Antidiabetic Activity of Compounds Isolated from the Kernel of *Mangifera indica* in Alloxan Induced Diabetic Rats

Md. Shah Amran\(^1\), Md. Zakir Sultan\(^2\), Asma Rahman\(^2\) and Mohammad A. Rashid\(^1\)

\(^1\)Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Dhaka University, Dhaka-1000, Bangladesh
\(^2\)Centre for Advanced Research in Sciences (CARS), University of Dhaka, Dhaka-1000, Bangladesh

**ABSTRACT:** Diabetes is one of the major causes of death in Bangladesh. A lot of medicinal plants are available in Bangladesh and they possess remarkable antidiabetic activities. The main purpose of this project was to find out the new antidiabetic compound(s) from plants available in our locality. As such an attempt has been made to isolate the antidiabetic compounds from the kernel of mango. Solvent extractions followed by column and thin layer chromatographic techniques were used to purify the compounds. \(^1\)H-NMR spectroscopy was used to identify the purified compounds which might have antidiabetic property.

**Key words:** Diabetes, Mango, Kernel, Isolation, \(^1\)H-NMR, Antidiabetic.

**INTRODUCTION**

Bangladesh is considered as the home of medicinal/herbal plants where over 546 species of herbal plants grow, out of those 206 plants are used for producing phyto/herbal medicines.\(^1\) Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products that contain parts of plants, or other plant materials, or combinations as active ingredients. Diabetes is a major health hazard now-a-day in Bangladesh. Different types of herbal medicines are used in the treatment of diabetes\(^2\) and a number of attempts have been made to develop suitable antidiabetic drugs through complexation\(^3\)\(^-\)\(^6\) for long time. Beside complexation, search for antidiabetic molecules from natural sources are going on.\(^7\)\(^,\)\(^8\) In this research work, we have tried to find out the chemical compounds present in the kernel of the hexane-extract of *Mangifera indica* that exhibit antidiabetic activity by studying the blood sugar lowering effect of the extractive(s) in alloxan-induced diabetic rats.

**MATERIALS AND METHODS**

**Instrumentation.** \(^1\)H-NMR spectra of samples were recorded in the Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhanmondi, Dhaka. The spectra were taken in the CDCl\(_3\) as the solvent and tetramethylsilane (TMS) as internal standard. Glucometer (ACCU, Germany) with appropriate kit were used for checking glucose level.

**Drugs, chemicals and selection of animals.** Alloxan (Merck, Germany) was obtained as a kind gift from Professor Dr. M. Abdur Rashid, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka and glimepiride was collected from Square Pharmaceuticals Ltd., Bangladesh. Other chemicals, reagents and kits were purchased from the local market/suppliers. Total 56 long Evan's rats of either sex weighing about 150-200 g and aged 2 months were purchased from Department of Pharmacy, Jahangirnagar University, Savar, Bangladesh.

**Extraction and isolation.** Ripe mangoes (about 40 kg) was purchased and mango seeds were collected from them. The seeds were crushed and the kernels were collected during the May-June period of
the year 2011. A voucher specimen has been deposited in the Phytochemical Laboratory of the Department of Pharmaceutical Chemistry, University of Dhaka. The kernels were cut into pieces and dried in sun. After proper drying, the pieces of kernels were pulverized to make coarse powders which are suitable for solvent extraction process. 500.0 g powdered kernel were subjected to extraction with 90% aqueous methanol (2 L) at room temperature (about 27°C). The extraction procedure was repeated two times more with the same volume of aqueous MeOH for 7 days. The resulting aqueous methanolic extracts were combined and concentrated under vacuum using rotary evaporator to give a brownish gummy residue (about 97.1 g) and used for isolations of compounds. The residue was partitioned between water (300 ml) and chloroform (300 ml x 4). The combined chloroform layers were dried under vacuum using rotary evaporator and it was 10.2 g. The chloroform extract was subjected to chromatography over a silica gel column eluted with a mixture of hexane, ethyl acetate, and methanol of increasing polarities and monitored by TLC to give sixteen fractions (fraction A: hexane 100%, 579.6 mg; fraction B: hexane-EtOAc 9:1, 293.7 mg; fraction C: hexane-EtOAc 8:2, 364.2 mg; fraction D: hexane-EtOAc 7:3, 514.3 mg; fraction E: hexane-EtOAc 6:4, 376.5 mg; fraction F: hexane-EtOAc 5:5, 398.2 mg; fraction G: hexane-EtOAc 4:6, 504.3 mg; fraction H: hexane-EtOAc 3:7, 681.3 mg; fraction I:hexane-EtOAc 2:8, 180.1 mg; fraction J: hexane-EtOAc 1:9, 214.25 mg; fraction K: EtOAc 100%, 100.4 mg; fraction L: EtOAc-MeOH 7:3, 81.2 mg; fraction M: EtOAc-MeOH 5:5, 68.6 mg; fraction N: MeOH 100%, 89.4 mg). Further purification of fraction A and B by column chromatography afforded few compounds. The flow chart given in Figure 1 shows the schematic protocol of the overall extraction and isolation processes of the mango kernel. Another hexane extract was made by using the same procedures.

