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Short Communication

Phytochemical Screening of *Solenostemon monostachyus* and the Effect of Extract and Fractions on Castor Oil-Induced Diarrhoea in Rats

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ABSTRACT

Solenostemon monostachyus P. Beauv (Lamiaceae) is among plants reportedly used in West and Central African ethnomedicine for the treatment and management of diabetes, malaria, hypertension and sickle cell anaemia. The effect of ethanol leaves extract and fractions (chloroform and aqueous) from *Solenostemon monostachyus* on castor oil-induced diarrhoea was studied in rats. The extract (75-225 mg/kg) and fractions (150 mg/kg) were evaluated for their effect on experimentally induced diarrhoea using different models; castor oil-induced diarrhoea, castor oil-induced fluid accumulation and small intestinal propulsion. The extract inhibited significantly ($p < 0.05$) diarrhoea induced by castor oil in a non-dose-dependent fashion. The extract (150 mg/kg) and aqueous fraction (150 mg/kg) showed considerably higher inhibition of castor oil-induced diarrhoea, small intestine transit time and castor oil induced fluid accumulation *in vivo* although the effect was not comparable to the standard drugs, Loperamide and Atropine. Results of phytochemical screening showed the presence of alkaloids, saponins, tannins and cardiac glycosides. The observed antidiarrhoeal property of the ethanol leaves extract of the plant may be linked to the presence of these phytoconstituents.

***Keywords*:** *Solenonstemon monostachyus*, Antidiarrhoea, Castor oil-induced, Phytochemical.

Introduction

Diarrhoea is a leading cause of malnutrition, and a second leading cause of death of children under five years globally.[1] The World Health Organisation (WHO) has estimated that about 1.7 billion cases of diarrhoea occur annually in developing countries with death of over half a million children yearly.[1,2] Considerable progress in the treatment of diarrhoea have met setbacks, therefore necessitating the search for therapeutic agents of plant origin. Medicinal plants have largely been utilised recently because of the significant physiological response they exert on biological systems. They have served either as sources for the development of novel drug compounds or for the application of its phytomedicinal properties for treatment of various diseases and ailments.[3] In Africa for example, the utilisation of these plants for medicine and for food is a source of relief since earnings and income of the populace are poor and meagre. *Solenostemon monostachyus* P. Beauv (Lamiaceae) is among plants reportedly used in West and Central African ethnomedicine for the treatment and management of various health problems.[4] It occurs as an annual weed in anthropogenic habitats and rocky savannahs. It is slightly succulent, aromatic, and has been reported to reach a height of 100 cm.[5] The aerial parts of the plant have so far been made into decoctions and utilised traditionally by the Ibibios of the South East Nigeria for the treatment of stomach ulcer, fever/malaria,haemorrhoid, and other inflammatory diseases.[6,7] The plant decoction has also showed antihypertensive and antidiuretic potential.[8] The plant has been reported to possess antioxidant,[9,10] antihypertensive,[11] antimicrobial,[12] antiulcer,[13] antidiabetic and hypolipidemic,[14] antipyretic and antimalarial,[15] anti-inflammatory and antinociceptive,[16] hepatoprotective and nephroprotective[17] and antidepressant properties.[18] Phytoconstituents including diterpenoids,[19] flavonoids, coumarin, and polyphenol[9] have so far been isolated. The leaf essential oil contains *β*-pinene, oct-1-en-3-ol, β caryophyllene, octan-3-ol, and (*E, E*)-α-farnesene.[5] The study report, antidiarrhoeal potential of *S. monostachyus* to provide scientific basis for its use in ethnomedicine for diarrhoeal conditions.

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Materials and Methods

Plant materials

Leaves of the plant were harvested from a forest in Akwa Ibom State, Nigeria, on the 24th October 2014. The plant was identified and authenticated in the Department of Botany, University of Uyo, Uyo. Nigeria. Herbarium specimen (FPUU 573) was deposited at Faculty of Pharmacy Herbarium. The plant was dried on laboratory table for 2 weeks and reduced to powder. The powder (700g) was macerated in 95% ethanol (300ml) for 72 hours. This was further partitioned using chloroform to obtain the chloroform and aqueous extracts. The liquid filtrates obtained were concentrated *in vacuo* at 40°C. The extracts were stored in a refrigerator until used for experiments.

Phytochemical analyses

Preliminary phytochemical analysis for the determination of Alkaloids, Flavonoids, Tannins, Anthraquinone, Deoxy-sugar, Cardiac glycosides, Terpenes and Saponin was conducted according to methods described by Harborne; Trease and Evans.[20, 21]

Animals

Wistar rats (105-165g) of either sex were obtained from the University of Uyo animal house. They were maintained on standard animal pellets and water *ad libitum*. The approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo (UU/CS/AE/14/63).

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Castor oil induced diarrhoea.

