Effects of *Averrhoa bilimbi* leaf extract on blood glucose and lipids in streptozotocin-diabetic rats

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Abstract

The present study was designed to investigate the hypoglycemic and hypolipidemic activities of an ethanolic extract of *Averrhoa bilimbi* Linn. leaves (Oxalidaceae, Common name: Bilimbi) in streptozotocin (STZ)-diabetic rats. The optimal hypoglycemic dose (125 mg kg\(^{-1}\)) was determined by performing the oral glucose tolerance test (OGTT) in both normal and STZ-diabetic rats. To investigate the effect of repeated administration of an ethanolic extract of *Averrhoa bilimbi* (ABe) leaves, diabetic rats were treated with vehicle (distilled water), ABe (125 mg kg\(^{-1}\)) or metformin (500 mg kg\(^{-1}\)) twice a day for 2 weeks. Like metformin, ABe significantly lowered blood glucose by 50% and blood triglyceride by 130% when compared with the vehicle. ABe also significantly increased the HDL-cholesterol concentrations by 60% compared with the vehicle. ABe thus significantly increased the anti-atherogenic index and HDL-cholesterol/total cholesterol ratio. However, like metformin, ABe did not affect total cholesterol and LDL-cholesterol concentrations, but significantly reduced the kidney lipid peroxidation level. These data show that ABe has hypoglycemic, hypotriglyceridemic, anti-lipid peroxidative and anti-atherogenic properties in STZ-diabetic rats.

Keywords: *Averrhoa bilimbi* Linn.; Diabetes mellitus; Hypoglycemia; Hypolipidemia; Streptozotocin; Sprague–Dawley rat

1. Introduction

The use of medicinal plants for the treatment of diabetes mellitus dates back from the Ebers papyrus of about 1550 BC. Many traditional plant treatments for diabetes mellitus are used throughout the world. After the introduction of insulin therapy the use of the traditional treatments for diabetes mellitus greatly declined in the occidental societies, although some traditional practices are continued for prophylactic purposes and as adjuncts to conventional therapy (Swanson Flatt et al., 1990). Few of the traditional plant treatments for diabetes have received scientific scrutiny, and the World Health Organization has recommended that this area warrants attention (WHO, 1980).
This paper describes the study of *Averrhoa bilimbi* Linn. (Oxalidaceae, common name: Bilimbi), a common plant in Asia, which has been widely used in traditional medicine as a cure for cough, cold, itches, boils, rheumatism, syphilis, diabetes, whooping cough, and hypertension (Goh et al., 1995). In addition, *A. bilimbi* has been widely reported for its multiple ethnopharmacological properties such as anti-inflammatory, anti-scorbutic, astringent, anti-bacterial, and postpartum protective properties (Goh et al., 1995). In Indonesia it has a considerable medicinal reputation as a potent folk remedy in the treatment of diabetes mellitus (Wee Yeow Chin, 1992). A preliminary study in our laboratory showed the reduction of blood glucose and food intake in diabetic rats given with extracts of *A. bilimbi* fruits and leaves (Tan et al., 1996).

This study was thus initiated with the aim of evaluating the effects of an ethanolic extract of *A. bilimbi* leaves on the blood glucose level and serum lipid profile in streptozotocin-diabetic Sprague–Dawley (SD) rats.

### 2. Materials and methods

#### 2.1. Materials

The plant was collected from a private garden and identified as *Averrhoa bilimbi* by Dr Ruth Kiew, Keeper of Herbarium and Library, Singapore Botanic Gardens. A dried specimen was deposited in the herbarium (Voucher specimen No. BT 2).

#### 2.2. Preparation of the plant extract

The fresh leaves of *A. bilimbi* (1 kg) were blended and extracted with 80% ethanol (10 l) until exhaustion. The mixture was filtered with Whatman No. 1 filter paper (Whatman International, UK). The filtrate was centrifuged for 10 min at 10 000 × g to remove particulate substances. The clear supernatant was concentrated at 40°C by a rotavapor (Buchi Labortechnik, Switzerland) to 1 l. The concentrate was freeze-dried, yielding about 40 g of yellowish-green powder. The extract was suspended in distilled water before use.

