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Antidiarrheal activity of aqueous ethanol extract of *Cyperus esculentus* tuber in albino rats

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ABSTRACT

Cyperus esculentus is used in the treatment of diarrhea in traditional medicine. Antidiarrheal activity of aqueous ethanol extract of *C. esculentus* tuber was investigated in albino rats with castor oil induced and charcoal meal assays. Acute toxicity and preliminary phytochemical constituents were determined. Fifty albino rats were divided into five groups of five animals each for each assay. The extract was administered at doses of 250, 500, and 1,000 mg/kg, loperamide at 2 mg/kg, and atropine at 0.1 mg/kg (positive control groups), while 1% tracaganth mucilage was given to negative control group. The LD50 was above 5,000 mg/kg. Phytochemical evaluation indicated the presence of steroids, carbohydrates, alkaloids, and saponins. A non-statistically significant ($p > 0.05$) decrease in mean weight of wet feces (1.71 ± 1.23 g, 1.75 ± 0.77 g) and mean frequency of watery defecation (1.6 ± 1.03, 1.8 ± 0.97) at 500 and 1,000 mg/kg extract doses. Percentage inhibition of defecation was 46.7% and 40% at 500 and 1,000 mg/kg doses of the extract, respectively, while that of loperamide was 46.7% relative to the negative control in castor oil induced test. The findings have shown that the aqueous ethanol extract of *C. esculentus* tuber seems to possess anti-secretory effect but does not have anti-motility effect.

1. INTRODUCTION

Diarrhea is the passage of three or more loose or liquid stools per day, or more frequent passage than is normal for an individual occurring when various factors interfere with the normal intestinal physiology, resulting in decreased absorption, increased secretion of fluid and electrolytes, or increased bowel motility. The most severe threat posed by diarrhea is dehydration [1] occurring when the losses in fluid and electrolytes are not replaced.Plants have long been a very important source of new drugs in the management of several conditions, including diarrhea with approximately 25% of drugs derived from them [2]. In many parts of Africa, medicinal plants are the most easily accessible health resource available to the community [3]. Traditionally, people consider them efficacious against disorders without a scientific basis [3]. In addition, they are most often preferred option for the patients [3] as antidiarrheal medications in contemporary medicine may present with adverse effects and microbial resistance tends to develop in the use of antibiotics. *Cyperus esculentus* (Tiger nut) is an annual or perennial plant, a species of sedge, native to warm temperate to subtropical regions of the Northern Hemisphere, belonging to the family Cyperaceae [4]. It is cultivated in Western Africa [4] and popularly consumed by humans. It possesses several medicinal properties, such as anti-inflammatory [5], hepatoprotective [6], antioxidant [7], antibacterial [5], antifungal [5], and anticonvulsant [5]. In Ayurvedic medicine, it is used in the treatment of flatulence, indigestion, colic, diarrhea, dysentery, debility, and excessive thirst [8]. There has been no previous scientific evaluation of its antidiarrheal activity. Hence, this study aims to investigate the antidiarrheal activity of *C. esculentus* tuber.

2. MATERIALS AND METHODS

2.1. Collection of the Plant Materials

The dried tubers of *C. esculentus* (Tiger nut) were purchased from Sangana market, Diobu in Port Harcourt city, Rivers State and were authenticated by Dr. Ekeke Chimezie of the Department of Plant Science and Biotechnology, University of Port Harcourt. A voucher specimen assigned with UPH/C/103 was deposited in the herbarium of the above named department.

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2.2. Plant Extract Preparation

The dried tubers were cleaned and pulverized using a mechanical grinder. The powdered tuber (3.83 kg) of *C. esculentus* was subjected to solvent extraction for 72 hours with 9.2 l of 50% ethanol. The extract was concentrated using a rotary evaporator, carefully evaporated to dryness over a water bath at 45°C and then placed in a desiccator using silica gel. The percentage yield was determined and the dried extract was stored in a refrigerator.

