Original Article

Study on hypoglycemic effect of Berberis aristata on type 2 diabetic model rats

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Abstract

Introduction: Berberis aristata is one of the herbs mentioned in all ancient scriptures of Ayurveda. It is a well-known medicinal plant in hilly areas of Nepal and its occurrence is reported from middle altitude areas. It is used traditionally for treating various disorders including diabetes mellitus.

Methods: The experiment was carried out for duration of 28 days on a total of 27 rats. The rats were divided into 4 groups – type 2 water control, type 2 glibenclamide treated, type 2 extract treated and normal treated group. Blood glucose, lipid profile and serum insulin level of all groups were compared at 0 day and 28 day. The renal function, liver function liver glycogen, MDA and GSH concentration value of all groups were compared on 28 day.

Results: The study revealed that type 2 rats treated with extract showed decrease in blood glucose level at 120 mins. The significant decrease was found in normal rat treated with extract in comparison to water control, glibenclamide and type 2 rats extract treated group when fed simultaneously with glucose on the 15 day. There was significant decrease in fasting glucose level in both type 2 and normal rat treated with extract on 28 day in comparison to 0 day.

Conclusion: Berberis aristata dry stem powder 80% ethanolic extract possesses significant hypoglycemic activity in Type 2 diabetic model rats and normal rats.

Key Words: Berberis aristata, Ethanolic extract, Hypoglycemia, Hyperglycemia.

Introduction

Nepalese medicinal plants have been well known in the regional and overseas markets. Over 21,000 plant names that have medicinal uses are reported by WHO and more than 1,600 species of wild plants in Nepal are used in traditional medicinal practice and majority of which await proper documentation[1].

Management of hyperglycemia with low side effects is still a challenge to the current medical system though some newer and more efficient antidiabetic agents are currently in clinical use[2]. Berberis aristata is one of the herbs mentioned in all ancient scriptures of Ayurveda. It is a well-known medicinal plant in hilly areas of Nepal and its occurrence is reported from middle altitude areas. It belongs to family Berberidaceae. The genus Berberis contains 450 species.

Methods

Study design

The study was prospective, case control study conducted

in department of pharmacology, Bangladesh Institute of Research and Rehabilitation in Diabetic, Endocrine and Metabolic Disorders, Dhaka.

Plant material:

The barks of Berberis ariststa were collected from hilly part of Surkhet district, western Nepal. They were cut in to small pieces and then dried. The plant was identified in Pharmacognosy laboratory, department of Pharmcy, Tribhuwan University, Kathmandu, Nepal.

Preparation of ethanol extract of Berberis ariststa bark powder:

At first 600 g of dried powder was soaked in 10 lit of 80 % aqueous ethanol for 24 hours. It was filtered and the filtrate was collected. The residue was again soaked in 5 lit of 80% ethanol for 24 hr in second time and finally third time. The collected filtrate was evaporated at 430C. Finally the concentrated solution was freeze dried to get extract crystal (Brownish color crystal).

Induction of type 2 diabetes:

Type 2 diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ, at a dose of 90mg/kg body weight) in Citrate Buffer (10ml) to the 48 hours old rat pups (average weight 7 gm) as described by Bonner et al.[3]

Testing of model for type 2 diabetic rats.

Three months after STZ injection, the models were screen for type 2 diabetes. The 12 hour fasted rats were made unconscious by using diethylether and blood was collected from tail vain. Then the rats were feed dextrose 2.5 gm/kg body weight/10 ml and the samples were again collected after half an hour of glucose loaded. The samples (fasting and after dextrose feeding) were analyzed and the rats having blood glucose level 8-12 mmol/l at fasting conditions was considered as diabetic and were taken to carry out the experiments.

