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Effect of the fractions of *tamarindus indica* L. (Caesalpiniaceae) on experimentally induced hyperglycaemic wistar rats.

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Abstract
Introduction: World Health Organization defines diabetes mellitus as "a metabolic disorder of multiple etiologies, characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both.
Objective: The stem-bark extract of *Tamarindus indica* L. was investigated for its hypoglycaemic action on experimentally induced hyperglycaemic Wistar rats.
Methodology: A single dose of alloxan (150 mg/kg IP) and fructose (10% w/v *ad libitum* for 20 days). LD_{50} and phytochemical screening were conducted using Lorke's method 1983 and Trease and Evans 1989 respectively.
Results: The oral LD_{50} of the extract was found to be 3,800 mg/kg. The fractions of the extract lowered the elevated blood glucose significantly with the 1000 mg/kg dose at the 8th, 16th, and 24th hours. The 500 mg/kg dose also lowered the glucose level throughout the study but only significantly at the 1st, 16th, and 24th hours. The ethyl acetate fraction also lowered the elevated blood glucose with all the doses used. The 250 mg/kg dose did not show significant decrease in the blood sugar concentration.
Conclusion: The fractions of the stem-bark extract of *T. indica* L. significantly lowered elevated blood glucose concentration (BGL) in alloxan and fructose-induced hyperglycaemic Wistar rats.
Keywords: *T. indica,* alloxan, fructose, hyperglycaemia.

1. Introduction
Diabetes is the largest endocrine disease worldwide[1]. World Health Organization defined diabetes mellitus as "a metabolic disorder of multiple etiologies, characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs"[2]. Therefore, the metabolic aberration of diabetes results from insufficient insulin action, due to deficient insulin secretion or insensitivity to its action, or a combination of both[3,4].

In developing countries adherence to therapies is very low, resulting in poor health outcomes at an expensive cost for society, governments and families[5]. If not successfully managed, diabetes along with other chronic diseases will become the most expensive problem faced by the health care systems. Several herbal preparations have been studied in the search for an effective management of diabetes mellitus and most of them have therapeutic claims[6]. However, several other medicinal plants in use today in Nigeria for the management of diabetes mellitus remain invalidated scientifically and one of such is the stem-bark of *Tamarindus indica* L.

The WHO has recommended and encouraged the use of alternative therapy especially in countries where access to the conventional treatment of diabetes is not adequate. The World Health Organisation also approved the use of plant-based drugs for different ailments, including diabetes mellitus[7]. Also in 2002, WHO Expert Committee on diabetes mellitus recommended an urgent and further evaluation of the folkloric methods of managing the disease[8]. In response to this recommendation, several medicinal plants are currently being investigated for their hypoglycaemic efficacies and one of such plants is *Tamarindus indica.* Many of the medicinal plants in use today, employed for the local management of diabetes mellitus remain invalidated including the stem-bark of *Tamarindus indica.*

1.1 Hypothesis
Fractions of the stem-bark extract of *Tamarindus indica* L. possess hypoglycaemic activity.

1.2 Aim of the Study
To investigate the hypoglycaemic effect of the fractions of the stem-bark extract of *Tamarindus indica* L. on experimentally induced hyperglycaemic Wistar rats.

2.0 Materials and Method

2.1 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.

2.2 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 Nigeria 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.

2.3 Preparation of the extract

Fifty grams of methanol extract was dissolved in distilled water and then filtered; the filtrate was shaken vigorously with 200 ml of butanol in a separating funnel. The resulting mixture was allowed to stand for about an hour then the aqueous and the butanol layers were collected separately. This process was repeated until enough quantity of the butanol fraction was obtained and was evaporated to dryness on a water bath maintained at 62^{O}C. The aqueous layer was kept and used later. This same procedure above was done to obtain the ethyl acetate fraction.

2.4 Phytochemical screening

The screening was carried out in accordance with the standard protocol as described by Trease and Evans[9].