Diarrhoea was induced in rats using a modified method described by Sunil *et al* and Nwafor *et al*. [22, 23] Animals were fasted for 24 h but allowed free access to water. They were randomly allotted into five groups of six rats each. Group 1 (control) received 10% Tween 80 (5 mL/kg, p.o.), Groups 2-4 were treated with *S. monostachyus* ethanol extract (75,150 and 225 mg/kg, p.o. respectively); Group 5 and 6 were treated with chloroform and aqueous extracts (150 mg/kg p.o. each) respectively. Group 7 was treated with atropine (0.1 mg/kg,i.p). After 1h, each rat received 2 mL of castor oil (p.o.) and was then observed for consistency of faecal matter and frequency of defecation for 3h.

Small intestinal propulsion

The effect of the extract on intestinal propulsion in rats was tested using a charcoal meal method.[23] Animals were fasted for 24 h but allowed free access to water only. They were randomly allocated into six groups of six rats each. Group 1 (control) received 10% Tween 80 (5 mL/kg, p.o.); groups 2-5 were treated with *S. monostachyus* extract (75-225 mg/kg, p.o. respectively); Group 6 received atropine (0.1mg/kg, i.p). After 1h, each rat was administered 1ml charcoal meal (5% activated charcoal suspended in 10% aqueous tragacanth) orally. The animals were sacrificed 30 min later by cervical dislocation and bled, and the small intestines were rapidly dissected and placed on a clean surface. The small intestine of each animal was carefully inspected, and the distance traversed by the charcoal meal from the pylorus was measured. The length of the whole small intestine was also measured. The distance traversed by the charcoal meal from the pylorus was expressed as a percentage of the distance from the pylorus to the ileocaecal junction.

Effect of S. monostachyus extract on castor oil induced fluid accumulation in rats

Castor oil induced fluid accumulation was conducted in rats according to a previously described method.[24] Animals were deprived of food for 24 h but allowed free access to water. They were randomly allocated into eight groups of six rats each. Group 1 (control) received 10% Tween 80 (5 mL/kg, p.o.), Group 2 received castor oil (2mL/rat), Groups 3-5 were administered with the extract (75, 150 or 225 mg/kg, p.o), Group 6 received chloroform fraction (150 mg/kg, p.o.), group 7 was administered aqueous fraction (150 mg/kg, p.o.) and group 8, loperamide (3.0 mg/kg, p.o). After 1h, each rat received 2mL castor oil (p.o.), 30 minutes later, the rats were sacrificed by cervical dislocation and exsanguinated, and the small intestine of each rat was ligated at both pyloric sphincter and at the ileocaecal junctions. The entire small intestine was dissected, its contents were expelled into a graduated measuring cylinder and the volume of the contents recorded.

Statistical analysis

The data were statistically analysed with one-way ANOVA, followed by Tukey-Kramer multiple comparison post-test. Values of $p < 0.001$ were considered significant.

Results and Discussion

The results of the percentage weight of *S. monostachyus* leaves extract were as follows; ethanol extract (2.5%), chloroform fraction (0.9%) and aqueous (0.7%) (Table 1). The phytochemical screening of the ethanol extract revealed the presence of alkaloids, saponins, tannins, and cardiac glycosides. Saponin and cardiac glycosides were found in the chloroform fraction (Table 2).

Antidiarrhoeal activity of extract and fractions from *Solenonstemon monostachyus* was assessed by castor oil-induced diarrhoea in rats. Ethanol extract, aqueous and chloroform fractions (75-225 mg/kg) exhibited antidiarrhoeal effects on experimentally induced diarrhoeal rats as observed in this study. The extract reduced the castor oil-induced diarrhoea significantly ($p<0.05$-0.01) in a non-dose-dependent fashion. Exhibition of the inhibitory activity at the dose of 150 and 225 mg/kg body weight were 74.62 and 50.00% respectively, while atropine (0.1 mg/kg body weight) showed 80.03% inhibition. The aqueous fraction (150 mg/ kg) was more potent (47.14%) than the chloroform fraction (24.92%) (Table 3). *Solenostemon monostachyus* leaves extract and

fractions also inhibited the intestinal propulsion of the rats in a non-dose dependent manner with the extract (150 mg/kg) exerting an inhibition of 59.33%, followed by aqueous fraction (150 mg/kg) of 54.32%. The activity of the extract and fractions were not comparable to that of atropine (0.1 mg/kg body weight) (Table 4).

