Antidiabetic and Hepatoprotective Activities of Bombax ceiba Young Roots in Alloxan-Induced Diabetic Mice

Rokshana Sharmin¹, Maruf-ul-Islam¹, Md. Hasibul Hasan Joarder¹, Md. Mohiuddin Alamgir¹, Md. Golam Mostofa² and A. H. M. Khurshid Alam²*

¹Department of Pharmacy, Jessore University of Science and Technology, Jessore-7408, Bangladesh
²Department of Pharmacy, University of Rajshahi-6205, Bangladesh

Abstract
Bombax ceiba (B. ceiba), a member of the Malvaceae family, has widely been used by rural practitioners for various ailments from ancient civilization. Although different parts of this plant are known to have several biological activities, very little is known about the effects of young roots of B. ceiba (BCYR) on diabetes and hepatic toxicity. Therefore, the present study was undertaken to investigate the effects of the BCYR on diabetes and hepatic toxicity in Alloxan-Induced Diabetic Mice (AIDM). The dried coarse powder of the BCYR was extracted with ethanol (Et-BCYR) by cold extraction method. Qualitative phytochemical screening of the Et-BCYR revealed the presence of flavonoids, phenolics, tannin, steroids, alkaloids and glycosides. Administration of the Et-BCYR (400 mg/kg bw) intraperitoneally in AIDM, it significantly (p>0.05) reduced the blood glucose level compared to untreated AIDM at different time points (0-24 hours). Strikingly, the most action was 78.36% in 16 hours, which was higher than that of the standard met for min (72.36%). Treatment with Et-BCYR significantly (p>0.0001) elevated the HDL level; on the contrary, it reduces LDL, TC and TG levels when compared to untreated AIDM. Additionally, Et-BCYR treatment significantly (p>0.0001) decreased the hepatotoxicity by detecting the reduced level of SGOT and SGPT (hepatotoxic markers) compared to untreated AIDM. Our findings show that the young roots of B. ceiba have potential hypoglycemic, hypolipidemic and hepatoprotective activities and confirm the traditional uses of this plant to manage diabetes and its associated liver toxicity.

Keywords: Diabetes mellitus; Bombax ceiba; Hypoglycemic; Hypolipidemic; Hepatoprotective;

Introduction
Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism, which is characterized by high blood sugar level due to either inadequate synthesis of insulin or insulin resistance [1]. It is the most leading causes of death globally and more than 25% of the total population are affected with this disease, and it will be increased to 50% by 2025 [2]. Nisha et al., (2014) reported that hyperlipidemia is one of the major complications of diabetes mellitus due to abnormal lipid metabolism [3]. In chronic hyperglycemia, excess formation of free radicals leads to the development of the diabetic nephropathy [4]. The higher level of lipid profile, especially Total Cholesterol (TC) synthesis during hyperglycemic condition contributes to accelerate the atherosclerosis in diabetes mellitus [5]. The International Diabetes Federation (IDF) estimated more than 7.1 million people suffer from diabetes in Bangladesh and an equal number of people remain unknown with their diabetic condition and the number of affected people will be 15 million by 2025. For this, treatments, created along the standards of western prescription (allopathic) are regularly constrained inadequacy, carry the risk of adverse effects and are often too costly, particularly in the developing world. Thus, it is essential to discover a potent, safe and cost-effective hypoglycemic agent. Since ancient time, diabetes treatment has been done orally with several medicinal plants or their extracts based on their folkloric reputation. Recently, WHO has also been recommended to use of traditional plants as a treatment for diabetes patient [6]. For the last few years, herbal medicine and herbal drugs used were gradually increasing in developing and developed countries [7]. Hence, the search for potent pharmacologically active agents from natural sources such as from medicinal plants or their extracts that have
led to the discovery of many clinically useful drugs might play a major role in the treatment of human diseases [8]. Keeping this in mind, we have chosen a plant, Bombax ceiba (B. ceiba), which has different biological activities, including hypoglycemic activity.

