Changes in organ and body weight, serum amylase and antidiabetic effects of tannins from *Spondias mombin* on streptozotocin-induced diabetic rats

**Introduction**

Diabetes is a disease of the pancreas characterised by multiple defects in its pathophysiology and abnormalities in protein, fat and carbohydrate metabolism. It is evident that this disease leads to hyperglycaemia and to many other complications such as hyperlipidaemia, hypertension, atherosclerosis, retinopathy, neuropathy and nephropathy. The World Health Organization (WHO) projects that diabetes will be the seventh leading cause of death in 2030. Statistics also show the disease will affect more than the current 347 million people with diabetes by 2030. The Diabetes Association of Nigeria had put the diabetic population in Nigeria at about 10 million as of 2004. The high cost of diabetic medications, their unavailability in poor developing countries and the high cost, where the majority of people in developing countries can barely afford such costs, coupled with the side effects from these drugs, have led to a search for a number of plants used to manage diabetic patients with folk medicines, and the innovation will result in isolating plant active secondary metabolites that can be cost-effective and safer than synthetic products.

Though the mechanism of these plants is not well documented, the majority of studies are being conducted to uncover the mechanism of action of these plants and their isolates. Hence, more attention is being paid to study the proper mechanism of these plants’ actions along with the bioactive compounds responsible for these activities.

Tannins, a secondary metabolite from plants, have been described as developing glucose uptake through mediators of the insulin-signalling pathways, such as phosphoinositide 3-kinase and p38 mitogen-activated protein kinase activation, GLUT-4 translocation and antihyperglycaemic effects.
agent in diabetic rats. Iweala et al. have shown that the seeds of *Spondias mombin* possess hypoglycaemic effects in alloxan-induced diabetic rats, while Nkanu et al. have shown that the leaves of *S. mombin* produce hypoglycaemic and hypolipidemic effects in alloxan-induced diabetic rats. However, little or no work is available on the effect of the tannin extract of *S. mombin* leaves on streptozotocin-induced diabetic rats. The present study seeks to evaluate the serum amylase activity and changes in organ and body weight of diabetic rats treated with tannin extract from *S. mombin* leaves.

**Materials and methods**

**Isolation of tannins**

The leaves of *S. mombin* were collected from a field around the University of Benin campus, Benin City, Nigeria. The leaves were authenticated by a botanist in the department of Department of Plant Biology and Biotechnology of the University of Benin, Benin City, Nigeria. A herbarium specimen was deposited at the University of Benin Herbarium with herbarium number UBH 345. It was air dried and pulverised. The ground leaves (500 g) were weighed into sealable glass flasks and 4 L of 80% acetone (v/v) was poured in. The flaskes were placed in a water bath at 70°C and shaken for 15 min. After cooling, the supernatant was decanted and the extraction was repeated twice more. The supernatants were combined, the acetone was evaporated using a rotary evaporator at 40°C and the aqueous residue was lyophilised.

The crude tannin extract (2 g), dissolved in 100 mL of ethanol, was applied on a column (5 cm × 40 cm) packed with Sephadex LH-20 gel (Sigma Aldrich, Darmstadt, Germany). Ethanol (1 L), used as the first eluent, allowed removal of low molecular weight phenolic compounds. Then 800 mL of 70% acetone (v/v) was used to elute tannins. Collected fractions from the stepwise gradient were tested for the presence of tannins by adding 0.1 M FeCl₃ and monitoring for brownish green or blue-black coloration. Fractions that gave a positive test for tannins were bulked together. Solvent in the tannin fractions was removed using a rotary evaporator. The tannin extract was then stored in the freezer until used.

**Test animals**

A total of 24 male albino Wistar rats weighing 190 g – 220 g were bought and kept in galvanised cages in the Department of Biochemistry animal house. They were divided into four groups containing six rats each. They were allowed access to feed and water *ad libitum* on a 12-hr light – 12-hr dark cycle. The animals were acclimatised for 2 weeks before induction of diabetes.

- **Group A** served as the normal control and was given standard feed only.
- **Group B** were the experimentally induced diabetic rats without tannins extract treatment (diabetic control).
- **Group C** were the experimentally induced diabetic rats treated with the standard drug glibenclamide 5 mg/kg body weight (positive control).
- **Group D** were the experimentally induced diabetic rats and treated with 100 mg/kg body weight of tannins extract.

- Tannins extract was administered to the tannin-treated rats daily for 21 days using orogastric cannula.

