Antihyperglycaemic activity of aqueous extract of *Embelia ribes* Burm in streptozotocin-induced diabetic rats

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Forty days of orally feeding the aqueous *E. ribes* extract (100 and 200 mg/kg) to streptozotocin (40 mg/kg, iv, single dose) induced diabetic rats produced significant decrease in heart rate, systolic blood pressure, blood glucose, blood glycosylated hemoglobin, serum lactate dehydrogenase, creatine kinase and increase in blood glutathione levels as compared to pathogenic diabetic rats. Further, the extract significantly decreased the levels of pancreatic lipid peroxides and increased the levels of pancreatic superoxide dismutase, catalase and glutathione. The results suggest that aqueous *E. ribes* extract exhibits a significant blood glucose and blood pressure lowering potential. Further, it enhances endogenous antioxidant defense against free radicals produced under hyperglycaemic conditions, thereby, seemingly protects the pancreatic β-cells against loss in streptozotocin induced diabetic rats.

**Keywords**: Blood pressure, Diabetes mellitus, *Embelia ribes*, Streptozotocin

Diabetes mellitus (DM) is a heterogenous metabolic disorder characterized by hyperglycaemia resulting from defective insulin secretion, resistance to insulin action or both. Type 2 diabetes usually occurs in obese individuals and is associated with hypertension and dyslipidemia. The capacity of nutrients to stimulate insulin release from the pancreatic β-cell reflects their capacity to augment oxidative fluxes in the islet cells. Also, oxidant stress associated with insulin resistance and non-insulin-dependent diabetes mellitus contributes to poor insulin action. Thus, the treatment aims to reduce insulin resistance (diet, exercise and drug therapy) and to stimulate insulin secretion. In DM, oxidative stress seems mainly to be due to an increased production of free radicals and/or a sharp reduction of antioxidant defenses. Jang et al. found that increased oxidative stress involved in the pathogenesis and progression of diabetic tissue damage. Further, there is evidence that diabetes induced changes in the activities of antioxidant enzymes in various tissues.

Blood pressure (BP) is also a major risk factor for cardiovascular events, such as myocardial infarction and stroke, as well as for microvascular complications in diabetes. Recent studies have shown that systolic BP is more closely related to end-organ damage than diastolic BP, and it has been shown that increased pulse pressure is an independent cardiovascular risk factor. Tight blood pressure control reduces the risk of both micro- and macrovascular complications in patients with hypertension and type II diabetes.

Herbal medicines have been used for the treatment of diabetic patients since long and they are currently accepted as an alternative therapy for diabetic treatment. More than 1200 plants have been described in the scientific and popular literature as hypoglycaemic agents.

*Embelia ribes* Burm (Myrsinaceae) is commonly known as Vidanga, Vayavidang, Babrang, Bibidang (Hindi), Bavding (Gujarati), Vaayu vilanga, Hulimeese (Kannada), Vivilangam, Vaivelangum (Tamil), Vaividungalu (Telugu) False Black Pepper, (English), and Krimighna, Tandula (Sanskrit). It is a woody shrub widely distributed in India, Sri Lanka, Malaysia and South China. It is highly esteemed in Ayurveda as a powerful anthelmintic. Ayurveda also describes vidanga as pungent and cures flatulence and colic. In diabetic phytotherapy, the effect of aqueous *E. ribes* extract has never been demonstrated experimentally in diabetes mellitus. However, in a preliminary study, Tripathi has demonstrated the only blood glucose lowering activity of decoction of the *E. ribes* fruits in glucose-fed albino rabbits. Recently, Bhandari et al. have reported the cardiprotective activity of aqueous extract of *E. ribes*.
in isoproterenol induced myocardial infarction in albino rats. The object of this study is to investigate the pharmacological effects of *E. ribes* on blood glucose, glycosylated hemoglobin, blood pressure and oxidative stress in streptozotocin-induced diabetes in rats. The effect of *E. ribes* extract was compared to gliclazide, used as a reference hypoglycaemic drug.