#### 2.3. Animals

All experiments were performed on male Sprague–Dawley (SD) rats aged 10 weeks (200–250 g) obtained from the Laboratory Animal Holding Unit, National University of Singapore, Singapore. The animals were acclimatized for 1–2 weeks before being used for the experiments. Standard pelleted diet (Glen Forest, WA, Australia) and water were given ad libitum. Animals were maintained under a constant 12-h light and dark cycle and an environmental temperature of 21–23°C (Niyonzima and Vlietinck, 1993).

#### 2.4. Induction of experimental diabetes mellitus

The overnight fasted SD rats were made diabetic with streptozotocin (STZ) (Sigma, St Louis, MO; 60 mg kg⁻¹, i.p). The STZ was freshly dissolved in citrate buffer (0.01 M, pH 4.5) and maintained on ice prior to use; the injection volume was 1 ml kg⁻¹ (Hamilton et al., 1998). Diabetes was confirmed in the STZ-treated rats by measuring the fasting blood glucose concentration 72 h after injecting STZ. The SD rats with blood glucose level above 350 mg dl⁻¹ were considered to be diabetic and were used in the experiment (Lisa et al., 1998). Animals had free access to food and water after the STZ injection.

#### 2.5. Experimental procedure

##### 2.5.1. The OGTT in normal and STZ-diabetic SD rats

Prior to an oral glucose tolerance test (OGTT) rats were fasted for 16 h. Distilled water (control), a reference drug metformin (500 mg kg⁻¹), or each of three different doses of ABe (125, 250 and 500 mg kg⁻¹) were then orally administered to groups of 4–5 rats each. Thirty minutes later, glucose (3 g kg⁻¹) was orally administered to each rat with a feeding syringe (Al-awadi et al., 1985). Blood samples were collected from the tail vein by tail milking at −30 (just before the ABe and metformin administration), 0 (just before the
oral administration of glucose), 60, 120, and 180 min after glucose load for the assay of glucose (Trinder method, Sigma Diagnostics, Sigma, St Louis, MO).

The OGTTs were performed in STZ-diabetic rats using the same procedure as described for the normal rats. Six animals were used for distilled water (control), metformin (positive control) and ABe-treated groups.

2.5.2. Repeated administration of the ABe in STZ-diabetic SD rats

One week after STZ induction of diabetes in male SD rats, the fasting blood glucose levels were measured. The hyperglycemic rats (blood glucose > 350 mg dl\(^{-1}\)) were divided on day zero into three groups (each with 5–6 rats). The fasting blood glucose level, total cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol concentrations were also measured on day zero. Distilled water, metformin (500 mg kg\(^{-1}\)) and ABe (125 mg kg\(^{-1}\)) were then administered orally twice a day to control, positive control and the treatment groups, respectively for 2 weeks. Body weight, food and water intakes were monitored daily for 2 weeks. On day 15, after 16 h fasting, the rats were decapitated and the blood was collected for estimation of the fasting blood glucose, total cholesterol, triglycerides, LDL-cholesterol, and HDL-cholesterol concentrations. The organs such as liver and kidney were isolated, weighed and stored at \(-70^\circ\)C for the assay of hepatic cytochrome P\(_{450}\) and thiobarbituric acid reactive substances (TBARS) in both liver and kidney.

2.6. Liver microsomal cytochrome P\(_{450}\) content

2.6.1. Preparation of liver microsomes

After 14 days of treatment, all the rats were decapitated and the blood was collected for estimation of the fasting blood glucose, total cholesterol, triglycerides, LDL-cholesterol, and HDL-cholesterol (using diagnostic kits, Boehringer Mannheim, Germany). The organs such as liver and kidney were isolated, weighed and stored at \(-70^\circ\)C for the assay of hepatic cytochrome P\(_{450}\) and thiobarbituric acid reactive substances (TBARS) in both liver and kidney.

