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Research Article

In vivo and *In vitro* Antidiabetic Characterization of *Nymphaea alba* Leaves

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ABSTRACT

The study was aimed to trace out antihyperglycemic potentials of *Nymphaea alba* leaves, using *in vitro* and *in vivo* approaches. In *in vitro* study, determination of IC₅₀ of *N. alba* extracts was done using α -amylase and α -glucosidase inhibition assay. In α -amylase, *N. alba* methanol extract (NAME) exhibited maximum inhibition of $56.77 \pm 1.23\%$ at 125 $\mu\text{g/mL}$, in comparison to $66.7 \pm 0.94\%$ of standard acarbose. In α -glucosidase, NAME exhibits 59.89 ± 0.92 , while standard acarbose showed $70.31 \pm 1.25\%$ inhibition. The *in vivo* study was performed on diabetic rats, made diabetic by streptozotocin (STZ). A 200 and 400 mg/kg NAME was administered orally, which significantly ($p < 0.001$) reduces blood glucose of treated animals in contrast to diabetic control. A distinguishable linear rise of body weight and high-density lipoprotein (HDL) was observed, while there was a remarkable reduction in cholesterol, triglycerides (TG), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL). Reduction in liver function serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT) with serum creatinine, and urea results was also observed in contrast to diabetic control rats. A 400 mg/kg of NAME reflected the most significant results in comparison to that of standard. In addition, an antioxidant study of all three extracts of *N. alba* was done by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and H₂O₂ assay. Methanol extracts were found to possess the highest antioxidant capacity among chloroform and petroleum ether extracts. The whole study gives insight that NAME probably has antidiabetic potentials.

INTRODUCTION

Today's modern society is facing a variety of health issues and diabetes mellitus is one of them. It is considered a metabolic disorder, diagnosed by an increase in blood glucose levels.^[1] It is associated with the deregulation of insulin production or secretion or depletion of cellular responses for insulin stimulus.^[2] This could be due to abnormality of insulin receptors, signal transducer system, effector enzymes, or genes.^[3] Diabetes mellitus has been a threat to mankind from very old times. The evidence of its historical existence was reported in the Egyptian manuscript three thousand years ago,^[4,5] and now it a global issue, recent research reports that diabetes mellitus ubiquity is exponentially rising throughout the

world.^[6] Although, diabetes can be treated by several therapeutic approaches, yet no such drug has been identified which can provide complete relief without side effects.^[7,8] Incorporation of traditional medicinal plants has always been an imperative tool in drug discovery.^[9] The hypoglycemic potential of numerous plants, used in diabetes treatment, has been proved already.^[10] Plants derived formulations have lower toxicity and side effects in contrast to synthetic drugs.^[11,12] Furthermore, taking care of World Health Organisation (WHO) recommendation on diabetes mellitus, it becomes necessary to investigate the hypoglycemic potentials of medicinal plants.^[13] Therefore, documentation and validation of antidiabetic therapeutic efficacy of medicinal plants, as well as, characterization of phytoconstituents is increasing.^[14]

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The genus *Nymphaea* comprises about forty-fifty species,^[15] out of which five species are present in India.^[16] *N. alba* belongs to the Nymphaeaceae family. *N. alba* is frequently referred to as "European White Water Lily or Nenuphar."^[17] It is a perennial hydrophyte having a black, sturdy, almost horizontal, and scarcely branched rhizome submerged in the mud.^[17] It grows at depth of about 0.5 to 3 meters, in water over mud, silt, or peat.^[17-20] It spreads extensively throughout Europe, North Africa, north and central Asia, southwest Asia, Finland, India, China, Russia, Turkey, and Poland.^[21] *N. alba* has a history of ethnobotanical applications in different cultures.^[22-24] Other species of *Nymphaea* are known to possess potent antidiabetic properties. Moreover, as no scientific evidence of antidiabetic activity is there in *N. alba* leaves; the present study was carried to trace out its hidden antidiabetic potentials. A collaborated approach, including *in vivo* and *in vitro* techniques, was employed for the exploration of the antidiabetic properties of *N. alba* in the current research work.

