Pharmacological studies of anti diarrhoeal activity of *Casuarina equisetifolia* (L.) in experimental animals

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**ABSTRACT**

The purpose of the present study was to evaluate scientifically the anti-diarrhoeal effects of ethanolic (90%) extract of *Casuarina equisetifolia* Linn (EECE) was studied against castor oil-induced diarrhoea model in rats. Anti-diarrhoeal activity of 90% ethanol extract of *Casuarina equisetifolia* was investigated in this study using castor oil-induced diarrhoea, enteropooling and Small intestinal transit models in rats. The weight and volume of intestinal content induced by castor oil were studied by enteropooling method. Standard drug diphenoxylate (5 ml/kg, p.o) was significant reductions in fecal output and frequency of droppings whereas EECE at the doses of 200 and 400 mg/kg p.o significantly (P<0.001) reduced the castor oil induced frequency and consistency of diarrhoea and enteropooling. The gastrointestinal transit rate was expressed as the percentage of the longest distance travelled by the charcoal divided by the total length of the small intestine. EECE at the doses of 200 and 400 mg/kg significantly inhibited (P<0.001) the castor oil induced charcoal meal transit. The EECE showed marked reduction in the number of diarrhoea stools and the reduction in the weight and volume of the intestinal contents, as well as a modest reduction in intestinal transit. The results obtained establish the efficacy and substantiate the folklore claim as an anti-diarrheal agent. Further studies are needed to completely understand the mechanism of anti-diarrhoecal action of *Casuarina equisetifolia* Linn.

**Keywords:** Antidiarrhoeal Activity, *Casuarina equisetifolia* L., Traditional medicine, Castor Oil-induced diarrhoea, Enteropooling Method, Small intestinal transit.

**INTRODUCTION**

*Casuarina equisetifolia* L. (Casuarinaceae) is a tree to 25 m high with drooping branches and needle-like branchlets. Common along the coast on beaches, rocky coasts, limestone outcroppings, dry hillsides and open forests in both wet and dry zones from sea-level to mid-montane. Native to South-East Asia, Australia and Polynesia. The chief chemical constituents include Ellagic acid, beta-sitosterol, kaempferol and glycosides, quercetin, cupressuflavone, isoquercitrin, several common triterpenoids, trifolin, catechin and epicatechin, cholesterol, stigmasterol, campesterol, cholest-5-en-3-beta-ol derivatives, tannin, proantho-cyanidins, juglanin, citrulline and amino acids, afzelin, casuarine, gallicin, catechol derivatives, gentisic acid, hydroquinone, nictoflorin, rutin, trifolin [1-3]. Phytosterol from the leaves of the plant shows antibacterial activity, hypoglycemic, antifungal, molluscicidal, cytotoxic [2-6]. In Tahiti, the plant is used to treat nervous disorders, diarrhoea and gonorrhoea [7-8]. Tongans use it to treat coughs, ulcers, stomachaches and constipation. Dysuria and menorrhagia are treated with an infusion of the bark. An infusion of the bark, in Tonga, is used as an emetic to treat throat infections. However there are no reports on the anti diarrheal activity of the plant. Hence, the present study was designed to verify the claims of the native practitioners.

**MATERIALS AND METHODS**

**Plant collection**

The Plant material of *Casuarina equisetifolia* used for investigation was collected from Tirunelveli District, in the Month of August 2008. The plant was authenticated by Dr.V. Chelladurai, Research Officer Botany. C.C.R.A.S., Govt. of India. The voucher specimen (CHE-SA-GS-01) of the plant was deposited at the college for further reference.
Preparation of extracts

Inner bark of the whole plants were dried in shade, separated and made to dry powder. It was then passed through the 40 mesh sieve. A weighed quantity (60gm) of the powder was subjected to continuous hot extraction in Soxhlet Apparatus. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample. Percentage yield of ethanolic extract of *Casuarina equisetifolia* was found to be 17.5 % w/w.

Preliminary Phytochemical screening

The phytochemical examination of ethanolic (90%) extract of *Casuarina equisetifolia* was performed by the standard methods [9].

Animals used

Albino wistar rats (150-230g) of either sex were obtained from the animal house in Cherrana’s College of Pharmacy, Coimbatore. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. The animals were fed with standard pellet feed (Hindustan Lever Limited., Bangalore) and water was given *ad libitum*. Ethical committee clearance was obtained from IAEC.

