Anti-diabetic activity of stem bark of Berberis aristata D.C. in alloxan induced diabetic rats
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Citation

Abstract
Object: To evaluate antidiabetic activity of stem bark of Berberis aristata D.C. (Berberidaceae) in alloxan induced diabetic rats.
Materials and Method: Male Wistar albino rats (150–250 g) were taken and study for glucose tolerance test and alloxan induced diabetic rats. The ethanolic extract of B. aristata were selected for antidiabetic activity. Blood glucose levels were estimated in all groups on 1st, 4th, 7th and 15th day of the treatment with B. aristata. The biochemical parameters and body weight were estimated all treated groups and compared against diabetic control group.
Results: The ethanolic extract of stem bark of B. aristata showed a significant hyperglycemic effect in alloxan induced diabetic rats. It reduces blood glucose level 60.4% and 75.46 % at the dose of 25 mg/kg and 50 mg/kg in diabetic rats. B. aristata has a significant antidiabetic activity in glucose tolerance test
Conclusion: The results justify the traditional use of bark in the treatment of diabetes.

INTRODUCTION
Diabetes mellitus is one of the common metabolic disorders with micro and macro vascular complications that results in significant morbidity and mortality. It is considered as one of the five leading causes of death in the world, About 150 million or 1.3% people are suffering from diabetes world wide which is almost five times more than the estimates ten years ago and this may double by the year 2030. Diabetes was discovered as early as 700-200 BC; until the time insulin was invented, this disorder was managed principally by the traditional practices by using medicinal plants. 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), Ocimum santum (Tulsi) many of these are less effective in lowering glucose levels in severe diabetes.

Berberis aristata D.C. (Berberidaceae) is an edible plant commonly used in ayurvedic system of medicine as in diarrhoea, hypoglycemic, anticancer, gastro-irritant, antipyretic, hypotensive, CNS depressant and diaphoretic. However no scientific study on anti-diabetic activity of this plant has been reported. The present investigation was undertaken to study the anti-diabetic activity of stem bark of B. aristata in alloxan induced diabetic rats.

MATERIALS AND METHODS
PLANT MATERIAL
Plant material used in this study consisted of the stem bark of B. aristata, collected from the local area of Rishikesh, Uttrakhand. The plant was authenticated by Dr. H. J. Chaudhary, Director of Botanical Survey of India, Govt. of India, Dehradun Bsi/Nc/6(4)/06-07. The stem bark was dried in shade at room temperature. Dried stem bark was coarsely powdered by using grinder, and latter was packed into soxhlet column and that was defatted with petroleum ether then extracted with ethanolic and dried in vacuum. The presence of alkaloids were confirmed by qualitative examination.

ANIMALS
Male Wistar albino rats (150–250 g) housed in a spacious cage for ten days after obtaining approval from 'Institutes Ethical Committee' 997/c/06/CPCSEA. During the experiments rats were fed with standard pellet diet. After randomization into various groups, the rats were acclimatized for 2–3 days in environment before initiation of experiment.
GLUCOSE TOLERANCE TEST
Rats were divided into four groups containing six animals in each group. All animals fasted before treatment. Group I was kept as vehicle control which received 5% Tween 80 p.o., group II received glucose only, group III received ethanolic extract 25 mg/kg and group IV received only extract only in a vehicle respectively. The rats of group II and III were loaded with glucose (3 g/kg, p.o.) 30 min after drug administration. Blood samples were collected from puncturing the retro orbital sinus just prior to drug administration, and 30, 90, 150 min after loading glucose. Serum glucose level was measured immediately by using glucose estimation kit (Span Diagnostic Pvt. Ltd. Surat, India) 8.

INDUCTION OF DIABETES
All the animals were randomly divided into five groups with six animals in each group. Group I was used as control. Group II, III, IV and V were made diabetic by single intraperitoneal injection of alloxan monohydrate (125 mg/kg; Rollex, India) and served as diabetic control, standard and treatment groups respectively. Rats exhibited in plasma glucose levels >250 mg/dl, 48 h after administration of alloxan were included in the study. Treatment for diabetes (B. aristata extract 25 and 50 mg/kg / p.o.) was started from 7th day of alloxan administration.