2.6.2. Assay of liver microsomal cytochrome P\(_{450}\) content

An aliquot of microsomal preparation of 1 mg protein ml\(^{-1}\) was obtained by adding 0.5 ml of 1 M potassium phosphate buffer and the required volume of 1.15% KCl. A modified technique (Omura and Sato, 1964) was adopted in this assay to eliminate the absorption peak at 420 nm due to contamination by hemoglobin in the sample. The microsomal preparation was placed in two cuvettes and initially saturated with carbon monoxide. A small amount of sodium dithionite (not more than 2 mg) was added to the sample cuvette only. The microsomal P\(_{450}\) content was then determined from the difference in absorbance values between the dithionite-reduced and control microsomal preparations using a Shimadzu UV-dual-beam spectrophotometer. The molar extinction coefficient of microsomal P\(_{450}\) at the \(\lambda_{\text{max}}\) of 450 nm was 91 mM\(^{-1}\) cm\(^{-1}\).

2.7. Assay of TBARS

The liver and kidney samples were minced and homogenized by a polytron homogeniser in ice-cold 1.15% KCl to make a 25% (w/v) homogenate. The determination of the lipid peroxidation level in the liver and kidneys was performed by the thiobarbituric acid method (Uchiyama and Mihara, 1978). Briefly to 0.1 ml of 25% homogenate, 0.2 ml of 8.1% sodium dodecyl sulfate (SDS), 1.5 ml of 1% phosphoric acid and 0.2 ml of distilled water were added, followed by 1 ml of 0.6% 2-TBA. The mixture was heated for 45 min in a boiling water bath. After the
reaction, the mixture was cooled in an ice bath; the cold TBA reactants were extracted with 4.0 ml of n-butanol. After centrifugation at 1000 × g for 10 min, the absorbance of the n-butanol layer was determined at 535 nm. The TBARS values thus obtained were expressed as nmol of malonaldehyde per 25 mg wet tissue.

2.8. Statistical analysis

The results are presented as means ± S.E.M. The changes in body weight, food and water intakes during the 14 days of treatment period were compared by two-way ANOVA. The data on blood glucose, total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol, TBARS, and anti-atherogenic index were analysed by the Student’s t-test (two-tailed). P-values of < 0.05 were considered to be statistically significant.

3. Results

3.1. Effects of ABe on glucose tolerance in normal and STZ-diabetic SD rats

The blood glucose levels of the normal rats reached a peak 60 min after the oral administration of glucose and gradually decreased to the pre-glucose load level (Table 1A). Of the three different doses, viz. 125, 250 and 500 mg kg⁻¹, the lowest dose, i.e. 125 mg kg⁻¹ caused a significant attenuation in the blood glucose at 180 min when compared to the vehicle-treated control group (P < 0.05). Metformin (500 mg kg⁻¹) produced a significant decrease (P < 0.01) in blood glucose level 180 min after the administration of an oral glucose load.

In the diabetic rats, the fasting blood glucose levels were 4–5 times higher than that of the normal SD rats. The ABe at a dose of 125 mg kg⁻¹ produced a significant attenuation (P < 0.05) in the blood glucose (P < 0.01) after the oral glucose load (Table 1B). There was no significant attenuation in the rats administered 250 mg of ABe kg⁻¹, even at 180 min. However, ABe at a dose of 500 mg kg⁻¹ caused a significant attenuation (P < 0.01) in the blood glucose only at 180 min when compared to the vehicle-treated group. Metformin (500 mg kg⁻¹) caused significant attenuation at 60 min (P < 0.001), 120 min (P < 0.01) and 180 min (P < 0.01) when compared to the vehicle-treated group. Of the three doses of ABe tested, the lowest dose (125 mg kg⁻¹) was found to be most effective in improving glucose tolerance (P < 0.01). Hence it was selected for the 2-week study.

