ANTIDIABETIC EFFECT OF THE SAPONIN-RICH FRACTION OF THE EXTRACT OF TAMARINDUS INDICA L. ON EXPERIMENTAL HYPERGLYCAEMIA

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Keywords: Alloxan, fructose, Hyperglycemia, metformin, Tamarindus indica

ABSTRACT: It is estimated that more than 170 million people are suffering from diabetes globally and this number is expected to double by 2030, and the greatest increase in prevalence is, however, expected to occur in Asia and Africa. Diabetes is the most common endocrine disease and its prevalence is reaching epidemic proportion worldwide. Tamarindus indica is a slow growing tree that is resistant to strong winds and perennial. The stem-bark extract of the plant is used locally for the management of diabetes. The saponin-rich portion of the stem-bark extract of Tamarindus indica L. was investigated for its hypoglycaemic action on experimentally induced hyperglycaemic Wistar rats. The oral LD₅₀ of the extract was found to be 1,265 mg/kg. The extract lowered the Blood Glucose Level (BGL) in the three doses used (100, 200 and 400 mg/kg) and was significant at 400 mg/kg dose after the 8th and 16th hours. The 200 mg/kg dose significantly lowered the BGL at 24 hours p < 0.02. The saponin-rich portion of Tamarindus indica Linn significantly lowered elevated BGL in the experimental animal models.

INTRODUCTION: More than 10% of the population is affected by diabetes mellitus and it is the fifth most common cause of death worldwide. Diabetes mellitus is often linked with abnormal lipid metabolism and dyslipidemia and hyperlipidemia are recognized complications of diabetes mellitus characterized by increased levels of cholesterol, triglycerides and phospholipids and alterations in lipoprotein composition ¹, ². Diabetes is the world’s largest endocrine disease with deranged carbohydrate, fat and protein metabolism ³. The increasing prevalence of diabetes is reaching epidemic proportion worldwide. According to the World Health Organization (WHO) report, approximately 150 million people have diabetes worldwide, and this figure may double by the year 2025. As part of the pathogenesis of noninsulin-dependent diabetes mellitus (NIDDM), the skeletal muscle, liver and adipose tissue become resistant to the hormonal effects of insulin, which in turn leads to decrease insulin-mediated glucose disposal, hepatic glucose overproduction and a marked increase in lipolysis ⁴. Fructose feeding leads to insulin resistance and a compensatory hyperinsulinemia responses ⁵, ⁶.

Nigeria is among the top five countries with the highest cost of diabetic care in Sub Saharan Africa ⁷. Tamarindus indica Linn, belongs to the family Caesalpiniaeae, which is a sub-family in Leguminosae; a dicotyledonous. Caesalpiniaeae, is the third largest family of flowering plants ⁸. The tamarind tree grows slowly and is resistant to strong winds and it is perennial. The stem-bark of
the plant is used locally in the management of diabetes mellitus but there is no scientific evidence to support this claim. The extract does not lower a normoglycaemic BGL. The plant is also reported to possess potent hypolipidemic activity, it also has the potential of restoring altered liver enzyme parameters.

This study aims to scientifically validate the hypoglycaemic activity of the plant.

**METHODOLOGY**

**Plant Collection**

A sample of the plant was collected from Namaye in Bunkure Local Government Area of Kano state Nigeria. Botanical identification was done at the herbarium unit of the Department of Biological Sciences, Ahmadu Bello University Zaria. Mallam U. S. Gallah of the herbarium unit compared the sample with voucher specimen 00026.

**Animals used in the study**

Male and female Wistar albino rats (weighing 150-200 g) obtained from the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria were used. The rats were housed in polypropylene cages at room temperature and maintained on standard laboratory animal feed obtained from the Department and water *ad libitum*, throughout the study. These studies were carried out in Ahmadu Bello University in accordance with the rules governing the use of laboratory animals as accepted internationally.

**Saponin-rich fraction**

The method described by Woo *et al.*, (1980) was followed. The method involves deffatting initially with petroleum ether followed by extraction with hydroalcoholic solution. Polar compounds were further removed by dissolving the hydroalcoholic extract in diethyether solution with subsequent addition of water. Butanol was added to the water residue and the mixture shaken vigorously. The two distinct layers were then separated and 1% potassium hydroxide (KOH) solution was added to the butanol residue and gently shaken. The butanolic portion contains the saponin-rich portion. The KOH solution (alkaline fraction) was neutralized with diluted hydrochloric acid (HCl) then partitioned with n-butanol. The n-butanol fraction was removed, concentrated and tested for the presence of flavonoids. This fraction was subsequently referred to as saponin-rich fraction.