Chronic experiment:

The chronic experiment was carried out for duration of 28 days on a total of 27 rats. The rats were divided into 4 categories and they were fed as following specific doses:

Group-1 (n=7)	Type 2 water control group [10 ml water/kg body weight]
Group-2 (n=6)	Type 2 glibenclamide group [5 mg/10 ml (9.9 ml H_2O + 0.1 ml Twin 20)/kg body weight]
Group-3 (n=8)	Type 2 treated group Ethanolic extract of *berberis aristata* (1.25g/kg/10 ml).
Group-4 (n=6)	Normal treated group Ethanolic extract of *berberis aristata* (1.25g/kg/10 ml)

Experimental groups

Dose and route of administration:

For the evaluation of the antidiabetic activity, the extract of Berberis aristata was administrated orally to the rats for 4 weeks at a dose of 1.25g/kg body weight/10 ml. For all the pharmacological studies, the drug glibenclamide was administrated orally at a dose of 5 mg/10 ml (9.9 ml H2O + 0.1 ml Tween 20)/kg body weight for Type 2 model rats. For the control groups, 10ml water was administrated per kg body weight.

Duration of study

The study was conducted in 3 month from Dec 2011 to March 2012.

Place of study: The study was conducted in the Biomedical Research Group (BMRG), Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), Dhaka, Bangladesh and Department of Chemistry, University of Dhaka, Bangladesh.

Statistical analysis: Data from the experiments were analyzed using the Statistical Package for Social Science(SPSS) software for windows version 12. All the data were expressed as Mean ± SD or as Median (Range) as appropriate. Statistical analysis of the results was performed by using the student's t-test (paired and unpaired) or ANOVA (analysis of variance) followed by

Bonferroni post hoc test or Mann Whitney (u) test. The limit of significance was set at $p<0.05$.

Results

Effect on the blood glucose level of Diabetic Model rats fed simultaneously with glucose

The significant decrease was found in normal rats treated with extract (6.40±0.61) in comparison to water control (12.22±2.20; p=0.000), glibenclamide (12.93±2.25, p=0.000) and type 2 extract treated group (9.27±1.14,p=0.02). Type 2 extract treated group also showed a significant (p=0.003) decrease of fasting glucose level when it compared with glibenclamide treated group at 120 minutes. The result is shown in table 1.

Table 1: Effect on the blood glucose level of Diabetic Model rats fed simultaneously with glucose

Group	Glucose (mmol/l) Mean ± SD			
	0 min	30 min	60 min	120 min
WC (n = 7)	7.52±0.99	18.28±1.46	17.25±2.23	12.22±2.20
Glib (n = 6)	6.92±0.88	14.80±2.76	16.61±2.33	12.93±2.25
T2_ext (n=8)	7.02±0.59	11.47±2.28	11.71±3.21	9.27±1.14
N_ ext (n=6)	6.30±0.68	6.57±0.31	6.87±0.59	6.40±0.61

Results are expressed as Mean ±SD. Statistical analysis between group comparison was done by using one way ANOVA with post hoc Bonferroni test.*= $p<0.05$; **= $p<0.005$. WC = Type 2 Water Control; Glib = Type 2 Glibenclamide treated group; T2_ext = T2DM b aristata EtOH ext ; N_ ext= Normal b aristata EtOH ext.

Effect on fasting serum glucose level of type-2 diabetic rats.

The significant decrease in fasting glucose level was seen in both type 2 (8.74±0.97 vs 6.35±0.95; p=0.003) and normal rat (7.93±0.53 vs 5.97±0.50; p=0.001) extract treated group on 28 day in comparison to baseline values. The standard drug glibenclamide treated group also showed a significant decrease (p=0.000) of fasting blood glucose level on 28 day when it compared to 0 day values. The result is shown in table 2.

Table 2: Effect on fasting serum glucose level of type-2 diabetic rats

Groups	Glucose (mmol/l)		
	0 day	14 day	28 day
WC (n = 7)	8.33±1.27 (100%)	7.53±0.99 (90.4%)	6.87±0.93* (82.5%)
Glib (n = 6)	9.19±1.00 (100%)	6.92±0.88 (75.23%)	5.80±0.71*** (63.11%)
T2_ext (n=8)	8.74±0.97 (100%)	7.02±0.59* (80.5%)	6.35±0.95** (72.65%)
N_ ext (n=6)	7.93±0.53 (100%)	6.30±0.68** (79.45%)	5.97±0.50** (75.28%)

Results are expressed as Mean ±SD. Statistical analysis between group comparison was done by using one way ANOVA with post hoc Bonferroni test and paired t test. *= $p<0.05$; **= $p<0.005$,***$p<0.001$. WC = Type 2 Water Control; Glib = Type 2 Glibenclamide treated group; T2_ext = T2DM b aristata EtOH ext ; N_ ext= Normal b aristata EtOH ext.