MATERIALS AND METHODS

Animals

Wistar rats were incorporated into the study (180 ± 20 grams). Proper hygiene and sanitation were maintained for animals. Normal conservation conditions provided in clean polypropylene cages at 23 ± 1°C, equal hours light:dark period, and relative humidity were regulated at 60 ± 4%. Normal animal feed with water was maintained. The animals had been acclimatized to the laboratory environment prior to the start of the experimentation.^[25] Guidelines provided for animal care and experimentations were followed throughout the period. The experimental protocol was accepted by the Institutional Animal Ethics Committee (IAEC) at M. M. College of Pharmacy, M. M. U. Mullana (IAEC/19/20).

Plant Collection and Authentication

Plant material was collected during July-September from crocodile breeding park Kurukshetra. It was authenticated by Dr. B. D. Vashishta, Botany Department, Kurukshetra University, Haryana, India.

Extracts Preparation

After shade drying, plant material was coarsely powdered and then successively extracted using different solvents with increasing polarity in the Soxhlet apparatus. Initially, pet-ether (60–70°C) was used to remove fats from the plant material, as extracts containing lipid content cause hindrance in the spectroscopic evaluation of isolated compound, followed by extraction with chloroform and methanol. The whole extraction procedure was carried at the same temperature. After the collection of the extracts, a rotary evaporator was then employed to concentrate them, so that a concentrated crude mass can be formed for further study.

In vitro Studies

α -Amylase Activity

To investigate the amylase inhibitory potential of all the three extracts of *N. alba*, α -amylase inhibition assay was used, the method followed as per Ali *et al.*, with some alterations. In brief, 30 μ L of varying concentrations of plant extracts that is 8, 15, 30, 60, and 125 μ g/mL, 200 μ L α -amylase was added, followed by incubation for 20 minutes at 37°C. Then, the addition of 100 μ L of the (1%) starch solution was done and again subjected to incubation for 10 minutes at 37°C. To stop the reaction, 200 μ L 3,5-dinitrosalicylic acid (DNSA) was added. Readings were taken at 540 nm wavelength.^[26,27] Acarbose was taken as standard. To enhance the reproducibility and resolution of the results, experiments were repeated thrice using the same protocol.

α -Glucosidase Activity

In the α -glucosidase assay, 95 μ L of the phosphate buffer (100 mM) was added to 96-well microplates. 25 μ L of the α -glucosidase (0.5 U/mL) was added, and then 30 μ L of plant extracts (8, 15, 30, 60, and 125 μ g/mL) and acarbose in the same concentrations were added. Acarbose was taken as a standard for the experiment. Then, the above reaction mixtures were subjected to incubation for 20 minutes at 37°C. After incubation, 50 μ L of 5 mM p-nitrophenyl-D-glucopyranoside (p-NPG) was added and subjected to incubation for 10 minutes at 37°C. A 2 mL of Na₂CO₃ (0.1 M) was added for termination of the reaction.^[28,29] Absorbance was taken at 415 nm, using iMark microplate reader. The quantity of α -nitrophenol released from p-NPG was used to estimate percent inhibition.

$$\% \text{ inhibition} = 100 \times \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}}$$

Antioxidant Activity

DPPH Antioxidant Assay

DPPH stock solution was made by mixing 3.3 mg of DPPH in 100 mL of methanol. 1 mL of different concentrations of the test solution (8, 15, 30, 60, and 125 μ g/mL) was put in 2.5 mL of stock solution. After keeping for half an hour, readings were taken at 517 nm in an ultraviolet-visible (UV) spectrophotometer in contrast to the standard at varying concentrations (2, 4, 6, 8, and 10 μ g/mL).^[30] Ascorbic acid was taken as a reference. The calculation was done using the formula given below.

$$\% \text{ inhibition} = 100 \times \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}}$$

Hydrogen Peroxide Assay

For the evaluation, a solution was made by mixing hydrogen peroxide (40 mM) and phosphate buffer with pH 7.4. to 0.6 mL of this solution, varying concentration (25, 50, 100, 200, and 400 μ g/mL) of samples and (10, 20, 40, 60,



and 80 µg/mL) standard was added.^[31] After 10 minutes, readings of hydrogen peroxide were taken at 230 nm. Blank contained only buffer solution. The same above-mentioned formula was employed to find the percentage inhibition of hydrogen peroxide in samples, as well as, standard compounds.

