

PHARMACOLOGICAL STUDIES OF NEOPICRORRHIZA SCROPHULARIIFLORA AND ITS ANTIDIABETIC EFFECT

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Abstract

Neopicrorrhiza srophulariiflora (NS), locally known as “kutki / katuki” in nepali is available in 3500-4800 m of Nepal. The present study was carried out to evaluate the antidiabetic property of NS in streptozotocin (STZ) induced type 2 diabetic model rats. NS dried rhizomes, 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. Serum glucose was estimated by GOD PAP method. Ethanol extract of *N. srophulariiflora* significantly (p<0.05) improved oral glucose tolerance in type 2 rats in comparison to control group. 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 control group. *N. srophulariiflora* is beneficial for treating Type 2 diabetes and therefore needs 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.

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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, a study found 9.1% in urban areas and 1.3% in rural areas.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.⁴

Neopicrorrhiza srophulariiflora (Family: *Scrophulariaceae*), 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 contains C-9 iridoid glycosides-Picroside I and Kutakosid, Apocynin. 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



Fig. 1: *Neopicrorrhiza srophulariiflora* plant and rhizomes

METHODS

Preparation of the extract: The powdered *N. srophulariiflora* rhizomes (2000gms) were extracted with 80% ethanol by cold percolation method. Similarly, the water extract was also prepared using the same method.

Animal models: Adult male Long-Evans rats, weighing (180-250) gms were used throughout the study, maintained at ambient room temperature.

Preparation of Type 2 diabetes (NIDDM) Model rats: 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

Chronic experiment

The chronic experiment was carried out for duration of 21 days 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 (dose 10 ml/kg bw).
- NIDDM Glibenclamide positive control group (n=4): This group was fed with glibenclamide (dose of 5 mg/kg bw¹).
- NIDDM Treated group (n=10): This group was fed with 80% ethanolic extract of *Picrorrhiza* (dose of 1.25 g/ kg bw).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.

Acute Experiment

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

Biochemical Procedures: Serum glucose was estimated on the same day by GOD-PAP method.¹¹

Data and Statistical Analysis: Data were analyzed using the SPSS version 12. All the data were expressed as Mean \pm 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

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
<i>Gliben</i> (n = 6)	223±17	218±14	219±14	211±80
<i>Picirro_et</i> (n = 8)	221±33	208±22	214±19	216±18

Data are presented as Mean±SD WC= Water control, *Gliben*= Glibenclamide, *Picirro_et*= *Picrorrhiza* 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 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
<i>Gliben</i> (n = 6)	7.95±1.14	17.86±3.50	13.98±4.67*
<i>Picirro_et</i> (n = 8)	7.80±0.85	15.92±2.60*	14.40±4.16*
<i>Picirro_wt</i> (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, *Picirro_et*= *Picrorrhiza* ethanol extract *Picirro_wt*= *Picrorrhiza* water extract ANOVA (Bonferroni test) was done as the test of significance. *p< 0.01 - 0.05

Table 3: Acute effect of *Picrorrhiza 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
<i>Gliben</i> (n = 6)	7.41±1.18	14.73±7.61*	14.28±5.20*
<i>Picirro_et</i> (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, *Picirro_et*= *Picrorrhiza* 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 plant has significant glucose lowering effect (Table 3). Glibenclamide as a standard drug 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 (\pm SD), 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 \pm SD	
	Glu_0 day (mMol/l)	Glu_21 st day (mMol/l)
WC(n = 6)	7.20 \pm 0.66	6.58 \pm 1.50
Gliben (n = 6)	7.44 \pm 1.17	5.86 \pm 1.05
Picirro_et (n= 8)	7.89 \pm 0.86	5.95 \pm 0.79*

Data are presented as Mean \pm SD WC= Water control, Gliben= Glibenclamide, *Picirro_et*= *Picrorrhiza* 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

Type 2 diabetes was induced using streptozotocin to neonates rats described by Boiner et al. to evaluate the activity of antidiabetic agents.¹² These results demonstrates, that both ethanolic extract (80%) and water extract of *N. scrophulariflora* in type 2 diabetic rats showed significant hypoglycemic effect ($p < 0.01-0.05$) in type 2 model rats, when the extracts were fed simultaneously with oral glucose load. 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). It indicates that the plant rhizomes might contain some hypoglycemic principle(s) which probably act by initiating the release of insulin from pancreatic β -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 prandial reduction in glucose level by the *N. scrophulariflora* suggests that it may also interfere with intestinal glucose absorption or stimulation of glycogenesis (enhanced by feeding) and improving the insulin-secretory capacity or enhancement of insulin action by the extract.

CONCLUSION

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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