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Research article

Evaluation of anti-diabetic potential of leaves of *Nelumbo nucifera* in streptozotocin-induced diabetic rats

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ABSTRACT

Nelumbo nucifera Gaertn. (Nymphaeaceae), also known as sacred lotus, is a well known medicinal plant. *Nelumbo nucifera* (family Nymphaeaceae) are free floating plants.The methanolic extract of Nelumbo nucifera leaves was obtained by soxhlet extraction apparatus. The extract was subjected to preliminary phytochemical screening by using standard procedures.The toxicity studies and dose fixation were carried out by using OECD 425 guideline. According to OECD 425 guideline toxicity study no toxic symptoms were observed up to dose 2000 mg/kg.The anti diabetic effect of Nelumbo nucifera leaf methanolic extract given in streptozotocin induced diabetic rats. Oral administration of methanolic extract for 15 days in diabetic mice exhibits highly significant ($P < 0.01$) antidiabetic activity and also alters the body weight significantly . The data were analyzed using analysis of variance followed by Dunnett's test.The observations confirm that methanolic extract of NELUMBO NUCIFERA leaf and stem has antidiabetic activity due to presence of alkaloids,aminoacids, saponins, glycosides, triterpenoid, vitamins etc There is a need of further investigation to isolate and identify the principle chemical constituents for its anti diabetic property.

KEYWORDS: Antidiabetic activity, *Nelumbo nucifera* , Hypogycemic, Weight variation, streptozotocin

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder which affects a significant portion of the population worldwide.[1] DM is a group of metabolic diseases characterized by hyperglycemia, hypertriglyceridemia and hypercholesterolemia, resulting from defects in insulin secretion, its action or both. [2] Both type 1 and type 2 diabetes are known to be multifactorial diseases caused by a combination of genetic (inheritance) and environmental (diet and lifestyle) factors.[3] Non-insulin-dependent diabetes mellitus (NIDDM) is a multifactorial disease, which is characterised by hyperglycemia and lipoprotein abnormalities.[4] 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).[5] Two groups of oral hypoglycemic drugs, sulphonylureas and biguanides, have been used in the treatment of DM. They act by lowering blood glucose thereby delaying or preventing the onset of diabetic complications.[6] Nelumbo nucifera (Family Nymphaeaceae) are free floating

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plants . Leaves are to be useful in vomiting, dysentery, cholera, diarrhea, ringworm affection and dyspepsia,fever, intermittent fever, cough, burning sensation, dysuria and hyperdipsia, hemorrhoids, haemoptysis, menorrhagia, inflammation, bleeding piles and leucorrhoea, skin diseases, leprosy and pruritus.

Aim and Objective

The main aim of the present work was to evaluate the anti diabetic activity of Nelumbo leaves and stem in streptozotocin induced diabetis by using methanol as a solvent.

MATERIALS AND METHODS

Collection and authentication of plant Leaves of the plant were collected from local region of Pallavaram,Chennai, India in the month of March 2010. The botanical identity was confirmed by a taxonomist Prof. Kamal, Department of Botany; Gorakhpur University, Gorakhpur where voucher specimen (No. GU0309186) has been deposited.

Preparation of plant extract

The leaves and stem of N. nucifera were washed, shade dried and powdered. The powdered material was defatted with petroleum ether (60-80ºC) and then extracted with methanol in Soxhlet apparatus (40 cycles). The extract was concentrated for further studies at reduced pressure and temperature in a rotary evaporator. Methanolic extract was tested for the presence of secondary metabolites by various phytochemical tests.In this extract more amount of phytochemical constituents was isolated and the yield of the extract was very high when compared with the other extracts.

Acute toxicity test

Acute oral toxicity study for the test extract of the plant was carried out using OECD/OCED guideline 425. The test procedure minimizes the number of animals required to estimate the oral acute toxicity. The test also allows the observation of signs of toxicity and can also be used to identify chemicals that are likely to have low toxicity.

