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Effect of Stem-bark Extract of *Tamarindus indica* L. on Serum Lipid Profile, Liver Enzymes and Blood Glucose Level of Experimentally Induced Hyperglycaemic Wistar Rats

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The increasing prevalence of diabetes is reaching epidemic proportion worldwide. Diabetes is a major threat to global public health. 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. Many of the medicinal plants in use today, employed for the local management of diabetes mellitus do not have scientific validation including the stem-bark of *Tamarindus indica*. The stem-bark extract of *Tamarindus indica* is used locally for the management of diabetes. Acute toxicity test LD₅₀ and phytochemical screening were conducted on the extract. The stem-bark extract of *Tamarindus indica* L. was investigated for its hypoglycaemic action on experimentally induced hyperglycaemic Wistar rats using dexamethasone (10 mg kg⁻¹ SC for ten days) and fructose (10% w/v *ad libitum* for 20 days). The preventive effect of the extract on the development of insulin resistance was also investigated. The oral LD₅₀ 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 stem-bark extract of *Tamarindus indica* Linn significantly lowered elevated Blood Glucose Concentration (BGL) in fructose and dexamethasone-induced hyperglycaemia in experimental animal models. The extract also restored some of the altered lipid profile parameters and not much change was seen in the liver enzyme levels.

Key words: Hyperglycemia, *Tamarindus indica*, dexamethasone, fructose

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INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disorder of carbohydrate, protein and lipid metabolism characterized by persistent elevations in fasting blood glucose above 11 mmol L⁻¹, due to insufficient or complete lack of insulin synthesis or secretion and/or peripheral resistance to insulin action (Murray and Pizzorno, 1997). DM is a common and serious metabolic disorder throughout the world. As a result of side effects associated with the currently available antidiabetics, there is the need for continues search for newer agents with less side effects.

Diabetes mellitus as been defined as a metabolic disorder of various etiologies and is characterized by chronic hyperglycaemia with abnormalities in carbohydrate, fat and protein metabolism resulting from relative or absolute defects in insulin secretion, insulin action, or both (WHO, 1999). Metabolic abnormalities of diabetes could result from deficiency of insulin, inadequacy of its action, or insensitivity to its action or a combination of both (International Diabetes Federation, 2003; WHO, 1994).

Although diabetes has been recognized for long and treatments of varying efficacies have been known since the Middle Ages, the full explanation was only made in the 20th century (Nathan *et al.*, 2005). The discovery of the action and importance of insulin in the metabolism of glucose, led to the understanding that lack of insulin was an important factor in the development of the disease (Patlak, 2002).

Tamarindus indica Linn, is of the Caesalpiniaceae which is the third largest family of flowering plants, which is a sub-family in Leguminosae; a dicotyledonous. (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.

MATERIALS AND METHODS

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 LD₅₀ 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 1, 2 and 3 were treated with 10, 100 and 1000 mg kg⁻¹ body weight 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 LD₅₀ value was calculated by taking geometric mean of the lowest dose that caused death and the highest dose that did not produce death.

Dexamethasone induced insulin resistance model: In this model described by Kaur *et al.* (1989), Wiesenbergs *et al.* (1998) and Mahendran and Deva (2001), thirty-six male rats were divided into 6 groups of 6 animals. The grouping is as follows:

- **Group 1:** Dexamethasone sodium phosphate 10 mg kg⁻¹, once daily/SC+Normal saline
- **Group 2:** Dexamethasone 10 mg kg⁻¹/SC+250 mg kg⁻¹/oral of methanol stem bark extract of *T. indica*
- **Group 3:** Dexamethasone 10 mg kg⁻¹/SC+500 mg kg⁻¹/oral of methanol stem bark extract of *T. indica*
- **Group 4:** Dexamethasone 10 mg kg⁻¹/SC+1000 mg kg⁻¹/oral of methanol stem bark extract of *T. indica*
- **Group 5:** Dexamethasone 10 mg kg⁻¹/SC+1 mg kg⁻¹ glibenclamide
- **Group 6:** Normal saline only

Animals in all the groups were treated daily for ten consecutive days. The blood glucose levels, triglyceride levels and changes in body weight were recorded on the 5th and 10th days.

