

Full Length Research Paper

Antidiabetic activity of hydro-ethanolic extracts of *Nymphaea Stellata* flowers in normal and alloxan induced diabetic rats

K. Rajagopal¹ and K. Sasikala²

¹Aizant Drug Research Solutions Pvt Ltd, RR District, Hyderabad, Andra Pradesh, 500014, India.

²Institute of Rehabilitation Medicines and Allied Sciences (IRMAS), New Delhi-110017, India.

Accepted 21 October, 2008

The antidiabetic effect of hydro-ethanolic extract (HEE) of *Nymphaea stellata* Willd flower was investigated in normal and alloxan-induced diabetic rats. In the present study, the animals were divided into normal control, diabetic control, diabetic treated and control treated group (n = 6). Effect of oral administration of HEE (300 mg/kg) for 30 days on the level of blood glucose, glycosylated hemoglobin (HbA_{1c}), total cholesterol (TC), triglycerides (TG), phospholipids, low density lipoprotein (LDL), very low density lipoprotein (VLDL), high density lipoprotein (HDL), Hexokinase, lactate dehydrogenase (LDH) and Glucose-6-phosphatase in normal and alloxan-induced diabetic rats were evaluated. When comparing the values of the HEE treated group with those of the control diabetic group, we found that the HEE significantly decreased the elevated blood glucose level, glycosylated hemoglobin, cholesterol, triglycerides, phospholipids, LDL, VLDL and it showed a significant increase in liver glycogen, insulin and HDL level. Treatment with HEE in diabetic rats increased the Hexokinase, LDH activity and decreased the glucose 6-phosphatase activity. These results clearly indicated that *N. stellata* flowers possess promising antidiabetic effect in diabetic rats.

Key words: Antidiabetic, alloxan, blood glucose, *Nymphaea stellata*.

INTRODUCTION

Diabetes mellitus is a heterogeneous metabolic disorder characterized by altered carbohydrate, lipid and protein metabolism (Mutalik et al., 2003). It is a common endocrine disorder in which there occur increased food and water intake (Pal et al., 2001) and characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both (Gavin, 2003). India has today become the diabetic capital of the world with over 20 million diabetics and this number is set to increase to 57 million by 2025 (Sridhar, 2000). This astronomical increase in the prevalence of diabetes has made diabetes a major public health challenge for India.

Nymphaea stellata wild (Nymphaeaceae) is a perennial aquatic herb generally found in tanks and ponds through-

out the warmer parts of India and Africa. In Sanskrit it is called as Kumuda and in southern India it is well known as Alli or Nilotpalam (Anon, 2001). All parts of the plants are used in folk medicine. The powder of rootstock is given to treat dyspepsia, diarrhoea and piles. An infusion of the rhizomes and stem is considered to be an emollient, diuretic and used for treatment of blennorrhagia and diseases of the urinary tract. The flower has astringent, bitter-sweetish taste; removes impurities from blood, refrigerant and alleviative of cough, biliousness, aphrodisiac, vomiting, giddiness, worm infestation and burning of the skin. The decoction of the flower is used in palpitation of heart, and as a narcotic; syrup of the flower is used in high fever, apoplexy, inflammatory diseases of the brain as also in dysuria. The filaments of the plants work as astringent and cooling agent, used in treating burning sensation of the body, bleeding piles and menorrhagia. Leaves are applied topically in erysipelas, whereas the macerated leaves are used as a lotion in eruptive fevers. The seeds are said to be stomachic and restorative

*Corresponding author. E-mail: rajagopal_pharmacy@rediffmail.com. Tel.: +914343 250049. Fax: +9111 26053320.

(Satyavati, 1987; Kirtikar and Basu, 2001).

Protein, pentosan and tannins were reported to present in seeds of *N. stellata* (Gujral et al., 1955). *N. stellata* seeds are prescribed as diet in diabetes mellitus in Aurvedic system of medicine (Achariya et al., 1996; Subbulakshmi and Naik, 2001). Phenolic constituents were found to present in flowers of *N. stellata* (Kizu and Tamimori, 2003). Recently *N. stellata* flowers have also been reported to have hepatoprotective activity against CCl₄ induced hepatic damage (Bhandarkar and Khan, 2001).