SELECTION ANIMALS

Healthy, young adult albino Wistar rats (200-250 g) were used for this study. Animals were fasted (food but not water was withheld overnight) prior to dosing. The fasted body weight of each animal was determined, and the dose was calculated according to the body weight.

Limit test at 2000 mg/kg

The drug was administered in the dose of 2000 mg/kg body weight orally to one animal. This first test animal survived. Then, four other animals were dosed sequentially; therefore, a total of five animals were tested. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h (with special attention given during the first 4 h), and daily thereafter, for a total of 14 days. No animal died. Therefore, the LD_{50} is greater than 2000 mg/kg. [12] An investigation with $1/20^{th}$,$1/8^{th}$, $1/4^{th}$ of 2000 mg/kg, i.e.100,250 and 500 mg was done in pre-screening. Only 250mg/kg and 500 mg/kg was found to be effective against diabetes, hence this dose was used in final screening.

Phytochemical screening

The plant may be considered as biosynthetic laboratory for the chemical compounds such as carbohydrates, protein, glycosides, Saponin etc. The compounds that are responsible for therapeutic effect are usually the secondary metabolites. A systematic study of a crude drug embraces thorough consideration of both primary and secondary metabolites derived as result of plant metabolism. The plant material may be subjected to preliminary phytochemical screening for detection of various plant constituents. Standard screening test of the extract was carried out for various plant constituents. The crude extract was screened for the presence or absence of secondary metabolites such as carbohydrate, polysacharides, saponins, etc. by using standard procedures. [10-11]

Experimental animals

Healthy Wistar albino rats of either sex (150-200 g) were housed in polypropylene cages in an air-conditioned area at 25 ± 2º&C with 12/12 h light-dark cycle. All animals had free access to standard pellet diet (Mahavir Industries, Delhi) and clean water ad libitum. The norms for Good Laboratory Practice (GLP) were followed for care of laboratory animals. The study was approved by Institutional Animal Ethical Committee (IAEC, clearance no: 003/2009/IAEC/anu).

Drugs and chemicals used Metformin, streptozotocin (STZ), and sodium citrate buffer were used in this study. Other chemicals used for extraction purpose and phytochemical tests were of laboratory grade.

Antidiabetic activit: [13–15]

After fasting, DM was induced by intra peritoneal injection of STZ dissolved in 0.1 M cold sodium citrate buffer (pH 4.4) at a dose of 70 mg/kg b.w. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. After 72 h, STZ-treated animals were considered as diabetic when the fasting plasma levels were observed above 200 mg/dL with glucosuria. The experiments were conducted on animal groups to see the effect of MENn on diabetic rats.

Six rats were used in each of the five groups which were as follows:

Group I: Normal control (vehicle).

Group II: Diabetic control (vehicle).

Group III: Diabetic rats treated with MENn (250 mg/kg p.o.).

Group IV: Diabetic rats treated with MENn (500 mg/kg p.o.).

Group V: Diabetic rats treated with Metformin(0.25 mg/kg p.o.).

Vehicle, MENn, and Metformin, were adminis-tered once daily for 15 days from the day of induction. Blood was drawn from Retero orbital puncture , and blood glucose level was estimated on 0, 10 th and 15 th day of experiment with the help of glucometer (one touch ultra, Johnson and Johnson Ltd.) using strip method.

Statistical analysis

All results were expressed as mean ± SEM. The data were analyzed using analysis of variance (ANOVA), and the group means were compared by Dunnett's test. Values were considered statistically significant with $P < 0.05$. GraphPad Instat was used for the analysis of data.

Preliminary phytochemical screening

Phytochemical screening was done using color forming and precipitating chemical reagents to generate preliminary data on the constituents of the plant extract. The chemical tests revealed the presence of major secondary metabolites such as carbohydrate, poly sacharids, saponins, etc. in the extract of the leaves and stem of N.nucifera The results indicated the presence of saponins and carbohydrate, poly sacharides compounds in methanolic extract of N.nucifera.

