The Effects of Oral Administration of an Aqueous Extract of Ficus bengalensis Stem Bark on Some Hematological and Biochemical Parameters in Rats with Streptozotocin-Induced Diabetes

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Abstract: The effects of sub-chronic administration of an aqueous extract of Ficus bengalensis stem bark on hematological and biochemical parameters in rats with streptozotocin (STZ)-induced diabetes were evaluated. The possible protective effect of the F. bengalensis (500 mg/kg of body weight per day) extract on STZ-induced changes was evaluated over 12 weeks. The parameters evaluated were plasma proteins, including total protein, albumin, and globulin, non-protein nitrogenous substances, including urea, uric acid, and creatinine, and hematological indices, including total hemoglobin content, erythrocytes, leucocytes, and platelets counts. The aqueous extract had a significant (P < 0.05) protective effect against STZ-induced changes in terms of the markers of protein metabolism and hematological indices. Furthermore, increases in levels of non-protein nitrogenous substances were normalized in response to the administration of the extract. Thus, the aqueous extract of F. bengalensis had a significant protective effect on STZ-induced changes to biochemical and hematological parameters in the diabetic rats.

Key Words: Ficus bengalensis, streptozotocin, non-protein nitrogenous substances, hematological indices
death by necrosis (4). It was previously reported that high
doses of STZ produce marked steatosis in the liver and
tubular injury in the kidneys, as well as increased blood
eric nitrogen and creatinine levels in experimental animals
(5). STZ produces dose-dependent mortality in
experimental animals and was reported to cause 15%-20% mortality at a dose of 65 mg/kg of body weight (6). Ficus bengalensis (Banyan tree) is a large tree with many
aerial roots that grows wild in the lower region of the
Himalayas and throughout India. Different parts of the tree
are reported to possess medicinal properties. The leaves
are good for ulcers, aerial roots are useful in the treatment
of gonorrhea, and seeds and fruit are known for their
cooling effect and are often used as a tonic (7). A water
extract of the bark of this plant was shown to have a
hypoglycemic effect (8), as well as hypocholesterolemic and
hypolipidemic effects (9,10).

The objective of the present study was to investigate
the possible protective effect of an aqueous extract F.
bengalensis on STZ-induced changes in levels of total
protein, albumin, and non-protein nitrogenous substances,
as well as hematological indices in diabetic rats.

Materials and Methods

Animals

Male albino rats (Wistar strain, 150-200 g) were
purchased from Tamil Nadu Veterinary and Animal Sciences
University, Madhawaram, Chennai. The rats were housed
under standard husbandry conditions (30 ± 2 °C, 60%-70%
relative humidity, and 12:12 h day/night cycle), and
were allowed standard pellet rat feed and water ad libitum.
Animal experiments were designed and conducted in
accordance with the guidelines of the Institutional Animal
Ethical Committee (IAEC, VIT University).

Preparation of Plant Extract

The bark of F. bengalensis was collected from the
Morappur Forest, Dharmapuri District, Tamil Nadu, during
April 2006. A voucher specimen was prepared and
submitted to the Forest Department. The bark was washed
with distilled water, shade dried, powdered, and stored in
an air-tight container until further use.

The aqueous extract was prepared as described by Vats
et al. (11). F. bengalensis stem bark (100 g) was
powdered in an electric grinder. The powder was then
soaked in 500 ml of sterile water, stirred intermittently,
and left overnight. The macerated pulp was then filtered
through a coarse sieve and the filtrate was dried at reduced
temperature. This dry mass served as the aqueous extract
of F. bengalensis used for experimentation. To increase the
shelf life and uniformity of the extract, it was completely
lyophilized by a continuous freeze drying operation for 54
h (Christ freeze dryer, Germany) and the yield was
calculated as 3.7% (w/v).

Induction of Diabetes Mellitus

A single dose of a freshly prepared solution (35 mg/kg
of bodyweight in 0.1 M cold citrate buffer, pH 4.5) of STZ
(Sigma, USA) was injected (intraperitoneally) into the rats.
After 72 h, blood was collected from the tail vein under
ether anesthesia. Blood glucose levels were determined
using a Microlab 2000 autoanalyzer (Hamilton, UK). The
animals were considered to be diabetic if blood glucose
values were > 250 mg/dl and only those animals were used
for the study. STZ-induced diabetes developed and
stabilized over a period of 7 days (12). Untreated control
rats were given 0.1 M citrate buffer (pH 4.5) only.

