

Original Research Article

Anti-diarrhoeal effect of methanol leaf fraction of *Cola hispid* Brenan and Keay in rats

Ugochi Olivia Njoku*, Obinna Martins Ogugofor

Department of Biochemistry, University of Nigeria, Nsukka, Nigeria

*For correspondence: **Email:** ugochi.njoku@unn.edu.ng; **Tel:** +234 8037461525

Sent for review: 5 February 2021

Revised accepted: 23 June 2021

Abstract

Purpose: To evaluate the anti-diarrhoeal activity of 20 % methanol leaf fraction of *Cola hispid* (MLFC) using castor oil-induced diarrhoea model in rats.

Methods: The activity of MLFC against castor oil-induced diarrhoea, intestinal fluid accumulation (enteropooling), and gastrointestinal motility were evaluated. The experimental animals were grouped into five, each with four animals. Group 1 received distilled water, while group 2 received loperamide and served as negative and standard control groups respectively. Groups 3 - 5 were administered 200, 400 and 600 mg/kg of MLFC respectively. One-hour post-treatment, castor oil (p.o.) was used to induce diarrhoea in all the groups. The activity of MLFC was tested against castor oil-induced looseness of stool, fluid accumulation in the intestine and intestinal motility of charcoal meal. Its effect on electrolyte concentration in the small intestinal content was assessed; furthermore, its effect on the electrolyte concentrations of Na⁺, Cl⁻, K⁺, and HCO₃⁻ in supernatants obtained from the intestinal loop effluents of the rats was evaluated.

Results: The MLFC produced a significant ($p < 0.05$) and dose-dependent decrease in the severity of diarrhoea and frequency of watery stool passed in castor oil-induced diarrhoea, enteropooling, gastrointestinal transit of charcoal meal in rats and serum concentrations of sodium and chloride ions, while significant ($p < 0.05$) increase in potassium and bicarbonate ion concentrations were observed when compared with diarrhoeal control group. Similar inhibitory effects were obtained for loperamide control group.

Conclusion: The results of the study indicate that methanol leaf fraction of *Cola hispid* possesses anti-diarrhoeal properties by inhibiting intestinal peristalsis and hypersecretion of water and electrolytes. Thus, the findings lend some support to the traditional use of the plant for the treatment of diarrhoea

Keywords: *Cola hispid*, Antidiarrhoeal activity, Castor oil, Diarrhoea

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

In Nigeria, many indigenous plants are used as sources of affordable medicine, food and spices, and are regarded as powerful potential therapeutic agents due to their pharmacological

properties [1]. Extracts from the roots, barks, stem, seeds and fruits of these plants are used in the preparation of syrups in traditional medicine as cough suppressants and in the treatment of oxidative related diseases [2]. Diarrhoea is an alteration of bowel movement characterized by

an increase of volume, fluidity, frequency and passage of loose or watery stool with abdominal pain and bowel sounds [3]. It is the most common clinical manifestation of gastrointestinal disease and can be caused by infectious and non-infectious agents [4]. The onset of the disease may be abrupt and self-limiting in immune-competent individuals, but chronic diarrhoea may be persistent even with therapy, especially in people with an underlying debilitating clinical condition such as HIV/AIDS and diabetes mellitus or individuals with an aging immunity [5].

Diarrhoea is a leading killer in children, accounting for approximately 8 percent of all deaths among children under age 5 worldwide in 2017. This translates to over 1,300 young children dying each day, or about 480,000 children a year, despite the availability of a simple treatment solution [6]. The majority of deaths occur in rural African communities where healthcare facilities are inadequate and the majority of the people lack access to safe and pure water, a key mode of diarrhoeal disease transmission access to clean and safe water, a major vehicle for transmission of diarrhoeal diseases [7].