B. ceiba, a member of the Malvaceae family, is a lofty, deciduous tree. It is popularly known as silk cotton tree and widely distributed in temperate and tropical Asia, Africa and Australia. Different parts of this plant are reported to have therapeutic potentials against different diseases such as diabetes, hepatic toxicity, infections, asthma, polyurea and glycosuria [9-12]. Although different parts of B. ceiba are known to have different biological activities including antioxidant, antimicrobial, anti-inflammatory and analgesic, hypotensive and hypoglycemic activity, very little work has been done by taking an ethanolic extract of young roots of B. ceiba (BCYR) on diabetes and hepatic toxicity [9,10,13-15]. Therefore, the present study was undertaken to investigate the effects of the BCYR on diabetes and hepatic toxicity in Alloxan-Induced Diabetic Mice (AIDM).

Materials and Methods

Plant Materials

According to Ayurveda, B. ceiba has proven medicinal properties and is the ingredient of many formulations. The roots are sweet, cooling, stimulant, restorative, astringent, alternative, aphrodisiac, demulcent, emetic and tonic and they are used in the treatment of diarrhea, hepatotoxicity, dysentery, menorrhagia, stypitic and for wounds [16-20]. The chemical investigations worldwide suggest that the roots of B. ceiba are used for the medicinal purpose because they are rich in lupeol, β-sitosterol and sesquiterpenes, which are beneficial for health in treating some diseases. Apart from the above active constituents, the stem, root, flower, fruit and leaves of B. ceiba have been reported to contain many important phytoconstituents, including alkaloids, glycosides, flavonoids, steroids, saponins, phytosterols and triterpenoids (lupeol and beta-sitosterol), phenolic compounds and tannins [21]. From ancient civilization, tips of young root have been used as a vegetable for patients suffering from impotency. Jain et al., (2009) reported that young roots of B. ceiba (BCYR) are used traditionally as an ethnomedicine for various ailments, including calculous affections, chronic inflammation and ulceration of the bladder and kidneys. Moreover, younger roots of B. ceiba are more nutritious than the older ones and are roasted in the fire to eat like roasted sweet potato during famine or otherwise also [22]. Based on above mentioned reasons, young roots instead of adult roots were selected for this study. The fresh BCYR were collected from rural area of Kustia and Jessore district in Bangladesh. The species was identified by an expert taxonomist of Bangladesh national herbarium, Dhaka. After collection, the fresh roots were thoroughly washed with distilled water and kept apart barking layer from the roots and sliced into small pieces. The roots were sun dried under shade and ground with an electric grinder into coarse powder and were used for cold extraction.

Preparation of ethanolic extract

The dried coarse powders were soaked with 1.5 L of 95% ethanol in amber-coloured extraction bottle. The bottles were sealed and kept for the 7 days at room temperature with occasional shaking and stirring. The extracts were filtered through cotton followed by Whatman No.1 filter paper and were concentrated with a rotary evaporator (Bibby Sterlin Ltd, UK) under reduced pressure at 50°C to afford brown-coloured, powdery crude extract.

Qualitative Phytochemical Profiles

Test for Carbohydrate (Biuret’s test)

To 5 ml of Benedict’s reagent, 1 ml of Et-BCYR solution was added and boiled for 2 minutes and cooled. Formation of a red precipitate shows the presence of carbohydrate.

Test for Saponins (Foam test)

Ten (10) mg of Et-BCYR was taken in a test tube and shaken vigorously with 5 ml of water. Production of persistent foam indicates the presence of saponins.

Test for Flavonoid, Phenol and Tannin (Ferric chloride test)

Five (5) mg of Et-BCYR was dissolved in 2 ml of water. 1 ml of neutral 5% ferric chloride solution was added in this solution. A dark green color indicates the presence of flavonoid, phenols and tannin.

Test for Proteins (Biuret Test)

Three to five (3-5) mg of Et-BCYR was added in 4% NaOH and few drops of 1% CuSO4 solution was added in this solution. A violet or pink color indicates the presence of protein.