Acute toxicity studies

A preliminary toxicity study was designed to demonstrate the appropriate safe dose range that could be used for subsequent experiments rather than to provide complete toxicity data on the extract. Acute toxicity studies conducted revealed that the administration of methanolic extract (up to a dose of 2000 mg/kg) of N.nucifera did not produce significant changes in behavior of the animals. No death was observed up to the dose of 2000 mg/kg b.w. The rats were physically active. These effects were observed during the experimental period (15 days). The results showed that in single dose the plant extract had no adverse effect, indicating that the medium lethal dose (LD_{50}) could be greater than 2000 mg/kg body weight in rats. In acute toxicity study, no toxic symptoms were observed for MENn up to dose of 2 g/kg body weight. All animals behaved normally. No neurological or behavioral effects could be noted. No mortality was found up to 15 days study.

Blood glucose level

In STZ -induced diabetic rats, the blood glucose levels were in the range of 279-281 mg/dL, which were considered as severe diabetes. In the Metformin(0.25 mg/kg) and methanolic extract (250mg/kg & 500 mg/kg) treated groups, the peak values of blood sugar significantly decreased from 281.2 mg/dL to 102.34 mg/dL and from 280.6 mg/dL to 109.13 mg/dL on the 15 th day, respectively [Table 1] . Hence, in this study observations showed that the methonalic n reduced the blood glucose level in diabetic rats but values did not return to those of normal controls. Therefore, methanolic extract possesses significant ($P < 0.05$) antidiabetic activity, when compared with diabetic control. There was a marked reduction in blood glucose level (in 15 days) in STZ -diabetic animals. This effect of the methanolic extract (250mg/kg & 500 mg/kg) is nearly equal to, if not better than, that of Metformin (0.25 mg/kg) [Table 1].

Body weight variation

Body weight of streptazosin-induced NIDDM rats were found to be statistically less ($p<0.05$) compared to normal rats at basal level (before drug treatment).After one week of drug treatment, methanolic extract did not improve the body weight of NIDDM rats ($p>0.05$) compare to NIDDM control. Progress in weight gain of animals in drug treated group was absorbed up to 21 days. Body