**Materials and Methods**

Plant material — The dried fruits of *E. ribes* Burm were purchased locally during October, 2006 and identified by the Department of Botany, Faculty of Science, Hamdard University, New Delhi, India (voucher specimen No. UB 2). The dried and coarsely powdered drug (100 g) was packed in a soxhlet apparatus and was subjected to extraction with water over 72 hr. The filtrate was evaporated under vacuum drier and brown mass residue obtained was stored at 4ºC for further use. The average yield of the aqueous *E. ribes* extract was approximately 5.26%. For experimental study, the weighed amount of aqueous *E. ribes* extract (100 and 200 mg/kg) was dissolved in 1% Tween 80 in normal saline.

Experimental induction of diabetes — Diabetes was induced by single intravenous injection (40 mg/kg body weight) of streptozotocin (Sigma, St Louis, Mo, USA) into the tail vein of rat. Streptozotocin (STZ) was dissolved in 0.1 M cold sodium citrate buffer, *pH* 4.5 immediately before use. After injection rats had free access to food and water and were given 5% glucose solution to drink overnight to counter hypoglycemic shock. Diabetes in rats were confirmed by moderate polydipsia and marked polyuria.

After 3 days, the fasting blood glucose levels were determined by ortho-toluidine method. The rats showing fasting blood glucose more than 200 mg/dl were considered diabetic and selected for the experiment.

Animals — The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Hamdard University, New Delhi, which is registered with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, India (Registration no. 173/CPCSEA, dated 28 January, 2000). Albino rats of either sex, weighing between 200-250 g, procured from the Central Animal House Facility, Hamdard University, New Delhi and acclimatized under standard laboratory conditions at 25±2°C, 50 ± 15 % RH and normal photoperiod 12:12 hr light : dark cycle for 7 days, were used. Commercial pellet diet (Nav Maharashtra Chakan Oil Mills Ltd, Delhi, India) and water were provided *ad libitum*. After acclimatization, 50 rats were randomly divided into 5 groups of 10 animals each and treated as follows: Group I: normal control. Group I and II rats received 1% Tween 80 in normal saline orally once a day for 40 days. Group II: Pathogenic diabetic control; STZ administered rats. Group III: ER-100 treated; diabetic rats received aqueous ER extract (100 mg/kg body wt) orally for 40 days. Group IV: ER-200 treated; diabetic rats received aqueous ER extract (200 mg/kg body wt) orally for 40 days. Group V: Gliclazide treated; diabetic rats received gliclazide (25 mg/kg body wt) orally for 40 days.

The experiment was terminated at the end of 40 days and the animals were fasted overnight.

Hemodynamic measurement — The animals were trained for 3 weeks for measurement of heart rate and blood pressure. The hemodynamic parameters viz heart rate and blood pressure were recorded on 0 day and 41st day of all the groups of rats using non-invasive method of rat’s tail cuff plethysmography using LE 5001 pressuremeter (LETICA Scientific Instruments, USA).

Biochemical analysis — After hemodynamic measurement, blood samples were collected from the retro-orbital plexus using micro-capillary technique from all the groups of overnight fasted rats. Whole blood was collected for the estimation of blood glucose, glycosylated hemoglobin and glutathione. Serum was separated by centrifugation for biochemical estimation of marker enzymes, lactate dehydrogenase and creatine kinase. After blood collection, all animals were sacrificed by cervical dislocation and pancreas were dissected out and immediately frozen in liquid nitrogen. Six hearts from each group were chosen for lipid peroxides, glutathione, superoxide dismutase and catalase measurements. The protein content was determined via the method described by Lowry *et al*.