**Acute Toxicity Studies**

The oral median lethal dose (*LD₅₀*) of the extract in rats was conducted according to the method of Lorke (1983) with modifications. The method was divided into two phases. In the initial phase, 3 groups of three rats each were treated with the extract at doses of 10, 100 and 1000 mg/kg body weight orally and the rats were observed for clinical signs and symptoms of toxicity within 24 hours and death within 72 hours.

In the second phase, 4 groups each containing one fresh rat was administered with three more specific doses of the extract based on the result of the initial phase. The animals were also observed for clinical signs and symptoms of toxic effects and mortality for 14 days.

The *LD₅₀* value was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived (0/1 and 1/1).

**Alloxan-Induced Hyperglycaemia**

Hyperglycaemia was induced by a single intraperitoneal injection of 150 mg/kg body weight of alloxan to 12 hours fasted rats. Six hours after the alloxan administration, the rats were maintained on 5% glucose solution for the next 24h to prevent hypoglycaemia that may result from acute massive pancreatic release of insulin.

Seventy-two hours after drug administration, the rats were examined for hyperglycaemia by cutting the tail tip and using a one touch glucometer with compatible strips. Animals with fasting blood glucose of 180 mg/dL and less than 550 mg/dL were used in the study. Blood samples for blood glucose determination were collected from the tail at intervals of 0, 1, 4, 8, 16 and 24 hours. Determination of blood glucose level was done by the glucose-oxidase principle using the one touch Basic.

**Group I**: Received normal saline orally

**Group II**: Received 100 mg/kg body weight of saponin-rich fraction of methanol stem bark extract of *T. indica* orally.
**Group III:** Received 200 mg/kg body weight of Saponin-rich fraction of methanol stem bark extract of *T. indica* orally.

**Group IV:** Received 400 mg/kg body weight of Saponin-rich fraction of methanol stem bark extract of *T. indica* orally.

**Group V:** Received metformin 250mg/kg body weight orally\(^{17, 18}\).

**Fructose-induced Insulin Resistance Model**

For this model the method\(^{19, 20}\) was adopted. The animals were divided into six groups of five rats each.

**Group I:** Received 10%w/v Fructose solution *ad libitum* and 100 mg/kg body weight methanol stem bark extract of *T. indica* orally daily for 28 days.

**Group II:** Administered 10%w/v Fructose solution *ad libitum* and 200 mg/kg body weight of methanol stem bark extract of *T. indica* orally daily for 28 days.

**Group III:** Received 10%w/v Fructose solution *ad libitum* and 400 mg/kg body weight of methanol stem bark extract of *T. indica* orally daily for 28 days.

**Group IV:** Fructose – fed with 10%w/v fructose solution *ad libitum* in their drinker for 28 days only.

**Group V:** Received normal saline only.

**Group VI:** Received 10%w/v fructose solution *ad libitum* and metformin 250mg/kg

All rats were fasted for half an hour prior to extract administration every day.

**Data Analysis**

Results were expressed as mean ± SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA). Student’s t-test at 95% level of significance was used to assess significant difference between the control and treated group. The results are presented in tables and charts.

**RESULTS:**

The extract gave a yield of 6.2%, the oral LD\(_{50}\) in rats for the saponin-rich fraction was calculated to be 1,265 mg/kg body weight. The saponin-rich fraction lowered the BGL in the three doses used (100, 200 and 400 mg/kg) and was significant at 400 mg/kg dose after the 8th and 16th hours. The 200 mg/kg dose significantly lowered the BGL at 24 hours and metformin after one hour p < 0.02 (Fig. 1b).

![Graph](image-url) **FIG 1a:** THE EFFECT OF SAPONIN-RICH FRACTION EXTRACTED FROM THE STEM-BARK EXTRACT OF *T. INDICA* ON BLOOD GLUCOSE LEVELS OF ALLOXAN INDUCED HYPERGLYCAEMIA

n = 6

** = sig at p < 0.02 Vs Normal saline group

*Student’s T-test*

S.F = Saponin Fraction

MFN = Metformin
**FIG 1b: THE EFFECT OF SAPONIN-RICH FRACTION EXTRACTED FROM THE STEM-BARK EXTRACT OF T. INDICA ON BLOOD GLUCOSE LEVELS OF ALLOXAN INDUCED HYPERGLYCEAMIA**

All doses of the saponin-rich fraction used lowered the BGL on the 10th and 20th days. But it was significantly lowered on both days at 400 mg/kg and 100 mg/kg doses. However, the 200 mg/kg dose only lowered the BGL significantly on the 20th day (Table 1).

