Effect of aqueous leaf extract of *Irvingia gabonensis* on gastrointestinal tract in rodents

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Effect of the aqueous leaf extract of *I. gabonensis* on the gastrointestinal tract was investigated on isolated rabbit jejunum, guinea pig ileum, gastrointestinal motility, castor oil-induced diarrhoea in mice and castor oil-induced fluid accumulation in rats. The results showed that the extract exhibited a concentration-dependent relaxation of spontaneous pendular movement of isolated rabbit jejunum and guinea pig ileum, and attenuated both acetylcholine-induced contraction of rabbit jejunum and histamine-induced contraction of guinea pig ileum. The extract (100, 200 and 400 mg/kg) also caused a significant dose-dependent decrease of gastrointestinal motility in mice (40.12, 39.45 and 37.45 %), intestinal fluid accumulation in rats (71.43, 81.63 and 83.27 %), and remarkably protected mice against castor oil-induced diarrhoea [58.33, 75 and 91.67 % (Di Carlo score)] respectively. Preliminary phytochemical screening of the aqueous leaf extract of *I. gabonensis* revealed the presence of saponins, tannins, phenols and phlobatans.

**Keywords**: Diarrhoea, Gastrointestinal tract, *Irvingia gabonensis*

**IPC Code**: Int. Cl. 7, A 61 P

Diarrhoea is a condition characterized by an abnormal increase in liquidity, frequency of defecation and stool weight exceeding 200 g daily and containing 60-95 % water. It is a common disease in tropics and in some parts of the world. It is a prime cause of illness and mortality especially in infants and children. Diarrhoea occurs at all ages of human, but its incidence is higher in children between 6 month and 3 year. On a worldwide basis, 750 million cases are reported in children below 5 years in Asia, Latin America and Africa resulting in 4-5 million deaths. However, there are seasonal variations in the incidence of diarrhoea. In an effort to tackle the problems of diarrhoea, the World Health Organization (WHO) has established a diarrhoea disease control programme (DDC) which includes studies of traditional medicinal practices together with the evaluation of health education and prevention approaches. In most parts of the developing countries, particularly Africa, the use of herbal remedies in management of diarrhoea is a common practice. *Irvingia gabonensis* (Simaroubaceae) is a tree plant popularly known as wild or African Mango. The plant occurs freely in many parts of Africa. Local names include goron biri (Hausa), Ogbono (Ibo). The plant is commonly used as food supplement. In Nigeria, oily seeds of the plant are used in soup as a seasoning for various native dishes.

In addition, a decoction of the leaf is employed in southern Nigeria to treat diarrhoea (personal communication/oral testimony). Recently, Raji et al. have reported anti-ulcer properties of *I. gabonensis*. The present study was designed to evaluate the antidiarrhoeal effect of *I. gabonensis*.

**Materials and Methods**

Animals—New Zealand rabbits (1.5-3.0 kg), Swiss albino mice (18-25g), Adult Wistar rats (180-220g) and adult guinea pigs (300-400g) of either sex were maintained at the Animal Facility Centre, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja. These animals were fed with standard diet (Ladokun Feeds Ltd, Ibadan) and given water *ad libitum*.

Experimental procedures were conducted following the Principles of laboratory animal care (NIH Publication 85-23, revised 1985), as adopted by the
Ethical review committee of National Institute for Pharmaceutical Research and Development

**Plant material—**Leaves of *Irvingia gabonensis* were collected from Etim Osam, Akpabuyo, Cross River State, Nigeria in September 2002. The plant was identified and authenticated by Mallam Ibrahim Muazzam and Grace Ugbabe of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja. A voucher specimen of the plant 5395 was deposited at NIPRD herbarium.

**Preparation of extract—**The freshly collected leaves of *I. gabonensis* were washed with water and dried under sun. The dried leaves were ground using a mechanical grinder. The powder (84 g) was then cold macerated with 1.5 l of water for 24 hr with continuous shaking using GMB shaker. The extract was filtered through Whatman paper (No. 1) and the filtrate was dried completely over water bath to give a solid residue (14.29% w/w yield).

