

Evaluation of inhibitory Effect of *Solenostemon monostachyus* on alpha amylase and alpha glucosidase enzymes of rats

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ABSTRACT

Background: *Solenostemon monostachyus* (P. Beauv.) Brig. (Lamiaceae) 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.

Methods: The leaf extract (75, 150, 225 mg/kg) of *Solenostemon monostachyus* 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.

Results: The leaf extract caused significant ($p < 0.05$) and non-dose-dependent reduction in blood glucose levels of treated rats with the various substrates used.

Conclusion: The results suggest that the leaf extract of *Solenostemon monostachyus* have the potentials to inhibit alpha amylase and glucosidase in rats.

Keywords: *Solenostemon monostachyus*, alpha amylase, alpha glucosidase, diabetes.

1. INTRODUCTION

Solenostemon monostachyus P. Beauv (Lamiaceae), a medicinal plant, is well distributed in West and Central Africa. It is an annual succulent weed, which can grow up to 100 cm tall [1]. The aerial parts of the plant are used ethnomedicinally by the Ibibios of the Niger Delta of Nigeria to treat stomach ulcer, fever/malaria [2,3], hemorrhoid, and other inflammatory diseases. Phytoconstituents such as diterpenoids [4], flavonoids, coumarin, and polyphenol [5] have been isolated from the leaves. Compounds such as b-pinene, oct-1-en-3-ol, b caryophyllene, octan-3-ol, and (E,E)-a-farnesene have been identified in the essential oils from the leaves [1]. Biological activities such as antioxidant [5,6,7], antihypertensive [8], antimicrobial [9], antiulcer [10], antidiabetic and hypolipidemic [11,12], antipyretic and antimalarial [13], antiinflammatory and antinociceptive [14], hepatoprotective and nephroprotective [15], antidepressant [16] have been reported on the leaves. Inhibitory potentials of leaf extract of *Solenostemon monostachyus* on alpha amylase and alpha glucosidase activities in rats was evaluated in this study.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Equipment

The materials used include; metallic cannula, weighing balance (Ohaus, USA) glucometer and strips (Accu-answer, UK).

2.1.2 Chemicals and reagents

The chemicals and reagents used include; Acarbose (Sigma-Aldrich USA; standard drug) Starch, sucrose, maltose (Sigma- Aldrich , USA), Ethanol (Sigma-Aldrich, USA).

2.1.3 Biological Materials

Albino wistar rats (120 -135 g) of either sex were used for these experiments. The animals were housed in standard cages and were maintained on a standard pelleted feed (Guinea feed) and water *ad libitum*.

2.2 Methods

2.2.1 Plants collection and identification

The plant material *Solenostemon monostachyus* (leaves) were collected in compounds in Uruan area, Akwa Ibom State, Nigeria in January 2022. The plant was identified and authenticated by a taxonomist in Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria.

2.2.2 Extraction

The leaves were washed and shade-dried for two weeks. The dried plants' materials were further chopped into small pieces and reduced to powder using electric grinder. The powdered leaves material (1.5 kg) was macerated for 72 h in 50% ethanol. This was thereafter filtered and the liquid filtrate was concentrated and evaporated to dryness in vacuo 40°C using a rotary evaporator (BuchiLab, Switzerland). The extract was stored in a refrigerator at -4°C, until used for the proposed experiments.

2.2.3 In vivo alpha-amylase and glucosidase inhibition study

2.2.3.1 Alpha-Amylase inhibitory study

Thirty-five Wistar rats were divided into 7 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 *Solenostemon monostachyus* leaf extract at 75, 150 and 225 mg/kg respectively. All administrations were done orally and blood glucose concentration was monitored at 30, 60, 90, 120 and 180 min [17]. The blood glucose level was used to assess the effect of the extract on the enzyme activity

2.2.3.2 Glucosidase inhibitory study

The procedure as described above was used for this study but with sucrose and maltose used as substrates [17].

2.2.3.3 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-check, 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 [18].

2.3 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, (San Diego, USA). Differences between means were considered significant at 5% level of significance ie $p \leq 0.05$.

3. RESULTS

Table 1: Effect of ethanol leaf extract of *S. monostachyus* on Blood Glucose Level of rat after oral administration of starch load

TREATMENT	DOSE mg/kg	BLOOD GLUCOSE LEVEL mg/dL IN MIN					
		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±5.45a(63.18)	115.66±1.33a(57.72)	104.66±2.60a(42.72)	95.66±3.75a (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	75	79.66±5.36	92.33±16.81(15.90)	92.66±11.28(16.31)	86.66±1.45(8.78)	83.33±5.20(4.60)	73.33±4.17(0)
	150	83.66±3.38	103.6±12.33(23.83)	88.0±4.50(5.18)	83.00±7.20(0)	77.00±6.11(0)	83.33±2.35(0)
	225	94.38±1.76	104.0±19.34(9.62)	111.33±5.81(17.95)	95.66±9.20(1.35)	85.66±12.37(0)	83.0±3.21(0)

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.

Table 2: Effect of ethanol leaf extract of *S. monostachyus* on Blood Glucose Level of rat after oral administration of sucrose load

TREATMENT	DOSE mg/kg	BLOOD GLUCOSE LEVEL mg/dL IN MIN					
		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±2.90b(46.01)	128.66±5.45a (39.84)	117.33±4.66a(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	75	84.66±2.40	100.0±5.50(18.11)	86.66±3.33(2.36)	84.66±7.05(0)	81.33±5.19	81.0±4.58
	150	95.0±2.08	88.66±5.78(0)	82.00±0.57(0)	74.66±2.72(0)	71.0±2.08(0)	77.33±6.33(0)
	225	95.00±5.50	104.36±6.36(9.85)	94.66±3.18(0)	90.66±3.38(0)	87.0±4.04(0)	72.66±5.78(0)

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.