Experimental Design

Animals were divided into 4 groups, each consisting of
6 animals. Group 1 served as a control, group 2 included
rats with STZ-induced diabetes, group 3 served as a
positive control and received the standard hypoglycemic
drug, tolbutamide (100 mg/kg of body weight), and group
4 included rats with STZ-induced diabetes that were
treated with the aqueous extract (500 mg/kg of body
weight per day) for 12 weeks via oral intubation. Rats
were sacrificed at the end of 12 weeks and blood samples
were collected to estimate plasma proteins, non-protein
nitrogenous substances, and hematological indices.

Biochemical and hematological Parameters

Total hemoglobin was estimated by the
cyanmethemoglobin method (13). Plasma total protein was
estimated by the method of Lowry et al. (14) and albumin
by the method of Peters et al. (15). Plasma urea and uric
acid were estimated by the method of Tomas (16), and
creatinine was estimated by the method of Fossati et al.
(17). Hematological indices, including erythrocytes count,
leucocytes count, and platelets count, were also determined
using standard methods (18).

Acute Toxicity

The F. bengalensis aqueous extract was tested for acute
toxicity by administering oral doses ranging from 100 mg
to 1 g/kg of body weight per day and from 2 to 5 g/kg of body weight per day for 7 weeks to 2 groups of rats (n = 6) prior to the present investigation. The rats were monitored for any lethal effects due to administration of the drug.

**Statistical Analysis**

Statistical analysis was performed using SPSS v.9.05. Data were analyzed by one-way analysis of variance (ANOVA), followed by Duncan’s multiple range test. All the results were expressed as mean ± SD for the 6 rats in each group. A P value < 0.05 was considered significant.

**Results and Discussion**

The extract (administered at 20-50 times the effective dose) was observed to be non-toxic to the rats. Experimental animals appeared normal throughout the 7-week study period; there were no physical or behavioral changes observed, and there were no deaths in any of the experimental groups.

The levels of total protein and albumin in plasma decreased significantly (P < 0.05) in the rats with STZ-induced diabetes, in comparison to the untreated control rats (Table 1). The levels of total protein and albumin returned to normal after the administration of the extract (500 mg/kg of body weight per day) for 12 weeks; however, a mild globulin level increase (P < 0.05) in the diabetic rats was normalized by the administration of the plant extract. Blood levels of urea, uric acid, and creatinine increased significantly (P < 0.05) in the rats with STZ-induced diabetes, in comparison to the untreated control rats. Administration of the plant extract (500 mg/kg of body weight per day) for 12 weeks normalized levels of non-protein nitrogenous substances. The observed effect of the *F. bengalensis* aqueous extract was equivalent to the effect of tolbutamide.

Decreases in erythrocyte, leucocyte, and platelet counts were observed, and hemoglobin levels returned to normal following administration of the extract (500 mg/kg of body weight per day) for 12 weeks (Table 2). The decrease in hemoglobin level in the diabetic rats may have been due to a reduction in protein synthesis in all tissues, with decreased production of ATP and absolute or relative insulin deficiency (19).

The observed increase in plasma levels of urea, uric acid, and creatinine may have been due to STZ-induced metabolic disturbances, as well as renal dysfunction. Levels of non-protein nitrogenous substances are always used as significant markers for the assessment of renal dysfunction (20). Increased protein glycation in STZ-induced diabetes was reported to be associated with increased muscle wasting and, thereby, increased release of purines. The elevated levels of purine in diabetes are reported to be the main source of uric acid, in addition to xanthine oxidase activity (21). Our data revealed that the *F. bengalensis* aqueous extract significantly reduced the levels of serum urea, uric acid, and creatinine in rats with STZ-induced diabetes.