SAMPLE COLLECTION
Blood samples were collected retro-orbitally from the inner canthus of the eye under light ether anesthesia using capillary tubes (Micro Hematocrit Capillaries, Mucaps). Blood was collected in fresh micro centrifuge tube and plasma separated in a T8 electric centrifuger (Remi Udyog, New Delhi) at 2000 rpm for 15 min.

COLLECTION OF LIVER
After 15 days of daily feeding of extracts orally the animals were killed by decapitation liver was collected.

ESTIMATION OF BIOCHEMICAL PARAMETER
Serum glucose, serum cholesterol serum total lipids, serum protein, serum urea, SGOT (Serum glutamate oxaloacetate transaminase) and SGPT (Serum glutamate pyruvate transaminase) were estimated by commercially available kits (Span Diagnostic Pvt. Ltd. Surat, India). Liver glycogen content was estimated by the method of Carrol et al.,1955 9.

STATISTICAL ANALYSIS
All results were expressed as the mean ± Standard Deviation (S.D) Statistical analysis was performed with Graph Pad Instat software (version 3.00, Graph Pad Software, San Diego, California, USA) by using one way analysis of variance (ANOVA) comparison was done by using Dunnett test. P value <0.05 was considered as significant.

RESULTS

GLUCOSE TOLERANCE
The effect of extract of B. aristata (25 mg/kg) on glucose tolerance test are shown in Table 1. The supplementation of B. aristata improved the glucose tolerance in the fasted normal rats. After that serum glucose level was lowered significantly (P<0.05) at 90 mins and varied significantly (P<0.01) lowered at 150 mins. Extract also showed significant hypoglycemic effect after 90 mins of treatment.

Table 1: Effect of ethanolic extract ethanolic extract on glucose tolerance test

<table>
<thead>
<tr>
<th>S. No</th>
<th>Groups</th>
<th>Time(min)</th>
<th>30</th>
<th>50</th>
<th>150</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control Group</td>
<td></td>
<td>67.2±2.2</td>
<td>68.4±3.4</td>
<td>67.2±2.6</td>
</tr>
<tr>
<td>2</td>
<td>Glucose</td>
<td></td>
<td>65.4±2.4</td>
<td>100.4±2.4</td>
<td>100.4±3.6</td>
</tr>
<tr>
<td>3</td>
<td>Ethanolic extract 25 mg/kg</td>
<td></td>
<td>65.2±3.2</td>
<td>100.4±7.2</td>
<td>69.2±4.2</td>
</tr>
<tr>
<td>4</td>
<td>Ethanolic extract 50 mg/kg</td>
<td></td>
<td>66.2±3.4</td>
<td>58.2±3.2</td>
<td>56.2±4.2**</td>
</tr>
</tbody>
</table>

Values are mean ± SD of 6 in each group. Compared to control group
Very significant **P<0.01

EFFECT ON ALLOXAN-INDUCED DIABETIC RATS
Administration of alloxan monohydrates (125 mg/kg) led to elevation of blood glucose level. The anti-hyperglycemic effect of the ethanolic extract of stem bark of B. aristata (25 mg/kg, 50 mg/kg) and glibenclamide (5 mg/kg) on blood sugar levels of diabetic rats were shown in Table 2. The antidiabetic effect of the B. aristata extract 25 mg/kg, 50 mg/kg and Glibenclamide 5 mg/kg exhibited effect in dose dependent manner. The percent reduction of hyperglycemia was insignificant on the 1st day of the treatment by B. aristata (25, 50 mg/kg) but it was reduced very significantly by 41% on 1st day of glibenclamide (5 mg/kg) treatment. The percent reduction of hyperglycemia was significant (P<0.01) on 4th, 7th and 15th day after treatment of the 25 mg/kg of B. aristata which was 36.6, 40.4, 60.4 % respectively. The percent reduction of hyperglycemia was significant (P<0.01) on 4th, 7th and 15th day after treatment with the 50 mg/kg of B. aristata which was 43.30, 61.40,
75.46% respectively. The percent reduction of blood glucose levels were significant (P<0.01) 45, 61.50, 74.3% on 4th, 7th, and 15th day respectively after treatment by glibenclamide 5 mg/kg (Table 2).

