ANTIDIARRHOEAL ACTIVITY OF *CYMBOPOGON CITRATUS* AND ITS MAIN CONSTITUENT, CITRAL

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Summary

Cymbopogon citratus (DC) Stapf is one of the most commonly used plants in the folk medicine of native tribes of northeast India to cure diarrhoeal disorders. Hence the present study was carried out to verify the antidiarrhoeal claims of *C. citratus* extract and its main constituent, citral using three experimental diarrhoeal models in mice: castor oil induced-diarrhoea, $MgSO_4$ - induced enteropooling and charcoal meal test. The effects of extract and citral were also observed on faecal out put in mice. The 800 mg/kg, p.o. dose of plant extract significantly reduced the production of faecal matters by 53.44%, and conferred protection to diarrhoeal episodes after castor-oil challenge by 59.00%. The same dose of extract also significantly inhibited the intestinal fluid secretion induced by $MgSO_4$ and gastrointestinal motility in charcoal meal test. In all the experimental models, the citral showed an almost comparable efficacy with that of standard antidiarrhoeal drug, Loperamide. The study thus authenticates the presence of antidiarrhoeal activity in *Cymbopogon citratus*, which may have therapeutic benefits in humans encountering diarrhoeal disorders.

Keywords: Antidiarrhoeal activity, *Cymbopogon citratus,* citral, folk medicine, India.

The rich floral diversity of India has provided traditional practitioners in the country with an impressive pool of '*natural pharmacy*' from which plants are selected as ingredients to prepare herbal remedies to treat various diseases. The northeastern region of India, in particular is inhabited by approximately 130 major tribal communities. These tribes have a good faith in their traditional medicine system and thus they use many plant based medicines to cure various ailments, including diarrhoea. However, the purported efficacies of many of these plants have not been scientifically evaluated. During course of our studies on experimental validation of folk medicines of northeast India [1, 2, 3, 4], we collected information through a random survey in the region that *Cymbopogon citratus* (DC) Stapf. (Poaceae: Gramineae), locally known as *Harvosing*, is one of the most commonly used herbal plants to cure diarrhoeal disorders. In the literature there is neither any mention nor any experimental study pertaining to its antidiarrhoeal activity, though other studies on this plant indicate that it possesses scientifically proven antibacterial [5, 6, 7], antifungal [8, 9, 10, 11], antimalarial [12], anticancer [13] and larvicidal activities against *Aedes aegypti* [14].

C. citratus (DC) Stapf. is a perennial grass that grows up to 1.5 meters high. It is found distributed in tropical Asia, including Penisula of India and Naga Hills in northeast India [15]. The main chemical constituent of the plant is citral, which accounts for 75 to 80% of its oil's volume [8, 16]. In practice, the whole stalk and the leaf are boiled and the decoction is drunk to relieve the diarrhoea. In view of its popular use in traditional medicine system, it was felt necessary to scientifically evaluate the antidiarrhoeal potentials of *C. citratus* as claimed by indigenous tribes in India. The present study reports the antidiarrhoeal efficacy of *C. citratus* stalk decoction and its main chemical constituent citral, using experimental diarrhoeal models in mice.

Methods

Plant material and preparation of extracts:

The plant material was collected in the month of August from surroundings of cultivated lands in Manipur by V. Tangpu and later duly identified by Dr. P. B. Gurung, Department of Botany, NEHU, Shillong. The leaves and stalk were air-dried under shade and pulverized into husky powder. The powdered material was extracted by maceration in 2 L of cold distilled water for 24 h. The material was filtered and freeze dried to obtain the extract as a solid residue. The w/w yield in terms of drying starting material was 10.50%.

Drugs and chemicals:

Loperamide (Axar Pharmaceuticals, Baroda), Castor oil (S. D. Fine, Mumbai), Activated Charcoal (E. Merck, India), Gum Acacia (S. D. Fine Chem, Boisar) were used in this study.

Experimental Animals:

Six to eight weeks old mice (20-30 g) were used. The animals were acclimatized for 15 days under the standard laboratory conditions following procurement from Pasteur's Institute, Shillong. All the animal experiments were carried out in accordance with the Rules and Regulations approved by the Institutional Animal Care and Use Committee.

