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Antidiabetic Activity of *Nerium indicum* Leaf Extract in Alloxan-induced Diabetic Rats

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ABSTRACT

This research aims to investigate the antidiabetic activity of *Nerium indicum* (Family: Apocynaceae) leaf extract in alloxan induced diabetic albino rats. A comparison was made between the action of *Nerium indicum* extracts and a known antidiabetic drug glibenclamide (600 µg/kg body wt.). An oral glucose tolerance test (OGTT) was also performed in experimental diabetic rats. The petroleum ether, chloroform, alcohol and aqueous extracts of *Nerium indicum* were obtained by simple maceration method and subjected to standardization by following pharmacognostical and phytochemical screening methods. Dose selection was made on the basis of acute oral toxicity study (50mg to 5000 mg/kg body weight) as per OECD guidelines. *Nerium indicum* chloroform extract (NICE) and ethanolic extract (NIEE) showed significant ($P<0.001$) antidiabetic activity. In alloxan induced model, blood glucose level of these extracts on the seventh day of study were NICE (113.33±6.662) and NIEE (169.33±9.735) in comparison of diabetic control (413.50 ±4.752) and petroleum ether extract (337.83±25.515). In OGGT model (glucose loaded rats), NICE exhibited glucose level after 30 min. (164.33±5.661) and 90 min. (121.00±2.966) whereas NIEE after 30 min. (174.16±3.380) and 90 min. (128.00±5.266). These extracts also prevented body weight loss in diabetic rats. The antihyperglycemic action of the extracts may be due to improving the glycemic control mechanisms. The drug has the potential to act as antidiabetic drug.

Key words: Acute toxicity, Alloxan monohydrate, Antidiabetic activity, *Nerium indicum*

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INTRODUCTION

Diabetes mellitus is a serious complex chronic condition that is a major source of ill health worldwide. This metabolic disorder is characterized by hyperglycemia and disturbances of carbohydrate, protein and fat metabolisms, secondary to an absolute or relative lack of the hormone insulin. Besides hyperglycemia, several other factors including dislipidemia or hyperlipidemia are involved in the development of micro and macrovascular complications of diabetes, which are the major causes of morbidity and death.[1] According to World Health Organization (WHO) projections, the prevalence of diabetes is likely to increase by 35%. Currently there are over 150 million diabetics worldwide and this is likely to increase to 300 million or more by the year 2025. Statistical projection about India suggests that the number of diabetics will rise from 15 million in 1995 to 57 million in the year 2025, the highest number of diabetics in the world.[2] Reasons for this rise include increase in sedentary lifestyle, consumption of energy rich diet, obesity, higher life span etc. Other regions with greater number of diabetics are Asia and Africa,

where there could be a two-three fold rise in diabetes mellitus cases.[3] Evaluation of plant products to treat diabetes mellitus is of growing interest as they contain many bioactive substances with therapeutic potential. In recent years several authors evaluated and identified the antidiabetic potential of traditionally used Indian medicinal plants using experimental animals. Previous studies confirmed the efficacy of several medicinal plants in diabetes mellitus. Although a large number of medicinal plants have been already tested for their antidiabetic effects, these effects remain to be investigated in several other Indian medicinal plants.[4]

Nerium indicum (Family: Apocynaceae) is a large evergreen shrub with milky juice. The leaves are mostly in whorls of three, sometimes two, linear-lanceolate, acuminate and coriaceous. This tree is popularly known as Karavira in Sanskrit, Indian oleander in English and Kanagale in Kannada. *Nerium indicum* found in the Himalayas from Nepal westwards to Kashmir up to 1,950 m. and in the upper Gangetic plain and Madhya Pradesh. It grows wild in many other states of India. *Nerium indicum* have been used in Ayurvedic medicine since the glycosides present in the plant is having paralyzing action on the heart, like digitalin, and a stimulating action on the spinal cord, like strychnine. [5] Previous studies have demonstrated that *Nerium indicum* is rich in cardio-active glycosides, formerly designated as neriodorin, neriodorein and karabin; the bark also contains scopoletin and scopolin. The alcoholic extract of the root bark showed the presence of α-amyrin, β-sitosterol; the ether and the chloroform fraction showed kaempferol and odoroside respectively.[6] Yassin MM *et al.* assessed the protective potential of glimepiride and *Nerium oleander* extract on lipid profile, body growth rate, and renal function in streptozotocin-induced diabetic rats.[7] Ishikawa Akiko *et al.* reported that hot-water extract of *Nerium indicum* leaves was found to reduce the postprandial rise in the blood glucose when maltose or sucrose was loaded in rats.[8] To our best knowledge no scientific data regarding the antidiabetic effect of *Nerium indicum* leaves are available except in the treatise of Ayurvedic medicine. Thus, the present study was undertaken to evaluate the antidiabetic effect of *Nerium indicum* leaves in alloxan induced diabetic rats.

