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Evaluation of Antidiarrheal Activity of Aqueous Extract of *Cucurbita moschata* (Pumpkin) Leaf in Castor Oil Induced Diarrhea Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Diarrhea is one of the major health problems in developing countries leading to mortality and morbidity among children under 5 years of age. This study evaluated the antidiarrheal activities of *Cucurbita moschata* on castor oil-induced diarrheal wistar rats.
Methods: Thirty (30) wistar rats were divided into six groups of five rats each. All rats except group 1 received 1mL castor oil to induce diarrhea. Groups I and II served as the normal and negative control. Group III received the standard drug (loperamide), groups IV-VI were treated with 100, 200 and 300 mg/kg b.wt of aqueous leaf extract of *Cucurbita moschata* respectively. Stool inhibition, castor oil-induced enteropooling, and gastrointestinal motility test were determined to evaluate the antidiarrheal effect of the extract.

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Results: Percentage stool inhibition significantly ($p < 0.05$) increased with increase in the dose of the extract. The percentage distance travelled by the charcoal of the groups treated with the plant extract significantly decreased when compared to the negative control. This decrease was comparable with the group administered loperamide. Administration of aqueous extract of *Cucurbita moschata* leaf significantly decreased the volume of the intestinal fluid when compared with the volume of intestinal fluid of the negative control and the group administered loperamide. **Conclusion:** Aqueous leaf extract of *Cucurbita moschata* possess antidiarrheal activity against castor oil-induced diarrhea.

Keywords: Diarrhea; stool inhibition; gastrointestinal motility; enteropooling; Cucurbita moschata.

1. INTRODUCTION

Diarrhea is a gastrointestinal disorder characterized by changes in bowel movement, frequency, and changes in the consistency of feces with increased water content, making it liquid or pasty, which can manifest acutely or chronically [1]. Diarrhea is one of the major health problems worldwide especially in developing countries for children under 5 years of age [2]. It occurs mainly due to poor hygiene practices, lack of improved sanitation facilities and low hygienic status of shared sanitation facilities [3,4]. Diarrhea etiology is associated with a wide array of putative pathogens such as *rotavirus, Cryptosporidium, E. coli, Shigella, Adenovirus, Aeromonas, V. cholera, C. jejuni, Norovirus, Rotavirus, Salmonella enterica, Sapovirus, Astrovirus and Clostridium difficile* with *E. coli* being the most common among infants [5,6,7]. Enteropathogenic *E. coli* (EPEC) continues to be the most important cause of diarrhea in children under 2 years of age while enterotoxic *E. coli* (ETEC) is the most common in children over 2 years [8]. Diarrheal disease is classified based on its duration either as acute (1–13 days), persistent (14 days and above), or chronic (above 30 days), or based on the physiological mechanisms as secretory, inflammatory, osmotic, and motor, where the majority of the etiologies possess complex pathophysiology involving one or more of these mechanisms [9,10,11]. The principles of managing diarrhea diseases are correct diagnosis and treatment of the specific agent that caused the diarrhea [12]. Oral rehydration therapy (ORT) containing electrolytes are administered to prevent or treat dehydration caused by persistent diarrhea, and medications such as antimotility agents like loperamide, antimicrobial agents, antibiotics, and nitazoxanide an anti-parasitic agent are used for the treatment of diarrheal disease when an accurate diagnosis is done and the causative agent is known [13]. However, a recent report establishes that loperamide induces toxic cardiac arrhythmias and death when taken in high doses [14]. The side effects of other antidiarrheal drugs include nausea, drug toxicity, dizziness, dependency, sedation, respiratory depression, and constipation. Hence, there is need to search for new antidiarrheal drug with fewer side effects. *Cucurbita moschata* (pumpkin) is a major and important annual dicotyledonous vegetable crop from the family Cucurbitaceae, extensively cultivated worldwide [15]. It grows up to 5 m with creeping and climbing stems bearing tendrils. The stems and leaves are fairly hairy. The stems are strong and cylindrical or perpendicular with petioles measuring 12-30 cm while the leaves are circular, having a kidney shape, heart shape, or triangular shape. The flowers grow up to 12 cm long, are bell-shaped and largely yellow [16]. Pumpkins can be cultivated in warm areas all over the world due to their cheap growth and production of high nutrient content [17,18]. Due to the rich nutritional composition of *Cucurbita moschata* such as carbohydrates, flavonoids, phenolics, vitamins, and amino acids [19], it is believed to have functional and health benefits [20]. Thus, it has been proven to possess various medicinal properties that include wound healing [21], antidiabetic, anti-carcinogenic, antioxidant [22], antibacterial [23], antidepressant, andante-inflammatory properties without notable ulcerogenic effect often associated with antiinflammatory drugs [24]. Traditionally, the plant is used to treat diarrhea. There is no scientific report on the antidiarrheal activity of the leaves of *Cucurbita moschata*. Therefore, this study evaluated the antidiarrheal effects of *Cucurbita moschata* on diarrheal wistar rats.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Fresh matured leaves of *Cucurbita moschata* were collected in May 2022 and authenticated at the Dept. of Botany, Adamawa State University,

Mubi. It was washed and air-dried under shade at room temperature and then pulverized using mortar and pestle into powder form. The powdered sample was stored in a well-tight container and kept at room temperature until required.