***In vivo* Studies**

Acute Toxicity Studies

For the acute toxicity evaluation, the guidelines of the Organization of Economy and Cooperation Development (OECD 423) were followed. 8 to 12 weeks old albino mice weighing 20 to 30 grams were included for toxicity studies. Animals were fasted for 3 to 4 hours prior to dosing. After that, weighing of the animals was done followed by administration of the extracts using gastric intubation.^[32,33] Even after the administration of the extracts, animals were not provided any food for the next couple of hours. A dose of 2,000 mg/kg was given to six animals initially.^[34] In the initial 30 minutes, animals were monitored independently, upon dosing, and periodically over 24 hours, out of which, the first four hours were considered crucial, hence, monitored vigilantly and regularly afterward for a duration of 14 days.

Oral Glucose Tolerance Test (OGTT)

Four groups of rats were made on a random basis, having six animals each. Animals were made to fast overnight. Normal control group-I animals were only administered with the vehicle; group-II animals were treated with 2.5 mg/kg of standard drug glibenclamide; groups III and IV received NAME (200, 400 mg/kg) and served as test groups.^[35] Rats of every group were administered with 2,000 mg/kg of glucose that is 30 minutes prior to the oral administration of the test and standard compounds.^[36] After 30, 60, and 120 minutes of oral glucose, blood was withdrawn from the tail on to the strip of glucometer, and concentrations of glucose in the blood was measured (using Accu-Chek, Roche Diagnostics, USA).

STZ-induced Diabetic Study^[37,38]

A single dose of STZ (55 mg/kg) was administered intraperitoneally to the overnight fasted rats. Preparation of dose was carried spontaneously, using very cold citrate buffer with pH 4.5. Group-I animals were given no treatment or STZ throughout the study. For the next 12 days, animals were kept under observation with proper feed and water and on the 12th day, animals were screened for blood glucose.^[39,40] Animals were believed to have diabetes, if blood glucose levels were found to be 200 mg/dL or higher, and incorporated into the study.

Experimental Design

Five groups (G) of rats were assigned, containing six rats (n = 6) each.

Group-I: Normal healthy rats administered 0.9 percent sodium chloride (NaCl).

Group-II: Diabetic rats were given only 0.9 percent NaCl.

Group-III: Diabetic rats + glibenclamide (2.5 mg/kg).

Group-IV: Diabetic rats + NAME (200 mg/kg).

Group-V: Diabetic rats + NAME (400 mg/kg).

Both test and standard drugs were administered for the next 21 days and a dose of methanol extract of *N. alba* and glibenclamide were made in the vehicle solution (0.9% NaCl) at room temperature.

Biochemical Estimations

For tracing blood glucose levels, a one-touch glucometer (Accucheck, Roche Diagnostics) was used at weekly intervals. After completion of the study, blood samples were procured in ethylenediaminetetraacetic acid (EDTA) tubes, by cardiac puncture, as well as, retro-orbital plexus method, from all animals. After that, for 20 minutes at 3,000 rpm centrifugation of blood samples was done. Serum separation and storage at -20°C was done till tests were performed. Determination of cholesterol, HDL, LDL, VLDL, triglycerides, urea, and creatinine were done from collected serum samples with the help of an autoanalyzer, using ERBA diagnostic kits.

