



Hypoglycaemic and anti-diabetic effect of *Setaria megaphylla* on normal and alloxan induced diabetic rats

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Abstract

Objective: To investigate the hypoglycaemic and anti-diabetic activities of ethanolic extracts of leaves of *Setaria megaphylla*. **Materials and Methods:** The hypoglycaemic activity of the ethanolic leaves extract was evaluated in normal as well in as alloxan (150mg/kg i.p) induced hyperglycaemic rats. Preliminary phytochemical screening and acute toxicity studies of the leaves were carried out. The potency of the ethanolic extract (100, 200 and 300mg/kg b.w. p.o) was evaluated after a single dose (acute study) and after prolonged treatment (chronic study) in normal and alloxan induced diabetic albino rats. The activity of the extract was compared with that of reference drug, chlorpropamide (100mg/kg b.w.p.o). The blood glucose level was measured by using a glucometer. **Results and Discussion:** The extract produced a significant ($P < 0.01$) reduction in Blood glucose level (BGL) of both normal and diabetic rats after a single dose of the extract and in prolonged treatment (i.e. for a week). The hypoglycaemic activity was comparable to that of the reference drug, chlorpropamide. **Conclusion:** Ethanolic leaves extract of *Setaria megaphylla* exhibited a significant hypoglycaemic activity in rats and this confirms its use in ethnomedical diabetic management.

Keyword: *Setaria megaphylla*, hypoglycaemic, anti-diabetic, Alloxan.

1. 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 [1]. About 140 million people world wide suffer from diabetes [2], with the real problem in the third world countries where there is increasing prevalence besides inadequate and costly treatment [3]. Due to the inability of the existing

modern therapies to control all the pathological aspects of the disorders coupled with the high cost and unavailability of the modern therapies especially for many rural population in developing countries, the World Health Organisation recommended and encouraged the use of alternative therapy [4]. The employment of plant in the treatment of diabetes is a valuable alternative for the control of this disease.

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Setaria megaphylla (Steud) Dur & Schinz (family-Poaceae) also called broad leafed brittle grass is a very tall, robust, 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 [5, 6]. The plant is used 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 possess antiparasitic activity *in vitro* [7]. Till date there are no report on its anti-diabetic activity. Therefore, it was thought worthwhile to evaluate its hypoglycaemic as well as its antidiabetic effects using normal and alloxan induced diabetic rats.

2. Materials And Methods

2.1 Plant Materials

Fresh leaves of *Setaria megaphylla* were collected in November, 2004 from Anwa forest in Uruan, Akwa Ibom State, Nigeria. The plant was identified and authenticated by Dr. Margaret Bassey, 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 leaves (2kg) of the plant were dried on laboratory table for 2 weeks and reduced to powder. The powder (100g) was macerated in 95% ethanol (300ml) for 72 hours. The liquid extract obtained was concentrated in vacuum at 40°C. The yield was 2.08% w/w. The extract was stored in a refrigerator at 4°C until used for experiment reported in this study.

2.2 Animals

Albino wistar rats (105-165g) and albino swiss mice (25-32g) of either sex were obtained from the University of Uyo animal house. They were maintained on standard animal pellets and water and *ad libitum*. Permission and approval for

animal studies were obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo.

2.3 Determination of LD₅₀

The LD₅₀ of the extract was estimated using swiss albino mice by intraperitoneal (i.p) route using the method of Lorke [8]. This method involved the administration of different doses of the extract 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 hours was recorded. The LD₅₀ was calculated as the geometrical mean of the maximum dose producing 0% mortality and the minimum dose producing 100% mortality.

2.4 Phytochemical Screening

A preliminary phytochemical screening of the ethanolic extract was carried out employing the standard phytochemical procedures [9,10] to reveal the presence of saponin, flavonoids, tannins, alkaloids and glycosides.

2.5 Induction of diabetes

Alloxan monohydrate dissolved in sterile normal saline immediately before use was injected intraperitoneally to 18 hours fasted rats at a dose of 150 mg/kg body weight. After alloxanisation, the animals were given feed *ad libitum* and 5% dextrose solution for the next 24 hrs to overcome initial hypoglycaemic phase due to massive pancreatic insulin release caused by alloxan. The Blood Glucose Levels (BGL) were monitored after alloxanisation in blood samples collected by tail tipping method. The blood was dropped on the detrostix reagent pad and inserted into microprocessor digital blood glucometer and the reading noted. After 72 hours, rats having BGL beyond 150 mg/dl of blood were selected for the study.

