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Antidiarrhoeal evaluation of rhizomes of *Cryptocoryne spiralis* Fisch. ex Wydler: Antimotility and antisecretory effects

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The antidiarrhoeal activity of *Cryptocoryne spiralis* rhizomes extract (250, 500, 750 mg/kg, po) was evaluated using faecal excretion, castor oil-induced diarrhoea, small intestinal transit, intestinal fluid accumulation, gastric emptying and PGE_2 induced enteropooling models in rats. In addition, various biochemical estimations, histopathological studies and antibacterial evaluations on strains responsible for diarrhoea were also performed. The results illustrated a significant reduction in normal faecal output rate after 5^{th} and 7^{th} h of treatment, while castor oil-induced diarrhoea model depicted a protection of 55.44% at same dose level from diarrhoea. The other models except, gastric emptying test demonstrated more pronounced effect at same dose level. A significant inhibition in nitric oxide, increase in carbohydrates, protein, DNA, Na^+ and K^+ level with minimum degeneration of colonic fibrous tissues and potent antibacterial activity were also observed. The antidiarrhoeal potential of *C. spiralis* may be as a result of antimotility and antisecretory type effect mediated through nitric oxide pathway.

Keywords: *Aconitum heterophyllum*, Antidiarrhoeal activity, Castor oil induced diarrhoea, *Cryptocoryne spiralis*, PGE_2 induced enteropooling, Small intestinal transit

Diarrhoea is a gastrointestinal disorder/infection characterized by an increase in stool frequency, consistency (fluidity and volume), or movement of bowel (gut motility) resulting in loss of fluids, electrolytes and nutrients. Acute watery diarrhoea accounts for approximately 80% of such episodes, persistent diarrhoea for 10%, and dysentery for up to 10%[1]. Around nine million children under the age of five years die every year because of diarrhoea, where 88% of diarrhoeal-related deaths are attributed to inadequate sanitation and poor hygiene[2].

Herbal treatment remains an important home remedy for diarrhoea where traditional healers, practitioners and patients rely heavily on available phytomedicines that fulfills the objectives, such as increased resistance to flow (segmental contraction, decreased propulsion and peristalsis) and increased mucosal absorption or decreased secretion[3]. *Cryptocoryne spiralis* Fisch. ex Wydler*., family:* Araceae, is a common weed found in rice fields of India and Ceylon. In India, it is widely available in West Bengal, Calicut and Coromandal coast[4]. The rhizomes of the plant are easily available at low cost and are widely used as a substitute for highly expensive *Aconitum heterophyllum* Wall. (Ranunculaceae) for the treatment of diarrhoea, which is also well documented in Siddha system of medicine[5,6]. It is also used in the form of decoctions in combination with other drugs as a remedy for infantile vomiting, cough and in case of adults for abdominal complaints and fever[4]. So far only two steryl esters i.e. 5a-stigmast-11-en-3b-yl palmitate and 24-ethyl-5a-cholesta-8(14),25-dien-3b-yl stearate with one oxo fatty acid have been isolated from the rhizomes of *C. spiralis*[7]. Even though, the rhizomes of the weed are used by the traditional healers for the treatment of diarrhoea, still there is no scientific data available justifying its traditional use. Therefore, the present study has been carried out to validate traditional claims of *C. spiralis* rhizomes as an antidiarrhoeal agent.

Materials and Methods

Plant material and extraction procedure—The rhizomes of *C. spiralis* were obtained from Panakudi, Tirunelveli district of Tamil Nadu and were authenticated by Prof. V. Chelladurai [Retd. Botanist, Central Council for Research in Ayurvedic Sciences (CCRAS), Department of AYUSH, Chennai]. For future reference a voucher specimen (COG/CS/12) of the plant was deposited in Department. The rhizomes

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of the plant (500 g) were grinded and extracted with ethanol (1.5 L) using Soxhlet apparatus until the whole drug was exhausted. Further, the extract (ECS; 9.57%, w/w) was concentrated, evaporated in a Rota evaporator and kept in a desiccator until use.

Experimental animals—Healthy Charles Foster albino rats of either sex weighing between 150-200 g were procured from the Central Animal House (Reg. No. 542/02/ab/CPCSEA), Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. The certified pathogen free animals were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycle at an ambient temperature of 25 ±1°C and 45-55% RH). They were fed with commercially available rat feed ((Amrut Rat & Mice Feed Pvt. Ltd., Sangli, India) and water *ad libitum*. The animals were allowed to acclimatize to the environment for 7 days before the commencement of experiments. All experimental protocols were performed after approval from Central Animal Ethical Committee of Banaras Hindu University (No. Dean 10-11/60 dated 07/01/2011) and were conducted in accordance with accepted standard guidelines of National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985).

Quantitative estimations—Total alkaloid[8], total carbohydrates[9], total phenolic[10] and tannin[10], total flavonoid[11] and flavonol[11] and total saponin[12] contents were estimated in ECS.

Acute toxicity study—The OECD (Organization for Economic Co-operation and Development) guideline 425 was used to determine the oral acute toxicity study of plant extract. Nulliparous and non pregnant healthy female rats were used for this study. To the overnight fasted rats, ethanolic extract of *C. spiralis* (ECS) was administered orally as per the guidelines and the rats were observed individually up to 48 h for any behavioural and neurological changes such as tremors, convulsions, salivation, diarrhoea, sleep, lacrimation and feeding behaviour in extract treated rats as a sign of acute toxicity. The observation was further extended up to 14 days to see any sign of mortality[13].

