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Antidiabetic and regenerative effects of alcoholic corm extract of *Nervilia aragoana* gaud. in streptozotocin-nicotinamide induced NIDDM rats

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Abstract

Nervilia aragoana Gaud. (Orchidaceae) has long been used in the antidiabetic medicinal preparations of traditional healers of Wayanad (Kerala), but antidiabetic and regenerative potential of the plant remain unravelled. The aim of the present study was to investigate the recuperative and regenerative potential of alcoholic stem extract of *Nervilia aragoana* Gaud. on streptozotocin-nicotinamide induced type 2 diabetic models. Administration of 5mg/kg of plant extract, blood glucose levels of the NIDDM rats showed 65.91 and 76.58 % decrease in the blood glucose levels on 0 and day 30 days respectively. Damages caused to the kidney tissue were negligible or not seen. Serum urea and creatinine levels showed 65.00 % and 71.00% decrease on day 30. LPP levels of kidney and pancreas showed 76.47 % and 74.19% decrease respectively. These results demonstrate significant antidiabetic and regenerative potential of the *Nervilia aragoana* Gaud. justifying the use of plant in the indigenous system of medicine. Isolation and characterisation of the compound(s) playing pivotal role in the cure would open new vistas in the therapy of type 2 diabetes.

Keywords: Nervilia aragoana Gaud; Wayanad district; Streptozotocin-nicotinamide induced type 2 diabetes; Antidiabetic activity; Regeneration of kidney tissue.

Introduction

Nervilia aragoana Gaud. (Orchidaceae) commonly known as Orilathamara (Lotus with single leaf) in Malayalam (Regional language) is widely distributed in sand rich forest soils of Western Ghats. The plant produces spherical underground corm, leaves appear on flowering. Leaves are orbicular to sub reniform. Flowers purple, dried tuber yield white powder when pound, which is used in combination with local jin or coconut milk for medicinal application. Ancient texts describe this plant as one which reduces diabetes and cure kidney related diseases. The drug is reportedly sweet, cool, increases breast milk, uses as vitaliser, cures anaemia and kidney diseases [1]. According to Bhavaprakasha nighantu the drug is cool, astringent, rejuvenator and cures diabetes, pitta (Anaemia), mental diseases, epilepsy, hemiplegia, diarrhoea, asthma, and vomiting. Satavari Ghrtam is the preparations using this medicinal herb. This does not find mention in the Samhitas (Texts) of Charaka and Susrutha, but is a constituent drug of Rasayana (Ayurvedic preparation) of Vagbhata [2]. Considering the wide use of this herb in folk therapeutics for the treatment of diabetes, present study was conducted to investigate the anti diabetic activity of Nervilia aragoana Gaud.in streptozotocin-nicotinamide induced NIDDM rats.

Materials and Methods

Plant material

The corm of *Nervilia aragoana* Gaud. Was obtained from a medicine vendor, Kerala (India), identified at the University referral herbarium, Department of Studies in Botany, University of Mysore. A voucher specimen (O-102) has been deposited at the department Herbarium.

Preparation of alcoholic extract

Dried under ground corm (300g) was powdered in a fire proof blender and extracted with ethanol in a separating funnel for 48 hours. The separated extract was concentrated in a vacuum evaporator and lyophilized. The residue (30g.) was stored in a dessicator and was used for the experiments.

Animal groups

Healthy obese albino rats aged between 2-3 months, weigh 250-300g were used for the pharmacological studies. The animals were housed in polypropylene cages, maintained under standard conditions (12/12 hrs. light and dark cycles) at 25±3ºC and 35-60%

RH, fed with standard rat pellet diet and water *ad libitum*. The Institutional Animal Ethical Committee (No.122-1999/CPCSEA), Department of Studies in Zoology, University of Mysore, Karnataka, India approved the studies.

Acute toxicity studies

Healthy adult Wistar albino rats of both sex, starved overnight were divided in to five groups (n=6) and were orally fed with the alcoholic extract in the increasing dose of 2, 5, 10, 20 and 40 mg/kg body weight (BW)[3]. The rats were observed continuously for behavioural, neurological and autonomic profiles and any lethality up to 72 hrs. [4].