The decoction of flowers of *N. stellata* was used to treat diabetes mellitus in siddha system of medicine, which is practiced locally and it is popular in peninsular India. One of the reasons might be that the original texts of siddha system medicine are written in Tamil language, translations of which are not readily available in other parts of the country. To our knowledge, no scientific studies have been done to establish the antidiabetic effect of *N. stellata*. Hence, the present investigation was carried out to study the effect of *N. stellata* on alloxan induced diabetic rats.

MATERIALS AND METHODS

Plant material

Fresh white colored flowers of *N. stellata*, abundant during rainy season were collected from 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.

Preparation of the plant extract

About 3 kg of flowers of *N. stellata* were shade dried at room temperature and pulverized using a mixer grinder. About 1 kg of coarse powder was chopped in (1:1 v/v) ethanol and cold macerated for 3 days. During the maceration period occasional stirring was done. After 3 days the suspension was filtered through a fine muslin cloth. The residue was removed and the extract was concentrated on rotavapour under reduced pressure and then lyophilized. Finally a dark brown coloured crystal was obtained (yield: 6.8 %w/w, dry weight basis).

Animals

Male Wistar strains of rats, weighing about 150 – 200 g obtained from the small animal's breeding centre of Kerala Agricultural University, Mannuthy, Trichur, Kerala, India were used for study. Animals were kept in the animal house at room temperature of 25 - 30°C and at 45 - 55% relative humidity for 12 h, each of dark and light cycle. The animals were fed with rat pellets feed (Hindustan Lever Limited, Bangalore, India) and filtered water. Animal studies in the work have been strictly performed as per the Institutional Animal Ethical Committee (IAEC) constituted under the guidelines of Committee for the Purpose of Control and Supervision on experimental Animal (CPCSEA), Ministry of Environment, Govt. of India, New Delhi.

Induction of diabetes

Alloxan monohydrate (Sigma-Aldrich Co., USA) was used to induce diabetes mellitus in normoglycemic rats. Animals were allowed to fast for 16 hours and were injected intraperitoneally (i.p.) with freshly prepared alloxan monohydrate in sterile normal saline in a dose of 120 mg / kg body weight (El-Demerdash et al., 2005). Blood glucose was measured after 72 hours of alloxanisation by one-touch glucometer (Accu-chek sensor) of Roche Diagnostics, Germany, and it was confirmed by testing for glucosuria using glucose indicator sticks (Traisman and Greenwood, 1973; Jaouhari, 2000). Rats showing fasting blood glucose levels (>250 mg / dl) were selected for the study.

Experimental design

The normoglycemic animals were divided in to four groups of six animals in each group. The animals were fasted overnight before the experimental schedule began but allowed free access to water.

Group I – Normal Control

Normal healthy rats received water saline only.

Group II – Diabetic control

The rats were made diabetic by an i.p. injection of single dose of alloxan monohydrate (120 mg/kg) in normal saline.

Group III – Diabetic treated

Diabetic rats treated with hydro-ethanolic extract (HEE) of *N. stellata* flowers (300 mg / kg body weight) orally for 30 days.

Group IV – Control treated

Normoglycemic rats received only HEE (300 mg/ kg body weight, Oral route) for 30 days. The dose of 300mg/kg was selected for the study based on our preliminary screening tests.

Collection of rat liver, kidney and blood

After the experimental regimen, the animals were fasted overnight and sacrificed by cervical dislocation under mild anesthesia. Blood was collected on decapitation and serum was separated by centrifugation at 2500 rpm for 15 min. The liver and kidney were excised immediately and thoroughly washed with ice-cold physiological saline. The serum collected was used for biochemical estimations.

Preparation of tissue homogenate

The tissues of 100 mg were homogenized in 0.1 M cold Tris – HCl buffer (pH 7.4) in a potter-Elvehjam homogenizer fitted with a Teflon plunger at 600 rpm for 30 min. The homogenate was centrifuged at 10,000 g for 20 min at 4°C and the supernatant was used for enzyme assays. Lipids were extracted from liver and kidney tissues by the method of Folch (Folch et al., 1957) and used for assaying biochemical parameters.