Histopathology

Once the whole animal study was completed, the pancreas of dissected rats was fixed in 10% formalin solution, after rinsing in ice-cold normal saline.^[41] Further, tissues were subjected to embedding in paraffin wax. With the help of microtome, 4 to 5 µm thick sections were made. For staining of the sections, hematoxylin-eosin was used. Stained tissues were examined under a light microscope.

Statistical Analysis

For tracing significant difference ($p < 0.05$), one-way analysis of variance (ANOVA) was applied and comparative analysis was done with Dunnet's t test *in vivo* studies, for instance, blood glucose, body weight, biochemical parameters, and *in vitro* inhibition assays. For the representation of results, the mean \pm standard error of the mean pattern was used.

RESULTS

In vitro

α-Amylase Activity

Concentrations including 8, 15, 30, 60, and 125 µg/mL were used for screening of *N. alba* petroleum ether, chloroform, and methanol extract (Fig. 1). The NAME exhibited IC₅₀ 74.56 \pm 1.872 in comparison to IC₅₀ 24.16 \pm 1.383 of standard acarbose. IC₅₀ of both other extracts of *N. alba* was found to be insignificant in comparison to IC₅₀ of standard.

α -Glucosidase Activity

N. alba extracts were studied at 8, 15, 30, 60, and 125 $\mu\text{g/mL}$ concentrations and NAME exhibited significant inhibition in comparison to standard acarbose, while *N. alba* chloroform extract (NACE) and *N. alba* petroleum

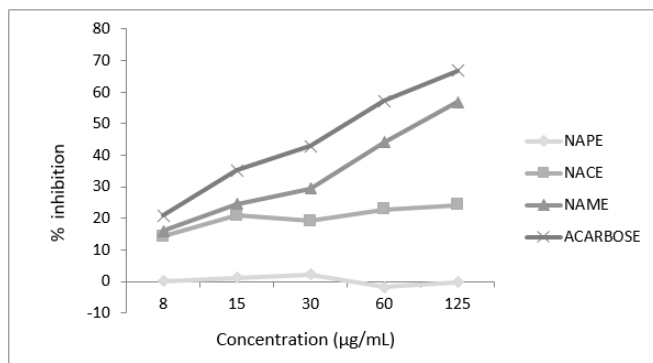


Fig. 1: α -amylase inhibition activity of *N. alba*
NAPE: *N. alba* petroleum extract; NACE: *N. alba* chloroform extract;
NAME: *N. alba* methanol extract

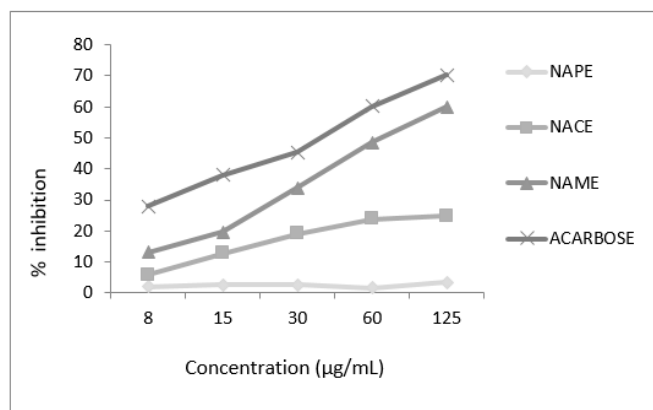


Fig. 2: α -glucosidase inhibition activity of *N. alba*
NAPE: *N. alba* petroleum extract; NACE: *N. alba* chloroform extract;
NAME: *N. alba* methanol extract

extract (NAPE) showed minimum inhibition (Fig. 2). With an increase in concentration, percentage inhibition was found to be increased. The maximum enzyme inhibition with standard drug, i.e., acarbose was 70.31 ± 1.25 at 125 $\mu\text{g/mL}$.

Antioxidant Activity

DPPH Assay for Radical Scavenging Activity

In DPPH assay, NAME showed 63.97 ± 0.62 percent inhibition at 125 $\mu\text{g/mL}$, while NAPE and NACE exhibit only 12.35 ± 1.86 and 41.39 ± 1.86 , respectively. NAME exhibited comparable inhibition to that of standard ascorbic acid (Table 1).

