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Glucose lowering efficacy of the aqueous stem bark extract of *Trema orientalis* (Linn) Blume in normal and streptozotocin diabetic rats

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The glucose-lowering efficacy of the aqueous stem bark extract of *Trema orientalis* (Ulmaceae) was evaluated both in normal and streptozotocin-induced diabetic rats. In normoglycemic rats, the single oral administration of the aqueous extract of *T. orientalis* failed to reduce blood glucose levels while in STZ-diabetic rats, the plant extract (38–300 mg/kg) exhibited significant hypoglycaemic activity with a maximum effect of 29.67%, 5 hours after administration of the 75 mg/kg dose when compared with the diabetic untreated group. Glibenclamide was not able to lower blood glucose in STZ-diabetic rats, while it significantly lowered the blood sugar in normoglycemic rats. The hypoglycaemic property of *T. orientalis* was also assessed by an oral glucose tolerance test (OGTT) in STZ-diabetic rats. The aqueous extract of *T. orientalis* and the reference drug, glibenclamide, (10 mg/kg) produced significant blood glucose lowering effects in the diabetic rats when compared to the diabetic controls. One week after repeated administration of *T. orientalis* extract, blood glucose levels were significantly decreased ($p < 0.05$) and still remained low after 2 weeks ($p < 0.01$). The results indicated that *T. orientalis* stem bark extract significantly reduces blood glucose in STZ-induced diabetic rats by a mechanism different from that of sulfonylurea agents. The present investigation provides pharmacological evidence that the use of this plant extract in traditional medicine for cardiovascular disease can be of benefit particulary in diabetic patients.

1. Introduction

In spite of available therapeutic strategies involved in the management and prevention of diabetes including glycaemia control either with insulin (Type 1 diabetes) or antidiabetic drugs and antihypertensive agents, there is an increased demand by patients for medicinal plants (Venkatesh et al. 2003). Approximatively, 80% of people in rural African communities still use phytotherapy to control or treat diabetes mellitus (Ojewole 2002). Plants have been an exemplary source of drugs and many of the currently available drugs have been derived directly from them (Grover et al. 2000). This interest in medicinal plants for the treatment of different diseases is due to the fact that plant drugs are frequently considered to be less toxic and relatively low cost than synthetic ones (Jouad et al. 2001; Stanley et al. 2004).

T. orientalis (Linn) Blume is a tropical tree belonging to the family Ulmaceae, which is reported to grow on poor soil (Samantaray et al. 1995). The tree is found in modified forest, widespread in all tropical Africa, in Arabia and Madagascar (Adjanouhoun et al. 1996). The plant is used in traditional medicine to cure diseases like asthma, jaundice, dental pain (White and Abernethy 1996). The leaves are used in Ghana for treating jaundice, oliguria, bronchitis, diabetes mellitus and pharyngitis (Mshana et al. 2000). In Cameroon, the leaves are used to treat male sexual impotence (Adjanouhoun et al. 1996). Roots and barks are used in Cameroon for the treatment of cardiovascular disease (Noungoue et al. 2001). Compelling evidence suggests a causal link between hyperglycemia and the development of macrovascular and microvascular complications of diabetes. It is well known that compounds which can reduce both macrovascular and microvascular complications in diabetes may improve insulin sensitivity and glucose metabolism (McFarlane et al. 2003). Phytochemical study of *T. orientalis* revealed the presence of xanthones and epicatechin, a secondary metabolite with cardiovascular and antidiabetic properties (Basnet 1994, 1995; Noungoue et al. 2001).

The present investigation was undertaken to evaluate the glucose-lowering efficacy of the stem bark aqueous extract of *T. orientalis* in normoglycemic and streptozotocin-induced insulin-dependent diabetes mellitus.

2. Investigations and results

The glucose-lowering efficacy of the stem bark aqueous extract of *T. orientalis* was assessed in normoglycaemic and streptozotocin-diabetic rats following a method previously described (Cam et al. 1993; Mukherjee et al. 1997).

