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Alpha-Amylase, Maltase and Sucrase Inhibitions by Aqueous Leaf Extracts of *Anacardium occidentale* (Anacardiacea) and *Piliostigma reticulatum* (Caesalpiniaceae) in Rats

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ABSTRACT

Amylases, maltase and sucrase enzymes are involved in digestion of carbohydrate meals and are therefore targets for inhibition to prevent postprandial blood glucose rise in diabetes mellitus. Extracts of the leaves of *Anacardium occidentale* and *Piliostigma reticulatum* are reported to possess antidiabetic activity. In this study, the inhibitory potential of the aqueous extracts of the leaves of two plants on the carbohydrate digestive enzymes *in vivo* was investigated to establish their possible mechanism of action. The extracts at 100 and 200 mg/kg body weight were co-administered to normoglycaemic rats with starch for the α-amylase and maltose or sucrose for the maltase and sucrase inhibitions, respectively. Acarbose was used as standard drug for comparison. Increases of 27%, 30%, and 22% in blood glucose levels were seen after 30 minutes following starch, maltose and sucrose administrations, respectively to the experimental rats. Co-administration of starch or maltose with the aqueous extract of *Anacardium occidentale* at 200 mg/kg body weight completely inhibited the rise in blood glucose. Similar pattern was observed when the aqueous extract of *P. reticulatum* at 200 mg/kg body weight was co-administered with the starch or maltose. The results therefore suggest that the antidiabetic potential of the plants' leaf extracts may be associated with amylases and maltase inhibitions.

***Keywords*:** *Anacardium occidentale, Piliostigma reticulatum*, antidiabetic, amylase, enzymes.

Introduction

Diabetes mellitus (DM) is a disease associated with hyperglycaemia, polyuria, polyphagia, polydipsia, ketosis, nephropathy and cardiovascular disorders.[1] Acute complications can include diabetic ketoacidosis, hyperosmolar hyperglycaemic state, or death.[2] Serious long-term complications include cardiovascular disease, stroke, chronic kidney disease, foot ulcers, and damage to the eyes.[3] DM is an endocrine disease caused primarily by diminished insulin secretion by the pancreatic β-cells or to reduced insulin sensitivity by cells ultimately leading to hyperglycaemia; the hallmark of the disease.[4] Broadly, DM is classified into Types 1 and 2. The Type 2 diabetes (T2D) accounts for more than 90% of cases. Factors believed to be responsible for the development of T2D are genetic predisposition, foods and life style changes among others.[5]

The hyperglycaemia in DM is known to be responsible for the long-term diabetic complications.[6] Postprandial hyperglycaemia results from dietary carbohydrates. Carbohydrate digestive enzymes hydrolyse starch and other complex polysaccharides to oligosaccharides, which are further broken down to liberate absorbable monosaccharides. The α-amylases and α-glucosidases are respectively responsible for these enzymatic activities. One of the chemotherapeutic strategies for the control of postprandial hyperglycaemia is the inhibition of α-amylase and α-glucosidase enzymes.[7,8] Inhibition of the enzymes will delay the digestion of dietary carbohydrates, reduce rate of glucose absorption and effectively lower postprandial blood glucose rise.[9]

Conventional α-glucosidase inhibitors includes acarbose and miglitol which are reported to have some serious side effects including flatulence, diarrhoea, abdominal pain and many other intestinal disturbances necessarily leading to non-compliance and early patients' withdrawal.[10]

The use of herbal medicines (phytotherapy) has recently gained worldwide popularity for their efficacy in Type 2 diabetes and have been used for this treatment since ancient times. Plant therapy is frequently used due to its low costs and easy accessibility especially in third world countries,[11] however, for most of the plants studied for possible anti-diabetic activity, their mechanisms of action have not been evaluated. The need to establish the mode of action of the plants based antidiabetic agents will further increase their scientific basis and acceptability.