2.3. Animals Used

Sixty-eight albino rats with average weight of 195 g were made use of in this study. They were kept in plastic cages with wire mesh under normal room temperature in the Department of Experimental Pharmacology and Toxicology laboratory and provided with food and water *ad libitum*.

2.4. Drugs and Chemicals Used

Absolute ethanol, Diethyl ether (JHD Guangdong Guanghua Sci-Tech.Co. Ltd. Shantou, Guangdong, China); Castor oil B.P. (Bell, Sons & Co Druggists Limited, United Kingdom); Loperamide tablet, 2 mg (Imodium, Janssen Pharmaceuticals, Belgium); Atropine sulfate injection USP 0.6 mg/ml; Tragacanth gum; Activated charcoal.

2.5. Qualitative Phytochemical Analysis

Phytochemical analysis was done according to established protocols as described by Harborne [9].

2.6. Acute Toxicity Study

Acute toxicity of the tuber of *C. esculentus* was determined using 18 albino rats. The rats were divided into six groups of three animals each. The first three groups were treated with extract doses of 10, 100, and 1,000 mg/kg aqueous ethanol extract of *C. esculentus* respectively. The animals were under observation for 24 hours for signs of mortality or morbidity or death. The animals showed no sign of adverse effects after the period of observation. Doses of 1,600, 2,900, and 5,000 mg/kg were then administered to the remaining three groups and kept under observation for another 24 hours [10].

2.7. Castor Oil-Induced Diarrhea

Twenty-five rats were used in this experiment which did not have access to feed for 18 hours. They were randomized into five groups of five animals each. Group A animals were administered with 0.1 ml of aqueous 1% tragacanth suspension, group B animals received loperamide (2 mg/kg), while groups C, D, and E were given the three test doses (250, 500, and 1,000 mg/kg) of the extract, respectively, through the oral route with the aid of a cannula. Each animal was then treated with 1 ml of castor oil orally after a period of 1 hour.

After 1 hour, all groups received 1 ml of castor oil each orally. The animals were then placed in cages lined with Whatmann filter papers and observed for characteristic diarrheal droppings for 4 hours.

The number of defecation, weight of paper before and after defecation were noted [11]. The activity was expressed as % inhibition of defecation.

% inhibition of defecation = $[(A-B)/A] \times 100$

A = Mean number of watery diarrhea of negative control.

B = Mean number of watery diarrhea of drug/ extract.

2.8. Charcoal Meal Test

Twenty-five albino rats that did not have access to feed for 18 hours prior to the experiment were used. The animals were allotted into groups of five animals per group. The control group received 0.1 ml aqueous 1% tragacanth suspension while the second group was administered with 0.1 mg/kg atropine (reference drug) intraperitoneally. Animals in groups C, D, and E were orally administered 250, 500, and 1,000 mg/kg of the extract, respectively. Each group was later given 1 ml of 10% activated charcoal suspended in 10% aqueous tragacanth powder through the oral route, 30 minutes after the treatment. Animals were euthanized 30 minutes after charcoal meal administration by diethyl ether anesthesia. The abdomen was incised, and the small intestine carefully removed. The distance travelled by the charcoal plug from pylorus to caecum and the total length of the intestine was then measured [11]. The percentage transit and percentage of inhibition were calculated by using the following formula:

$$\text{Percentage transit} = \frac{\text{Distance travelled by charcoal meal}}{\text{Total length of intestine}} \times 100$$

Percentage intestinal inhibition = $(T_0 - T_1/T_0) \times 100$

T_0 = Mean total length of intestine

T_1 = Mean distance travelled by charcoal in intestine.

2.9. Statistical Analysis

The results are presented as mean ± standard error of mean (SEM). The one-way analysis of variance test with Dunnett's post hoc test was used to analyze and compare the data using IBM SPSS version 21 software, while $p < 0.05$–0.001 was taken as statistically significant**.**

3. RESULTS

3.1. Preliminary Qualitative Phytochemical Analysis

Preliminary qualitative phytochemical evaluation of the aqueous ethanol extract of *C. esculentus* showed the presence of alkaloids, steroids, cardiac glycosides, carbohydrate, and saponins. However, flavonoids, tannins, anthraquinones, and phlobatannins were observed to be absent in this extract.