Cola hispida (sterculiaceae) is a small tree about 12 m high or a shrub native to West Tropical Africa. It is a shrub of the evergreen forest in Northern, Southern Nigeria, West Cameroons, and extending into Zaïre. In Nigeria, it is commonly called Oji-ogodo (Igbo), Ikpa obuko (yoruba) Òbò kpéhé (Igala). *Cola hispida* is an edible fruity medicinal plant used as a source of food. In folklore medicine, the leaves are used in the treatment of ear infection, pulmonary troubles and decoction of the leaf is taken as a tonic to ease cough and stomach upsets in Congo. Roots of *Cola hispida* are used as genital stimulants and depressants [8]. In Nigeria, the ethanol extract of the leaves is used for the treatment of threatened abortion [9]. Among the locals of OzomMgbagbuOwa, in Ezeagu Local Government Area, *hispida* are used in the treatment of diarrhoea. Despite the fact that this plant has been used in African folklore medicine for the relieve of diarrhoea, there is a paucity of studies to validate this claim. Hence, the main objective of this study was to ascertain the effectiveness of 20 % methanol leaf fraction of *Cola hispida* in the treatment of diarrhoea disease. Enugu State of Nigeria, the leaves of *Cola* Therefore, the aim of the research was to scientifically validate the anti-diarrhoeal properties of the 20 % methanol leaf fraction of *Cola hispida* (MLFC).

EXPERIMENTAL

Chemicals and reagents

The chemicals and reagents used in the study were of analytical grades. They include: chloroform, absolute ethanol, acetylacetate and Methanol (Sigma Aldrich, Germany), glacial acetic acid, n-hexane and ferric chloride (Merck, USA), lead acetate solution, ethylacetate, concentrated sulphuric acid (Kernel, China), activated charcoal, gum acacia, castor oil (Bell Sons and Co., England), loperamide (Janssen, Germany), Distilled water (STC, UNN), Grower's mash (Grand Cereals Ltd., Enugu)

Animals

Adult male Wistar albino rats bred in the laboratory animal facility of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Nigeria were used for this study. The animals were maintained freely on normal rat feed (Pecco foods, Enugu, Nigeria) and water *ad libitum* in a clean, well-ventilated cage. They were exposed to a 12-h light-dark cycle and handled according to standard protocol. The study was approved by the Ethical Committee of Faculty of Biological Sciences, University of Nigeria, Nsukka (no. UNN/FBS/EC/1040) in accordance with "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised 1985) [10].

Plant material

The leaves of *Cola hispida* were harvested from Ozom Mgbagbu Owa, Enugu State of Nigeria and identified by a taxonomist, Mr A Ozioko of the Bioresources Conservation and Development Center (BCDC), Enugu State. The leaves were air-dried at room temperature and pulverized into powder using a miller.

Extraction and fractionation

The pulverized leaf sample was extracted with methanol by maceration and allowed to stand for 72 h at room temperature with thorough shaking. The resulting solution was filtered using filter cloth and subsequently concentrated at 45 °C in rotary evaporator under reduced pressure to afford 124.52 g of extract (11.49 %). The methanol leaf extract of *Cola hispida* was further re-extracted by the process of solvent partitioning with n - hexane, ethylacetate and 20 % methanol in the order of increasing polarity. The respective fractions were concentrated in a rotary evaporator under vacuum and stored at - 4 °C in a freezer. The pilot study was carried out with the

three respective fractions and 20 % methanol fraction gave the optimum anti-diarrhoeal activity using castor oil-induced diarrhoea model in rats. Consequently, 20 % methanol fraction was used for the experiment.

Activity of 20 % MLFC on castor oil-induced diarrhoea in rats

Twenty male albino rats divided into 5 groups of four animals each were fasted for 24 h with free access to water prior to the experiment and maintained under standard conditions of 12 h light-dark cycle. To induce diarrhoea, 1 mL of castor oil was orally administered to all the experimental rats. Group 1 received distilled water (2 ml/kg) and served as the negative control, while group 2 received loperamide and served as the standard group. The 20 % MLFC was given orally to groups 3, 4, and 5 at doses of 200, 400, and 600 mg/kg respectively.

