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Antihyperglycaemic Activity of the Stem-Bark Extract of *Tamarindus indica* L. on Experimentally Induced Hyperglycaemic and Normoglycaemic Wistar Rats

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Abstract: Diabetes is the most common endocrine disease and its prevalence is reaching epidemic proportion worldwide. In 2002, WHO Expert Committee on diabetes mellitus recommended an urgent and further evaluation of the folkloric methods of managing the disease. In response to this recommendation, several medicinal plants are currently being investigated for their hypoglycaemic activity and one of such plants is *Tamarindus indica*. *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 stem-bark extract of *Tamarindus indica* L. was investigated for its hypoglycemic action on experimentally induced hyperglycaemic Wistar rats using a single dose of alloxan monohydrate (150 mg kg^{-1} IP). The oral LD_{50} of the extract was found to be greater than 5,000 mg kg^{-1}. Phytochemical screening revealed the presence of carbohydrates, glycosides, saponins, flavonoids, cardiac glycosides, tannins, alkaloids and triterpenes. The 1000 mg kg^{-1} dose of the extract lowered the blood glucose level significantly ($p<0.05$) at the 4th, 8th and 16th h. The 500 mg kg^{-1} lowered the BGL significantly ($p<0.05$) throughout the study. In the oral glucose load method the 1000 mg kg^{-1} dose of the extract significantly ($p<0.05$) lowered elevated blood glucose at the 3rd and 5th. The 500 mg kg^{-1} lowered the blood glucose from the 1st to the 5th, while the 250 mg kg^{-1} also lowered the blood glucose level but only significantly at the 5th h. The extract is practically non toxic when administered orally. The stem-bark extract of *Tamarindus indica* Linn significantly lowered elevated Blood Glucose concentration (BGL) in the experimental animal models, while the crude extract was able to prevent an elevation in BGL when used in the oral glucose load model.

Key words: Hyperglycemia, *Tamarindus indica,* metformin, glibenclamide

INTRODUCTION

According to World Health Organisation (WHO), the global burden of diabetes was estimated to be about 177 million in the year 2000 (WHO, 2003). The International Diabetes Federation in its second edition of the Diabetes Atlas estimated that 194 million people had diabetes in 2003 and over 60% of these people lived in less developed countries including Nigeria (International Diabetes Federation, 2003). However, countries in the Sub-Saharan region of Africa continue to regard diabetes as uncommon over the 5 to 6 decades. This increases in the incidences of diabetes is as a result of increases in populations with older people, increasing urbanization (WHO, 1998) and associated changes in risk-factor levels, such as tobacco smoking, obesity and physical inactivity (Hunter *et al*., 2000; Kaufman *et al*., 1999; Pavan *et al*., 1997). Sub-Saharan Africa countries are in various stages of their epidemiological development with a multiple burden of diseases.

Diabetes mellitus can be classified into four principal types, type 1 or Insulin Dependent Diabetes mellitus (IDDM), type 2 or Non Insulin Dependent Diabetes Mellitus (NIDDM), other specific types of diabetes and gestational diabetes mellitus. Type 2 and 1 are the most commonly seen in Sub-Saharan Africa (WHO, 1999).

Tamarindus indica Linn, belongs to the family Caesalpiniaceae, which is a sub-family in Leguminosae; a dicotyledonous. Caesalpiniaceae, is the third largest family of flowering plants (Lewis *et al*., 2005). 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. This study aims to scientifically validate the hypoglycaemic activity of the plant.

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MATERIALS AND METHOD

Plant collection: A sample of the plant (stem-bark of *Tamarindus indica* L.) was collected by scrapping the trunk 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.

The stem-bark was cleaned and air dried under shade for 26 days. It was then pulverized using a pestle and mortar and then sieved to obtain the fine powder. The powder was weighed and kept in an air tight container.

Animals used in the study: Male and female Wistar albino rats (weighing 150-200 g) obtained from the animal house facilities of 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.

Preparation of the extracts: To 500 g of the powder 1 L of 90% methanol was added and allowed to soak for 48 h in a separating funnel. The filtrate was then collected in a conical flask and transferred to an evaporating dish where it was evaporated to dryness on a water bath at a temperature of 62°C. The extract was collected in an air tight container and labeled as methanol stem-bark extract of *Tamarindus indica* and it was kept in a desiccator until ready for use.