weight of different group of animals at basal level & at the end of 0, 7, 14, 25 days of drug treatment [Table 2]. The various numbers of plants have been traditionally used to treat diabetes, and some have been proven to have hypoglycemic effects. These studies have identified that compounds such as polysaccharides, flavonoids, terpenoids and tannins, and steroids are responsible for antidiabetic effect. Methanolic extract also contains flavonoids, saponins and carbohydrate, steroids, tannins, and phenolic compounds. The observed hypoglycemic effects of this plant could have resulted from the combined activity of these compounds present in the extract.Administration of STZ caused rapid destruction of pancreatic β-cells in rats, which led to impaired glucose stimulated insulin release and insulin resistance, both of which are marked feature of type II diabetes.Oral hypoglycemic agents and insulin are currently available for treating DM. There is, however, a growing interest in herbal remedies due to the side effects associated with the existing drugs. The present investigation indicates the hypoglycemic and also protective effects of methanolic extract on serum lipid profile of STZ-diabetic rats. We have observed a significant ($P < 0.05$) decrease in blood glucose in methanolic extract -treated diabetic rats, when compared with diabetic control rats. The possible mechanism of extract on hypoglycemic action may be through potentiation of pancreatic secretion of insulin from b-cell of islets and/or due to enhanced transport of blood glucose to the peripheral tissue or by other mechanisms such as stimulation of glucose uptake by peripheral tissue, inhibition of endogenous glucose production or activation of gluconeogenesis in liver and muscles. Diabetes is associated with hyperlipidemia. It is well known that insulin activates enzyme lipoprotein lipase, which hydrolyzes triglyceride under normal conditions. Destruction of β-cells leads to depletion of plasma insulin, which results in hyperlipidemia. The significant control of plasma lipid levels suggests that the extract may produce its action by improving insulin secretion. A significant reduction in bodyweight was observed in the STZ- induced diabetic rats. the decrease in the weight in diabetes is due to continuous excretion of glucose and decrease in peripheral uptake of glucose and glycogen synthesis. the decreace in weight was arrested on administration of methanolic extract.Diabetogenic agents significantly increase the cholesterol and TG levels. The abnormally high concentration of serum lipids in DM 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 hyperlipidemia that characterises the diabetic state may, therefore, be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots. Excess of fatty acids in plasma produced by STZ 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. As a result, serum phospholipids are elevated. Administration of MENn to diabetic rats improved the cholesterol and TG . This effect may be due to low activity of cholesterol biosynthesis enzymes and/or low level of lipolysis which are under the control of insulin. [25] Defects in carbohydrate metabolizing machinery and consistent efforts of the physiological system to correct the imbalance in carbohydrate metabolism place an overexertion on the endocrine system, which leads to the deterioration of endocrine control. Continuing deterioration of endocrine control exacerbates the metabolic disturbances and leads primarily to hyperglycemia.The most significant findings of this study is that the MENn has shown beneficial effect not only on blood glucose, but also on glucose and ketone levels of urine in STZ -induced diabetic rats. Urine analysis on 0 day showed the presence of glucose and traces of ketone in the entire group except normal control. However, on 15 thday glucose and ketone traces were absent in methanolic extract and Metformin-treated groups while they were present in diabetic control [Table 2]. Therefore, results obtained from this study are quite promising and comparable with Metformin, a standard drug used to treat DM.The observations confirm that methanolic extract of the leaf and stem of the plant has antidiabetic activity and is also involved in correction of altered biological parameters. It also warrants further investigation to isolate and identify the hypoglycemic principles in this plant so as to elucidate their mode of action.

TABLE 1: Effect of MENn on blood glucose level in STZ- induced diabetic rats

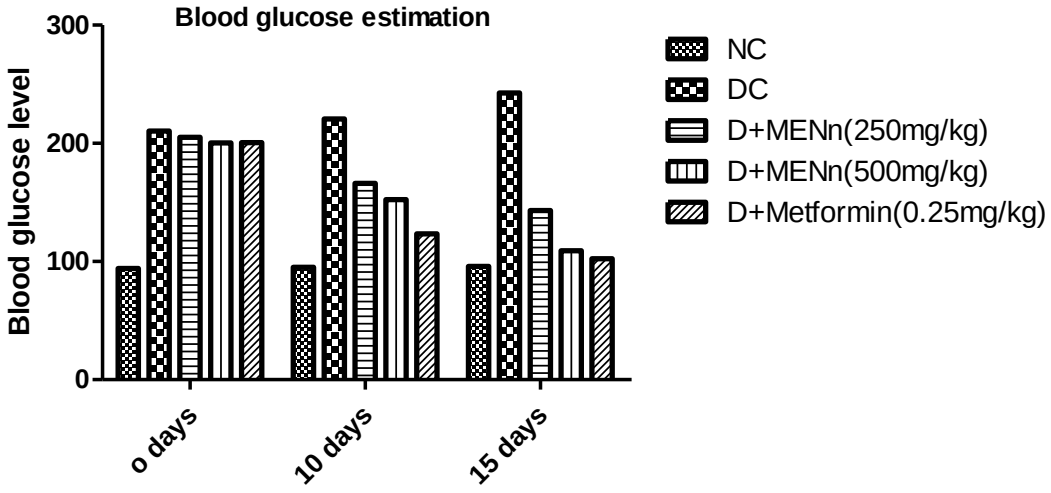
Groups	Blood glucose (mg/dl)		
	0day	10day	15day
Normal control (-)	94.2±3.65	95.0±2.7	95.8±3.06
Diabetic control(-)	210.35±1.41	220.56±2.35	242.56±2.53
Diabetic rats + MENn(250mg/kg)	205.05±1.45	166.23±1.81	143.23±2.13
Diabetic rats + MENn(500mg/kg)	200.38±4.84	152.44±1.56	109.13±2.42
Diabetic rats + Metformin(0.25mg/kg)	200.74±8.41	123.43±3.21	102.34±1.36

