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

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. **Result:** Percentage stool inhibition 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*

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.

MATERIALS AND METHOD

Collection of plant material

Fresh 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.

Extraction of the plant material

The powdered plant material was extracted using the maceration method described by Azwanida (2015). The plant material was soaked in distilled water in the ration 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.

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.

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 induce diarrhea using an orogastric cannula. Thirty (30) minutes

after castor oil administration, rats of group II (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 stem bark 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.

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 animals. 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 stem bark 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.

Castor oil-induced enteropooling

Castor oil (1 mL) was administered orally to these rats. 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 stem bark 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.

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.

RESULTS AND DISCUSSION

Table 1 shows the effect of aqueous leaf extract of *Cucurbita moschata* on castor oil-induced diarrhea. The plant extract reduced the number of wet stool more than the standard drug, loperamide, and the negative control. The percentage inhibition of the treatment group increased as the dose of the extract increased. The percentage stool inhibition by the extract was greater than that of the group treated with the standard drug. 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 1. Effect of aqueous leaf extract of *Cucurbita moschata* on castor oil-induced diarrhea

Groups	Wet stool	% Stool inhibition
Group I (Normal control)	0	100
Group II (Negative control)	12	0
Group III (2 mg/kg loperamide)	8	49
Group IV (100 mg/kg b.wt. extract)	5	72
Group V (200 mg/kg b.wt. extract)	3	83
Group VI (300 mg/kg b.wt. Extract)	2	90

Values are presented as mean ± S.D.

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

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. Values with different superscript down the column are significantly different at ($p < 0.05$).

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 decrease when compared to the negative control. The volume of the intestinal fluid of the groups treated with the extract was significantly lower than that of the group treated with loperamide. 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. 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 and peristalsis. It may also be due to the fact that the extract enhances water reabsorption by decreasing the intestinal motility to allow for 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].

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. Values with different superscript down the column are significantly different at ($p < 0.05$).

CONCLUSION

The results revealed that *Cucurbita moschata* aqueous leaf extract is a remarkable antidiarrheal agent with antimotility and antienteropooling effects, and could be useful in the management of diarrhea.

REFERENCES

1. Smieja, M., Goldfarb, D. M., Molecular detection of diarrheal pathogens. *Clin. Microbiol. Newsl.* 2016; 38 (2016): 137–145.
2. Asrie, A. B., Abdelwahab, M., Shewamene, Z., Gelayee, D. A., Adinew, G. M., Birru, E. M. Antidiarrheal activity of methanolic extract of the root bark of *Cordia africana. Journal of Experimental Phamacology*. 2016; 8: 53–59.
3. Semba, R. D., Kraemer, K., Sun, K., De Pee, S., Akhter, N., Moench-Pfanner, R., Bloem, M. W. Relationship of the presence of a household improved latrine with diarrhea and under-five child mortality in Indonesia. *The American Journal of Tropical Medicine and Hygiene*. 2011; *84*(3): 443.
4. Simiyu, S., Swilling, M., Cairncross, S., Rheingans, R. Determinants of quality of shared sanitation facilities in informal settlements: case study of Kisumu, Kenya. *BMC Public Health*. 2017; *17*(1): 1-13.
5. Nataro, J. P., Mai, V., Johnson, J., Blackwelder, W. C., Heimer, R., Tirrell, S., Hirshon, J. M. Diarrheagenic escherichia coli infection in Baltimore, Maryland, and New haven, Connecticut. *Clinical Infectious Diseases*. 2006; *43*(4): 402 - 407.
6. Kotloff, K. L., Nataro, J. P., Blackwelder, W. C., Nasrin, D., Farag, T. H., Panchalingam, S., Levine, M. M. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *The Lancet*. 2013; *382*(9888): 209 - 222.

