Phytochemical, Antidiabetic, and Cytoprotective Properties of *Berberis aristata* DC. Root Extracts

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**Abstract:** The present study was aimed to evaluate the phytochemical, antidiabetic, and cytoprotective properties of *Berberis aristata* DC. (*Berberidaceae*) root extracts. After phytochemical evaluation of various extracts of *B. aristata* roots, the ethanol extract was found to be rich in phytochemicals with the presence of alkaloid determined by the thin layer chromatography; hence ethanol extract was selected for further study. Administration of ethanol extract of *B. aristata* roots in diabetic rats showed dose dependent reduction in hyperglycemia. The levels of serum total cholesterol, triglyceride, AST (aspartate aminotransferase), ALT (alanine aminotransferase), serum creatinine and blood urea were significantly decreased in diabetic rats when compared with diabetic control rats.

**Keywords:** Alloxan, antidiabetic, *Berberis aristata* DC., cytoprotective, glimepiride, phytochemical.

**INTRODUCTION**

Diabetes is rapidly emerging as a global health problem which could reach pandemic levels by 2030. The number of worldwide diabetes cases is projected to increase from 171 million in 2000 to 366 million in 2030. Diabetes mellitus (DM) is rapidly increasing in developing countries of the world, it is being recognized that the incidence and prevalence are increasing at an alarming rate in young person, particularly in obese children [1].

DM is one of the oldest common metabolic disorders that affect the whole body system. Several types of DM exist and result by a complex interaction of genes and drugs, gestational diabetes, environmental factors like stress and sedentary life-style choices. DM is the leading cause of end-stage renal disease, non-traumatic lower extremity amputations, and adult blindness. With increasing incidences worldwide, DM will likely continue to be a leading cause of morbidity and mortality for the foreseeable future [2].

From the ancient time, various ethnic and traditional plant medicines have been used to treat diabetes and some of them were clinically proven by various medicinal systems like Ayurveda and Chinese medicines. These herbal drugs were found to be effective in controlling blood glucose levels after thorough investigations and provide active hypoglycemic principles [3].

The world health organization (WHO) has also recommended the evaluation of the effectiveness for various plants’ treatments of disease conditions where we lack safe modern drugs [4]. Plants have long been a principal source of drugs and now many of the available drugs have been derived directly or indirectly from plants. More than 800 plants may possess anti-diabetic potential according to ethnobotanical information reports [5].

*Berberis aristata* DC. belongs to family Berberidaceae and widely distributed in evergreen regions of temperate and sub-tropical. Berberis has about 650 species worldwide, of which 54 have been reported from Indian Himalaya, especially in state of Himachal Pradesh. *Berberis aristata*, known as Daruhaldi, is a large deciduous shrub usually in 1.8–3.6 meter height. Its leaves are obovate or elliptic, entire, base gradually narrowed with reticulate nerves and glossy dark green color. Its flowers are numerous and stalked. Its roots are thick woody, yellowish brown, cylindrical, knotty and covered with a thin brittle bark [6] and have valuable isoquinoline alkaloid berberine. *Berberis aristata* is used in Ayurveda medicines from very long time. An important ayurvedic preparation Rashut is prepared by this plant [7]. Berberine has predominant clinical uses in bacterial diarrhoea, intestinal parasite infections, ocular trachoma infection [8], eye infection, skin diseases, jaundice, antifungal, antipyretic [9] and as antiarrhythmic, antiinflammatory, immunostimulative, antitumor [8], astringent, tonic, febrifuge, laxative and also for menorrhagia [10].

The objective for this study was to evaluate the phytochemical, antidiabetic, cytoprotective effect of *B. aristata* roots on diabetic rats.

**MATERIALS AND METHODOLOGY**

**Plant Material**

The roots of *B. aristata* were collected in November 2009 from higher regions of Mussoorie, Uttarakhand, India, and were identified and authenticated by botanist Dr. Veena at Forest Research Institute, Dehradun. A voucher specimen
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(voucher no. Dis/2010-Bot./4-12 herb) was deposited at Forest Research Institute Herbarium for future reference.

Extraction

The fresh roots were shade dried at room temperature (25 ± 2 °C) and then ground to a coarse powder. The powdered roots were extracted successively with petroleum ether, benzene, chloroform, methanol, ethanol, and water in a soxhlet assembly. Each extract solution was concentrated under reduced pressure in a rotor evaporator, the furnished concentrated extracts were stored in the air tight container.

Phytochemical Screening

The different solvent extracts of the roots of B. aristata were subjected to preliminary phytochemical screening for the identification of their chemical constituents using the method suggested by Harbone [11] as shown in Table 1.