**Experimental induction of diabetes.** Prior to commencement of the experiment, all the rats were acclimatized to the new environment for a period of one week. During the experimental period, the rats were kept in a well ventilated animal house at room temperature of 25 °C. They were supplied with standard pellets purchased from local market and fresh drinking water. All the rats were kept in cage and maintained with natural 12 h light and 12 h dark cycle in the animal house of Institute of Nutrition and Food Sciences (INFS), Dhaka University, Dhaka-1000, Bangladesh. 56 Long Evan's rats of either sex were randomly assigned into 8 groups with seven rats in each group as follows: Group 1 (control group), Group 2 (diabetic rats - positive control group), Group 3, 4 and 5 (hexane extract treated group), Group 6, 7 and 8 (purified compound treated group). The rats of Group 1 were used as negative control group and they were treated with normal diet, water and saline.

Group 1-8 animals were allowed to fast for 12 hours. Diabetes was induced in Group 2-8 by intraperitoneal injection of freshly prepared solution of alloxan (150 mg/kg) in normal saline after base line glucose level determination. The alloxan treated animals were allowed to food and glucose solution over night to overcome drug induced hyperglycemia. After that, blood glucose level was measured with a glucometer using blood sample from the tail vein of the rats. When the blood glucose level raised to above 11.1 mmol/l, then it was assumed that diabetes has been established in animals according to our previous study.8 The normal blood glucose level was 6.68 ± 0.72 mmol/l and after administration of alloxan alone at a dose of 150 mg/kg, the blood glucose level raised to 21.45 ± 1.39 mmol/l in alloxan induced rats. These values were used as standard in the subsequent experiments from Groups 2-8.

Group 2 was selected for glimepiride control group. After induction of diabetes by alloxan, they were given glimepiride orally at a dose of 15 µg/kg body weight. Alloxan induced diabetic rats of Groups 3-5 received the hexane extract of kernel of *M. indica* at doses of 2.5 mg/kg, 5 mg/kg and 10 mg/kg body weight, respectively. Alloxan induced diabetic rats of Groups 6-8 received the purified compounds obtained from kernel of *M. indica* at a maximum dose of 10 mg/kg body weight.
Statistical analysis. The results were expressed as mean ± Standard deviation, where n = 6 in each group.

RESULTS AND DISCUSSION

The resulting crude extract was concentrated under vacuum and subjected to a series of column chromatographic purification procedure. Compounds 3, 4 and 8 were obtained in yield of 14.4, 6.0 and 11.6 mg, respectively (Figure 1).

\[ \text{Aquoeus Chloroform layer} \]
\[ 10.2 \text{ g} \]

\[ \text{Crude methanolic extract} \]
\[ 97.10 \text{ g} \]

Exp# 4 and 5: Silica column chromatography:
Column size:35 cm x 5 cm (id)
Silica: 60 mesh size