2.5 Acute toxicity study

The oral LD_{50} of the extract in rats was conducted according to the method described by Lorke[10]. 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 body weight orally of the extract and observed for signs of toxicity and death for 24 hours. 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.

2.6 Alloxan-Induced Hyperglycaemia

Hyperglycaemia was induced by a single intraperitoneal injection of 150 mg/kg body weight of alloxan to 12 hours fasted rats[11, 12]. 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[13]. 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 [Lifescan, Milpitas, CA]. 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[14].

The alloxan-induced hyperglycaemic Wistar rats were randomly divided into five groups of five rats each as shown below,

Group I : Received normal saline orally
Group II : Received 250 mg/kg body weight of methanol stem bark extract of *T. indica* orally
Group III : Received 500 mg/kg body weight of methanol stem bark extract of *T. indica* orally
Group IV : Received 1000 mg/kg body weight of methanol stem bark extract of *T. indica* orally
Group V : Received metformin 250mg/kg body weight orally[15, 16].

2.7 Fructose-induced Insulin Resistance Model

For this model the method Dai *et al.*, 1995[17] and Vikrant *et al.*, 2001[18] was adopted. The animals were divided into six groups of five rats each.

Group I: Received 10%w/v Fructose solution *ad libitum* and 250 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 500 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 1000 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

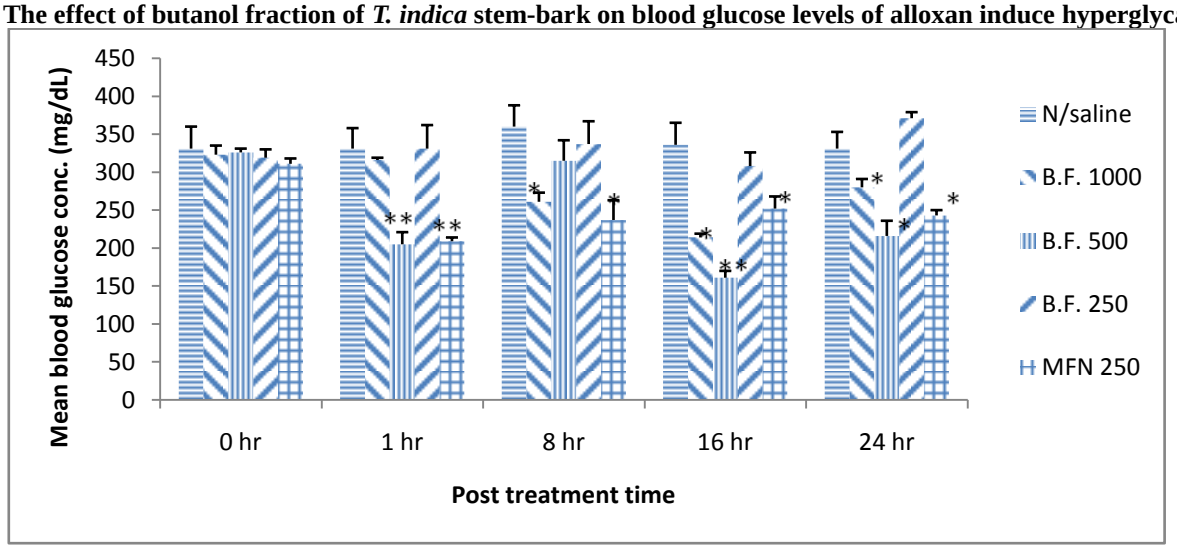
All rats were fasted for half an hour prior to extract administration every day. On the 29th day of extract administration the animals were sacrificed under slight chloroform aneasthesia. Blood was collected from the jugular vein upon sacrifice. Serum was separated by centrifugation at 3000 rpm for 10 minutes and it was used to analyze changes in the lipid profile and liver enzymes. The blood glucose level was monitored on the tenth and twentieth days.

