Original article

Antidiabetic effect of *Neopicrorrhiza scrophulariiflora* on type 2 diabetic model rats

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Abstract

Introduction: *Neopicrorrhiza srophulariiflora* (NS), which is locally known as "kutki / katuki" in Nepali is available at 3500-4800 m, east to west Himalayan region of Nepal. It is being used as indigenous antidiabetic plant in Nepal by the local people in the treatment of diabetes. The present study was carried out to evaluate the antidiabetic property of NS in streptozotocin (STZ) induced type 2 diabetic model rats.

Methods: NS dried rhizomes, collected from mountainous region of Nepal, was extracted with 80% ethanol and water by cold percolation method. The extracts were administered at a dose of 1.25gkg^{-1} body weight for 21 consecutive days to type 2 diabetic male Long-Evans rats, bred at BIRDEM animal house. Type 2 diabetes was induced by a single intraperitoneal injection of STZ to 48 hour old pups (Long Evans) from BIRDEM Animal house. Serum glucose was estimated by GOD POP method, on 0 day and by decapitation on 21st day by tail tip method.

Results: Ethanol extract of *N. srophulariiflora* significantly ($p<0.05$) improved oral glucose tolerance in type 2 rats in comparison to control group at the end of the study period. It was also found that the water extract and ethanol extracts significantly lowered serum glucose level of type 2 diabetic rats in both prandial states (simultaneously with oral glucose load $p<0.05$; at 75min and 30 minutes prior to oral glucose load $p<0.05$; at 105min) compared to water fed control group. Administration of Glibenclamide (5 mgkg^{-1}) also produced significant reduction ($p<0.01$) in serum glucose concentration in type 2 diabetic rats.

Conclusions: *N. srophulariiflora* is beneficial for treating Type 2 diabetes and therefore merits further exploration and researches both chemically and biologically to identify the active principle(s) and mechanism of action.

Key words: Anti-hyperglycemic, hypoglycemic, *Neopicrorrhiza srophulariiflora.*

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. It is the most common endocrine disorder affecting mankind all over the world, prevalence of which is increasing day by day.[1] Type 2 Diabetes Mellitus is more prevalent and account for about 90% to 95% of all diagnosed cases of diabetes. By 2030, it is estimated that the number of people with diabetes >64 years of age will be >82 million in developing countries and >48 million in developed countries.[2] In case of Nepal too, S.Haruka et.al study showed a surprisingly rapid increase in the prevalence of diabetes in the Nepalese population. The study found 9.1% in urban areas and 1.3% in rural areas. This phenomena appears to have been influenced more by rapid urbanization and changes in lifestyles after the ongoing democratic movements that have taken place

since 1990 in Nepal.[3]

Considering the limitations of existing therapies in restoring the quality of life to normal as well as reducing the risk of chronic diabetic complications, search for alternating sources is a requirement. Traditional preparations from plant sources are widely used almost everywhere in the world to treat diabetes and plant materials are considered to be the alternative sources for finding out new leads as hypoglycemic agents.[4]

Neopicrorrhiza srophulariiflora (Family: Scrophulariaceae), which is popularly known as "kutki" in nepali, east to west Himalayan region (3500-4800 m) of Nepal. It is a prostrate herb with perennial woody rhizomes covered with old leaves at the base.[5] It constitutes kutkin, a bitter glycosidal principle, D-mannitol, vanillic acid and some steroids are reported. Kutkin was shown to be a stable mixed crystal of two C-9 iridoid glycosides-Picroside I and Kutakosid. Apocynin has been isolated from the plant. Picroside II has been isolated and shown to have hepatoprotective activity.[6] It promotes secretion of bile, improves appetite and stimulates gastric secretion.[6] In traditional medicine it has been used to treat liver, dropsy, antiperiodic fever, anemia jaundice and bronchial problems.[7] It is being used as endogenous antidiabetic plant in Nepal by the local people in the treatment of diabetes.

Fig. 1: *Neopicrorrhiza srophulariiflora* plant and rhizomes

In this present study, our objective was to screen the possible hypoglycemic and anti-hyperglycemic activity of *N.srophulariiflora rhizomes* in type 2 diabetic model rats following a standardized approach.

