Antihyperglycemic and antidiabetic effects of *Morinda tinctoria* Roxb using streptozotocin-induced diabetic rats

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**Background:** Metabolic surgical procedures have been shown to improve diabetes, but the mechanism of action is poorly understood.

**Objective:** To evaluate the antihyperglycemic and antidiabetic effects of *Morinda tinctoria* Roxb (MTR) fruit extract in streptozotocin (STZ)-induced diabetic rats.

**Methods:** Albino wistar rats with STZ-induced diabetes were divided into four groups: citrate buffer, troglitazone (TGZ; 36 mg/kg), methanolic fruit extract of MTR (50 mg/kg, 100 mg/kg body weight)-administrated groups. Five, 10, and 15 days after administration of each drug, the fasting blood glucose (FBG), blood glutathione (GSH), and serum ceruloplasmin levels were measured.

**Results:** MTR at the high dose (100 mg/kg bodyweight) produced a significant reduction in the FBG level with increase in blood GSH level. This reduction was much less than that in the FBG produced by TGZ. Treatments with TGZ or MTR at both doses did not alter the ceruloplasmin level significantly.

**Conclusion:** MTR fruit extract contains compounds that could be effective in glucose tolerance impairment during diabetes.

**Keywords:** Blood glutathione, fasting blood glucose, Morinda tinctoria Roxb, streptozotocin, Troglitazone.

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**Materials and methods**

**Preparation of methanolic extracts of MTR**

The fresh fruits of MTR were collected from Western Ghats of Southeastern part of India during June 2006. The identity of the species was authenticated by Plant Anatomy Research Centre (Chennai, India).

According to Duduku et al. [8], we first washed the fruits of MTR (1 kg) to remove any adhering foreign particles and soil materials. These were dried under shade, coarsely powdered, and extracted with methanol using the soxhlet extraction method. The solvent was then removed by filtration, which was added to the plant material. The extract process was twice repeated. The filtrates were then evaporated under a reduced pressure to give a dark colored viscous mass. The extract was stored at 0-4°C and the percentage yield was 16%w/w.

**Phytochemical screening.** Phytochemical screening of the MTR extract was performed using the reagents and chemicals as follows.

- Alkaloids with Mayer’s, Hager’s, and Dragendorff’s reagent,
- Flavonoids with the use of sodium acetate, ferric chloride, amyl alcohol,
- Phenolic compounds and tannins with lead acetate and gelatin,
- Carbohydrate with Molish’s, Fehling’s and Benedict’s reagent [9],
- Proteins and amino acids with Millon’s, Biuret Xanthoprotein test,
- Saponins test using the hemolysis method,
- Sterols with 5% potassium hydroxide,
- Steroids with Libermann Burchard’s test,
- Saponins with foam test,
- Terpenes with thionyl chloride,
- Glycosides with ferric chloride, acetic acid and concentrated sulphuric acid,
- Gum tested using Molish’s reagent and Ruthenium red,
- Coumarin by 10% sodium hydroxide and Quinones by concentrated sulphuric acid.

These were identified by characteristic color changes using standard procedures [10].

The screening results were as follows: Alkaloids +; Carbohydrates +; Proteins and amino acids +; Steroids -; Sterols +; Phenols +, Flavonoids +; Gums and mucilage +; Glycosides +; Saponins -; Terpenes +, and Tannins -, where + and - indicates the presence and absence of compounds.

**Acute toxicity study.** This was performed for the extracts to ascertain safe dose by the acute oral toxic class method by the Organization of Economic Cooperation and Development (OECD) [11]. A single administration of starting dose of 2000 mg/kg body weight/po of the MTR was administered to three female mice, and the mice were observed for three days to evaluate considerable changes in body weight, before and after treatment, and signs of toxicity. Repeating the experiment with the same dose level of MTR for more seven days, we observed the body weight change and toxicity sign for totally fourteen days.

**Animal preparation**

Albino wistar male rats weighing (150-200g body weight) were obtained from the animal house of C.L.Baid Metha College of Pharmacy. This study was approved by the Institutional Animal Ethical Committee.

The animals were maintained in colony cages at 26±2°C, relative humidity 45-55%. The animals were fed a standard animal feed (Hindustan Lever, Chennai, India) and water ad libitum. Sixteen hours before the experiments, they were fasted overnight, but allowed free access to water.