Hydrogen Peroxide Activity

N. alba extracts at 25, 50, 100, 200, and 400 $\mu\text{g/mL}$. The NAME showed good hydrogen peroxide radical scavenging activity when compared to standard ascorbic acid, which showed 79.91 percent inhibition at 400 $\mu\text{g/mL}$ concentration, as shown in Table 2.

In vivo

Toxicity Study

No mortality was observed in all groups. No clinical signs were observed in selected animals for study. The overall study showed that LD_{50} of oral toxicity of extract to be above 2,000 mg/kg in mice of both sexes.

Oral Glucose Tolerance Test (OGTT)

The blood glucose concentration of group-I normal control rises significantly in comparison to groups III and IV. The mean blood glucose level after 30 minutes in group-I (normal control) was 145.71 mg/dL (Table 3). Oral administration of extracts and standard drug caused a comparable fall in blood glucose in contrast to the

Table 1: DPPH scavenging activity of extracts of *N. alba*

Drug	8 $\mu\text{g/mL}$	15 $\mu\text{g/mL}$	30 $\mu\text{g/mL}$	60 $\mu\text{g/mL}$	125 $\mu\text{g/mL}$
NAPE	1.24 ± 0.36	3.39 ± 0.95	4.11 ± 1.29	9.49 ± 1.56	12.35 ± 1.86
NACE	10.2 ± 0.62	16.66 ± 0.62	24.9 ± 0.95	35.65 ± 1.29	41.39 ± 1.86
NAME	24.9 ± 1.56	34.58 ± 1.29	46.41 ± 0.95	53.57 ± 0.95	63.97 ± 0.62
-	2 $\mu\text{g/mL}$	4 $\mu\text{g/mL}$	6 $\mu\text{g/mL}$	8 $\mu\text{g/mL}$	10 $\mu\text{g/mL}$
Ascorbic acid	37.09 ± 0.62	44.25 ± 0.36	53.22 ± 0.62	66.12 ± 0.62	75.08 ± 1.29

NAPE: *N. alba* petroleum ether extract; NACE: *N. alba* chloroform extract; NAME: *N. alba* methanol extract

Table 2: H_2O_2 scavenging activity of extracts of *N. alba*

Drug	25 $\mu\text{g/mL}$	50 $\mu\text{g/mL}$	100 $\mu\text{g/mL}$	200 $\mu\text{g/mL}$	400 $\mu\text{g/mL}$
NAPE	11.84 ± 0.61	15.35 ± 1.27	20.61 ± 0.93	22.02 ± 0.93	24.12 ± 1.95
NACE	18.51 ± 1.27	21.67 ± 1.95	26.58 ± 0.61	33.6 ± 0.93	42.72 ± 0.35
NAME	29.04 ± 1.27	35.7 ± 0.35	39.56 ± 0.35	48.68 ± 0.61	60.26 ± 0.61
-	10 $\mu\text{g/mL}$	20 $\mu\text{g/mL}$	40 $\mu\text{g/mL}$	60 $\mu\text{g/mL}$	80 $\mu\text{g/mL}$
Ascorbic acid	40.61 ± 0.7	48.33 ± 1.27	53.6 ± 0.93	64.12 ± 0.93	79.91 ± 0.35

NAPE: *N. alba* petroleum ether extract; NACE: *N. alba* chloroform extract; NAME: *N. alba* methanol extract



result obtained from group-I. Glibenclamide (2.5 mg/kg) along with NAME (400 mg/kg) exhibited a reduction of blood glucose concentrations to a maximum after 30 minutes of glucose administration (121.1 ± 1.6 mg/dL, 127.61 ± 1.67 , respectively).

