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

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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: Antidiabetic 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 homeostasis [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, 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...
temperature. Dried material was coarse powdered and packed in soxhlet apparatus and extracted with petroleum ether (60-80°C), chloroform (61°C) and methanol (65°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 humidity) in standard cage and maintained on standard pellets and water ad libitum. The test was carried out on small group of male wistar albino rats (150–200 g) and albino mice (25-30g) 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 single 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],

\[ C = \frac{cv}{m} \]

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

\[ C = \text{concentration of gallic acid (mg/ml from calibration curve)} \]

\[ v = \text{volume of extract (ml)} \]

\[ m = \text{wt of pure plant extract in gram} \]

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

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

Potassium ferricyanide (1 % w/v). The mixture was incubated at 50°C 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 %) were measured with spectrophotometer at 700 nm. The absorbance at 680 nm was used to estimate the concentration of reducing sugars. The total phenolic content was calculated using the standard curve of gallic acid. The total phenolic content of methanolic extract was 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.

Table 3. Total Phenolic Content of B. aristata stem bark (methanolic extract)

<table>
<thead>
<tr>
<th>Extract/Drug</th>
<th>Absorbance at 680 nm</th>
<th>Total Phenolic Content of dry extract (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanolic</td>
<td>0.304 0.305 0.305</td>
<td>0.305</td>
</tr>
<tr>
<td>Extract (10mg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gallic Acid</td>
<td>0.176 0.175 0.176</td>
<td>0.176</td>
</tr>
<tr>
<td>(50 µg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gallic Acid</td>
<td>0.458 0.460 0.460</td>
<td>0.460</td>
</tr>
<tr>
<td>(100 µg/ml)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 the known standard BHT.
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


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