Biochemical parameters

Blood glucose was estimated by GOD-POD method (Trinder, 1969) using a commercial kit (Span Diagnostics, India). Plasma insulin was assayed by Axsym autoanalyser (Abbott Laboratory, Abbott Park, IL, USA) TC, TG and HDL were analyzed by kits (Roche Diagnostics, GmbH, D-68298 Mannheim, Germany) on Hitachi auto analyzer. LDL and VLDL, Liver glycogen (Carroll et al., 1956),

Table 1. Effect of HEE on BGL, liver glycogen, HbA_{1C} and insulin in control and experimental rats.

Groups	Glucose Mg/dl	Liver Glycogen mg/g wet tissue	HbA _{1C} mg/dl	Insulin µ/ml
I	80.08 ± 0.35	47.96 ± 0.33	3.98 ± 0.32	25.58 ± 1.51
II	275.68 ± 0.35***	19.61 ± 0.83 ***	9.05 ± 0.34 ***	16.00 ± 0.41***
III	89.88 ± 0.44***	41.91 ± 1.35***	6.41 ± 0.65***	20.11 ± 0.55***
IV	79.65 ± 0.31	48.53 ± 0.85	4.03 ± 0.26	23.48 ± 0.51

Values are mean ± S.E.M ($n = 6$).

Statistical Comparison: I vs. II; II vs. III; I vs. IV. *** $P < 0.001$.

Table 2. Concentration of cholesterol level in serum, liver and kidney.

Groups	Cholesterol		
	Serum Mg/dl	Liver mg/g wet tissue	Kidney mg/g wet tissue
I	167.85 ± 1.06	22.50 ± 0.28	18.40 ± 0.46
II	355.40 ± 1.62***	27.73 ± 0.61 ***	28.14 ± 0.67 ***
III	183.31 ± 1.72***	22.72 ± 0.46***	23.37 ± 1.65**
IV	150.36 ± 9.39	20.45 ± 0.56 *	17.69 ± 0.39

Values are mean ± S.E.M ($n = 6$).

Statistical Comparison: I vs. II; II vs. III; I vs. IV. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

Phospholipids (Zilversmit and Davis, 1950), HbA_{1C} (Nayak and Pattabiraman, 1981), Hexokinase (Brandstrup et al., 1957), LDH (Cabaud and Wroblewski, 1958) and Glucose-6-phosphatase (Koida and Oda, 1959) were evaluated.

Statistical analysis

All values are expressed as the mean of six experiments ± S.E.M. statistical significance was estimated by analysis of variance (ANOVA). Tukey's test was used for multiple comparisons, $p < 0.05$ implies significance.

RESULTS

Effect on glucose, liver glycogen, plasma insulin and HbA_{1C}

Treatment with HEE of *N. stellata* on Blood Glucose Level (BGL), insulin, glycogen and HbA_{1C} are depicted in Table 1. The rats exposed to alloxan developed diabetes as evident from the significant elevation in BGL as compared to normal control rats. A significant decrease ($p < 0.001$) in BGL was observed in diabetic rats treated with HEE. The extract failed to produce hypoglycemic activity in normal treated animals as it shows non significance. Liver glycogen level was significantly decreased in diabetic rats as compared to normal control rats. Administration of HEE significantly ($p < 0.001$) increased the liver glycogen level. The insulin levels in diabetic control rats were decreased significantly compared to those in normal rats. In diabetic treatment group insulin levels are significantly increased ($p < 0.001$). The normal rat treated with the HEE alone has no significant change.

Alloxan induced diabetic rats shows a significant ($p <$

0.001) increase in the levels of HbA_{1C} compared to normal control rats indicating poor glycemic control. Treatment with 300 mg/kg body weight HEE decreased HbA_{1C} significantly. However the normal control rats and control treated rats with HEE have no significant difference between them.

Effect on lipid profile

Comparison of results obtained from control rats and diabetic rats revealed that there was a significant increase of TC and TG levels in serum, liver and kidney. Administration of *N. stellata* HEE shows a significant decrease in TC and TG level in diabetic rats (Table 2 and 3). However the control rats treated with HEE shows a significant decrease ($p < 0.05$) in liver TC levels.

A significant increase ($p < 0.001$) in LDL, VLDL levels were observed in the serum of alloxan induced diabetic rats compared to control rats, whereas the HDL level was decreased significantly ($p < 0.01$). The treatment with HEE of *N. stellata* flowers reduced LDL, VLDL and improved HDL significantly, in diabetic treated rats (Table 4).