Anacardium occidentale is a tropical ornamental evergreen tree that can grow to a height of about 14 m (46 ft) and belongs to the Anacardiacea family. It is known as cashew in English and produces the cashew seed and the cashew apple.[12] *Anacardium occidentale* is known to have a lot of medicinal values. Its fruits were shown to exhibit antibacterial activity against *Helicobacter pylori* that is commonly implicated in acute gastritis and stomach ulcers.[13-14] It has also been found to possess anti-viral[15] and anti-fungal[16] activities. The stem-bark and leaves of *A. occidentale* have been reported to possess hypoglycaemic activities[17] while its anti-oxidative stress activities have been reported for the shoot [18] and the leaves.[19] In Nigeria, the decoction of the root and stem is used as anti-inflammatory, anti-diarrheal[20] as well as anti-hypertensive and anti-diabetic agent.[21,22]

Piliostigma reticulatum is a tropical forage plant that grows to about 8 m high. In English, it is known as Camel's foot. It is traditionally used in the treatment of many diseases such as dysentery,diarrhoea,

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inflammation, infections, neuralgia, smallpox, malaria, rheumatism and diabetes. In addition, the leaves and barks are used to aid digestion.[23,24]
In this study we report the possible α-amylase, maltase and sucrase inhibitory activity of aqueous extracts of the leaves of *A. occidentale* and *P. reticulatum* on rats treated with starch, maltose or sucrose as substrates.

Materials and Methods

Plants' material and extracts' preparation (AOE, PRE)
Anacardium occidentale and *Piliostigma reticulatum* were collected from University of Maiduguri, Works Department in January, 2018 and were identified and authenticated by a Taxonomist (Prof. S. S. Sanusi) from the Department of Biological Sciences, University of Maiduguri. Voucher specimen numbers of 14/003A and 13/004, respectively were obtained. The leaves of both *A. occidentale* and *P. reticulatum* were separately shade dried and processed into powder using pestle and mortar. The separate powders (300 g each) of the leaves were boiled in 1.5 L each of distilled water for 10 min. The extracts were cooled and filtered using muslin cloth and evaporated to dryness using a rotary evaporator.

Experimental Animals
Wistar strain albino rats of both sexes weighing between 100-200 g were used for this study. The rats were obtained from the Animal House of the Department of Biochemistry, University of Maiduguri, Borno State. They were maintained under standard laboratory conditions with free access to water and standard rodent feed (Growers mash, BFFM, Ewu, Nigeria). They were kept to acclimatize for two weeks. The rats were treated in accordance with the principles of laboratory animal care.[25]

Alpha-Amylase inhibitory study
Thirty-five Wistar rats were divided into 7 groups (I-VII) 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 (1 mL/kg). Group II rats were orally administered starch at 2 g/kg body weight (orally with distilled water as vehicle) and distilled water (1 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 and V were administered simultaneously, starch (2 g/kg) and *Anacardium occidentale* extract at 100mg/kg and 200 mg/kg respectively while groups VI and VII rats were administered starch (2 g/kg) and *Piliostigma reticulatum* extract at 100 mg/kg and 200 mg/kg respectively. All administrations were done orally and blood glucose concentration was monitored at 30, 60, 90, 120 and 180 min.[26]

Glucosidase inhibitory study
The procedure as described above was used for this study but with maltose and sucrose used as substrates.

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.[27]

Statistical analysis
The data obtained were analysed using one way ANOVA and bonferonni *post hoc* test was used to indicate the significance between means when compared to the controls. ANOVA and Bonferronni were conducted using SPSS version 21.0. Values were presented as mean ± SEM and $P \leq 0.05$ was considered as level of significance.

