PHARMACOLOGICAL STUDIES OF ANTI-DIARRHOEAL ACTIVITY OF MALACHRA CAPITATA (L.) IN EXPERIMENTAL ANIMALS

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ABSTRACT

The purpose of the present study was to evaluate scientifically the anti-diarrhoeal effects of aqueous extract of roots of Malachra capitata Linn (AMC) was studied against castor oil-induced-diarrhoea model in rats. Antidiarrhoeal activity of aqueous extract of Malachra capitata 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 AMC 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. AMC at the doses of 200 and 400 mg/kg significantly inhibited (P<0.001) the castor oil induced charcoal meal transit. The AMC 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-diarrhoeal action of Malachra capitata.

KEY WORDS: Antidiarrhoeal Activity, Malachra capitata, Traditional medicine, Castor Oil-induced diarrhoea, Enteropooling Method, Small intestinal transit.

INTRODUCTION

Malachra capitata (L.) is a herb belongs to family: Malvaceae. Description: Mostly erect, coarse, annual or perennial herb 1-2 m tall, throughout densely whitish- or yellowish-tomentose with stellate hairs and usually also moderately to copiously hispid with simple or stellate hairs to 2 mm long; roots long-petioled; stipules lanceolate, 5-15 mm long; blades orbicular to ovate, 2-10 cm long, pinnately sinuate to 3-, 5-, or 7-lobed, lobes mostly obtuse, crenate to serrate, the base obtuse or truncate; flowers in axillary, pedunculate, bracteate heads, bracts 1-2 cm long, stipitate and subtended by paired, filiform bracteoles, conduplicate, suborbicular to ovate, obtuse or acute, entire or once or twice dentate, obtuse to cordate at base, prominently veined and whitish basocentrally; involucral bracts wanting; calyx tubular-campanulate, 4-8 mm long, 5-lobed to below middle, lobes ovate-lanceolate, white with brownish or reddish nerves; petals yellow, obovate, 10-15 mm long, slightly exceeding staminal column; mericarps 3-3.5 mm long, muticous, reddish veined, puberulent; seed obovoid-cuneate, about 2.5 mm long, black, whitish-pubescent about hilum. The root of the Malachra capitata (L.) is traditional
remedies for the many disease condition such as pain, hepatic cirrhosis, inflammation, diarrhea, convulsion, dementia, pyrexia, ulcer, healing of wounds [1-4]. However there are no reports on the antidiarrheal activity of the plant. Hence, the present study was designed to verify the claims of the native practitioners.

MATERIALS AND METHODS

Collection and authentication of plant material

The Plant material of Malachra capitata (L.) roots was collected from Tirunelveli District, in the Month of August 2011. The plant was authenticated by Dr.V.Chelladurai, Research Officer Botany. C.C.R.A.S., Govt. of India. The voucher specimen of the plant was deposited at the college for further reference.

Preparation of plant extract

The roots of the Malachra capitata (L.) are properly washed in tap water and then rinsed in distilled water. The rinsed roots are dried in an oven at 35°C for 4 days. The dried roots of Malachra capitata was crushed to obtain powder. These powdered samples are then stored in air tight polythene bags protected from sunlight until use. The aqueous extract of each sample was prepared by soaking 10g of powdered sample in 200ml distilled water for 12h. The extracts are then filtered using Whatmann filter paper. Percentage yield of aqueous extract of Malachra capitata was found to be 10.5 % w/w. The aqueous extract was administered to the animals by suspending each time in 1% CMC.

Phytochemical Screening

The phytochemical examination of aqueous extract of Malachra capitata (L.) was performed by the standard methods [5].

Experimental animals

Adult Wistar rats of either sex weighing 180-250 gms were used in pharmacological and toxicological studies. The inbred animals were taken from the animal house and maintained in a well-ventilated room with at 12:12 hr light, dark cycle in polypropylene cages and maintained at 22±1°C with humidity at 55±5%. They were fed balanced rodent pellet diet from Poultry Research station, Nandanam, Chennai-35 and tap water ad libitum throughout the experimental period. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of CPCSEA (Committee for the Purpose of Control and Supervision of Experimental Animals).

Acute toxicity study

The acute toxicity of aqueous extract of Malachra capitata (L.) roots was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not lethal to the rats even at 2000mg/kg dose. Hence, 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for further study [6].

Castor oil-induced diarrhoea

Diarrhoea was induced by Nwodo and Alumanah (1991) and Nwafor et al., (2005) [7, 8]. 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 AMC (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) [9, 10]. 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 AMC 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 AMC 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 to caecum was measured and expressed as percentage of the total length of the intestine from the pylorus to caecum [11].

Statistical analysis

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

RESULTS

Phytochemical investigation

The results of preliminary phytochemical investigation of the aqueous extract of Malachra capitata
(L.) roots (AMC) shows the presence of carbohydrates, phenols, flavonoids, glycosides, terpenes, alkaloids, tannins, and Saponins.

**Acute toxicity study**
Acute toxicity study in which the animals treated with the AMC at a higher dose of 2000 mg/kg did not manifest any significant abnormal signs, behavioral changes, body weight changes, or macroscopic findings at any time of observation. There was no mortality in the above-mentioned dose at the end of the 14 days of observation.

**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). A similar marked reduction in the number of defecations over four hours was achieved with *G. speciosa* at the doses of 200 or 400 mg/kg p.o. AMC 200 and 400 significantly inhibited the defecation AMC 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. AMC 200 and 400 produced a dose-dependent reduction in intestinal weight and volume. AMC 200 and 400 mg/kg, p.o dose produced inhibit the 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).

