



International Journal of Pharmacology

ISSN 1811-7775

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Antidiabetic and Hypolipidaemic Effects of Ethanolic Root Extract of *Setaria megaphylla*

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Abstract: Evaluation of antidiabetic and hypolipidaemic activity of ethanolic root extract of *Setaria megaphylla* as well as its acute toxicity was carried. The extract was found to be slightly toxic with LD₅₀ value of 2074.8 mg kg⁻¹. Treatment of alloxan-induced diabetic rats with the extract caused a significant ($p < 0.01$) reduction in fasting Blood Glucose Levels (BGL) of the diabetic rats both in acute study and prolonged treatment (15 days). The activity of the extract was comparable to that of the reference drug, Glibenclamide. *Setaria megaphylla* treatment showed a considerable lowering of serum total cholesterol, triglycerides, LDL cholesterol, VLDL cholesterol and an increase in HDL cholesterol in the treated diabetic group. This results suggest that the root extract of *Setaria megaphylla* possesses antidiabetic and hypolipidaemic effect in alloxan-induced diabetic rats.

Key words: *Setaria megaphylla*, antidiabetic, hypolipidaemic, alloxan

INTRODUCTION

Diabetes mellitus is a disease of disordered metabolism of carbohydrate, protein and fat which is caused by the complete or relative insufficiency of insulin secretion and/or insulin action (Balkau *et al.*, 2000). According to Zimmet (2000) there are about 150 million diabetic patients world wide and the number is likely to double by the year 2025. Besides hyper glycaemia, several other factors such as hyperlipidaemia contribute to the development of cardiovascular complications related to diabetes which are the major causes of death (Nabel, 2003; Nagappa *et al.*, 2003). The disease constitutes a major health problem in the developing countries because of expensive and inadequate treatments (Djrolo *et al.*, 1998), coupled with the side effects associated with these drugs, hence search for a new drug with low cost, more potentials and without adverse effects is being pursued in several laboratories around the world (Kumar *et al.*, 2006). A great number of medicinal plants have been used in the treatment of diabetes in different parts of the world, some of which are without scientific or medical scrutiny although World Health Organisation (WHO) has recommended and encouraged the use of plants as an alternative therapy for diabetes (WHO, 1980). Evaluation of the antidiabetic potentials of these plants is therefore necessary to provide scientific proof and justify their use in ethnomedicine.

Setaria megaphylla (Steud) Dur and Schinz (family-Poaceae) also called broad leafed brittle grass is a very tall, tufted, perennial grass used mainly as pasture grass. It occurs in tropical and subtropical areas of Africa, America and India where there is high rainfall (Van Oudtshoorn, 1999; Lowe, 1989). The plant (leaves and roots) are use traditionally by the Ibibios in Akwa Ibom State, Nigeria in the treatment of various ailments such as inflammation and diabetes. The plant has also been reported to posses antiplasmodial activity *in vitro* (Clarkson *et al.*, 2004). Okokon and Antia (2005) reported on the antidiabetic activity of the leaves. The present study aims to evaluate the effects of ethanolic root extract of *Setaria megaphylla* on alloxan diabetic rats during both single and repeated oral administrations to observe acute and chronic effects of the extract.

MATERIALS AND METHODS

Plant materials: Fresh roots of *Setaria megaphylla* were collected in October, 2005 at Anwa forest in Uruan, Akwa Ibom State, Nigeria. The plant was identified and authenticated by Dr. Margaret Bassey, a taxonomist in the department of Botany, university of Uyo, Uyo, Nigeria. Herbarium specimen was deposited at Faculty of Pharmacy Herbarium, University of Uyo, Uyo with voucher no FPHU 221. The fresh roots (2 kg) of the plant were dried on laboratory table for 2 weeks and reduced to powder. The

powder 100 g was macerated in 95% ethanol (300 mL) for 72 h. The liquid extract obtained was concentrated in vacuo at 40°C. The yield was 2.68% w/w. The extract was stored in a refrigerator at 4°C until used for experiment reported in this study.

Animals: Albino wistar rats (115-148 g) and albino swiss mice (20-31 g) of either sex were obtained from the University of Uyo animal house. They were maintained on standard animal pellets and water *ad libitum*. Permission and approval for animal studies were obtained from the College of Health Sciences Animal ethics Committee, University of Uyo.