Table 1
The mean percentage changes in the blood-glucose levels over the 0-h control, ABe and metformin-treated normal and diabetic rats after oral glucose load (3 g kg⁻¹) in the oral glucose tolerance test (OGTT)

<table>
<thead>
<tr>
<th>Group</th>
<th>Percentage change in blood glucose level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60 min</td>
</tr>
<tr>
<td>A. Normal rat</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>76 ± 16</td>
</tr>
<tr>
<td>ABe 125 mg kg⁻¹, p.o.</td>
<td>39 ± 8.3</td>
</tr>
<tr>
<td>250 mg kg⁻¹, p.o.</td>
<td>67 ± 16.1</td>
</tr>
<tr>
<td>500 mg kg⁻¹, p.o.</td>
<td>60 ± 7.9</td>
</tr>
<tr>
<td>Metformin 500 mg kg⁻¹, p.o.</td>
<td>-2 ± 9.7*</td>
</tr>
<tr>
<td>B. STZ-diabetic rat</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>64 ± 9.6</td>
</tr>
<tr>
<td>ABe 125 mg kg⁻¹, p.o.</td>
<td>23 ± 3.5</td>
</tr>
<tr>
<td>250 mg kg⁻¹, p.o.</td>
<td>47 ± 6.5</td>
</tr>
<tr>
<td>500 mg kg⁻¹, p.o.</td>
<td>36 ± 8.5</td>
</tr>
<tr>
<td>Metformin 500 mg kg⁻¹, p.o.</td>
<td>2 ± 6.7***</td>
</tr>
</tbody>
</table>

* Values are expressed as the mean ± S.E.M. for 4-6 rats in each group. The percentage change in blood glucose at 60, 120 and 180 min was calculated from the corresponding 0-h value (just before the oral administration of glucose) in each group.
* P < 0.05 compared with the corresponding control.
** P < 0.01 compared with the corresponding control.
*** P < 0.001 compared with the corresponding control.
Table 2

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Body weight (g)</th>
<th>Water intake (ml rat(^{-1}) day(^{-1}))</th>
<th>Food intake (g rat(^{-1}) day(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>Vehicle</td>
<td>222 ± 11</td>
<td>205 ± 14</td>
<td>149 ± 27</td>
</tr>
<tr>
<td>ABe</td>
<td>259 ± 18</td>
<td>282 ± 31*</td>
<td>92.6 ± 30</td>
</tr>
<tr>
<td>Metformin</td>
<td>258 ± 13</td>
<td>323 ± 47*</td>
<td>101.1 ± 27</td>
</tr>
</tbody>
</table>

*Values are expressed as mean ± S.E.M. (n = 5 or 6).
*Significantly different from vehicle at \(P < 0.001\).

3.2. Effects of 2-week administration of ABe and metformin on STZ-diabetic SD rats

The body weights in the ABe-treated and the metformin-treated groups were increased significantly \((P < 0.001)\) on day 14 when compared with the vehicle-treated group (Table 2). The food intake was significantly lowered in the ABe-treated and metformin-treated groups \((P < 0.001)\) when compared with the vehicle-treated group. Similarly, the water intake was significantly reduced \((P < 0.001)\) in both ABe-treated and metformin-treated groups (Table 2).