Fasting serum insulin level

The fasting serum insulin level was measured on 0 day and 28 day in all groups of rat. The insulin values were unchanged on final day. The result is shown in table 3.

Table 3: Serum Insulin of Type-2 Diabetic Model Rats.

Groups	Insulin (pg/dl)	
	0 Day	28 Day
WC (n = 7)	0.34±0.13 (100%)	0.32±0.15 (94%)
Glib (n = 6)	0.44±0.18 (100%)	0.30±0.10 (68%)
T2_ext (n=8)	0.40±0.15 (100%)	0.35±0.26 (87%)
N_ ext (n=6)	1.08±0.74 (100%)	0.37±0.20 (34%)

Results are expressed as Mean ±SD. Statistical analysis between group comparison was done by using one way ANOVA with post hoc Bonferroni test.*= $p<0.05$; **= $p<0.005$. WC = Type 2 Water Control; Glib = Type 2 Glibenclamide treated group; T2_ext = T2DM b aristata EtOH ext ; N_ ext= Normal b aristata EtOH ext.

Antihyperlipidemic Effect

There was no decrease in serum cholesterol and triglycerides level in extract treated group on final day in comparison to baseline values. Glibenclamide treated group showed increase in serum cholesterol as well as TG level by 30% and 21 % on final day in comparison to baseline values respectively. LDL-cholesterol level was increased by 8% and 19% in type2 and normal extract treated group on 28 day when it was compared with 0 day values. The results have been shown in table 4 and 5.

Table 4: Effect on total serum cholesterol and TG of type-2 diabetic model rats

Group	Cholesterol (mg/dl)		TG (mg/dl)	
	0 Day	28 Day	0 Day	28 Day
WC (n = 7)	65±5 (100%)	78±12 (120%)	59±7 (100%)	81±18 (137%)
Glib (n = 6)	59±6 (100%)	80±15 (135%)	56±7 (100%)	68±16 (121%)
T2_ext (n=8)	67±7 (100%)	76±4 (113%)	58±13 (100%)	73±19 (125%)
N_ ext (n=6)	64±6 (100%)	84±20 (131%)	56±12 (100%)	59±12 (105.3%)

WC = Type 2 Water Control; Glib = Type 2 Glibenclamide treated group; T2_ext = T2DM b aristata EtOH ext; N_ ext= Normal b aristata EtOH ext

Table 5: Effect on HDL-C and LDL-C of type-2 diabetic model rats

Groups	HDL-C (mg/dl)		LDL-C (mg/dl)	
	0 Day	28 Day	0 Day	28 Day
WC (n = 7)	41±6 (100%)	46±5 (109%)	95±8 (100%)	108±13 (102%)
Glib (n = 6)	48±7 (100%)	49±3 (102%)	85±9 (100%)	115±14 (135%)
T2_ext (n=8)	42±5 (100%)	44±7 (104%)	97±6 (100%)	105±9 (108%)
N_ ext (n=6)	44±2 (100%)	43±6 (79%)	97±8 (100%)	116±23 (119%)

WC = Type 2 Water Control; Glib = Type 2 Glibenclamide treated group; T2_ext = T2DM b aristata EtOH ext ; N_ ext= Normal b aristata EtOH ext

Estimation of liver and kidney function

There were 10% and 24% decrease in serum ALT level in type 2 and normal extract treated rats on final day level in comparison to baseline level. In control, glibenclamide and both extract treated group, serum creatinine levels were remaining unchanged.