One hour after pretreatment, rats in all the groups received 1 mL of castor oil (p.o). The rats were placed in individual metal cages lined with a blotting paper and observed for severity and consistency of diarrhoea for 4 h after administration of castor oil. After each hour the blotting paper with stools was changed. The total number of faeces (both wet and dry) expelled in the groups pretreated with the plant fraction was recorded every hour for 4 hours and compared with the negative control group. The total diarrhoeal faeces for the control group were considered to be 100 %. The results were expressed as inhibition of diarrhea (D) as shown in Eq 1.

$$D (\%) = \{(T_0 - T_1 / T_0)\} 100 \dots\dots\dots (1)$$

where T₀ = number of wet faeces in positive control group, and T₁ = number of wet faeces in test group

Severity of diarrhea (S) was evaluated as in Eq 2.

$$S (\%) = \frac{W_s}{T_s} \times 100 \dots\dots\dots (2)$$

Ws = weight of wet stool while Ts = total weight of stools

Activity of 20 % MLFC on castor oil-induced gastrointestinal enteropooling in rats

Twenty male albino rats were divided into five groups of four animals each and maintained under standard conditions. Group 1 received distilled water (2 ml/kg) and served as negative control group. Group 2 received loperamide (2

mg/kg) and served as standard group, while groups 3, 4 and 5 received 20 % methanol leaf fraction of *Cola hispida* MLFC at doses of 200, 400 and 600 mg/kg respectively and served as test groups. Experimental rats were fasted for 24 h but allowed free access to water prior to the experiment. One hour after pretreatment with respective drugs, each rat was orally administered 1 mL of castor oil. One hour later, the animals were anaesthetized and sacrificed by inhalation of chloroform. The abdomen of the animals was cut open and small intestine of each rat from the pylorus to the caecum was isolated. The intestinal content of each rat was milked into a graduated test tube and the volume was determined.

$$\text{Intestinal fluid inhibition (\%)} = (T_c - T_t / T_c) \times 100 \dots\dots\dots (3)$$

T_c = Mean fluid accumulation in negative control group; T_t = Mean fluid accumulation in test group

Effect of 20 % MLFC on intestinal propulsion in rats

The effect of MLFC on intestinal motility in rats was determined using the charcoal meal test, with slight modification [11,12]. Five groups of 4 animals each were fasted for 24 h, with free access to water. Group 1 received distilled water (2 ml/kg) orally and served as control group. Group 2 received loperamide (2 mg/kg) and served as standard drug group, while groups 3, 4, and 5 received 200, 400 and 600 mg/kg of 20 % MLFC respectively and served as test groups. After pretreatment, castor oil (1 mL) was orally administered to all the groups to produce diarrhoea. Thirty minutes later, charcoal meal (5 % activated charcoal suspended in 10 % aqueous solution of gum acacia) was administered orally to each animal. The animals were sacrificed in a chloroform chamber 30 min after administration of the charcoal meal and their abdomens were sliced open. The distance covered by the charcoal meal in the intestine, from the pylorus to the caecum, was measured and expressed as the percentage of the total length of the small intestine from pylorus to the caecum. The extent of intestinal propulsion (%) of the charcoal meal was calculated using the relation:

$$\text{Intestinal propulsion (\%)} = \text{DTCM} / \text{TLSI} \times 100 \dots\dots\dots (4)$$

where DTCM = distance traversed by the charcoal meal, and TLSI = total length of the small intestine.

Inhibition of propulsion (P, %) was computed as in Eq 5.

$$P (\%) = 100[1-(a/b)] \dots\dots\dots (5)$$

where a = IP (intestinal propulsion) of treated animals
 b = IP of control animals

