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

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**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 Cameroonto[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, antitheatropotoxic, trypanocidal, anti-inflammatory, anti-diabetic, gastric antisecretory, smooth muscle relaxant and antispasmytic 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.

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 filtrate was concentrated in a hot air oven at 4 °C and the hydromethanolic C. dolichopetalum extract was stored in a refrigerator at 4 °C until required for the experiment. The percentage yield was calculated by using the formula below:

\[
\text{Weight of extracted material} \times \frac{100}{\text{Weight of starting plant material}}
\]

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:

\[
\text{Mean number of wet faeces} \times \frac{100}{\text{Total number of faeces}}
\]

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 2000 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 2000 mg/kg.

3.3. Effect on castor oil-induced diarrhea

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

**Table 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% small intestine travelled</th>
<th>% inhibition</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water 10 mL/kg</td>
<td>64.71 ± 3.68</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loperamide 5 mg/kg</td>
<td>49.13 ± 3.71*</td>
<td>24.08</td>
<td></td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Extract 50 mg/kg</td>
<td>55.21 ± 2.38*</td>
<td>14.68</td>
<td></td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Extract 100 mg/kg</td>
<td>51.75 ± 2.68*</td>
<td>20.03</td>
<td></td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Extract 200 mg/kg</td>
<td>32.95 ± 4.57*</td>
<td>49.08</td>
<td></td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

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

### 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 2**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Volume of intestinal fluid (mL)</th>
<th>% inhibition</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water 10 mL/kg</td>
<td>0.71 ± 0.18</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loperamide 5 mg/kg</td>
<td>0.63 ± 0.21</td>
<td>11.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extract 50 mg/kg</td>
<td>0.61 ± 0.38</td>
<td>14.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extract 100 mg/kg</td>
<td>0.55 ± 0.26</td>
<td>22.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extract 200 mg/kg</td>
<td>0.49 ± 0.17</td>
<td>30.99</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 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).

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 enteroooling models in mice. The extract was well tolerated by the mice and the LD₅₀ 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 LD₅₀ 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 antiserotective activities that have been reported by previous workers[3]. Intestinal smooth muscle relaxant and antiserotective 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.

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References


