Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Original article doi: 10.12980/jclm.3.2015j5-111

Antidiarrheal effects of hydromethanolic extract of *Combretum dolichopetalum* leaves in mice

Samuel Okwudili Onoja*, Nkeiruka Emmanuela Udeh

Department of Veterinary Physiology, Pharmacology, Biochemistry and Animal Health and Production, College of Veterinary Medicine, Micheal Okpara University of Agriculture, PMB 7267, Umudike, Abia State, Nigeria

ARTICLE INFO

Article history:
Received 8 Jul 2015
Accepted 20 Oct 2015
Available online 9 Nov 2015

Keywords:
Antidiarrheal
Loperamide
Castor oil
Charcoal meal
Enteropooling

ABSTRACT

Objective: To evaluate the antidiarrheal activity of the hydromethanolic extract of *Combretum dolichopetalum* (*C. dolichopetalum*) leaves.

Methods: The antidiarrheal activity of the hydromethanolic extract of *C. dolichopetalum* leaves was evaluated by inducing diarrhea with castor oil, testing small intestinal motility and establishing enteropooling models in mice. Five groups of animals were used for each model and were treated as follows: Group A received 10 mL/kg of distilled water, and Group B received loperamide (5 mg/kg) while Groups C, D, E received 50, 100 and 200 mg/kg of hydromethanolic *C. dolichopetalum* extract, respectively.

Results: The pre-treatment of the mice with the extract (50, 100 and 200 mg/kg) caused a significantly dose-dependent decrease in the mean percentage of wet faeces ($P < 0.05$), compared with the negative control in diarrhea induced by castor oil. The extract reduced the distance travelled by the charcoal meal in the small intestine in a dose-dependent manner in the treated groups, compared with the negative control. The charcoal meal travelled 64.71%, 49.13%, 55.21%, 51.75% and 32.95% of the small intestine length in the groups treated with distilled water, loperamide, 50 mg/kg, 100 mg/kg and 200 mg/kg of *C. dolichopetalum* extract, respectively. The extract treatment produced a dose-dependent decrease in the mean small intestinal fluid volume, but there was no significant difference ($P > 0.05$), compared with the negative control.

Conclusions: The study shows that *C. dolichopetalum* leaves possess antidiarrheal activity and validate its use in ethnomedicine for that purpose.

1. Introduction

Combretum dolichopetalum Engl and Diels (*C. dolichopetalum*) belonging to the family Combretaceae is commonly known as "achicha nza" (food of the sun bird) in Igboland and "okoso" in Edo Nigeria[1]. It is a scandent shrub or forest liane of deciduous forest, and usually occurs along banks from Sierra Leone to West Cameroon[2]. The leaves and roots are extensively used in ethnomedical practices of many cultures. In Nigeria, the plant is used in the treatment of burns and gastrointestinal disorders[3]. In Ghana, an infusion of the leaves, roots and stem is used in the management of "garil" (Fula), a condition of "stomach stagger" in cattle[1]. The antiulcer, antihepatotoxic, trypanocidal, anti-inflammatory, antidiabetic, gastric antisecretory, smooth muscle relaxant and antispasmolytic activities of *C. dolichopetalum* have been reported by previous workers[1,3-7]. The presence of alkaloids, saponins, glycosides, flavonoids, tannins, steroids and terpenoids in *C. dolichopetalum* has been documented[1]. Our literature search did not yield any reports on the *in vivo* antidiarrheal activity of *C. dolichopetalum*, despite its extensive folkloric use for this purpose. The present study aimed at the investigation of the antidiarrheal activity of hydromethanolic extract of *C. dolichopetalum* leaves.

