IJPT
1735-2657/10/91-21-24
IRANIAN JOURNAL OF PHARMACOLOGY & THERAPEUTICS

IJPT 9: 21-24, 2010

RIDR

RESEARCH ARTICLE

Blood Glucose Lowering Potential of Stem Bark of *Berberis aristata* Dc in Alloxan-Induced Diabetic Rats

J. K. GUPTA, P. MISHRA, A. RANI and P. MITRA MAZUMDER

For author affiliations, see end of text.

Received March 9, 2009; Revised September 13, 2009; November 5, 2009

This paper is available online at *http://ijpt.iums.ac.ir*

ABSTRACT

The present investigation explores the blood glucose lowering potential of *Berberis aristata* stem bark extract (methanolic extract) in alloxan-induced diabetic rats as well as its *in vitro* antioxidant property. It is observed that methanolic extract of *B. aristata* stem bark exhibits significant antidiabetic activity in a dose-dependent manner, but not better than glibenclamide. The extract also has enough reducing power to manifest its antioxidant nature.

Keywords: *Antidiabetc activity, Antioxidant, Berberis aristata, Methanolic extract, Alloxan, Phenolic content*

Diabetes mellitus along with its associated complication has become the common problem in contemporary world. It is a metabolic disorders characterized by hyperglycemia due to absolute or relative deficiency of insulin and results in significant morbidity and mortality. Lack of insulin affects the metabolism of carbohydrates, protein and fat, and causes a significant disturbance of water and electrolyte homeostatis [1]. Diabetes, by itself, increases the production of tissue damaging oxidative stress. Therefore, in diabetes, the oxidative stress is referred as a case of double jeopardy for any beta cells that survive the disease [2]. Management of diabetes with minimal side effects is still a complicated medical challenge and there is an increasing demand by patients to use the natural products with antidiabetic activity, because both insulin and oral hypoglycemic drugs possess undesirable side effects [3].

Berberis aristata DC (family – Berberidaceae) is a large deciduous shrub usually 1.8- 3.6 m high having leaves 3.8-10x1.5-3.3 cm, obovate or elliptic, entire or spinous-toothed, base gradually narrowed with prominent reticulate venation , glossy dark green above and glossy pale green beneath. Flowers are numerous and stalked having simple raceme inflorescence. Bark is internally yellowish brown, rough, closely and rather deeply furrowed. Cut surface has bright yellow colour with rough and fibrous texture.

Berberis aristata DC, known as 'Daruharidra' in Ayurvedic system of medicine, is extensively used in various systems of indigenous medicine for treating a variety of ailments such as eye and ear diseases, rheumatism, jaundice, diabetes, stomach disorders, skin disease, malarial fever and as tonic etc [4, 5]. The reported constituents are berberine, berbamine, aromoline, karachine, palmatine, oxyacanthine and oxyberberine [6]. The species *Berberis aristata* is known for its hepatoprotective activity [7]. In the present study we investigated antidiabetic effect of the stem bark of *Berberis aristata* considering its antioxidants property in alloxan-induced diabetic rats. Though the antihyperglycemic activity of the same plant as herbo-mineral preparation in streptozotocin-induced diabetic rats has been shown [8], but its antioxidant activity is not reported.

MATERIALS AND METHODS

Plant Material

Berberis aristata is an erect, glabrous, spinescent shrub collected from Dehradun (India). It is commonly known as Daruhaldi. The plant specimen was authenticated by Botanical Survey of India, Government of India, Howrah, (Ref. voucher no.BSI/CDM/052). The stem bark was isolated and dried in shade at room

Table 1. Determination of LD_{50} Value for *BERBERIS ARISTATA* extarct

S.No.	Extract	LD50 in mice (mg/kg ± SEM)
1	Petroleum ether extract	1710 ± 35
2	Chloroform extract	1330 ± 38
3	Methanolic extract	1540 ± 21

All values are represented as Mean ± SEM

temperature. Dried material was coarse powdered and packed in soxhlet apparatus and extracted with petroleum ether (60-80^0 C), chloroform (61^0 C) and methanol (65^{0}C).

Animals

Male wistar albino rats (150–200 g) and albino mice (25-30g) were obtained from the Animal house of Arya College of Pharmacy, Jaipur, (India) after obtaining approval from Institute's Ethics Committee. They were housed in standard environmental condition (at room temperature and 50% relative humadity) in standard cage and maintained on standard pellets, germinated grams, and water *ad libitum*. Prior to experimentation, the animals were fasted for 18 hours but free access to drinking water.

