Effect of *Aframomum melegueta* Seed Extract on Castor Oil–Induced Diarrhea

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Abstract

The effect of the aqueous (aq) seed extract of *Aframomum melegueta* K. Schum. (Zingiberaceae) on castor oil-induced diarrhea, intestinal fluid secretion, and gastrointestinal transit was investigated in the study. Castor oil (10 ml/kg, p.o.) induced copious diarrhea in all rats 3 h after treatment. Furthermore, it produced a significant increase in the volume of intestinal fluid secretion in rats and also enhanced intestinal transit in mice. The aq seed extract of *A. melegueta* (100–500 mg/kg, p.o.) offered significant protection against diarrhea induced by the oil. At a dose range of 250–500 mg/kg, the extract reduced significantly the volume of fluid secretion in castor oil–treated rats. At these doses, it also demonstrated a significant antitransit activity in a dose-related manner. Acetylsalicylic acid (100 mg/kg, p.o.) delayed diarrhea and reduced the number of animals with diarrheal droppings to 20%. At the same dose level, acetylsalicylic acid reduced significantly the volume of intestinal fluid secretion but lacked antitransit property in castor oil–treated animals. *N*-Nitro-l-arginine methyl ester (l-NAME) (2.5–10 mg/kg, i.p.) dose-dependently reduced the number of animals with diarrhea. At 50 mg/kg i.p., it offered 100% protection against diarrhea induced by the oil. Furthermore, l-NAME (10 mg/kg, i.p.) significantly inhibited both the intestinal fluid secretion and gastrointestinal transit induced by castor oil. However, l-NAME (10 mg/kg, i.p.) did not significantly modify the antidiarrheal effect of *A. melegueta*. l-Arginine, a substrate of nitric oxide synthase or isosorbide dinitrate, a nitric oxide donor, did not alter the effect of *A. melegueta* on diarrhea. Ascorbic acid (100 mg/kg, p.o.) and r-tocopherol (20 mg/kg, p.o.) reduced the number of animals with diarrhea to 80% and 70%, respectively. However, they both lacked significant activities on intestinal fluid secretion and gastrointestinal transit induced by castor oil. The combination of ascorbic acid (100 mg/kg, p.o.) or r-tocopherol (20 mg/kg, p.o.) with *A. melegueta* (500 mg/kg) offered higher protection against diarrhea than the extract alone. Considering these results together, it may be inferred that *Aframomum melegueta* seed extract may be a useful antidiarrheal agent.

Keywords: *Aframomum melegueta*, antidiarrheal, castor oil, inhibition, prostaglandins.

Introduction

The seeds of *Aframomum melegueta* K. Schum. (Zingiberaceae) are widely used as condiments as well as a flavoring for dishes in Nigeria (Enyikwola, 1994). Various parts of this plant have been claimed to possess medicinal properties. The seeds are used for diarrhea, stomachache, as a carminative, and for inflammatory painful conditions (Kokwaro, 1976; Houghton & Osibogun, 1993; Rafatullah et al., 1995). The natives of Kenya used the roots as a remedy for dysentery or snake bite (Kokwaro, 1976). The juice extracted from the stems and leaves are used by the Gabonese for wound and intestinal infections (Akendengue & Louis, 1994). Experimental studies have demonstrated its antiulcer, cytoprotective, and antimicrobial properties (Rafatullah et al., 1995; Galal, 1996). Our earlier investigations revealed that the aqueous (aq) seed extract of *A. melegueta* possesses anti-inflammatory activity (Umukoro & Ashorobi, 2001). A survey of literature revealed absence of detailed experimental studies that suggest the use of this plant in diarrhea. In view of this, the current study sought to investigate the effect of the aqueous

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Antidiarrheal effect of *Aframomum melegueta* seed extract of *Aframomum melegueta* on diarrhea, intestinal fluid secretion, and gastrointestinal transit induced by castor oil.

**Materials and Methods**

**Animals**

Albino rats of both sexes (150–250 g) and albino mice of both sexes (18–25 g) used in the study were purchased from the Laboratory Animals Center, College of Medicine, University of Lagos, Nigeria. They were fasted for 18 h before the commencement of the experiment.