**Drug—**Acetylcholine chloride, histamine, atropine, and castor oil were obtained from Sigma Chemical Company, USA; mepyramine from May & Baker Pharmaceuticals, Nigeria.

**Phytochemical tests—**The freshly prepared extract was subjected to a standard phytochemical screening test for various constituents. The extract was screened for the presence of alkaloids, glycosides, saponins, tannins, flavonoids and phlobatannins etc using conventional protocols.

**Studies on rabbit jejunum—**The rabbits were killed by a blow to the head, exsanguinated and their abdomens were opened. Segments of the jejunum about 2-3 cm long were removed and dissected free of adhering mesentery. The intestinal content was removed by flushing with Tyrode’s solution comprising (mM) - NaCl, 136.8; KCl, 2.7; CaCl₂, 1.3; NaHCO₃, 12.0; MgCl₂, 0.5; Na₂PO₄, 0.14; and glucose, 5.5. The tissue was mounted in organ bath (20 ml) containing Tyrode solution at 37°C±1°C aerated with O₂ (95%) and CO₂ (5%). This solution was comprised (mM) - NaCl, 136.8; KCl, 2.7; CaCl₂, 1.3; NaHCO₃, 12.0; MgCl₂, 0.5; Na₂PO₄, 0.14; and glucose, 5.5. The initial tension was 0.5g. An equilibration period (60min) was allowed during which the physiological solution was changed for every 15min. At the end of the equilibration period, effect of graded concentrations of histamine, extract and mepyramine were investigated. Contact time for each concentration of drug was 60 sec, which was followed by washing three times. The tissue was allowed a resting period of 15min in between drug addition. Inhibitory effect of the extract and mepyramine on histamine-induced contraction was also investigated. Responses were recorded isometrically on an Ugo Basile Unirecorder (7050), through isometric transducer (7004)⁸.

**Studies on guinea pig ileum—**Adult guinea pigs were killed and bled out. Their abdomens were opened and the ceacum exposed. For this experiment, the terminal portions (2-3 cm in length) were used after discarding the 10 cm portion nearest to the ileocecal junction. The tissue was suspended in organ bath (20 ml) containing Tyrode solution at 37°C±1°C aerated with O₂ (95%) and CO₂ (5%). This solution was comprised (mM) - NaCl, 136.8; KCl, 2.7; CaCl₂, 1.3; NaHCO₃, 12.0; MgCl₂, 0.5; Na₂PO₄, 0.14; and glucose, 5.5. The initial tension was 0.5g. An equilibration period (60min) was allowed during which the physiological solution was changed for every 15min. At the end of the equilibration period, effect of graded concentrations of histamine, extract and mepyramine were investigated. Responses were recorded on Ugo Basile Unirecorder (7050)⁹.

**Studies on gastrointestinal motility test in mice—**Effect of the extract on small intestinal transit in unanaesthetised mice were tested using the charcoal method¹⁰. In brief, overnight fasted mice were randomly divided into four groups of 6 animals each. Mice in group I were given normal saline (20 ml/kg) intraperitoneally (ip), while those in groups II, III and IV received the extract (100, 200 and 400 mg/kg; ip) respectively. Five minutes after drug administration, 0.5ml of charcoal suspension (5%) in suspension of tragacanth powder (10%) was administered per oral (po) to each mouse. All the mice were killed 30 min after treatment. The abdomen opened and the distance travelled by the charcoal plug from pylorus to ceacum was determined and expressed as a percentage of the total length of the small intestine¹ⁱ.

**Studies on castor oil-induced diarrhoea—**This experiment was carried out using mice. The animals were fasted for 16 hr prior to experiment and were randomly divided into five groups having 6 animals in each group. Animals in group I were pretreated with normal saline (20 ml/kg; ip), groups II, III and IV animals received the extract (100, 200 and 400 mg/kg;
of drug administration, diarrhea was induced by oral administration of castor oil to mice (0.5 ml/animal) \(^\text{11}\). The animals were placed in individual cages and over clean filter paper. Four hours after oil challenge, the mice were inspected (by an observer unaware of the particular treatment) for the characteristic droppings; their absence was recorded as a protection from diarrhea \(^\text{11,12}\) and the percentage protection calculated.