Table 6: Effects on the serum ALT and Creatinine level of type 2 diabetic model rats

Group	ALT (mg/dl)		Creatinine (mg/dl)	
	0 day	28 day	0 day	28 day
WC (n = 7)	69±16 (100%)	58±23 (84%)	0.64±0.05 (100%)	0.69±0.15 (107%)
Glib (n = 6)	59±16 (100%)	49±8 (83%)	0.68±0.07 (100%)	0.66±0.05 (97%)
T2_ext (n=8)	65±14 (100%)	59±12 (90%)	0.66±0.07 (100%)	0.65±0.05 (98%)
N_ ext (n=6)	68±15 (100%)	52±7 (76%)	0.66±0.08 (100%)	0.65±0.05 (98%)

WC = Type 2 Water Control; Glib = Type 2 Glibenclamide treated group; T2_ext = T2DM b aristata EtOH ext ; N_ ext= Normal b aristata EtOH ext

Measurement of glycogen

In comparison to control, hepatic glycogen content among the test groups was not increased after 28 days of chronic oral administration of extract.

Table 7: Effects on the Liver Glycogen of Type-2 Diabetic Model Rats

Groups	Glycogen (mg/g) on 28th Day
WC (n = 7)	2.54±1.87 (100%)
Glib (n = 6)	2.76±2.10 (108%)
T2_ext (n=8)	1.76±0.66 (69%)
N_ ext (n=6)	1.66±1.60 (65%)

WC = Type 2 Water Control; Glib = Type 2 Glibenclamide treated group; T2_ext = T2DM b aristata EtOH ext ; N_ ext= Normal b aristata EtOH ext

Estimation for plasma Malondialdehyde (MDA) level and reduced Glutathione (GSH) concentration.

After 28 days, normal and type 2 extract treated rats showed no change in MDA level as compared to control group. Effect of B aristata extracts on concentration of reduced glutathione (GSH), a lipid peroxidation product of erythrocyte is shown in same table. In T2DM extract treated group, erythrocyte GSH concentration was increased by 13% [20.56 (14.97-24.98) mg/g Hb] as compared to control. The concentration of MDA is shown in Table-8.

Table 8: Effect on serum malondialdehyde (MDA) of type-2 diabetic model rats

Groups	MDA (µmol/ml) 28 day	GSH (mg/g Hb) 28 day
WC (n = 7)	1.12 (0.82-3.22)	18.17 (17.13-21.35)
Glib (n = 6)	1.08 (0.58-1.51)	18.97 (15.41-20.86)
T2_ext (n=8)	1.49 (1.05-2.11)	20.56 (14.97-24.98)
N_ ext (n=6)	1.26 (0.52-1.79)	18.07 (15.22-21.67)

WC = Type 2 Water Control; Glib = Type 2 Glibenclamide treated group; T2_ext = T2DM b aristata EtOH ext ; N_ ext= Normal b aristata EtOH ext

Effects on body weight of type-2 DM model rat

It was found that body weight of normal rat with extract treated group was decreased although the reduction was not statistically significant.

Table 9: Effect on body weight of type-2 DM model rat

Group	Body weight (gm)				
	0 day	7 day	14 day	21 day	28 day
WC (n = 7)	171±29	163±33	179±43	183±46	190±49
Glib (n = 6)	182±22	175±19	178±23	185±23	190±19
T2_ext (n=8)	173±18	164±20	176±21	183±23	184±24
N_ ext (n=6)	182±8	168±13	173±19	177±22	177±23

WC = Type 2 Water Control; Glib = Type 2 Glibenclamide treated group; T2_ext = T2DM b aristata EtOH ext ; N_ ext= Normal b aristata extract.

