Research

Effect of Ethanolic Extract of *Embelia ribes* on Dyslipidemia in Diabetic Rats

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Diabetes mellitus has been treated orally with herbal remedies based on folk medicine since ancient times. *Embelia ribes* burm (Myrsinaceae), known commonly as vidanga, was used in Ayurveda for its anthelmintic activity. Ayurveda describes vidanga as pungent, causes increase in digestive fire, and cures flatulence and colic. A single study reported the antihyperglycemic activity of decoction of *E. ribes* in glucose-induced hyperglycemic albino rabbits. In the present study, the lipid-lowering and antioxidant potential of ethanolic extract of *E. ribes* burm was investigated in streptozotocin (40 mg/kg, IV, single injection)-induced diabetes in rats. Twenty days of orally feeding the extract (200 mg/kg) to diabetic rats resulted in significant (*P* < 0.01) decrease in blood glucose, serum total cholesterol, and triglycerides, and increase in HDL-cholesterol levels when compared to pathogenic diabetic rats. Further, the extract also lowered the liver and pancreas thiobarbituric acid–reactive substances (TBARSs) values (*P* < 0.01) when compared to TBARS values of liver and pancreas of pathogenic diabetic rats. The results of test drug were comparable to gliclazide (25 mg/kg, orally), a standard antihyperglycemic agent. This is the first pilot study to provide biochemical evidence of potential of *E. ribes* in diabetic dyslipidemia.

**Keywords** Diabetes; Dyslipidemia; *Embelia ribes*; Lipid Peroxidation; Streptozotocin

Improved control of hyperglycemia does moderate diabetes-associated dyslipidemia; therefore, lipid-modifying treatment is warranted in many diabetic patients. There is also considerable evidence that oxidative damage is increased in diabetes, though the mechanisms are not clear [2, 3].

Efforts continue in the field of medicine to find insulin substitutes from synthetic or plant sources for the treatment of diabetes. In traditional medicine, several medicinal plants or their extracts have been used to treat diabetes [4].

*Embelia ribes* burm (family, Myrsinaceae), known commonly as vidanga, is used in Ayurveda as anthelmintic [5]. Ayurveda describes vidanga as pungent, causes increase in digestive fire, and cures flatulence and colic. One Ayurvedic formulation, vidangadya curna (powder of vidanga), containing vidanga as main ingredient is taken with honey to alleviate obesity [6]. In a preliminary study, Tripathi [7] reported the antihyperglycemic activity of decoction of the *E. ribes* fruits in glucose-fed albino rabbits. The present study was undertaken to investigate the effect of ethanolic extract of *E. ribes* on diabetic dyslipidemia induced by streptozotocin (STZ) in wistar rats.

**MATERIALS AND METHODS**

**Preparation of the Extract**

Dried *E. ribes* fruits, 200 g, were purchased locally from a grocery shop in New Delhi, India (in India, it is commonly available) and authenticated by a pharmacognosist, Prof. Mohd. Ali in our institute. A voucher specimen was retained in the department (UB # 04).

The fruits were soxhlet extracted with 90% ethanol in a soxhlet apparatus for 72 hours. The solvent was removed under reduced pressure to give a dry extract, 7% yield w/w (with respect
to the crude material) and dose equivalent to 200 mg of the crude
drug per kilogram body weight was calculated, and suspended
in 2% \( v/v \) Tween 80 solution for the experiment.

**Experimental Induction of Diabetes in Rats**

Experiments on animals were conducted after obtaining ap-
proval from Hamdard University Animal Ethics Committee,
which is registered with Committee for the Purpose of Con-
trol and Supervision of Experiments on Animals (CPCSEA),
Government of India, India (Registration no. 173/CPCSEA,
dated 28 January, 2000).

Wistar rats of either sex (150 to 200 g) were obtained from the
central animal house facility of Hamdard University of Delhi.
They were acclimatized in an air-conditioned room at 22\(^\circ\)C ±
2\(^\circ\)C for 7 days and provided with free access to food (Gold
Mohur rat pellet diet, Lipton India, Bangalore, India) and water.

