± 0.44†	11.53 ± 2.23†	20.11 ± 0.55†	9.25 ± 0.72†	38.63 ± 3.58†	Nil
Diabetic + <i>N. stellata</i> (400 mg/kg)	158.50 ± 14.32†	08.59 ± 3.98†	17.87 ± 0.78†	8.52 ± 1.08†	47.76 ± 4.53†	+
Diabetic + Glibenclamide (2 g/kg)	110.65 ± 0.31†	10.65 ± 5.40†	21.20 ± 0.53†	9.01 ± 1.58†	39.50 ± 5.64 †	Trace

Data is the mean ± SEM (n = 6).

† p < 0.001; * p < 0.05 when compared to diabetic control rats; ** p < 0.001 when compared to normal control rats.

In diabetic control rats, the urine sugar was (+ + +) but in the treatment group at 200 mg/kg and 400 mg/kg body weight urine sugar was decreased to (+ +) and (+), respectively. But the *N. stellata* flower extract at 300 mg/kg body weight showed no urine sugar as compared with glibenclamide.

Fig. 1 depicts the levels of TL, TC, TG, phospholipids and FFA in the serum of control and experimental groups of rats. There was significant increase in these lipids in diabetic control rats (p < 0.001). *N. stellata* flower extract and glibenclamide significantly reduced the lipid levels (p < 0.001). The levels of LDL, VLDL and AI in diabetic control rats were significantly increased compared to normal control rats (Fig. 2). HDL was, however, significantly decreased in diabetic control rats compared to normal control rats (p < 0.001). Oral administration of flower extract significantly reversed all these changes (p < 0.001).

DISCUSSION

Hyperglycaemia and hyperlipidaemia are important characteristics of diabetes mellitus; an endocrine disorder is one of the most common chronic diseases worldwide. Alloxan, a β-cytotoxin, induces diabetes mellitus by

damaging the insulin secreting β-cells of the pancreas, resulting in decreased endogenous insulin release. Alloxan-administered rats become hyperglycaemic in a short period of time, followed by hepatic glucose overproduction.⁽¹⁶⁾ Intraperitoneal administration of alloxan (120 mg/kg body weight) effectively induced diabetes mellitus in normal rats as reflected by glycosuria, hyperglycaemia, polyphagia, polydipsia and body weight loss compared with normal rats. The aim for the present work is to explore the scientific basis of the utility of the ethanolic extract of *N. stellata* for correction of hyperglycaemia and hyperlipidaemia in diabetes mellitus.

It was evident from the results that *N. stellata* flower extract reduced the FBG level in alloxan-induced diabetic rats. The antihyperglycaemic effect of *N. stellata* extract could be linked to more than one mechanism. The possible mechanism includes the stimulation of β-cells and subsequent release of insulin and activation of the insulin receptors. The plant's antihyperglycaemic action may be by potentiation of pancreatic secretion of insulin, which was clearly evidenced by the increased level of insulin in diabetic rats treated with *N. stellata* flower extract. In this context, a number of other plants

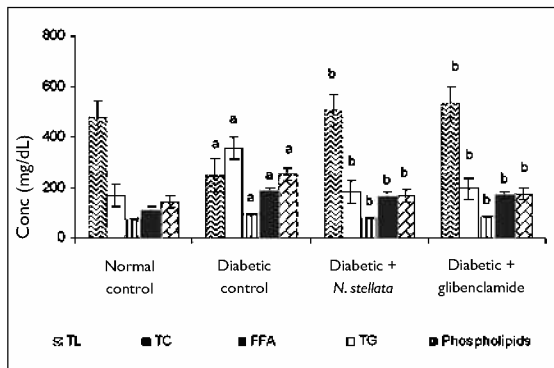


Fig. 1 The effects of *Nymphaea stellata* (300 mg/kg bw) on TL, TC, FFA, TG, phospholipids in normal and alloxan-diabetic rats.

Data is the mean \pm SEM (n = 6); G: glibenclamide

^a p < 0.001 when compared to diabetic control rats; ^b p < 0.001 when compared to normal control rats

have also been reported to have antihyperglycaemic and insulin release stimulatory effect.⁽¹⁷⁻¹⁹⁾ *N. stellata* also acts as a hepatoprotective agent,⁽⁴⁾ so this evidently improves the function of the liver and maintains glucose uptake, enhances the transport of blood glucose to peripheral tissues and its utilisation, which may be another mechanism of action.