Exp. 1
Mango seed 40 kg

Exp. 2: Aqueous methanol 2 L x 3 = 6 L

Crude methanolic extract 97.10 g

Exp. 3: Partition between water and chloroform

Exp. 1
Karnel 1 kg

Exp. 4

A

1

2

3

4

5

6

7

8

9

10

Aqueous

Exp. 5
Chloroform layer

10.2 g

B

C

D

E

F

G

H

I

J

K

L

M

N

Exp# 4 and 5: Silica column chromatography:
Column size:35 cm x 5 cm (id)
Silica: 60 mesh size

\[ \text{Figure 1. Scheme for isolation} \]

\[ ^1\text{H-NMR study revealed four compounds}, \text{ viz compounds 3, 4, 8A and 8B. A brief description of these compounds are given below:} \]

\[ \text{Compound 3} \]

\[ \text{Compound 3 was isolated as off-white semisolid mass (14.4 mg). The} \]
\[ ^1\text{H NMR spectrum revealed signals for anomeric proton at } \delta 5.32 \text{ and two aromatic protons at } \delta 6.9 (\text{m}). \text{ Two singlets at } \delta 6.1 \text{ and } \delta 6.2 \text{ indicated the presence of un-substituted protons at H-1 and H-4 of a xanthene ring. Signals between } \delta 3.5 \text{ to } \delta 4.5 \text{ demonstrated the presence of a sugar moiety. Based on the above discussion and comparison of the} \]
\[ ^1\text{H NMR data with that of 6-O-galloyl mangiferin the tentative structure of compound 3 was proposed as 6-O-galloyl-5'-hydroxy mangiferin.} \]
Compound 4 was obtained as a white gum (6.0 mg). The $^1$H NMR spectrum showed signals for anomic proton at $\delta$ 5.32. Signals at $\delta$ 6.1, $\delta$ 6.2 and $\delta$ 6.9 revealed the presence of un-substituted protons at H-1, H-4 and H-8 of a xanthene ring, respectively. The presence of a sugar moiety was indicated by the signals between $\delta$ 3.5 to 4.5. Comparison of the $^1$H-NMR data of compound 4 with those of compound 3 suggested that both compounds contain sugar moiety. On this basis, the structure of compound 4 was tentatively assigned as mangiferin.\(^9\)

Compound 8 was isolated as white gum (11.6 mg). The $^1$H NMR spectrum displayed signals for anomic proton at $\delta$ 5.32 and two aromatic protons $\delta$ 6.9 (m). Two singlets at $\delta$ 6.1 and $\delta$ 6.2 also indicated the presence of H-1 and H-4 of un-substituted protons of a xanthene ring. The presence of a sugar moiety was clearly evident from $^1$HNMR signals for oxymethine protons between $\delta$ 3.5 to 4.5. A singlet of three proton intensity at $\delta$ 3.46 revealed the presence of a carboxymethyl group. The signal at $\delta$ 9.9 could be assigned to chelated hydroxyl group. Thus compound 8 was characterized as a mixture of two compounds 5-hydroxy mangiferin (compound 8A) and methyl gallate (compound 8B).

All the structures were elucidated tentatively because of lack of sufficient data, especially $^{13}$C-NMR, COSY, HSQC, HMBC, ROESY etc.

In the present study, diabetes was induced in rats by intraperitoneal injection of alloxan (150 mg/kg body weight) which is a cytotoxic agent and induced diabetes by damaging insulin secreting beta cells. The normal blood glucose level was $6.68 \pm 0.72$ mmol/l and after administration of alloxan alone, the blood glucose level raised to $21.45 \pm 1.39$ mmol/l. These values were used as standard in the subsequent experiments.