Fructose-induced insulin resistance model: For this model described by Dai *et al.* (1995) and Vikrant *et al.* (2001), the animals were divided into six groups of five rats each.

- **Group 1:** Received 10% w/v Fructose solution *ad libitum* and 250 mg kg⁻¹ body weight methanol stem bark extract of *T. indica* orally daily for 28 days
- **Group 2:** Administered 10% w/v Fructose solution *ad libitum* and 500 mg kg⁻¹ body weight of methanol stem bark extract of *T. indica* orally daily for 28 days
- **Group 3:** Received 10% w/v Fructose solution *ad libitum* and 1000 mg kg⁻¹ body weight of methanol stem bark extract of *T. indica* orally daily for 28 days
- **Group 4:** Fructose B fed with 10% w/v fructose solution *ad libitum* in their drinker for 28 days only
- **Group 5:** Received normal saline only
- **Group 6:** Received 10% w/v fructose solution *ad libitum* and metformin 250 mg kg⁻¹

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 anaesthesia. Blood was collected from the jugular vein upon sacrifice. Serum was separated by centrifugation at 3000 rpm for 10 min 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.

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.

RESULTS

The extract gave the yield of 17.6%, the oral acute toxicity test (LD₅₀) of the extract was found to be greater than 5,000 mg kg⁻¹ body weight. Phytochemical test of the methanol stem-bark extract of *Tamarindus indica* L. showed the extract to contain carbohydrate, saponins, flavonoids, alkaloids and steroids.

Dexamethasone-induced insulin resistance model: On Table 1 the 1000 mg kg⁻¹ dose significantly (p<0.05) lowered the Blood Glucose Level (BGL) when compared with the group that was administered dexamethasone alone. The triglyceride level was also lowered significantly with the 1000 mg kg⁻¹ dose. Glibenclamide the standard agent used gave us a similar result to the 1000 mg kg⁻¹ dose. The 250 mg kg⁻¹ dose of the extract slightly lowered the glucose and triglyceride level. The 500 mg kg⁻¹ only showed a significant reduction in the glucose level, though there was also a slight reduction in the triglyceride level.

Fructose-induced insulin resistance model (lipid profile):

All the doses of the extract used showed significant reduction in the total cholesterol level when compared with the group that received only fructose (group 2) but slightly and non-significantly increased when compared with the normal saline group. All doses of the extract also lowered the TG levels when compared with the fructose alone group. The HDL-C was elevated significantly (p<0.05) in the 1000 and 500 mg kg⁻¹ groups compared to fructose alone group but the 250 mg kg⁻¹ group did not show any significant elevation compared to the fructose group. There was significant lowering of the LDL-C levels at all the doses of the extract used when compared with the fructose group (Table 2).

Table 1: Effect of methanol stem-bark extract of *T. indica* on blood glucose, triglycerides and body weight changes on dexamethasone-induced insulin resistance

Group	Dose	Mean blood glucose levels (mg dL ⁻¹)	Triglyceride (mg dL ⁻¹)	Body weight change (g)
1	Extract (1000 mg kg ⁻¹) +Dexa	115.5±2.5*	22.4±3.3*	-10.8±1.1
2	Extract (500 mg kg ⁻¹) +Dexa	110.2±2.6*	36.2±1.2	-1.7±2.1
3	Extract (250 mg kg ⁻¹) +Dexa	135.1±2.3	33.6±4.5	-12.8±2.3
4	Glibenclamide (1 mg kg ⁻¹) +Dexa	105.7±3.3*	21.4±2.3*	-8.7±4.5
5	Dexamethasone alone	158.6±4.6	52.9±6.4	-15.4±6.0
6	Normal saline alone	81.6±1.9	17.1±1.5	-2.6±2.3

n = 5, *Significant at p<0.05 vs. Dexamethasone alone group Student's t-test, Dexa.: Dexamethasone