*Corresponding author: Samuel Okwudili Onoja, Department of Veterinary Physiology, Pharmacology, Biochemistry and Animal Health and Production, College of Veterinary Medicine, Micheal Okpara University of Agriculture, PMB 7267, Umudike, Abia State, Nigeria.
Tel: +2348030613032, +2348113140624
E-mail: samonreal@yahoo.com

2. Materials and methods

2.1. Plant collection and identification

The leaves of *C. dolichopetalum* were collected in May, 2014 from Umuahia North, Local Government, Abia State, Nigeria. They were identified by Mr A. O. Ozioko, a taxonomist at Bioresource Development and Conservation Programme, Enugu State, Nigeria. A voucher specimen catalogued MOUAU/VPP/2014/013 was deposited in the departmental herbarium for reference purposes.

2.2. Preparation of the plant material

The leaves of *C. dolichopetalum* were dried at room temperature

on a laboratory bench and pulverized into coarse powder. The powdered plant material was extracted by using cold maceration method in 80% methanol for 48 h with intermittent shaking at 3-hour interval. The extract was filtered by using Whatman No. 1 filter paper. The filterate was concentrated in a hot air oven at 40 ℃ and the hydromethanolic *C. dolichopetalum* extract was stored in a refrigerator at 4 ℃ until required for the experiment. The percentage yield was calculated by using the formula below:

$$\frac{\text{Weight of extracted material}}{\text{Weight of starting plant material}} \times \frac{100}{1}$$

2.3. Experimental animals

Sixty-five mice (28–34 g), sourced from the Laboratory Animal Unit of the Department of Veterinary Physiology, Pharmacology and Biochemistry, Michael Okpara University of Agriculture Umudike, Abia State, were used for the study. The animals were housed in aluminium cages at room temperature and under natural light/darkness cycles. The mice were supplied with clean drinking water and fed *ad libitum* with standard commercial pelleted grower feed (Vital feed®, Nigeria). The mice were acclimatized for two weeks prior to the study. They were maintained in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals and the experimental protocol was approved by the institution's ethical committee[8].

2.4. Oral acute toxicity study

The oral acute toxicity study of hydromethanolic *C. dolichopetalum* extract was determined by using "up and down" method as described by Organization for Economic Co-operation and Development[9].

2.5. Effect on castor oil-induced diarrhea

The modified method as described by Atta and Mouneir was adopted for the study[10]. Thirty mice were divided randomly into five groups of six mice each and were fasted for 16 h before the experiment. Group A received 10 mL/kg of distilled water, and Group B received loperamide (5 mg/kg) while Groups C, D, E received 50, 100 and 200 mg/kg of hydromethanolic *C. dolichopetalum* extract, respectively. About 0.3 mL of castor oil (Bell Sons and Co., Southport, England) was administered to all the animals 30 min after treatment. The animals were kept in individual cages with the floors lined with blotting papers, and the number of both normal and watery droppings was counted every hour over a period of 4 h. Mean number of the stools passed by the treated groups was compared with that of the control. The percentage of wet faeces was calculated as follows:

$$\frac{\text{Mean number of wet faeces}}{\text{Total number of faeces}} \times \frac{100}{1}$$

2.6. Effect of hydromethanolic C. dolichopetalum extract on intestinal motility

The method described by Vogel *et al.* was used in this experiment[11]. Thirty mice were randomly divided into five groups of six mice each and were fasted for 16 h before the experiment. Group A received 10 mL/kg of distilled water and Group B received loperamide (5 mg/kg) while Groups C, D, E received 50, 100 and 200 mg/kg of hydromethanolic *C. dolichopetalum* extract, respectively. The standard charcoal meal was administered to all the animals 1 h after treatment. The animals were sacrificed 30 min after administration of charcoal meal by cervical dislocation and the intestines were immediately isolated and ligated at the pyloric sphincter and ileocaecal junction. The small intestinal transit was expressed as percentage of distance travelled by the charcoal meal relative to the total length of the small intestine from the pyloric sphincter to the ileocaecal junction.

2.7. Effect of hydromethanolic C. dolichopetalum extract on enteropooling

The method of Hassan *et al.* was used[12]. Briefly, thirty mice were randomly divided into five groups of six mice each and were fasted for 16 h before the experiment.