Test for Steroids (Liebermann-Burchard test)

The Et-BCYR was dissolved in 1 ml of chloroform. 2 ml acetic anhydride and 1 ml concentrated sulfuric acid were added. Formation of greenish color solution indicates the presence of steroids.

Test for Phytosterol (Salkowski’s reaction)

To 2 ml of Et-BCYR, 2 ml of chloroform and 2 ml of concentrated H2SO4 were added and shaken vigorously. Chloroform layer shows greenish yellow fluorescence, which indicates the presence of phytosterol.

Test for Amino Acids (Ninhydrin test)

To an aliquot of diluted extract, 2 ml of ninhydrin solution was added. A violet color formation indicates the presence of amino acid.

Test for Glycosides (Keller- Killani Test)

About 2 ml of extracts was taken in a test tube followed by 1 ml of glacial acetic acid containing trace amount of FeCl3 and 1 ml of concentrated H2SO4 were added to the extract carefully. A reddish-brown colour is formed at the junction of two layer and
the upper layer turns bluish green in presence of glycosides.

**Test for Alkaloids (Wagner’s Test)**

The Et-BCYR was taken in a test tube. 5 ml of 1% HCl was added to the test tube and stirred on a stream bath. After filtering the solution, few drops of Wagner’s reagent was added. Formation of a reddish brown precipitate indicates the presence of alkaloids.

**Preparation of Dose**

The dose (120 mg/kg body weight) of standard metformin is prepared by using sterilized water. BCYR extract is dissolved in 10 % DMSO to prepare dose at a concentration of 400 mg/kg body weight, bw.

**Induction of Diabetes**

For the development of diabetic model mice, the mice were grouped into 4 classes (Group I-IV). All the mice in group II-IV were kept overnight fasting and a freshly prepared solution of alloxan monohydrate (150 mg/kg bw in 0.9% normal saline) was administered intraperitoneally. The group I mice was kept as a normal control group. After 48 hours of alloxan induction, blood glucose content was measured by a Glucometer (SAFE TOUCH Glucometer, HMD Bio Medical Inc., Taiwan Technology of USA). Mice with blood glucose levels above 11.1 mmol/L were selected for the study. Baseline blood glucose level of group II-IV mice was also measured just prior to the administration of alloxan.

**Grouping of Experimental Mice**

Swiss Webster mice were randomly assigned into five groups (n=4).

- **Group 1** (Normal control) received 0.9% normal saline (1 mL/kg, bw).
- **Group 2** (Diabetic control) mice received alloxan in 0.9% normal saline.
- **Group 3** (positive control, standard) mice received metformin hydrochloride (120 mg/kg, bw) in 0.9% normal saline.
- **Group 4** (Et-BCYR 400) mice received Et-BCYR extract (400 mg/kg, bw) in 10% DMSO.

**Ethical Permission**

The protocol used in this study for the use of rat as an animal model for diabetes research was approved by the Rajshahi University Animal Ethical committee (27/08/RUCMB). This research work was approved by the Ethical Review Committee of Research Cell of Rajshahi Medical College, Bangladesh (ref. RMC/ER/2010-2013/01).

**Biochemical Analysis**

Blood samples were withdrawn by cutting the tail-tip of each group of mice on the 0, 4, 8, 12, 16, 20 and 24 hours of the day for estimating the blood glucose level. At the end of treatment, the mice were sacrificed and the levels of HDL, LDL, TG, TC, SGOT and SGPT were determined using commercial kits according to the manufacturer protocols by a semi-auto analyzer.

**Statistical Analysis**

Data were expressed as mean ± standard error of mean (SEM). Statistical comparisons were performed by two-way analysis of variance (ANOVA) for blood glucose analysis and two–way ANOVA analysis. The results were considered to be significant when p values were less than 0.05 (p<0.05). Statistical calculations and graphs were prepared using Graph Pad Prism Version 6.00 for Windows (Graph Pad Software, San Diego, CA, USA).

**Results**

**Phytochemical Screening of Et-BCYR Extract**

Freshly prepared ethanolic extract of the BCYR (Et-BCYR) was subjected to preliminary phytochemical screening for various constituents. Table 1 shows the differential distribution of phytoconstituents in the Et-BCYR extract. Et-BCYR contains large amounts of flavonoids, phenolics, tannins, and steroids. It also contains cardiac glycosides and alkaloids in the moderate level.