Determination of LD₅₀: The LD₅₀ of the extract was estimated using swiss albino mice by intraperitoneal (i.p) route using the method of Lorke (1983). This method involved the administration of different doses of the extract (1000-5000 mg kg⁻¹) to groups of five mice each. The animals were observed for manifestation of physical signs of toxicity and the number of death in each group within 24 h was recorded. The LD₅₀ was calculated as the geometrical mean of the maximum dose producing 0% mortality and the minimum dose producing 100% mortality.

Phytochemical screening: A preliminary phytochemical screening of the ethanolic root extract of *Setaria megaphylla* was carried out employing the standard phytochemical procedures (Harbone, 1983; Evans, 1987) to reveal the presence of saponin, flavonoids, tannins, alkaloids and glycosides.

Evaluation of antidiabetic activity: The rats were fasted for 18 h prior to the experiment allowing access to water only. They were rendered diabetic by injecting freshly prepared alloxan monohydrate in normal saline (150 mg kg⁻¹ b.w.) intraperitoneally (Yanardag and Colak, 1988). One hour after alloxan administration, the animals were given feed *ad libitum* and 5% dextrose solution was given in feeding bottle for a day to over come the early hypoglycaemic phase. The Blood Glucose Level (BGL) of the rats administered with alloxan were monitored from the blood samples collected from the animals by tail tipping method. The blood was dropped on the dextrostix reagent pad. The strip was inserted into microprocessor digital blood glucometer and the readings were noted (WHO, 1980). After 72 h, rats having BGL above 150 mg dL⁻¹ of the blood were selected for the study. The diabetic rats were divided into five groups of 6 rats each. The extract 200, 400, 600 mg kg⁻¹ were orally administered to animals in groups I, II and III, while animals in group IV received glibenclamide (10 mg kg⁻¹ b.w). Animals in Group V (negative control) received distilled water. The treatments

were carried out in each group of diabetic animals for 14 consecutive days. The BGL was monitored after 1, 2, 3, 4 and 6 h of administration of a single dose of the extract (for acute study) and at the end of 1, 2, 3, 5, 7 and 15 days (prolonged treatment).

Evaluation of hypolipidaemic activity: This was carried out in five groups (n = 6) of alloxan induced diabetic rats treated as described above with various doses of the extract (200, 400 and 600 mg kg⁻¹), Glibenclamide (10 mg kg⁻¹) and distilled water for the negative control group. Treatment of the animals lasted for 14 days. Twenty four hours after the last dose, the animals were anaesthetized with diethyl ether vapour and blood collected through cardiac puncture into sample bottles devoid of anticoagulant. The samples were centrifuged at 1000 rpm for 10 min to obtain the sera. Serum cholesterol, triglyceride and high-density lipoprotein (HDL) levels were measured by enzymatic colorimetric methods using Randox kits. All samples were analysed with a wine-light-unicam spectrophotometer. The concentrations of low-density lipoprotein (LDL) and very low density lipoprotein (VLDL) were calculated by the formula of Friedewald *et al.* (1972).

Statistical analysis: Data are expressed as mean±SEM. Data were statistically analysed using student's t-test and ANOVA (one way or two ways) followed by Tukey-Kramer post test and p<0.01, 0.05 were considered significant.

Drugs and reagents: The drugs and reagents used included alloxan monohydrate (Sigma- Aldrich, USA), Daonil (glibenclamide) (Aventis, Germany), ethanol analar grade (Sigma-Aldrich, USA).

RESULTS

Acute toxicity: The extract (500-2100 mg kg⁻¹) produced physical signs of toxicity ranging from writhing, decreased motor activity, decrease body and limb tone, decreased respiration to death. The intensities of these effects were proportional to the dose administered. The i.p LD₅₀ of the extract was calculated to be 2074.8 mg kg⁻¹.

Phytochemical screening: The result of the phytochemical screening of the ethanolic leaf extract of *Setaria megaphylla* showed that the roots contains flavonoids, deoxy-sugar, terpenes, saponins, tannins, anthraquinones and cardiac glycosides.