3.0 Results

3.1 Alloxan Induced Hyperglycaemia (n-butanol fraction)

The n-butanol fraction of the extract lowered the elevated blood glucose significantly with the 1000 mg/kg dose at the 8th, 16th, and 24th hours. The 500 mg/kg dose also lowered the glucose level throughout the study but only significantly at the 1st, 16th, and 24th hours. The 250 mg/kg dose only lowered the glucose level at the 16th hour however not significantly, at the 1st, 8th and 24th the blood glucose level was slightly elevated (figures 1).

Fig 1: The effect of butanol fraction of *T. indica* stem-bark on blood glucose levels of alloxan induce hyperglycaemia



n = 6 ** P<0.02 Vs Normal saline group *Student's T-test*
B.F. = n-Butanol fraction values are mean ±SEM MFN = Metformin

3.2 Fructose-Induced Insulin Resistance Model on Blood Glucose Level (n-Butanol Fraction)

All the dose of n-butanol fraction used lowered the blood glucose significantly on the 10th and 20th days for the 1000 mg/kg and on the 20th day for the 500 mg/kg dose (Table 1).

Table 1: Effect of n-butanol fraction of *T. indica* on blood glucose level of fructose induced insulin resistance in Wistar rats after 10 and 20 days

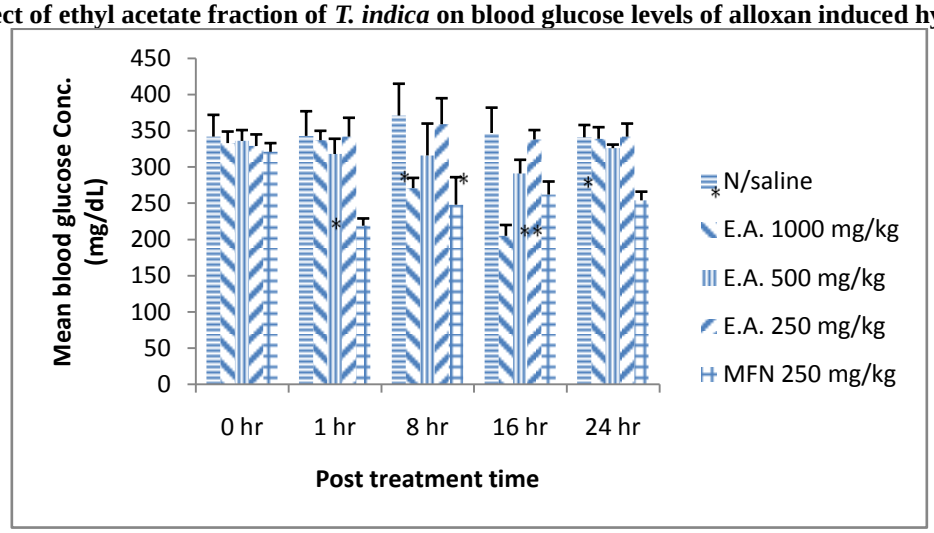
Groups	Dose	Mean blood glucose level (mg/dl)	
		(10 days)	(20 days)
i	Normal saline	89 ± 0.6	92 ± 0.9
ii	B.F. 1000mg/kg + fructose	98 ± 0.4*	87 ± 0.7*
iii	B.F. 500mg/kg + fructose	153 ± 1.2	113 ± 0.8*
iv	B.F. 250mg/kg + fructose	147 ± 0.9	154 ± 1.1
v	Fructose only	161 ± 1.7	173 ± 2.1
vi	MFN 250 mg/kg + fructose	96 ± 0.8*	91 ± 1.2*

n = 5 * = sig. at p < 0.05 Vs fructose only group *Student's T-test*
B.F. = Butanol fraction values are mean ±SEM MFN = Metformin

3.3 Alloxan Induced Hyperglycaemia (Ethyl Acetate Fraction)

The ethyl acetate fraction lowered the elevated blood glucose with all the doses, but only significantly at 1000 mg/kg (8th and 16th hours) and 500 mg/kg (16th hour). The 250 mg/kg dose did not show significant decrease in the blood sugar concentration. Metformin however, lowered the sugar level throughout the duration of study. The 1000 mg/kg dose increased the glucose concentration by the first hour but lowered it at the 8th and 16th hours but increased it at 24 hours. The 500 mg/kg progressively decreased the glucose concentration for the first 16 hours but increased also after 24 hours. The 250 mg/kg dose increased the glucose concentration throughout the 24hours (Figures 2).