Histopathological studies — The remaining four pancreatic tissues from all the groups were subjected to histopathological studies as described by Luna...
et al.\textsuperscript{35} The tissues were fixed using 10% formalin, routinely processed and embedded in paraffin wax. Paraffin sections (5 μm thick) were cut on glass slides and stained with hematoxylin and eosin (H & E) after dewaxing, and examined under a light microscope by a pathologist blinded to the groups studied.

Statistical analysis — Statistical analysis was carried out using Graphpad Prism 3.0 (Graphpad software; San Diego, CA). All data were expressed as mean±SE. Groups of data were compared with an analysis of variance followed by Dunnett t-test. Values were considered statistically significant at $P < 0.01$

Results
Rats administered with streptozotocin alone (group II) showed significant increase in heart rate and systolic blood pressure as compared to normal control rats i.e. group I. Pretreatment with aqueous ER extract (group III and IV) and gliclazide (group V) showed a significant decrease in heart rate and systolic blood pressure as compared to pathogenic control rats (Table 1).

The results of blood glucose, HbA\textsubscript{1c}, blood GSH, serum LDH and CK levels are shown in Table 2. A significant increase in blood glucose, HbA\textsubscript{1c}, serum LDH and CK levels and a decrease in blood GSH levels was observed in STZ induced diabetic (untreated) rats. In turn, the diabetic rats given aqueous $E$. \textit{ribes} extracts (100 and 200 mg/kg) reversed above biochemical markers significantly in both the doses. A dose of 100 mg/kg was more effective than 200 mg/kg.

The pancreatic LPO, SOD, catalase and GSH levels in normal and experimental rats are shown in Table 3. The pancreatic LPO levels in diabetic animals increased significantly and further, pancreatic SOD, catalase and GSH levels were decreased significantly in same animals. After treatment with aqueous $E$. \textit{ribes} extracts, the pancreatic LPO levels decreased significantly and pancreatic SOD, catalase and GSH levels were increased significantly in the diabetic rats in both the doses given for 40 days.

Histopathological studies — The results of hemodynamic and biochemical observations were supplemented by histopathological examination of rat’s pancreas sections. Figure 1(a-e) depicts the islets of the pancreas of rat in different groups. Photomicrograph of normal healthy control group shows globules of acini with normal islet cells (Fig. 1a). However, in the STZ only treated rats, there was atrophy of islet cells. And they appeared to be irregular. It’s β-cell mass appeared to be reduced as

| Table 1 — Effect of aqueous extract of $E$. \textit{ribes} (ER) on STZ - induced changes on heart rate and systolic blood pressure |

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Heart rate</th>
<th>Systolic BP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal healthy control</td>
<td>417.29 ± 1.19</td>
<td>115.00 ± 1.25</td>
</tr>
<tr>
<td>Pathogenic control</td>
<td>467.20 ± 8.12\textsuperscript{a}</td>
<td>127.70 ± 2.15\textsuperscript{a}</td>
</tr>
<tr>
<td>STZ (40 mg/kg, iv) + ER extract (100 mg/kg, po)</td>
<td>388.16 ± 9.49\textsuperscript{b}</td>
<td>105.30 ± 3.93\textsuperscript{b}</td>
</tr>
<tr>
<td>STZ (40 mg/kg, iv) + ER extract (200 mg/kg, po)</td>
<td>400.16 ± 5.29\textsuperscript{b}</td>
<td>110.30 ± 2.83\textsuperscript{b}</td>
</tr>
<tr>
<td>STZ (40 mg/kg, iv) + gliclazide (25 mg/kg, po)</td>
<td>421.50 ± 4.55\textsuperscript{b}</td>
<td>116.80 ± 1.01\textsuperscript{b}</td>
</tr>
</tbody>
</table>

Heart rate = beats/min; Systolic BP = systolic blood pressure (mm Hg) $P$ values < 0.01; when compared with \textsuperscript{a}normal healthy control group, \textsuperscript{b}pathogenic control group.

| Table 2 — Effect of aqueous extract of $E$. \textit{ribes} (ER) on STZ - induced changes in the levels of blood glucose, HbA\textsubscript{1c}, blood GSH, serum CK and serum LDH levels |