2.2 Extraction of the Plant Material

The powdered plant material was extracted using the maceration method described by Azwanida [25]. The plant material was soaked in distilled water in the ratio of 1:4 in a stoppered container and allowed to stand at room temperature with frequent agitation for 3 days. The mixture was pressed and strained by filtration using Whatman filter paper no. 1 after 3 days and the filtrate was evaporated to dryness using a crucible and water bath at 40^0C.

2.3 Experimental Animals

Adult albino rats with a weight range of 150 – 180 g were purchased from the Animal Resource Unit, National Veterinary Research Institute (NVRI) VOM, Plateau State, Nigeria and were housed in wired cages well ventilated, which were allowed free access to drinking water and fed with standard laboratory diet. Guidelines for the protection and handling of laboratory animals by the International Council for Laboratory Animal Science (ICLAS) rats were used in handling the rats. Animals were allowed to acclimatize to the laboratory environment for one week before the experiment commenced.

2.4 Stool Inhibition

Six groups of five animals each were fasted for 12 hours and thereafter castor oil at a dose of 1 mL/rat was administered to rats in groups II – VI to induce diarrhea using an orogastric cannula. Thirty (30) minutes after castor oil administration, rats of group II (negative control) received 1.0 mL of 0.9% NaCl in distilled water (normal saline), and group III received 2 mg/kg b. wt. loperamide (standard drug), groups IV-VI received 100, 200, and 300 mg/kg b.wt. of aqueous leaf extract of *Cucurbita moschata*, p.o. respectively. The animals were placed separately in metabolic cages over white clean Whatman filter paper, which was changed every hour. The severity of diarrhea was assessed each hour for 4 hours. The total number of diarrhea feces in the control group was considered 100%.

% inhibition = (Control - Test) × 100/Control.

2.5 Measurement of Gastrointestinal Transit

Six groups of five animals each were fasted for 12 hours and thereafter castor oil (1 mL) was administered orally to the rats in groups II - VI. One hour later, group III received the standard drug loperamide (2 mg/kg p.o) while rats of groups IV-VI received 100, 200, and 300 mg/kg b.wt. of leaf extract of *Cucurbita moschata,* p.o. respectively. After 30 min of the administration, 1 mL of charcoal meal, (10% suspension in 5% gum acacia) was orally administered to rats in each group. The rats were sacrificed by ether (20% v/v) anesthesia and the small intestine was carefully separated from mesentery to avoid being stretched. For each animal, gastrointestinal transit was calculated as the percentage distance traveled by charcoal meal to the total length of the intestine. The inhibitory effect of the extracts on gastrointestinal transit was calculated relative to the respective group.

2.6 Castor Oil-induced Enteropooling

Thirty adult albino rats were randomly divided into six groups. Castor oil (1 mL) was administered orally to rats in groups II - VI. One hour later, group III received the standard drug, loperamide (2 mg/kg p.o.). Rats of groups IV-VI received 100, 200, and 300 mg/kg b.wt. of aqueous leaf extract of *Cucurbita moschata*, p.o. respectively. After 2 hours of treatment, the rats were sacrificed by ether anesthesia. The edges of the intestine from the pylorus to the caecum were tied with thread and the intestine was removed and weighed. Intestinal fluid was milked into a graduated tube, and the intestinal fluid volume was taken. The intestine was reweighed and differences between full and empty intestines were calculated.

2.7 Statistical Analysis

The mean and statistical analysis computation was done using SPSS software version 24.0. Data are expressed as the mean ± S.D for a group of five animals. It was statistically analyzed with one-way analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT). For all the tests, results with p values < 0.05 was considered significant.

3. RESULTS AND DISCUSSION

Table 1 shows the effect of aqueous leaf extract of *Cucurbita moschata* on castor oil-induced

diarrhea. The plant extract significantly ($p < 0.05$) decreased the number of wet stool when compared with the standard drug, loperamide, and the negative control. The percentage inhibition of the treatment group significantly ($p < 0.05$) increased as the dose of the extract increased. The percentage stool inhibition of the group treated with the extract significantly ($p < 0.05$) increased when compared with the group treated with the standard drug and the negative control. Induction of diarrhea by castor oil is due to the most active component, ricinoleic acid, which causes inflammation and irritation of the intestinal mucosa leading to the release of prostaglandins which contributes to the pathophysiological functions in the gastrointestinal tract resulting in the stimulation of secretion by increasing the intestinal volume contents and prevention of re-absorption of water [26,27,28]. "Loperamide which acts by increasing the colonic phasic segmenting activities through inhibition of the presynaptic cholinergic nerves in the submucosal and myenteric plexus was used as a positive control in this study" [29]. "These effects of loperamide results in the reduction of the postprandial flow of digesta and absolute net colonic water absorption, while the relative digesta flow remains unchanged or is transiently reduced thereby reducing the frequency of defecation" [30].

