Antidiarrhoeal effect of the crude methanol extract of the dried fruit of *Adansonia digitata* L. (Malvaceae)

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**Abstract**

**Aim:** The study was designed to evaluate the antidiarrhoeal property of the methanol extract of the fruit of *Adansonia digitata* using laboratory animal models.

**Materials and Methods:** The acute oral toxicity (LD₅₀) of the extract was determined in mice, while the antidiarrhoeal effect of different doses of the extract was evaluated in both mice and rats. The effect of the extract at doses of 300, 700 and 1000 mg/kg were tested against intestinal transit time, magnesium sulphate-induced gastrointestinal motility test and castor oil-induced diarrhoea in mice. Similarly, the extract at the doses mentioned above was evaluated against castor-oil induced enteropooling in rats.

**Results:** The methanol extract of *A. digitata* was shown to have no toxic effect in mice at doses up to 5000 mg/kg. At doses of 300 and 700 mg/kg the extract significantly (p<0.01) decrease intestinal transit time in mice. In addition, the extract at doses of 300, 700 and 1000 mg/kg, significantly (p<0.05) lowered diarrhoea induced by magnesium sulphate and castor oil in mice. Similarly, the extract produced a significant (p<0.05) inhibition of enteropooling caused by castor oil in rats. The antidiarrhoeal effect of the extract was shown to be dose-dependent.

**Conclusion:** These findings revealed the potential of the extract of fruit of *A. digitata* as an antidiarrhoeal agent. The detailed mechanism of the extract antidiarrhoeal action is yet to be ascertained. However, one or more of the phytoconstituents contained in the extract could be responsible for the observed antidiarrhoeal effect.

**Keywords:** *Adansonia digitata* L., antidiarrhoeal activity, castor oil, charcoal meal, mice.

**Introduction**

Diarrhoeal disease is a potential cause of morbidity and mortality especially in children and young animals in the developing countries [1]. Traditional medicinal plants are widely employed in most developing countries for treating diarrhoea [2]. Treatment of diarrhoea is generally non-specific and usually aimed at reducing the discomfort and inconvenience of frequent bowel movements [3]. Most modern antidiarrhoeal agents are beyond the reach of the common man in developing countries. In addition, they have some levels of toxic effect [4,5]. To overcome the menace of diarrhoeal disease in developing countries, the World Health Organization (WHO) has included a programme for the control of diarrhoea, which involves the use of traditional herbal medicine [6].

*Adansonia digitata* L. (Malvaceae), popularly called baobab tree is widely distributed in hot savannah regions of sub-Saharan Africa. In India, powdered leaves are used to reduce excessive perspiration. The fruit is used internally with butter milk to manage cases of diarrhoea and dysentery. The efficacy of *A. digitata* on microbial infection had been demonstrated [7]. The plant has a variety of medicinal uses which have been well documented [8]. In Nigeria, the plant is found mostly in the northern part of the country. Aqueous extract prepared from the bark of the plant is used in Nigerian traditional medicine for the treatment of fever [9]. The aqueous extract of the stem-bark of *Adansonia digitata* produced a significant chemosuppressive effect against *Plasmodium berghei* infection in mice [10]. The powder obtained from the fruit of the plant is used traditionally to treat dysentery and diarrhoea in Nigeria [11].

The present study was aimed at investigating the potential antidiarrhoeal effect of the fruit of *A. digitata* L. in *in vitro* and *in vivo* model.

**Materials and Methods**

**Ethical approval:** All experimental protocols described in this study were approved by the Ethics Review Committee for Animal Experimentation of Ahmadu Bello University, Zaria (U05VM10341087).