Preliminary acute toxicity test:

The *C. citratus* stalk extract was administered orally in the doses of 100, 200, 400, 800, 1600 and 3200 mg/kg, p.o. to six animals in each group. The animals were observed for mortality, if any, and adverse signs in terms of body weight, body temperature, and food and water intake for 72 h post administration of extract.

Antidiarrhoeal Activity:

A. Measurement of faecal output: Six groups of mice (n = 6) were housed singly in separate cages. Group I served as the control and received 2% gum acacia (0.5 ml); Groups II – IV mice were treated with 200, 400 and 800 mg/kg of plant extract. Group V mice received citral while, Group VI mice were given 0.5 ml of 5 mg/kg Loperamide HCl, the standard antidiarrhoeal drug. The faecal materials collected for 12 h post treatment, were dried in an incubator and their weights measured. The faecal output of animals were calculated and expressed in terms of percentage reduction [17].

B. Castor oil model:

Overnight-fasted mice were randomly divided into six groups (n = 6). Group I received 0.5 ml of 2% gum acacia suspension; groups II – IV were treated with 200, 400 and 800 mg/kg of plant

extract; Group V mice were given 5 mg/ kg of citral while group VI received 0.5 ml of 5 mg/kg of Loperamide. 1 h later, diarrhoea was induced in all groups by inoculating castor oil (0.5 ml/mouse, p.o.). The numbers of diarrhoeal episodes were recorded for each time and cumulative values were calculated for 4 h post induction of diarrhoea [18, 19].

C. Enteropooling assay:
Overnight-fasted mice were randomized into seven groups (n = 6). The animals received a diarrhoeal agent (0.5 ml of 10% $MgSO_4$/per mouse p.o.). Group I served as the control (0.5 ml; 2% gum acacia); Group II served as a vehicle control (10% $MgSO_4$ + 2% gum acacia); groups III – V received 200, 400 and 800 mg/kg of plant extract, respectively; group VI received 5 mg/kg dose of citral and group VII animals were given 5 mg/kg Loperamide. All these treatments were done 1 h prior to diarrhoeal induction. 30 min later, animals were sacrificed and their small intestines were ligated from pyloric sphincter to ileocaecal junction, and assessments of the accumulation of intestinal fluid secretion induced by $MgSO_4$ were made and expressed as percentage reduction in fluid secretion [20].

D. Gastrointestinal transit test:
The animals were starved for 16 h prior to the experiment. The test extract (200, 400 and 800 mg/kg) was given orally to groups II – IV of mice (n = 6). Group I served as the control, group V animals received 5 mg/kg of citral, while group VI was given 5 mg/kg Loperamide. 5 min later, 0.5 ml of charcoal meal was orally inoculated to each mouse. All the mice were sacrificed 30 min later, their small intestines from pylorus to caecum cut out and distance travelled by the charcoal marker measured, and expressed as a percentage of the total length of small intestines. The percentage inhibition of the marker transit in the intestine was calculated as described by Akah & Offiah [21].

Statistical analysis:
The results are expressed as mean ± standard error of mean (SEM). Significance of the result was analyzed using Student's *t*-test. *P* values < 0.05 were considered as significant.

Results

Acute toxicity test:
The plant extract administered orally to the mice up to 3200 mg/kg, p.o. did not show any mortality or any adverse signs in the animals in terms of body weight, body temperature, and food and water in take during 72 h period of observation.

Antidiarrhoeal activity:
Effect on faecal output:
The 800 mg/kg dose of extract reduced the faecal output by 53.44%. The reduction in faecal output by Loperamide and citral was 57.01 and 45.37%, respectively. The results (Table 1) were significantly different from the control value at $P < 0.05$ (200 mg/kg) and at $P < 0.001$ (400, 800 mg/kg extract and 5 mg/kg Loperamide HCl).

Table 1 Effect of *Cymbopogon citratus* extract and citral on faecal output in mice

Treatment (mg/kg, p.o.)	Wt. (g) of the dried faecal matter per 100 g mouse*	% Faecal output	% Reduction in faecal output
Control (2% gum acacia)	0.842 ± 0.058	100.00	0.00
Plant extract			
200	0.686 ± 0.021 [a]	81.47	18.53
400	0.445 ± 0.037 [b]	52.85	47.15
800	0.392 ± 0.007 [b]	46.56	53.44
Citral 5	0.460 ± 0.010 [b]	54.63	45.37
Loperamide			
5	0.362 ± 0.021 [b]	42.99	57.01

* Data represent mean ± SEM from six animals. [a]$P < 0.05$, and [b]$P < 0.001$ as compared with control group.