Antidiabetic activity: Significant weight loss was observed in untreated diabetic rats. Treatment with

Table 1: Effect of treatment with ethanolic root extract of *S. megaphylla* on body weight of alloxan induced diabetic rats

Drug	Dose (mg kg ⁻¹)	Average body weight (g)	
		Day 0	Day 15
Control		177.5±0.67	155.5±57
Extract	200	170.5±1.12*	215.5±1.57*
	400	163.0±0.44*	211.0±45*
	600	174.0±0.89*	223.0±0.60*
Glibenclamide	10	180.0±0.67*	253.0±1.66*

Values are expressed as mean±SEM, *p<0.05 (n = 6) (Students' t-test)

Table 2: Effect of ethanolic root extract of *Setaria megaphylla* on blood glucose levels of alloxan diabetic rats

Drug	Dose (mg kg ⁻¹)	Blood Glucose level mg dL ⁻¹ (mean±SD)					
		Initial	1 h	2 h	3 h	4 h	6 h
Control		220.3±1.05	245.2±1.39*	250.2±0.89	262.9±0.75	264.7±0.98	266.2±0.95
Extract	200	225.1±0.83*	192.2±0.15*	188.2±1.53*	182.2±2.09*	153.2±1.92*	141.3±2.15*
	400	227.2±2.12*	189.5±1.03	128.3±0.97*	109.5±0.93*	93.2±1.31*	86.6±1.64*
	600	223.4±1.77*	190.2±3.14*	115.0±1.47*	100.0±3.74*	89.5±2.01*	79.2±2.00*
Glibenclamide	10	223.8±1.42*	169.4±2.38*	162.4±2.11*	148.2±0.51*	130.3±0.75*	125.5±1.21*

<0.01 vs control F = 3.12, 13.36. df = 5,20. * p<0.01 (Two-way ANOVA) n = 6

Table 3: Effect of *Setaria megaphylla* on Blood Glucose levels of alloxan diabetic rats during prolonged treatments

Drug	Dose (mg kg ⁻¹)	Blood Glucose level mg dL ⁻¹ (mean±SD)						
		Initial	1st day	2nd day	3rd day	5th day	7th day	15th day
Control		220.3±1.05	224.5±2.13	230.1±1.51*	243.3±0.99	251.2±0.91	250.3±1.56	257.4±1.13
Extract	200	225.1±0.83*	133.1±0.92*	97.9±0.87*	86.2±0.34*	72.9±2.42*	69.5±2.33*	66.3±1.22*
	400	227.2±2.12*	122.0±3.85*	91.2±1.03*	77.1±0.72*	71.6±5.08*	68.8±1.37*	64.2±0.82*
	600	223.4±1.77*	115.2±1.03*	81.4±1.44*	72.3±1.03*	65.2±1.03*	64.2±2.01*	60.7±1.10*
Glibenclamide	10	223.8±1.42*	128.3±4.13*	82.6±3.17*	76.3±1.42*	62.3±1.27*	61.4±3.18*	59.6±0.97*

*p<0.01 when compared to control, F = 10.05, 26.88 df = 5,20 p<0.01 (Two-way ANOVA) n = 6 per group

Table 4: Effect of ethanolic root extract of *S. megaphylla* on serum total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol and TC/HDL C of alloxan diabetic rats

Drug	Dose (mg kg ⁻¹)	Average serum lipids profile (mmol L ⁻¹)					
		Total cholesterol	Triglycerides	HDL cholesterol	LDL cholesterol	TC/HDL C	VLDL
Control		4.05±1.15	2.32±0.22	0.83±0.86	3.68±0.14	4.87±1.34	0.46±0.31
Extract	200	3.9±0.6*	2.13±0.30*	1.28±0.73*	3.05±0.31*	3.05±0.82*	0.43±0.11*
	400	2.9±0.30*	2.12±0.01*	1.89±0.02*	1.43±0.87*	1.53±0.15*	0.42±0.14*
	600	2.8±0.30*	2.0±0.20*	2.02±0.32*	1.18±0.39*	1.38±1.36*	0.40±0.08*
Glibenclamide	10	2.60±0.20*	1.29±0.57*	1.63±0.13*	1.23±0.12*	1.59±0.92*	0.23±0.19*
F-value		27.2	10.5	65.5	82.00	89.40	80.65

Values are expressed as mean±SEM, p<0.05 (N-6) df = 4,20 (one way ANOVA)

ethanolic root extract of *S. megaphylla* or glibenclamide improved the weight gain compared to untreated diabetic rats (Table 1). Dose-dependent reduction in blood glucose was observed in alloxan induced diabetic rats treated with *S. megaphylla*. After a single dose of the extract on the alloxan diabetic rats, there was a significant (p<0.01) reduction in BGL of the diabetic rats within the period of the study (6 h) (Table 2) compared to control. The effect was significantly (p<0.01) more than that of the standard drug (Glibenclamide). During prolonged study (15 days), the extract (200-600 mg kg⁻¹) produced a sustained significant (p<0.01) antidiabetic activity (Table 3) and this was comparable to that of the reference drug, Glibenclamide, which also produced a significant (p<0.01) reduction in BGL of the diabetic rats compared to control.