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean Defecation in 4hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Castor oil (2ml p.o) + saline (2ml/kg p.o)</td>
<td>24.29±1.52</td>
</tr>
<tr>
<td>II</td>
<td>Castor oil (2ml p.o) + diphenoxylate (5 ml/kg p.o)</td>
<td>7.42±0.21**</td>
</tr>
<tr>
<td>III</td>
<td>Castor oil (2ml p.o) + AMC (200mg/kg p.o)</td>
<td>13.58±0.52*</td>
</tr>
<tr>
<td>IV</td>
<td>Castor oil (2ml p.o) + AMC (400mg/kg p.o)</td>
<td>8.64±0.46**</td>
</tr>
</tbody>
</table>

Effect of AMC on castor oil-induced diarrhoea in rats: AMC 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 AMC on castor oil induced enteropooling in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Weight of Intestinal Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Castor oil (2ml p.o) + saline (2ml/kg p.o)</td>
<td>2.89±0.43</td>
</tr>
<tr>
<td>II</td>
<td>Castor oil (2ml p.o) + diphenoxylate (5 ml/kg p.o)</td>
<td>1.60±0.28**</td>
</tr>
<tr>
<td>III</td>
<td>Castor oil (2ml p.o) + AMC (200mg/kg p.o)</td>
<td>1.68±0.25*</td>
</tr>
<tr>
<td>IV</td>
<td>Castor oil (2ml p.o) + AMC (400mg/kg p.o)</td>
<td>1.24±0.15**</td>
</tr>
</tbody>
</table>

Effect of AMC on castor oil-induced enteropooling in rats: AMC 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 3. Effect AMC on castor oil-induced small intestinal transit in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Total Length of Intestine</th>
<th>Distance Travelled By Marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>saline (2ml/kg p.o)</td>
<td>89.57 ± 1.24</td>
<td>44.19 ± 1.27</td>
</tr>
<tr>
<td>II</td>
<td>Castor oil (2ml p.o) + saline (2ml/kg i.p)</td>
<td>81.64 ± 2.37</td>
<td>75.29 ± 1.33</td>
</tr>
<tr>
<td>III</td>
<td>Castor oil (2ml p.o) + atropine (3mg/kg i.p)</td>
<td>92.18 ± 2.21</td>
<td>34.69 ± 1.36**</td>
</tr>
<tr>
<td>IV</td>
<td>Castor oil (2ml p.o) + AMC (200mg/kg i.p)</td>
<td>82.31 ± 1.42</td>
<td>53.49 ± 1.54*</td>
</tr>
<tr>
<td>V</td>
<td>Castor oil (1ml p.o) + AMC (400mg/kg i.p)</td>
<td>84.62 ± 1.21</td>
<td>43.17 ± 1.22**</td>
</tr>
</tbody>
</table>

Effect of AMC on castor oil-induced small intestinal transit in rats: AMC 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.

**Small intestinal transit**
The percent intestinal transit was increased with castor oil, but it was reduced in both doses of extract, and much more markedly by atropine. AMC 200 mg/kg, p.o dose of extract produced significant intestinal transit induced by castor oil respectively. Whereas, AMC 400 mg/kg, p.o dose significantly produced castor oil induced charcoal meal transit (Table 3).
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 aqueous extract of Malachra capitata showed significant anti-diarrhoeal activity against castor oil-induced diarrhoea as compared with the control group (Table 2). The AMC 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 diarrhoea 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 adenylyl cyclase [13], stimulation of prostaglandin formation [16], platelet-activating factor and recently nitric oxide was contribute to the diarrhoeal effect of castor oil [17, 18, 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].

AMC may act an above any one of the mechanism. It is also noted that AMC 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 profuse watery diarrhoea [20]. The involvement of muscarinic receptor effect was confirmed by increased production of both gastric secretion and intraluminal fluid accumulation induced by castor oil. The AMC may inhibit the secretion of water into the intestinal lumen and this effect is partly mediated by both α2-adrenoceptor and muscarinic receptor systems. The significant inhibition of the castor oil-induced enteropooling in mice suggests that the extract of Malachra capitata produced relief in diarrhoea by spasmylytic activity in vivo and anti-enteropooling effects [10].

The AMC 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 AMC increased the reabsorption of NaCl and water by decreasing intestinal motility as observed by the decrease in intestinal transit by charcoal meal.

Anti-dysentric and antidiarrhoeal properties of medicinal plants were found to be due to tannins, alkaloids, saponins, flavonoids, sterols and/or triterpenoids and reducing sugars [22-29]. The phytochemical analysis of AMC revealed the presence of alkaloids, flavonoids, triterpinoids carbohydrates, tannins, phenols, gums and mucilage. These constituents may mediate the antidiarrhoeal property of the AMC.

In conclusion, the present study has shown that Malachra capitata is a potential therapeutic option in the effective management of diarrhoea, thus justifying its widespread use by the local population for these purposes. Concerted efforts are being made to fully investigate the mechanisms involved in the pharmacological activities of Malachra capitata and phytochemical studies are also in progress to isolate and characterize the active constituents of Malachra capitata. The isolated compound may serve as useful prototypes of anti-diarrhoeal drugs of natural origin possessing the desired pharmacological activities while lacking certain untoward effects.

REFERENCES
7. Nwafor PA, Jacks TW, Ekanem AU, Ching FP. Nigerian Journal of Natural Product and Medicine, 9, 2005, 66–70.