Castor oil-induced intestinal fluid accumulation was also assessed in the rats. The extract (75 -225 mg/kg) demonstrated a significant ($p <$ 0.01-0.001) reduction in the intestinal fluid accumulation with inhibition, though non dose-dependent, being comparable to that of loperamide (3.0 mg/kg body weight). However, the aqueous fraction produced the highest reduction (70.33%) at the dose of 150 mg/kg body weight, which, was more than that of the standard antidiarrhoeal agent Loperamide (3.0 mg/kg) of 60.16% (Table 5). Castor oil induces diarrhoea due to active ingredient, ricinoleic acid, which is liberated as a resulting action of lipases on castor oil. Consequently, causing peristaltic activity in the small intestine, and leading to changes in the electrolyte permeability of the intestinal mucosa that is being stimulated. It also stimulates the release of endogenous prostaglandins.[25,26] Castor oil has been reported to cause secretory and motility diarrhoea.[27] Inhibitors of prostaglandin synthesis are known to delay diarrhoea induced with castor oil.[22] The leaves extract and fractions from *S. monostachyus* may have caused the inhibition of prostaglandin synthesis in the tested rodents thereby leading to inhibition of diarrhoea. It has been reported that plant phytochemical constituents are capable of inhibiting small intestinal movement through mechanism such as antagonism of α-2-adrenoceptor stimulation.[28,29] Plant bioactive substances have been reported to possess a broad spectrum of pharmacological activities with curative effects.[30,31,32] Availability of flavonoid, tannins and phenols for example, in plant is an indication that the plant may have a significant pharmacological property that can alter the reaction in living systems to allergies, ulcers, anti-inflammatory, antimicrobial, antidiarrhoeal and antidysentery activities.[33,34] Havagiray[35] reported antidiarrhoeal and antidysentery properties of medicinal plants as a result of the presence of tannins, alkaloids, saponins, flavonoids, steroid. The phytochemical constituents in the leaves extract and fractions of *S. monostachyus* may be responsible for the observed *in vivo* antidiarrhoeal activity of the plant.

Table 1: Extraction of *Solenonstemon monostachyus.*

S/N	Extract	Weight of extract (g)	Percentage yield (%)
1.	Ethanol	17.41	2.5
2.	Chloroform	6.06	0.9
3	Aqueous	4.80	0.7

Yield (%) were calculated from triplicate measurement

Table 2: Phytochemical screening of extracts

S/N	Phytochemicals	Ethanol	Chloroform
1.	Alkaloids	+	-
2.	Saponin	+	+
3.	Flavonoids	-	-
4.	Tannins	+	-
5.	Terpenes	-	-
6.	Cardiac glycosides	+	+
7.	Anthraquinones	-	-
8.	Deoxy sugar	-	-

+ Present; -Absent

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Table 3: Effect of *S. monostachyus* extract on castor oil induced diarrhoea in rats

Treatment	Dose (mg/kg)	Onset of diarrhoea (min)	Mean faecal matter	% inhibition
Control (10% Tween 80)	-	28.40 ± 3.93	6.66 ± 0.15	-
S. monostachyus	75	46.49 ± 2.46	6.00 ± 1.00	9.90
	150	54.30 ± 2.70	1.69 ± 0.66**	74.62
	225	30.26 ± 3.92	3.33 ± 0.20*	50.00
Chloroform fraction	150	45.31 ± 3.92	5.00 ± 0.57	24.92
Aqueous fraction	150	44.42 ± 3.37	3.53 ± 0.36*	47.14
Atropine	0.1	126.0 ± 2.53	1.33 ± 0.50**	80.03

Data were expressed as mean ± SEM significant at *p < 0.01; **p < 0.001 when compared to control, n = 6.

Table 4: Effect of *S. monostachyus* extract on small intestinal propulsion in rats

Treatment	Dose (mg/kg)	Intestinal Transit %
Control (10% Tween 80)	-	74.23 ± 4.36
S. monostachyus	75	70.0 ± 4.36
	150	65.30 ± 3.97
	225	59.33 ± 3.84*
Chloroform fraction	150	63.33 ± 2.45
Aqueous fraction	150	54.32 ± 2.33*
Atropine	0.1	36.30 ± 2.48**

Data were expressed as mean ± SEM significant at *p < 0.01; **p < 0.001 when compared to control, n = 6.

Table 5: Effect of *S. monostachyus* extract on castor oil induced fluid accumulation in rats

Treatment	Dose (mg/kg)	Mean volume of intestinal fluid (mL)	% Reduction
Control (10% Tween 80)	-	1.18 ± 0.52	-
Castor oil	-	4.82 ± 0.24	-
S. monostachyus	75	2.34 ± 0.35	51.45
	150	1.88 ± 1.04	60.99
	225	2.10 ± 1.14	56.43
Chloroform fraction	150	2.50 ± 1.64	48.13
Aqueous fraction	150	1.43 ± 0.22*	70.33
Loperamide	3.0	1.92 ± 0.18**	60.16

Data were expressed as mean ± SEM significant at *p < 0.01;**p < 0.001 when compared to control, n = 6.

Conclusion

The results of the study show that the ethanol leaves extract and fractions of *S. monostachyus* possess antidiarrhoeal properties which justify the ethnobotanical uses of this plant for the treatment of diarrhoea.

Conflict of interest

The authors declare 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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