**Drugs**

Acetylsalicylic acid (Sigma, St. Louis, MO, USA), α-tocopherol (Teva Pharmaceuticals Ltd, Israel), L-arginine (Sigma), isosorbide dinitrate (Ayerst Laboratories Inc. USA) and N^G^-nitro-L-arginine methyl ester (Sigma) were used in the study.

**Preparation of the extract**

The dried fruits of *A. melegueta* were obtained from Mushin Market, Lagos, Nigeria, and identified by Prof. J.D. Olowokudejo of the Department of Botany, University of Lagos, Nigeria. A voucher specimen of the fruits was deposited in the herbarium of the Department of Pharmacognosy, College of Medicine, University of Lagos, Nigeria. The seeds were ground into fine powder, and 150 g of the powered seeds was soaked in 500 ml of distilled water for 48 h. This was kept in the refrigerator at 5°C and was stirred periodically. Thirty minutes after removal from the refrigerator, the solution was filtered and the filtrate was evaporated to dryness at less than 40°C. The yield of the extract was 10.6% with reference to the powdered seeds. On any experimental day, 500 mg of the residue was dissolved in 10 ml of distilled water and ready for use.

**Castor oil diarrhea**

The castor oil test was performed as described by Awounters et al. (1978). Adult rats of both sexes were pretreated orally with acetylsalicylic acid (100 mg/kg), ascorbic acid (50–100 mg/kg), α-tocopherol (10–20 mg/kg), *A. melegueta* (100–500 mg/kg), normal saline and L-NAME (2.5–50 mg/kg), individually, 30 min before oral administration of 10 ml/kg of castor oil. One hour later and each hour for 3 h, the individual rat cages were examined for the presence or absence of characteristic diarrheal droppings. Absence of diarrheal dropping was recorded as a positive result, indicating protection. In the interaction studies, the animals (6 rats/group) were pretreated with *A. melegueta*(500 mg/kg, p.o.) 30 min before administration of either L-arginine (200 mg/kg, i.p.), isosorbide dinitrate (100 mg/kg, p.o.), ascorbic acid (100 mg/kg, p.o), α-tocopherol (20 mg/kg, p.o.) or L-NAME (2.5 mg/kg, i.p.). Thirty minutes later, the animals were challenged with castor oil, and the presence or absence of diarrhea was assessed as described above.

**Intestinal fluid volume**

The intraluminal fluid accumulation was carried out according to the method of Robert et al. (1976). The animals (6 rats/group) were pretreated with either acetylsalicylic acid (100 mg/kg, p.o.), *A. melegueta* (100–500 mg/kg, o.p.), L-NAME (10 mg/kg, i.p.), ascorbic acid (100 mg/kg, p.o.) or α-tocopherol (20 mg/kg, p.o.), 30 min before oral administration of 10 ml/kg of castor oil. Thirty minutes later, the animals were killed, the small intestine was removed, its contents were collected, and the volume was measured. Control group received normal saline alone.

**Small intestinal transit (SIT)**

The animals (8 mice/group) were pretreated with either L-NAME (10 mg/kg, i.p.), *A. melegueta* (100–500 mg/kg, kg, p.o.), α-tocopherol (20 mg/kg, p.o.), or acetylsalicylic acid (100 mg/kg, p.o.). Thirty minutes later, the animals were given a mixture of 10 ml/kg of castor oil and black ink as described by Ruvart et al. (1980). The animals were killed 30 min after by cervical dislocation, and the gastrointestinal tract was rapidly removed. The distance traveled by the marker was measured and expressed as percentage of total length of the intestine from the pylorus to caecum.

**Statistical analysis**

The χ² test was used to determine the significance between groups with or without diarrhea. Small intestinal transit and intestinal fluid volume were expressed as mean ± SEM and compared by using Student’s t-test.

**Results**

**Castor oil diarrhea**

Castor oil produced copious diarrhea in all the animals at the 3-h interval (Table 1). However, *A. melegueta* (100–500 mg/kg) significantly (p<0.05) protected the animals against diarrhea induced by the oil (Table 1). L-NAME (2.5–10 mg/kg) dose-dependently reduced the number of rats with diarrhea with 100% protection at a dose of 50 mg/kg (Table 1). Furthermore, acetylsalicylic acid (100 mg/kg) delayed the onset of diarrhea and
Table 1. Effect of *Aframomum melegueta* on castor oil–induced diarrhea.