Fig 2:The effect of ethyl acetate fraction of *T. indica* on blood glucose levels of alloxan induced hyperglyceamia



n = 6 * = sig at $p < 0.05$ Vs Normal saline group *Student's T-test*
E.A. = Ethyl acetate portion value are mean ±SEM MFN = Metformin

3.4 Fructose-Induced Insulin Resistance Model on Blood Glucose Level (Ethyl Acetate Fraction)

The blood sugar concentration was significantly lowered ($p < 0.05$) on the 10th and 20th days at 1000 mg/kg and 250 mg/kg dose levels. The 500 mg/kg dose also lowered the glucose concentration but only significantly at 20 days.

Table 2: The effect of ethyl acetate fraction of *T. indica* on blood glucose level of fructose induced insulin resistance in Wistar rats after 10 and 20 days

Groups	Dose	Mean blood glucose level (mg/dl)	
		(10 days)	(20 days)
i	Normal saline	89 ± 0.6	90±0.6*
ii	E.A. 1000mg/kg + fructose	93±0.7*	87 ± 0.7*
iii	E.A. 500mg/kg + fructose	156±1.2	117±1.1*
iv	E.A. 250mg/kg + fructose	104±1.8*	115±2.1*
v	Fructose only	161±2.2	173 ± 2.1
vi	MFN 250 mg/kg + fructose	96 ± 0.8*	91 ± 1.2*

n = 5 * = sig. at $p < 0.05$ Vs fructose only group *Student's T-test*
E.A. = Ethyl acetate portion values are mean ±SEM MFN = Metformin

4.0 Discussion

Higashino and co-workers used the n-butanol soluble fraction of *Mormodica charantia* to effectively lower BGL of both oral and intraperitoneal induced-glucose load[19]. Other studies also showed that the n-butanol extract of some other plants e.g. *Ophiopogonis tuber* reduce the level of blood glucose in healthy mice[20]. The n-butanol fraction of *T. indica* reduced the elevated BGL after the first hour and was significant only at the 500 mg/kg dose and metformin significantly reducing the BGL in the alloxan induced hyperglycaemic animals. At the 8th hour 1000 mg/kg of the extract and the standard drug had a significant ($p < 0.05$) reduction in BGL. At the 16th and 24th hours post administration there was significant ($p < 0.05$) reduction in BGL with the 1000 and, 500 mg/kg doses of the extract and metformin groups. In fructose induced hyperglycaemia all doses of the extract reduced the BGL on both the 10th and 20th days dose independently. The 1000 mg/kg dose was the most active with significant ($p < 0.05$) reduction on both days. The 500 mg/kg dose showed a significant reduction only on the 20th day, while 250mg/kg dose did not show significant reduction in BGL on both days.

The ethyl acetate fraction of the extract was also investigated for its hypoglycaemic and antihyperglycaemic activity on alloxan and fructose induced hyperglycaemia. The fraction lowered the elevated BGL only eight hours post extract administration and was significant at 1000 mg/kg dose of the extract. Sixteen hours after the administration of the extract, 1000 and 500 mg/kg gave a significant reduction in the BGL. However, after 24 hours the there was no significant reduction with the extracts. Metformin significantly reduced the BGL throughout the study period. In the fructose induced hyperglycaemic model, graded dose of the extract prevented significant elevation in the BGL on the 10th (1000 and 250mg/kg) and the 20th days. Several other investigators have used the ethyl acetate fraction of plants to lower or prevent elevation of BGLs[21, 22,23]. In conclusion, the fraction of the stem-bark extract of *T. indica* potent hypoglycaemic activity.

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