**TABLE 1: THE EFFECT OF SAPONIN-RICH FRACTION EXTRACTED FROM THE STEM-BARK EXTRACT OF T. INDICA ON BLOOD GLUCOSE LEVEL OF FRUCTOSE INDUCED INSULIN RESISTANCE IN WISTAR RATS AFTER 10 AND 20 DAYS**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean blood glucose level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(10 days)</td>
</tr>
<tr>
<td>Normal saline</td>
<td>8 ± 0.8</td>
</tr>
<tr>
<td>S.F. 400mg/kg + fructose</td>
<td>97 ± 0.7*</td>
</tr>
<tr>
<td>S.F. 200mg/kg + fructose</td>
<td>124 ± 1.4</td>
</tr>
<tr>
<td>S.F. 100mg/kg + fructose</td>
<td>109 ± 1.2*</td>
</tr>
<tr>
<td>Fructose only</td>
<td>182 ± 2.3</td>
</tr>
<tr>
<td>MFN 250 mg/kg + fructose</td>
<td>87 ± 2.1**</td>
</tr>
<tr>
<td></td>
<td>(20 days)</td>
</tr>
<tr>
<td>Normal saline</td>
<td>9 ± 0.5</td>
</tr>
<tr>
<td>S.F. 400mg/kg + fructose</td>
<td>90 ± 0.8*</td>
</tr>
<tr>
<td>S.F. 200mg/kg + fructose</td>
<td>115 ± 1.3*</td>
</tr>
<tr>
<td>S.F. 100mg/kg + fructose</td>
<td>102 ± 1.4*</td>
</tr>
<tr>
<td>Fructose only</td>
<td>178 ± 2.1</td>
</tr>
<tr>
<td>MFN 250 mg/kg + fructose</td>
<td>92 ± 1.9*</td>
</tr>
</tbody>
</table>

DISCUSSIONS: The study seeks to demonstrate the efficacy of saponin-rich portion of *Tamarindus indica* Linn in lowering an elevated blood glucose concentration as well as to investigate the ability of the portion at preventing a rise in blood glucose level. The stem-bark extract has been reported to have blood glucose lowering activity in hyperglycaemic animals. The oral median lethal dose of the fraction was calculated to be 1,265 mg/kg. A scale proposed by Lorke, (1983) roughly classifies substance as; only slightly toxic (LD$_{50}$ up to 1000 mg/kg), LD$_{50}$ values greater than 1000 mg/kg are considered safe. Alloxan is one the various chemical methods of inducing experimental diabetes, alloxan diabetes has been commonly utilized as an animal model of insulin dependent diabetes mellitus (IDDM). In the present study, alloxan caused a significant increase in blood glucose concentration when compared to normal animals. It can be suggested that *T. indica* lowers the elevated glucose level by increasing peripheral glucose uptake. This is supported by the fact that metformin also lowered glucose level meaning that there is still some residual function of the β-cells.

High fructose intake over a long period has been shown to lead to rapid stimulation of lipogenesis and triglyceride accumulation; resulting in reduced insulin sensitivity and hepatic insulin resistance or glucose tolerance.

The saponin-rich fraction reduced elevated BGL in both the alloxan and fructose-induced hyperglycaemia at all the doses used and the hours monitored. However, BGL was only significantly (p < 0.05) lowered after 8 and 16 hours for 400
mg/kg dose and after 24 hours for the 200 mg/kg dose. All doses of the fraction significantly (p < 0.05) lowered the BGL after 20 days in the fructose-induced hyperglycaemic model, and both the 400 mg/kg and 100 mg/kg significantly lowered the BGL after 10 days.

Shane-McWhoeter in 2001 reported that extracts with high triterpenoid saponin content mediate their hypoglycaemic effect through inhibition of intestinal glucose uptake, increase hepatic glucose deposition and enhanced hyperinsulinemia. Since *T. indica* contains saponins, it is possible that the reduction in glucose concentration seen in both the alloxan and fructose induced hyperglycaemia could be due to the presence of this phytoconstituent. Other studies further showed that saponin was used to decrease experimental hyperglycaemia induced by adrenaline, glucose and alloxan. Abdel-Hassan and co-workers reported that saponin components have been used to reduce glycaemia induced by alloxan in rabbits and suggested that saponin glycoside components could be responsible for the observed hypoglycaemic effect.

REFERENCES:


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