Effects of 20 % MLFC on electrolytes concentration

Twenty (20) adult albino rats were fasted for 24 hrs and divided into five groups of four rats each. Group 1 received distilled water (2 ml/kg b.w.) orally and served as negative control group. Group 2 received loperamide (2 mg/kg b.w.) orally and served as standard group, while groups 3, 4, and 5 received 200, 400 and 600 mg/kg b.w. of 20 % methanol leaf fraction of *Cola hispida* respectively and served as test groups. After duration of 1 h, animals in all the groups were orally administered castor oil (1 ml) using suitable stomach tube. Two hours later, the rats were anaesthetized and sacrificed by inhalation of chloroform. Following the induction of diarrhoea in all of the rats in the groups and sacrificing of the animals, the small intestines of the rats were located and tied at the pylorus and ileocaecal junction, then sliced open and the contents discharged into a measuring cylinder. The effluents of the intestinal loops (serosal solutions) of the rats were centrifuged at 3,000 g for 30 min. The supernatants were obtained and analyzed procedurally for concentrations of Na^+ , K^+ , Cl^- and HCO_3^- [13-15].

Statistical analysis

The results are expressed as mean \pm SD and tests of statistical significance were carried out using one-way analysis of variance (ANOVA). The analysis was carried out using Statistical Package for Scientific Solution (SPSS) version 20. Data obtained from the test groups were compared with the negative control and the differences were considered significant at $p < 0.05$.

Table 1: Effect of 20 % MLFC on castor oil-induced diarrhoea in rats

Group	Total number of faeces	No of diarrhoeal faeces	Severity of diarrhoea (%)	Inhibition of diarrhoea (%)
Control (distilled water (2 ml/kg) + castor oil (1 mL)	10.33 \pm 0.88 ^d	8.67 \pm 0.67 ^d	83.93	-
Loperamide (2 mg/kg) + castor oil (1 mL)	5.33 \pm 0.67 ^{ab}	1.67 \pm 0.33 ^a	31.33	80.74
MLFC (200 mg/kg) + castor oil (1 mL)	9.33 \pm 0.88 ^{cd}	5.00 \pm 0.58 ^c	53.59	42.33
MLFC (400 mg/kg) + castor oil (1 mL)	8.00 \pm 0.58 ^{bcd}	3.00 \pm 1.15 ^{bc}	37.50	65.40
MLFC (600 mg/kg) + castor oil (1 mL)	6.67\pm1.67^{abc}	2.33\pm0.88^b	34.93	73.13

Values are expressed as mean \pm S.D. (n=4). Groups with different superscripts down the column are significantly different from each other at $p < 0.05$

RESULTS

According to the findings of this study, the negative control group developed copious diarrhoea 4 h after being given castor oil orally. Rats given 20 % methanol leaf fraction of *Cola hispida* (MLFC) showed dose-related and significant ($p < 0.05$) reductions in the intensity and consistency of defecation by 42.33 (200 mg/kg), 65.40 (400 mg/kg), and 73.13 % (600 mg/kg) in relation to the diarrhoeal control group. In contrast, loperamide inhibited castor oil-induced diarrhoea by 80.74 % (Table 1).

In addition, at same doses (200, 400 and 600 mg/kg), MLFC significantly ($p < 0.05$) inhibited castor oil-induced intestinal fluid accumulation and volume of intestinal content by 71.43 %, 76.74 % and 86.05 % respectively, relative to the negative control group, while loperamide inhibited intestinal fluid accumulation caused by castor oil by 86.05 % (similar to the effect of fraction at 600 mg/kg) as shown in Table 2.

In the result of effect of methanol fraction of *Cola hispida* leaf on intestinal transit of charcoal meal in rats, the fraction achieved significant ($p < 0.05$) reduction in propulsion of charcoal meal in rats gastrointestinal tract in a dose dependent pattern, with percentage inhibitions of 37.74, 43.57 and 49.27 % at doses of 200, 400 and 600 mg/kg respectively when compared to the negative control, while loperamide achieved 61.49 % (Table 3).