Experimental Method

Acute toxicity study

The LD50 values were determined by Miller and Tainter (1944) method [9]. Acute toxicity studies were conducted for all the extracts through intraperitonial route. The test was carried out on small group of male mice and nearly 50 % mortality was observed. The dose of 500 mg/kg body weight by oral route did not produce any toxic effect (Table 1).

Antidiabetic activity [10, 11]

Male wistar albino rats (150-200g) were randomly divided into five groups with six animals in each group:

Group I (normal control): Carboxy methyl cellulose 1% w/v (0.5ml/100g of body wt, OD) was administered orally.

Group II (diabetic control): Alloxan (125 mg/kg of body wt.) was injected intraperitonially as a single dose and kept without any treatment to study the diabetic nature of rat.

Group III (standard): Alloxan (125 mg/kg of body wt.) was injected intraperitonially as a single dose and glibenclamide (10 mg/kg of body wt, OD) orally after 72 hours of Alloxan treatment.

Group IV (petroleum ether extract): Alloxan (125 mg/kg of body wt.) was injected intraperitonially as a sinsle dose and petroleum ether extract (500 mg/kg, OD) in 1% w/v CMC through oral route after 72 hours of Alloxan treatment.

Group V (chloroform extract): Alloxan (125 mg/kg of body wt.) was injected intraperitonially as a single dose and chloroform extract (500 mg/kg, OD) in 1 % w/v CMC orally after 72 hours of Alloxan treatment to study the diabetic nature of rat.

Group VI (methanolic extract): Alloxan (125 mg/kg of body wt.) injected intraperitonially as a single dose and methanolic extract (500 mg/kg of body wt, OD) in 1 % w/v CMC orally after 72 hours of Alloxan treatment to study the antidiabetic nature of abstract.

Alloxan monohydrate (125 mg/kg of body wt) was injected intraperitonially as a single dose in 18-hr previously-fasted rats to induce diabetics. After one hour of Alloxan administration, the rats were fed standard pellets and water *ad libitum*. The extracts in CMC (1% w/v) were given after 72 hours of Alloxan treatment. Study was carried out for four days and blood glucose was determined. The fasting blood glucose level was determined after one hour of the extract / drug treatment and the blood sample was collected from tail vein.

Another group of animals were treated with alloxan (125 mg/kg, i.p.) and methanolic extract (250 mg/kg p.o.) and the study was extended for 15 days to determine the plasma level of cholesterol, total lipids, protein, urea, SGOT, SGPT and glucose using commercial available kits [12] (Span diagnostic Pvt. Ltd. Surat, India).

Antioxidant activity

Determination of Phenolic Content:

The modified form of Folin Ciocalteu method [13] was used to determine total phenolic content of dried methanolic extracts. A calibration curve was made in the range 50-100 µg/ml of alcoholic gallic acid, for which 1 ml of alcoholic gallic acid solution was mixed to ten fold diluted Folin Ciocalteu reagent and the volume was made up to 6 ml and further mixed with 4 ml of sodium carbonate (0.7 M).

One ml of methanolic solution of dried extract (conc., 100 mg in 10 ml) was mixed with the same reagent in a similar manner and after one hour, the absorbance was measured at 680 nm spectrophotometrically for the determination of total phenolic content using following formula [14],

$C = cv / m$

Where C = total phenolic content (mg/g of plant extract)

c = concentration of gallic acid (mg/ml from calibration curve)

v = volume of extract (ml)

m = wt of pure plant extract in gram

The recorded absorbance of the extract manifested nearly 72.7 µg / ml of Gallic acid (from calibration curve).

Determination of Change in Absorbance due to Reducing Power

Reducing power of the extract was determined by Butyl Hydroxy Toluene (BHT) method of Yen and Chen [15]. The extract (20, 40 and 60 mg/ml in methanol) was mixed with an equal volume of 0.2 M Phosphate buffer (pH 6.6) and aqueous solution of

Table 2. Effect of *BERBERIS ARISTATA* Extracts, Vehicle and Standard Drug on Blood Glucose Level of Alloxan-induced Diabetic Rats

Treatment	Blood Glucose Level in mg/100 ml ± SEM					
	Initial	1 hr	2 hr	4 hr	8 hr	4 th Day
Normal control	90.84 ± 1.21	87.51 ± 1.58	85.12 ± 1.25	82.15 ± 1.05	84.15 ± 1.52	85.06 ± 2.01
Diabetic control	169.15±2.12	180.00±2.21	174.12 ± 1.56	185.12 ± 3.21	195.12 ± 2.14	110.56 ± 1.50
Glibenclamide	170.41±1.15	165.11±1.56	142.22* ± 1.21	112.12** ± 1.56	105.50** ± 1.50	92.58**± 1.25
Petroleum ether extract	180.45±1.15	174.15±1.25	168.15 ± 2.02	165.12 ± 1.25	158.05 ± 1.85	150.01 ± 1.50
Chloroform extract	174.12±1.28	168.10±2.85	156.26 ± 2.18	155.15 ± 2.52	145.12* ± 2.12	141.01*± 3.18
Methanolic extract	171.14±1.54	162.18±1.52	145.16* ± 3.15	124.18** ± 3.12	98.98** ± 2.12	94.20**± 3.14

n = 6, * $p < 0.05$ (significant), ** $p < 0.01$ (more significant) compared with diabetic control

