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In vivo Alpha-amylase and Alpha-glucosidase Inhibitory Potentials of *Panicum maximum Jacq*. (*Guinea grass*) Leaf Extract on Wister Rats

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Abstract

Panicum maximum Jacq. (Guinea grass) a medicinal plant used traditionally in the treatment of diseases including diabetes was evaluated for its effect on alpha amylase and alpha glucosidase enzymes *in vivo*. The crude ethanol extracts (150, 300 and 450 mg/kg) of *P. maximum* were investigated using starch, sucrose, glucose and maltose as substrates and acarbose as reference drug. The leaf extract caused significant ($p<0.05$) reduction in blood glucose levels of the treated rats with the four substrates used. The findings show that the leaf extract of *Panicum maximum* has the potentials to inhibit alpha amylase and alpha glucosidase in rats.

Keywords: *Panicum maximum*; alpha amylase; alpha glucosidase; phytochemicals.

INTRODUCTION

Plants have therapeutic substances because of their potency and huge benefits, and have been widely researched and adopted in our society for the treatment and management of some diseases. In the last few decades, bioactive compounds have been trapped, purified and characterized into active drugs and, administered in rural and urban communities of the world (Coulibaly *et al.*, 2023). Recent findings demonstrate that the world sales of medicinal plant-based products have escalated to 100.9 billion dollars representing 7.2% annual turn-over and that global population of up to 80% are utilizing medicinal plants to treat various diseases (Yusupova et al., 2023; Karahan et al., 2020). With the increasing rate of spread of diseases and infections, there is need to explore more plant-based materials for possible discoveries and publications of new and efficient remedies for these challenges. One of such herbs which could find possibility in combating current health challenges such as diabetes is *Panicum maximum* commonly called "Guinea grass".

Panicum maximum Jacq. is a perennial grass of the *Poacace* family distributed widely in Africa and other tropical regions of the world (Van Oudtshoorn, 1999). The ethnopharmacology of the plant indicates that, the plant has been employed for the treatment of malaria, microbial infections, rheumatic pain, inflammation and diabetes (Antia *et al*., 2010). In Nigeria, particularly in Ibibio ethnomedicine, the leaf is used to treat malaria, microbial infections and rheumatism. Other biological activities of the leaves and roots include antidiabetic (Antia *et al*., 2010), antimalarial and analgesic (Okokon *et al*., 2012), antibacterial (Gothandam *et al*., 2010; Doss *et al*., 2011a; Doss *et al*., 2011b), anti-inflammatory and antipyretic (Okokon *et al*., 2011), antifungal (Kanife, 2012), anticancer, antioxidative and antileishmanial (Okokon *et al.*, 2014). Phytochemical studies of the root have shown the presence of alkaloid, flavonoid, tannins, terpenes, saponin, and cardiac glycosides (Okokon *et al*., 2016). In this study, we investigated the inhibitory activities of *Panicum maximum* Jacq. ethanol leaf extract on alpha-amylase and alpha-glucosidase in Wister rats.

MATERIALS AND METHODS

Materials

The materials used include *Panicum maxima* powdered leaf extract, oral gastric gavage, weighing balance, gloves, scissors, glucometer and strips (fine test), distilled water, acarbose (Aldrich sigma, USA; standard drug), stirrer, beakers, 1mL syringe, starch, sucrose, maltose (Aldrich sigma, USA).

Plant Collection

Fresh leaves of *Panicum maxima* were harvested from a farmland in Use offot, Uyo, Akwa Ibom State, Nigeria, in May, 2023. The plant was identified and authenticated by Dr. Margaret Bassey, a taxonomist in the Department

of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. Herbarium specimen was deposited at the Department of Botany and Ecological Study, University of Uyo.

Extraction
The fresh leaves (2 kg) of the plant were dried on a laboratory table for 2 weeks and reduced to powder. Powdered sample (500 g) was macerated in 95% ethanol (5000 mL) for 72 hours. The liquid filtrate obtained was concentrated *in vacuo* at 40˚C to completely remove the ethanol. The yield was calculated, and the extract was stored in a refrigerator at 4˚C until used.

Phytochemical Screening
Phytochemical screening for the presence of saponins, tannins, flavonoids, alkaloids, cardiac glycosides and anthraquinones was conducted following previously reported standard procedures (Enin *et al*., 2023).

Animals
Albino Wistar rats (120 -135 g) of either sex maintained at animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Nigeria were used for the study. The animals were housed in standard cages and were maintained on a standard pelleted feed (Guinea feed) and water *ad libitum*.