<table>
<thead>
<tr>
<th>Drug and dose (mg/kg)</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline)</td>
<td>40</td>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td><em>A. melegueta</em> (100)</td>
<td>20</td>
<td>40</td>
<td>60*</td>
</tr>
<tr>
<td><em>A. melegueta</em> (250)</td>
<td>20</td>
<td>40</td>
<td>40*</td>
</tr>
<tr>
<td><em>A. melegueta</em> (500)</td>
<td>20</td>
<td>30*</td>
<td>40*</td>
</tr>
<tr>
<td>Acetylsalicylic acid (100)</td>
<td>0*</td>
<td>0*</td>
<td>20*</td>
</tr>
<tr>
<td>t-NAME (2.5)</td>
<td>20</td>
<td>40</td>
<td>70</td>
</tr>
<tr>
<td>t-NAME (10)</td>
<td>0*</td>
<td>0*</td>
<td>20*</td>
</tr>
<tr>
<td>t-NAME (50)</td>
<td>0*</td>
<td>0*</td>
<td>0*</td>
</tr>
<tr>
<td>Ascorbic acid (50)</td>
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<td>50</td>
<td>80</td>
</tr>
<tr>
<td>Ascorbic acid (100)</td>
<td>20</td>
<td>50</td>
<td>80</td>
</tr>
<tr>
<td>a-Tocopherol (10)</td>
<td>20</td>
<td>40</td>
<td>70</td>
</tr>
<tr>
<td>a-Tocopherol (20)</td>
<td>20</td>
<td>40</td>
<td>70</td>
</tr>
<tr>
<td><em>A. melegueta</em> (500) + ascorbic acid (100)</td>
<td>0</td>
<td>10</td>
<td>10*</td>
</tr>
<tr>
<td><em>A. melegueta</em> (300) + a-tocopherol (20)</td>
<td>0</td>
<td>0</td>
<td>10*</td>
</tr>
<tr>
<td><em>A. melegueta</em> (500) + t-arginine (100)</td>
<td>20</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td><em>A. melegueta</em> (500) + isosorbide dinitrate (100)</td>
<td>20</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td><em>A. melegueta</em> (500) + t-NAME (2.5)</td>
<td>10</td>
<td>20</td>
<td>30</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SEM from 6 to 10 animals in each group.

*p < 0.05 compared to saline control group. **p < 0.05 compared to castor oil group (Student’s t-test).

Reduced significantly the number of rats with diarrheal droppings (Table 1). The role of ascorbic acid and α-tocopherol in castor oil–induced diarrhea was investigated in the study. The experimental data revealed that these agents slightly modify the diarrheagenic action of castor oil (Table 1). In the interaction studies, the results revealed that no significant difference in inhibitory action of *A. melegueta* (500 mg/kg) on castor oil–induced diarrhea (Table 1) was shown by t-NAME (2.5 mg/kg), isosorbide dinitrate (100 mg/kg), or t-arginine (200 mg/kg). In contrast, ascorbic acid (100 mg/kg) and α-tocopherol (20 mg/kg) significantly (p < 0.05) enhanced the ability of the extract to inhibit castor oil diarrhea (Table 1).

**Intestinal fluid volume**

*A. melegueta* (250–500 mg/kg) dose-dependently and significantly reduced castor oil–induced intestinal fluid secretion in rats (Table 2). Furthermore, both acetylsalicylic acid (100 mg/kg) and t-NAME (10 mg/kg) reduced significantly the volume of intestinal fluid produced by castor oil. However, ascorbic acid (100 mg/kg) or α-tocopherol (20 mg/kg) did not significantly alter the volume of intestinal fluid secretion caused by castor oil (Table 2).

**Intestinal transit**

*A. melegueta* (100–500 mg/kg) dose-dependently reduced intestinal transit in castor oil–treated mice (Table 2). Furthermore, t-NAME (10 mg/kg) significantly inhibited (p < 0.05) castor oil–induced intestinal transit in mice (Table 2). However, acetylsalicylic acid (100 mg/kg), α-tocopherol (20 mg/kg), and ascorbic acid did not demonstrate significant antitransit activity in castor oil–treated animals (Table 2).