**Induction of diabetes.** STZ was purchased from Sigma (Aldrich, USA). It was dissolved in 0.1M sodium citrate buffer at pH 4.5 just before use. The STZ solution (40 mg/kg body weight) was injected intraperitoneally to the rats that were fasted overnight. The blood glucose level was measured using a glucometer (Accu-chek sensor, Roche Diagnostics, Germany). Rats with blood glucose level greater than 150mg/dL were considered as diabetic.

**Experimental procedure**

Rats were divided into normal, diabetic and non-diabetic groups (each six rats) as follows:

- **Normal group.** Received 1% citrate buffer
- **Diabetic group (group 1-4, using STZ-induced diabetic rats)**
  - Group 1. Received 1% citrate buffer as the control of diabetic rats.
  - Group 2. Received Troglitazone (TGZ) (36 mg/kg body weight) once a day via intragastric tubing for 15 days.
  - Group 3. Received the methanolic fruit extract of MTR at a low dose of 50 mg/kg body weight once a day orally for 15 days.
  - Group 4. Received the methanolic fruit extract of MTR at a high dose of 100 mg/kg body weight once a day orally for 15 days.
- **Non-diabetic group (group 5-7)**
  - Group 5. Received 1% citrate buffer as the control of non-diabetic rats.
  - Group 6. Received the methanolic fruit extract of MTR at a low dose of 50 mg/kg body weight once a day orally for 15 days.
  - Group 7. Received the methanolic fruit extract of MTR at a high dose of 100 mg/kg body weight once a day orally for 15 days.

**Measurement of blood glucose, glutathione, and ceruloplasmin level**

Rats were fasted overnight, and the blood was withdrawn by means of tail-vein puncture on 5th, 10th, and 15th day after administration of each drug. The fasting blood glucose (FBG) and ceruloplasmin levels in serum and blood glutathione (GSH) in heparinized blood were determined. For the FBG measurement, we used the glucometer (Accu-chek sensor, Roche Diagnostics, Germany). We used the method of
Beutler et al. [14] and the Diamine oxidase method [13] for the measurement of blood GSH and ceruloplasmin level, respectively.

Statistical analysis

All values are expressed as mean \(\pm\) SEM. Data were analyzed by non-parametric ANOVA followed by Dunnett’s multiple comparison tests, and other data were evaluated using Graph Pad PRISM software. A p-value<0.05 was considered significantly different.

Results

Table 1 shows the fasting blood glucose, glutathione, and serum ceruloplasmin levels measured in normal and STZ-induced diabetic rats on 5th, 10th and 15th days after the induction.

Interestingly, the FBG levels in diabetic rats were significantly higher (p <0.05) compared to the normal level. MTR administration at the low dose (50 mg/kg body weight) did not decrease the level on the 10th and 15th day (Group 3), but at the high dose (100 mg/kg body weight), the level decreased significantly (p <0.05) (Group 4).

The GSH levels decreased significantly in STZ-induced diabetic rats, compared to the normal level, at 10th and 15th days after the induction. TGZ administration increased the level significantly on the 10th and 15th days (Group 2). Diabetic rats administrated with MTR showed a significant increase in the GSH level at the high dose (Group 3), but at the low dose, the increase was not significant (Group 4).

Table 1. The fasting blood glucose (FBG), blood glutathione (GSH) and serum ceruloplasmin levels measured in normal and diabetic rats administrated with citrate buffer (Group 1), TGZ (Group 2) or MTR (Group 3 and 4). Values are expressed as means±SEM of six determinations.

(A) Fasting blood glucose (FBG) level

<table>
<thead>
<tr>
<th>Group</th>
<th>Days after the induction of diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5(^{th})</td>
</tr>
<tr>
<td>Normal</td>
<td>61.0±1.9</td>
</tr>
<tr>
<td>Group 1</td>
<td>247.3±1.2*</td>
</tr>
<tr>
<td>Group 2</td>
<td>208.0±15.6</td>
</tr>
<tr>
<td>Group 3</td>
<td>228.0±18.4</td>
</tr>
<tr>
<td>Group 4</td>
<td>240.0±13.1</td>
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</table>

*p <0.05 by the Dunnett test, compared to the normal level.

(B) Blood glutathione (GSH) level

<table>
<thead>
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</tr>
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<tr>
<td></td>
<td>5(^{th})</td>
</tr>
<tr>
<td>Normal</td>
<td>21.6±0.2</td>
</tr>
<tr>
<td>Group 1</td>
<td>11.1±0.8*</td>
</tr>
<tr>
<td>Group 2</td>
<td>16.8±1.0</td>
</tr>
<tr>
<td>Group 3</td>
<td>14.3±1.6</td>
</tr>
<tr>
<td>Group 4</td>
<td>15.8±1.2</td>
</tr>
</tbody>
</table>

*p <0.05 by the Dunnett test, compared to the normal level.