Results and Discussion

There was a 37.8%, 31.6% and 18.6% increase in blood glucose concentrations after 30, 60 and 90 minutes of starch administration respectively (Table 1). However, co-administration of the starch with acarbose significantly inhibited the rise in the blood glucose concentrations. The aqueous extracts of *Anacardium occidentale* and *Piliostigma reticulatum* when co-administered with the starch at the 200 mg dose also significantly inhibited the glucose concentration rise. Administration of *Piliostigma reticulatum* extract at 100 mg/kg dose showed a slight, 26.5% and 27.8% increases in blood glucose concentration after 30 and 60 minutes as against the 37.8% and 31.6% increases observed with starch administration at the same time points.
The maltose study (Table 2), produced a 28.9% increase in blood glucose concentration 30 minutes post-administration of the maltose in the control group. The blood glucose concentration returned to normal values as from 60 minutes post-administration. Acarbose completely inhibited any rise in blood glucose concentration. Notable inhibitions were only observed at the 200 mg/kg dose of the two extracts. At 30 minutes post-administration of the extracts with maltose, *A. occidentale* extract showed 18.8% increase, *P. reticulatum* produced a 12.4% increase as against the 28.9 % increase observed in the maltose control group.
The two extracts co-administered with sucrose did not inhibit rise in blood glucose concentrations. Increases of 23.8% and 17.3 % were seen when sucrose was administered to the control group at 30 and 60 minutes respectively. The 100 mg/kg body weight of *A. occidentale* extract showed a 40.1 % and 12.9 % increases respectively at the 30 and 60 minutes post-administration. For the same time intervals, the increases following administration of the 200 mg/kg were 43.9 % and 37.6 % respectively. Similar patterns were observed when different doses of the *P. reticulatum* extract was administered however, acarbose was able to inhibit the rise in blood glucose concentration when co-administered with sucrose (Table 3).
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.[28-30] Inhibitions of these enzymes delay the digestion of ingested carbohydrates thereby resulting in a small rise in blood glucose concentrations following carbohydrate meals. As a target for managing Type 2 diabetes mellitus, many medicinal plants have been reported to possess α-amylase and α-glucosidase inhibitory potential.[9,31]
Acarbose, the standard drug used in this study completely inhibited blood glucose rise when co-administered with starch (Table 1) and maltose (Table 2). At 200 mg/kg dose, the two aqueous extracts of *Anacardium occidentale and Piliostigma reticulatum* similarly showed a 100 % inhibition in blood glucose rise following their co-administration with the starch. The lower dose (100 mg/kg) of the extracts did not produce the same degree of inhibition in blood glucose concentration. Co-administration of the extracts 200 mg/kg body weight with maltose also repressed the rise in blood glucose to about half of the depression caused by acarbose. But this depressive effect was not observed at the lower dose of 100 mg/kg for both extracts and also when the extracts were co-administered with sucrose (Table 3).
Alpha-amylase and α-glucosidase inhibitions by plants extracts have been reported severally.[9,32,7] Phytochemicals implicated as anti-diabetic agents, do so possibly through α-amylase and glucosidase inhibition. The phytochemicals in question include flavonoids, saponins, tannins and terpenoids.[31,33,34] Phytochemicals screening of he leaves of *Anacardium occidentale* [35,36] and *Piliostigma reticulatum* [37] revealed the presence of saponins, tannins, flavonoids, alkaloids and terpenes. Polyphenolic compounds from plants are known to cause several effects on the biological systems which include enzymes inhibitions.[38,39] The phenolic compounds are known to be strong metal ion chelators and

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Table 1: Blood Glucose Concentration (mg/dL) at Different Time Points after Oral Administration of Aqueous Leaf Extracts of *Anacardium occidentale* and *Piliostigma reticulatum* on Starch Load in Wistar Rats.