As shown in Table 3, the daily administration of the ABe (125 mg kg\(^{-1}\) twice a day) for 14 days in STZ-diabetic SD rats caused a significant reduction in the blood glucose level when compared with the vehicle-treated control \((P < 0.01)\) rats and day zero value \((P < 0.05)\). Similarly, repeated administration of metformin (500 mg kg\(^{-1}\) twice a day) for 14 days caused a significant reduction \((P < 0.01)\) in the blood glucose level in STZ-diabetic SD rats when compared to vehicle and day zero values. There was a significant decrease in serum triglycerides \((P < 0.05)\) and a significant increase in HDL-cholesterol \((P < 0.05)\) in the ABe-treated SD rats when compared to the vehicle-treated control SD rats. However, ABe did not decrease the serum cholesterol and LDL-cholesterol significantly \((P > 0.05)\). The daily administration of metformin (500 mg kg\(^{-1}\) twice a day) for 14 days to STZ-diabetic SD rats caused a significant decrease in the serum triglycerides \((P < 0.01)\) when compared to the vehicle-treated control rats. Metformin, however, did not decrease serum cholesterol and LDL-cholesterol. It also failed to increase the HDL-cholesterol. The anti-atherogenic index was significantly increased in the ABe-treated group \((P < 0.001)\) when compared to the vehicle-treated group of rats. However, there was no significant difference in the anti-atherogenic index of the metformin and the vehicle-treated groups \((P > 0.05)\). The HDL-cholesterol/total cholesterol ratio was significantly increased in the ABe-treated group when compared to the vehicle-treated group \((P < 0.01)\). However, there was no significant difference \((P > 0.05)\) in the HDL-cholesterol/total cholesterol ratio in the metformin-treated group when compared to the vehicle-treated group.

4. Discussion

Our present studies show that ABe possesses definite hypoglycemic, hypotriglyceridemic, anti-
Table 3
Metabolic variables in STZ-diabetic rats before and after oral treatment with vehicle (distilled water), ABe (125 mg kg⁻¹) and metformin (500 mg kg⁻¹) twice a day for 2 weeks

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Fasting blood glucose (mg dl⁻¹)</th>
<th>Total cholesterol (mg dl⁻¹)</th>
<th>Triglycerides (mg dl⁻¹)</th>
<th>LDL-cholesterol (mg dl⁻¹)</th>
<th>HDL-cholesterol (mg dl⁻¹)</th>
<th>Antiatherogenic index (%)</th>
<th>HDL-TC ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>Vehicle</td>
<td>498.4 ± 57.1</td>
<td>522.9 ± 62.6</td>
<td>81.5 ± 8.2</td>
<td>110 ± 13.1</td>
<td>173.7 ± 9.7</td>
<td>185.8 ± 37.6</td>
<td>26 ± 6.1</td>
</tr>
<tr>
<td>ABe</td>
<td>394.3 ± 46.5</td>
<td>258.6 ± 40.2b,e</td>
<td>70.5 ± 13.1</td>
<td>78.9 ± 6.5</td>
<td>130.6 ± 24.5</td>
<td>44.2 ± 10.2f</td>
<td>21.4 ± 1.8</td>
</tr>
<tr>
<td>Metformin</td>
<td>509.7 ± 20.4</td>
<td>202.4 ± 31.2f</td>
<td>57.5 ± 9.1</td>
<td>72.4 ± 9.4</td>
<td>194.1 ± 7.0</td>
<td>59.1 ± 14.2g</td>
<td>19.7 ± 7.9</td>
</tr>
</tbody>
</table>

a Values are expressed as mean ± S.E.M. (n = 5 or 6).
b Significantly different from vehicle at P < 0.05.
c Significantly different from vehicle at P < 0.01.
d Significantly different from vehicle at P < 0.001.
e Significantly different from zero day at P < 0.05.
f Significantly different from zero day at P < 0.01.

Table 4
Liver cytochrome P₄₅₀ content and lipid peroxidation level in the kidney and liver of STZ-diabetic rats after 2 weeks of oral treatment twice a day with vehicle (distilled water), ABe (125 mg kg⁻¹) and metformin (500 mg kg⁻¹)

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Liver cytochrome P₄₅₀ content (nmol mg⁻¹ protein)</th>
<th>Lipid peroxidation level (nmol of malonaldehyde per 25 mg of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>Vehicle</td>
<td>1.2 ± 0.07</td>
<td>3.3 ± 0.25</td>
</tr>
<tr>
<td>ABe</td>
<td>1.1 ± 0.08</td>
<td>2.9 ± 0.04</td>
</tr>
<tr>
<td>Metformin</td>
<td>1.01 ± 0.03*</td>
<td>2.8 ± 0.13</td>
</tr>
</tbody>
</table>