After fasting for 18 hours, the rats were injected intravenously
through tail vein with a single dose of 40 mg/kg STZ (Sigma,
St. Louis, MO, USA), freshly dissolved in citrate buffer (pH 4.5).
After injection, the rats had free access to food and water and
were given 5% glucose solution to drink overnight to counter
hypoglycemic shock. Diabetes in rats was identified by moderate
polydipsia and marked polyuria.

After 3 days, the fasting blood glucose levels were determined
by \( \text{ortho-toluidine method} \) \([8, 9]\). The rats showing fasting blood
sugar more than 200 mg/100 dL were considered diabetic and
selected for the experimentation \([10, 11]\).

**Experimental Procedure**

Normal and diabetic rats (\( n = 10 \) each) were randomly di-
vided into 4 groups of 10 rats each: Group I, normal healthy
control; group II, pathogenic diabetic control (STZ treated only);
group III, STZ + ethanolic \( E. \) ribes extract treated (200 mg/kg);
group IV, STZ + gliclazide treated (25 mg/kg), a standard con-
trol. The test and standard drug were fed orally for 20 days.
Groups I and II rats received 2% Tween 80 solution orally once
a day for 20 days.

**Blood Collection and Biochemical Estimations**

On the 21st day, fasting blood samples were collected from
tail vein of all the groups of rats. Whole blood was collected
for estimation of blood glucose \([8, 9]\). Serum was separated
for the estimation of total serum cholesterol \([12]\), high-density
lipoprotein (HDL)-cholesterol \([13]\), and triglycerides \([14]\).

**Measurement of Tissue Lipid Peroxidation**

On the evening of the 21st day, all fasted rats were killed by
decapitation under light ether anaesthesia. Liver and pancreas
were removed immediately and washed with ice-cold normal
saline. Ten-percent homogenate of above tissues were prepared
separately at 10,000 rpm in cooling centrifuge. Supernatant
thus obtained was used for measurement of thiobarbituric acid–
reactive substances (TBARSs), which can be measured by the
formation of malondialdehyde (MDA) after the breakdown of
polyunsaturated fatty acids \([15]\). Liver and pancreas protein con-
tents were evaluated by the method of Lowry using bovine serum
albumin (BSA) as standard \([16]\).

**Statistical Analysis**

The results are presented as mean ± SEM using 1-way anal-
ysis of variance test (ANOVA) followed by Dunnett’s \( t \) test. \( P < \)
0.01 was considered significant.

**RESULTS**

The mean blood glucose levels in rats fed on normal diet
(group I) alone was stable throughout the experimental period.
Conversely, in the STZ-treated group, group II, there was signif-
cicant rise in blood glucose level, as compared to group I. Drug
treatment in STZ-treated rats for 20 days significantly reduced
\( (P < 0.01) \) blood glucose levels in groups III and IV when com-
pared to pathogenic group II rats (Table 1).

The ethanolic extract of \( E. \) ribes also significantly \( (P < 0.01) \)
reduced serum total cholesterol and triglycerides and increased

**TABLE 1**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose (mg/dl)</th>
<th>Serum total cholesterol (mg/dl)</th>
<th>Serum HDL cholesterol (mg/dl)</th>
<th>Serum triglycerides (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Normal control</td>
<td>92.1 ± 6.0</td>
<td>69.1 ± 3.4</td>
<td>45.7 ± 1.4</td>
<td>51.9 ± 3.9</td>
</tr>
<tr>
<td>II STZ (40 mg/kg, IV)</td>
<td>573.9 ± 29.6*</td>
<td>101.1 ± 2.47*</td>
<td>33.2 ± 4.39*</td>
<td>123.1 ± 4.5*</td>
</tr>
<tr>
<td>III STZ + Ethanolic ( E. ) ribes extract</td>
<td>243.9 ± 41.3*</td>
<td>81.5 ± 1.25*</td>
<td>69.2 ± 4.0*</td>
<td>56.9 ± 4.9*</td>
</tr>
<tr>
<td>(200 mg/kg, PO)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV STZ + gliclazide (25 mg/kg/day, PO)</td>
<td>248.1 ± 25.8*</td>
<td>87.2 ± 1.5*</td>
<td>53.9 ± 3.7*</td>
<td>67.0 ± 3.0*</td>
</tr>
</tbody>
</table>