Hypolipidaemic activity: Serum total cholesterol, triglycerides, LDL cholesterol, VLDL and (T.C/HDL.C)

were significantly (p<0.01) elevated in the untreated diabetic rats as compared to the treated animals (Table 4). All lipids parameters tested were reduced after the treatment with ethanolic root extract of *S. megaphylla* and Glibenclamide for 2 weeks except HDL which was significantly (p<0.01) elevated in the treated animals compared to control.

DISCUSSION

Evaluation of antidiabetic and hypolipidaemic activity of *Setaria megaphylla* was carried out in alloxan induced diabetic rats. The extract which showed slight toxicity was observed to demonstrate significant antidiabetic and hypolipidaemic activity in alloxan diabetic rats.

Phytochemical compounds such as polysaccharides (Tomoda *et al.*, 1985), flavonoids (Shimizu *et al.*, 1984) terpenoids and tannins (Reher *et al.*, 1991), Steroids

(Ivorra *et al.*, 1989), glycoprotein (Hikino *et al.*, 1989), polypeptides (Khanna and Jain, 1981) and alkaloids (Karawya and Wahab, 1984) have been implicated in the antidiabetic activity of plants.

Phytochemical studies of the root extract of *Setaria megaphylla* revealed the presence of flavonoids, terpenes, saponins, tannins and alkaloids who are likely to be responsible for the observed significant activity of this extract, either singly or in synergy with one another. Sulphonylureas cause hyperglycemia by stimulating insulin secretion from pancreas and these compounds are potent in mild alloxan induced diabetes and inactive in intense alloxan diabetes, where nearly all β -cells have been destroyed (Yallow *et al.*, 1960). The observed reduction in BGL of diabetic rats by glibenclamide in our study portrays an in severe state of diabetes. In the present study, the continuous treatment with root extract of *S. megaphylla* for a period of 2 weeks caused a significant decrease in BGL of treated diabetic rats compared to the negative control (untreated diabetic rats). These results correlate well with the findings of Okokon and Antia (2005) who reported antidiabetic activity of the leaves of this plant. The hypoglycaemic action of this extract maybe by potentiating the insulin effect, either by increasing the pancreatic secretion of insulin from the cells of islets of langerhans or its release from bound insulin (Pari and Amarnath, 2004). However, the antidiabetic effect of this extract cannot be completely ascribed to be intra pancreatic since the insulin level of the extract treated rats as well as those of the control were not determined. The antidiabetic activity of this extract can also an effect through extra pancreatic mechanisms by inhibition of hepatic glucose production (Edduoks *et al.*, 2003), inhibition of intestinal glucose absorption (Youn *et al.*, 2004) or correction of insulin resistance (Hu *et al.*, 2003).

Serum lipids are known to be elevated during severe diabetes and have been implicated in the development of artherosclerosis (Mironava *et al.*, 2000; Kaplan, 1989). In this study, the serum levels of untreated diabetic rats were elevated, while that of the treated diabetic rats were reduced significantly ($p < 0.01$) after 2 weeks of administration of the extract. Diabetes-induced hyperlipidaemia is attributable to excess mobilization of fat from the adipose due to the under utilization of glucose (Krishnakumar *et al.*, 2000). The regression of the diabetic state due to ethanolic root extract of *S. megaphylla* administration may have increased the utilization of glucose, thereby depressing the mobilization of fat. Moreso, lowering of lipid levels in rats have been reported

to be due to antioxidant activity of phytochemical compounds such as flavonoids (Igarasi and Onhuruma, 1995) which is present in this extract.

CONCLUSION

The findings of this study confirms that the ethanolic root extract of *Setaria megaphylla* has anti diabetic and hypoglycaemic activity on alloxan induced diabetic rats and also justify its ethnomedical use in the treatment of diabetes.

ACKNOWLEDGMENT

The authors are grateful to Mr. Nsikan Malachy of pharmacology and toxicology Department, University of Uyo, Uyo, for his technical assistance.

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