TLC Profile

Thin layer chromatography (TLC) profile of the ethanol extract was done with the help of some selected solvent systems, detection reagents and wavelength as shown in Table 2 [12].

Animal

Male wistar albino rats (150-200 g, 6-8 week) were housed in spacious cage and allowed one week to adapt to their new environment. The animals were maintained in an environment of controlled temperature (25 ± 2 °C) under a 12 hr. light–dark cycle. For rats, standard rodent chow and water were provided throughout the experimental period. All animal procedures used were in strict accordance with the committee for the purpose of control and supervision of experiments on animals (CPCSEA) and all experimental protocols were approved by the institutional ethics committee.

Induction of Diabetes

Animals were made diabetic by a single intraperitoneal injection of alloxan monohydrate (Rolex chemical Limited, Mumbai). Experimental diabetes was induced by alloxan which was first weighed individually for each animal according to 120 mg/kg body weight (b. wt.) and then dissolved in sterile normal saline just prior to injection [13] and as diabetic control, standard and test groups respectively. After three days alloxan injection, rats with plasma glucose levels excess of 150 mg/dl were used in the study [14].

Experimental Design

Thirty male wistar rats were divided into five groups.

Group- I (Normal Control): Rats were given only vehicle (water) (diabetes free rats).

Group- II (Diabetic control): Rats were given alloxan monohydrate to induce diabetes (diabetic rats).

Group- III (Standard): Glimepiride (1mg/kg b. wt., Intas Pharmaceuticals Ltd.) was administered to alloxan monohydrate induced diabetic rats.

Table 1. Phytochemical Evaluation of Berberis aristata DC. Roots Extracts

<table>
<thead>
<tr>
<th>Tests</th>
<th>Pet. Ether</th>
<th>Chloroform</th>
<th>Methanol</th>
<th>Ethanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>- -</td>
<td>+ ++</td>
<td>++</td>
<td>+- + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>- -</td>
<td>- -</td>
<td>+ +</td>
<td>+ +</td>
<td>- -</td>
</tr>
<tr>
<td>Tannins</td>
<td>- -</td>
<td>- -</td>
<td>+ +</td>
<td>+ +</td>
<td>- -</td>
</tr>
<tr>
<td>Protein &amp; Amino acid</td>
<td>- -</td>
<td>+ +</td>
<td>+ +</td>
<td>+ +</td>
<td>- -</td>
</tr>
<tr>
<td>Glycosides</td>
<td>- -</td>
<td>+ +</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>- -</td>
<td>+ +</td>
<td>- -</td>
<td>+ +</td>
<td>+ +</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>- -</td>
<td>+ +</td>
<td>+ +</td>
<td>+ +</td>
<td>+ +</td>
</tr>
<tr>
<td>Volatile oils</td>
<td>+ +</td>
<td>- -</td>
<td>+ +</td>
<td>+ +</td>
<td>- -</td>
</tr>
<tr>
<td>Fixed oils &amp; fats</td>
<td>+ + +</td>
<td>- -</td>
<td>+ +</td>
<td>+ +</td>
<td>- -</td>
</tr>
<tr>
<td>Saponin</td>
<td>- -</td>
<td>+ +</td>
<td>- -</td>
<td>- -</td>
<td>+ +</td>
</tr>
</tbody>
</table>

pH (1%) 7.6

+++ positive result, - - negative result

Table 2. TLC Profile of Ethanol Extract of Berberis aristata DC. Roots

<table>
<thead>
<tr>
<th>Solvent system</th>
<th>Extracts</th>
<th>Rf value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclohexane:Chloroform:Glacial acetic acid (45:45:10)</td>
<td>Ethanol</td>
<td>0.70, 0.73</td>
</tr>
<tr>
<td>Toluene:Ethylacetate:Diethylamine (70:20:10)</td>
<td>Ethanol</td>
<td>0.61, 0.67</td>
</tr>
</tbody>
</table>

Detection reagent: Dragendroffs reagent, 5% sulphuric acid; Detection: UV 365 nm.
Group- IV (Test 1): Ethanol extract of *B. aristata* roots (50 mg/kg b. wt.) was administered to alloxan monohydrate induced diabetic rats.

Group- V (Test 2): Ethanol extract of *B. aristata* roots (100 mg/kg b. wt.) was administered to alloxan monohydrate induced diabetic rats.

Treatment with the standard drug glimepiride and the ethanol extracts were started 72 hr. after alloxan injection. Blood samples were collected at 1st, 10, 20, and 30th day intervals through retro-orbital route. Fasting blood glucose estimation was done on the 1st, 10, 20, and 30th day of the study.