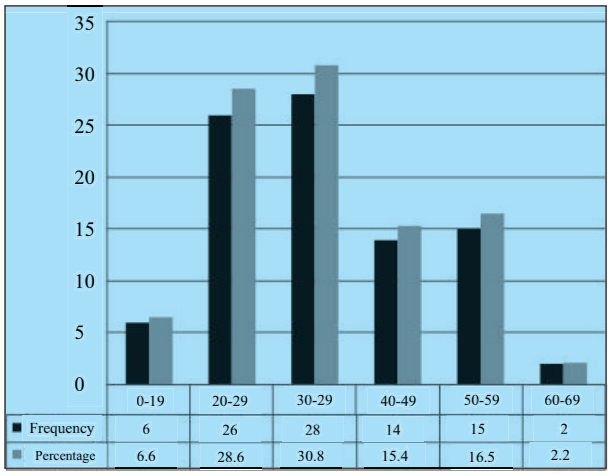

Figure 1: Effect on body weight of type-2 DM model rats

Discussion

Though various categories of hypoglycemic agents and insulin are currently in use for diabetes therapy, none of them are free from side effects[4].

Plant is one of the most potential sources to treat hyperglycemia. However research on antidiabetic plant material has been limited and appropriate methodology is also lacking. In particular, finding appropriate animal model with proper characterization is a big challenge[5]. In present

study type II diabetic model rat has been used to explore the hypoglycemic action of 80% aqueous ethanol extract of the dry stem bark powder of Berberis aristata. The chronic effect of B aristata was evaluated on serum glucose, serum lipids, serum insulin, serum creatinine, serum alanine aminotransferase (ALT), liver glycogen content in type 2 diabetic model rats. It was found that after 28 days of consecutive oral administration of ethanol extract, type 2 treated group and normal treated group showed significant ($p<0.050$) reduction in fasting serum glucose (FSG) level in both in day 14 and 28. Glibenclamide is the standard drug for type 2 diabetes and acts by stimulating the β cells of pancreas to release insulin[6]. It significantly ($p=0.000$) decreased FSG in type 2 treated group. Hence, the finding demonstrates that ethanol extracts of B. aristata may improve glycemic status in type 2 diabetes. Several studies reported that berberine reduces hyperglycemia [7], serum cholesterol, triglycerides and low density lipoprotein (LDL)-cholesterol 8 in animal model. Clinical studies showed that oral administration of berberine cause significant reduction of blood cholesterol, triglyceride as well as glucose in patients with hyperlipidemia and type 2 diabetes; with no side-effects on liver, kidney and muscle[9] Berberine acts by multiple mechanisms within multiple cellular energy metabolisms. It sensitizes the effect of insulin, reduced insulin resistance, increase glucose uptake and utilisation during insulin deficiency or even in the absent of insulin. In addition to this, it stimulates glycolysis pathway and finally enhanced the metabolism of glucose[10]. It has also been reported that berberine inhibit the enzyme alpha-glucosidase which is responsible for conveting carbohydrate into simple sugars (monosaccharides) in small intestine and finally reduces the rate of digestion of carbohydrates[11].

Hypolipidemic effect of ethanol extracts was evaluated in type 2 diabetic rats. After 28 days of chronic treatment there was no effect on lipid profile. Serum cholesterol, TG and LDL cholesterol levels raised which is in consistent to the result of study conducted by Upwar et al.[7] but serum HDL cholesterol increased in extracts treated groups.

Increased serum creatinine level in diabetes reflects deterioration of kidney function associated with persistent hyperglycemia. In this study, serum creatinine level was increased in the water control group, which suggests that kidney damage may be associated with persistent hyperglycemia. But kidney function is preserved in extract as well as glibenclamide treated group because in both group serum glucose level was controlled. Liver function, serum alanine aminotransferase (ALT) level was measured at 0 and 28 day. It was found that ALT level was decreased but non-significant in both extract treated and normal treated group. Therefore, it may be stated that it has no toxic effect on kidney and liver function.

Conclusion

Thus it can be concluded that, B. aristata possess significant anti-hyperglycemic effects in type 2 diabetic model rats. It was also found that the extract doesn't exhibit any significant toxic effect in liver and kidney. However, comprehensive chemical and pharmacological researches are required to find out the exact mechanism of its hypoglycemic effect and to identify the active constituent(s) responsible for this effect.

Conflict of interests

None Declared

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