**Induction of diabetes**

To overnight-fasted rats, streptozotocin (STZ) was dissolved in cold citrate buffer (0.1 M, pH 4.5) and was administered intraperitoneally at a dose of 65 mg/kg body weight. Blood glucose level was measured on Day 0 and on the third Day after STZ induction using an Accu-Chek® Active Blood Glucose Meter (Roche Diagnostics, Mannheim, Germany); only rats with fasting blood glucose (FBG) level greater than 250 mg/dL (FBG ≥ 250 mg/dL) were selected and used for the study. Thereafter blood glucose was monitored on Day 10 and Day 21 before sacrifice.

**Blood parameters**

Blood was collected from the tail on days 0, 3, 10 and 21 from the control and experimental rats for FBG assay. At the end of the 21-Day experimental period the animals were fasted overnight and blood samples were collected and allowed to clot for 30 min, after which they were centrifuged at 3000 rpm for 15 min. The serum was collected separately and used for serum amylase assay. Serum amylase activity was measured by the method of Wallenfels et al. as described in the manual of the TECO diagnostic α-amylase kit.

Tissues (liver, pancreas, kidney and heart) were also excised and were homogenised in ice-cold normal saline (1:4 w/v), centrifuged at 1000 g for 15 min and the supernatant stored in the freezer until analysis.

**Statistical analysis**

Data for all groups were expressed as mean ± standard error of mean. Statistical analyses were performed using one-way analysis of variance and Student’s *t*-test. Differences were considered to be statistically significant when the *p*-value was less than 0.05 using SPSS statistical package.

**Ethical considerations**

Treatment of the animals was in accordance with the principle of laboratory animal care (NIH publication 85-93, revised 1985).

**Results and discussion**

Table 1 shows the effect of tannins on the body weight of the rats. A significant decrease in body weight was seen in

| TABLE 1: Effects of tannins on body weight of control and diabetic rats. |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Groups                      | Day 0                       | Week 1 (after STZ administration) | Week 2                      | Week 3                      |
| A (normal control)          | 190.0 ± 5.7                 | 206.6 ± 8.82                 | 220.0 ± 11.56               | 230.0 ± 5.77                |
| B (diabetic control)        | 210.0 ± 10.0                | 162.0 ± 2.00                 | 170.0 ± 0.01                | 165.0 ± 5.00                |
| C (positive control)        | 210.0 ± 2.14                | 164.0 ± 2.05                 | 198.5 ± 0.05                | 202.0 ± 6.00                |
| D (tannin-treated)          | 197.5 ± 8.54                | 167.8 ± 7.94                 | 185.5 ± 11.41               | 201.5 ± 2.50                |

STZ, streptozotocin.

*Statistically significant difference when compared with the diabetic control.
*Statistically significant difference when compared with the normal control.
TABLE 2: Effects of tannins on organ weight (grams) of control and diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver</th>
<th>Heart</th>
<th>Kidney</th>
<th>Pancreas</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (normal control)</td>
<td>6.87 ± 0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.78 ± 0.01</td>
<td>1.56 ± 0.09</td>
<td>6.61 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>B (diabetic control)</td>
<td>9.33 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.65 ± 0.03</td>
<td>1.58 ± 0.02</td>
<td>3.5 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C (positive control)</td>
<td>7.01 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.78 ± 0.08</td>
<td>1.48 ± 0.05</td>
<td>0.58 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>D (tannin-treated)</td>
<td>6.29 ± 0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.69 ± 0.03</td>
<td>1.45 ± 0.13</td>
<td>0.58 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Statistically significant difference when compared with the normal control.

TABLE 3: Effects of tannins on blood glucose levels (mg/dL) of diabetic treated and control rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 0</th>
<th>Day 3 (after STZ administration)</th>
<th>Day 10</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (normal control)</td>
<td>88.0 ± 1.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>89.2 ± 2.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>94.25 ± 2.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>93.3 ± 1.37&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>B (diabetic control)</td>
<td>87.0 ± 2.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>328.3 ± 15.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>365.3 ± 9.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>363.5 ± 8.72&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C (positive control)</td>
<td>92.3 ± 5.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>328.4 ± 6.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>262.0 ± 7.79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>145.5 ± 6.44&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>D (tannin-treated diabetic rats)</td>
<td>93.0 ± 4.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>337.0 ± 6.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>298.8 ± 9.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>179.0 ± 9.68&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

STZ, streptozotocin.

TABLE 4: Effects of tannins on serum amylase level (IU/L) of control and diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum amylase (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (normal control)</td>
<td>36.27 ± 1.37&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>B (diabetic control)</td>
<td>8.90 ± 1.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C (positive control)</td>
<td>40.61 ± 1.38&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>D (tannin-treated diabetic rats)</td>
<td>25.88 ± 1.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

IU/L, international units per litre.