**Estimation of Biochemical Parameter**

Blood samples from animals were collected on 1st and 30th day of study for serum total cholesterol, serum triglycerides, and serum creatinine, blood urea, AST and ALT. All these values were estimated by commercially available kits (Span diagnostic Pvt. Ltd. Surat, India).

**Statistical Analysis**

All the values of body weight, blood sugar and biochemical estimations were expressed as mean ± S.E.M. (Standard Error Mean) and analyzed with SigmaStat® software for ANOVA & Tukey’s test. Differences between groups were considered significant at *p*<0.05 levels.

**RESULTS AND DISCUSSION**

**Phytochemical Evaluation**

This study found after phytochemical screening that the ethanol extract of *B. aristata* roots contains maximum phytoconstituents with the presence of carbohydrate, alkaloid, tannins, phytosterols, flavonoid, volatile oils, fixed oils and fats as shown in the Table 1. On the basis of phytochemical screening the ethanol extract was selected for...
Antihyperlipidemic Effect

Diabetes nephropathy is a common complication associated with diabetes. As seen in our study after 30 days the levels of serum creatinine and blood urea were increased in diabetic control indicating the kidney damage. The levels of serum creatinine and blood urea were controlled significantly ($p<0.05$) in the groups treated with 50 mg/kg and 100 mg/kg b. wt. dose of ethanol extract when compared with diabetic control rats (Table 4). The results showed the dose dependent effect in controlling serum creatinine and blood urea.

### Table 4. Cytoprotective and Antilipidemic Effect Ethanol Extract of Berberis aristata DC. Roots

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal</th>
<th>Diabetic control</th>
<th>B.aristata Extract (50 mg/kg body wt.)</th>
<th>B.aristata Extract (100 mg/kg body wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood urea (mg/dl)</td>
<td>31.80 ± 0.39</td>
<td>95.77 ± 1.07</td>
<td>71.46 ± 1.27$^a$</td>
<td>59.85 ± 0.45$^a$</td>
</tr>
<tr>
<td>S.Creatinine (mg/dl)</td>
<td>0.48 ± 0.01</td>
<td>1.48 ± 0.01</td>
<td>0.77 ± 0.01$^a$</td>
<td>0.58 ± 0.001$^a$</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>37.40 ± 0.56</td>
<td>99.15 ± 1.01</td>
<td>39.13 ± 0.59$^a$</td>
<td>34.90 ± 0.44$^a$</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>47.46 ± 0.65</td>
<td>107.52 ± 0.54</td>
<td>70.70 ± 0.78$^a$</td>
<td>51.68 ± 0.34$^a$</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>66.40 ± 0.61</td>
<td>183.22 ± 0.45</td>
<td>68.86 ± 0.38$^a$</td>
<td>55.43 ± 0.33$^a$</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>87.42 ± 1.39</td>
<td>150.70 ± 1.51</td>
<td>64.27 ± 0.60$^a$</td>
<td>58.26 ± 0.30$^a$</td>
</tr>
</tbody>
</table>

Effect of ethanol extract of *B. aristata* roots on serum profile in alloxan induced diabetic wistar rats after 30 days of treatment. Values are given as mean ± S.E.M. for groups of six animals; $^a$: statistically significant Vs Diabetic control $p<0.05$; one way ANOVA Tukey’s test.

CONCLUSION

Our study revealed that ethanol extract of *B. aristata* has antidiabetic activity because it has significant dose dependent reduction effect on the blood glucose levels of diabetic rats. This study also revealed that this ethanol extract decreases the levels of some biochemical parameters such as AST, ALT, total cholesterol, triglyceride, creatinine, and blood urea, which always be found to be increased in diabetic rats. Therefore, the ethanol root extract of *B. aristata* would be beneficial for the patients with diabetes because of its antidiabetic and cytoprotective activities.

### ABBREVIATIONS

- ALT = Alanine aminotransferase
- AST = Aspartate aminotransferase
- CPCSEA = Committee for the purpose of control and supervision of experiments on animals
- DM = Diabetes mellitus
- TLC = Thin layer chromatography
- WHO = World Health Organization

### Footnotes

1. WHO = World Health Organization
2. DM = Diabetes mellitus
3. ALT = Alanine aminotransferase
4. AST = Aspartate aminotransferase
5. CPCSEA = Committee for the purpose of control and supervision of experiments on animals
6. TLC = Thin layer chromatography
7. DC. Roots = Decaneolaceous roots
8. WHO = World Health Organization
REFERENCES