In the intestinal content in the experimental rats, concentration of the electrolytes; sodium, potassium, chloride and bicarbonate ions were measured. In diarrhoeal control group, sodium ions concentration was 139.75 \pm 1.50 mEq/L. Treatment with 20 % methanol leaf fraction of *Cola hispida* (MLFC) reduced sodium ions concentration, significantly ($p < 0.05$) at doses of 400 mg/kg (133.05 \pm 1.46 mEq/L) and 600 mg/kg (129.90 \pm 2.20 mEq/L) when compared with

Table 2: Effect of 20 % MLFC on castor oil-induced enteropooling in rats

Treatment/dose(mg/kg)	Volume of intestinal fluid (mL)	Inhibition of intestinal fluid (%)
Control (distilled water 2 ml/kg) + castor oil (1 mL)	3.01 ± 0.01 ^c	-
Loperamide (2 mg/kg) + castor oil (1 mL)	0.42 ± 0.06 ^a	86.05
MLFC (200 mg/kg) + castor oil (1 mL)	0.86 ± 0.26 ^b	71.43
MLFC (400 mg/kg) + castor oil (1 mL)	0.70 ± 0.42 ^{ab}	76.74
MLFC (600 mg/kg) + castor oil (1 mL)	0.42 ± 0.04 ^a	86.05

Values are expressed as mean ± S.D. (n = 4). Groups with different superscripts down the column are significantly different from each other at $p < 0.05$

Table 3: Effect of 20 % MLFC on intestinal transit of charcoal meal in rats

Group	Length of Intestine (cm)	Distance travelled by charcoal meal (cm)	Peristalsis index (%)	Inhibition of propulsion (%)
Control (distilled water 2ml/kg) + castor oil (1 mL)	54.33 ± 2.52 ^a	35.27 ± 3.17 ^d	64.91	-
Loperamide (2 mg/kg) + castor oil (1 mL)	54.67 ± 3.06 ^a	13.67 ± 1.04 ^a	25.00	61.49
MLFC (200 mg/kg) + castor oil (1 mL)	56.50 ± 2.29 ^a	22.83 ± 1.46 ^c	40.41	37.74
MLFC (400 mg/kg) + castor oil (1 mL)	53.87 ± 3.11 ^a	19.73 ± 1.10 ^{bc}	36.63	43.57
MLFC (600 mg/kg) + castor oil (1 mL)	55.30 ± 3.20^a	18.21 ± 1.77^b	32.93	49.27

Values are expressed as mean ± S.D. (n = 4). Groups with different superscripts down the column are significantly different from each other at $p < 0.05$

Table 4: Effect of 20 % MLFC on electrolyte concentration in intestinal content in rats

Groups	Sodium (mEq/L)	Potassium (mEq/L)	Chloride (mEq/L)	Bicarbonate (mEq/L)
Negative control (dist. Water 2ml/kg) + castor oil (1 mL)	139.75 ± 1.50 ^e	3.95 ± 0.06 ^a	91.50 ± 4.12 ^e	34.74 ± 2.24 ^d
Loperamide (2 mg/kg) + castor oil (1 mL)	105.50 ± 4.65 ^a	4.00 ± 0.01 ^b	74.58 ± 1.89 ^b	19.95 ± 0.79 ^b
MLFC (200 mg/kg) + castor oil (1 mL)	136.50 ± 2.08 ^{de}	4.24 ± 0.14 ^d	88.00 ± 3.16 ^{cd}	33.13 ± 2.96 ^d
MLFC (400 mg/kg) + castor oil (1 mL)	133.05 ± 1.46 ^{cd}	4.20 ± 0.06 ^{de}	82.78 ± 2.49 ^c	25.42 ± 1.97 ^c
MLFC (600 mg/kg) + castor oil (1 mL)	129.90 ± 2.20 ^c	4.13 ± 0.17 ^c	74.58 ± 1.89 ^b	12.66 ± 0.30 ^a