Induction of non insulin dependent diabetes mellitus (NIDDM)

NIDDM was induced in overnight fasted animals by an IMI of 50mg/kg STZ (Sigma Aldrich, Germany) and thereafter 120mg/kg nicotinamide after 5 min. Hyperglycaemia was confirmed by the elevated blood glucose level determined on day 0 and day 10 after injection. The rats found with permanent NIDDM were used for antidiabetic study [5].

Experimental design

Animals were divided in to two groups, diabetic and the normal. Diabetic animals were designated as untreated diabetic (control), diabetic treated with alcoholic corm extract (10mg/kg) and the diabetic treated with glibenclamide (10mg/kg) is a known hypoglycaemic agent. Hypoglycaemic potential of the compound was studied estimating the blood glucose levels during treatment days (10 days) and post treatment days (20 days) at regular intervals of 5 days [6].

Each experimental animal group was orally administered daily during first 10 days (treatment days), the medicinal extract by means of a catheter under mild ether anaesthesia. The animals were sacrificed on day 30 by decapitation, blood samples were taken by cardiac puncture, sera were separated for urea and creatinine analysis. Kidney tissues were taken for histological evaluation and lipid peroxidation product (LPP) determination. Serum urea and creatinine levels were determined by diacetylmonooxime method and Jaffe reaction [7, 8] respectively.

For histological observations, tissues of the kidney were fixed in Bouins's solution and subsequently embedded in paraffin. Sections of 5 µm thickness were taken and stained with Masson's tri-dye and Periodic Acid Schiff (PAS). The sections were microscopically examined for histological parameters [9].

LPP was determined as thiobarbituric acid reactive substance levels [10]. Kidney and the pancreas tissues were washed with saline and kept frozen until the day of the experiment, homogenised in saline (1/10/w/v) using ultrasonic homogeniser. The homogenates were centrifuged in a refrigerated centrifuge (4000 rpm/10min.) at 4º C. The clear supernatant was used for LPP analysis.

Statistical analysis

Data were analysed statistically by applying one way ANOVA, followed by Schiff's post hoc test using 7.5 version of SPSS computer software. The values were considered significant when $p<0.05$.

Results and Discussion

At any selected dose no lethality or toxic reactions were observed up to the end of the study. On administration of alcoholic corm extract and glibenclamide, blood glucose levels of the NIDDM rats showed a significant reduction from day 0 onwards. Manifestations of the drug were found incessant in the animal body till the end of post treatment days. STZ-nicotinamide induced NIDDM rats administered with alcoholic extract (10mg/kg) showed a 65.91 and 76.58 % decrease in blood glucose level on 0 day of the treatment day and day 30 of the post treatment days respectively in contrast with diabetic control (Table 1).

Alcoholic corm extract has revived structural integrity of the tubular epithelium in kidney tissues. The damage to the kidney tissue was routine in the diabetic animals administered glibenclamide, while tissue damage was negligible in the diabetic animals administered with alcoholic corm extract. Serum urea and creatinine levels, kidney and pancreas LPP were significantly reduced NIDDM rats administered with alcoholic corm extract in comparison with diabetic control (Table.2). The changes were statistically significant at $p < 0.05$.

NIDDM rats given alcoholic corm extract (10mg/kg) showed 65.00 % decrease in the serum urea levels on day 30 of the post treatment day when compared to diabetic control. Serum creatinine levels of the NIDDM rats were decreased to normal level by day 30 of the post treatment days. The decline was 71.00%, when compared to diabetic control (Table 2).

LPP levels were higher in rats treated with glibenclamide as observed by Sehnaz et al., 2003. NIDDM rats administered with alcoholic corm extract (10 mg/kg) showed a 76.47 % decline in the kidney LPP levels on day 30 of the post treatment days (Table 2). Where as administration of same dose of alcoholic stem extract causes a 74.19% decrease in the pancreatic LPP levels when compared to diabetic control on day 30 of the post treatment days (Table 2).

Present investigation provides evidence that, the alcoholic corm extract and the glibenclamide has lowered the high blood glucose level in diabetic animal to the normal level from day 0 onwards. The effect of the drug was retained in the animal till last day of the post treatment days, designate longer tolerance of the medicine in the biological system with no toxic effects.

Table 1:Effect of plant extract (10 mg/kg) on blood glucose level in NIDDM rats during treatment and post treatment.