**Effect of Et-BCYR on Blood Glucose Level in Alloxan-Induced Diabetic Mice (AIDM)**

The Et-BCYR (400 mg/kg bw), and the standard metformin (150 mg/kg bw) were administered i.p. in AIDM and the blood samples were collected on the 4, 8, 12, 16, 20 and 24 hours of treatment. Et-BCYR extract significantly reduced the blood glucose levels at different time points (4, 8, 12, 16, 20 and 24 hours) when compared with the untreated AIDM. Interestingly, the reduction activity of glucose level in blood was the maximum of 16 hours and found to be 78.36%, whereas the standard metformin reduced the blood glucose level to 72.36% by 16 hours suggest that Et-BCYR has higher hypoglycemic activity than that of a standard (Figure 1). All the experiments were done in three different times and values are expressed as mean ± SEM (n=3).
Effect of Et-BCYR on Lipid Profile in AIDM

After the measure of glucose level in blood, the mice were sacrificed and blood samples were collected to measure the lipid profile by a semi bio-analyzer. The standard metformin reduced the Total Cholesterol (TC) level to a maximum of 76.69% when compared to AIDM. On the other hand, treatment with Et-BCYR, in comparison to AIDM, reduced the TC level to 78% which was higher than that of standard metformin (76.69%) (Figure 2A).

The treatment of the Et-BCYR and the standard metformin reduced the Triglyceride (TG) level to 75.24% and 79.81%, respectively in AIDM when compared with the untreated AIDM. The activity of the Et-BCYR was higher than that of the standard drug (Figure 2B). Beside these, Et-BCYR reduced the low lipid density (LDL) level to 92.71%, on the other hand, it significantly increased the high density lipoprotein (HDL) to 255.78%, which was higher than standard metformin (204.95%) (Figure 2C-2D). All the results together suggest that the Et-BCYR shows a potent dislipidemic activity. All the experiments were done in triplicate and values were expressed as mean ± SEM (n=3).

Effect of Et-BCYR on Hepatic Toxicity in AIDM

The level of hepatotoxicity markers, Serum Glutamic Pyruvate Transaminase (SGPT) and Serum Glutamic Oxalate Transaminase (SGOT) in AIDM and treated AIDM were examined by a semi bio-analyzer. Et-BCYR extract and the standard metformin declined the SGOT and SGPT level to 58% and 81.11%, respectively (Figure 3A-3B). Values are expressed as mean ± SEM of 3 independent experiments.

Figure 2: Effect of Et-BCYR on lipid profile in AIDM. * indicates significant change (p<0.0001) of TC (Fig. 2A), TG (2B), LDL (Fig. 2C) and HDL (Fig. 2D) levels in Et-BCYR-treated AIDM compared to untreated AIDM. All the experiments are carried out in three individual times and the results are expressed as means ± SEM (n=3).
Discussion

Alloxan monohydrate destroys the β-cells of islets of Langerhans of the pancreas and inhibits the production of insulin, which affect to push glucose into the body tissues, resulting high level of glucose, decreased protein content and increased cholesterol and triglyceride level in the blood [23]. In this study, alloxan (150 mg/kg bw in 0.9% normal saline) was administered intraperitoneally in mice and the mice, which have a base line blood glucose level above 11 mmol/L was selected for the study. The treatment with standard metformin reduced blood glucose level of 5.58 mmol/L, whereas the Et-BCYR significantly reduced blood glucose level to 5.38 mmol/L, (Figure 1), which was similar to that of the standard suggesting that the extract might control diabetes by increasing the production of insulin. Qualitative phytochemical analysis of the Et-BCYR revealed the presence of alkaloid, glycoside, tannins, and flavonoids (Table1) (Rani N, 2012) reported that flavonoids put down glucose level significantly by inhibiting α-glucosidase enzyme [24]. Generally alkaloids inhibit α-glucosidase enzyme and decrease glucose transport through the intestinal epithelium cell [25]. In this study, we assume that phytochemicals, which have been detected in Et-BCYR, reduced glucose level by inhibiting the α-glucosidase enzyme leading to decrease glucose transport through the intestinal epithelium.