2.1. Body weight, food and water intake

There was a significant reduction in body weight of the rats in the diabetic control group in comparison to non

Table 1: Body weight, water and food intake in STZ-diabetic rats before and after oral treatment with *T. orientalis* extract once a day for two weeks

Treatment	Body weight (g)		Water (mL/rat/day)		Food (g/rat/day)	
	Before	After	Before	After	Before	After
Control	225.52 ± 4.80	232.50 ± 6.65	19.14 ± 0.8	20.00 ± 0.84	21.54 ± 0.8	19.62 ± 2.18
Diabetic	219.63 ± 9.40	191.53 ± 3.55*	93.12 ± 4.12	110.80 ± 9.07[b]	30.07 ± 0.72	41.23 ± 3.12
Extract (38 mg/kg)	221.58 ± 8.05	216.23 ± 7.19*	87.56 ± 3.00	47.26 ± 6.70*	33.00 ± 1.32	28.68 ± 1.44*
Extract (75 mg/kg)	216.21 ± 5.45	221.01 ± 10.62*	88.85 ± 4.60	35.89 ± 3.00*	35.20 ± 2.17	32.29 ± 2.64*
Extract (150 mg/kg)	222.06 ± 8.81	212.22 ± 10.72*	91.15 ± 2.10	48.97 ± 6.85*	32.00 ± 3.43	29.18 ± 2.45*
Extract (300 mg/kg)	217.07 ± 4.08	222.10 ± 11.19*	92.14 ± 0.37	43.09 ± 4.58*	32.82 ± 2.79	27.81 ± 1.15*
Glib (10 mg/kg)	227.43 ± 6.46	204.71 ± 4.75*	97.66 ± 2.50	85.83 ± 3.60	31.53 ± 1.55	31.09 ± 5.60

Data is expressed as mean ± S.E.M., n = 5, Glib. = glibenclamide
*$p < 0.05$ *T. orientalis*-treated diabetic rats compared with the untreated diabetic rats

diabetic controls during two weeks of observation. After aqueous stem bark extract of *T. orientalis* and glibenclamide treatment for 14 days, the body weights were recovered but not back to the control levels (Table 1). The induction of STZ-diabetes resulted in elevated food and fluid intakes. The food intake and water intake of *T. orientalis*-treated diabetic rats were reduced significantly when compared to the distilled water-treated diabetic rats.

2.2. Effects of a single oral administration of T. orientalis extract

The aqueous extract of *T. orientalis* (38–300 mg/kg) did not affect, significantly, the blood glucose concentration in normoglycemic rats at various time intervals when compared to the control group and initial blood glucose levels. Glibenclamide (10 mg/kg) reduced, significantly, blood glucose concentrations of normoglycemic rats at each of the time points examined. Five hours after administration, glibenclamide reduced the blood glucose levels by 69% (data not shown).

Blood glucose levels after oral administration of a single dose of *T. orientalis* extract in STZ-rats are shown in Fig. 1. In STZ rats, the extract at a dose of 300 mg/kg caused a significant reduction of blood glucose within 1 h, with the maximal reduction in the basal blood glucose level of 34.33% at 5 h. The plant extract at the dose of 75 and 150 mg/kg also produced significant reduction (41.37% and 36.83%, respectively) in the glycaemia 5 h after administration. However, glibenclamide (10 mg/kg) did not reduce the blood glucose of STZ-diabetic rats.