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Blood glucose</th>
<th>HbA\textsubscript{1c}</th>
<th>Blood GSH</th>
<th>CK</th>
<th>LDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal healthy control</td>
<td>69.30±0.78</td>
<td>5.06±0.25</td>
<td>3.21±0.07</td>
<td>60.74±3.10</td>
<td>191.11±7.40</td>
</tr>
<tr>
<td>Pathogenic control</td>
<td>344.00±11.34\textsuperscript{a}</td>
<td>18.50±0.61\textsuperscript{a}</td>
<td>1.12±0.08\textsuperscript{a}</td>
<td>235.35±4.81\textsuperscript{a}</td>
<td>477.17±34.86\textsuperscript{a}</td>
</tr>
<tr>
<td>STZ (40 mg/kg, iv) + ER extract (100 mg/kg, po)</td>
<td>83.20±0.60\textsuperscript{b}</td>
<td>7.14±0.96\textsuperscript{b}</td>
<td>3.36±0.10\textsuperscript{b}</td>
<td>71.68±3.81\textsuperscript{b}</td>
<td>213.64±8.63\textsuperscript{b}</td>
</tr>
<tr>
<td>STZ (40 mg/kg, iv) + ER extract (200 mg/kg, po)</td>
<td>102.40±0.79\textsuperscript{b}</td>
<td>10.10±1.47\textsuperscript{b}</td>
<td>2.73±0.33\textsuperscript{b}</td>
<td>102.23±4.14\textsuperscript{b}</td>
<td>353.78±5.90\textsuperscript{b}</td>
</tr>
<tr>
<td>STZ (40 mg/kg, iv) + gliclazide (25 mg/kg, po)</td>
<td>79.05±1.26\textsuperscript{b}</td>
<td>8.81±0.65\textsuperscript{b}</td>
<td>2.08±0.34\textsuperscript{b}</td>
<td>79.84±4.42\textsuperscript{b}</td>
<td>315.00±0.31\textsuperscript{b}</td>
</tr>
</tbody>
</table>

Blood glucose (mg/dl); HbA\textsubscript{1c} = blood glycosylated haemoglobin (%); GSH = blood glutathione (mg/dl); CK = creatine kinase (IU/L); LDH = lactate dehydrogenase (IU/L) $P$ values < 0.01; when compared with \textsuperscript{a}normal healthy control group, \textsuperscript{b}pathogenic control group.
compared to pancreatic islet cells of group I rats (Fig. 1b). Treatment of diabetic rats with the test drug in group III and IV showed normal islet cells (Fig. 1c and 1d). However, gliclazide treatment in group V showed moderate expansion of islets cells (Fig. 1e).

Discussion

The present study was undertaken to investigate the antihyperglycaemic activity of aqueous extract of *E. ribes* fruits in STZ induced diabetes in rats. The aqueous extract of *E. ribes* produced a marked decrease in blood glucose levels in both the dose levels i.e. 100 and 200 mg/kg. It is to be seen whether the antidiabetic effect of *E. ribes* may be due to increased release of insulin from the existing β-cells of pancreas similar to that observed after gliclazide administration.

Glycosylated hemoglobin (HbA1C) is the measurement of the mean blood glucose levels over the previous 6-8 weeks, during the life span of RBC. It has been shown to be an important parameter of chronic glycaemic control in patients with diabetes mellitus (DM), an elevated HbA1C almost always indicate DM. Our results showed that, there was significant increase in HbA1C levels in STZ induced diabetic rats as compared to normal control rats. Further, we observed significant decrease in glycated hemoglobin treated with aqueous extracts of *E. ribes* which is not reported earlier.

Further, lowering blood pressure has been shown to be highly effective in reducing cardiovascular events in clinical trials including diabetic patients. In present study, chronic administration of aqueous extract of *E. ribes* at the dose of 100 mg/kg has shown to offer optimal reduction in systolic blood pressure and heart rate, thus, providing protection in diabetes.