Alpha-Amylase inhibitory study
Thirty Wistar rats were divided into 6 groups of 5 rats each. The rats in all groups were fasted for 18 h and fasting blood glucose concentration was first taken at 0 min before administration. Group I, as the normal control, received distilled water (10 mL/kg). Group II rats were orally administered starch at 2 g/kg body weight (orally with distilled water as vehicle) and distilled water (10 mL/kg) simultaneously. Rats in group III were administered starch (2 g/kg) and the standard drug (acarbose) at 100 mg/kg simultaneously. Groups IV, V and VI were administered simultaneously, starch (2 g/kg) and *Panicum maximum* leaf extract at 150, 300 and 450 mg/kg respectively. All administrations were done orally and blood glucose concentration was monitored at 30, 60, 120 and 180 min (Gidado *et al.*, 2019). The blood glucose level was used to assess the effect of extract on the enzyme activity.

Alpha Glucosidase inhibitory study.
The procedure as described above was used for this study but with sucrose and maltose used as substrates (Gidado et al., 2019).

Blood Glucose Determination
Drops of blood from tip of rats tails were dropped on stripes and glucose concentration was measured using a glucometer according to manufacturer's specifications (fine test). The glucometer works with the following principle; the blood sample is exposed to a membrane covering the reagent pad (strip), which is coated with an enzyme (glucose oxidase, glucose dehydrogenase). The reaction causes a colour change and the intensity of this change is directly proportional to the amount of glucose in the blood sample. Light from an LED strikes the pad surface and is reflected to a photodiode, which measures the light intensity and converts it to electrical signals. An electrode sensor measures the current produced when the enzyme converts glucose to gluconic acid. The resulting current is directly proportional to the amount of glucose in the sample (WHO, 2011).

Statistical Analysis
Data obtained were analyzed statistically using one –way ANOVA followed by Tukey-Kramer multiple comparison test using InstatR Graphpad software, (San Diego, USA). Differences between means were considered significant at $p< 0.05$ and very significant at $p < 0.001$.

RESULTS AND DISCUSSION

Extraction
The extraction yield was 6.56% w/w.

Phytochemical screening
The results of the phytochemical screening revealed the presence of saponins, tannins, flavonoids, alkaloids, cardiac glycosides. The presence of anthraquinone was not observed in the study.

In vivo alpha amylase and glucosidase inhibition assay
Administration of starch (2 g/kg) to fasted rats caused varying percentages of increase in blood glucose levels of the treated animals after 30 mins. The percentages were starch (66.56%), *P maximum* leaf extract-treated groups (37.17 - 47.26%), and acarbose-treated group (17.97%). These increases were reduced after 60 min with only the groups treated with higher doses of the extract (300 and 450 mg/kg) having percentage increases of 3.82 and 18.44 % respectively. All the extract-treated groups had their BGL reduced to a normal level at 120 min and this was sustained throughout the study. Also, co-administration of the starch with acarbose prominently inhibited the rise in the blood glucose concentrations (Table 1).

Table 1. Effect of ethanol leaf extract of *Panicum maximum* on Blood Glucose Level of rat after oral administration of starch load.

TREATMENT	DOSE	BLOOD GLUCOSE LEVEL mg/dL IN MIN				
	mg/kg	0 min	30 min	60 min	120 min	180 min
Control normal saline	-	86.00±11.53	87.66±7.12(1.93)	87.66±7.62(1.93)	91.0±7.50(5.81)	80.00±6.02
Starch		80.0±4.54	133.25±6.86[a](66.56)	112.25±4.73(40.31)	92.50±1.70(15.62)	87.25±6.52(9.06)
Acarbose	100	72.33±2.69	85.33±12.97(17.97)	80.33±7.21(11.06)	74.0±1.00(2.30)	72.33±8.68(0)
Extract	150	95.75±5.20	133.50±7.83(39.42)	92.0±9.71[a]()	82.50±3.22[a]()	67.25±3.42[a]()
	300	91.50±5.56	134.75±8.49(47.26)	95.0±9.44(3.82)	88.50±5.23[b]()	72.75±8.94[a]()
	450	86.75±12.41	119.0±2.12(37.17)	102.75±1.48(18.44)	85.50±6.89[a]()	85.25±5.88()

Data is expressed as MEAN ± SEM, Significant at [a]p<0.05, [b]p< 0.01, when compared to control (n=6). Values in parenthesis are percentage increases in blood glucose concentrations compared to 0 min in the same group.