In diabetes induced rats the serum phospholipid level was significantly increased ($p < 0.001$) whereas the phospholipids in liver and kidney were decreased significantly when compared to the normal control rats (Table 5). Treatment with HEE was found to reverse back the condition closer to normal level. The control treated rats have no significant difference.

Hepatic hexokinase, LDH and glucose 6-phosphatase

The activities of carbohydrate enzymes are represented

Table 3. Effect of oral administration of HEE on triglycerides.

Groups	Triglycerides		
	Serum Mg/dl	Liver mg/g wet tissue	Kidney mg/g wet tissue
I	106.47 ± 0.88	30.84 ± 0.46	17.81 ± 0.37
II	180.68 ± 2.75***	43.36 ± 2.58 ***	39.31 ± 0.52 ***
III	164.14 ± 4.58**	37.39 ± 0.82*	34.52 ± 1.40**
IV	108.57 ± 0.86	32.69 ± 0.63	17.64 ± 0.55

Values are mean ± S.E.M ($n = 6$).

Statistical Comparison: I vs. II; II vs. III; I vs. IV; *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

Table 4. Effect of HEE on serum HDL, LDL and VLDL.

Groups	HDL mg/dl	LDL mg/dl	VLDL mg/dl
I	41.49 ± 1.78	82.58 ± 0.91	13.93 ± 0.79
II	34.92 ± 0.79**	133.59 ± 1.53 ***	35.53 ± 1.15 ***
III	39.40 ± 0.20*	117.71 ± 1.51***	21.35 ± 0.62***
IV	41.50 ± 0.89	80.49 ± 0.77	14.33 ± 0.38

Values are mean ± S.E.M ($n = 6$).

Statistical Comparison: I vs. II; II vs. III; I vs. IV; *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

Table 5. Effect of oral administration of HEE on phospholipids.

Groups	Phospholipids		
	Serum Mg/dl	Liver mg/g wet tissue	Kidney mg/g wet tissue
I	142.90 ± 2.43	46.09 ± 2.43	29.30 ± 0.96
II	251.16 ± 4.61***	31.57 ± 1.20 ***	20.44 ± 0.88 ***
III	168.15 ± 2.35***	39.95 ± 1.39**	25.37 ± 0.53**
IV	154.39 ± 2.85	41.54 ± 1.08	27.28 ± 0.91

Values are mean ± S.E.M ($n = 6$).

Statistical Comparison: I vs. II; II vs. III; I vs. IV; *** $P < 0.001$, ** $P < 0.01$.

in Table 6. Activity of hexokinase and LDH in liver is markedly decreased while glucose 6-phosphatase activity increased significantly in diabetic control rats. Treatment with HEE in diabetic rats increased the hexokinase, LDH activity and decreased the glucose 6-phosphatase activity.

DISCUSSION

Diabetes mellitus is a collection of disorders, which results from either lack of insulin or factors, which interfere with the action of this hormone. In animals, it can be induced by partial pancreatectomy or by the administration diabetogenic drugs such as alloxan, streptozotocin, ditizona and anti-insulin serum (Carvalho et al., 2003). Alloxan causes massive destruction of the β -cells of islets of langerhans, not only destroys pancreatic β -cells but also damages the kidney (Gupta et al., 2005). The disease is progressive and is associated with high risk of

atherosclerosis, kidney and nerve damage as well as blindness. Abnormalities in the regulation of peroxide and transition metal metabolism are postulated to result in the development of the disease as well as its long-term complications (Bartosikova et al., 2003). The mechanism of alloxan has been fully described much earlier (Colca et al., 1983; Lazarow, 1954).

The present study for the first time reports the antidiabetic activity of HEE of the flowers of *N. stellata*. The results indicate that the plant extract was found to reduce the BGL in alloxan induced diabetic rats. The results of our study is supported by the earlier reports on hypoglycemic activity of *Zygophyllum gaetulum* extracts (Jaouhari et al., 2000), *Artemisia herba alba* (Shamaony et al., 1994), against alloxan induced diabetes mellitus.

The antidiabetic 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 re-

Table 6. Activities of hexokinase, LDH and glucose-6-phosphatase in liver of normal and experimental animals.