**Discussion**

The results of the current study demonstrate that the aq seed extract of *A. melegueta* possesses antidiarrheal activity in castor oil–treated animals. The extract protected the animals against diarrhea as well as reduced the volume of intestinal fluid secretion induced by the oil. Furthermore, it inhibited castor oil–induced intestinal transit in a dose-related manner. Previous studies carried out by Tackie et al. (1975) revealed the presence of 6-paradol, 7-paradol, 6-shogoal, gingerol, and zingerone as the active chemical constituents in the seed of this plant. These chemical substances have been shown to be responsible for the biological activities demonstrated by *A. melegueta* (Rafatullah et al., 1995; Galal, 1996; Umukoro & Ashorobi, 2001). We have in our earlier study suggested that the anti-inflammatory activity demonstrated by *A. melegueta* was due to the inhibition of prostaglandin biosynthesis (Umukoro & Ashorobi, 2001). It is our suggestion that the antidiarrheal action exerted by the extract may also be related to the inhibition of prostaglandin formation. This suggestion is validated by the facts that castor oil–induced diarrhea...
is related to the release of prostaglandin by the colonic cells (Capasso et al., 1986; Mascolo et al., 1994). Furthermore, the delay of castor oil-induced diarrhea and inhibition of intestinal fluid secretion have been shown to characterize nonsteroidal anti-inflammatory drugs (Robert et al., 1976; Awounters et al., 1978; Singh et al., 1996). Moreover, Van Loon et al. (1992) reported that indomethacin reduced intestinal fluid secretion and inhibited the luminal release of prostaglandin E in patients with acute diarrhea. Awounters et al. (1978) tested 44 aspirin-like drugs and observed a positive correlation in their ability to prevent diarrhea induced by castor oil and inhibition of inflammation caused by carrageenan.

The results of our study also revealed the ability of aspirin to inhibit diarrhea and reduce intraluminal fluid secretion caused by castor oil, which indicate possible inhibition of prostaglandin synthesis. However, aspirin did not significantly inhibit intestinal transit induced by castor oil. This observation suggests that the effect of prostaglandin on gastrointestinal motility, unlike its secretory actions, plays little or no role in the production of watery diarrhea.

Further studies have also shown that castor oil causes the release of nitric oxide (NO), which in turn provokes the generation of prostaglandin by colonic cells thereby worsening the diarrheal conditions (Mascolo et al., 1994). Accordingly, L-NAME, an inhibitor of nitric oxide synthesis, prevented diarrhea and reduced the intraluminal fluid secretion induced by the oil. It further inhibited the increased intestinal transit caused by castor oil. In similar studies carried by Mascolo et al. (1994), L-arginine, a substrate of nitric oxide, and isosorbide dinitrate, a nitric oxide donor, reversed the actions of L-NAME in castor oil–treated animals. Thus, the role of nitric oxide in the diarrheagenic actions of castor oil was confirmed. However, the results of the study showed that L-arginine, isosorbide dinitrate, or L-NAME did not significantly modify the effects of A. melegueta on castor oil–induced diarrhea.

Several studies have shown that ascorbic acid and α-tocopherol reduce prostaglandin concentrations possibly through inhibition of peroxidation of phospholipids (Stickel et al., 1997; Child et al., 1999). Considering the role of prostaglandin in the regulation of intestinal fluid secretion, these antioxidant nutrients may offer beneficial effects in castor oil–induced diarrhea. Our results revealed that these agents slightly reduced the efficacy of castor oil to evoke copious diarrhea. Moreover, ascorbic acid or α-tocopherol delayed the onset of diarrhea in the animals pretreated with the extract before castor oil challenge. The protective ability of A. melegueta against diarrhea was enhanced by ascorbic acid and α-tocopherol, respectively. This finding further reinforces the possibility that A. melegueta exerts its anti diarrheal action through inhibition of prostaglandin formation.

In conclusion, the current study provides evidence that supports the use of Aframomum melegueta seed extract in diarrheal conditions.

References