**Plant collection, identification and processing:** The dried fruits of *A. digitata* were purchased from a local market in Zaria (Samaru), Nigeria and were identified...
Intestinal transit time in mice:

Experimental animals:
Animal experiments were performed in fifteen groups of five mice each. Animals were divided at random into five groups of five mice each. Before the start of the experiment all mice were fasted for 24 h. Animals in groups 1, 2, and 3 were dosed orally with the extract at 10, 100 and 1000 mg/kg, respectively. Animals in group 4 and 5 received loperamide (1 mg/kg) and normal saline (5 ml/kg), respectively. One h after treatment, all animals were given 5 ml/kg body weight of castor oil by mouth. Mice in each group were kept in individual cages whose floor was covered with a clean white paper. Faeces were collected on the paper for 8 h and the severity of diarrhoea in each group was graded as follows: Grade 0 (normal pelleted faeces), Grade 1 (discrete soft-formed faeces), Grade 2 (soft-formed faeces), Grade 3 (soft watery stool) and Grade 4 (watery stool) [16]. The diarrhoeal index was calculated by multiplying the number of mice in each grade by the number of grade divided by the number of mice in each group.

Effect of A. digitata on Castor oil-induced enteropooling:
Intraluminal fluid accumulation was determined by method described by Robert et al [17]. Rats were at divided at random into five groups of five animals each. Groups 1 and 2 were given normal saline (5 ml/kg) and loperamide (1 mg/kg) and served as non-treated and treated control animals, respectively. Animals in groups 3, 4 and 5 received the extract at 300, 700 and 1000 mg/kg, respectively. All treatments were administered orally. One h after the administration of normal saline, loperamide and extract, 2 ml of castor oil was given to each rat. Two hours after castor oil administration, the rats were sacrificed and the small intestine from the pylorus to the caecum was

Magnesium sulphate-induced gastrointestinal motility test:
Mice were divided at random into five groups of five mice each. All mice were deprived of food for 24 h before the experiment. Group 1 (untreated control) was given normal saline (5 ml/kg), while group 2 (treated control) received loperamide (1 mg/kg). Groups 3, 4 and 5 were administered with the extract of A. digitata at 300, 700 and 1000 mg/kg, respectively. All treatments were given per os. After 30 minutes, each mouse received magnesium sulphate (2 g/kg) by oral route [15]. The animals were placed individually in cages over white filter paper. The number of wet faeces was recorded for a period of 6 h.

Diarrhoea induced by castor oil in mice:
Twenty five mice were randomly assigned to five groups of five mice each. Before the start of the experiment all mice were fasted for 24 h. Animals in groups 1, 2, and 3 were dosed orally with the extract at 300, 700 and 1000 mg/kg, respectively. Animals in group 4 and 5 received loperamide (1 mg/kg) and normal saline (5 ml/kg), respectively. One h after treatment, all animals were given 5 ml/kg body weight of castor oil by mouth. Mice were kept in individual cages whose floor was covered with a clean white paper. Faeces were collected on the paper for 8 h and the severity of diarrhoea in each group was graded as follows: Grade 0 (normal pelleted faeces), Grade 1 (discrete soft-formed faeces), Grade 2 (soft-formed faeces), Grade 3 (soft watery stool) and Grade 4 (watery stool) [16]. The diarrhoeal index was calculated by multiplying the number of mice in each grade by the number of grade divided by the number of mice in each group.

Intestinal transit time in mice:
Twenty five mice were randomly distributed into five groups of five mice each. Animals were denied food for 24 h but allowed free access to water before the start of the experiment. Mice in groups 1, 2 and 3 received the extract orally at 300, 700 and 1000 mg/kg body weight, respectively. Animals in groups 4 and 5 received loperamide (1 mg/kg) and normal saline (5 ml/kg), respectively. All treatments were given per os. One hour after treatment, each mouse was given 1 ml of 5% charcoal meal in 2% carboxymethylcellulose orally [14]. An h after the charcoal meal was administered, all the mice were sacrificed in a closed chamber with the aid of chloroform. Mice were placed on dorsal recumbency on a dissecting board and a ventral mid-line incision was made on their abdomens, the intestines were exteriorized and carefully removed from the cardia to the ileocaecal junction. The intestines were immediately immersed in formalin to stop peristalsis and subsequently washed carefully using clean tap water to clearly see the intestinal contents. The distance traversed by the charcoal meal in each mouse was measured using a graduated ruler.