Vales are mean ± SEM; n=6 ; **P<0.01as compare to NIDDM control (one way ANOVA followed by Dunnet multiple comparison test); MENn = methanolic extract of Nelumbo , NIDDM = non insulin dependent diabetic mellitus.

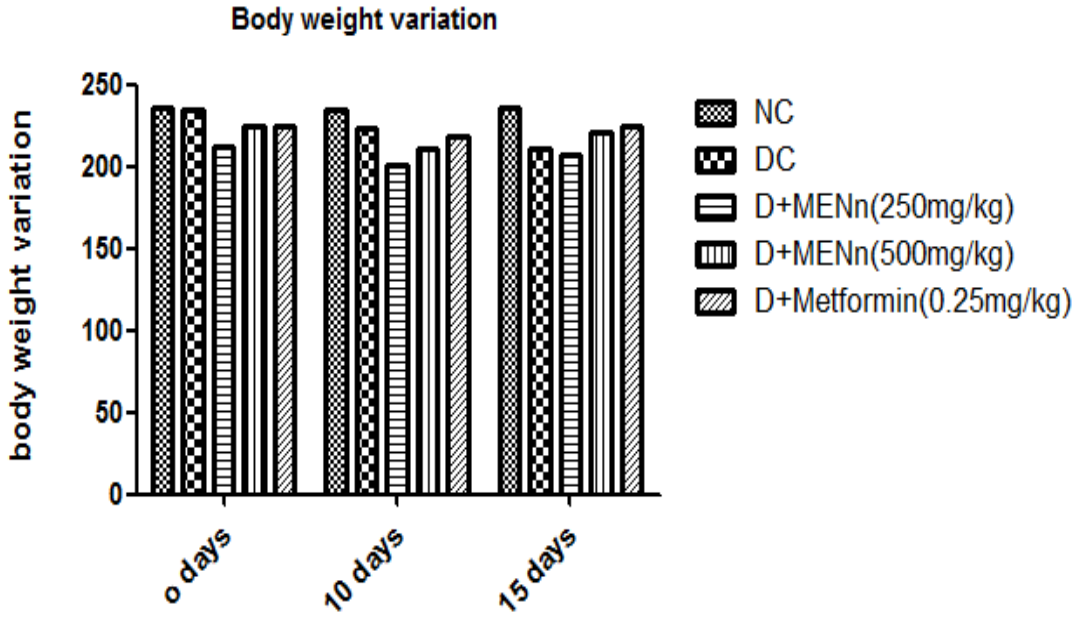
TABLE 2: Effect of MENn on body weight in STZ- induced diabetic rats

Groups	Body weight variation		
	0 day	10day	15day
Normal control (-)	236. 13±1.19	235.12±1.92	236.23±1.43
Diabetic control(-)	235.12 ± 1.42	223.14 ± 2.11	210.25 ±1.64
Diabetic rats + MENn(250mg/kg)	212.22 ±1.21	200.32 ±0.51	207.41 ±1.12
Diabetic rats + MENn(500mg/kg)	224.10 ±2.14	210.43 ±0.18	220. 35 ±0.44
Diabetic rats + Metformin(0.25mg/kg)	225.12 ±1.31	218.32 ±1.45	224.35 ±0.65

Data-1: Blood glucose Estimation



Data-2: Body weight variation



CONCLUSION

The observations confirm that methanolic extract of the leaf and stem of the plant has antidiabetic activity and is also involved in correction of altered biological parameters. It also warrants further investigation to isolate and identify the hypoglycemic principles in this plant so as to elucidate their mode of action.

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