Phytochemical screening: The screening was carried out in accordance with the standard protocol as described by Trease and Evans (1989).

Acute toxicity study: The oral LD_{50} of the extract in rats was conducted according to the method described by Lorke (1983). Briefly, the method was divided into two phases. In the initial phase, animals were randomly divided into 3 groups of three rats each. Group I, II and III were treated with 10, 100 and 1000 mg kg^{-1} b.wt. orally of the extract and observed for signs of toxicity and death for 24 h. In the second phase, 4 groups each containing one mouse was administered with four more specific doses of the extract based on the results obtained during the first phase. The LD_{50} value was calculated by taking geometric mean of the lowest dose that caused death and the highest dose that did not produce death.

Alloxan-induced hyperglycaemia: Hyperglycemia was induced by a single intraperitoneal injection of 150 mg kg^{-1} alloxan monohydrate diluted in citrate buffer to rats previously fasted for 12 h (Dunn and McLetchie, 1943; Goldner and Gomori, 1944). Six hours after the induction of hyperglycaemia, the rats were maintained on 5% glucose solution for 24 h to prevent hypoglycaemia that may result from acute massive pancreatic release of insulin (Owu *et al*., 2006). Animals with blood glucose level of 10 mmol L^{-1} and above but less than 31 mmol L^{-1} were used in the study. Blood samples for blood glucose determination were collected from the tail by cutting the tail tip and getting a drop of blood at intervals of 0, 2, 4, 8 and 24 h. Blood glucose level was evaluated by the glucose-oxidase principle (Beach and Turner, 1958) using the one touch Basic (Lifescan, Milpitas, CA) and results were expressed as mmol L^{-1} (Rheney and Kirk, 2000).

The hyperglycaemic Wistar rats were randomly divided into five groups of five animals each. The first group received normal saline while groups II-IV were administered 100, 200 and 400 mg kg^{-1} b.wt. of the extract, respectively. The fifth group was administered with metformin 250 mg kg^{-1} (Santure *et al*., 2000; Solskov *et al*., 2008). All treatments were given orally.

Effect of extract on normoglycaemic animals: For this experiment thirty rats of either sex were used, they were divided into five groups of six rats. Group I received normal saline, groups II-IV were administered graded doses of the extract while group V received metformin 250 mg kg^{-1}.

Oral glucose-induced hyperglycaemia model: In this model described by Sepici *et al*. (2004), 12- 14 h fasted rats were randomly divided into 6 groups of 6 rats each. Group VI which served as untreated control received distilled water 1 h before treatment at a dose of 10 mL/kg/oral. Group V served as the model control and were pretreated with 1 mg kg^{-1} glibenclamide 1 h before the oral administration of 3 g kg^{-1} of D-glucose. Groups I-IV were administered 10 mL/kg/oral distilled water, 250, 500 and 1000 mg kg^{-1} of methanol stem bark extract of *T. indica*, respectively, 1 hour before treatment with 3 g/kg/oral D-glucose.

The blood glucose level of the animals was recorded at 0 hour and then after every one hour for the following six hours using an acucheck

glucometer with compatible strips. A drop of blood was collected from the tail tip of the animals.

RESULTS

The extract gave the yield of 17.6%, the oral acute toxicity test (LD_{50}) of the extract was found to be greater than 5,000 mg kg^{-1} b.wt. Phytochemical test of the methanol stem-bark extract of *Tamarindus indica* L. showed the extract to contain carbohydrate, saponins, flavonoids, alkaloids and steroids.

Effect of the extract on normoglycaemic animals: The extract showed a slight non significant increase in the blood glucose level only at the first hour after its administration (Table 1). Four hours after extract administration, there was a gradual reduction of the blood glucose level to normal and 24 h post administration the blood glucose levels were back to normal.