In case of alloxan induced diabetic rats, glimepiride at a dose of 150 mg/kg reduced the blood glucose level to $19.57 \pm 1.76$, $15.36 \pm 1.62$, $8.81 \pm 1.07$ and $7.3 \pm 1.79$ mmol/l in 24, 48, 72 and 96 h, respectively. When the hexane extract of kernel of \textit{M. indica} at a dose of 2.5 mg/kg was administered, the blood glucose level was reduced to $17.88 \pm 1.39$, $14.48 \pm 1.62$, $10.41 \pm 1.45$ and $9.05 \pm 0.81$ mmol/l in 24, 48, 72 and 96 h, respectively. When the hexane extract of kernel of \textit{M. indica} at a dose of 5.0

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time</th>
<th>Blood glucose level in mmol/l ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>48 h</td>
</tr>
<tr>
<td>Alloxan (150 mg/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glimepiride (150 mg/kg)</td>
<td>19.57±1.76</td>
<td>15.36±1.62</td>
</tr>
<tr>
<td>Hexane extract (2.5 mg/kg)</td>
<td>17.88±0.75</td>
<td>14.48±1.62</td>
</tr>
<tr>
<td>Hexane extract (5 mg/kg)</td>
<td>17.40±2.59</td>
<td>11.52±1.10</td>
</tr>
<tr>
<td>Hexane extract (10 mg/kg)</td>
<td>15.88±1.39</td>
<td>11.01±1.61</td>
</tr>
<tr>
<td>Compound - 3 (10 mg/kg)</td>
<td>18.63±1.98</td>
<td>16.43±3.32</td>
</tr>
<tr>
<td>Compound - 4 (10 mg/kg)</td>
<td>2.51±2.43</td>
<td>18.83±2.64</td>
</tr>
<tr>
<td>Compound - 8 (10 mg/kg)</td>
<td>20.17±1.72</td>
<td>17.50±1.10</td>
</tr>
</tbody>
</table>
mg/kg was administered, the blood glucose level was reduced to 17.40 ± 2.59, 11.52 ± 1.10, 8.10 ± 0.98 and 7.62 ± 0.65 mmol/l in 24, 48, 72 and 96 h, respectively. When the hexane extract of kernel of *M. indica* at a dose of 10.0 mg/kg was administered, the blood glucose level was reduced to 15.88 ± 1.39, 11.01 ± 1.61, 7.70 ± 0.51 and 7.20 ± 0.39 mmol/l in 24, 48, 72 and 96 h, respectively.

Compounds 3, 4 and 8 were also administered in the alloxan induced rats at a high dose of 10 mg/kg b.w. In comparison to the results of the hexane extract, these compounds showed mild antidiabetic property. When compound 3 was administered at a dose of 10.0 mg/kg, the blood glucose level was reduced to 18.63 ± 1.98 mmol/l, 16.43 ± 3.32, 14.98 ± 2.1 and 11.5 ± 2.16 mmol/l in 24, 48, 72 and 96 h, respectively. When 4 was administered at a dose of 10.0 mg/kg, the blood glucose level was reduced to 2.51 ± 2.43, 18.83 ± 2.64, 15.33 ± 3.26 and 12.67 ± 2.34 mmol/l in 24, 48, 72 and 96 h, respectively. When the compound 8 was administered at a dose of 10.0 mg/kg, the blood glucose level was reduced to 20.17 ± 1.72, 17.50 ± 1.10, 15.35 ± 2.07 and 9.46 ± 1.56 mmol/l in 24 h, 48 h, 72 h and 96 h, respectively (Table 1).

Although 10 compounds were determined but structures of only three compounds were and tested for antidiabetic property. Further studies are needed with the remaining compounds for structure determined and screening for antidiabetic property.

**ACKNOWLEDGEMENTS**

This work was supported by the grant from the University Grants Commission of Bangladesh, Agargaon, Dhaka-1207, Bangladesh [Grant No. 6(75/UGC/B&P/BIKA/BIO(2)/2008-2009/11236)]. We would also like to express our gratitude to - Professor Dr. Shahabuddin Kabir Choudhury, Department of Pharmacy, Jahangirnagar University for kindly supplying us with rats and Professor Dr. Sheikh Nazrul Islam, Institute of Nutrition and Food Sciences, Dhaka University, Dhaka-1000, Bangladesh for permitting us to use their Animal House.

**REFERENCES**