	Time					
Groups	**0 min**	**30 mins**	**60 mins**	**90 mins**	**120 mins**	**180 mins**
DW	76.60 ± 5.44	86.20 ± 6.50	77.00 ± 3.44	72.40 ± 4.08	80.20 ± 3.83	72.20 ± 3.45
Starch + DW	86.20 ± 8.43	118.80 ± 6.70[a] (37.8%)	113.40 ± 9.40[a] (31.6%)	102.20 ± 11.65[a] (18.6%)	100.00 ± 9.37[a] (16.0%)	86.20 ± 11.11
Starch + Acarbose 100 mg/kg	102.60 ± 4.01	91.20 ± 3.73	86.80 ± 3.46[a]	90.80 ± 4.73	77.60 ± 4.20[a]	78.40 ± 4.18[a]
Starch + AOE 100 mg/kg	98.00 ± 4.63	132.60 ± 7.20[a] (35.3%)	132.20 ± 3.70[a] (34.9%)	127.00 ±7.80[a] (29.6%)	108.80 ± 8.90 (11.0%)	112.60 ± 6.20[a] (14.9%)
Starch + AOE 200 mg/kg	108.60 ± 3.07	108.00 ± 4.38	106.00 ± 5.64[a]	105.60 ± 3.94[a]	100.00 ± 3.06	89.60 ± 3.70[a]
Starch + PRE 100 mg/kg	90.60 ± 1.86	114.60 ± 11.80[a] (26.5%)	115.80 ± 7.60a (27.8%)	107.60 ± 6.73[a] (18.8%)	91.60 ± 12.95 (1.1%)	98.40 ± 3.04 (8.6%)
Starch + PRE 200 mg/kg	106.60 ± 4.31	105.80 ± 4.28	106.20 ± 4.20	102.60 ± 3.48	100.00 ± 3.38	93.60 ± 5.03

Values are expressed as mean ± SEM, n = 5. AOE = *Anacardium occidentale* extract; PRE = *Piliostigma reticulatum* extract; DW = Distilled water. Values in parenthesis are percentage increase in blood glucose concentrations compared to 0 min in the same group. Values with the superscript letter 'a' are significantly different from the zero (0) min value ($p < 0.05$).

Table 2: Blood Glucose Concentration (mg/dL) at Different Time Points after Oral Administration of Aqueous Leaf Extracts of *Anacardium occidentale* and *Piliostigma reticulatum* on Maltose Load in Wistar Rats.

	Time					
Groups	**0 min**	**30 mins**	**60 mins**	**90 mins**	**120 mins**	**180 mins**
DW	76.60 ± 5.44	86.20 ± 6.00	77.00 ± 3.44	72.40 ± 4.08	80.20 ± 3.83	72.20 ± 3.45
Maltose + DW	95.60 ± 4.42	123.20 ± 5.33[a] (28.9 %)	87.80 ± 13.79	85.40 ± 6.17	85.60 ± 4.88	82.00 ± 3.68
Maltose + Acarbose 100 mg/kg	104.20 ± 3.59	106.60 ± 2.11 (2.3 %)	104.40 ± 4.00	104.40 ± 2.90	101.40 ± 3.94	92.20 ± 2.85a
Maltose + AOE 100 mg/kg	92.60 ± 6.32	153.80 ± 21.29[a] (66.1%)	120.20 ± 6.68[a] (29.8%)	99.60 ± 2.73 (7.6%)	95.80 ± 2.26 (3.5%)	82.80 ± 4.67
Maltose + AOE 200 mg/kg	91.40 ± 2.08	108.60 ± 2.85[a] (18.8%)	101.20 ± 4.07[a] (10.71%)	94.60 ± 3.07 (3.5%)	88.40 ± 2.31	85.20 ± 2.00
Maltose + PRE 100 mg/kg	109.60 ± 4.89	170.40 ± 27.30[a] (55.5%)	149.80 ± 19.44[a]	135.60 ± 18.09[a] (36.7%)	116.80 ± 15.46 (6.6%)	97.40 ± 8.46
Maltose + PRE 200 mg/kg	103.00 ± 1.70	115.80 ± 6.36[a] (12.4%)	112.20 ± 3.87[a] (8.9%)	97.20 ± 11.65	95.60 ± 0.92	80.80 ± 43.40

Values are expressed as mean ± SEM, n = 5. AOE = *Anacardium occidentale* extract; PRE = *Piliostigma reticulatum* extract; DW = Distilled water. Values in parenthesis are percentage increase in blood glucose concentrations compared to 0 min in the same group. Values with the superscript letter 'a' are significantly different from the zero (0) min value ($p < 0.05$).