Groups	Hexokinase Unit ^a / g protein	LDH Unit ^b /g tissue	Glucose-6-phosphatase Unit ^c /mg protein
I	135.67 ± 4.28	2192 ± 152.45	0.172 ± 0.06
II	98.92 ± 1.89***	1536 ± 79.63 ***	0.236±0.038 ***
III	123.48 ± 2.78***	1842 ± 85.78***	0.193 ± 0.028***
IV	142.78 ± 3.21	2126 ± 146.78	0.168 ± 0.02

a, μ moles of glucose phosphorylated / min

b, IU/g: international unit, the amount of enzyme that catalyzes one mole of substrate/min/g tissue.

c, μ moles of Pi liberated / min

Values are mean \pm S.E.M ($n = 6$).

Statistical Comparison: I vs. II; II vs. III; I vs. IV; *** $P < 0.001$, ** $P < 0.01$.

receptors. The plants antihyperglycemic 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 HEE. In this context a number of other plants have also been reported to have antihyperglycemic and insulin release stimulatory effect (Kaleem et al., 2006; Pari and Maheswari, 1999; Prince et al., 1998). *N. stellata* also acts as a hepatoprotective agent so this evidently improves the function of liver and maintains glucose uptake, enhanced transport of blood glucose to peripheral tissue and utilization, which may be another mechanism of action.

Liver is an insulin dependent tissue, which plays a pivotal role in glucose and lipid homeostasis and is severely affected during diabetes. Decreased glycolysis impeded glycogenesis and increased gluconeogenesis are some of the changes of glucose metabolism in the diabetic liver (Baquer, 1998). Increase in liver glycogen can be brought about by an increase in glycogenesis and/or decrease in glycogenolysis (Babu et al., 2003). So the HEE might have stimulated glycogenesis and/or inhibited glycogenolysis in the diabetic rat liver.

HbA_{1C} was found to increase in patients with diabetes mellitus to about 16% (Koenig et al., 1976) and the amount of increase is directly proportional to the fasting BGL (Jackson et al., 1979; Al-Yassin and Ibrahim, 1981). The HEE reduces the elevated HbA_{1C} in diabetic rats. Under normal circumstances, insulin activates enzyme lipoprotein lipase and hydrolysis triglycerides (Sharma et al., 1997). In uncontrolled type-II diabetes mellitus, observed an increase in TC, TG, LDL and VLDL cholesterol with decrease in HDL cholesterol, which contribute to coronary artery disease (Arvind et al., 2002). The abnormal high concentration of serum lipids in the diabetic subject is mainly due to increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase (Pari and Latha, 2002). The marked hyperlipidaemia that characterize the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots. Administration of HEE of *N. stellata* reduced TC, TG, LDL, VLDL and improved

HDL level.

Excess of fatty acids in plasma produced by the alloxan-induced diabetes promotes the liver conversion of some fatty acids in to phospholipids and cholesterol. These two substances along with excess of TG formed in the liver may be discharged in to the blood in the lipoproteins (Bopanna et al., 1997). As a result serum phospholipid is elevated whereas the phospholipids in the liver and kidney were decreased. Treatment with HEE normalized the condition.

The reduction in hepatic hexokinase and LDH are mainly due to leakage of these enzymes in to the blood as a result of alloxan toxicity. Higher activity of glucose 6-phosphatase provides H⁺ which binds with NADP⁺ to form NADPH which is helpful in the synthesis of fats from carbohydrates. When glycolysis slows down because of cellular activity, pentose phosphate pathway that is still active in liver provides NADPH, which converts acetyl radicals in to long chain fatty acids during diabetes mellitus. Similar results were reported by other researchers in experimental diabetes (Grover et al., 2000). However treatment of alloxan diabetic rats with HEE for 30 consecutive days could restore the normal metabolism by shifting the balance from lipids metabolism to carbohydrate metabolism.

Thus the results of the present investigation clearly indicate that the flowers of *N. stellata* possess possible usefulness in the treatment of diabetes mellitus. Further, fractionation of the active principle and comprehensive pharmacological investigation to elucidate the exact mechanism of action is in progress.

ACKNOWLEDGEMENT

We are thankful to Mr. R. S. Sujeeth Bala for his great help in ethnobotanical and ethnopharmacological aspects.

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