MATERIALS AND METHODS

Animals

Adult Albino rats of wistar strain (150-200g) of either sex were procured from the Government Veterinary College, Bangalore, and housed in the animal house of KLES College of Pharmacy, Ankola with 12 hour light and 12 hour dark cycles. Standard pellets obtained from Goldmohar rat feed, Mumbai, India, were used as a basal diet during the experimental period. The control and experimental animals were provided food and drinking water ad libitum. All the experiments on animals were conducted according to the ethical norms approved by CPCSEA, Ministry of social justice and empowerment, Government of India and ethical clearance was granted by institutional ethical committee in resolution no. 1/18/2007 held on 23rd November 2007 at J N Medical college, Belgaum (Ethical committee IAEC reg. no.: 627/02/a/CPCSEA).

Chemicals

Alloxan monohydrate (Spectrochem Pvt. Ltd. Mumbai), Glibenclamide (Aventis Pharma Ltd, Verna, Goa), Dextrose (Emkay Labs, India), Tween 80 (S. D. Fine-chem limited, Mumbai), Anesthetic Ether (Ozone International, Mumbai) were used.

Accu-chek® Active Glucometer (Roche Diagnostic Corporation Germany), blood gluco-strips (Roche Diagnostic Pvt. Ltd. Mumbai) were also used.

All other chemicals and reagents used were of analytical grade.

Plant material

Leaves of *Nerium indicum* were collected in and around the local forest area of Sirsi in the Western Ghats, Karnataka, and authenticated by the Botanist Prof. G. S. Naik, Department of Botany, G. C. Science and Art College, Ankola. A voucher herbarium specimen number GCSAC/NI/01 was also preserved in the same college. The collected leaves were dried under shade and powdered to coarse consistency in a grinder mill. The powder passed through 40 # mesh particle size and stored in an airtight container at room temperature.

Preparation of plant extract

About 2.5 kg of the fresh air-dried, powered crude drug of *Nerium indicum* was extracted with petroleum ether ($60\text{-}80^0$), chloroform, 95% ethanol and chloroform water I. P. by adopting a simple maceration procedure at room temperature, for seven days, in a conical flask with occasional shaking and stirring. The extract was filtered and concentrated to dryness at room temperature to avoid the decomposition of the natural metabolites.[9] The yield of the extracts was 1.20%, 4.95%, 22.56% and 19.78% w/w

for pet. Ether, chloroform, ethanol and water respectively. All the extracts were preserved in a refrigerator till further use. Preliminary phytochemical analysis was carried out in all 4 extracts by different methods of phytochemical analysis.[10] A known volume of extract was suspended in distilled water and was orally administered to the animals by gastric intubation using a force feeding needle during the experimental period.

Acute oral toxicity studies[11]

The acute oral toxicity studies of extracts were carried out as per the OECD guidelines, draft guidelines 423 adopted on 17th December 2001 received from CPCSEA, Ministry of social justice and empowerment, Govt. of India. Administration of the stepwise doses of three extracts of *Nerium indicum* from 50 mg/kg b.w. up to the dose 5000 mg/kg b.w. caused no considerable signs of toxicity in the tested animals except alcoholic extract which show toxicity at this particular dose. The dose of alcoholic extract of *Nerium indicum* bark was reduced 3000 mg/kg and observed for three days. This particular dose was found to be safe and no toxicity was observed. One-tenth of the upper limit dose was selected as the level for examination of antidiabetic activity.

Experimental models

Oral glucose tolerance test (OGTT)[12]

Fasted rats were divided into groups of six. Group I served as normal control and received distilled water with Tween 80. Group II received the standard drug Glibenclamide as an aqueous suspension at a dose of 600µg/kg body weight. Group III received 300mg/kg and IV to VI received different extracts at a dose of 500mg/kg body weight as a fine tween 80 suspension. After 30 minutes of extract administration, the rats of all groups were orally treated with 2g/kg of glucose. Blood samples were collected from the rat tail vein just prior to glucose administration and at 30, 60 and 90 minutes after glucose loading. Blood glucose levels were measured immediately by using Gluco-meter.

Alloxan induced diabetic model[13]

Alloxan monohydrate was first weighed individually for each animal according to the weight and then solubilized with 0.2 ml saline just prior to injection. Diabetes was induced by injecting it at a dose of 150 mg/kg b.w. intraperitonially. After one hour of alloxan administration the animals were given feed ad libitm and 5% dextrose solution were also given in feeding bottle for a day to overcome the early hypoglycemic Phase. The animals were kept under observation and after 48 hours blood glucose was measured by a gluco-meter. The diabetic rats (glucose level > 300 mg/dl) were separated and divided into six different groups for experimental study; each group contained six animals.