The percentage inhibition of the castor oil-induced diarrhea by the leaf extract of the *Cucurbita moschata* may indicate that the extract is an effective inhibitor of diarrhea. The observed decrease in the number of stools by the plant extract indicates the antidiarrheal potential of the extract. Comparing the results of the groups treated with the extract and the group treated with the standard drug suggests that the plant extract is an effective agent for inhibition of diarrhea than the synthetic drug, loperamide.

Table 2 shows the percentage distance travelled by charcoal. "All the treated groups including the group treated with the standard drug were not significantly ($p < 0.05$) different from each other however, there was a significant ($p < 0.05$) decrease when compared with the negative control group. This significant ($p < 0.05$) decrease observed in the gastrointestinal transit by the extract may indicate that the extract has antimotility activity responsible for decreasing the peristaltic movement and secretion" [31,32]. "It may also indicate that the plant extract possess anticholinergic compounds since castor oil has been suggested to be indirectly mediated by the cholinergic system" [33].

Table 3 shows the effects of *Cucurbita moschita* leaf extract on castor oil-induced enteropooling. "When compared to the negative control group, the volume of intestinal fluid of all the treated groups significantly ($p < 0.05$) decreased. The volume of intestinal fluid of the group that received 300 mg/kg b.wt. of the extract decreased significantly from the other treated groups. The significant reduction observed in the intestinal fluid volume, especially at a higher dose of the extract suggests that the extract has an inhibitory effect on ricinoleic action. It may also be due to the fact that the extract enhances water reabsorption by decreasing the intestinal

Table 1. Effect of aqueous leaf extract of *Cucurbita moschata* on castor oil-induced diarrhea

Groups	Wet stool	% Stool inhibition
Group I (Normal control)	0 ± 0.00^{a}	100 ± 0.00^{e}
Group II (Negative control)	12 ± 0.15^{e}	0 ± 0.00^{a}
Group III (2 mg/kg loperamide)	8 ± 0.23^{d}	49 ± 0.12^{b}
Group IV (100 mg/kg b.wt. extract)	5 ± 0.09^{c}	72 ± 0.09^{c}
Group V (200 mg/kg b.wt. extract)	3 ± 0.13^{b}	83 ± 0.95^{d}
Group VI (300 mg/kg b.wt. Extract)	2 ± 0.15^{b}	90 ± 1.23^{d}

Values are presented as mean ± S.D. n=5.
Values with different superscript down the column are significantly different at ($p < 0.05$).

Table 2. Effect of aqueous leaf extract of *Cucurbita moschata* on castor oil induced gastrointestinal motility

Groups	% Distance travelled by the charcoal
Group I (Normal control)	30.51 ± 4.01^{a}
Group II (Negative control)	46.53 ± 2.74^{c}
Group III (2 mg/kg loperamide)	35.43 ± 3.70^{b}
Group IV (100 mg/kg b.wt. extract)	38.90 ± 3.57^{b}
Group V (200 mg/kg b.wt. extract)	34.24 ± 4.31^{b}
Group VI (300 mg/kg b.wt. Extract)	40.31 ± 2.23^{b}

Values are presented as mean ± S.D. n = 5. Values with different superscript down the column are significantly different at ($p < 0.05$)

Table 3. Effect of aqueous leaf extract of *Cucurbita moschata* on castor oil-induced enteropooling

Groups	Volume of intestinal fluid
Group I (Normal control)	1.46 ± 0.15^{ab}
Group II (Negative control)	2.42 ± 0.27^{c}
Group III (2 mg/kg loperamide)	1.84 ± 0.12^{b}
Group IV (100 mg/kg b.wt. extract)	1.54 ± 0.06^{ab}
Group V (200 mg/kg b.wt. extract)	1.55 ± 0.12^{ab}
Group VI (300 mg/kg b.wt. Extract)	1.20 ± 0.04^{a}

Values are presented as mean ± S.D. n = 5. Values with different superscript down the column are significantly different at ($p < 0.05$)

motility to allow for proper reabsorption" [34]. The antienteropooling effect of the extract may indicate that the extract is potent in phytochemicals such as steroids that are capable of stimulating the Na^{+} absorption by stimulating any of the apical transporters and reducing mucosal secretion [35]. The antidiarrheal activity of *Cucurbita moschata* observed in this study may indicate the presence of phytochemicals in the extract since earlier studies revealed that bioactive components such as tannins, saponins, flavonoids, alkaloids, sterols, and reducing sugar from medicinal plants possess anti-diarrheal property [31,36,37].

4. CONCLUSION

The results revealed that *Cucurbita moschata* aqueous leaf extract reduced the number of wet stools. It has antimotility and antienteropooling effects. It is more efficient than loperamide. The leaf extract has antidiarrheal potential.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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