Group A received 10 mL/kg of distilled water and Group B received loperamide (5 mg/kg) while Groups C, D, E received 50, 100 and 200 mg/kg of hydromethanolic *C. dolichopetalum* extract, respectively. One hour after the treatment, the animals were sacrificed by cervical dislocation, then were laparotomized and the intestines were immediately isolated and ligated at the pyloric sphincter and ileocaecal junction.

The small intestines were weighed, the content of each intestine was milked out, and the empty intestines were reweighed. The difference in weight between the full and empty intestines was recorded as the weight of the intestinal content.

2.8. Statistical analysis

Data obtained were presented as mean ± SEM and analyzed by using ANOVA. The variant mean was separated by the least significant difference of the different groups. Significance was accepted at the level of $P < 0.05$.

3. Results

3.1. Yield of the extract

The extraction of the plant material yielded 8.53% of dark green extract.

3.2. Oral acute toxicity study

The oral administration of hydromethanolic *C. dolichopetalum* extract was well tolerated at a high dose of 2 000 mg/kg, as no death and clinical signs of toxicity were observed throughout the 48-hour observation. Therefore, LD_{50} of the extract was greater than 2 000 mg/kg.

3.3. Effect on castor oil-induced diarrhea

The result of the antidiarrheal effects of hydromethanolic *C.*

dolichopetalum extract on castor oil-induced diarrhea was presented in Figure 1. The pre-treatment of the mice with the extract (50, 100 and 200 mg/kg) caused a significantly dose-dependent decrease in the mean percentage of wet faeces, compared with the negative control ($P < 0.05$).

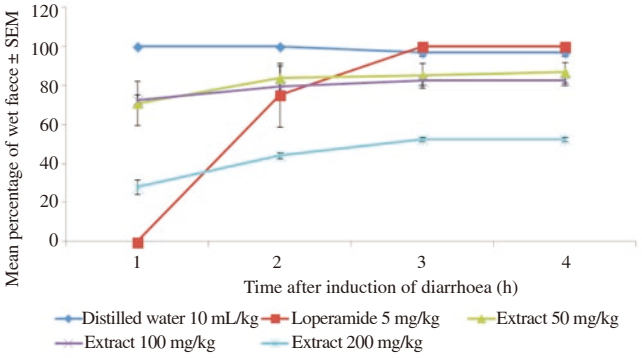

Figure 1. Effects of treatment with hydromethanolic *C. dolichopetalum* extract on castor oil-induced diarrhea in mice.

3.4. Effect on gastrointestinal motility

The extract (50, 100 and 200 mg/kg) and loperamide (5 mg/kg) produced a significant reduction in the distance travelled by the charcoal meal in the small intestine in a dose-dependent manner in the treated groups, compared with the negative control group treated with distilled water ($P < 0.05$). The charcoal meal travelled 49.13%, 55.21%, 51.75% and 32.95% of the small intestine length in the groups treated with loperamide, hydromethanolic *C. dolichopetalum* extract of 50, 100 and 200 mg/kg, respectively (Table 1).

Table 1

Effect of hydromethanolic *C. dolichopetalum* extract on intestine motility.

Treatment	% of small intestine travelled	% inhibition
Distilled water 10 mL/kg	64.71 ± 3.68	-
Loperamide 5 mg/kg	49.13 ± 3.71*	24.08
Extract 50 mg/kg	55.21 ± 2.38*	14.68
Extract 100 mg/kg	51.75 ± 2.68*	20.03
Extract 200 mg/kg	32.95 ± 4.57*	49.08

*: $P < 0.05$ when compared with distilled water-treated group.

3.5. Effect on small intestinal fluid volume

The extract treatment produced a dose-dependent decrease in the mean small intestinal fluid volume, but there was no significant difference, compared with the negative control ($P > 0.05$) (Table 2).

Table 2

Effect of hydromethanolic *C. dolichopetalum* extract on enteropooling.