2.6 Experimental design

Two sets of experiments involving a total of 50 rats (25 diabetic and 25 normal rats) were carried out: for single and multiple dose studies.

2.7 Single dose studies (Acute study)

This experiment involved testing for hypoglycaemic effect of the plant in fasted normal and diabetic rats after a single oral administration. 10 groups of fasted normal and diabetic rats of 5 rats/group were used. The negative control in both normal and diabetic rats received 3ml of distilled water. Three groups each of normal and diabetic rats were given po. 100, 200 and 300mg/kg of the plant extract respectively. Their positive control groups had chlorpropamide (100mg/kg), a standard oral hypoglycaemic agent for

comparison. Blood glucose levels were determined after 1, 3, 5 and 12 h of administration of a single dose of the extract.

2.8 Multiple dose studies (Prolonged treatment)

The experiment consisted of three groups of diabetic rats administered orally with 100, 200, and 300 mg/kg of the extract daily for 7 days. Negative and positive diabetic control groups received 3 ml of distilled water and chorpropamide (100 mg/kg) respectively daily for 7 days. Fasting blood glucose levels were monitored at the end of 1, 2, 3, and 7 days.

2.9 Statistical analysis

Data obtained was subjected to two- way Anova followed by Dunnet's *t*-test to determine the statistical significance of the change in BGL.

Table 1: Effect of *Setaria megaphylla* on Blood Glucose Levels of Normal Rats after a Single dose.

Drug	Dose mg/kg	Blood Glucose Level mg/dl (Mean \pm S.D)					
		Initial	1hr	3h	6h	12h	24h
Control	-	70.7 \pm 8.19	70.2 \pm 9.14	71.3 \pm 6.37	70.4 \pm 5.42	70.3 \pm 8.31	76.2 \pm 8.72
Extract	100	68.3 \pm 3.05	51.2 \pm 9.13*	50.5 \pm 9.08**	65.6 \pm 3.91	68.2 \pm 1.18	68.1 \pm 3.07
	200	72.9 \pm 2.26	47.5 \pm 7.05**	45.3 \pm 0.13**	60.3 \pm 4.55*	66.4 \pm 3.54	71.3 \pm 7.98
	300	71.7 \pm 3.71	43.4 \pm 4.50**	42.3 \pm 6.74**	61.2 \pm 5.83*	67.9 \pm 7.61	69.9 \pm 8.14
Chlorpropamide	100	72.3 \pm 5.42	42.3 \pm 8.18**	40.9 \pm 5.39**	58.9 \pm 5.46*	65.5 \pm 8.91	70.3 \pm 4.67

N = 5 in each group, **P < 0.01, *P < 0.05 Vs control, F = 760 and 5.45, df = 4, 16; P<0.01 (two-way ANOVA)

Table 2: Effect of *Setaria megaphylla* on Blood Glucose Levels of alloxan diabetic rats after a Single dose.

Drug	Dose mg/kg	Blood Glucose Level mg/dl (Mean \pm S.D)				
		Initial	1hr	3h	6h	12h
Control	-	231.3 \pm 8.12	239.8 \pm 11.21	235.4 \pm 8.01	221.3 \pm 4.79	206.4 \pm 8.14
Extract	100	238.7 \pm 9.05	219.5 \pm 12.33*	173.3 \pm 8.51**	124.1 \pm 3.15**	130.3 \pm 4.36**
	200	235.8 \pm 4.18	211.7 \pm 6.80**	169.4 \pm 10.2**	119.2 \pm 7.81**	122.9 \pm 9.78**
	300	237.8 \pm 6.80	207.2 \pm 7.31**	148.6 \pm 13.41**	107.3 \pm 2.83**	119.2 \pm 5.68**
Chlorpropamide	100	234.6 \pm 3.52	197.4 \pm 11.71**	145.8 \pm 7.15**	101.5 \pm 11.31**	110.5 \pm 6.81**

N = 5 in each group, **P < 0.01, *P < 0.05 Vs control; F = 23.34 and 8.58; df = 4, 16; P<0.01 (two-way ANOVA)

Table 3: Effect of *Setaria megaphylla* on Blood Glucose Levels of alloxan diabetic rats after prolonged treatment.