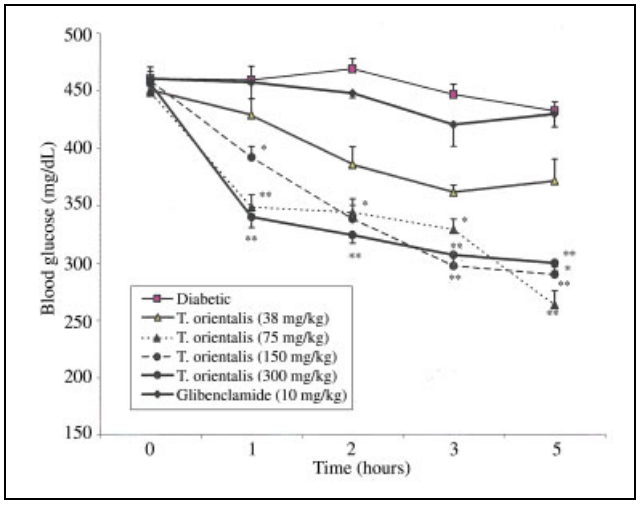

Fig. 1: Acute effect of the stem bark aqueous extract of *T. orientalis* on blood glucose levels in STZ-diabetic rats. Values are means ± SEM, n = 5. * ($p < 0.05$) ** ($p < 0.01$) compared with untreated diabetic rats

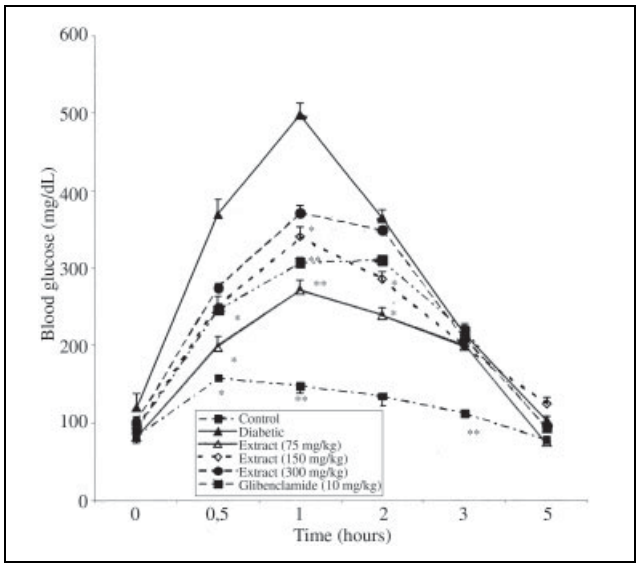

Fig. 2: Effect of *T. orientalis* extract on blood glucose levels in glucose-fed hyperglycaemic rats. Values are means ± SEM, n = 5. * $p < 0.05$; ** ($p < 0.01$) as compared with untreated diabetic rats

Fig. 2 shows the blood glucose levels of non diabetic control, diabetic control, *T. orientalis* and glibenclamide-treated rats after oral administration of glucose (5 g/kg body weight). In diabetic control rats, the peak increase in blood glucose concentration was observed after 1 h. *T. orientalis* (75 and 150 mg/kg) and glibenclamide (10 mg/kg)-treated rats showed significant decrease in blood glucose concentrations at 1 and 2 h when compared with diabetic control rats. Aqueous extract of *T. orientalis* at a dose of 75 mg/kg was found to be more effective than glibenclamide.

2.3. Repeated administration of T. orientalis extract in STZ-diabetic rats

The aqueous stem bark extract of *T. orientalis* caused a dose-dependent and significant hypoglycaemic effect after once daily administration for 7 and 14 days (Table 2). Diabetic rats treated with the plant extract at the oral doses ranging from 38 to 300 mg/kg for 14 days showed 55.63% to 78.70% reduction in blood glucose in comparison to untreated diabetic rats. Blood glucose levels of control diabetic and extract-treated animals (300 mg/kg) were 360.00 ± 10.50 and 76.67 ± 5.83 mg/dL, respectively. The aqueous extract of *T. orientalis* showed 40.86% and

Table 2: Effect of repeated administration of *T. orientalis* on blood glucose (mg/dl) in diabetic rats

Days groups	J1	J7	J14	% Variation for day 1
Control	87.00 ± 3.31	71.40 ± 2.20	72.40 ± 3.00	19.00
Diabetic	378.50 ± 9.83	372.50 ± 19.83	360.00 ± 40.50	5.00
T. orientalis 300 mg/kg	381.83 ± 37.60	225.80 ± 28.64* [b]	76.67 ± 5.83** [b]	80.00
T. orientalis 150 mg/kg	329.10 ± 31.24	201.79 ± 53.00* [a]	124.20 ± 35.13* [b]	62.30
T. orientalis 75 mg/kg	324.00 ± 33.50	167.40 ± 43.50* [b]	141.48 ± 34.64** [b]	56.33
T. orientalis 38 mg/kg	321.16 ± 32.71	237.67 ± 42.41* [b]	159.73 ± 31.78* [a]	50.26
Glibenclamide 10 mg/kg	338.16 ± 27.19	193.83 ± 33.36** [b]	135.73 ± 24.37**	52.92