Effect on Bodyweight

Bodyweight of group-II diabetic control rats was reduced remarkably, in contrast to group-I (normal control) and treated groups III to V, as given in Table 4. The normal control group was observed to have a mean weight of 178.83 ± 1.54 grams at the beginning of the study that got raised to 189.56 ± 1.63 grams after 21 days. Bodyweight of group-II rats was significantly and sequentially reduced from 187.67 ± 1.48 to 141.81 ± 1.03 grams from beginning to the end of the study. The mean body weight in group-III at day 1 was 191 ± 2.73 grams, while these values were 195.55 ± 3.08 , 209.24 ± 3.3 , and 206.28 ± 2.95 grams on the 7th, 14th, and 21st day, respectively. 400 mg/kg NAME showed a remarkable rise in body weight from 185.75 ± 1.71 to 209.9 ± 1.94 grams.

STZ-induced Diabetic Study

The remarkable rise of blood glucose was analyzed in diabetic control (group-II) animals in contrast to the groups-I, and III to V. In normal control (group-I), mean blood glucose was 103.66 ± 3.31 mg/dL on the 1st day and 109.33 ± 2.35 mg/dL on the 21st day. In group-II (diabetic control), blood glucose level on the 1st day was

269.67 ± 3.31 mg/dL and increased to 305.83 ± 2.91 mg/dL on the 21st day. The mean body glucose in group-III at day 1 was 270.17 ± 3.02 mg/dL, while these values were 121.58 ± 1.36 , 113.47 ± 1.27 , and 108.07 ± 1.21 mg/dL on the 7th, 14th, and 21st day, respectively. NAME exhibited a comparable fall in blood glucose levels in contrast to the result obtained from diabetic animals. Animals administered 400 mg/kg of NAME reflected a fall in blood glucose from 264 ± 3.06 to 137.19 ± 1.56 ; details are given in Table 5.

Biochemical Parameter

There was a marked increase in all the biochemical parameters except HDL in group-II (Diabetic control). Standard drug glibenclamide (2.5 mg/kg) effectively controlled the levels of all the biochemical parameters. Doses of NAME 400 mg/kg were more effective than NAME 200 mg/kg in decreasing cholesterol levels and enhancing HDL level (Table 6). 21 days of NAME administration caused a remarkable reduction of SGOT, SGPT, serum creatinine, and serum urea level, in a dose-dependent manner of diabetic animals.

Histopathology

Microscopic structural alterations in the pancreas of experimental animals are shown in Fig. 3. The pancreas of normal rats has normal exocrine acinar tissues and endocrine β -cells, shown in Fig. 3A. On the contrary, histopathology of the pancreas of diabetic control group

Table 3: OGTT study results of *N. alba* methanol extract

Groups <i>n</i> = 6	Treatment	Glucose concentration (mg/dL)			
		0 min	30 min	60 min	120 min
I	Normal control	98.83 ± 1.77	145.71 ± 2.73	129.83 ± 1.53	118.33 ± 0.87
II	Glibenclamide (2.5 mg/kg)	100.91 ± 1.34	$121.1 \pm 1.6^{***}$	$111.01 \pm 1.47^{***}$	$105.96 \pm 1.4^{***}$
III	NAME 200	100.14 ± 1.41	$135.19 \pm 1.91^{**}$	$118.6 \pm 2.13^{***}$	113.66 ± 2.04
IV	NAME 400	98.16 ± 1.28	$127.61 \pm 1.67^{***}$	$112.89 \pm 1.47^{***}$	$110.92 \pm 1.45^*$

Readings represented as mean \pm standard error mean (*n* = 6); * *p* < 0.05; ** *p* < 0.01; *** *p* < 0.001, in comparison to normal control animals (one-way ANOVA with Dunnet's *t* test); NAME: *N. alba* methanol extract

Table 4: Liver, pancreas, and bodyweight analysis of rats after treatment with NAME