Elevated serum LDH and CK levels in diabetic rats indicate cardiac muscular damage. Similar increase in the activity of these two enzymes in serum of the STZ diabetic rats was observed in the present study. The quantity of enzyme released from damaged tissue is a measure of the number of necrotic cells. Furthermore, there was a significant attenuation of serum LDH and CK levels with the test drug treatment indicating the cardioprotective potential of aqueous extract of *E. ribes*.

The levels of antioxidant defense system are altered in STZ induced diabetic rats which is in good correlation with the present observation. Non protein thiols like glutathione are one of the important primary defenses that counteract the oxidative stress. Further, we observed lower levels of blood glutathione as well as decrease in the activity of glutathione, SOD and CAT in pancreatic tissues of STZ diabetic rats which is in consistent with earlier reports. The observed decrease may be due to utilization of non protein thiols by increased oxygen free radicals produced in hyperglycemic conditions associated with diabetes mellitus.

Lipid peroxidation is one of the characteristic features of chronic diabetes. In the present study, a marked increase in the concentration of LPO was observed in the pancreatic tissue of diabetic rats.

The treatment with aqueous extract of *E. ribes* in diabetic rats significantly increased the levels of SOD, CAT as well as blood and pancreatic glutathione and significantly decreased the levels of lipid peroxides. The treatment showed normal pancreatic β-cells (Fig. 1c and 1d). The protection might have been mediated through ER extract induced increase in basal pancreatic SOD and catalase activities.

However, it was observed in present study that the aqueous *E. ribes* extract in 100 mg/kg dose exhibited

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LPO (nmol MDA/mg protein)</th>
<th>SOD (IU/mg protein)</th>
<th>CAT (nmol H2O2 consumed/min/mg protein)</th>
<th>GSH (µmol of phosphorous liberated/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal healthy control</td>
<td>0.47±0.05</td>
<td>1.73±0.14</td>
<td>0.58±0.01</td>
<td>56.26±2.86</td>
</tr>
<tr>
<td>Pathogenic control</td>
<td>6.80±0.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.16±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.32±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.3±5.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>STZ (40 mg/kg, iv) + ER extract</td>
<td>0.65±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.21±0.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.47±0.73&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48.89±6.41&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>STZ (40 mg/kg, iv) + ER extract</td>
<td>0.88±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.64±0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.79±1.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.83±1.75&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>STZ (40 mg/kg, iv) + gliclazide</td>
<td>2.16±0.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.62±0.48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.66±1.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>46.10±2.40&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

LPO=lipid peroxides (nmol MDA/mg protein); SOD=superoxide dismutase (IU/mg protein); CAT=catalase (nmol H2O2 consumed/min/mg protein); GSH=glutathione (µmol of phosphorous liberated/min/mg protein).

*P* values < 0.01; when compared with *normal healthy control group*, *pathogenic control group.*
Fig. 1 — Histological examination of pancreas in experimental animals. (a) - Normal healthy control group (Group I) rat showing globules of acini with normal islet cells (NIC) (10×); (b) - Pathogenic control group (Group II) rat showing atrophy of islets cells (AIC) with inflammatory infiltrate with edema (IIE) (10×); (c) - Aqueous ER extract (100 mg/kg) treated group (Group III) rat showing normal pancreatic islets (NPI) (10×); (d) - Aqueous ER extract (200 mg/kg) treated group (Group IV) rat showing normal islet cells (NIC) (10×); (e) - Gliclazide (25 mg/kg) treated group (Group V) rat showing moderate expansion of islets cells (MEIC) (10×).
more significant antihyperglycaemic and blood pressure lowering activity against STZ induced diabetes and offered significant protection to pancreas against the STZ induced oxidative stress in terms of preservation of endogenous antioxidants.

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References