Experimental design

The animals were divided in to seven groups and each group consisted of 6 rats.

1. Normal control (vehicle only)
2. Diabetic control (untreated rats)
3. Diabetic rats treated with Glibenclamide 600µg/kg
4. Diabetic rats treated with *Nerium indicum* aqueous extract 500mg/kg b.w (NIAE)
5. Diabetic rats treated with *Nerium indicum* ethanolic extract 300mg/kgb.w (NIEE)
6. Diabetic rats treated with *Nerium indicum* chloroform extract 500/kg b.w (NICE)
7. Diabetic rats treated with *Nerium indicum* pet. ether extract 500/kg b.w (NIPEE)

This experimental design was followed in both the alloxan induced model and OGGT model but in the latter model, normal rats loaded with glucose were used instead of diabetic rats.

Body weight measurement[14]

Body weight was measured four times during the course of the study period (i.e., on, before alloxan induction (initial values), days 1, 4, 7 of the treatment period), using a digital weighing scale obtained from KERN (EMB), Germany.

Statistical analysis

The results of the study were subjected to one-way analysis of variance followed by Dunnett's t-test for multiple comparisons. Values with $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Standardization parameters for *Nerium indicum* leaves were determined and all the parameters were found to be within pharmacopoeial standards limit. Crude powder taken for extraction was of green color with faint odor and slightly bitter taste. Loss on drying, total ash, acid insoluble ash, water soluble ash was found to be 8.02, 4.90, 3.14 and 0.89%w/w respectively. Thin layer chromatography of *Nerium indicum* leaves revealed 6 blue florescent spots at 254 and 366 nm. Phytochemical screening of all the extract of *Nerium indicum* showed the presence of various

phytochemical constituents like alkaloids, steroids, triterpenoids, carbohydrates and amino acids. In acute toxicity study, petroleum ether, chloroform and water extracts of *Nerium indicum* leaves at the tested dose level of 5000 mg/kg body weight and alcoholic extract at the tested dose level of 3000 mg/kg body weight. did not show significant toxicity signs when observed for the parameters during the first four hours and followed by daily observations for 14 days. No mortality was observed and the drug was found to be safe.

In order to ascertain a scientific base for the usefulness of this plant in the treatment of diabetes, it was decided to evaluate experimental design of antidiabetic activity by following glucose tolerance test and alloxan-induced model. As expected, in the diabetic control, there was severe hyperglycemia as compared to the normal animals. It was observed that the standard drug glibenclamide lowered the blood glucose level significantly bringing it nearly back to normal. The single dose of ethanolic extract (300 mg/kg b.w.) of *Nerium indicum* has more significantly ($P<0.01$) reduced the blood glucose level as compare to diabetic control at 7th day of the study. Chloroform extract (500 mg/kg b.w shown significant reduction of blood glucose after one hour whereas ethanolic extract showed significant reduction at three hours [Table 1]. Aqueous extract of the same plant could not reduce glucose level at sub acute level, though it did showed reduction of glucose level at seven hours as compared to diabetic control [Figure 1].

The effects of different extracts on glucose tolerance test in normal rats were evaluated. At 30 min after glucose administration the peak of blood glucose level increased rapidly from the fasting value and then subsequently decreased. Glibenclamide treated group (600µg/kg) prevented glucose induced hyperglycemia significantly

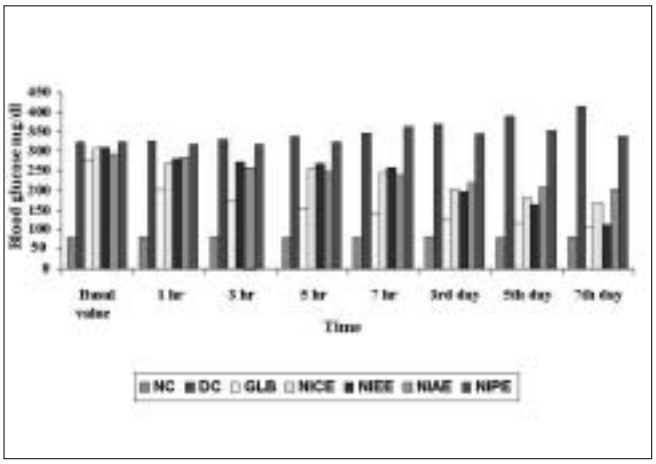

Figure 1: Blood glucose level of alloxan induced diabetic albino rats after treatment with *Nerium indicum* leaves extracts

Table 1: Effect of Nerium indicum extracts on blood glucose level of alloxan induced diabetic albino rats after sub-acute treatment

