Antidiarrhoeal Evaluation of the aqueous leaves extract of Costus lucanusianus—Family Costaceae

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Abstract: The aim of this work was to investigate the antidiarrhoeal activity of the aqueous extract of Costus lucanusianus, J. Braun (Costaceae) leaves on castor oil-induced diarrhoea and small intestinal transit in mice. Costus lucanusianus at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg caused a marked inhibition of the diarrhoea response following castor oil administration (P<0.0001). It also significantly (p<0.0001) inhibited the small intestinal transit in mice. Its effect was however not dose dependent as lower doses; 200 and 100 mg/kg gave the highest effect in the castor oil-induced diarrhoea and small intestinal transit respectively. In comparison with Atropine, its antidiarrhoeal effect at 100 mg/kg was found to be 179% and 165% respectively on castor oil induced diarrhoea and on small intestinal transit.

Key words: Costus lucanasianus, Antidiarrhoeal activity, castor oil-induced diarrhoea, small intestinal transit.

INTRODUCTION

Diarrhoea literally means in (Greek and Latin: dia, through and rhoein: to flow or run) Diarrhoea is defined by scientists as excessive fluid weight, with 200g per day representing the upper limit of normal stool water weight for healthy adults. Since stool weight is largely determined by stool water, most cases of diarrhoea result from disorders of intestinal water and electrolyte transport.

In most instances, multiple processes are simultaneously affected leading to a net increase in stool volume and weight accompanied by changes in percentage water content. The strategy of oral rehydration which has now been available for several years, utilizing the ability of the small intestine to absorb water and salt during glucose absorption even in patients with cholera, can significantly reduce the mortality but not the morbidity, of acute diarrhoea. Therefore, there is a need for drugs that decrease intestinal hypersecretion, to be used in combination with rehydration solution.

Ethno medicine has played an important role in Africa and Western societies and in Nigeria particularly. Herbal medicine is an integral part of Traditional medicine.

In traditional medicine, many plants are claimed to have an antidiarrhoeal effect without any scientific basis. The leaves of the plant Costus lucanasianus locally called monkey sugar cane in the Niger delta region of Nigeria is a herb up to 3 m tall, clumping; stem green, base with leaves smaller toward base, larger above; and dark green, has a wide reputation in folk medicine for the treatment of malaria, rheumatism, ulcers and diarrhoea.

To our knowledge there are no available reports on the bioactivity of the aqueous leaf extract of Costus lucanusianus. In the present study, we evaluated the aqueous extract for a possible antidiarrhoeal activity in animal models of secretory diarrhoea and its inhibitory effect on intestinal transit.

MATERIALS AND METHODS

Collection and Identification of Plant Material: The leaves of Costus lucanusianus were collected in Amarata, Yenagoa, Bayelsa State in May, 2007. The botanical identity of the plant and its leaves was by Dr B.A Ayinde of the Department of Pharmacognosy, Faculty of Pharmacy, university of Benin, Benin City. Immediately after collection, the leaves were air dried for one week. This was further subjected to another one week of drying in an oven maintained at 40°C.

Extraction and Preparation of the Extract: The leaves were pulverized into a smooth powder using impact mill. The pulverized material (150g) was mixed with distilled water (3.0 litres) and left for 72 hours.
The mixture was stirred at 6 hours intervals using a sterile glass rod. At the end, the extract was passed through filter paper. The filtrates were concentrated with the aid of a vacuum pump and rotavapour at 40°C, giving a yield of 5.53%.

The concentrated extract was stored in universal bottles, labelled and refrigerated at -4°C prior to use.

Phytochemical Screening: The aqueous extract was subjected to phytochemical screening testing for the presence of alkaloids, Tannins, saponions, reducing sugars and carbohydrate using the method of Trease and Evans[10].

The aqueous extract (5 g) was boiled with water on a steam bath for 30 min. After filtration, the filtrate obtained was tested for the presence of alkaloids using alkaloidal reagents like Dragendorff’s, Mayer’s, Wagner’s and Hager’s reagents.

For the saponins, about 0.1 g of the extract was boiled with 5 mL of water for 2 min and filtered. About 0.1 mL of the filtrate was diluted to 1 mL with water. The mixture was shaken vigorously for 2 min and observed for frothing.

In order to test for the Tannins, portion of the extract was dissolved in chloroform, shaken vigorously with 50 mL distilled water, boiled for 5 min and filtered.

Volume was made up to 50 mL. To a few drops of filtrate was added an equal volume of 15% ferric chloride and observed for colour change.

The test for the presence of flavonoids involves the addition of 2 mL of the extract to a solution of dilute NaOH and concentrated HCL. The colour change was also observed.

Antidiarrhoeal Activity: Animals: Albino Swiss mice weighing between 20-30g of either sex fed on standard diet (Ladokun feeds, Ibadan, Oyo State) were obtained from the Animal house, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin. The animals were allowed free access to water. They were maintained in the laboratory for a minimum period of 10days prior to experimentation. All experiments were performed after an overnight fast.

Castor-oil Induced Diarrhoea: Mice were divided into five groups of four animals each. diarrhoea was induced by administering 0.3ml of castor oil orally to mice as described by Awouters et al., [3]. Group one served as control (distilled water 10ml/kg), groups 2, 3 and 4 received the aqueous extract (100, 200 and 400mg/kg respectively) while group 5 received atropine (0.1mg/kg I.p). This was done 30 minutes before castor oil administration.