Table 3. Effect of Methanolic Extraxt on Body Weight, Serum Urea, Protein, Cholesterol, SGOT, SGPT and Total Lipids in Diabetic Rats

Group	Initial body weight 1st day	Final body weight 15th day	Blood glucose mg/kg	Urea mg/dl	Protein mg/dl	Cholesterol mg/dl	SGOT IU/dl	SGPT IU/dl	Total lipids mg/dl
Normal control	198.5 ± 3.02	205.8 ±1.15	84.21 ± 5.17	31.91±1.18	2.85 ± 1.05	71.50 ± 5.79	46.15 ± 2.20	64.25 ± 1.25	150.50 ± 5.25
Diabetic control	185 ± 1.59	180.1 ± 2.25	258.12 ± 5.52	135.10 ± 1.25	0.57±0.95	233.31 ± 2.15	241.50 ±2.05	189.15 ± 1.50	350.25±15.25
Glibenclamide 10 mg/kg	185.5 ± 1.25	197.0 ± 2.95	103.14** ± 2.98	49.50** ± 3.15	0.80 ± 1.01	78.50** ± 3.15	99.5** ± 1.65	115.5** ± 3.17	175.10**± 9.01
B. aristata 250 mg/kg	176.15 ± 2.15	189.19 ± 5.61	135.17** ± 2.15	85.14** ± 4.16	0.95 ± 0.50	74.15** ± 1.85	155.15** ± 3.12	170.5* ± 1.50	178.20** ± 5.1
B. aristata 500 mg/kg	184.5 ± 6.5	188.80 ± 5.10	115.12** ± 6.12	58.12** ± 8.1	0.98 ± 0.31	61.71** ± 0.17	115.5** ± 1.95	141.5 **± 1.50	152.51** ± 5.15

Values are Mean ± SEM compared to diabetic control group, significant $^{*}p<0.05$ and very significant $^{**}p<0.01$

Potassium ferricyanide (1 % w/v). The mixture was incubated at 50^0C for 20 min. An equal volume of 1 % w/v of aqueous solution of trichloroacetic acid was added to the mixture and centrifuged at 6000 rpm for 10 min. Supernatant: distilled water: ferric chloride (0.1 % w/v) were mixed in the ratio 1:1:2 and the absorbance were measured with spectrophotometer at 700 nm. The increase in absorbance signified the increase in reducing power.

Statistical Analysis

The results were analyzed with one way analysis of variance followed by Dunnett t-test and p values < 0.05 were considered significant. Groups were compared with control group.

RESULTS

Acute toxicity studies were conducted for all the extracts through intraperitonial route. The test was carried out on small group of male mice and nearly 50 % mortality was observed. The dose of 500 mg/kg body weight by oral route did not produce any toxic effect (Table 1).

The antidiabetic activity of methanolic extract of the stem bark of *Berberis aristata* was significantly observed at 1, 2, 4 and 8 hr and 4th day of treatment compared to diabetic control (Table 2). Administration of alloxan monohydrates (125 mg/kg) led to elevation of blood glucose level. The hypoglycemic effects of the methanolic extract of the stem bark of *B. aristata* and glibenclamide (10 mg/kg) on diabetic rats are shown in Table 3. Daily treatment with 250 mg/kg and 500 mg/kg of methanolic extract of *B. aristata* led to a dose-dependent fall in blood sugar levels.

Changes in body weight of diabetic rats were observed which was normalized by *B. aristata* extract significantly but not better then glibenclamide. Administration of *B. aristata* extract even at a dose of 250 mg/kg showed marked decrease in the level of blood urea, total protein, SGOT, SGPT, cholesterol and lipids.

The total phenolic content of methanolic extract was observed to determine the antioxidant property. It was found to be 7.27 mg/g of dried extract (Table 4). The reducing power of the extract was found to be less than the known standard BHT.