Treatment	Volume of intestinal fluid (mL)	% inhibition
Distilled water 10 mL/kg	0.71 ± 0.18	-
Loperamide 5 mg/kg	0.63 ± 0.21	11.28
Extract 50 mg/kg	0.61 ± 0.38	14.08
Extract 100 mg/kg	0.55 ± 0.26	22.54
Extract 200 mg/kg	0.49 ± 0.17	30.99

4. Discussion

The antidiarrheal activity of the hydromethanolic extract of *C. dolichopetalum* leaves was evaluated by inducing diarrhea with castor oil, determining charcoal meal transit distance in small intestine and establishing enteropooling models in mice.

The extract was well tolerated by the mice and the LD_{50} was greater than 2000 mg/kg. This is in agreement with the report of Uzor *et al.*[1]. The result of the oral acute toxicity study is at variance with the report of Udem *et al.*[6]. They reported LD_{50} of 246 mg/kg for ethanolic extract of *C. dolichopetalum* root administered intraperitoneally. The variation in our reports may be due to the difference in the route of administration, vegetative part of the plant, weather condition and genetic make-up of the animals used[13-15].

Diarrhea occurs due to imbalance in the absorptive and secretory mechanism in the intestinal tract which involves motility and secretory functions[16]. Castor oil-induced diarrhea is a sensitive model for screening antidiarrheal agents[17,18]. Castor oil is a triglyceride of ricinoleic acid and its hydrolysis in the small intestine liberates the ricinoleic acid, which primarily acts on the small intestine to stimulate secretion of fluid, electrolyte and speed intestinal transit[19,20].

The extract significantly reduced castor oil-induced diarrhea ($P < 0.05$) (Figure 1), slowed the movement of the charcoal meal (Table 1) and reduced the volume of intestinal content in a dose-dependent manner (Table 2). The extract demonstrated potent antidiarrheal activity which could be mediated by the phytochemical constituents of the plant[21-23]. The antidiarrheal activity may be due to intestinal smooth muscle relaxant and antisecretory activities that have been reported by previous workers[3]. Intestinal smooth muscle relaxant and antisecretory agent reduces intestinal peristalsis and can be used as antidiarrheal drug[15,24-28]. Loperamide (standard antidiarrheal drug) slows gastrointestinal motility by affecting the circular and longitudinal muscles of the intestine, through interaction with the μ-opioid receptors in the intestine, and can be employed in the control of chronic diarrhea[29-31].

Another possible mechanism for the antidiarrheal effects of *C. dolichopetalum* may be through the inhibition of lipase and nitric oxide synthase activities. Agents that reduce the nitric oxide synthase and lipase activities can prevent the laxative effects of castor oil[32]. The extract also may have acted through the inhibition of prostaglandin biosynthesis. Secretion of endogenous prostaglandins is stimulated by ricinoleate and agents that inhibit prostaglandins biosynthesis are considered to delay castor oil-induced diarrhea[33,34]. Studies have shown that diarrheal effect of castor oil is due to the induction of contraction of intestinal smooth muscles mediated by the activation of E prostanoid 3 receptors on intestinal smooth muscles[32,35].

In conclusion, the extract demonstrated a potent antidiarrheal activity comparable to loperamide and validated the traditional use of the plant in diarrhea management. The plant is a useful source for the development of plant-based antidiarrheal drug and further work should be encouraged to identify the active principle and the mechanism of action.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors are grateful to Rev Fr Kingsley Ndubueze for plant

collection and Mr A. O. Ozioko for the identification of the plant sample.

References

[1] Uzor PF, Osadebe PO, Omeje EO, Agbo MO. Bioassay guided isolation and evaluation of the antidiabetic principles of *Combretum dolichopetalum* root. *Br J Pharm Res* 2014; **4**(18): 2155-71.

[2] Burkill HM. *The useful plants of west tropical Africa. Vol 5.* Kew: Royal Botanic Garden; 1985.

[3] Asuzu IU, Njoku JC. The pharmacological properties of the ethanolic root extract of *Combretum dolichopetalum*. *Phytother Res* 1992; **6**(3): 125-8.