Values are expressed as mean ± S.D. (n = 4). Groups with different superscripts down the column are significantly different from each other at $p < 0.05$

animals in diarrhoeal control group. The decrease elicited in animals treated with loperamide was 105.50 ± 4.65 mEq/L. Relative to the diarrhoeal control group that produced potassium ions concentration of 3.95 ± 0.06 mEq/L, animals treated with MLFC significantly ($p < 0.05$) enhanced potassium ions concentration at tested doses of 200 mg/kg (4.24 ± 0.14 mEq/L), 400 mg/kg (4.20 ± 0.06 mEq/L), and 600 mg/kg (4.13 ± 0.17 mEq/L), while animals treated with loperamide achieved 4.00 ± 0.01 mEq/L (Table 4). Chloride ions concentration in animals pretreated with MLFC and loperamide was significantly ($p < 0.05$) suppressed when compared with diarrhoeal control group (91.50 ± 4.12 mEq/L). The methanol leaf fraction of *Cola hispida* (MLFC) elicited chloride ions concentrations of 88.00 ± 3.16 mEq/L (200 mg/kg), 82.78 ± 2.49 mEq/L (400 mg/kg) and 74.58 ± 1.89 mEq/L (600 mg/kg), while animals treated with reference

drug, loperamide achieved 74.58 ± 1.89 mEq/L. Treatment with MLFC at doses of 400 and 600 mg/kg afforded significant ($p < 0.05$) decreases in bicarbonate with 25.42 ± 1.97 mEq/L and 12.66 ± 0.30 mEq/L respectively, while treatment with loperamide achieved 19.95 ± 0.79 mEq/L when compared with diarrhoeal control group which produced 34.74 ± 2.24 mEq/L.

DISCUSSION

With over a decade of the practice and promotion of oral rehydration therapy, diarrhoea is still the second among the causes of child death. However, during intense diarrhoeal state, these management options often fail, coupled with undesirable side effects of antidiarrhoeal drugs in world market. To avert these threats, the WHO has initiated a programme which supports traditional herbal medicine [3].

Depending on its etiology, diarrhoea may be characterized by increased secretion of electrolytes (secretory diarrhoea), increased luminal osmolality (osmotic diarrhoea), decreased electrolyte absorption, and/or increased intestinal motility responsible for reduced transit time. Castor oil causes diarrhoea by the action of its active component ricinoleic acid, which acts by enhancing intestinal motility, fluid secretion, and permeability of the intestinal mucosa to electrolytes [16], inhibiting intestinal Na⁺/K⁺ ATPase activity [17], and stimulating the biosynthesis and release of diarrhoea-causing endogenous prostaglandins [16].

Inhibition of the frequency of defecation, reduction in fecal output and wetness of faeces are possible indices of antidiarrhoeal action [16]. In this study, increases in total faecal output and diarrhoeal stools observed in negative control group could be attributed to diarrhoea induced by the active component, ricinoleic acid, liberated from the enzymatic breakdown of castor oil in the gut. Treatment with 20 % MLFC significantly inhibited the severity and frequency of defecation, as well as the number of wet stools in a dose-dependent manner, with optimal effect observed at highest tested dose of 600 mg/kg when compared with diarrhoea control group in the 4 h observation. The percentage inhibition of diarrhoea produced at the highest tested dose of the fraction was similar to that obtained by the standard drug, loperamide.

Apart from controlling the gastrointestinal tract, loperamide, which is currently one of the most effective and commonly used antidiarrhoeal drugs [16], has also been documented to slow intestine transit, reduce colon flow rate, and thus any impact on colonic motility. The antimotility and antisecretory properties of loperamide are thought to be responsible for its therapeutic activity [16]. Loperamide exerts its antisecretory effect by activation of Na⁺/K⁺ ATPase which catalyzes the efflux of Na⁺ ions and influx of K⁺ ions in order to maintain their low and high intracellular concentrations respectively [17], or by stimulating co-transport of sodium chloride from the intestine and inhibiting calmodulin [18].

Possibly, *Cola hispida* fraction elicited this significant inhibition of diarrhoea by stimulating Na⁺/K⁺ ATPase activity mediated by antisecretory mechanism similar to that of loperamide, consequently, activating all active transport mechanisms in the intestine.