DISCUSSION

The study reports the blood glucose lowering potential and *in vitro* antioxidant activity of methanolic extract of stem bark of *Berberis aristata* DC. Though the anti diabetic activity of the same plant was shown by

Table 4. Total Phenolic Content of *B. aristata* stem bark (methanolic extract)

Extract/Drug	Absorbance at 680 nm				Total Phenolic Content of dry extract (mg/g)
	I	II	III	Average	
Methanolic Extract (10mg/ml)	0.304	0.305	0.305	0.305	7.27
Gallic Acid (50 µg/ml)	0.176	0.175	0.176	0.176	
Gallic Acid (100 µg/ml)	0.458	0.460	0.460	0.460	

other workers [16], but its antioxidant activity is still not reported. Due to the antioxidant property of the extract, the diabetic rats got a significant protection from the reactive oxygen species produced in alloxan-induced diabetic rats.

The present study, for the first time looked into the antioxidant potential of the *Berberis aristata* methanolic extract. The blood glucose-lowering potential of a drug becomes noble if it exhibits an antioxidant property too. The present study confirms the same for *B. aristata* extract. On the whole, results of present study support the blood glucose lowering potential of methanolic extract of *Berberis aristata.* Further studies at cellular level are being carried out in the laboratory to establish the actual mechanism of action.

REFERENCES

1. Frier BM, Fischer BM. Diabetes mellitus. In: Davidson's Principles and Practice of Medicine, ed. 19, Pub. Churchill Livingstone, 2002; p 644.
2. Robertson RP, Harman JS. Diabetes, glucose toxicity and oxidative stress: a case of double jeopardy for the pancreatic islets βcell. *Free Radic Biol Med* 2006; 41:177-84.
3. Kameswra RB, Appa Rao CH. Hypoglycemic and antihyperglycemic activity of alternifolium (Wt.) Walp. seed extracts in normal and diabetic rats. *Phytomedicin*e 2001; 8:88-93.
4. Anonymous. The Wealth of India, PID_5CSIR; New Delhi, 1965; 1: pp 114-9.
5. Kirtikar KR, Basu BD. Indian Medicinal Plants I, Lalit Mohan Basu and Co., Allahabad; 1933.
6. Rastogi RP, Mehrotra BN. Compendium of Indian Medicinal Plants.Vol.3 CDRI, Lucknow and PID CSIR, New Delhi; 1993.
7. Sohni YR, Bhatt RM. Activity of a crude extract formulation in experimental hepatic amoebiasis and in immunomodulation studies. *J Ethnopharmacol* 1996**;** 54: 119-24.
8. Mitra SK, Gopumadhavan S, Muralidhar TS, Anturlikar SD, Sujatha MB. Effect of a herbomineral preparation D-400 in streptozotocin-induced diabetic rats. *J Ethnopharmacol* 1996; 54:41-6.
9. Miller LC, Tainter ML. Estimation of the LD-50 and its error by means of logarithmic–robit graph paper. *Proc Soc Exp Biol Med* 1944; 57:261.
10. Muller G. Drug Discovery and Evaluation. In: Pharmacological Assays. Vogel HG, Vogel WH. Springer-Verlag Berlin Heidelberg, 2002; pp 950-951.
11. Badole S, Patel N, Bodhankar S, Jain B, Bhardwaj S. Antihyperglycemic activity of aqueous extract of leaves of *Cocullus hirsutus* (L) *Diels* in alloxan-induced diabetic mice. *Ind J Pharmacol* 2006; 38: 49-53.
12. Godkar BP, Godkar DP. Textbook of Medical Laboratory Technology, ed. 2, Publ. Bhalani Publishing House, Mumbai, India, 2006; pp 48,190,235,251,340,352,387,77.
13. Folin O, Ciocalteu V. On Tyrosine and Tryptophane determinations in proteins. *J Biol Chem*. 1927; 73:627-50.
14. Mallurwar VR, Pathak AK. Hepatoprotective and antioxidant activity of fresh aerial roots of *Ficus bengalensis*. *Ind J Nat Prod* 2006; 23:8-11.
15. Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J Agric Food Chem* 1995; 46:849-54.
16. Semwal BC, Shah K, Chauhan NS, Badha R, Divakar K. Anti-diabetic activity of stem bark of Berberis aristata D.C. in alloxan-induced diabetic rats. *Internet J Pharmacol* 2008; 6, November 1.

CURRENT AUTHOR ADDRESSES

J. K. Gupta, G.L.A., Institute of Pharmaceutical Research, Mathura , U.P. India. E-mail: jkgupta81@rediffmail.com (Corresponding author)

P. Mishra, G.L.A., Institute of Pharmaceutical Research, Mathura , U.P. India.

A. Rani, Arya College of Pharmacy, Jaipur, Rajasthan, India.

P. Mitra Mazumder, Birla Institute of Technology, Mesra, Ranchi, Jharkhand, India.