ORIGINAL ARTICLE

Antihyperglycemic activity of root of *Berberis aristata* D.C. in alloxan-induced diabetic rats

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The aim of this study is to examine the antihyperglycemic activity of root of *Berberis aristata* D.C. in alloxan-induced diabetic rats. Five groups of albino Wistar rats were used (n = 6). The two dose of 71.42 and 100 mg/kg body weight ethanol extract of *B. aristata* were selected for antidiabetic activity. Blood glucose levels were estimated in all the groups by the commercial kit (Span diagnostic Pvt. Ltd, Surat) on 1st, 5th, 10th and 20th day of the treatment with *B. aristata*. The serum cholesterol, triglycerides, HDL, liver glycogen and body weight were estimated on 20th day of treatment in all the groups compared against diabetic control group. The different extracts of root of *B. aristata* were also tested for glucose tolerance test in normal fasted rats. The ethanol extract of root of *B. aristata* 71.42 and 100 mg/kg body weight showed a significant (P<0.01) reduction of serum glucose level in alloxan induced diabetic rats at 15th day as compared to diabetic control group. Cholesterol and triglycerides level were increased very significantly (P<0.01), in diabetic animal when compared with normal control group. The level of cholesterol and triglycerides reduced very significantly (P<0.01), when compared with diabetic control group. The level of HDL cholesterol was significantly (P<0.05) increased in the extract treated group when compared to diabetic control group. In oral glucose tolerance test ethanol extract of *B. aristata* increase the glucose tolerance. It is concluded that the ethanol extract of *B. aristata* possess anti-diabetic activity in alloxan induced diabetic rats. The ethanol extract of *B. aristata* is very promising to develop standardized phytomedicine for diabetes mellitus.

Key words: *Berberis aristata*, Indian berbery, daruharidra, alloxan-induced diabetes

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and alternation in carbohydrates, fat and protein metabolism associated with absolute or relative deficiencies in insulin secretion or insulin action. It is considered as one of the five leading causes of death in the world.[1] About 150 million or 1.3% people are suffering from diabetes worldwide which is almost five times more than the estimates 10 years ago and this may double by the year 2030.[2] Different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes mellitus but their long-term use produces undesirable side effects such as skin rashes, transient leucopenia, thrombocytopenia, severe hypoglycemia, and increase chances of cardiovascular death of unknown mechanism. There are numerous traditional medicinal plants reported to have hypoglycemic properties, such as *Allium sativum* (garlic), *Azadirachta indica* (neem), *Vinca rosea* (nayantara), *Trigonalla foenum* (fenugreek), *Momordica charantia* (bitter ground), and *Ocimum santum* (tulsi), and many of them proved to be not very effective in lowering glucose levels in severe diabetes.[3]

Berberis aristata D.C. (Berberidaceae) is an edible plant commonly used in Indian system of medicine as a anti-diarrhea, hypoglycemic, anti-cancer, gastro-irritant, anticoagulants, antipyretic, hypotensive, CNS depressant, and diaphoretic etc.[4-6] However no scientific study on the anti-diabetic activity of this plant has been reported. The present investigation was undertaken to study the anti-diabetic activity of root of *B. aristata* in alloxan-induced diabetic rats.

MATERIALS AND METHODS

Plant Material

After proper identification by Taxonomists of the Botanical Survey of India, Dehradun, India. The root of *B. aristata* was collected from surrounding area of Rishikesh, Uttaranchal. The roots was dried in shade at room temperature and coarsely powdered by using a mechanical grinder. The powdered drug was then extracted successively with petroleum ether, chloroform, and ethanol each for 24 hours. The extracts were concentrated under reduced pressure. The dried extracts were stored in the airtight container.

Animals

Male Wistar albino rats (150–200 g) were housed in a spacious cage for 10 days after obtaining approval from

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'Institute Ethics Committee'. During the experiment, rats were fed standard chow diet. After randomization into various groups, the rats were acclimatized for 2 to 3 days in the new environment before initiation of experiment. Animals had free access to food and drinking water till before 30 minutes of sampling.

Chemicals

Alloxan monohydrates a most widely used chemical diabetogen was procured from Rolex chemical Ltd, Mumbai India. Glibenclamide, a standard antidiabetic drug was purchased from Total health care Pvt. Ltd, Baddhi, Himanchal Pradesh**.**

Glucose Tolerance Test

Rats were divided into four groups. Six fasted animals were used in each group. Group I was kept as vehicle control which received 5% v/v Tween 80 per oral, group II received ethanol extract (100 mg/kg) with distilled water, groups III and IV received (100 mg/kg) petroleum ether extract and chloroform extract respectively .The rats of all the groups were loaded with glucose (3 g/kg, per oral) 30 minutes after drug administration. Blood samples were collected from puncturing the retro orbital sinus just prior to drug administration and 30, 90, and 150 minutes after glucose loading.[7] Serum glucose level was measured immediately by using glucose estimation kit (Span diagnostic Pvt. Ltd, Surat, India).