Table 3: Effect of ethanol leaf extract of *S. monostachyus* on Blood Glucose Level of rat after oral administration of maltose load

TREATMENT	DOSE mg/kg	BLOOD GLUCOSE LEVEL mg/dL IN MIN					
		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±1.90b(60.78)	130.22±2.45(58.22)	120.66±3.22a(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±2.20c(0.77)	85.33±2.15c(0)	84.26±1.14a(0)	82.28±2.26a(0)
Extract	75	84.33±12.99	120.33±7.17(42.68)	109.33±5.48a(29.64)	98.66±4.84 a(16.99)	96.33±6.06b(14.22)	79.33±3.84(0)
	150	86.0±3.46	124.0±3.78b(44.18)	117.66±1.85(36.81)	109.66±5.78(27.51)	98.66±9.17a(14.72)	93.00±6.50(8.13)
	225	86.66±18.65	123.0±12.34(41.93)	117.0±13.65 b(35.01)	109.33±18.26b (26.15)	95.0±16.05 b(9.62)	80.66±2.82c(0)

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.

3.1 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 concentrations of the treated animals after 30 mins. The percentages were starch (63.18%), extract-treated groups (9.92 - 23.83 %) and acarbose-treated group (17.97%). These increases were reduced after 60 min in groups treated with 60 mg/kg (5.18 %), 30 mg/kg (16.31%), 90 mg/kg (17.95%) of the extract respectively. At 90 min, BGL increments of 0%, 1.35% and 8.78% were recorded for 150, 225 and 75 mg/kg of the extract respectively. All the extract-treated groups had their BGL reduced to normal level without any further increase at 180 min. Also, 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. BGL increment of 9.85 and 18.11 % were also recorded in groups treated with 75 and 225 mg/kg of extract, while the group treated

with 150 mg/kg had no increment in BGL. The blood glucose concentrations were reduced to normal in 150 and 225 mg/kg treated groups after 60 mins post-administration of sucrose. Group treated with 75 mg/kg had 2.36% increment in BGL. There was no increment in BGL of all the extract-treated groups from 90 -180 min (Table 2). There was 60.78% increase in blood glucose concentration 30 min following maltose administration in the control group. However, 41.93 - 44.18 % increases were observed in the extract-treated groups. At 60 min, the BGL levels of the extract-treated groups were significantly reduced with groups treated with 75, 150, and 225 mg/kg having percentage increments in BGL ranging from 29.64 -35.01%. These reductions were sustained and significant throughout the duration of the study with only the group treated with 150 mg/kg of the extract recording 8.13% increment in BGL at 180 min (Table 3).

4. DISCUSSION

Solenostemon monostachyus parts are used in Ibibio traditional medicine in the treatment of diseases such as diabetes among others. This work investigated the effect of *S. monostachyus* leaf extract on alpha amylase and alpha glucosidase activities in rats. The leaf extract was found to inhibit increases in blood glucose concentration following starch administration independent of the dose. 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 [19,20]. 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 [21,22]. Similarly, the leaf 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 leaf extract and further suggest this activity to be one of the mechanisms of antidiabetic action of the plant. The inhibitory activities of plant extracts are linked to their phytochemical constituents especially polyphenols. The leaf of *S. monostachyus* have been reported to be rich in phytoconstituents such as diterpenoids [4], flavonoids, coumarin, polyphenol[5,23]. Mve-Mba et al.[1], reported that the leaf essential oil of *Solenostemon monostachyus* contains β -pinene, oct-1-en-3-ol, β -caryophyllene, octan-3-ol and (E, E)- α -fanesene. These compounds have been variously reported to inhibit alpha glucosidase and alpha amylase activities [24,25,26]. Moreso, monoterpenes which richly found in the leaf essential oil of this plant similarly have been reported to inhibit alpha amylase and alpha glucosidase [27]. Coumarins in particular have been reported to inhibit alpha glucosidase and alpha amylase activities [28,29].The presence of these compounds in the leaf extract could have contributed to the observed activity of this study and therefore explains the antidiabetic mechanism of the leaves of *S. monostachyus*. Alpha-amylase and α -glucosidase inhibitions by plants extracts have been reported severally [30,31]. Phytochemicals implicated as anti-diabetic agents, do so possibly through α -amylase and glucosidase inhibition. The phytochemicals implicated include; flavonoids, saponins, tannins and terpenoids [30,32,33]. Also, polyphenolic compounds from plants are known to cause several effects on the biological systems which include enzymes inhibitions [34,35]. 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 [30]. The presence of the polyphenolic compounds in the leaf extract in addition to the monoterpenes may suggests that their inhibitory potential on α -amylase and the membrane-bound intestinal α -glucosidase enzymes.

5. 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 fractions of *Solenostemon monostachyus* which can be attributed to the activities of its phytochemical constituents.

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Conflict of Interest

The authors declare that there are no conflicts of interest related to this article

Author's contribution

JEO, EIE,MOA, - Research concept and design; LOA, MOA- Animal studies,JEO, Data analysis and interpretation; JEO,MOA, EIE- Writing the article.

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