[4] Asuzu IU, Adimorah RI. The antiinflammatory activity of extracts from the root of *Combretum dolichopetalum*. *Phytomedicine* 1998; **5**(1): 25-8.

[5] Asuzu IU, Onu OU. Anti-ulcer activity of the ethanolic extract of *Combretum dolichopetalum* roots. *Pharm Biol* 1990; **28**(1): 27-32.

[6] Udem SC, Madubunyi I, Asuzu IU, Anika SM. The trypanocidal action of the root extract of *Combretum dolichopetalum*. *Fitoterapia* 1996; **67**(1): 31-7.

[7] Udem SC, Madubunyi I, Okoye JOA, Anika SM. Anti-hepatotoxic effects of the ethanolic extracts of *Combretum dolichopetalum* root bark and *Morinda lucida* leaf. *Fitoterapia* 1997; **68**(1): 21-4.

[8] Committee for the Update of the Guide for the Care and Use of Laboratory Animals. *Guide for care and use of laboratory animals.* 8th ed. Washington D.C.: the National Academies Press; 2011.

[9] Organization for Economic Cooperation and Development. OECD guidelines for the testing of chemicals, acute oral toxicity - up-and down-procedure. No. 425. Paris: Organization for Economic Cooperation and Development; 2008. [Online] Available from: http://www.oecd-ilibrary.org/docserver/download/9742501e.pdf?expires=1437730561&id=id&accname=guest&checksum=0B6C86B8F1B2E751997E5F54CFDF8F5E [Accessed on 5th May, 2015]

[10] Atta AH, Mouneir SM. Antidiarrhoeal activity of some Egyptian medicinal plant extracts. *J Ethnopharmacol* 2004; **92**(2-3): 303-9.

[11] Vogel HG, Vogel WH, Schölkens BA, Sandow J, Műller G, Vogel WF. *Drug discovery and evaluation: pharmacological assays.* 2nd ed. Berlin: Springer-Verlag; 2002.

[12] Hassan KA, Brenda AT, Patrick V, Patrick OE. *In vivo* antidiarrheal activity of the ethanolic leaf extract of *Catharanthus roseus* Linn. (Apocyanaceae) in Wistar rats. *Afr J Pharm Pharmcol* 2011; **5**(15): 1797-800.

[13] Braide VB, Anika SM. *Environmental toxicology*. Enugu: SNAAP Press Ltd.; 2007.

[14] Evans WC. *Trease and Evans' pharmacognosy.* 16th ed. Edinburgh: Saunders Ltd.; 2009.

[15] Rang HP, Dale MM, Ritter JM, Moore RK. *Pharmacology.* 6th ed. Edinburgh: Churchill livingstone; 2007.

[16] Eke IG, Anaga AO. Anti-diarrheic properties of the aqueous methanolic extract of *Palisota hirsuta* leaves and its fractions in mice. *Int J Recent Sci Res* 2013; **4**(4): 410-4.

[17] Lin J, Puckree T, Mvelase TP. Anti-diarrhoeal evaluation of some medicinal plants used by Zulu traditional healers. *J Ethnopharmacol* 2002; **79**(1): 53-6.

[18] Ezekwesili JO, Nkemdilim UU, Okeke CU. Mechanism of antidiarrhoeal effect of ethanolic extract of *Psidium guajava* leaves. *Biokemistri* 2010; **22**(2): 85-90.

[19] Brunton LB, Parker KL, Lazo JS. *Goodman & Gilman's the pharmacological basis of therapeutics.* 11th ed. New York: McGraw-Hill Education/Medical; 2005.

[20] Sadraei H, Asghari G, Shams M. Antidiarrheal action of hydroalcoholic extract of *Pycnocycla spinosa* in comparison with loperamide and dicyclomine. *Iran J Pharm Res* 2011; **10**(4): 835-41.