Methods

It is a prospective, case control study, conducted in the Biomedical Research Group (BMRG), Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), Dhaka, during the period of Dec 2009 to Jan 2010.

The rhizomes of *N. srophulariiflora* were collected from local market of Kathmandu, Nepal. The powdered N. srophulariiflora rhizomes (2000gms) were extracted with 80% ethanol for 3 consecutive days by changing solvent after every 24hrs and then filtered. Similarly, the water extract was also prepared using the same method. The collected ethanol extract and water extracts were evaporated to dryness at reduced pressure using a rotary vacuum evaporator. The extract was further dried in a freeze drier at (-55)^{o}C temperature and stored in a reagent bottle at (2-8)^{o}C in a freezer. The total amount of ethanol extract was found to be 76.55gms and water extract 57.32 gms, utilized for biological screening at BIRDEM.

Adult male Long-Evans rats, weighing (180-250) gms were used throughout the study. The animals were bred at BIRDEM Animal house, Dhaka, Bangladesh maintained at ambient room temperature of 22±5^{0}C with humidity of 40-70 % and the natural 12 hours day-night cycle, fed with pellet diet and water ad libium.

Type 2 diabetes was induced by intraperitoneal injection of streptozotocin (STZ) at a dose of 90 mg/kg body weight/10 ml, pH 4.5 citrate buffer (0.1 M)) to the 48 hours old rat pups as described by Bonner *et.al*[8] and experiments were performed after 3 months of STZ injection. Before starting the experiments, rats were checked whether they have developed type2 diabetes. For this purpose their fasting and 30 min after (an oral glucose load) blood glucose levels will be determined. Rats with fasting value ranging from (7.5-10mmol/l) and 30 min after value more than 15mmol/l and above will be used for experiment.

The chronic experiment was carried out for duration of 21days on a total of 16 rats. These rats were divided into 3 groups, they are as follows:

- Normal Water Control group (n=4) : This group was fed with deionized water at a dose of 10 ml/kg body weight.
- NIDDM Glibenclamide positive control group (n=4) : This group was fed with glibenclamide at a dose of 5 mg/10 ml (9.9 ml H_2O + 0.1 ml Twin 20)/kg body weight[1].
- NIDDM Treated group (n=10): This group was fed with 80% ethanolic extract of Picrorrhiza at a dose of 1.25 g/ 10 ml/ kg body weight.[9]

After the treatment, they were finally sacrificed on the 21st day and the antihyperglycemic/ hypoglycemic properties were deduced from the blood glucose level on the 0th and 21st day's.

The acute effects of the on hypoglycemic activity of 80% Ethanol and water extract of *Neopicrorrhiza Scrophulariiflora* rhizomes, were observed in two different

prandial states as described below-

Acute effect on serum glucose level when fed simultaneously with glucose: The extracts (1.25 g/kg bw) were fed with glucose (2.5g / 10 ml / kg BW) to overnight fasted rats at 0 minute and blood samples were drawn at 0, 30, 75 minutes. Both positive control and water control rats were fed with glucose solution at a dose of 2.5g / 10 ml / kg bw.[9]

Acute effect on serum glucose level when fed 30 minute before glucose load: The extracts (1.25 g/kg bw) were fed to overnight fasting (12 hrs) rats at 0 minute and glucose load (2.5g / 10 ml / kg BW) were given at 30 minutes. Blood samples were drawn at 0, 60, 105 minutes. The control group received water (10 ml/kg bw) following glucose load of 2.5g / 10 ml / kg bw.[9]

Biological Test: The freeze dried extract (1.25 mg/kg bwt dissolved in deionized water in addition with 250 ml of Tween 20) was fed to type 2 diabetic rats in fasting and postprandial states (simultaneously with glucose and 30 min before glucose load) by a metallic tube under mild ether anesthesia.[10] The control rats were given equal volume of distilled water and positive control rats were given Glibenclamide (5mg/kg bwt). Blood samples were collected by amputation of tail tip at 0, 30 and 75 min for simultaneous feeding of extract with glucose and at 0, 60 and 105 min when the extract was fed 30 min before glucose load.