Effect of extract on alloxan-induced hyperglycaemia: The 1000 mg kg^{-1} dose of the extract lowered the blood glucose level significantly ($p<0.02$) at the 4th hour and ($p<0.05$) at the 8th and 16th hours. However, after 24 h the blood glucose level was low but not significantly when compared with the normal saline group. In the group that received 500 mg kg^{-1}, the blood glucose level was significantly lowered ($p<0.05$) throughout the study. The dose of 250 mg kg^{-1} of the extract gave a similar result to the 1000 mg kg^{-1} dose that is significant difference ($p<0.05$) at the 16th hour. The standard drug used (metformin) lowered the blood glucose level significantly ($p<0.05$) also at the 4th, 8th and 24th h and ($p<0.02$) at the 16th h after oral administration (Fig. 1).

$$\text{Glycaemic change (\%)} = \frac{\text{Glucose conc. (1,4,8,16 or 24 h)} - \text{fasting blood glucose 0h)}}{\text{Fasting blood glucose}} \times 100$$

The oral glucose-induced hyperglycaemic model: The dose of 1000 mg kg^{-1} of the extract significantly ($p<0.05$) prevented elevation in blood glucose at the 3rd and 5th h in the rats. The 500 mg kg^{-1} lowered the blood glucose from the 1st to the 5th hour but only significantly at the 2nd and 3rd h. The 250 mg kg^{-1} also lowered the blood glucose level but only significantly at the 5th h. The standard drug (glibenclamide) significantly ($p<0.05$) lowered the blood glucose level at the 2nd, 3rd and 4th (Fig. 2).

Table 1: Effect of single administration of methanolic stem-bark extract of *T. indica* on blood glucose levels of normoglycaemic Wistar rats

Dose (mg kg^{-1})	Mean blood glucose levels (mg dL^{-1})				
	0 h	1 h	4 h	8 h	24 h
N/saline	86.3±4.4	86.2±6.1	84.2±5.9	86.0±4.8	84.5±1.6
T.I. 250	83.1±5.1	91.3±4.7	93.3±1.8	86.1±9.1	85.3±1.6
T.I. 500	85.1±4.0	90.8±4.3	82.7±2.8	79.8±4.3	83.1±6.2
T.I. 1000	84.8±4.1	88.8±2.7	82.5±8.1	83.5±2.7	80.3±1.3
MFN	82.1±6.9	83.9±6.2	83.5±4.2	80.3±4.8	83.3±2.4

n = 6, T.I.: *Tamarindus indica* L., MFN: Metformin

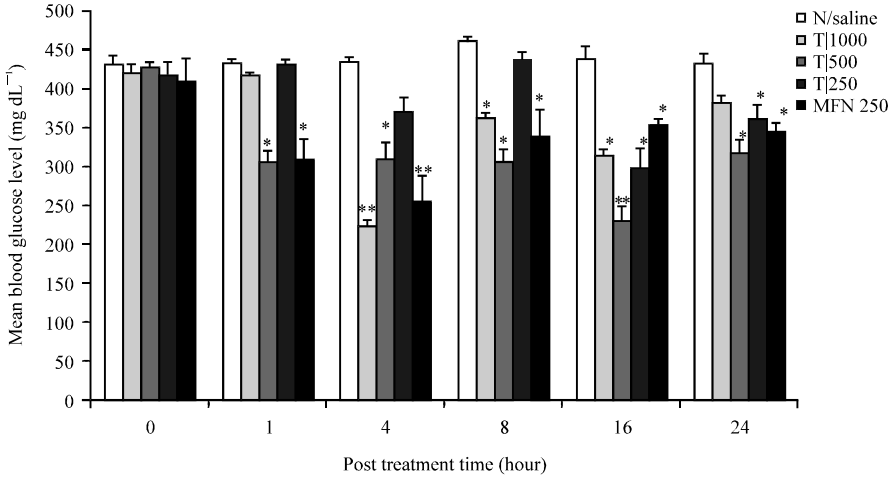

Fig. 1: Effect of methanolic stem-bark extract of *T. indica* on blood glucose levels of alloxan induced hyperglycaemia, n = 6, *significant at $p<0.05$ vs. Normal saline group, **Significant at $p<0.02$ vs. Normal saline group

