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

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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.
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). All values are represented as Mean ± SEM.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Extract</th>
<th>LD50 in mice (mg/kg ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Petroleum ether extract</td>
<td>1710 ± 35</td>
</tr>
<tr>
<td>2</td>
<td>Chloroform extract</td>
<td>1330 ± 38</td>
</tr>
<tr>
<td>3</td>
<td>Methanolic extract</td>
<td>1540 ± 21</td>
</tr>
</tbody>
</table>

Mean ± SEM

**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)

\(c\) = 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...
Blood Glucose Lowering Potential of Stem Bark of *Berberis aristata* Dc in Alloxan-Induced Diabetic Rats

### 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>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td>Normal control</td>
<td>90.84 ± 1.21</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>169.15±2.12</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>170.41±1.15</td>
</tr>
<tr>
<td>Petroleum ether extract</td>
<td>180.45±1.15</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>174.12±1.28</td>
</tr>
<tr>
<td>Methanolic extract</td>
<td>171.14±1.54</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM compared to diabetic control group, significant *p* < 0.05 (significant), **p** < 0.01 (more significant) compared with diabetic control.

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial body weight 1s day</th>
<th>15s day</th>
<th>Blood glucose mg/kg</th>
<th>Urea mg/dl</th>
<th>Protein mg/dl</th>
<th>Cholesterol mg/dl</th>
<th>SGOT IU/dl</th>
<th>SGPT IU/dl</th>
<th>Total lipids mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>198.5 ± 3.02</td>
<td>205.8 ± 1.15</td>
<td>84.21 ± 5.17</td>
<td>31.91±1.18</td>
<td>258.12±5.52</td>
<td>135.10±1.05</td>
<td>71.50±2.25</td>
<td>46.15±2.20</td>
<td>150.50±5.25</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>185 ± 1.59</td>
<td>180.1 ± 2.25</td>
<td>258.12±5.52</td>
<td>135.10±1.05</td>
<td>258.12±5.52</td>
<td>135.10±1.05</td>
<td>71.50±2.25</td>
<td>46.15±2.20</td>
<td>150.50±5.25</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>185.5 ± 1.25</td>
<td>197.0 ± 2.95</td>
<td>103.14**±2.98</td>
<td>49.50**±3.15</td>
<td>3.15±0.81</td>
<td>78.50**±1.01</td>
<td>115.5**±3.17</td>
<td>175.10**±9.01</td>
<td></td>
</tr>
<tr>
<td>250 mg/kg</td>
<td>176.15±2.15</td>
<td>189.19±5.61</td>
<td>135.17**±2.15</td>
<td>85.14**±4.16</td>
<td>9.50±0.95</td>
<td>74.15**±3.15</td>
<td>155.15**±3.17</td>
<td>170.5±5.10</td>
<td></td>
</tr>
<tr>
<td>500 mg/kg</td>
<td>184.5±6.5</td>
<td>188.80±5.10</td>
<td>115.12**±6.12</td>
<td>58.12**±8.1</td>
<td>9.80±0.31</td>
<td>61.71**±1.95</td>
<td>141.5**±1.50</td>
<td>152.51**±5.15</td>
<td></td>
</tr>
</tbody>
</table>

Values are Mean ± SEM compared to diabetic control group, significant *p* = 0.05 and very significant **p** = 0.01.

### Results

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 % w/v) were mixed in the ratio 1:1:2 and the absorbance was 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.

### 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 *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.

### Table 4. 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>0.176 0.175 0.176</td>
<td>0.176</td>
</tr>
<tr>
<td>Gallic Acid</td>
<td>0.458 0.460 0.460</td>
<td>7.27</td>
</tr>
<tr>
<td>(50 µg/ml)</td>
<td>0.458 0.460 0.460</td>
<td>0.460</td>
</tr>
<tr>
<td>Gallic Acid</td>
<td>0.458 0.460 0.460</td>
<td>7.27</td>
</tr>
<tr>
<td>(100 µg/ml)</td>
<td>0.458 0.460 0.460</td>
<td>0.460</td>
</tr>
</tbody>
</table>
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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