*Note. Values are mean ± SEM (\( n = 10 \)).

*\( P < 0.01 \) when compared with group I; \( \# P < 0.01 \) when compared with group II.*
the HDL-cholesterol levels as compared to pathogenic diabetic rats, i.e., Group II. Furthermore, the results of the test drug were comparable to gliclazide, a standard antihyperglycemic agent. There was no significant change in food consumption during the administration of ethanolic extract of E. ribes in dose of 200 mg/kg. However, the experimental animals showed marked polyuria and moderate polydipsia.

STZ treatment also induced a statistically significant increase in liver and pancreas lipid peroxide levels ($P < 0.01$) as compared to group I. E. ribes and gliclazide treatments lowered the liver and pancreas TBARS values ($P < 0.01$) as compared to group II diabetic rats (Table 2).

**DISCUSSION**

Cardiovascular diseases constitute the main cause of morbidity and mortality in diabetes mellitus. Diabetic individuals have a 2- to 4-fold increased risk of clinical atherosclerotic disease [17]. Dyslipidemia has been proven to be the most important modifiable risk factor contributing to atherosclerosis in diabetes [18]. Furthermore, there is widespread acceptance of a possible role for reactive oxygen species, generated as a result of hyperglycemia, in causing many of the secondary complications of diabetes, such as nephropathy, retinopathy, and neuropathy [19].

The inability of the modern synthetic approach to provide a satisfactory answer has led to a shift in focus to alternative forms of therapy based on drugs derived from plants. The present study was an effort to investigate the effect of ethanolic extract of E. ribes on diabetic dyslipidemia induced by STZ in rats. The study revealed the significant antihyperglycemic activity ($P < 0.01$) of ethanolic extract of E. ribes. Furthermore, extract treatment also produced significant fall in serum total cholesterol and triglyceride levels, indicating profound lipid-lowering activity of the test drug. The study also indicated the presence of antioxidant principles in the extract.

In conclusion, the present study shows that increased oxidative stress is apparent in STZ-induced diabetic animals. The ethanolic extract of E. ribes can protect tissues from lipid peroxidation. The extract also exhibits a significant lipid-lowering activity in these rats. Further studies are being undertaken to explain more fully the mechanism(s) of the lipid-lowering and antioxidant effects of E. ribes.

**REFERENCES**


**TABLE 2**

Effect of ethanolic extract of E. ribes on tissues lipid peroxidation in STZ-diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lipid peroxides (mol/mg protein) in liver</th>
<th>Lipid peroxides (mol/mg protein) in pancreas</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Normal control</td>
<td>0.0441 ± 0.008</td>
<td>0.0303 ± 0.008</td>
</tr>
<tr>
<td>II STZ diabetic control (40 mg/kg, IV)</td>
<td>0.1861 ± 0.007*</td>
<td>0.2019 ± 0.010*</td>
</tr>
<tr>
<td>III STZ + ethanolic E. ribes extract (200 mg/kg)</td>
<td>0.0798 ± 0.001#</td>
<td>0.0883 ± 0.006#</td>
</tr>
<tr>
<td>IV STZ + gliclazide (25 mg/kg)</td>
<td>0.0635 ± 0.001#</td>
<td>0.0811 ± 0.006#</td>
</tr>
</tbody>
</table>

*Note. Values are mean ± SEM (n = 10).

*P < 0.01 when compared with group I; #P < 0.01 when compared with group II.