Dehydration and loss of body weight have been associated with diabetes mellitus.⁽²⁰⁾ In diabetic rats, increased food consumption and decreased body weight were observed. This indicates the polyphagic condition and loss of weight due to excessive breakdown of tissue proteins.⁽²¹⁾ The decrease in body weight in diabetic rats could be due to dehydration and catabolism of fats and proteins.⁽²²⁾ Increased catabolic reactions leading to muscle wasting might also be the cause for the reduced weight gain by diabetic rats.⁽²³⁾ Oral administration of *N. stellata* flower extract for 30 consecutive days to diabetic rats decreased their food consumption and improved body weight. This could be due to a better control of the hyperglycaemic state in the diabetic rats. Decreased FBG could improve body weight in alloxan-induced diabetic rats.^(24,25) The total haemoglobin level was found to be decreased in diabetic animals. During diabetes mellitus, the excess glucose present in the blood leads to glycation of tissue proteins.⁽²⁶⁾ Administration of flower extract to diabetic rats significantly increased the level of total haemoglobin and this might be due to the decreased level of blood glucose.

In diabetes mellitus, a variety of proteins are subjected to non-enzymatic glycation and this is thought to contribute to the long-term complications of the disease.⁽²⁷⁾ The levels of serum total proteins were found to be decreased in this study. This decrease in diabetic rats may be ascribed to (i) decreased amino acid uptake; (ii) greatly decreased concentration of variety of essential amino acids; (iii) increased conversion rate of glycogenic amino acids to carbon dioxide and water;

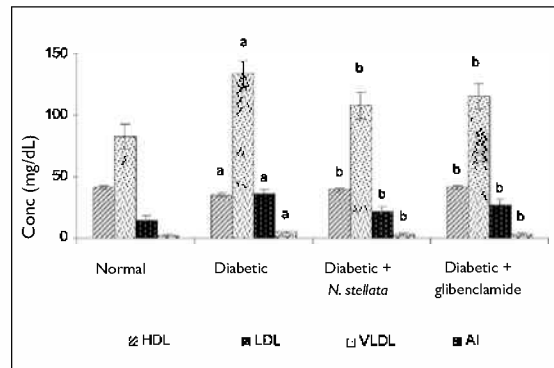


Fig. 2 The effects of *Nymphaea stellata* (300 mg/kg bw) on HDL, LDL, VLDL and AI in normal and alloxan-diabetic rats.

Data is the mean \pm SEM (n = 6); G: glibenclamide

^a p < 0.001 when compared to diabetic control rats; ^b p < 0.001 when compared to normal control rats

and (iv) reduction in protein synthesis secondary to a decreased amount and availability of mRNA.⁽²⁸⁾ Oral administration of flower extract improved total protein concentration in the serum. Diabetes mellitus is also associated with hyperlipidaemia with profound alteration in the concentration and composition of lipid.⁽²⁹⁾ Changes in the concentrations of the lipid with diabetes mellitus contribute to the development of vascular disease.^(30,31) Fatty acids, an important component of cell membranes, are eicosanoid precursors and are therefore required for both the structure and function of every cell in the body.⁽³²⁾

Alloxan significantly increased TL, TC, TG, FFA, phospholipids, LDL, VLDL and AI levels. The abnormally high concentration of serum lipids in diabetes mellitus is mainly due to an increase in the mobilisation of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. The marked hyperlipidaemia that characterises the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots.⁽³³⁾ Excess of fatty acids in plasma produced by alloxan promotes the liver conversion of some fatty acids to phospholipids and cholesterol. These two substances, along with excess of TG formed in the liver, may be discharged into lipoproteins in the blood.⁽³⁴⁾ As a result, serum phospholipid is elevated. Administration of *N. stellata* flower extract to diabetic rats reversed all the above-mentioned changes and improved the HDL levels.

The results of the present investigation clearly indicate that the flower extract of *N. stellata* have a glucose lowering effect on alloxan-induced diabetic rats. It was also found to be highly effective in managing the complications associated with diabetes mellitus, such as body weight maintenance and hyperlipidaemia and prevents the defects in lipid metabolism. Therefore *N. stellata* flowers show therapeutic promise as a protective agent against the development and progression of

atherosclerosis and possible related cardiovascular complications in diabetes mellitus. Further studies are in progress to isolate the active principle and elucidate the exact mechanism of action of *N. stellata* flowers.

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