7. Qadri, F., Svennerholm, A. M., Faruque, A. S. G., & Sack, R. B. Enterotoxigenic Escherichia coli in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. *Clinical Microbiology Reviews*. 2005; *18*(3): 465-483.
8. Nguyen, T. V., Le Van, P., Le Huy, C., Gia, K. N., & Weintraub, A. Etiology and epidemiology of diarrhea in children in Hanoi, Vietnam. *International Journal of Infectious Diseases*. 2006; *10*(4): 298 - 308.
9. Dantas, R. O., Diarreia, E. Constipação Intestinal. *Medicina.* 2004; 37 (2004): 262–266.
10. Patel, K., Thillainayagam, A.V., Diarrhoea. *Medicine*. 2009; 37 (2009) : 23 – 27.
11. Bhutta, Z., Syed, S. Diarrheal Diseases. *Encyclopedia of Food and Health* 2016; 361–372.
12. Camilleri, M., Sellin, J. H., & Barrett, K. E. Pathophysiology, evaluation, and management of chronic watery diarrhea. *Gastroenterology.* 2017; 152(3): 515 - 532.
13. DuPont, H. L. Persistent diarrhea: a clinical review. *Jama.* 2016; 315(24): 2712 - 2723.
14. Dierksen, J., Gonsoulin, M., Walterscheid, J. P. Poor man's methadone: a case report of loperamide toxicity. *The American Journal of Forensic Medicine and Pathology*. 2015; 36(4): 268-270.
15. Wu, T., Luo, S., Wang, R., Zhong, Y., Xu, X., Lin, Y., He, X., Sun, B., Huang, H. The first Illumina-based de novo transcriptome sequencing and analysis of pumpkin (Cucurbita moschata Duch.) and SSR marker development. *Mol Breeding.* 2014. DOI 10.1007/s11032-014-0128-x
16. Marie-Magdeleine, C., Mahieu, M., & Archimède, H. Pumpkin (Cucurbita moschata Duchesne ex Poir.) Seeds as an Anthelmintic Agent? In Nuts and seeds in health and disease prevention (pp. 933-939). Academic Press.Kazuo KOIKE, Wei LI, Lijuan LIU, Emiko HATA, and Tamotsu NIKAIDO. New Phenolic Glycosides from the Seeds of Cucurbita moschata. *Chem. Pharm. Bull.* 2011; 53(2): 225 – 228.
17. Kumar, S., Rattan, P., Samnotra, R. Squashes and gourds. In Pessarakli, M. (Ed.) Handbook of cucurbits: growth, cultural practices, and physiology. Boca Raton, FL: CRC Press. 2016; 513 - 531. ISBN-13 978-1-4822-3459-6.
18. Provesi, J. G., Amante, E. R. Carotenoids in pumpkin and impact of processing treatments and storage. *Processing and Impact on Active Components in Food*. 2015; 71-80. ISBN-13 978- 0-12-404699-3.