3.2 Acute Toxicity Study

The result of oral acute toxicity studies showed that the LD_{50} of the aqueous ethanol extract of *C. esculentus* tuber was above 5,000 mg/kg body weight. However, itching was observed at 1,000 mg/kg, sedation at 2,900 mg/kg and 5,000 mg/kg, and piloerection (standing fur) at 5,000 mg/kg.

Table 1. Effect of aqueous ethanol extract of *C. esculentus* tuber on castor oil induced diarrhea (Mean ± SEM).

Treatment	Dose (mg/kg)	Mean weight of wet feces (g)	Mean no. of watery diarrhea	Inhibition of defecation (%)
1% tragacanth	1 ml	2.07 ± 0.39	3 ± 0.91	0
Loperamide	2	1.34 ± 0.56	1.6 ± 0.68	46.7
Extract	250	3.69 ± 0.65	3 ± 0.32	0
Extract	500	1.71 ± 1.23	1.6 ± 1.03	46.7
Extract	1,000	1.75 ± 0.77	1.8 ± 0.97	40

Values are expressed as mean ± SEM ($n = 5$).

*$p < 0.05$, when compared to the control.

Table 2. Effect of aqueous ethanol extract of *C. esculentus* tuber on intestinal transit of charcoal meal (Mean ± SEM).

Treatment	Dose (mg/kg)	Mean total length of intestine (cm)	Mean distance travelled by charcoal (cm)	% Intestinal transit	% Intestinal inhibition
1% tragacanth	0.1 ml	87.80 ± 9.88	61.94 ± 4.94	72.5 ± 4.78	29.45
Atropine	0.1	100.74 ± 9.54	46.04 ± 6.56	46.38 ± 4.35	54.3
Extract	250	91.50 ± 3.47***	70.32 ± 4.19***	76.89 ± 2.41***	23.15
Extract	500	96.90 ± 5.50***	67.92 ± 3.78***	70.68 ± 4.70***	29.9
Extract	1,000	105.80 ± 2.00***	83.62 ± 4.04***	79.11 ± 4.0***	20.96

Values are expressed as mean ± SEM ($n = 5$).

***$p < 0.001$, when compared to the control.

3.3. Effect of the Extract on Castor Oil Induced Diarrhoea

The mean weight of wet feces showed a non-statistically significant ($p > 0.05$) reduction in the (1.71 ± 1.23 g, 1.75 ± 0.77 g) and mean frequency of watery defecation (1.6 ± 1.03, 1.8 ± 0.97) at extract doses of 500 and 1,000 mg/kg, respectively, when compared to the controls. Percentage inhibition of defecation was 46.7% and 40% at 500 and 1,000 mg/kg doses of the C. *esculentus*, respectively, while loperamide had 46.7% relative to the negative control group. The extract at 250 mg/kg showed no reduction in the mean weight of wet feces, frequency of watery defecation, and percentage inhibition when compared to the negative control (Table 1).

3.4. Effect of the Extract on Intestinal Transit of Charcoal Meal

Cyperus esculentus at the three test doses gave a statistically significant ($p < 0.001$) difference in gastrointestinal movement of charcoal meal, (70.32 ± 4.19, 67.92 ± 3.78, and 83.62 ± 4.04) cm, and % intestinal transit (76.89 ± 2.41%, 70.68 ± 4.70% and 79.11 ± 4.0%), respectively compared to the atropine (0.1 mg/kg). At these same doses, % intestinal transit was non-statistically significant ($p > 0.05$) compared to the negative control. Atropine showed a significant reduction in the gastrointestinal movement of charcoal meal, the lowest percentage intestinal transit and the highest percentage of intestinal inhibition (54%) of charcoal meal relative to all the extract treated groups of (23.15%, 29.9%, and 20.96%), respectively (Table 2).