Serum glucose was estimated on the same day by GOD-PAP method (Boehringer-Mannhein GmbH) using Auto Analyzer.[11]

Data were analyzed using the SPSS software for windows version 12 (SPSS Inc., Chicago, Illinois, USA). All the data were expressed as Mean ± standard deviation. Statistical analysis of the results had been analyzed by using the student's t-test (paired and unpaired), ANOVA (analysis of variance) to ensure an overall error rate of 5%. Differences were considered significant at $p<0.05$.

Results

STZ injection to adult rats (for simulation of type 2 diabetes), characterized by hyperglycemia, fasting blood glucose slightly higher (7.20 -8.44 mmol/L) on the 7th day, indicating the presence of functioning ??cells. It is seen from the Table 1 that the changes of the body weight of rats were not remarkable between different treated groups during treatment period.

Table 1: Chronic effect of N. *scrophulariflora* rhizome extract on body weight (gm) of Type 2 diabetic model rats

Treatment	Experimental period (day)			
	BW_0 (gm)	BW_7 (gm)	BW_14 (gm)	BW_21 (gm)
WC (n = 6)	218±25	218±24	221±17	220±12
Gliben (n = 6)	223±17	218±14	219±14	211±80
Picrro_et(n = 8)	221±33	208±22	214±19	216±18

Data are presented as Mean±SD WC= Water control, Gliben= Glibenclamide, *Picrro_et= Picrrorhiza* ethanol extract. Compared using paired 't' test. Between group comparison was done using one way ANOVA *p< 0.01, ** p< 0.001. n= number of rats

Regarding the acute effects of *N. scrophulariflora* rhizome ethanol and water extracts on serum glucose level of type 2 diabetic model rats when fed simultaneously with glucose load, the control drug glibenclamide showed significant hypoglycemic effects at 75 min. Similarly, the *P. scrophulariflora* rhizome ethanol and water extracts also showed significant glucose lowering effect (p< 0.01 - 0.05) at 30 min and 75 min.

Table 2: Acute effects of *N. scrophulariflora* rhizome ethanol and water extracts on serum glucose level of type 2 diabetic model rats when fed simultaneously with glucose load

Group	Glu_0 Min (mMol/l)	Glu_30Min (mMol/l)	Glu_75 Min (mMol/l)
WC (n = 6)	7.31±0.60	17.04±4.80	17.61±4.08
Gliben (n = 6)	7.95±1.14	17.86±3.50	13.98±4.67*
Picrro_et (n = 8)	7.80±0.85	15.92±2.60*	14.40±4.16*
Picrro_wt(n = 6)	8.11±1.26	13.85±2.33*	12.58±3.01*

Data are presented as Mean±SD WC= Water control, Gliben= Glibenclamide, *Picrro_et= Picrrorhiza* ethanol extract *Picrro_wt= Picrrorhiza* water extract ANOVA (Bonferroni test) was done as the test of significance. *p< 0.01 - 0.05

Table 3: Acute effect of *Picrrorhiza scrophulariflora* rhizome extract on serum glucose levels of type 2 rats when the extract was fed 30 minutes before to glucose load.

Group	Glu_0 Min (mMol/l)	Glu_60 Min (mMol/l)	Glu_105 Min (mMol/l)
WC (n = 6)	8.44±0.88	17.65±6.03	20.22±5.45
Gliben (n = 6)	7.41±1.18	14.73±7.61*	14.28±5.20*
Picrro_et(n= 8)	7.21±0.98	16.90±6.00	14.50±5.12*

Data are presented as Mean±SD WC= Water control, Gliben= Glibenclamide, *Picrro_et*= *Picrrorhiza* ethanol extract ANOVA (Bonferroni test) was done as the test of significance. *p< 0.01 - 0.05

When the extract was fed 30 minutes before the glucose load, the effect of 80% ethanol extract on serum glucose levels clearly demonstrated that the plant has significant glucose lowering effect in the type 2 model rats (Table 3). Glibenclamide as a standard drug for Type 2 diabetes significantly lowered serum glucose levels at both time points i.e. at 60 minutes and at 105 minutes (p< 0.01 - 0.05). And the 80% ethanol extract of *P. scrophulariflora* also lowered at 105 min (p< 0.01 - 0.05).