Discussion

Diabetes is the leading cause of mortality worldwide and STZ, which is a naturally occurring nitrosourea, has been widely used to induce insulin-dependent diabetes mellitus in experimental animals because of its toxic effects on islet beta cells.17

In the present study, induction of rats with STZ led to a significant decrease in body weight in all administered groups when compared with the normal control (Table 1). However, a significant increase in body weight was seen in the tannin-treated diabetic rats when compared with the control at Week 3. Significant decrease in body weight was seen in the diabetic control groups, which continued until the end of the experimental period. The significant reduction in total body weight seen in the diabetic control rats despite their increased feed intake is attributed to the fact that STZ-induced diabetes is characterised by severe loss of body weight,18 resulting from loss of fat from adipose tissue and catabolism of amino acids in muscle tissue. Moreover, there is loss or degeneration of structural proteins that are known to be a major contributor to body weight. Again, it is also an indication that the food being taken in is not being transformed into weight gain or growth attainment.

The findings from organ weight measurement in this study showed a significant increase in liver weight for the diabetic control rats and a non-significant change in the liver of diabetic tannin-treated rats when compared to the control liver (Table 2). The result also shows a non-significant change in the weight of the heart and kidney of treated and diabetic control rats. Moreover, a significant decrease was observed in the weight of the pancreas in the diabetic control rats when compared with the treated rats. The decrease in the weight of the pancreas could be attributed to the disruption and disappearance of pancreatic islets and selective destruction of insulin-producing cells.19,20 The significant increase in the weight of the liver seen in this study could be attributed to increased triglyceride accumulation leading to enlarged liver, which could be a result of the increased influx of fatty acids into the liver induced by hypoinsulinaemia. This finding is in agreement with previous work by Habibuddin et al.,21 Lee et al.,22 and Ohno et al.23

The destruction of the insulin-secreting β-cells starts 3 days post-STZ administration, reaching its peak at 3–4 weeks in rats, leaving less active cells, which results in a diabetic state.24,25 This explains the increased glucose concentration seen in the study on Day 3 after STZ administration (Table 3). Treatment with tannin extract from S. mombin leaves led to a significant decrease in blood glucose level on days 10 and 21; this decrease was comparable to that seen for the glibenclamide-treated
groups but not comparable to the normal control. This result is in line with work conducted by Velayutham et al. on the antihyperglycaemic effect of tannins from Ficus racemosa. Moreover, Babby et al. showed that tannic acid reduces blood glucose levels in STZ-induced rats; Praveen et al. showed that tannins from Jatropha gossypifolia exert antihyperglycaemic effects. Studies have demonstrated that tannic acid induces glucose transport through activation of the insulin-mediated signalling pathway in adipocytes, in human diabetic patients. A study conducted by Xueqing et al. showed that tannic acid may have the potential to become the lead compound in the development of new types of antidepressant pharmaceuticals that are able to reduce blood glucose levels without increasing adiposity.

Amylases are enzymes that hydrolyse the carbohydrates into small glucose particles. All the alpha amylase enzyme molecules act on alpha 1-4, 1-6 links of glucose residues. The enzyme alpha amylase is one of the most important in the human body, responsible for hydrolysis of starch into small sugar molecules. Amylase is one of the main enzymes produced in exocrine pancreatic cells and may be recognised as an adequate indicator of organ activity, both in physiological and pathological states. The significant decrease in serum amylase activity seen in the diabetic control rats could have resulted from the induction of the animals with STZ (Table 4). STZ has been proposed to cause a reduction in amylase activity by interfering with calcium and magnesium homeostasis and amylase gene expression. Administration of tannins extract to the diabetic rats led to a significant (p < 0.05) increase in serum amylase levels when compared to the diabetic control groups. This therefore shows that the tannins had a stimulant effect on this enzyme and probably exert a similar influence on other pancreatic hydrolases. It has been clearly demonstrated that a significant number of type I diabetes mellitus patients have impaired exocrine pancreatic function, as shown by a reduced duodenal output of enzymes in response to endogenous (meal, nutrients) and exogenous stimuli.

Conclusion
Tannin extract of S. mombin leaves has shown to be a potential agent in the treatment of hyperglycaemia resulting from diabetes as well as restoration of body weight and serum amylase level in STZ-induced diabetic rats.

Acknowledgements
Competing interests
The authors declare that they have no financial or personal relationships which may have inappropriately influenced them in writing this article.

Authors’ contributions
I.O. was the project supervisor, and was responsible for the experimental and project design and also prepared the write-up.

References