Administration of sucrose (2 g/kg) produced a 44.14% increase in blood glucose concentration 30 minutes post-administration of the sucrose in the control group. BGL increments of 12.07-34.60 % were also recorded in groups treated with 150, 300 and 450 mg/kg of *P maximum* leaf extract and 3.37% for acarbose treated group. At 60 min, percentage increases in BGL of groups treated with 150 and 450 mg/kg of extract were 19.94 and 9.22 % respectively, while the BGL of the group treated with 300 mg/kg was reduced to normal. Similarly, trend was also recorded at 120 min. There was no increment in BGL of all the extract-treated groups at 180 min (Table 2).

Table 2. Effect of ethanol leaf extract of *Panicum maximum* on Blood Glucose Level of rat after oral administration of sucrose load.

TREATMENT	DOSE	BLOOD GLUCOSE LEVEL mg/dL IN MIN				
	mg/kg	0 min	30 min	60 min	120 min	180 min
Control normal saline	-	100.00±4.25	88.33±1.85	92.33±4.25	89.0±4.35	87.33±3.84
Sucrose	2000	81.0±4.50	116.75±6.57[b](44.14)	112.66±1.45[a](39.08)	97.33±1.63(20.16)	94.15±4.81(16.23)
Acarbose	100	90.33±2.48	86.66±2.90	82.0±6.00	71.66±3.75	78.0±3.78
Extract	150	85.25±3.27	114.75±11.22[c](34.60)	102.25±4.82(19.94)	90.25±3.27(5.86)	83.50±4.48
	300	95.25±2.98	106.75±2.98[b](12.07)	89.50±4.94()	85.0±2.48()	72.75±5.93()
	450	84.0±5.87	101.25± 2.28(20.53)	91.75±4.55(9.22)	89.0±2.67(5.95)	76.50±3.52()

Data is expressed as MEAN ± SEM. Significant at [a]p<0.05, [b]p< 0.01, when compared to control (n=6). Values in parenthesis are percentage increases in blood glucose concentrations compared to 0 min in the same group.

Administration of glucose (2 g/kg) to fasted rats caused varying percentages of increase in blood glucose levels of the treated animals after 30 mins. The percentages were glucose (64.98%), *P maximum* leaf extract-treated groups (57.10 - 61.90%), and acarbose-treated group had no increment. All the extract-treated groups had their BGL reduced to a normal level at 120 min and this was sustained throughout the study (Table 3).

Table 3. Effect of ethanol leaf extract of *Panicum maximum* on Blood Glucose Level of rat after oral administration of glucose load.

TREATMENT	DOSE	BLOOD GLUCOSE LEVEL mg/dL IN MIN				
	mg/kg	0 min	30 min	60 min	120 min	180 min
Normal Control	-	100.00±4.25	88.33±1.85	92.33±4.25(1.80)	89.0±4.35(1.55)	87.33±3.84(3.98)
Glucose	2000	84.25±1.49	139.0±1.78[b](64.98)	126.75±0.47[b](50.44)	106.0±1.87[b](25.81)	96.75±2.78[a](14.83)
Acarbose	100	85.34±1.36	84.21±0.90	84.0±1.20	82.16±2.14[a]	80.00±1.10
Extract	150	84.0±2.16	136.0±2.16[a](61.90)	89.50±8.56[a](6.54)	84.0±7.70[a]()	76.50±6.38[a]()
	300	84.75±1.43	136.25±1.03[b](60.76)	92.50±4.17[a](9.14)	81.50±2.72[a]()	74.25±4.49()
	450	86.25±1.10	135.50±1.44[b](57.10)	88.0±2.04[b](2.02)	78.75±2.52[b]()	68.25±3.86[a]()

Data is expressed as MEAN ± SEM, Significant at [a]p<0.05, [b]p< 0.01, when compared to control. (n=6). Values in parenthesis are percentage increases in blood glucose concentrations compared to 0 min in the same group.

Administration of maltose (2 g/kg) to fasted rats caused varying percentages of increase in blood glucose levels of the treated animals after 30 mins. The percentages were maltose (65.86%), *P. maximum* leaf extract-treated groups (48.17-67.87%), and acarbose-treated group (3.37%). These increases were reduced

after 60 min with only the low dose (150 mg/kg) and middle dose (300 mg/kg) treated group having BGL increment of 14.28 and 53.93% respectively. All the extract-treated groups had their BGL reduced to a normal level at 180 min. Also, co-administration of the maltose with acarbose prominently inhibited the rise in the blood glucose concentrations (Table 4).