In addition, onset time and severity of diarrhea were noted. Severity were recorded as scores using Di Carlo et al. scoring method \(^\text{13}\) (++ for copious, + mild and 0 for lack of diarrhea).

**Studies on castor oil-induced fluid accumulation**—The method of Di Carlo et al. \(^\text{13}\) was used for this study. Briefly, overnight fasted rats were randomly divided into four groups having 5 animals in each group. Animals in group I were administered with normal saline (20 ml/kg; ip), while those in groups II, III, and IV received the extract (100, 200 and 400 mg/kg; ip) respectively. After 30 min of administration of normal saline and extract, castor oil (2 ml/rat; po) was given. The animals were killed by inhalation of chloroform 30 min later. The small intestine was ligated at both pyloric sphincter and ileocecal junctions. The entire small intestine was dissected out. The contents of the small intestine were expelled into a graduated measuring cylinder and the volume of contents was recorded.

**Statistical analysis**—The results were expressed as median and range. Chi-square test was used for diarrhea study, while Wilcoxon’s rank sum test was used for analysis of gastrointestinal transit test, studies on intraluminal fluid accumulation, isolated ileum of guinea pigs and jejunum of rabbits, and results were regarded as significant at \(P < 0.05\).

**Results**

**Phytochemical test**—The extract gave positive tests for saponins, tannins, phenols, alkaloids and phlobatamins.

**Effect on rabbit jejunum**—Acetylcholine \((2.5 \times 10^{-9} -1.0 \times 10^{-7} \text{ M})\) caused a concentration-dependent contraction of rabbit jejunum, while the extract produced a concentration-dependent inhibition of spontaneous contraction of jejunum (Fig. 1). The extract also attenuated the acetylcholine-mediated contraction of rabbit jejunum similar to the effect of atropine \((5.0 \times 10^{-9}\text{M})\) on acetylcholine-induced contraction (Fig. 2).

**Effect on guinea pig ileum**—The extract caused a concentration-dependent inhibition of guinea pig ileum. The extract also attenuated the histamine-mediated \((4.5 \times 10^{-7} -7.2 \times 10^{-6} \text{ M})\) contraction of guinea pig ileum in a concentration related manner similar to that of meperidine \((2.49 \times 10^{-9}\text{M})\) (Fig. 3).

**Effect on gastrointestinal transit studies**—The extract significantly decreased the gastrointestinal distance travelled by the charcoal plug in mice, compared with the control. The effect appeared to be dose related (Table 1).

**Effect on castor oil test**—The extract significantly protected mice against castor oil-induced diarrhea.

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**Fig. 1**—Concentration-dependent relaxant effect of the aqueous extract of *I. gabonensis* on the spontaneous contraction of the rabbit jejunum [*\(^*P < 0.05\) Wilcoxon’s rank sum test*].

**Fig. 2**—Effect of the aqueous extract of *I. gabonensis* on the contractile response induced by acetylcholine on rabbit jejunum. Acetylcholine (ACH) alone (●), ACH + *I. gabonensis* 0.8 mg/ml (▲), ACH + *I. gabonensis* 1.6 mg/ml (▲) and ACH + atropine (●) [*\(^*P < 0.05\) Wilcoxon’s rank sum test*].
when compared with the control as evident in the prolongation of onset, decrease in severity and reduction in the animal population with diarrhoea. Loperamide (5mg/kg; ip) also significantly protected mice against castor oil-induced diarrhoea. The extract effect was dose-dependent (Tables 2, 3).

Effect on castor oil-induced fluid accumulation test—There was dose-dependent decrease in intestinal fluid accumulation. The extract significantly reduced (62 to 84%) the intestinal fluid accumulation compared to control (Table 4).