Gr No.	Treatmenta	Blood glucose level mg/dl						
		Basal value(0hr)	1 hr	3 hr	5 hr	3rd day	5th day	7th day
I	Normal control (vehicle only)	80.00 ±1.693	80.83 ±1.721	80.83±1.424	79.83±1.376	81.33±0.988	79.83±0.833	81.16±0.7923
II	Diabetic control	322.33 ±7.775	327.50±7.945	329.50±7.388	336.67±6.515	369.00±6.110	388.33±16.591	413.50±4.752
III	Glibenclamide 600µg/kg	277.33 ±7.923	206.66±6.280**	174±7.095**	154.83±5.043**	125.33±6.960**	114.33±5.251**	105.66±5.097**
IV	Ethanolic extract 300mg (NIEE)	307.66 ±5.737	267.66±3.201**	260.5±4.129**	256.66±4.759**	203±12.946**	182.5±10.686**	169.33±9.735**
V	Chloroform extract 500 mg (NICE)	309.66 ±8.511	282.33±7.932*	273.5±8.160**	268±5.285**	196.33±3.547**	163±9.585**	113.33±6.662**
VI	Aqueous extract 500mg (NIAE)	293.50 ±12.790	283.66±12.529ns	266±12.022ns	248.5±13.178ns	219.83±12.413**	209.5±10.754**	201.5±8.098**
VII	Pet. ether extract 500 mg (NIPE)	324.16 ±12.411	317.5±17.500ns	316.66±13.386ns	324.5 ±16.211ns	342.66±20.537ns	351.5±32.212ns	337.83±25.515ns

amg/kg/day for 7 days. Values are means±SEM; N=6. Values are statistically significant at *P<0.05 and more significant at **P<0.01. ns= not significant, **P<0.01vs diabetic control. (ANOVA).

Table 2: Effect of Nerium indicum extracts on oral glucose tolerance test in normal rats

Sample	0 min	30 min	90 min
Normal Control (5% Tween 80 + glucose (2g /kg)	78.83±0.703	167.83±2.301	146.83±2.960
Giibenclamide (600µg/kg) + glucose (2g /kg)	76.50±1.522	171.83±4.214**	106.16±4.316**
NIAE (500mg/kg) + glucose (2g /kg)	75.50±2.986	177.16±3.439**	155.33±3.018**
NIEE (300mg/kg) + glucose (2g /kg)	75.16±2.738	174.16±3.380**	128.00±5.266**
NICE (500mg/kg) + glucose(2g /kg)	81.33±1.874	164.33±5.661**	121.00±2.966**
NIPEE (500mg/kg) + glucose(2g /kg)	83.83±2.548	165.16±4.301**	150.83±5.338**

One-way ANOVA followed by Dunnett's test. Values are expressed as mean±SEM. **P<0.01as compared to normal control group.

Table 3: Effect of various extracts on body weight after treatment in diabetic rats

Groups	Treatment (n=6)	Average body weight (g) ±SEM	
		Initial value	Day 7
1	Normal control (NC) (vehicle only)	166.33 ±2.974	182.3 ±2.525
2	Glibenclamide 600µg/kg (GLB)	183.5 ±2.078	205.33 ±1.65
3	Diabetic control(DC)	147.5 ±2.952	110.3 ±2.37
4	Aqueous extract 500mg (NIAE)	144.3 ±2.124	128.0 ±2.98
5	Ethanolic extract 300mg (NIEE)	147.8 ±2.915	163.3 ±3.48**
6	Chloroform extract 500mg (NICE)	143.5 ±2.964	160.7 ±2.108**
7	Pet. ether extract 500 mg (NIPE)	144.0 ±1.528	156.8 ±0.87

One-way ANOVA followed by Dunnett's test. Values are expressed as mean±SEM. **P<0.01as compared to normal control group; n= number of animals

at 30 min and 90min (171.83±4.214 and 106.16±4.316) as compare to normal control (167.83±2.301 and 146.83±2.960) respectively. Maximum glucose tolerance in *Nerium indicum* extracts was observed in chloroform extract (121.00 ±2.966) and minimum glucose tolerance was observed in aqueous extract (155.33 ±3.018) in 90 minutes compared with the normal control [Table 2].

In the present study, diabetic rats had lower body weights, high blood glucose level as compared to normal rats. However, orally administered NIEE and NICE significantly increased the body weight [Table 3] and decreased blood glucose level in diabetic rats. This may be due to improving the glycemic control mechanisms and insulin secretions from remnant pancreatic - cells in diabetic rats. The exact biologically active constitutents responsible for the said effect have not been reported nor was the exact mode of action of the antidiabetic activity reported earlier, with the lone observation that it is used in folklore diabetic treatments.

CONCLUSION

We conclude that the NICE and NIEE have potent antidiabetic effects in alloxan-induced diabetic rats. The present investigation has also opened avenues for further research especially with reference to the development of potent formulation for diabetes mellitus from *Nerium indicum* leaves. Activity guided fractionation, formulation and its evaluation is in progress and will be available in a short period of time.

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