Group	Treatment	Blood glucose concentration (mg/dl)				
		Treatment days		Post treatment days		
		Day-0	Day-10	Day-15	Day-25	Day-30
1	Normal Control	87.00±03.84a	86.33±04.17 a	94.83±06.67 a	87.16±05.34 a	87.16±03.81a
2	Diabetic Control	264.50±60.08b	312.16±82.39b	367.83±90.87b	382.66±62.0b	428.83±62.24b
3	Plant extract (10 mg/kg)	80.50±21.01a(65.91)	86.16±7.08 a(72.43)	78.66±5.39a (78.06)	78.66±6.97 a (79.44)	100.00±4.64 a (76.52)
4	Glibenclamide(10mg/kg)	105.16±03.97 a (60.24)	100.33±06.83a(67.85)	90.00±05.65a(75.53)	99.00±05.65a(74.12)	110.50±12.11a(74.23)

Each value represent mean± S.D., n=6.
Mean values with different superscripts are significantly different from each other as revealed by Schiff's posthoc test ($p<0.05$).
Values given in parenthesis represent percent decline in the blood glucose level in comparison with diabetic control.

Table 2:Effect of plant extract (10mg/kg) on levels of serum urea, creatinine and the TBARS in NIDDM rats.

		Serum		TBARS (m M/mg)	
Group	Treatment	Urea (mg %)	Creatinine (mg %)	Kidney	Pancreas
1	Normal Control	31.36±5.07 ^{a}	0.58±7.52 ^{a}	0.11±1.78 ^{a}	0.09±1.89 ^{a}
2	Diabetic Control	99.11±11.62^{d}	2.00±0.10 ^{c}	0.34±3.14 ^{c}	0.31±1.97 ^{c}
3	Plant extract (10 mg/kg)	34.68±2.74$^{a(65.00)}$	0.58±9.83 $^{a(71.00)}$	0.08±1.72 $^{a(76.47)}$	0.09 ±1.94^{a} $^{(74.19)}$
4	Glibenclamide (10 mg/kg)	70.38±9.51$^{c(28.98)}$	1.08±0.21$^{b(46.00)}$	0.26±5.35 $^{b(23.52)}$	0.24±5.30 $^{b(22.58)}$

Each value represent mean± S.D., n=6.
Mean values with different superscripts are significantly different from each other as revealed by Schiff's posthoc test $p<0.05$).
Values given in parenthesis represent percent decline in the serum urea, serum creatinine and the LPP in comparison with diabetic control.

Histology of NIDDM rat kidney showed narrowing of proximal tubules, picnotic nuclei at the brush border due to rupture and loss of structural integrity of the membrane. These anomalies cause functional disorders in the membrane dependant functions. Increased PAS (Periodic acid Schiff) positive reaction in the glomeruli may be an indication of thickened basement membrane associated with glomerular capillaries. Increased levels of urea and creatinine in the serum and high LPP levels in the kidney are indications of renal insufficiency. In the present study, morphological findings in the NIDDM rats were in agreement with the results of other studies [11].

Alcoholic corm extract (10mg/kg) has enhanced structure and function of kidney affected NIDDM. Regeneration of epithelium, expansion of glomeruli, disappearance of haemorrhages and cytoplasmic debris, decrease in amount of serum urea and creatinine were the changes observed besides lowering blood glucose. There were no histological regeneration and decrease in levels of serum urea and creatinine in NIDDM rats administered glibenclamide. Hence glibenclamide is hypoglycaemic, but no effect on damages caused to kidney because of NIDDM.

LPP levels were high in STZ- nicotinamide induced NIDDM rat kidney [12]. LPP values of kidney and pancreas was decreased significantly in rats administered with alcoholic corm extract compared to diabetic controls, whereas it was more in group treated with hypoglycaemic drug glibenclamide.

Conclusion

In summary, this study reports for the first time that *Nervilia aragoana* has anti NIDDM potential besides improving the structural integrity of the kidney affected NIDDM. The results presented in this study suggest the presence of one or more anti NIDDM constituent (s) in alcoholic corm extract of the plant. These bioactive compounds attributed to normalise the blood glucose, also cause regeneration of the kidney and pancreas. The results hence suggest that bioactive constituents responsible for improving the physiology of kidney in NIDDM rats need to be isolated and structurally elucidated to contribute immensely in the therapy of NIDDM.

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