Table 1: Phytochemical constituents of ethanolic extract of young roots of B. ceiba (Et-BCYR)

<table>
<thead>
<tr>
<th>No. of Tests</th>
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<th>Name of Phytochemicals</th>
<th>Et-BCYR</th>
</tr>
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<tbody>
<tr>
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<td>Benedicts Test</td>
<td>Carbohydrate</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Foam Test</td>
<td>Saponin</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Ferric chloride test</td>
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<tr>
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<td>Liebermann-Burchard test</td>
<td>Steroid</td>
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<td>Salkowski’s test</td>
<td>Phytosterol</td>
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<td>Amino acids</td>
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</tr>
<tr>
<td>10</td>
<td>Spot test</td>
<td>Fixed oils &amp; fatty acid</td>
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</tr>
<tr>
<td>11</td>
<td>Keller-Killani Test</td>
<td>Cardiac glycosides</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>Wagners Test</td>
<td>Alkaloid</td>
<td>++</td>
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- = absent; + = present in mild amount, ++ = moderate amount; +++ = large amount

Figure 3: Effect of Et-BCYR on liver toxicity in AIDM. * indicates significant change (p<0.0001) of SGOT (Fig. 3A) and SGPT (Fig. 3B) levels in Et-BCYR treated AIDM compared to untreated AIDM. All the experiments are carried out in three individual times and the results are expressed as means ± SEM (n=3).

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Our results are consistent with those of previously reported data [25]. The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia [26]. Hypertriglyceridemia is also associated with metabolic consequences of hypercoagulability, hyperinsulinemia, insulin resistance and glucose intolerance [27]. Administration of the Et-BCYR significantly (p<0.0001) reduced the TG (Figure 2A) and TC (Figure 2B) levels suggest that the observed hypolipidemic effect was due to decreased cholesterologenesis and fatty acid synthesis [28]. Moreover, the Et-BCYR treatment significantly (p < 0.0001) reduced LDL level (Figure 2C) and increased HDL level (Figure 2D). These values are a very desirable in the biochemical state for prevention of atherosclerosis and ischemic conditions [29]. Accumulating evidence suggests that a decrease in the ratio of the TC/HDL cholesterol (atherogenic index) lessens the risk of heart disease. Previous studies demonstrated that phytochemicals have the capacity for lipid-lowering properties [30&31]. Flavonoids prevent the oxidation of LDL, lower the TC and TG levels leading to minimize the risk of atherosclerosis [32&33]. Cardiac glycosides also used as diuretics and heart tonics because of its valuable effects on the heart and affect the availability of intracellular Ca2+for myocardial contraction [34]. SGPT is a better parameter than SGOT to identify the liver toxicity, since SGOT also found in kidney and cardiac muscle [35]. The Et-BCYR contains high level of flavonoids and phenolic compounds, which are natural antioxidants. They can scavenge off free radicals. So, the anti-oxidant principles may be involved in the hepatoprotective activity. In this study, Et-BCYR reduced SGOT (Figure 3A) and SGPT (Figure 3B) to 58% and 76.53%, respectively suggest that Et-BCYR can be considered as an agent for the treatment of liver toxicity.

Conclusion

The present study clearly indicates that the young roots of Bombax ceiba possess prolonged potential hypoglycemic activity evidenced by 24 hours. The effect of this plant on biochemical alterations reveals hypolipidemic and hepatoprotective activities and confirms the traditional uses of this plant in the management of diabetes and its associated liver toxicity. The phytochemicals present in this plant might be account for the observed pharmacological actions. Further study is necessary to characterize the potential compound(s) and their role in the control and management of diabetes and hepatotoxicity in molecular level.

Conflict of Interests

The author(s) declare(s) that there is no conflict of interests regarding the publication of this article.

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