Table 2: Effect of methanolic stem-bark extract of *T. indica* on the lipid profile of fructose induced insulin resistance in Wistar rats after 28 days administration

Groups	Mean serum level (mg dL ⁻¹)			
	TC	TG	HDL-C	LDL-C
N/saline	54.2±2.3	97.0±2.8	25.7±1.2	47.9±1.2
Fructose only	102.4±2.2 ^a	141.0±1.0 ^a	12.7±1.6 ^a	117.9±1.3 ^a
TI 1000 mg kg ⁻¹ +fructose	61.5±1.5 ^b	104.5±1.5 ^b	21.4±1.5 ^b	61.0±1.6 ^b
TI 500 mg kg ⁻¹ +fructose	63.3±1.8 ^b	106.2±2.8 ^b	20.7±1.2 ^b	63.8±1.3 ^b
TI 250 mg kg ⁻¹ +fructose	61.5±1.5 ^b	105.1±1.0 ^b	17.4±1.5	65.1±1.4 ^b

n = 5, Student's t-test, a: sig. at p<0.05 vs. N/saline group, b: sig. at p<0.05 vs. fructose group, TI: *Tamarindus indica*, TC: Total cholesterol, HDL-C: High density lipoprotein cholesterol, TG: Triglycerides, LDL-C: Low density lipoprotein cholesterol

Table 3: Effect of methanolic stem-bark extract of *T. indica* on the liver enzymes of fructose induced insulin resistance in Wistar rats

Groups	Mean serum level (mg dL ⁻¹)			
	AST	ALT	ALP	TB
N/saline	201.4±1.1	68.8±1.8	106.8±5.3	106.8±5.3
Fructose only	232.6±1.2	75.0±1.0	326.2±10.6 ^a	37.4±4.1 ^a
TI 1000 mg kg ⁻¹ +fructose	184.6±2.6	67.0±1.3	223±10.1 ^a	14.0±1.2 ^b
TI 500 mg kg ⁻¹ +fructose	184.0±1.8	60.2±1.5	317.8±14.8 ^a	24.4±2.4 ^a
TI 250 mg kg ⁻¹ +fructose	197.8±2.6	70.4±1.5	337.8±5.3 ^a	24.4±2.4 ^a

n = 5, Student's t-test, a: sig. at p < 0.05 vs. N/saline group, b: sig. at p<0.05 vs. fructose group, TI: *Tamarindus indica*, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, TB: Total bilirubin

Table 4: Effect of methanolic stem-bark extract of *T. indica* on blood glucose level after 20 days of fructose feeding in Wistar rats

Group Dose	Mean blood glucose level (mg dL ⁻¹)	
	(10 days)	(20 days)
1 Normal saline	84±4.2	87±5.3
2 TI 1000 mg kg ⁻¹ +fructose	101 ±2.3*	98±4.7*
3 TI 500 mg kg ⁻¹ +fructose	97±1.8*	103±5.2*
4 TI 250 mg kg ⁻¹ +fructose	103±1.6*	117±6.7*
5 Fructose alone	147±3.4	153±9.3
6 MFN 250 mg kg ⁻¹ +fructose	101±1.7*	98±2.1*

n = 5, Student's t-test, *: sig at p<0.05 vs. fructose group, TI: *Tamarindus indica*, MFN: Metformin

Fructose-induced insulin resistance model (liver enzymes): The serum levels of AST and ALT were lowered though not significantly at all the doses of the extract used when compared with the control groups however, not significantly. The 1000 mg kg⁻¹ dose lowered the ALP level when compared with the fructose group but elevated the ALP versus normal saline group, this result obtained (1000 mg kg⁻¹) is similar to the 500 and 250 mg kg⁻¹ group (Table 3).

Fructose-induced insulin resistance model (blood glucose level): The blood glucose level of the animals fed with extract for 20 days was significantly (p<0.05) lowered compared to the fructose alone group on the tenth and twentieth days (Table 4).