**Castor oil-induced diarrhoea**

Diarrhoea was induced by Nwodo and Alumanah (1991) and Nwafor et al., (2005) [10, 11]. Animals were fasted for 24 h but allowed free access to water. Rats were divided into four groups of six animals each, diarrhoea was induced by administering 2 ml of castor oil orally to rats. Group I treated as control (2 ml/kg, p.o. saline), group II received diphenoxylate (5 ml/kg p.o) served as standard and group III and IV received EECE (200 and 400 mg/kg, p.o) 1 h before castor oil administration. Then observed for consistency of faecal matter and frequency of defaecation for 4 hrs.

**Castor oil-induced enteropooling**

Intraluminal fluid accumulation was determined by the method of Robert et al., (1976) and DiCarlo et al., (1994) [12, 13]. Animals were fasted for 24 h but allowed free access to water. Rats were divided four groups of six animals each. Group I received normal saline (2 ml/kg, p.o served as a control, group II received diphenoxylate (5.0 mg/kg p.o.) and groups III and IV received EECE 200 and 400 mg/kg p.o respectively 1hr before the oral administration of castor oil. Two hours later the rats were sacrificed, the small intestine was removed after tying the ends with thread and weighed. The intestinal contents were collected by milking into a graduated tube and their volume was measured. The intestine was reweighed and the difference between full and empty intestines was calculated.

Small intestinal transit

Rats were fasted for 18 h divided into five groups of six animals each, Group I received 2 ml normal saline orally, group II received 2 ml of castor oil orally with saline 2 ml/kg p.o, group III received atropine (3 mg/kg, i.p.), group IV and V received EECE 200 and 400 mg/kg p.o respectively, 1 h before administration of castor oil. One ml of marker (10% charcoal suspension in 5% gum acacia) was administered orally 1 h after castor oil treatment. The rats were sacrificed after 1h and the distance traveled by charcoal meal from the pylorus was measured and expressed as percentage of the total length of the intestine from the pylorus to caecum [14].

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M). The Significance of differences among the groups was assessed using one way and multiple way analyses of variance (ANOVA). The test followed by Dunnett’s test P values less than 0.05 were considered as significance.

RESULTS

Phytochemical Screening

The results of preliminary phytochemical screening of the ethanolic extract of *Casuarina equisetifolia* Linn revealed that presence of alkaloids, flavonoids, triterpinoids, carbohydrates, tannins, phenols, gums and mucilage and absence of saponis and steroids.

**Castor oil-induced diarrhoea**

After 30 min administration of castor oil the diarrhoea was clinically apparent in all the animals of control group, for the next 4 h. This was markedly reduced by diphenoxylate (5 ml/kg p.o) (67.79%). A similar marked reduction in the number of defecations over four hours was achieved with *Casuarina equisetifolia* at the doses of 200 or 400 mg/kg p.o. EECE 200 and 400 significantly inhibited the defeccation (41.45% and 61.19%) EECE 200 and 400 mg/kg, p.o. dose of extract delayed the onset of diarrhoea and only 30% of animals showed diarrhoea at first hour (P<0.001) (Table 1).

**Castor oil-induced enteropooling**

Castor oil caused accumulation of water and electrolytes in intestinal loop. Castor oil-induced enteropooling is not influenced by diphenoxylate (5 ml/kg p.o) in rats. EECE 200 and 400 produced a dose-dependent reduction in intestinal weight and volume. EECE 200 and 400 mg/kg, p.o dose produced 34.43% and 54.77% inhibition of volume of intestinal content respectively with significance (P<0.001). The weight of intestinal content was also reduced significantly at both the doses (Table 2).
Small intestinal transit: The percent intestinal transit was increased with castor oil (90.77%), but it was reduced in both doses of extract, and much more markedly by atropine (37.02%). EECE 200 mg/kg, p.o dose of extract produced 62.54% intestinal transit induced by castor oil respectively. Whereas, EECE 400 mg/kg, p.o dose produced 48.80% of castor oil induced charcoal meal transit (Table 3).