Effect on castor oil-induced diarrhoea:

The number of diarrhoeal episodes at each time in extract treated mice showed significant difference from the control value. The onset of diarrhoeal droppings was significantly delayed in the treated mice following castor oil challenge, and also the numbers of diarrhoeal episodes (cumulative value) for 4 h were significantly inhibited in the treated groups (Table 2). The percentage protection of diarrhoea at the end of 4 h was 50.00% by 800 mg kg dose of plant extract, and 5 mg/kg doses of citral and loperamide, respectively.

Table 2 Effect of *Cymbopogon citratus* extract and citral on castor oil induced diarrhoea in mice

Treatment at – 60 h (mg/kg, p.o.)	No. of diarrhoeal episodes at time*				% Protection
	1 h	2 h	3 h	4 h	
Control (2% gum acacia)	4.33 ± 0.56	6.67 ± 0.71	8.33 ± 0.49	8.67 ± 0.42	0.00
Plant extract					
200	0.83 ± 0.65[a]	1.50 ± 0.72[b]	2.00 ± 0.89[b]	2.17 ± 0.83[b]	33.33
400	0.67 ± 0.49[b]	1.33 ± 0.71[b]	1.83 ± 0.70[b]	1.83 ± 0.70[b]	33.33
800	0 [b]	0.50 ± 0.34[b]	1.17 ± 0.65[b]	1.50 ± 0.96[b]	50.00
Citral 5	0 [b]	0 [b]	1.17 ± 0.54[b]	1.17 ± 0.54[b]	50.00
Loperamide					
5	0[b]	0.3 3± 0.21[b]	0.50 ± 0.22[b]	0.50 ± 0.22[b]	50.00

* Data represent mean ± SEM from six animals.[a]$P < 0.01$, and [b]$P < 0.001$ as compared with control group.

Effect on enteropooling assay:
The extract reduced significantly the diarrhoeal fluid accumulation, evoked by induction of $MgSO_4$, both in terms of small intestinal weights and measure of the accumulated fluids in small intestines. 800 mg/kg dose of extract yielded 22.82% reduction. Whereas the reduction was observed to be 26.51% by Loperamide. The citral (5 mg/kg) showed the maximum reduction of 27.85% (Table 3).

Table 3 Effect of *Cymbopogon citratus* extract and citral on $MgSO_4$ - induced enteropooling in mice

Treatment (mg/kg, p.o.)	Vol. (in ml) of the small intestinal fluids accumulated per 100 g mouse*	% Reduction
Normal Control (0.5 ml saline)	1.40 ± 0.16	-
Vehicle Control (0.5 ml saline+ $MgSO_4$	2.98 ± 0.17	0.00
Plant extract		
200	2.55 ± 0.07 [a]	14.43
400	2.34 ± 0.15 [b]	21.48
800	2.30 ± 0.09 [c]	22.82
Citral 5	2.15 ± 0.10 [c]	27.85
Loperamide 5	2.19 ± 0.16 [c]	26.51

* Data represent mean ± S.E.M. from six animals. [a]$P < 0.05$, [b]$P < 0.02$, and [c]$P < 0.01$ as compared with vehicle control group.

Effect on charcoal induced gastrointestinal transit:
The extract showed a dose-dependent inhibition of the charcoal marker in the small intestine of treated animals. The motility inhibition by 57.22% in 800 mg/kg extract treated animals was slightly higher to the inhibition observed (55.83%) for Loperamide. The values of the distance travelled by charcoal marker are significantly ($P < 0.001$) different from the control value (Table 4).

Table 4 Effect of *Cymbopogon citratus* extract and citral on gastrointestinal transit in mice

Treatment (mg/kg, p.o.)	Distance (cm) traveled by charcoal marker as % of the total length of small intestine *	% Inhibition
Control (2% gum acacia)	81.66 ± 0.54	0.00
Plant extract		
200	69.26 ± 1.40 [a]	15.18
400	53.29 ± 1.82 [a]	34.74
800	34.93 ± 0.43 [a]	57.22
Citral 5	42.74 ± 2.84 [a]	47.66
Loperamide 5	36.07 ± 1.71 [a]	55.83

Data represent mean ± SEM from 6 animals. [a]$P < 0.001$ as compared with control group.