DISCUSSION

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. 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.

Dexamethasone stimulates lipolysis and free fatty acids synthesis which is known to compete with glucose for intracellular glucose oxidation, causing insulin resistance through the glucose/fatty acid cycle (Randle *et al.*, 1963; Venkatesan *et al.*, 1987; Guillaume-Gentil *et al.*, 1993). Dexamethasone also increases triglyceride levels, leading to an imbalance in lipid metabolism leading to hyperlipidemia (Wiesenberg *et al.*, 1998) and hyperglycaemia (Mahendran and Deva, 2001).

In the present study, dexamethasone administration for 10 days resulted in increased triglyceride and BGL when compared to group 4 animals (untreated control) similar to a previous study by Dai *et al.* (1995) and Shalam *et al.* (2006). Administration of dexamethasone subcutaneously daily at a dose of 10 mg/kg/day for ten days elevated the triglyceride level in all the groups, however, these increased triglyceride levels were reduced by all the doses of the extract used when compared with group 5 animals treated with dexamethasone alone. The reduction was significant (p<0.05) with the 1000 mg kg⁻¹ dose and glibenclamide. The BGL was also significantly (p<0.05) reduced in all the doses and with glibenclamide except 250 mg kg⁻¹ dose. There was a significant reduction in the body weights of the dexamethasone alone treated animals when compared to normal control. Dexamethasone administration is known to cause a reduction in food consumption and a decrease in body weight (Shalam *et al.*, 2006). The decrease in body weight of hyperglycaemic rats could be due to dehydration and catabolism of fats and proteins (Hakim *et al.*, 1997). Treatment with *T. indica* extracts reversed dexamethasone-induced loss of weight in the rats most likely by preventing down-regulation of insulin receptor substrate (IRS)-1 expression and/or increasing insulin-stimulated GLUT4 translocation to the cell plasma membrane particularly of skeletal muscles.

Earlier studies have shown that prolonged administration of fructose is associated with significant hyperinsulinaemic, hyperglycaemic and hyperlipidaemic states in treated animals (Bezerra *et al.*, 2001; Fasanmade and Alabi, 2008). In this study, there was an increase in triglyceride, Low Density Lipoprotein and total cholesterol levels with a decrease in High Density Lipoprotein levels in the control animals. This corroborates with other studies that show lowered HDL

levels and elevated LDL in diabetes mellitus (Ruzaidi *et al.*, 2005). However, the increases in TC, TG and LDLC were reduced by all the doses of the extract though not significantly. A high serum total triglyceride level has earlier been implicated in diabetes (Anaja, 1995).

The reduced HDLC was also increased by the extract. It is well known that in uncontrolled diabetes, there will be increase in LDL, VLDL, total cholesterol and triglyceride with a decrease in HDL, all of which contribute to coronary artery disease seen in some diabetic patients (Arvind *et al.*, 2002).

In this study, alanine aminotransferase (ALT), aspartate aminotransferase (AST), Alkaline Phosphatase (AP) and Total Bilirubin (TB) were monitored. In animal models, chronic hyperinsulinemia is found to predispose the liver to relative resistance to insulin. This is characterized by a failure of insulin to signal an increase in insulin receptor substrate-2. Excess of free fatty acids found in the insulin-resistant state is known to be directly toxic to hepatocytes. Another possible explanation for the increase in elevated transaminases in insulin-resistant states include oxidative stress from lipid peroxidation, peroxisomal beta-oxidation and recruited inflammatory cells (Harris, 2005).

From this study, the aminotransferases were slightly elevated in group 2 (fructose alone) this elevation was however reduced in the extract treated groups but the reduction is not significant. All the treated groups have significant ($p < 0.05$) elevation in the ALP level versus normal saline treated group. The TB level was also affected as it was significantly reduced in comparison to the normal saline treated group.

CONCLUSION

In conclusion, it can be suggested that the stem-bark extract of *T. indica* L. possess potent hypolipidemic and hypoglycaemic activity. It also has the potential of restoring altered liver enzyme parameters.

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