Phytochemical analysis:
The methanol extract of the A. digitata was subjected to phytochemical screening tests to detect the presence of carbohydrates, anthraquinones, flavonoids, tannins, alkaloids, saponins, glycosides, sterols and triterpenes [12].

Experimental animals:
Adult Swiss albino mice of both sexes weighing about 18-22 g and albino rats weighing between 150-180 g were used for the study. The Animals were sourced from the Animal House, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. The animals were housed in locally constructed cages and kept under room temperature. All animals were preconditioned in the laboratory for two weeks before the commencement of the experiment. Wood shavings were used as beddings in the animal cages and changed once every week. Animals were allowed free access to standard feed and water ad-libitum. The animals were kept at an ambient temperature of 25 °C and relative humidity of 70-80% with 12 h/day light period.

Safety evaluation:
The method of Lorde [13] was used to determine the median lethal dose (LD₅₀) of the extract. In the first part of the trial, 9 Swiss albino mice were randomly allocated into three groups of three mice each. The animals were deprived of food for 24 h and water for 12 h. Groups 1, 2, and 3 received the extract at 10, 100 and 1000 mg/kg body weight, respectively. Mice were observed for signs of toxicity or mortality for 48 h. The second part of the trial involved the use of 3 mice which were randomly allocated into 3 groups of 1 animal each. Mice in groups 1, 2, 3 were given the extract at 1600, 2900 and 5000 mg/kg, respectively, and observed as described earlier.

and authenticated in the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. A voucher specimen number 2512 was deposited. The dried fruit was separated from the seed by gentle manual squeezing and made into fine powder. The powdered fruit (200g) was cold extracted using methanol (600 ml) in a separating funnel. The mixture was left to stand for 48 h before decanting the liquid extract. The process was repeated using 300 ml of fresh methanol and allowed to stand for 24 h before decantation. The liquid extracts were pooled and concentrated into a brown-waxy residue.

The animals were placed individually in cages over white filter paper. The number of wet faeces was recorded for a period of 6 h.

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isolated. The content of the intestines from each rat was collected in a graduated glass tube and the volume measured. Values obtained were expressed as mean ± S.E.M and then subjected to one-way analysis of variance (ANOVA) followed by Tukey’s post-hoc test using Graphpad Prism version 4.0 for Windows from Graphpad software, San Diego, California, USA. Values were considered significant if p < 0.05.

The plant gave a yield of 23.6% of the dried crude extract. The content of the intestines from each rat was collected in a graduated glass tube and the volume measured.

**Statistical analysis:** Values obtained were expressed as mean ± S.E.M and then subjected to one-way analysis of variance (ANOVA) followed by Tukey’s post-hoc test using Graphpad Prism version 4.0 for Windows from Graphpad software, San Diego, California, USA. Values were considered significant if p < 0.05.

**Results**

**Extract yield:** The plant gave a yield of 23.6% of the dried crude extract.

**Phytochemical tests:** The methanol extract of dried fruit of *A. digitata* tested positive for carbohydrate, glycosides, saponins, flavonoids, tannins, steroids and triterpenes.

**Acute toxicity test:** The extract did not produce any mortality or apparent toxic signs when tested at doses of between 10 to 5000 mg/kg.

**Effect of *A. digitata* on intestinal transit:** The extract at doses of 700 and 1000 mg/kg significantly (p<0.01) decreased intestinal transit time in mice (Figure-1).

**Magnesium sulphate-induced gastrointestinal motility test:** The extract at doses of 700 and 1000 mg/kg and loperamide (5mg/kg) significantly (p<0.001) inhibited the frequency of defaecation relative to the untreated control mice. Similarly, at the dose of 300 mg/kg, the extract produced significant (p<0.01) inhibition of diarrhoea caused by magnesium sulphate when compared with normal saline (untreated control). The extract produced an inhibition of diarrhoea caused by magnesium sulphate of between 36-75% (Table-1).

**Effect of *A. digitata* on castor oil-induced diarrhoea:** The extract at doses of 300, 700 and 1000 mg/kg protected the mice against castor oil-induced diarrhoea in a dose-dependent fashion (Figure-2).