Results are expressed as means ± SEM, n = 5
*$p < 0.05$, **$p < 0.01$ as compared with initial value
[a]($p < 0.05$), [b]($p < 0.01$) as compared with diabetic untreated rats

79.92% antidiabetic activity at the dose of 300 mg/kg given orally for 7 and 14 days, respectively, as compared to the basal blood glucose. Daily glibenclamide administration (10 mg/kg) for 2 weeks produced a statistically significant decrease in blood glucose levels (52.9%).

3. Discussion

Our results indicate that in normoglycaemic rats, the blood glucose levels were not significantly different in controls and in animals treated with *T. orientalis* stem bark extract. However, the results clearly demonstrated that *T. orientalis* aqueous extract reduced the blood glucose levels when administrated to STZ-diabetic rats. These findings are similar to those of earlier studies, which reported that *Bauhinia forticata* (Kameswara et al. 2001) and *Pterocarpus santalinus* (Pepato et al. 2002) had no effect on blood glucose of normoglycaemic rats but reduced hyperglycaemia induced in rats by alloxan. Glibenclamide (10 mg/kg) did not reduce the blood glucose in STZ-diabetic rats, but reduced blood glucose levels in normoglycaemic rats. It was reported that glibenclamide was not effective when destruction of β-cells has occurred (Gerich 1989; Hosseinzadeh et al. 2002). In normoglycaemic animals sulfonylurea agents have been found to induce hypoglycaemia by their ability to stimulate β-pancreatic cells to liberate more insulin (Pepato et al. 2002). The present results therefore indicate that the mechanism of hypoglycaemic action of the stem bark aqueous extract of *T. orientalis* is different from sulfonylurea agents. It was also observed in this study that the extract of *T. orientalis* effectively reduced the blood glucose levels in glucose-fed hyperglycaemic rats. Such an effect might be accounted for, in part, by a decrease in the rate of intestinal glucose absorption or by restoration of delayed insulin response as well as increased glucose uptake. In this context, other workers have reported that *Nelumbo nucifera* (Mukherjee et al. 1997) and cogen db (Pari and Saravanan 2001) have significant antidiabetic and glucose tolerance effects in experimentally induced diabetic rats.

Streptozotocin-induced diabetes, by damaging the insulin secreting cells of the pancreas, leads to hyperglycaemia (Szkudelski and Szkudelska 2002; Maiti 2004). Symptoms like loss of body weight, hyperphagia and polydipsia that accompany type 1 diabetes mellitus were significantly attenuated in our study by treatment with the plant extract. The improvement of body weight, food and water intake in *T. orientalis*-treated animals further support the antidiabetic effect of this extract as a diabetic condition is associated with loss of body weight and increased food and water intake. After 7 days and 14 days of repeated oral treatment, blood glucose levels were nearly normalized in STZ rats indicating that the hypoglycaemic effect of the plant extract is cumulative. Theoretically, hypoglycaemic plants act through a variety of mechanisms. At present, it is not possible to pin point the mechanism of the antidiabetic effect of the aqueous extract of the stem bark of *T. orientalis*. According to our findings, the plant extract may contain some active substance(s) that may sensitise the insulin receptors or stimulate the β stem cells of the islets of Langerhans in the pancreas in STZ-induced diabetic rats. This may improve carbohydrate metabolism towards the reestablishment of normal blood glucose levels. Hypoglycaemic effects have been reported for some plants containing (−)epicatechin (Males and Farnsworth 1995; Zaid et al. 2002). Since phytochemical analysis of the plant has revealed the presence of (−)epicatechin, it is possible that the antihyperglyceamic effect is related to this component.