* Values are expressed as mean ± S.E.M. (n = 5 or 6).
** Significantly different from vehicle at P < 0.01.
atherogenic, and anti-lipid peroxidative properties in STZ-diabetic rats after 2 weeks of treatment. The hypoglycemic activity of ABe was observed at the lowest dose (125 mg kg\(^{-1}\)) in normal as well as STZ-diabetic rats and was similar to the action of metformin. Metformin, a biguanide, (Bailey and Flatt, 1990) does not induce the secretion of insulin from the \(\beta\)-islet cells of pancreas, but it increases glucose utilisation in the extrahepatic tissues, reduces hepatic gluconeogenesis (Bailey, 1992) and increases the expression of insulin receptors in the liver plasma membranes (Kanigur-Sultuybek et al., 1995). Since ABe reduced the blood glucose potently in the STZ-diabetic SD rats like metformin, it may have hypoglycemic principle(s) that are similar in action to metformin.

The daily administration of ABe (125 mg kg\(^{-1}\)) and metformin (500 mg kg\(^{-1}\)) to STZ-diabetic rats twice a day for 2 weeks caused a statistically significant reduction in food and water intakes, and an increase in the body weight in STZ-diabetic rats. This could be the result of improved glycemic control produced by ABe and metformin. The ABe might reduce the triglycerides by decreasing the serum non-esterified fatty acids (NEFA) in the STZ-diabetic rats similar to masoprocol (nordihydroguaiaretic acid), a pure compound isolated from \textit{Larrea tridentata} (Reed et al., 1999). Since ABe increased HDL-cholesterol, it significantly increased the anti-atherogenic index and HDL-cholesterol/total cholesterol ratio. ABe thus has the potential to prevent the formation of atherosclerosis and coronary heart disease which are the secondary diabetic complications of severe diabetes mellitus (Fontbonne et al., 1989). In contrast, metformin failed to increase the HDL-cholesterol level and did not increase the anti-atherogenic index and HDL-cholesterol/total cholesterol ratio. However it has been reported that metformin can reduce the blood lipid parameters in non-diabetic patients with coronary heart disease (Carlsen et al., 1996). Hence, ABe may contain a hypolipidemic principle(s), which could act in a way different from that of metformin.

The cytochromes are the primary system (phase I detoxification enzymes) responsible for chemical defense in animals (Elizabeth Gillam, 1998). The cytochrome \(P_{450}\) content in the liver has been found to be increased in diabetic animals (Lucas et al., 1998). The reduction in the insulin levels in the diabetic state also causes an increase in the level of cytochrome \(P_{450}\) enzymes (Woodcroft and Novak, 1997). In this study cytochrome \(P_{450}\) content of the ABe-treated group was similar to that of the vehicle-treated group. However a reduction was found in the metformin-treated group. The mechanism by which metformin reduces the cytochrome \(P_{450}\) content is not known.

The hyperglycemia in the STZ-treated rats leads to the formation of hydrogen peroxide, which subsequently generates free radicals such as \(O_2^-\) and \(OH^\bullet\). These reactive compounds can cause peroxidation of lipids, resulting in the formation of hydroperoxy fatty acids and endoperoxides. This increases the formation of malonaldehyde (MDA) and thromboxane-B\(_2\) (TxB\(_2\)). The accumulation of TxB\(_2\) along with thromboxane-A\(_2\) (TXA\(_2\)) can cause platelet aggregation and promote thrombosis (Sushil Jain et al., 1998). Since ABe has the ability to reduce the formation of TBARS, it could potentially prevent platelet aggregation and thrombosis.

In conclusion, the hypoglycemic, hypotriglyceridemic, anti-lipid peroxidative as well as the anti-atherogenic properties of ABe might be due to different types of active principles, each with a single or a diverse range of biological activities. Further biochemical and pharmacological investigations are now in progress to isolate and identify the active compound(s) in ABe.

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