Discussion

The results of the present study suggested that the aqueous extract of Irvingia gabonensis had significant anti-diarrhoeal effect. The extract, in a dose-dependent manner, significantly protected mice against diarrhoea induced experimentally by castor oil in terms of severity and onset, and the population of animals with diarrhoea. Castor oil is a ricinoleate and its diarrhoea inducing property is known to be due to its active metabolite ricinoleic acid which increases peristaltic activity and alters permeability of the intestinal mucosa membrane to electrolytes, particularly Na⁺ and Cl⁻ (ref. 17) and water, effects associated with endogenous stimulation of prostaglandin release. Release of prostaglandins is also a major cause of arachidonic acid-induced diarrhoea. Further evidence of the anti-diarrhoeal activity of the extract is provided by the results of gastrointestinal transit studies in which the extract significantly inhibits the gastrointestinal motility in mice, dose-dependently. Agents that reduce intestinal motility are known to possess anti-diarrhoea effect. Our results also showed that intra-luminal fluid accumulation induced by castor oil was significantly inhibited in a dose-related fashion. Enteropooling assay has been proposed as a model for investigating diarrhoea produced by prostaglandins, and drugs

Table 1—Effect of aqueous leaf extract of I. gabonensis on gastrointestinal transit in mice.

<table>
<thead>
<tr>
<th>Drug/Conc. (ml/kg; i.p.)</th>
<th>Distance travelled (%)</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline 20</td>
<td>68.86</td>
<td>18.25</td>
<td></td>
</tr>
<tr>
<td>Extract 100</td>
<td>40.12</td>
<td>14.75*</td>
<td></td>
</tr>
<tr>
<td>Extract 200</td>
<td>39.45</td>
<td>14.23*</td>
<td></td>
</tr>
<tr>
<td>Extract 400</td>
<td>37.45</td>
<td>12.55*</td>
<td></td>
</tr>
</tbody>
</table>

*Statistical difference between treated groups and control.

Table 2—Effect of aqueous leaf extract of I. gabonensis on castor oil (0.5ml/mouse) induced diarrhoea in mice: population of animals with diarrhoea.

<table>
<thead>
<tr>
<th>Drug/Conc. (ml/kg; ip)</th>
<th>Mice with diarrhoea</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline 20</td>
<td>6/6</td>
<td>0*</td>
</tr>
<tr>
<td>Extract 100</td>
<td>3/6</td>
<td>30*</td>
</tr>
<tr>
<td>Extract 200</td>
<td>2/6</td>
<td>66.67*</td>
</tr>
<tr>
<td>Extract 400</td>
<td>1/6</td>
<td>83.33*</td>
</tr>
<tr>
<td>Loperamide 5</td>
<td>0/6</td>
<td>100*</td>
</tr>
</tbody>
</table>

*Statistical difference between treated groups and control.

Table 3—Effect of aqueous leaf extract of I. gabonensis on castor oil-induced diarrhoea.

<table>
<thead>
<tr>
<th>Drug/Conc. (ml/kg; ip)</th>
<th>Diarrhoea score</th>
<th>Total Score</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline 20</td>
<td>++</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Extract 100</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Extract 200</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Extract 400</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Statistical difference between treated groups and control.
affecting intestinal secretion also possess anti-diarrhoal activity\(^{13,17}\). Furthermore, the extract inhibited spontaneous and agonist (acetylcholine) mediated contraction of rabbit jejunum. It is well known that acetylcholine stimulates smooth muscles of the gastrointestinal tract through actions on the muscarinic receptors. Effect of the extract this way is indicative of possible cholinergic involvement. Similarly, the extract blocked the contractions induced by histamine on guinea pig ileum, suggesting that histaminergic receptors might be involved in the observed effect as well. The above finding is, therefore, suggestive of non-specific mechanism of action of anti-diarrhoal activity of the extract. Though, the compound responsible for the observed actions is unknown, tannins present in the extract might have made the intestinal mucosa more resistant and reduced the secretion\(^{15}\). The overall data presented suggested that the aqueous extract of \(I. \) gabonensis possesses anti-diarrhoea activity. Further studies to isolate the active components are in progress in our laboratory.

**Acknowledgement**

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**References**

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