In conclusion, the present study clearly demonstrates that *T. orientalis* stem bark extract significantly reduces blood glucose in STZ-induced diabetic rats. The extract has no effect in normoglycemic animals. The pharmacological effect of *T. orientalis* is different from that of sulfonylurea agents. Further experiments are needed to establish the exact mechanism of this extract's antidiabetic effect and to determine the active principle(s) responsible for this effect.

4. Experimental

4.1. Plant material

The stem bark of *T. orientalis* was collected in the month of July in Yaounde, centre province, Cameroon and the material was authenticated by Doctor Zapfack Louis, Department of Plant Biology and Physiology, University of Yaounde I.

4.2. Preparation of the stem bark aqueous extract of T. orientalis

The fresh stem bark of *T. orientalis* was cut into small pieces, sun-dried and powdered. An aqueous extract was prepared by boiling the powdered stem bark (200 g) in 1 L of distilled water for 15 min, and cooling to room temperature. The resultant extract was filtered, and then dried at reduced temperature (40 °C). The resulting dry mass served in the preparation of an aqueous extract of *T. orientalis* for experimentation. The extract yield was 24%.

4.3. Animals

Male albinos Wistar rats weighing 200–250 g raised in the animal house of the Faculty of Science, University of Yaounde I were used. They were housed in colony cages (five rats per cage), at an ambient temperature (25 ± 2 °C) with free access to standard food and water. The animals described as fasting had been deprived of food for at least 12 h but had been allowed free access to drinking water. Normoglycemic rats selected for this experiment had fasting blood glucose levels of 94 ± 6 mg/dL.

4.4. Sample collection

Blood samples were collected from the cut made on the rat tail. The blood collected was allowed to clot at room temperature and the serum obtained after centrifugation (3000 g for 10 min) was used for the estimation of blood glucose. The blood glucose level was determined using enzymatic kits (Randox).

4.5. Study of T. orientalis effect on normal blood glucose level

The method used here was described by Mukherjee et al. (1997). Rats were divided into six groups of five each. Four groups of animals received the aqueous extract at 38, 75, 150 and 300 mg/kg, p.o. while the two other groups of rats received distilled water (10 mL/kg) and the standard drug, glibenclamide (10 mg/kg), respectively, for assessing the comparative pharmacological significance. Blood glucose levels were determined before (0 h) and at 1, 2, 3 and 5 h after plant extract administration in 10 μL blood samples using the enzymatic method.

4.6. Study of T. orientalis effect on streptozotocin-induced diabetic rats

Hyperglycaemia was induced by a single intravenous injection of 55 mg/kg of streptozotocin (Sigma Chemical Co., USA) to non fasted rats. Control rats received a similar volume of the vehicle alone. Four days after STZ-injection, hyperglycaemic rats were selected (blood glucose level >250 mg/dL) and randomized into groups of five animals each. The stem bark aqueous extract of *T. orientalis* (38, 75, 150 and 300 mg/kg), glibenclamide (10 mg/kg) or distilled water were given orally and glucose levels were estimated in a manner similar to the above procedure.
In the second set of experiments, diabetic-rats were treated with the plant extract once a day for two weeks. Blood samples for 12 h fasted rats were drawn at weekly intervals until the end of the study (i.e. 15 days). Body weight, food and water intakes were evaluated every day.

4.7. Oral glucose tolerance test (OGTT) in diabetic-rats

Prior to an oral glucose tolerance test, rats were fasted for 18 h. The OGTT was performed by feeding glucose (5 g/kg), in the form of a solution, orally to diabetic rats and blood samples (100 μL) were collected at 0, 0.5, 1, 2, 3 and 5 h after glucose administration. Distilled water (diabetic-control), plant extract (75, 150 and 300 mg/kg) and a reference drug, glibenclamide (10 mg/kg) were given to groups of 5 rats each 1 h prior to glucose challenge and blood glucose levels were estimated as previously described.

4.8. Statistical analysis

Results are expressed as the mean ± S.E.M. Data were statistically evaluated by analysis of variance and Student's t-test (Mukherjee et al. 1997).

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