Drug	Dose mg/kg	Blood Glucose Level mg/dl (Mean \pm SD)				
		Initial	1st day	2nd day	3rd day	7th day
Control	-	231.3 \pm 8.12	228.7 \pm 3.47	237.3 \pm 11.37	230.2 \pm 7.43	228.7 \pm 2.07
Extract	100	238.7 \pm 9.05	167.8 \pm 9.31**	107.8 \pm 7.61**	99.3 \pm 4.94**	86.4 \pm 3.73**
	200	235.8 \pm 4.18	149.3 \pm 10.07**	98.6 \pm 5.31**	81.5 \pm 7.15**	76.8 \pm 5.42**
	300	237.8 \pm 6.80	131.6 \pm 7.28**	97.8 \pm 7.18**	77.4 \pm 9.12**	75.9 \pm 3.87**
Chlorpropamide	100	234.6 \pm 3.52	119.5 \pm 6.45**	92.9 \pm 3.35**	74.8 \pm 8.43**	72.2 \pm 2.27**

s N = 5 in each group, **P < 0.01, *P < 0.05 Vs control; F = 23.34 and 8.58; df = 4, 16; P<0.01(two-way ANOVA)

3. Results and Discussion

The result of the phytochemical screening of the ethanolic leaf extract of *Setaria megaphylla* showed that the leaves contain flavonoids, deoxy sugar, terpenes, saponins, tannins, anthraquinones, cardiac glycosides, while alkaloids were absent. These compounds are likely to be responsible for the observed significant (P < 0.01) anti-diabetic activity of this extract either singly or in synergy with one another.

The extract (100 – 3000mg/kg) produced physical signs of toxicity ranging from decreased motor activity, decreased respiratory rate, body and limb tone to death. The intensities of all these effects were proportional to the dose administered. The i.p LD₅₀ of the extract in mice was 2400 \pm 50mg/kg. This shows that the extract is slightly toxic [11].

The administration of ethanolic leaf extract of *Setaria megaphylla* (100 – 300mg/kg) produced a significant (P < 0.01) dose – dependent hypoglycemia in normal rats after a single dose of the extract compared to the control group. Maximum hypoglycaemic activity was reached at 3h, producing 35.03 \pm 6.45% reduction in blood glucose levels (table 1). Chlorpropamide (100mg/kg) produced a significant (P < 0.01) reduction in blood glucose level compared to control (43.5% 3h) (Table 1) and at 24h, the

blood glucose levels were found to revert back to the fasting levels.

Dose – dependent reduction in blood glucose was also observed in alloxan induced diabetic rats treated with *S. megaphylla*. The percent reduction in blood glucose levels tended to be higher in the diabetic condition than in normal state. 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 with maximum activity at 6h, producing 50.81 \pm 2.93% reduction in BGL compared to the control (Table2). The effect was sustained throughout the period of acute study. Chlorpropamide produced a significant effect (P < 0.01) which was comparable to that of the extract (56.8% 6h). During prolonged study (7 days), the extract (100 – 300mg/kg) produced a sustained significant (P < 0.01) antidiabetic activity (table 3) and this was comparable to that of the reference drug, chlorpropamide, which also produced a significant (P < 0.01) reduction in BGL of the diabetic rats compared to control. This observed effect can be attributed to the phytochemical compounds present in the extract studied as similar compounds found in various plants have been implicated in this activity and they include; polysaccharides [12], Flavonoids [13], terpenoids and tannins [14], steroids [15], glycoprotein [16], Polypeptides [17] and alkaloids [18]. Though the exact mechanism of action in

reducing blood glucose level is not well understood. The mechanism might be due to increased uptake of glucose peripherally and potentiation of insulin effect either by increasing the pancreatic secretion of insulin or its release from bound insulin [19]. The comparable pattern of hypoglycaemic activity of the extract under study with that of the reference drug, chlorpropamide, supports this suggestion. This observation confirms the use

of this plant in ethnomedical practice for diabetes management. It also warrants further investigation to isolate and identify the hypoglycaemic principles in this plant so as to elucidate its mode of action.

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