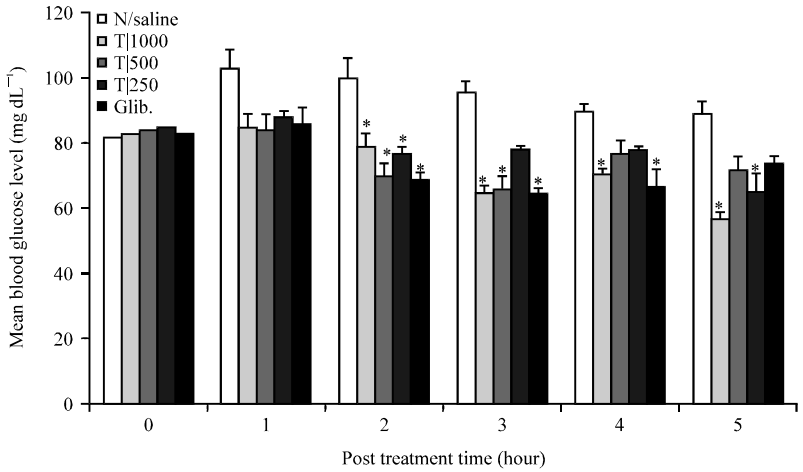

Fig. 2: Effect of methanolic stem-bark extract of *T. indica* on Oral Glucose-induced hyperglycaemic Wistar rats, n = 5, *Significant at $p<0.05$ vs. Normal saline group

DISCUSSION

The phytochemical screening of the methanolic stem-bark extract of *Tamarindus indica* revealed the presence of carbohydrate, glycosides, cardiac glycosides, steroids and terpenoid, flavonoids, tannins and alkaloids. Literature has shown that extracts that have a high content of triterpenoid saponin mediate their hypoglycaemic effect through prevention of intestinal glucose uptake, increase hepatic glucose deposition and enhanced hyperinsulinemia (Shane-McWhorter, 2001). The observed hypoglycaemic effects of *Tamarindus indica* could be due to the presence of one or combination of the active chemical constituents in the extract. An acute toxicity study in animals is important to drug development. The oral median lethal dose of methanol stem-bark extract of *Tamarindus indica* L. in rat was found to be greater than 5,000 mg kg^{-1}. This suggests that the stem-bark extract is non-toxic when administered orally.

The effect of the stem-bark extract was first investigated on normoglycaemic animals to test its effect at causing hypoglycaemia. The extract, unlike most oral antihyperglycaemic agents and like the standard drug used (metformin) did not lower the blood glucose concentration significantly. Use alloxan is one the various chemical methods of inducing experimental hyperglycaemia, it causes selective cytotoxicity of the pancreatic β-cells by disrupting the cell membrane when it accumulates in the cell (Palmer and Lernmark, 1997), these leads to a decrease in the secretion and release of endogenous insulin, which in turn leads to decreased glucose utilization by the tissues (Yamamoto *et al*., 1981; Szkudelski, 2001).

In the present study, there was an increase of over 400% from the normal blood glucose level which was sustained for seventy-two hours. Graded doses of the extract and metformin lowered the glucose concentration significantly ($p<0.05$) within the first 24 h with the 500 mg kg^{-1} dose of the extract being the most effective. The 500 mg kg^{-1} dose lowered the BGL throughout the hours of study. The greatest change in glycaemic concentration (40%) was seen with the 1000 mg kg^{-1} dose after 4 h followed by metformin (38%) also at 4 h. The animals in the first group were administered normal saline and the BGL increased from the first to the 16th hour. 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.

Following absorption of glucose, there is marked postprandial hyperglycaemia which is often accompanied by increased insulin secretion, particularly in the first few hours postprandial (Guyton, 1991). In this study, graded doses of the extract prevented an increase and at the 5th hour lowered the glucose concentration below the level at zero hour. The 500 mg kg^{-1} dose and glibenclamide gave significant reduction by the 2nd and 3rd hours but the 1000 mg kg^{-1} dose produced hypoglycaemic state at the 5th h.

All the doses of the extract and the standard drug used prevented a rise in post-absorptive blood glucose concentration. This preventive effect is seen after the first hour when compared with the normal saline group. It is hereby suggested that the extract produced its hypoglycaemic effect by inhibiting intestinal glucose uptake like the α-glucosidase inhibitors, this class of antidiabetic agents act by a competitive reversible inhibition of pancreatic α-amylase and membrane bound intestinal α-glucosidase hydrolase enzyme. The animals in group I that received normal saline had a low glucose

concentration at the 5th hour; which could be due to a secondary effect of hyperinsulinemia after the glucose load. This is a normal physiological response to hyperglycaemia following a high glucose intake (Hedeskov, 1980).

It can be said that the extract of *T. indica* has potentials to lower an elevated BGL and also prevent any increase in BGL; the extract does not lower a normoglycaemic BGL.

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