In vivo inhibitory effect of *Panicum maximum* root extract on alpha amylase and alpha glucosidase enzymes of rats

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Abstract

Panicum maximum Jacq, (Poaceae) a medicinal plant used traditionally in the treatment of diseases including diabetes was evaluated for effect on alpha amylase and alpha glucosidase enzymes *in vivo*. The leaf extract (137, 273, and 547 mg/kg) of *Panicum maximum* were investigated *in vivo* for inhibitory effect on alpha amylase and alpha glucosidase enzymes using starch, sucrose and maltose as substrates. Acarbose was used as reference drug. The leaf extract non dose-dependently caused significant ($p<0.05$) reduction in blood glucose levels of treated rats with the various substrates used. The results suggest that the leaf extract of *Panicum maximum* have the potentials to inhibit alpha amylase and glucosidase in rats.

Keywords:*Panicum maximum,* diabetes, alpha amylase, alpha glucosidase

Abbreviation:BGL (blood glucose level)

Introduction

Panicum maximum Jacq (Poacace) is a perennial grass which is distributed widely in Africa and other tropical regions of the world (Van Oudtshoorn, 1999). The leaves have been employed ethnomedically by the Ibibios of Akwa Ibom State, Nigeria in the treatment of various ailments such as malaria, microbial infections, rheumatism

pain, inflammation and diabetes. 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 burst and antileishmanial (Okokon *et al.,* 2014) activities of the leaf extract have been reported. Also, *Panicum maximum* root extract has been reported to possess analgesic and antimalarial properties (Okokon *et al*., 2016) with LD_{50} value of 2738.1 mg/kg, antidepressant and anticonvulsant (Okokon *et al.,* 2018) and anti-inflammatory activities (Udobang *et al.,* 2020) and antiulcerogenic (Okokon *et al.,* 2021). Phytochemical constituents of the root such as alkaloid, flavonoid, tannins, terpenes, saponin, and cardiac glycosides (Okokon *et al.,* 2016) have been reported. In this study, we investigated the effect of ethanol root extract of *Panicum maximum* on alpha amylase and alpha glucosidase activities of rats.

Materials and Methods

Plants collection

The plant materials, *Panicum maximum* (roots), were collected from a farmland in Uyo area, Akwa Ibom State, Nigeria in January, 2022. The plant was identified and authenticated by a Botanist, Mr Uwakwe B. and a voucher specimen (MUFP/58) was deposited at the herbarium of Department of Pharmacognosy,Faculty of Pharmacy, Madonna University, Elele Campus, Rivers State, Nigeria.

Extraction

The plant parts (roots) were washed and shade-dried for two weeks. The dried plants' materials were reduced to powder using mortar and pestle. The powdered material was soaked in 50% ethanol. The liquid filtrate was concentrated and evaporated to dryness *in vacuo* 40˚C using rotary evaporator and stored in a refrigerator at -4˚C.

Animals

Male Wistar rats (145 – 170 g) used for these experiments were gotten from animal

house of Department of Pharmacology and Toxicology, University of Uyo. The animals were housed in standard cages and were maintained on a standard pelleted feed (Guinea feed) and water *ad libitum*. Permission and approval for animal studies were obtained from Animal Research Ethics Committee, Madonna University (MUN/FP/AE/22/012).

In vivo alpha-amylase and glucosidase inhibition study

Alpha-Amylase inhibitory study

Thirty-five 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 *P. maximum* root extract at 137, 273 and 547 mg/kg respectively. All administrations were done orally and blood glucose concentration was monitored at 30, 60, 90, 120 and 180 min (Gidado *et al.,* 2019).

Glucosidase inhibitory study

The procedure as described above was used for this study butwith 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 (Accu-chek, Indiana). 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 from this work were analysed statistically using one –way ANOVA followed by Tukey-Kramer multiple comparison test using Instat Graphpad software Version 3, (San Diego, USA). Differences between means were considered significant at 5% level of significance i.e. $p \leq 0.05$.

Results

In vivo alpha amylase and glucosidase inhibition assay

Administration of starch (2g/kg) to fasted rats caused varying percentages of increase in blood glucose concentrations of the treated animals after 30 min. The percentages were starch (63.18%), extract-treated groups (20.59-26.31%) and acarbose-treated group (17.97%). These increases were reduced after 60 min with animals treated with the 137 mg/kg (10.11%), 547 mg/kg (28.21%) and 273 mg/kg (33.48 %). These decreases were significant and non dose-dependently sustained from 120- 180 min with all the extract-treated group recording 0% increment inBGL (blood glucose level). However, co-administration of the starch with acarbose prominently inhibited the rise in the blood glucose concentrations (Table 1). Administration of sucrose (2 g/kg) produced a 46.01% increase in blood glucose concentration 30 minutes post-administration of the sucrose in the control group and 19.90 - 32.25 % increases in blood glucose concentration of extract-treated groups. The blood glucose concentrations were non dose-dependently and significantly reduced in all the extract-treated groups (17.58-29.03%) after 60 min post-administration of sucrose. At 180 min,

the BGL increments were 1.84, 10.01 and 15.20 % for 547, 273 and 137 mg/kg of the extract-treated groups respectively (Table 2).There was 60.78% increase in blood glucose concentration 30 min following maltose administration in the control group. However, 0% increase was observed in the group treated with 547 mg/kg of the extract, while 137 and 273 mg/kg treated groups had 21.23 and 23.94% increases in BGL respectively. At 60 min, the middle dose (273 mg/kg)-treated group had no BGL increment while the low dose (137mg/kg) had 18.14% increment. The methanol fraction, n-hexane and ethyl acetate fractions-treated groups had percentage increments in BGL of 52.30, 62.93 and 61.02% respectively. The percent increase in BGL of low dose (137 mg/kg)-treated group was 1.76%) at 180 min. (Table 3).