Induction of Diabetes

All the animals were randomly divided into five groups with six animals in each group. Group I was normal and used as normal control. Groups II, III, IV, and V were made diabetic by a single intraperitoneal injection of alloxan monohydrate (125 mg/kg body weight) and served as diabetic control, standard, and treatment groups, respectively. Rats exhibiting plasma glucose levels of >250 mg/dl, 48 hours after administration of alloxan were included in the study. The 71.42 and 100 mg/kg/per oral of the extract were administered 7 days after induction of diabetes with alloxan.[8]

Estimation of Biochemical Parameter

Serum glucose, serum cholesterol, serum triglycerides, and serum HDL, were estimated by commercially available kits (Span diagnostic Pvt. Ltd. Surat, India) and liver glycogen content was estimated by the method of Carroll *et al*.[9]

Statistical Analysis

All results are expressed as the mean ± S.D. The results were analyzed for statistical significance using one-way analysis of variance (ANOVA); comparison was done by using Dunnett's test. *P* values <0.05 were considered as significant and *P* values <0.01 were considered as very significant.

RESULTS

Effect on Glucose Tolerance Test

The effects of various extracts of *B. aristata* (100 mg/kg) on glucose tolerance are shown in Table 1. The supplementation of *B. aristata* improved the glucose tolerance in the fasted normal rats. When the rats were first injected with glucose, the rate of increase in the blood glucose level was the same for normal, chloroform extract, and petroleum ether extract groups during the first 30 minutes but its rise was less for the ethanol extract group. After that serum glucose level lowered significantly ($P<0.05$) at 90 minutes and very significantly ($P<0.01$) lowered at 150 minutes in the ethanol group as compared to the normal control group. There was significant reduction ($P<0.05$) in the chloroform extract group compared to the control group. The petroleum. ether extract group did not show any significance reduction.

Effect on Alloxan-induced Diabetic Rats

Administration of alloxan monohydrates (125 mg/kg) led to elevation of blood glucose. The anti-hyperglycemic effects of the ethanol extract of root of *B. aristata* (71.42 mg/kg, 100 mg/kg) and glibenclamide (5 mg/kg) on the blood sugar levels of diabetic rats are shown in Table 2. After daily treatment with 71.42 mg/kg and 100 mg/kg of ethanol extract of *B. aristata* and glibenclamide 5 mg/kg led to a dose-dependent fall in blood sugar levels. The percent reduction of hyperglycemia was significant ($P<0.01$) on 5th, 10th, and 20th days after treatment with the 71.42 mg/kg of *B. aristata*, which was 36, 49, and 62%, respectively, as compared with the diabetic control group. The percent reduction of hyperglycemia was significant ($P<0.01$) on 5th, 10th, and 20th days after treatment with the 100 mg/kg of *B. aristata*, which was 53.24, 63, 76.04%, respectively, as compared with the diabetic control group. The percent reductions of blood

Table 1: Effect of different extracts of root of *B. aristata* on glucose tolerance in normal fasted rats

Group	Time (min) Serum glucose level (mg/dl)			
	Initial (0)	30	90	150
Normal control	66.00 ± 22.38	141.83 ± 32.56	126.67 ± 25.93	90.50 ± 16.81
Ethanol extract *B. aristata* (100 mg/kg)	55.33 ± 11.07	109.50 ± 50.57	71.66 ± 17.80*	46.50 ± 11.01**
Chloroform extract *B. aristata* (100 mg/kg)	68.16 ± 8.68	153.50 ± 34.98	92.83 ± 24.82^{NS}	71.33 ± 07.78*
Petroleum ether extract *B. aristata* (100 mg/kg)	60.16±6.76	130.80±34.98	126.67±25.93^{NS}	88.16±09.30^{NS}

The rats of the entire group were loaded with glucose (3 g/kg, P.O.) 30 minutes after the herbal drug administration. Values are mean ± SD, *n* = 6 in each group. **P* <0.05 is significant; NS- not significant vs the control group. ***P* <0.01 very significant.

glucose levels were significant (P <0.01) 50.52, 61.26, and 75% on 5th, 10th, and 20th days, respectively, after treatment with glibenclamide 5 mg/kg. The antihyperglycemic effect exhibited by 100 mg/kg of *B. aristata* was slightly higher than the glibenclamide 5 mg/kg.