Groups <i>n</i> = 6	Treatment	Bodyweight (gm)				Liver weight (g)	Pancreas weight (g)
		1st day	7th day	14th day	21st day		
I	Normal control + 0.1 mL vehicle	$178.83 \pm 1.54^*$	$185.37 \pm 1.76^*$	$185.9 \pm 1.6^{***}$	$189 \pm 1.63^{***}$	$5.4 \pm 0.06^{***}$	$0.84 \pm 0.03^{***}$
II	Diabetic control + 0.1 mL vehicle	187.67 ± 1.48	175.43 ± 1.57	157.9 ± 1.2	141.81 ± 1.03	4.05 ± 0.04	0.99 ± 0.03
III	Diabetic + glibenclamide (2.5 mg/kg)	191 ± 2.73	$195.55 \pm 3.08^{***}$	$209.24 \pm 3.3^{***}$	$206.28 \pm 2.95^{***}$	$5.45 \pm 0.04^{***}$	$0.86 \pm 0.03^{***}$
IV	Diabetic + NAME (200 mg/kg)	186.82 ± 2.07	$200.4 \pm 2.26^{***}$	$205.5 \pm 2.28^{***}$	$216.43 \pm 2.44^{***}$	$5.13 \pm 0.05^{***}$	$0.8 \pm 0.01^{***}$
V	Diabetic + NAME (400 mg/kg)	185.75 ± 1.71	$198.75 \pm 1.83^{***}$	$202.47 \pm 1.87^{***}$	$209.9 \pm 1.94^{***}$	$4.98 \pm 0.05^{***}$	$0.83 \pm 0.01^{***}$

Readings represented as mean \pm standard error mean (*n* = 6); * *p* < 0.05; ** *p* < 0.01; *** *p* < 0.001, in comparison to diabetic control animals (one-way ANOVA with Dunnet's *t* test); NAME: *N. alba* methanol extract

Table 5: Blood glucose levels of rats treated with NAME and standard drug

Groups n = 6	Treatment	Fasting blood glucose (mg/dL)			
		1st day	7th day	14th day	21st day
I	Normal control + 0.1 mL vehicle	103.66 ± 3.31***	109.83 ± 1.7***	107.16 ± 3.02***	109.33 ± 2.35***
II	Diabetic control + 0.1 mL vehicle	271.67 ± 3.31	285.5 ± 1.52	296.67 ± 1.74	305.83 ± 2.91
III	Diabetic + glibenclamide (2.5 mg/kg)	270.17 ± 3.02	121.58 ± 1.36***	113.47 ± 1.27***	108.07 ± 1.21***
IV	Diabetic + NAME (200 mg/kg)	269.66 ± 2.59	219.5 ± 3.83***	215.73 ± 2.07***	210.34 ± 2.02***
V	Diabetic + NAME (400 mg/kg)	264 ± 3.06	161.4 ± 1.83***	147.95 ± 1.68***	137.19 ± 1.56***

Readings represented as mean ± standard error mean (n = 6); * p < 0.05; ** p < 0.01; *** p < 0.001, in comparison to diabetic control animals (one-way ANOVA with Dunnet's t test); NAME: *N. alba* methanol extract