Table 4. Effect of ethanol leaf extract of *Panicum maximum* on Blood Glucose Level of rat after oral administration of maltose load.

TREATMENT	DOSE	BLOOD GLUCOSE LEVEL mg/dL IN MIN				
	mg/kg	0 min	30 min	60 min	120 min	180 min
Control normal saline	-	86.00±11.53	87.66±7.12(1.93)	87.66±7.62(1.93)	91.0±7.50(5.81)	80.00±6.02
Maltose		83.50±1.19	138.50±8.10[a](65.86)	120.25±2.95(44.01)	97.25±2.05(16.46)	87.25±2.13(4.49)
Acarbose	100	85.34±1.36	88.22±1.10(3.37)	86.0±2.20(0.77)	84.26±1.14[a]()	82.28±2.26()
Extract	150	75.25±5.13	111.50±9.64(48.17)	86.0±7.62(14.28)	72.25±3.98()	68.0±2.98()
	300	82.5±4.02	138.50±13.17(67.87)	127.0±18.35(53.93)	90.25±20.67(9.39)	68.75±6.83()
	450	83.50±1.19	138.50±8.10(65.86)	80.25±2.95()	67.25±2.05()	66.25±2.13()

Data is expressed as MEAN ± SEM, Significant at [a]p<0.05, [b]p< 0.01, when compared to control (n=6). Values in parenthesis are percentage increases in blood glucose concentrations compared to 0 min in the same group.

Discussion

The leaf ethanol extract was found to inhibit increases in blood glucose concentration significantly following starch administration though non-dose-dependently. It has been reported that complete digestion of dietary polysaccharides like starch is achieved by the combined action of α-amylases and α-glucosidase enzymes. The α-amylase enzyme digests α-bonds of the α-linked polysaccharides yielding disaccharides, like maltose, which are further reduced to monosaccharides by membrane bound α-glucosidase enzymes (Kalra, 2014; Alongi and Anese, 2018). Inhibitions of these enzymes delay the digestion of ingested carbohydrates thereby resulting in a small rise in blood glucose concentrations following carbohydrate meals as was observed in this study. As a target for managing Type 2 diabetes mellitus, many medicinal plants have been reported to possess α-amylase and α- glucosidase inhibitory potential (Ibrahim *et al*., 2014; Esimone *et al*., 2001). Similarly, the leaf extract significantly inhibited blood glucose rises when co-administered with maltose, glucose and sucrose. Acarbose, the standard drug used in this study significantly inhibited blood glucose rise when co-administered with starch, maltose and sucrose. The results of this study support the antidiabetic activity earlier reported on the root extract (Antia *et al*., 2010) and further suggest the involvement of inhibitory effects on alpha glucosidase and amylase as one of the modes of antidiabetic activity of the root extract. The inhibitory activities of plant extracts are linked to their phytochemical constituents. The leaf extract of P. maximum has been reported to be rich in flavonoids, terpenes, tannins amongst others (Okokon *et al*., 2011). Our results of the phytochemical screening also revealed the presence of: saponins, tannins, alkaloids, flavonoids and cardiac glycosides. These compounds have been variously reported to inhibit alpha glucosidase and alpha amylase activities (Proença *et al*., 2017; Su and Tang, 2019). Moreso, Phenols have been reported to inhibit alpha amylase and alpha glucosidase (Oboh *et al*., 2017). Also, polyphenolic compounds from plants are known to cause several effects on the biological systems which include enzymes inhibitions (Kalita *et al*., 2018; Funke and Melzig, 2005). The phenolic compounds are known to be strong metal ion chelators and protein precipitation agents forming insoluble complexes with proteins as well as acting as biological oxidants (Ishnava and Metisariya, 2018). The presence of the polyphenolic compounds and terpenes in the root extract may suggest that their inhibitory potential on α-amylase and the membrane-bound intestinal α-glucosidase enzymes. The presence of these compounds in the extract may have contributed to the observed activity of this study and therefore explains the antidiabetic mechanism of the leaf of P. maximum.

CONCLUSION

The results of this study suggest that inhibition of alpha amylase and alpha glucosidase enzymes maybe one of the modes of antidiabetic activity of the leaf extract of *Panicum maximum* which may be attributed to the activities of its phytochemical constituents.

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Competing Interests: The authors declare that there are no competing interests.

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