The observed changes in blood cell counts may have been due to the necrotic effect of STZ, which could also have been the cause of the drop in total hemoglobin. Some of the constituents present in the extract might reduce

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total Protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Urea (m mol/l)</th>
<th>Uric acid (m mol/l)</th>
<th>Creatinine (μ mol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>6.87 ± 0.81</td>
<td>4.60 ± 2.9</td>
<td>8.31 ± 0.27</td>
<td>65.43 ± 0.1</td>
<td>64.3 ± 2.3</td>
</tr>
<tr>
<td>Diabetic control*</td>
<td>4.25 ± 0.85*</td>
<td>3.90 ± 2.8*</td>
<td>9.41 ± 0.26*</td>
<td>142.8 ± 0.3*</td>
<td>74.8 ± 2.4*</td>
</tr>
<tr>
<td>Diabetic + Tolbutamide (100 g/kg per day)</td>
<td>6.10 ± 0.53</td>
<td>4.73 ± 1.9</td>
<td>8.75 ± 0.25*</td>
<td>112.9 ± 0.3*</td>
<td>63.2 ± 2.1</td>
</tr>
<tr>
<td>Diabetic + <em>F. bengalensis</em> (500 mg/kg per day)</td>
<td>6.45 ± 0.51*</td>
<td>4.42 ± 2.1*</td>
<td>8.25 ± 0.28*</td>
<td>65.24 ± 0.1*</td>
<td>64.0 ± 2.3*</td>
</tr>
</tbody>
</table>

Each value is the mean ± SD for 6 rats in each group.

*STZ: Streptozotocin (35 mg/kg body weight in 0.1 M cold citrate buffer, pH 4.5).

*Values are statistically significant when compared to controls at F > 0.05 (ANOVA) and P < 0.05 (DMRT).

*Values are statistically significant when compared to diabetic controls at F > 0.05 (ANOVA) and P < 0.05 (DMRT).
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Table 2. The effect of the F. bengalensis aqueous extract on hematological indices in rats with STZ-induced diabetes.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hb (g/dl)</th>
<th>RBC X10^12/l</th>
<th>WBC X10^9/l</th>
<th>Platelets X10^9/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>15.50 ± 0.5</td>
<td>5.10 ± 0.7</td>
<td>9.90 ± 0.7</td>
<td>163 ± 1.8</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>12.95 ± 1.0*</td>
<td>3.95 ± 0.6*</td>
<td>8.10 ± 0.5*</td>
<td>151 ± 1.3*</td>
</tr>
<tr>
<td>Diabetic + Tolbutamide (100 mg/kg per day)</td>
<td>14.10 ± 1.1</td>
<td>5.20 ± 0.7</td>
<td>8.62 ± 0.6</td>
<td>160 ± 1.2</td>
</tr>
<tr>
<td>Diabetic + F. bengalensis (500 mg/kg per day)</td>
<td>15.95 ± 0.9a</td>
<td>5.39 ± 0.6a</td>
<td>8.85 ± 0.5a</td>
<td>162 ± 2.0a</td>
</tr>
</tbody>
</table>

Each value is the mean ± SD for 6 rats in each group.
STZ: Streptozotocin (35 mg/kg body weight in 0.1 M cold citrate buffer, pH 4.5).
*Values are statistically significant when compared to controls at F > 0.05 (ANOVA) and P < 0.05 (DMRT).
Values are statistically significant when compared to diabetic controls at F > 0.05 (ANOVA) and P < 0.05 (DMRT).

STZ-induced changes in total protein, albumin, non-protein nitrogenous substances, and hematological indices. The antioxidant activity of polyphenolic flavonoids, leucopelargonidin, and leucocynidin isolated from F. bengalensis has been previously reported (22). Isolation of 3 ketones (20-tetratriacontene-2-one, 6-heptatriacontene-10-one, and pentatriacontan-5-one) and 2 other compounds (beta-sitosterol-alpha-D-glucose and meso inositol) from the stem bark of F. bengalensis have been reported (23). Thus, the ameliorative potential of the aqueous extract may be due to the synergistic effect of several of the phytochemicals present in the extract.

Conclusion

Findings from the present study show that the aqueous extract of F. bengalensis stem bark has some useful biological properties, as indicated by the significant changes observed in the biochemical parameters of rat plasma. Further studies are required to assess in greater detail the biochemical/pharmacological properties of purified F. bengalensis extract(s) on blood sugar levels in vivo.

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