**Figure 2**

Table 2: Effect of ethanolic extract on serum glucose level diabetic rats

<table>
<thead>
<tr>
<th>S. No</th>
<th>Groups</th>
<th>Days</th>
<th>Serum glucose level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>1</td>
<td>Normal control</td>
<td>66.6±4.2</td>
<td>69.2±3.2</td>
</tr>
<tr>
<td>2</td>
<td>Diabetic control</td>
<td>260.2±14</td>
<td>266.9±3.8</td>
</tr>
<tr>
<td>3</td>
<td>Glibenclamide 5 mg/kg</td>
<td>254.4±2.4</td>
<td>150.2±4.4</td>
</tr>
<tr>
<td>4</td>
<td>Ethanolic extract 25 mg/kg</td>
<td>253.8±3.2</td>
<td>260.4±3.4</td>
</tr>
<tr>
<td>5</td>
<td>Ethanolic extract 50 mg/kg</td>
<td>258.2±2.2</td>
<td>252.3±4.6</td>
</tr>
</tbody>
</table>

(EFFECT ON SERUM UREA, PROTEIN, CHOLESTEROL, TOTAL LIPIDS, SGOT AND SGPT, BODY WEIGHT AND LIVER GLYCOGEN)

At the end of 15 days of treatment the body weight of the untreated diabetic rats was found to be significantly decreased and significant increase in the liver glycogen compared with the control rats. The administration of B. aristata and glibenclamide to diabetic rats restored the changes in the body weight, liver glycogen to near normal levels.

The normal function of the kidney was assessed as blood urea level in normal, diabetic and treated animals and it was altered from 31.83 mg/dl (normal) against 131.51 mg/dl (diabetic control) after the 15th day of alloxan treatment. The results of serum urea level indicates that the animals administered with B. aristata (25 and 50 mg/kg) in diabetic rats reduced the urea levels dose dependently (P<0.01). The percent reduction of blood urea level was 34.4 and 58.31 with the treatment of ethanolic extract of stem bark of B. aristata at the dose of 25 and 50 mg/kg respectively and glibenclamide was reduced 68.3% in comparison with the diabetic control group. The blood urea and protein levels were reduced significantly (P<0.01) but which was slightly lesser in comparison with standard.

The treatment of alloxan induced diabetic rats by B. aristata decreased SGOT and SGPT levels significantly (P<0.01) and their reduction was slightly higher than the standard, which indicates the prevention of gluconeogenesis, ketogenesis and normal liver function. The percent reduction of SGPT was 61.52 and 63.46 and SGOT was 42.77 and 55.45 after the treatment of ethanolic extract of stem bark of B. aristata at the dose of 25 and 50 mg/kg respectively and glibenclamide which reduced 57.68% of SGPT and 50.93% of SGOT in comparison with the diabetic group (Table 3A and 3B).
Anti-diabetic activity of stem bark of *Berberis aristata* D.C. in alloxan induced diabetic rats

**DISCUSSION**

Management of diabetes with the agents devoid of any side effects is still a challenge to the medical system. This concern has led to an increase and demand for natural products with antihyperglycaemic activity having fewer side effects. Indian traditional medicine is one of the richest medicinal systems among those available around the world. *B. aristata* is folklore medicine used in the treatment of diabetes in Sikkim and Darjiling (India). Our results support its use as folklore medicine for treating diabetes. The ethanolic extract of stem bark of *B. aristata* exhibited dose dependent antidiabetic property. The antidiabetic effect of *B. aristata* at the dose of 50 mg/kg is even slightly higher than glibenclamide 5 mg/kg. It also exhibited potential glucose tolerance effect.

Plants may act on blood glucose through different mechanisms, some of them may have insulin-like substances, stimulation of β-cells to produce more insulin, and others may increase α-cells in the pancreas by activating regeneration of pancreatic cells. The fiber of plants may also interfere with carbohydrate absorption; thereby affecting blood glucose. The ethanolic extract of stem bark of *B. aristata* also inhibited hepatic gluconeogenesis in terms of prevention of proteolysis and lipolysis. The levels of total serum cholesterol, triglycerides and cholesterol were raised in diabetic rats but which were lowered significantly with the treatment of *B. aristata*. The total lipid content reduction in the dose of (30 mg/kg) of *B. aristata* treated group is greater than glibenclamide. It indicates that the ethanolic 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.