4. DISCUSSION

Quite a number of antidiarrheal agents act by decreasing either the gastrointestinal motility and/or the secretions. The acute toxicity profile of the extract indicates that it is relatively safe with an LD_{50} above 5,000 mg/kg although few signs of toxicity such as itching, sedation, and piloerection were observed at high doses above 1,000 mg/kg.

The plant extract exhibited a non-statistically significantly ($p > 0.05$) reduction in the occurrence of watery defecation at 500 and 1,000 mg/kg doses relative to the controls in castor oil induced diarrhea model. However, percentage inhibition of defecation produced at 500 mg/kg was the same as that of loperamide and slightly higher than that of 1,000 mg/kg. This showed that the plant extract was able to inhibit diarrhea more effectively at 500 mg/kg dose and so the effect can be said to be non-dose dependent.

Castor oil induces diarrhea through the release of ricinoleic acid, its metabolite [12]. Ricinoleic acid causes irritation of gastric intestinal mucosa, promoting subsequent release of prostaglandin which stimulates gastrointestinal motility and electrolyte secretion, reducing electrolyte absorption from the intestine, and colon thereby resulting in diarrhea. [13].

The plant extract at the three doses produced a statistically significant ($p < 0.001$) difference in gastrointestinal motility of charcoal meal and percentage intestinal transit when compared to the standard drug (atropine), whereas it exhibited a non-statistically significant ($p > 0.05$) difference relative to the negative control. Atropine had almost twice the value of the extract treated groups in percentage inhibition of intestinal transit. This indicates that the plant extract does not seem to possess anti-motility activity. This is because decrease in gastrointestinal motility increases the time of stay of gastrointestinal contents in the intestine which could lead to increase in intestinal water and electrolyte absorption [14] Atropine, the standard drug used in comparison, is an anticholinergic agent blocking M_1 receptors on gastric parietal cells and blocking M_3

receptors on visceral smooth muscles of stomach and intestine ultimately causing relaxation of these muscles [15].

Preliminary qualitative phytochemical evaluation of the aqueous ethanol extract of *C. esculentus* showed the presence of alkaloids, steroids, cardiac glycosides, carbohydrate, and saponins. However, flavonoids, tannins, anthraquinones, and phlobatannins were observed to be absent in this extract. Medicinal plants with antidiarrheal activity were reported to possess phytochemical constituents, such as tannins, flavonoids, alkaloids, and steroids/terpenoids [16] out of which only alkaloids and steroids were present in *C. esculentus* ethanol extract. The antidiarrheal properties of flavonoids could be as a result of their being able to reduce intestinal motility and hydro electrolytic secretions which are known to be altered in diarrheic conditions [17]. Moreover, according to Khalilur, flavonoids have been reported to inhibit prostaglandin E2 induced intestinal secretion and spasmogens induced contraction and inhibit release of prostaglandins and autocoids in biological assays [18]. Tannin's antidiarrheal effect as stated by Francesco is due to its involvement in the production of a coagulated protein on the mucosal membrane of the gut which offers some degree of protection, which may lead to a decrease in the sensitivity of nerve endings and peristaltic stimulation [19]. Alkaloids have been implicated in antidiarrheal effect with its reported antispasmodic, antimicrobial, and anticholinergic effect [20,21]. The absorption of hydro electrolytes in the intestinal lumen may be positively influenced by the presence of steroids [22]. Hence, lack of effective inhibition of gastrointestinal motility activity of this plant tuber can be attributed to the absence of flavonoids and tannins even though it exhibited some degree of anti-secretory activity through the inhibition of defecation in castor oil induced diarrhea test.

5. CONCLUSION

The findings have shown that the aqueous ethanol extract of *C. esculentus* tuber seems to possess anti-secretory effect but does not have anti-motility effect. Thus, the plant can be said to possess some antidiarrheal activity which may likely be due to the presence of alkaloids and steroids as its constituents.

ETHICAL APPROVAL

As per international standard, written approval of the University research ethics committee was obtained.

CONFLICT OF INTEREST

The authors hereby declare that there is no conflict of interest.

AUTHORS CONTRIBUTIONS

Author OAS designed the study and supervised the experimental work, while author DTD carried out the study.

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