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Table 3: Blood Glucose Concentration (mg/dl) at Different Time Points after Oral Administration of Aqueous Leaf Extract of *Anacardium occidentale* and *Piliostigma reticulatum* on Sucrose Load in Wistar Rats.

	Time					
Groups	**0 min**	**30 mins**	**60 mins**	**90 mins**	**120 mins**	**180 mins**
DW	76.60 ± 5.44	86.20 ± 6.50	77.00 ± 3.44	72.40 ± 4.08	80.20 ± 3.83	72.20 ± 3.45
Sucrose + DW	89.00 ± 2.25	110.20 ± 4.70[a] (23.8%)	104.40 ± 4.06[a] (17.3%)	97.20 ±1.93 (9.2%)	97.00 ±2.38 (8.0%)	92.80 ± 4.14
Sucrose + Acarbose 100 mg/kg	97.60 ± 3.50	108.60 ± 3.75[a] (11.3%)	95.60 ± 2.40	78.80 ± 3.55[a]	93.40 ± 3.27	92.04 ± 4.91
Sucrose + AOE 100 mg/kg	93.20 ± 7.14	130.60 ± 4.05[a] (40.1%)	105.20 ± 4.30[a] (12.9%)	105.40 ± 3.07[a] (13.0%)	97.60 ± 3.35 (4.7%)	94.40 ± 3.33 (1.3%)
Sucrose + AOE 200 mg/kg	92.40 ± 5.06	133.00 ± 5.72[a] (43.9%)	121.60 ± 2.92[a] (31.6%)	107.20 ± 3.20[a] (16.0%)	111.40 ± 2.46[a] (20.6%)	105.60 ± 2.11[a] (14.3%)
Sucrose + PRE 100 mg/kg	89.00 ± 3.00	119.80 ± 4.80[a] (34.6%)	99.80 ± 2.40 (12.1%)	95.80 ± 2.80 (7.6%)	92.80±3.78 (4.3%)	91.40 ± 3.63 (2.7%)
Sucrose + PRE 200mg/kg	92.80 ± 5.06	133.00 ± 5.72[a] (43.3%)	121.60 ± 2.92[a] (31.0%)	107.20 ± 3.20[a] (15.5%)	111.40 ± 2.46[a] (20.0%)	105.60 ± 2.11[a] (13.8%)

Values are expressed as mean ± SEM, n = 5. AOE = *Anacardium occidentale* extract; PRE = *Piliostigma reticulatum* extract; DW = Distilled water. Values in parenthesis are percentage increase in blood glucose concentrations compared to 0 min in the same group. Values with the superscript letter 'a' are significantly different from the zero (0) min value ($p < 0.05$).

protein precipitation agents forming insoluble complexes with proteins as well as acting as biological oxidants.[32] The presence of the polyphenolic compounds in the extracts of the two plants used in the study may suggests that their inhibitory potential on α-amylase and the membrane-bound intestinal α-glucosidase enzymes may be similar to the mechanism proposed generally for the polyphenolic compounds. However, further studies are necessary to confirm the mechanism(s) by which the extracts exhibit their blood glucose reducing effect.

Conclusion

In conclusion, the results of this study suggests that antidiabtetic potential of the leaves of *Anacardium occidentale* and *Piliostigma reticulatum* may be associated with α-amylase and α-glucosidase inhibition.

Conflict of Interest

The authors declare that there is no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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