Effect on Serum Cholesterol, Triglycerides, HDL, Atherogenic Index, and Liver Glycogen Content

The levels of serum cholesterol and triglycerides were increased very significantly and the levels of HDL were decreased in diabetic rats as compared with normal control rats. Treatment with ethanol extract of *B. aristata* at the doses of 71.42 mg/kg and 100 mg/kg reduced the cholesterol and triglycerides level very significantly (P<0.01), when compared with the diabetic control group. The reduction in cholesterol and triglycerides levels in the extract-treated group was slightly higher at the dose of 100 mg/kg as compared to standard drug glibenclamide. The level of HDL cholesterol was significantly (P<0.05) increased in the extract-treated group when compared to the diabetic control group. The HDL cholesterol improvement at the doses of 100 mg/kg of *B. aristata* treatment was also slightly higher than the glibenclamide 5 mg/kg; the data are shown in Table 3.

The results of the liver glycogen indicate that the liver glycogen concentration was found to be significantly (P<0.01) decreased in the diabetic group when compared with the normal control group. The ethanol extract of *B. aristata* 71.42 mg/kg, 100 mg/kg, and the glibenclamide 5mg/kg significantly increased the liver glycogen level to its normal level [Table 3].

Effect on Body Weight

The effects of the extract on body weight in the alloxan-induced diabetic rats are shown in [Table 4]. The results of the body weight analysis indicate that the body weight of the untreated diabetic rats was found to be significantly (P<0.05) decreased when compared with the normal control group. The body weight was slightly increased in the normal control group compared to initial weight. Ethanol extract of *B. aristata* 71.42 mg/kg, 100 mg/kg, and glibenclamide 5mg/kg treatment significantly (P <0.05) prevented this reduction in body weight. The data are shown in Table 4.

Table 2: Effect of ethanol extract of root *of B. aristata* on serum glucose level in alloxan-induced diabetic rats

Group	Time (days) serum glucose (mg/dl)			
	1st day	5th day	10th day	20th day
Normal	68.99 ± 6.03	70.48 ± 6.35	70.62 ± 5.51	71.20 ± 6.08
Diabetic	253.66 ± 3.57	260 ± 9.48	263 ± 4.58	270.51 ± 6.28
Glibenclamide 5 mg/kg	162 ± 6.47**	129 ± 8.96**	101 ± 7.96**	64 ± 9.75**
B. aristata 71.42 mg/kg	239 ± 10.39	162 ± 10.43**	129 ± 8.35**	95 ± 5.97**
B. aristata 100 mg/kg	248`± 8.75	121 ± 8.41**	95 ± 6.83**	62 ± 6.78**

Values are given as mean ± SD, n = 6 in each group except in the diabetic control group and *B. aristata* 71.42 mg/kg group, where n = 4 on the 10th day and 20th day. Two animals died on the 10th day. Compared to the diabetic control group. ** P <0.01 very significant and *P <0.05 is significant.Herbal drug or glibenclamide was administered daily for 19 days and the animals were sacrificed on the 20th day.

Table 3: Effect of ethanol extract of *B. aristata* root on serum cholesterol, triglycerides, HDL, atherogenic index, and liver glycogen weight

G Group	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL cholesterol (mg/dl)	Atherogenic index (total cholesterol-HDL cholesterol) HDL cholesterol (mg/dl)	Liver glycogen index weigh mg/g wet tissue
Normal	72.91 ± 9.12	47.88 ± 3.06	16.66 ± 1.26	3.78 ± 0.95	38.60 ± 4.83
Diabetic	121.0 ± 5.9	96 ± 3.00	10.25 ± 1.26	10.80 ± 1.20	12.99 ± 1.66
Glibenclamide 5 mg/kg	78.80 ± 8.03**	62.25 ± 3.19**	14.68 ± 1.99	4.36 ± 0.78	32.93 ± 8.08[a]
B. aristata 71.42 mg/kg	86.66±11.77**	65.45±3.11*	13.96±1.90	5.26±1.15	26.80±6.36[a]
B. aristata 100 mg/kg	69.84±23.16**	53.29±3.22**	18.11±1.15*	2.85±0.22**	35.03±9.89[a]

Values are given as mean ± SD, n = 6 in each group except in the diabetic control group and *B. aristata* 71.42 mg/kg group, where n = 4 on the 10th day and 20th day. Two animals died on the 10th day. Compared to diabetic control group. **P <0.01 very significant and *P <0.05 is significant.