19. Wang, P., Liu, J. C, Zhao, Q. Y. Studies on nutrient composition and utilization of pumpkin fruit. *J. Inner Mongolia Agric. Univ*. 2002; 23: 52 – 54
20. Zhang, F., Jiang, Z. M., Zhang, E. M. Pumpkin function properties and application in food industry. *Sci. Technol. Food Indus.* 2000; 21: 62 – 64
21. Bahramsoltani, R., Farzaei, M. H., Abdolghaffari, A. H., Rahimi, R., Samadi, N., Heidari, M., Amin, G. Evaluation of phytochemicals, antioxidant and burn wound healing activities of *Cucurbita moschata* Duchesne fruit peel. *Iranian Journal of Basic Medical Sciences.* 2017; 20(7): 798.
22. Yadav, M., Jain, S., Tomar, R., Prasad, G. B. K. S., Yadav, H. Medicinal and biological potential of pumpkin: an updated review. *Nutrition Research Reviews*. 2010; 23(2): 184-190.
23. El Zawane Kamarudin, Q. U. A., Helaluddin, A. B. M., Sirajudin, Z. N. M., Chowdhury, A. J. K. Studies on bactericidal efficacy of pumpkin (Cucurbita moschata Duchesne) peel. *Journal of Coastal Life Medicine.* 2014; 2(2): 146 -153.
24. Eleiwa, N. Z., Bakr, R. O., Mohamed, S. A. Phytochemical and pharmacological screening of seeds and fruits pulp of Cucurbita moschata Duchesne cultivated in Egypt. *International Journal of Pharmacognosy and Phytochemistry*. 2014; 29(1): 1226 - 1236.
25. Azwanida, N. N. A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength, and Limitation. *Medical and Aromatic Plants.* 2015; 4: 196. doi:10.4172/2167-0412.1000196.
26. Ammon, H. V., Thomas, P. J., Phillips, S. F. Effects of oleic and ricinoleic acids on net jejunal water and electrolyte movement. Perfusion studies in man. *The Journal of Clinical Investigation*. 1974; 53(2): 374-379
27. Luderer, J. R., Dermers, L. M., Nomides, C., Hayes, A. H. Mechanism of castor oil: A biochemical link to the prostaglandins. In: Samuelson B, Ramwell PW, Paoletti R. (eds). Advances in Prostagladin and Thromboxane Research, Raven Press, New York. 1980; 8: 1633 - 1635.
28. Nwachoko, N., Jack, I. R. Phytochemical screening and antidiarrhoeal activities of *Tetracarpidium conophorum* induce in albino rats. Sky Journal of Biochemistry *Research* 2015; 4(4): 021 – 024.

29. Okere, O. S., Sangodele, J. O., Tade, O. G., Obafemi, O. T., & Falode, J. A. Anti-diarrhea potential and acute toxicity studies of methanolic extract of *Vernonia amygdalina* and *Cymbopogon citratus* against castor oil induced diarrhea model in rats. *International Journal of Biochemistry Research & Review*. 2015; 6(2): 46.
30. Theodorou, V., Fioramonti, J., Hachet, T., Bueno, L. Absorptive and motor components of the antidiarrhoeal action of loperamide: an in vivo study in pigs. *Gut.* 1991; 32(11): 1355-1359.
31. Galvez, J., Zarzuelo, A., Crespo, M. E., Lorente, M. D., Ocete, M. A., Jimenez, J. Antidiarrhoeic activity of *Euphorbia hirta* extract and isolation of an active flavonoid constituent. *Planta medica.* 1993; 59(04): 333 - 336.
32. Brunton, L. L. Agents affecting gastrointestinal water flux and motility; emesis and antiemetics; bile acids and pancreatic enzymes. Goodman and Gilman's: the pharmacological basis of therapeutics. 1996; 917 - 936.
33. Brown, J. H. and P. Taylor. Cholinergic agonist In Brunton L.L. Lazo. J.S. Parker k.l. (Eds). The pharmacological basis of Therapeutics. 11th edn. McGraw-Hill. New York. 2006; 183 - 200.
34. Maiti, A., Dewanjee, S., & Mandal, S. C. In vivo evaluation of antidiarrhoeal activity of the seed of Swietenia macrophylla King (Meliaceae). *Tropical Journal of Pharmaceutical Research.* 2007; 6(2): 711 - 716.
35. Ahmed, M. U., Arise, R. O. and Umaru, I. J. Identification and biochemical characterization of anti-enteropooling compounds from *Annona senegalensis* root bark. *Scientific African*. 2022; 15(2022)e0128.
36. Galvez, J., Zarzuelo, A., Crespo, M. E., Utrilla, M. P., Jimenez, J., Spiessens, C., & de Witte, P. Antidiarrhoeic activity of Sclerocarya birrea bark extract and its active tannin constituent in rats. *Phytotherapy Research.* 1991; 5(6): 276 - 278.
37. Otshudi, A. L., Vercruysse, A., Foriers, A. Contribution to the ethnobotanical, phytochemical and pharmacological studies of traditionally used medicinal plants in the treatment of dysentery and diarrhoea in Lomela area, Democratic Republic of Congo (DRC). *Journal of Ethnopharmacology*. 2000; 71(3): 411 - 423.