Ghosh and Suryavanshi, 2001 observed elevation in transaminase activity (SGOT and SGPT) in liver and kidney in diabetic rats. Increased gluconeogenesis and ketogenesis observed in diabetes may be due to the high level in the activities of these transaminases. The restoration of SGOT and SGPT to their respective normal levels after treatment with both glibenclamide and alcoholic extract of *B. aristata* further strengthen the antidiabetogenic effect of this extract. Moreover, SGOT and SGPT levels also act as indicators of liver function and restoration of normal levels of these parameters indicate normal functioning of liver. Since the alloxan can also affect the liver by free radical mechanism.

Moreover, improvement of body weight of the extract treated animal further supports the antidiabetogenic effect of *B. aristata* extract as diabetic condition is associated with loss of body weight. Other possible mechanisms for antidiabetic potential of *B. aristata* is that it may contain berberine. Berberine may act as an alphaglucosidase inhibitor, which is its main mechanism in diabetes treatment. The inhibitory effect of berberine on diabetes might be associated with its hypoglycemic effect, modulating lipids metabolic effects and its ability to scavenge free radical. However, the hypoglycemic effect could also be due to inhibition of intestinal glucose absorption or stimulation of peripheral glucose uptake. Berberine activates AMPK activity in both adipocytes and myocytes, and within these cells type's berberine induces a variety of metabolic effects consistent with AMPK activation. These include activation of GLUT4 translocation; increased phosphorylation of

### Table 3A: Effect of ethanol extract of stem bark on biochemical parameter in alloxan induced diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
<th>Glucose (mg/dL)</th>
<th>Protein (mg/dL)</th>
<th>Cholesterol (mg/dL)</th>
<th>Total lipids (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>30.6±0.8</td>
<td>25.9±2.9</td>
<td>31.3±5.5</td>
<td>51.1±0.5</td>
<td>232±10</td>
<td>403±14</td>
</tr>
<tr>
<td>Diabetic</td>
<td>30.4±0.6</td>
<td>23.6±2.3</td>
<td>54.6±5.5**</td>
<td>54.6±5.5**</td>
<td>250±10</td>
<td>403±14</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>40.3±0.6</td>
<td>20.3±2.3</td>
<td>34.6±5.5</td>
<td>54.6±5.5**</td>
<td>250±10</td>
<td>403±14</td>
</tr>
<tr>
<td>L. aristata</td>
<td>40.3±0.6</td>
<td>20.3±2.3</td>
<td>34.6±5.5</td>
<td>54.6±5.5**</td>
<td>250±10</td>
<td>403±14</td>
</tr>
<tr>
<td>H. aristata</td>
<td>40.3±0.6</td>
<td>20.3±2.3</td>
<td>34.6±5.5</td>
<td>54.6±5.5**</td>
<td>250±10</td>
<td>403±14</td>
</tr>
</tbody>
</table>

Values are mean ±SD except in each group. Compared to diabetic control group very significant **P<0.01 significant *P<0.05

### Table 3B: Effect of ethanol extract of stem bark on biochemical parameter in alloxan induced diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver</th>
<th>GGT (IU/L)</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>30.6±0.8</td>
<td>31.3±5.5</td>
<td>31.3±5.5</td>
<td>31.3±5.5</td>
</tr>
<tr>
<td>Diabetic</td>
<td>30.4±0.6</td>
<td>54.6±5.5**</td>
<td>54.6±5.5**</td>
<td>54.6±5.5**</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>40.3±0.6</td>
<td>250±10</td>
<td>403±14</td>
<td></td>
</tr>
<tr>
<td>L. aristata</td>
<td>40.3±0.6</td>
<td>250±10</td>
<td>403±14</td>
<td></td>
</tr>
<tr>
<td>H. aristata</td>
<td>40.3±0.6</td>
<td>250±10</td>
<td>403±14</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ±SD except in each group. Compared to diabetic control group very significant **P<0.01 significant *P<0.05
AMPK, ACC, and p38 MAPK; reduced lipid content in adipocytes; increased expression of genes involved in lipid oxidation; and decreased expression of genes involved in lipid synthesis.

The data of our studies suggests that B. aristata has beneficial effects in diabetes mellitus holding the hope of a new generation for antihyperglycaemic drugs.

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