[21] Daswani PG, Brijesh S, Tetali P, Antia NH, Birdi TJ. Antidiarrhoeal activity of *Zingiber officinale* (Rosc.). *Curr Sci* 2010; **98**(2): 222-9.

[22] Konaté K, Yomalan K, Sytar O, Brestic M. Antidiarrheal and antimicrobial profiles extracts of the leaves from *Trichilia emetica* Vahl. (Meliaceae). *Asian Pac J Trop Biomed* 2015; **5**(3): 242-8.

[23] Tadesse WT, Hailu AE, Gurmu AE, Mechesso AF. Experimental assessment of antidiarrheal and antisecretory activity of 80% methanolic leaf extract of *Zehneria scabra* in mice. *BMC Complement Altern Med* 2014; **14**: 460.

[24] Islam AMT, Uddin ME, Chowdhury MAU, Rahman MM, Habib MR, Rahman MA. *In vivo* antidiarrheal and cytotoxic potential of different fractions of *Pandanus foetidus* leaves. *Am J Biomed Sci* 2013; **5**(3): 208-16.

[25] Agunu A, Ahmadu AA, Afolabi SO, Yaro AU, Ehinmidu JO, Mohammed Z. Evaluation of the antibacterial and antidiarrhoeal activities of *Heeria insignis* O. Ktze. *Indian J Pharm Sci* 2011; **73**(3): 328-32.

[26] Franca CS, Menezes FS, Costa LC, Niculau ES, Alves PB, Pinto JE, et al. Analgesic and antidiarrheal properties of *Ocimum selloi* essential oil in mice. *Fitoterapia* 2008; **79**(7-8): 569-73.

[27] Pérez-Gutiérrez S, Zavala-Mendoza D, Hernández-Munive A, Mendoza-Martínez A, Pérez-González C, Sánchez-Mendoza E. Antidiarrheal activity of 19-deoxyicetexone isolated from *Salvia ballotiflora* Benth in mice and rats. *Molecules* 2013; **18**(8): 8895-905.

[28] Tanko Y, Alladey O, Ahmed MK, Mohammed A, Musa KY. The effect of methanol leaves extract of *Ficus glumosa* on gastrointestinal motility and on castor oil induced diarrhea in laboratory animals. *J Nat Prod Plant Resour* 2012; **2**(3): 360-7.

[29] Mazumdar S, Akter R, Talukder D. Antidiabetic and antidiarrhoeal effects on ethanolic extract of *Psidium guajava* (L.) Bat. leaves in Wister rats. *Asian Pac J Trop Biomed* 2015; **5**(1): 10-4.

[30] Tzeng TF, Liu IM, Cheng JT. Activation of opioid μ-receptors by loperamide to improve interleukin-6-induced inhibition of insulin signals in myoblast C_2C_{12} cells. *Diabetologia* 2005; **48**(7): 1386-92.

[31] Wintola OA, Sunmonu TO, Afolayan AJ. The effect of *Aloe ferox* Mill. in the treatment of loperamide-induced constipation in Wistar rats. *BMC Gastroenterol* 2010; **10**: 95.

[32] Al-Fartosy AJM. Some pharmacological studies on the methanolic extract of *Inula graveolense* L. *J Biomed Sci Eng* 2013; **6**: 1040-9.

[33] Palombo EA. Phytochemicals from traditional medicinal plants used in the treatment of diarrhea: modes of action and effects on intestinal function. *Phytother Res* 2006; **20**(9): 717-24.

[34] Viswanatha GL, Hanumanthappa S, Krishnadas N, Rangappa S. Antidiarrheal effect of fractions from stem bark of *Thespesia populnea* in rodents: possible antimotility and antisecretory mechanisms. *Asian Pac J Trop Med* 2011; **4**(6): 451-6.

[35] Tunaru S, Althoff TF, Nüsing RM, Diener M, Offermanns S. Castor oil induces laxation and uterus contraction via ricinoleic acid activating prostaglandin EP3 receptors. *Proc Natl Acad Sci U S A* 2012; **109**(23): 9179-84.