Consistent with findings in castor oil-induced diarrhoea model, respective groups of experimental rats pretreated with graded doses

of MLFC showed significant decreases in volume of intestinal fluid compared with diarrhoeal control group. This result demonstrated the inhibitory effect of the fraction on castor oil-induced intestinal fluid accumulation (enteropooling). The percentage reduction in intestinal volume content was dose-dependent, optimal at the highest tested dose of 600 mg/kg and observed to be close to the inhibitory effect of the reference drug as shown in Table 2. The findings may indicate that the plant fraction has a significant antisecretory effect, thus contributing to its antidiarrhoeal effect.

In the gastrointestinal motility test model (Table 3), MLFC significantly ($p < 0.05$) reduced gastrointestinal motility as observed in all the groups that received the fraction, in a dose related pattern, evident by prolonged gastrointestinal charcoal meal transit when compared with the diarrhoeal control group. These findings indicate that the gastrointestinal motility was greatly decreased as the dose of the fraction was increased. The extension of charcoal meal transit time due to inhibition of small intestinal peristaltic movement caused by the fraction may be attributed to antimotility property of *Cola hispida*, thus, preventing prompt evacuation of gastrointestinal contents.

In the study on the effect of *Cola hispida* fraction on electrolyte concentration in intestinal content, increases in Na⁺ and Cl⁻ ions, as well as decreases in K⁺ and HCO₃⁻ ion concentrations seen in the negative control group (Table 4), could be attributed to castor oil-induced modification of the permeability of gastrointestinal mucosa to electrolytes and stimulation of both the biosynthesis and discharge of endogenous prostaglandins accountable for diarrhoea. Treatment with MLFC or loperamide ameliorated the electrolytes concentration imbalance triggered by the castor oil significantly ($p < 0.05$), probably by increasing Na⁺/K⁺ATPase activity, which is involved in electrolytes and intestinal fluid reabsorption, thus, preventing fluid accumulation in the intestine. As a result, MLFC improved water and sodium chloride reabsorption while also preventing potassium ion depletion and bicarbonate ion loss.

The effect of reduction in weight and volume of the intestinal content is a direct consequence of reduced water and electrolyte secretion into the small intestine [19], which suggests that the fraction may enhance electrolyte reabsorption from the intestinal lumen and is consistent with inhibition of hyper-secretion. However, since electrolyte absorption determines the efficiency

of nutrient absorption, it is likely that the enhanced electrolyte absorption by the fraction may have encouraged the absorption of other intestinal contents. More so, solute absorption in any region of the intestine is a function of the rate of water uptake in that region. These observations reasonably suggest that the fraction inhibits gastrointestinal hyper-secretion and enteropooling by enhancing electrolytes, solutes and water absorption from the intestinal lumen. It could also mean that at high dose, the fraction would possibly inhibit the biosynthesis of prostaglandins as they are agents implicated in intestinal fluid accumulation.

CONCLUSION

The findings of the present study reveal that 20 % MLFC possesses antidiarrhoeal properties mediated through inhibition of gastrointestinal hypersecretion, gastrointestinal motility and reabsorption of intestinal fluid and electrolytes. These results lend scientific credence to the conventional use of *Cola hispida* leaves as a diarrhoea remedy, which complements the sustained investigation of tropical plants as possible nutraceuticals.

DECLARATIONS

Acknowledgement

The authors wish to acknowledge the laboratory/technical assistance received from Mr Agbeze Kenneth Ebiale.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. The study and its experimental design are purely the original concept of Dr UO Njoku. In addition, the manuscript was written by her. Sourcing of the plant material, collection of data and its statistical analysis were carried out by Mr MO Ogugofor. The manuscript was read by both authors and approved by them for publication.