The following parameters were observed for a period of 4 hours, the time elapsed between the administration of the cathartic agent and the excretion of the first diarrhoeic faeces, the total number of both dry and wet diarrhoea droppings in 4 hours and the total weight of both the wet and dry diarrhoeal stool in that period of time and the onset which was measured as the time interval in minutes between the administration of castor oil and the appearance of the first diarrhoea stool.

Small Intestinal Transit in Mice: Groups of overnight fasted mice (n=4) received the aqueous extract (100, 200 and 400mg/kg), atropine (0.1 mg/kg) and 10ml/kg of distilled water, I.p). This was done 30 minutes before the charcoal meal was orally administered. (0.2ml /mouse of a 10 % charcoal suspension in 5% gum acacia solution) to determine their effect on normal intestinal transit. Twenty minutes after the charcoal meal administration the animals were sacrificed using ether, the stomach and small intestine from each animal were removed and extended on a clean glass surface. The distance traversed by the charcoal maker in relation to the total small intestinal length was measured and expressed as a percentage[10].

Statistical Analysis: All data were expressed as mean ±SEM and where applicable, the data were analysed statistically by Student’s t-test using graph pad instant version 2.05a. The level of significance was from P < 0.05.

RESULTS AND DISCUSSION

The phytochemical screening as shown in table 3, revealed the presence of saponins, tannins, reducing sugars and carbohydrates.

Flavanoids, Alkaloids and Anthracene derivatives were absent.

On its anti-diarrhoeal effect, the extract produced an inhibition of castor oil–induced diarrhoea with the 400mg/kg dose giving the highest effect on the number and weight of stool produced, however the 200 mg/kg dose gave the highest effect on the onset of diarrhoea, suggesting that its effect is not dose dependent. This is shown in Table 1.

The onset time of diarrhoea is in minutes, and it is the time interval between the administration of the cathartic agent and the first diarrhoeic stool. The pretreatment of mice with the aqueous extract of Costus lucanusianus delayed the onset of diarrhoea with a significant (p<0.0001) inhibition during the first 2hour period at all doses tested.

Costus lucanusianus (400 mg/kg dose) also significantly (p<0.0001) decreased the total number of stools passed (1.25±0.25) as compared to the castor oil
treated control groups (16.5 ± 0.65). Atropine at a dose of 0.1mg/kg (i.p) also produced a marked anti-diarrhoeal effect (8.25 ± 0.63). Hence the effect of the extract at all doses tested was significantly better than that produced by atropine (p<0.0001). Besides decreasing the number of stools passed, the extract also afforded protection against the castor oil-induced diarrhoea. Its effect was discernible at about the 2nd hour when 50% of the animals were protected and at the fourth hour 20% protection was observed.

The onset time of diarrhoea, total number of stools and total weight of stool were 155.3 ± 5.54, 1.5 ± 0.29 and 60 ± 0.02mg respectively for the 200mg/kg dose. In comparison to the control (distilled water treated group), its effect was significant, (p<0.0001). On its effect on small intestinal transit (Table 2), there was a reduction in the percentage distance travelled by the charcoal with the 100mg/kg dose giving the least percentage of 19.12 ± 2.12. In comparison with the control (distilled water treated group), its effect was significant (p<0.0001). This also suggest that the extract’s effect is not dose dependent.

Table 1: Antidiarrhoeal activity of the extract and atropine on castor-oil induced diarrhoea in mice.

<table>
<thead>
<tr>
<th>Treatments (mg/kg)</th>
<th>Onset time of diarrhoea (mins)</th>
<th>Total number of faeces</th>
<th>Total weight of faeces (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>34.8 ± 6.86</td>
<td>16.5 ± 0.65</td>
<td>995 ± 0.02</td>
</tr>
<tr>
<td>100</td>
<td>149.5 ± 9.60</td>
<td>5.8 ± 1.65</td>
<td>203 ± 0.06</td>
</tr>
<tr>
<td>200</td>
<td>155.3 ± 5.54</td>
<td>1.5 ± 0.29</td>
<td>60 ± 0.02</td>
</tr>
<tr>
<td>400</td>
<td>88.0 ± 1.41</td>
<td>1.3 ± 0.25</td>
<td>70 ± 0.02</td>
</tr>
<tr>
<td>Atropine (0.1)</td>
<td>74.3 ± 11.89</td>
<td>8.3 ± 0.63</td>
<td>210 ± 0.06</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. (n=5 per group).

The aqueous extract at doses of 100, 200 and 400mg/kg and the reference drug, atropine (0.1mg/kg) produced significant inhibition of normal transit. (Table 2). The 100mg/kg gave the highest percentage inhibition of 69.25% better than the effect produced by atropine.

The present study demonstrates that the extract inhibits castor oil-induced secretory diarrhoea in mice. The induction of diarrhoea is a well known action of castor oil attributed to its active ingredient ricinoleic acid[14] which stimulates the production of several mediator substances that include prostaglandins, nitric oxide, platelet activating factor, and tachykinins[9]. This model was therefore used to establish the anti-secretory effect of the aqueous leaf extract of Costus lucanusianus.

Besides producing an antisecretory effect, interestingly the extract was found to inhibit the intestinal transit in mice providing 69.25% inhibition at 100mg/kg dose.

Conclusion: The research was able to produce scientific basis for the traditional use of Costus lucanusianus leaves as a remedy for diarrhoea. The results suggests that the aqueous extract has a potential antidiarrhoeal effect that can be explored for therapeutic advantage as an alternative treatment for diarrhoea.

To improve the safety of this traditional herbal remedy, additional research is needed to define the stability and bioactivity of this product[13].
Therefore, further studies are therefore needed for the isolation and characterization of the active constituents.

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**REFERENCES**