Table 6: Effect of NAME biochemical parameter of diabetic rats

Parameters	Normal control + 0.1 mL vehicle	Diabetic control + 0.1 mL vehicle	Diabetic + glibenclamide (2.5 mg/kg)	Diabetic + NAME (200 mg/kg)	Diabetic + NAME (400 mg/kg)
Total cholesterol (mg/dL)	99.15 ± 1.79***	237.96 ± 4.3	112.54 ± 2.03***	151.7 ± 2.74***	125.92 ± 2.28***
Triglycerides (mg/dL)	77.87 ± 0.95***	179.09 ± 2.19	84.87 ± 1.04***	147.95 ± 1.81***	100.45 ± 1.23***
HDL (mg/dL)	47.4 ± 0.78***	27.44 ± 0.45	49.77 ± 0.82***	40.29 ± 0.67***	35.55 ± 0.59***
LDL (mg/dL)	36.18 ± 2.18***	174.7 ± 4.61	45.79 ± 2.43***	81.82 ± 3.1***	70.28 ± 2.57***
VLDL (mg/dL)	15.57 ± 0.19***	35.82 ± 0.44	16.97 ± 0.21***	29.59 ± 0.36***	20.09 ± 0.25***
SGOT (IU/L)	34.97 ± 1.24***	74.3 ± 2.65	41.96 ± 1.49***	57.7 ± 2.05***	44.43 ± 1.58***
SGPT (IU/L)	24.75 ± 1.03***	77.97 ± 3.23	32.18 ± 1.33***	44.56 ± 1.85***	37.87 ± 1.57***
Creatinine (mg/dL)	0.47 ± 0.05***	0.98 ± 0.11	0.56 ± 0.06**	0.89 ± 0.1	0.67 ± 0.07*
Serum urea (mg/dL)	34.55 ± 0.55***	107.12 ± 1.7	38.01 ± 0.6***	72.56 ± 1.15***	64.58 ± 1.03***

Readings represented as mean ± standard error mean (n = 6); * p < 0.05; ** p < 0.01; *** p < 0.001, in comparison to diabetic control animals (one-way ANOVA with Dunnet's t test); NAME: *N. alba* methanol extract

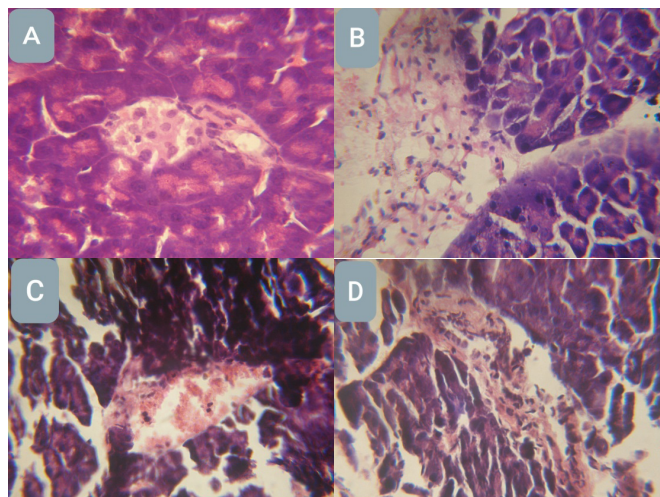


Fig. 3: Histology of pancreas; **A**-Normal group; **B**-Diabetic control; **C**-NAME 200 mg/kg treated; **D**-NAME 400 mg/kg treated

rats exhibited degranulation and disruption of the cell of islets of Langerhans (Fig. 3B). However, NAME 200 and 400 mg/kg treated animals were observed to have a better shape of tissue of the pancreas, as shown in Figs 3C and D.

DISCUSSION

The rationale of the whole study was to trace out antidiabetic tendencies of *N. alba* pre-clinically and by

α -amylase and α -glucosidase. Different methods were employed for the determination of goals, including *in vitro* and *in vivo* studies. In *in vitro* α -amylase and α -glucosidase brought foundation for the whole study by substantiating the antidiabetic potential of *N. alba* methanol extract over petroleum ether or chloroform extract. Further, *in vivo* study results provided the testimony of *N. alba* leaves methanol extract to possess antidiabetic potentials. Diabetic rats administered with 200 and 400 mg/kg of NAME gave comparable results to the group administered standard glibenclamide (2.5 mg/kg), in reducing blood glucose levels. Moreover, the results of other biochemical parameters, like total cholesterol, triglycerides, HDL, SGOT, SGPT, urea, and creatinine reflected a better picture in contrast to that of the control group. In addition, histopathological observations also indicate the activity of *N. alba* in the maintenance of the diabetic condition. Also, the antioxidant nature of the *N. alba* extracts, by DPPH and hydrogen peroxide assays, indicates the role of this plant in diabetes. Subsequently, even more research is required to analyze the molecular mechanism involved in the tendency of *N. alba* in the reduction and control of hyperglycemia.

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