**The effect of *A. digitata* on castor oil-induced enteropooling:** The extract at oral doses of 300 and 700 mg/kg significantly (p<0.05) inhibited castor oil-induced enteropooling in rats, in addition, the extract at 1000 mg/kg produced a more significant (p < 0.01) inhibition of enteropooling caused by castor oil (Table-2). The standard drug, loperamide (5 mg/kg), also significantly inhibited (p<0.01) intestinal fluid accumulation produced by castor-oil.

**Discussion**

The significance of plants in medicine has led to several scientific and ethnopharmacological studies that resulted in the discovery of many pharmacological properties in plants [18]. Several studies have confirmed the biological effects attributed to medicinal plants and the best way to utilize them [19]. Most of the newer drugs that were discovered in the last few decades originated from natural sources [20]. The use of
A. digitata reverses the pathological changes induced by castor oil. It is possible that the extract and enteropooling. The effect of the extract at doses of 700 mg/kg and 1000 mg/kg significantly (p<0.001) reduced the length traversed by charcoal meal in the gastro-intestinal tract of mice. Similarly, the extract attenuated the severity of castor oil-induced diarrhoea in a dose-dependent manner. The liberation of ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which stimulate gastrointestinal motility and secretion [25]. Furthermore, castor oil alters intestinal absorption causing decreased permeability of dissolved food and also the histology of the gastro-intestinal (GI) mucosa; that allows increased fluid loss across intestinal epithelial cells [25]. These findings indicate that the extract inhibits castor oil-induced diarrhoea and enteropooling. The effect of the extract at doses of 1000 mg/kg is similar to that of loperamide (standard antidiarrhoeal agent). It is possible that the extract reverses the pathological changes induced by castor oil.

The diarrhoeagenic effect of magnesium sulphate was significantly reduced (p<0.05) by the extract. In addition, detailed toxicity studies are required to understand the mechanism of its antidiarrhoeal action. However, further studies are needed in order to support its use in the traditional management of diarrhoea. Therefore, further studies are needed in order to understand the mechanism of its antidiarrhoeal action and also to identify the bioactive component(s) of the extract. In addition, detailed toxicity studies are required to justify its clinical use.

### Table-2: Effect of the extract of *Adansonia digitata* on castor oil-induced enteropooling in rats (n=5).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg) (oral)</th>
<th>Volume of intestinal fluid (ml) (mean ± S.E.M.)</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>5 ml/kg</td>
<td>4.64 ± 0.50</td>
<td>–</td>
</tr>
<tr>
<td>Loperamide</td>
<td>5</td>
<td>1.42 ± 0.26**</td>
<td>69.40</td>
</tr>
<tr>
<td><em>A. digitata</em></td>
<td>300</td>
<td>2.48 ± 0.23*</td>
<td>47.01</td>
</tr>
<tr>
<td><em>A. digitata</em></td>
<td>700</td>
<td>2.08 ± 0.21*</td>
<td>55.17</td>
</tr>
<tr>
<td><em>A. digitata</em></td>
<td>1000</td>
<td>1.80 ± 0.36**</td>
<td>61.21</td>
</tr>
</tbody>
</table>

* = p < 0.05; ** = p < 0.01, when compared with the non-treated (normal saline) control group.

Results from effects on gut smooth muscle (enhancement of transit) and mucosal electrolyte transport [26]. The inhibition of electrolyte absorption or stimulation of secretion causes the accumulation of fluid in the gut lumen [27].

**Conclusion**

From the present study, it can be concluded that the methanol extract of the fruit of *A. digitata* contains compound(s) with antidiarrhoeal property. This finding supports its use in the traditional management of diarrhoea. However, further studies are needed in order to understand the mechanism of its antidiarrhoeal action and also to identify the bioactive component(s) of the extract. In addition, detailed toxicity studies are required to justify its clinical use.

**Authors’ contributions**

MMS, IH and SG designed the study and collected materials for the experiments. MMS, MM and MUK drafted and revised the manuscript. MMS and PIK guided the research work. All authors read and approved the final manuscript.

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**Competing interests**

The authors declare that they have no competing interests.

**References**


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