Table 4 illustrates the level of blood glucose in the control and experimental group of rats on 0 day and 21st day. As it is seen at baseline the mean (±SD) fasting serum glucose (mmol/l) the Type 2 Water control, comparing with the Glibenclamide and 80% ethanol extract of *N. scrophulariflora* treated groups were almost similar, i.e. 6.58±1.50, 5.86±1.05, and 5.95±0.79 respectively. when compared between groups, Type 2 rats fed with ethanol extract of *N. scrophulariflora* showed a significant decrease while comparing within groups (p<0.01). As expected, glibenclamide also ameliorated the diabetic condition on 21st day.

Table 4. Chronic effect of *N. scrophulariflora* rhizome extract on fasting glucose level of type 2 diabetic model rats

Groups	Mean ± SD	
	Glu_0 day (mMol/l)	Glu_21st day (mMol/l)
WC(n = 6)	7.20±0.66	6.58±1.50
Gliben (n = 6)	7.44±1.17	5.86±1.05
Picrro_et (n= 8)	7.89±0.86	5.95±0.79*

Data are presented as Mean±SD WC= Water control, Gliben= Glibenclamide, *Picrro_et*= *Picrrorhiza* ethanol extract. Compared using paired 't' test. Between group comparison was done using one way ANOVA with post Hoc Bonferroni test. *p< 0.01. n= number of rats

Discussion

The present study was undertaken to screen the hypoglycemic and anti-hyperglycemic activity and to investigate the underlying mechanism of action of *N. scrophulariflora* rhizomes 80% ethanolic extract and water extracts, in type 2 diabetic model rats. Two different experimental approach has been followed ie. acute and chronic administration, which gives an approximate idea about the mechanism of action of the plant by analyzing the model, prandial states and timing of hypoglycemic effect activity.

Type 2 diabetes was induced using streptozotocin to neonates rats described by Boiner et al. Strptozotocin-induced diabetes has been described as an experimental model to evaluate the activity of antidiabetic agents.[12] Glibenclamide was used as a standard drug, a long-acting Sulfonylurea, act mainly by augmenting insulin secretion. Administration of Glibenclamide to Type 2 rats almost normalizes serum levels of glucose.

These results demonstrates, that both ethanolic extract (80%) and water extract of *N. srophulariiflora* in type 2 diabetic rats showed significant hypo-glycemic effect (p<0.01-0.05) in type 2 model rats, when the extracts were fed simultaneously with oral glucose load. Single oral administration of a dose of 250mg /kg body weight produces a potent and strong hypoglycemic effect in type 2 rats. Hypoglycemic activity that is found when given with a simultaneous glucose load in diabetic rats indicates that the extracts may interfere with the intestinal glucose absorption in the gut by various mechanisms.[13]

The extracts showed significant hypoglycemic effect in both prandial states (simultaneously with oral glucose load p<0.01-0.05; at 75min and 30 minutes, and prior to oral glucose load p<0.01-0.05; at 105min duration) (Table 2 and 3). Reduction in glucose level was greater than Glibenclamide treated group. It indicates that the plant rhizomes might contain some hypoglycemic principle(s) which probably act by initiating the release of insulin from pancreatic b-cells.[14] The hypoglycemic activity of the extract after 21 days of consecutive feeding, can postulate that the extract may act by reducing glycogenolysis in liver which reflects in reducing the blood glucose level.[15] Post pandrial reduction in glucose level by the *N. srophulariiflora* suggests that it may also interfere with intestinal glucose absorption or stimulation of glycogenesis (enhanced by feeding). The water extract was also effective in Type 2 model rats when fed 30 min prior to glucose load. This effect seems to be due to a systemic action, i.e. as a result of the stimulation of b-cells and improving the insulin-secretory capacity or enhancement of insulin action by the extract.

Thus the accumulating evidences suggests that both pancreatic and extra pancreatic mechanisms might be involved in *N. srophulariiflora* anti-diabetic or antihyperglycemic action, which can be beneficial for the

treatment of diabetes.

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