Table 4: Effect of ethanol extract of *B. aristata* root on body weight of alloxan-induced diabetic rats

Group	Base line	1st day	5th day	10th day	20th day
Normal	207.66 ± 5.26	207.80 ± 5.97	211.39 ± 4.22 ↑(2.49)	213.41 ± 5.29 ↑(2.76)	220.60 ± 3.26 ↑(6.23)
Diabetic	227.42 ± 9.47	227.61 ± 9.06	225.15 ± 5.65 ↓(1.05)	223.98 ± 5.00 ↓(1.69)	217.75 ± 8.11 ↓(4.90)*
Glibenclamide 5 mg/kg	210 ± 4.39	211.80 ± 4.15	209.05 ± 2.01 ↓(0.27)	207.75 ± 4.27 ↓(1.11)	203.60 ± 5.25 ↓(3.95)
B. aristata 71.42 mg/kg	213 ± 4.65	213.94 ± 4.23	211.84 ± 6.81 ↓(0.75)	212.01 ± 3.21 ↑(0.98)	220.42 ± 4.50 ↑(3.33)
B. aristata 100 mg/kg	215 ± 6.20	216 ± 6.15	217.65 ± 4.05 ↑(1.22)	220.00 ± 5.66 ↑(2.34)	225 ± 7.39* ↑(4.62)

** P <0.01 very significant and *P <0.05 is significant.Values are expressed as mean ± SD, n = 6 in each group.Values in parentheses represent % change (loss or gain) in body weight compared to initial body weight.↑Percentage increase in body weight; ↓Percentage decrease in the body weight.

DISCUSSION

Management of diabetes with the agents devoid of any side effects is still a challenge to the medical system. This has led to an increase in the demand for natural products with antihyperglycemic activity and fewer side effects. The ethanol extract of *B. aristata* exhibited dose-dependent antidiabetic property. The antidiabetic effect of it at the dose of 100 mg/kg is even slightly higher than glibenclamide 5 mg/kg. Our results are supporting its use as folklore medicine for the treatment of diabetes.

Plants may act on blood glucose through different mechanisms, some of them may have insulin-like substances and some may inhibit insulinase activity.[10-11] Stimulation of?-cells to produce more insulin[12] and others may increase? -cells in the pancreas by activating regeneration of pancreatic cells.[13]

Lipids play an important role in the pathogenesis of diabetes mellitus. Hyperlipidemia is a recognized consequence of diabetes mellitus demonstrated by the elevated levels of tissue cholesterol, phospholipids and free fatty acids.[12,13,14] Diabetes-induced hyperlipidemia is attributable to excess mobilization of fat from the adipose tissue due to the under utilization of glucose. The abnormal high concentration of serum lipids in diabetes is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots since insulin inhibits the hormone sensitive lipase. On the other hand, glucagons, catecholamine, and other hormones enhance lipolysis. The level of serum lipids is usually raised in diabetes and such an elevation represents a risk factor for coronary heart disease. The levels of serum cholesterol and triglycerides were raised in diabetic rats but which were lowered significantly with the treatment of *B. aristata*. It indicates that the ethanol extract of *B. aristata* is more useful in the treatment of diabetes as it has hypolipidemic effect. Moreover, its hypolipidemic effect could represent a protective mechanism against the development of atherosclerosis, which is usually associated with diabetes. The levels of HDL cholesterol were significantly increased in the extract treated group. Glycogen is the primary intracellular storable form of glucose and its levels in various tissues especially hepatic and skeletal muscle are a direct reflection of insulin activity as insulin promotes intracellular glycogen deposition by stimulating glycogen synthetase and inhibiting glycogen phosphorylase. Since destruction of β-cells of islets of Langerhans resulting in marked decrease in insulin levels, it is rational that glycogen levels in tissues (skeletal muscle and liver) decrease as they depend on insulin for influx of glucose.[15] Moreover, alteration in muscle and hepatic glycogen content is normalized by insulin treatment. A normal level of glycogen reflects the normalization insulin levels.

CONCLUSION

The data of our study revealed that the ethanol extract of *B. aristata* possess significant antidiabetic activity in alloxan-induced diabetic rats in a dose dependent manner. *B. aristata* is folklore medicine used in the treatment of diabetes in India Sikkim and Darjling. Our results are supporting its use as folklore medicine for the treatment of diabetes.

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