Open Access

This is an Open Access article that uses a funding model which does not charge readers or

their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

REFERENCES

1. Njoku UO, Nwodo OFC, Ogugofor MO. Cardioprotective potential of methanol extract of *Costus afer* leaf on carbon tetrachloride-induced cardiotoxicity in albino rats. *AJPRHC* 2017; 9(2): 51-58.
2. Farombi EO, Fakoya A. Free radical scavenging and antigenotoxic activities of natural phenolics compounds. *Mol Nutr Food Res* 2005; 49: 39-44.
3. Himanshu BS, Rakesh S, Anjan K, Amrita B, Subrat KB. Antidiarrhoeal investigation of *Apium leptophyllum* (Pers.) by modulation of $\text{Na}^+\text{K}^+\text{ATPase}$, nitrous oxide and intestinal transit in rats. *Biomed J* 2016; 39(6): 376-381.
4. Palambo EA. Phytochemicals from traditional medicine plants used in treatment of diarrhea: Mode of action and effect on intestinal function. *Phytother Res* 2006; 20: 717-724.
5. Nigro O, Milton AG, Ratnaike RN. Drug-associated diarrhoea and constipation in older people. *Aust J Hosp Pharm* 2000; 30: 165-169.
6. WHO. Diarrhoeal disease: Maternal Child Epidemiology Estimation (WHO-MCEE), 2018 [cited 2021 Apr 23]. Available from <https://data.unicef.org/topic/child-health/diarrhoeal-disease/>
7. Forsberg BC, Gwatkin D, Tomson G, Allebeck P, Petzold MG. Socioeconomic inequalities in the prevalence and management of childhood diarrhoea: Potential health gains to be achieved. *Open Infect Dis J* 2009; 3: 44-49.
8. Burkill HM. The useful plants of West Tropical Africa. Families A-D. Royal Botanic Gardens Kew vol. 1. Charlottesville VA: University Press of Virginia; 1985; p 960.
9. Nworgu ZAM, Owolabi OJ, Amaechina FC, Falodun A, Ojo OD. In vitro inhibitory evaluation of *Cola hispida*, Brennan and Keay (Streculiaceae) on the isolated rat uterus. *Can J Appl Sci* 2009; 3(2): 809-811.
10. Institute of Laboratory Animal Resources (U.S.). Guide for the care and use of laboratory animals. Bethesda, MD, U.S.A: Dept. of Health and Human Services, Public Health Services, National Institutes of Health; 1985.
11. Awouters F, Niemegeers CJ, Lenaerts FM, Janssen PA. Delay of castor oil diarrhoea in rats: A new way to evaluate inhibitors of prostaglandin biosynthesis. *J Pharm Pharmacol* 1978; 30: 41-45.
12. Rahman MM, Islam AMT, Chowdhury MAU, Uddin ME, Jamil A. Antidiarrheal activity of leaves extract of *Microcos paniculata* Linn in mice. *Int J Pharm* 2012; 2(1): 21-25

13. Ezike AC, Akah PA, Okoli CO, Udemezue N, Okoro O. Experimental evaluation of antiulcer and spasmolytic potentials of leaves of *Cajanus cajan*. *Indian J Pharmacol* 2011; 3: 144-148.
14. Trinder P. A rapid method for the determination of sodium in serum. *Analyst* 1951; 76: 596-599.
15. Henry RJ, Canon DC, Winkelman JW. *Clinical Chemistry: Principles and Technics*, ed 2 Harper and Row, Hagerstown, M.D, 1974.
16. Tietz NW. *Fundamentals of Clinical Chemistry*, Philadelphia, PA: W.B Saunder Co; 1976; p 874.
17. Agbon AN, Kwaneshie HO, Oliver W. Antidiarrheal activity of aqueous fruit extract of *Phoenix dactylifera* (DATE PALM) in Wistar Rats. *Br J Pharmacol Toxicol* 2013; 4(3): 121-127.
18. Keely SJ, Montrose MH, Barrett KE. Electrolyte secretion and absorption: Small intestine and colon. In Yamada T, editor. *Textbook of Gastroenterology*. 5th Ed. Oxford: Wiley-Blackwell; 2009. p. 330–367.
19. Huijghebaert S, Awouters F, Tytgat GNJ. Racecadotril versus loperamide: Antidiarrheal research revisited. *Dig Dis Sci* 2003; 48(2): 239-250.