Discussion

This study was aimed at evaluating the antidiarrhoeal potentials of *C. citratus* stalk decoction, which is widely used in the treatment of diarrhoeal diseases by indigenous tribes of northeast India.

The main feature of the small intestine is to absorb and secrete. Diarrhoea results from an imbalance between the absorptive and secretive mechanisms in the intestinal tract, accompanied by intestinal hurry, which results in an excess loss of fluid through the faeces [22]. Many animal-based studies have investigated the bioactivity and effects on intestinal function of plants traditionally used as treatment for diarrhoea [2, 17, 19]. Plant extracts can have antispasmodic effects, delay gastrointestinal transit, suppress gut motility, stimulate water adsorbption or reduce electrolyte secretion. These activities may explain the benefits of using a particular plants in the treatment of diarrhoeal diseases [23]. Some of these experimental models were therefore employed to evaluate the antidiarrhoeal potentials of *C. citratus* stalk extract in the present study.

The results demonstrated that the plant extract reduced the faecal output as compared to the control group in a dose-dependent manner. This indicates towards the presence of an antisecretory or proabsorbtive property in the extract. A significant inhibition in the diarrhoeal droppings after castor oil induction observed in the present study, further suggests that the test extract has the property to inhibit both secretory and motility mechanisms of diarrhoea. These results which showed ability of plant extract to suppress the production and accumulation of wet faeces and an inhibitory effect on gastrointestinal motility met the standard criterion to prove its efficacy as an antidiarrhoeal agent. Castor oil is metabolized into ricinoleic acid, which in turn irritates and causes inflammation in the intestinal mucosa, resulting in to release of prostaglandins. The prostaglandins thus released stimulate the motility and secretion in the small intestines [23]. The therapeutic effect of Loperamide is believed to be due to its antimotility and antisecretory properties [24]. A significant inhibition in the diarrhoeal droppings after castor oil induction observed in the present study, further suggests that the test extract has the property to inhibit both secretory and motility mechanisms of diarrhoea.

Reduction of gastrointestinal motility is one of the mechanisms by which many antidiarrhoeal agents can act [24]. It was observed that the extract suppresses the propulsion of charcoal marker in a dose-dependent manner. In the present study the percentage inhibition of charcoal marker by 800 mg/kg dose of extract was observed to be slightly higher when compared to Loperamide. This finding suggests that *C. citratus* extract has the ability to influence the peristaltic movement of intestine indicating thereby the presence of an intestinal antimotility activity in it. These results which showed ability of plant extract to suppress the production and accumulation of wet faeces and an inhibitory effect on gastrointestinal motility met the standard criterion to prove its efficacy as an antidiarrhoeal agent.

In the small intestinal transit test the study showed that the plant extract suppress the propulsion of charcoal marker in a dose-dependent manner. This finding suggests that the extracts appear to act on all parts of intestine. The percentage propulsion of charcoal marker by *C. citratus* extract was observed to be almost similar as that of Loperamide. The mode of action of loperamide is believed due to its direct effect on the circular and longitudinal muscles of the intestinal wall. A decrease in the motility of gut muscles increases the amount of time substances stay in the intestine [26]. This allows for more water to be absorbed out of the water. We therefore presume that the reduction in the intestinal propulsive movement in charcoal meal model may be due presence of similar antispasmodic properties of plant extracts. It may be mentioned here that *C. citratus* possesses essential oil, citral as one of its major active chemical components [16]. It is advocated that the plants that have essential oils, are generally used traditionally for gastrointestinal disorders. In several studies on relaxant effects of essential oils, including citral it has been reported that the inhibition of contractile over-activity or reduction of inflammatory response of the ileum is their basis for the treatment of gastro-intestinal disorders such as, diarrhoea [27, 28, 29, 30].

In the acute toxicity study, the plant extract up to a dose of 3200 mg/kg did not cause any mortality or any changes in body temperature and food and water intake in the animals. The preliminary observations indicate that the plant extract is non-toxic in nature. In conclusion, this study provides support to the folk medicinal use of stalk decoction of *C. citratus* in the treatment of diarrhoea.

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