TABLE 1: Effect of ethanol leaf extract of *Panicum maximum* root 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	90 min	120 min	180 min
Control normal saline	-	86.00±11.53	87.66±7.12(1.93)	87.66±7.62(1.93)	73.66±6.17	91.0±7.50(5.81)	80.00±6.02
Starch		73.33±8.25	119.66±5.45[a](63.18)	115.66±1.33[a](57.72)	104.66±2.60[a](42.72)	95.66±3.75[a] (30.45)	92.0±6.35(25.46)
Acarbose	100	72.33±2.69	85.33±12.97(17.97)	80.33±7.21(11.06)	76.33±3.48(5.53)	74.0±1.00(2.30)	72.33±8.68(0)
Extract	137	78.34±4.12	95.33±9.65(21.68)	85.66±5.81(10.62)	83.66±4.17(6.79)	75.16±8.68()	71.33±6.33()
	273	75.16±3.28	95.00±5.29(26.39)	100.33±3.75(33.48)	86.66±7.35(15.30)	74.33±5.17()	67.66±4.37()
	547	74.35±2.51	89.66±7.75(20.59)	95.33±2.96(28.21)	92.0±3.21(23.73)	76.66±2.40(3.10)	70.00±2.64()

Data is expressed as MEAN ± SEM, Significant at ap<0.05, bp< 0.01, when compared to control (n=5). Values in parenthesis are percentage increases in blood glucose concentrations compared to 0 min in the same group.

Table 2: Effect of ethanol leaf extract of *Panicum maximum* root 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	90 min	120 min	180 min
Control normal saline	-	100.00±4.25	88.33±1.85	92.33±4.25	90.33±2.33	89.0±4.35	87.33±3.84
Sucrose	2000	92.0±4.04	134.33±2.90[b](46.01)	128.66±5.45[a](39.84)	117.33±4.66[a](27.53)	97.66±0.66(6.15)	104.16±2.48(13.21)
Acarbose	100	90.33±2.48	86.66±2.90	82.0±6.00	79.33±2.96	71.66±3.75	78.0±3.78
Extract	137	73.65±3.05	97.3±5.46(32.11)	94.33±4.33(28.07)	89.36±10.17(21.33)	85.33±9.87(15.85)	83.66±11.34(10.01)
	273	72.33±7.83	95.6±8.11(32.25)	93.33±6.88(29.03)	91.33±5.38(26.38)	84.66±8.64(17.04)	83.33±8.41(15.20)
	547	72.00±1.15	86.33±1.36(19.90)	84.66±2.02(17.58)	80.33±2.90(11.56)	76.33±1.45(6.01)	73.33±1.20(1.84)

Data is expressed as MEAN ± SEM. Significant at ap<0.05, bp< 0.01, when compared to control (n=5). Values in parenthesis are percentage increases in blood glucose concentrations compared to 0 min in the same group.

TABLE 3: 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	90 min	120 min	180 min
Control normal saline	-	100.00±4.25	88.33±1.85	92.33±4.25(1.80)	90.33±2.33(3.62)	89.0±4.35(1.55)	87.33±3.84(3.98)
Maltose	2000	82.30±2.14	132.33±1.90[b](60.78)	130.22±2.45(58.22)	120.66±3.22[a](46.60)	115.0±2.46(39.73)	106.22±4.24(29.06)
Acarbose	100	85.34±1.36	88.22±1.10(3.37)	86.0±2.20[c](0.77)	85.33±2.15[c]()	84.26±1.14[a]()	82.28±2.26[a]()
Extract	137	75.33±9.32	91.33±8.65(21.23)	89.0±8.17(18.14)	84.0±7.17(11.59)	79.33±4.11(5.30)	76.66±11.97(1.76)
	273	73.66±2.72	91.30±2.40(23.94)	69.0±2.00()	63.66±2.96()	63.00±3.05()	56.66±1.33()
	547	73.33±3.18	71.0±3.60()	68.0±4.04()	65.0±3.51()	62.0±3.60()	57.0±3.78()

Data is expressed as MEAN ± SEM, Significant at ap<0.05, bp< 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 root extract was found to inhibit increases in blood glucose concentration significantly following starch administration though non dose-dependently. 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 root extract significantly inhibited blood glucose rise when co-administered with maltose 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 (Okokon*et al.,* 2014) 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 root extract of *P. maximum* has been reported to be rich in flavonoids, terpenes, tannins amongst others (Okokon *et al*., 2007). These compounds have been variously reported to inhibit alpha glucosidase and alpha amylase activities (Proença *et al*., 2017; Su and Tang, 2019). Moreso, terpenes have been reported to inhibit alpha amylase and alpha glucosidase (Obohet *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 root of *P. maximum.*

Conclusion

The results of this study suggest that the root extract has the potential to inhibit alpha amylase and alpha glucosidase activities in rats and this maybe one of the modes of antidiabetic activity of the root extract of *Panicum maximum* which can be attributed to the activities of its phytochemical constituents.

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