Table 1. Effect of EECE on castor oil-induced diarrhoea in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean Defecation in 4hr</th>
<th>% Inhibition of Defecation</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Castor oil (2ml p.o) + saline (2ml/kg p.o)</td>
<td>25.33±1.70</td>
<td>---</td>
</tr>
<tr>
<td>II</td>
<td>Castor oil (2ml p.o) + diphenoxylate (5ml/kg p.o)</td>
<td>8.16±0.30**</td>
<td>67.79</td>
</tr>
<tr>
<td>III</td>
<td>Castor oil (2ml p.o) + EECE (200mg/kg p.o)</td>
<td>14.83±0.91*</td>
<td>41.45</td>
</tr>
<tr>
<td>IV</td>
<td>Castor oil (2ml p.o) + EECE (400mg/kg p.o)</td>
<td>9.83±0.80**</td>
<td>61.19</td>
</tr>
</tbody>
</table>

Effect of EECE on castor oil-induced diarrhoea in rats: EECE was administered p.o 1 h before castor oil administration. Values are expressed as mean ± SEM from the experiments. *P<0.01, **P<0.001 when compared with Castor oil + saline-treated group.

Table 2. Effect of EECE on castor oil-induced enteropooling in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Weight of Intestinal Content</th>
<th>% Inhibition of Weight Intestinal Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Castor oil (2ml p.o) + saline (2ml/kg p.o)</td>
<td>2.41±0.12</td>
<td>---</td>
</tr>
<tr>
<td>II</td>
<td>Castor oil (2ml p.o) + diphenoxylate (5ml/kg p.o)</td>
<td>1.62±0.12**</td>
<td>32.78</td>
</tr>
<tr>
<td>III</td>
<td>Castor oil (2ml p.o) + EECE (200mg/kg p.o)</td>
<td>1.58±0.05*</td>
<td>34.43</td>
</tr>
<tr>
<td>IV</td>
<td>Castor oil (2ml p.o) + EECE (400mg/kg p.o)</td>
<td>1.09±0.12**</td>
<td>54.77</td>
</tr>
</tbody>
</table>

Effect of EECE on castor oil-induced enteropooling in rats: EECE was administered p.o 1 h before castor oil administration. Values are expressed as mean ± SEM from the experiments. *P<0.01, **P<0.001 when compared with Castor oil + saline-treated group.

DISCUSSION AND CONCLUSION

Diarrhoea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract, accompanied by hurry, resulting in an excess loss of fluid in the faeces. At doses of 200 and 400 mg/kg, the ethanol extract of Casuarina equisetifolia showed significant anti-diarrhoeal activity against castor oil-induced diarrhoea as compared with the control group it significantly(P<0.001) reduced the frequency of diarrhoea and consistency of defecations (Table 1). The EECE also showed a dose related decrease in castor oil-induced diarrhoea. Several mechanisms have been supposed to be involved in the diarrhoeal effect of castor oil [12]. These include Castor oil decreases fluid absorption, increases secretion in the small intestine and colon, and affects smooth muscle contractility in the intestine. Castor oil produces diarrhoeal effect due to its active component of recinoleic acid [13], inhibition of intestinal Na⁺,K⁺-ATPase activity to reduce normal fluid absorption [14, 15], activation of adenyl cyclase [13], stimulation of prostaglandin formation [16], platelet-activating factor and recently nitric oxide was contribute to the diarrhoeal effect of castor oil [17-19]. Despite the fact that number of mechanisms has been involved for the diarrhoeal effect of castor oil, it has not been possible to define its correct mechanism of action [11]. EECE may act above any one of the mechanism.

It is also noted that EECE significantly inhibited castor oil induced intestinal fluid accumulation and the volume of intestinal content (Table 2). The secretory diarrhoea is associated with an activation of Cl⁻ channels, causing Cl⁻ efflux from the cell, the efflux of Cl⁻ results in
massive secretion of water into the intestinal lumen and muscarinic receptor effect was confirmed by increased production of both gastric secretion and intraluminal fluid accumulation induced by castor oil. The EECE may inhibit the secretion of water into the intestinal lumen and this effect is partly mediated by both α-adrenoceptor and muscarinic receptor systems. The significant inhibition of the castor oil-induced enteropooling in mice suggests that the extract of Casuarina equisetifolia produced relief in diarrhoea by spasmolytic activity in vivo and anti-enteropooling effects. [10].

The EECE significantly reduced the castor oil induced intestinal transit as compared with control group (Table 3). In this study, atropine increased intestinal transit time possibly due to its anti-cholinergic effect [20]. In castor oil induced diarrhoea, the liberation of ricinoleic acid results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which results in stimulation of secretion [21] by prevents the reabsorption of NaCl and water [16]. Probably EECE increased the reabsorption of NaCl and water by decreasing intestinal motility as observed by the decrease in intestinal transit by charcoal meal.

REFERENCES
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