(C) Serum ceruloplasmin level

<table>
<thead>
<tr>
<th>Group</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5(^{th})</td>
</tr>
<tr>
<td>Normal</td>
<td>19.4±1.6</td>
</tr>
<tr>
<td>Group 1</td>
<td>11.2±2.2*</td>
</tr>
<tr>
<td>Group 2</td>
<td>10.8±1.5</td>
</tr>
<tr>
<td>Group 3</td>
<td>8.7±1.4</td>
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<tr>
<td>Group 4</td>
<td>6.8±1.1</td>
</tr>
</tbody>
</table>

*p <0.05 by the Dunnett test, compared to the normal level.
The serum ceruloplasmin level decreased significantly in STZ-induced diabetic rats compared to the normal levels. Treatments with TGZ and MTR at the both doses did not alter the levels significantly. Table 2 shows the fasting blood glucose and blood glutathione levels measured in non-diabetic rats. Note that non-diabetic rats treated with MTR at both low and high doses for 15 days did not exhibit any change in the FBG and GSH levels.

Table 2. Effects of the methanolic fruit extract of MTR on the fasting blood glucose (FBG) and blood glutathione levels (GSH) in non-diabetic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 5 (mg/dL)</th>
<th>Group 6 (mg/dL)</th>
<th>Group 7 (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBG level</td>
<td>71.0±4.3</td>
<td>67.7±2.9</td>
<td>71.7±4.1</td>
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<tr>
<td>GSH level</td>
<td>21.6±0.9</td>
<td>22.0±1.1</td>
<td>21.1±0.7</td>
</tr>
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</table>

Data are expressed as means±SEM.

Discussion

We studied the antihyperglycemic and antidiabetic activity of Morinda tinctoria Roxb using STZ-induced diabetic rats, in which blood glucose >.50 mg/dL measured before administration of MTR was used as the criteria. An important mechanism of the STZ diabetogenic action is an increased generation of oxygen free-radicals. The free-radical generation may decrease blood glutathione level (and accordingly the ratio of glutathione to glutathione synthesis) [15]. Since the glutathione functions as a free-radical scavenger [14], any drugs that can prevent the generation of the oxygen free-radicals or increase the free-radical scavenging enzyme, will have an effect on the STZ activity. In the present study, we observed a significant increase in blood glucose level and a decrease in blood glutathione level in STZ-induced diabetic rats administered with MTR. These changes might be due to the MTR activity by which the oxygen free-radicals generated under STZ action might be suppressed.

The flavonoid component in MTR is known to be efficient in scavenging the highly-reactive hydroxyl radical and superoxide anion. It inhibits the lipid peroxidation by quenching the peroxyl radicals [17]. In our study, a high dose of MTR (100 mg/kg body weight) reduced the fasting blood glucose level significantly up to the level by TGZ. Interestingly, this result was in contrast to a low dose (50 mg/kg body weight).

Ceruloplasmin forms a major part of the extracellular antioxidant defense. It inhibits iron- and copper-dependent lipid peroxidation, and also has a superoxide radical scavenging activity [18]. In our study, the ceruloplasmin level decreased significantly in rats administrated with MTR (or TGZ). This reduction may be due to their preventing increase in free-radicals generated under STZ-activity.

There are accumulating evidences to indicate that antioxidants (such as N-acetylcysteine) and dietary antioxidants (such as vitamin C) are beneficial in protecting the beta cells from glucose toxicity in diabetes [19]. There are a number of herbal drugs showing antioxidant properties. Previously, we showed antihyperglycemic and antidiabetic activity of leave extracts of Sapindus emarginatus Vahl using glucose-overloaded hyperglycemic and alloxan-induced diabetic rats [20]. MTR is an antioxidant. Considering the increased glutathione level by MTR, it may strengthen the antioxidant status of pancreas with a relatively weak defense system against oxidative stress. Further study using the measured insulin level will be needed to get a definite conclusion.

Conclusion

The methanol extract of MTR possessed significant inhibitory effect on blood glucose levels and increase in blood glutathione levels in STZ-induced diabetic rats. MTR could be beneficial for the protection and alleviation of diabetic complications.

Acknowledgement

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