Faecal excretion—The method described by Croci and Bianchetti[14] with some modification was adopted to study the effect of ECS on faecal excretion rate in rats. The rats were divided into following 5 groups: Group 1 was served as control and was given 0.5% carboxy methyl cellulose (CMC), while groups 2 to 4 were administered ECS suspended in 0.5% CMC at a dose level of 250, 500 and 750 mg/kg, po (equivalent to approximately 25, 50 and 75 mg/kg, po for humans)[15]. Group 5 was kept as positive control and was given loperamide at dose level of 2 mg/kg, po (Torrent Pharmaceuticals India Ltd., Ahmadabad, India). The dose of the extract was selected based upon the results of acute oral toxicity study. Food was withdrawn from the cages 3 h before commencement of the experiment. The pellets discharged by the rats at 1st, 3rd, 5th and 7th h after treatment were collected and weighed immediately followed by taking its dry weight (after drying for 24 h at 50 °C). Further, wet to dry ratio of pellets discharged 7 h after treatment was calculated to assess the effect of ECS on absorption of fluids.

Castor oil-induced gastrointestinal transit test using charcoal meal—The rats fasted for 18 h were divided into following 6 groups: Group 1 was treated as vehicle control and was given 0.5% CMC, groups 2, 3 and 4 were given ECS orally at dose mentioned before while groups 5 and 6 were administered standard loperamide (2 mg/kg, po) and atropine (0.1 mg/kg, sc). After 30 min of the above treatment the animals were administered 1 mL of castor oil orally by gavage. Followed by this, 1 mL of 5% deactivated charcoal suspended in 10% aqueous tragacanth gum po was given to rats 30 min after the castor oil administration. After 30 min of charcoal meal administration, rats were sacrificed by cervical dislocation, the abdomen was cut, opened and the small intestine was carefully removed and the distance travelled by the charcoal plug from the pylorus was measured to calculate the Peristaltic Index (PI)[16].

Castor oil-induced diarrhoea—In this method, rats fasted for 18 h were divided into following 6 groups: Groups 1 and 2 received 0.5% CMC, groups 3, 4 and 5 received ECS and 6 was given standard loperamide. One hour after the above treatment, the rats from groups 2 to 6 were administered 1 mL of castor oil orally by gavage. The animals were then transferred into cages containing plastic sheets at the base and then were kept for observation of different parameters up to 4 h with a change of sheets after every hour. In addition, an hourly effect of ECS on overall and wet faeces was observed for the first time and *in vivo* antidiarrhoeal index ($ADI_{in\ vivo}$) was also calculated[16,17].

Castor oil-induced intestinal fluid accumulation—Grouping and treatment of rats was same to that of castor oil-induced diarrhoea model. After 30 min of the above treatment the rats were given orally 1 mL of

castor oil through gavage. Later, 30 min after castor oil administration, all the rats were sacrificed by cervical dislocation and small intestine was dissected from the pylorus to caecum and the volume of its content was measured. The intestinal fluid was then collected and was analyzed for Na^+ and K^+ concentration using flame photometer (Elico CL-360, Elico Pvt. Ltd., India)[16,18].

PGE_2-induced enteropooling—For this study rats deprived for food and water for 18 h were divided in following 5 groups: Groups 1 and 2 were served as control and PGE_2 control and were treated with 2% (w/v) aqueous tragacanth suspension while groups 3, 4 and 5 were given ECS. Immediately after this, rats of all the groups except control group were administered 100 µg/kg po of PGE_2 (Astra Zeneca, Bangalore, India) in 5% (v/v) ethanol in normal saline. After 30 min of the above treatment the rats were sacrificed by cervical dislocation and the whole intestine from pylorus to the caecum was dissected out and the volume of the intestinal content was measured[17].

Gastric emptying—Rats fasted for 24 h were divided into four groups keeping group 1 as control and other three groups were treated with ECS. The rats were administered 3 mL of a semisolid meal based on methyl cellulose orally 1 h after the treatment. The rats were further sacrificed by cervical dislocation and laparatomized after 1 h and their stomachs were removed. The full stomach of each rat was weighed, opened, rinsed, reweighed and the difference in weight between the full and empty stomachs was subtracted from the weight of 3 mL of the test meal[16].

Biochemical estimations and histopathological studies—The intestine dissected out during the castor oil-induced fluid accumulation test was immediately taken and colonic portion of the intestine was removed and rinsed with tyrode solution to remove all the contents. The tissue was then homogenized with phosphate buffer, centrifuged and the supernatant was used for nitric oxide (NO) assay[19]. Total carbohydrate (reducing sugars) in the tissues was estimated by adopting the ferricyanide method[20], whereas DNA content in the tissue sample was determined using the method proposed by Burton[21]. The method proposed by Lowry *et al*.[22] was used to estimate total protein content in the tissue sample.

For histopathological studies the dissected colonic portion was immediately blotted, dried and fixed in 10% formalin. For sectioning the samples were first dehydrated in acetone and were embedded in paraffin wax. Section (4 µm thickness) of the tissues samples were taken using microtome and stained with haematoxylin-eosin (H & E). Photomicroscopic examination of the tissues was carried out on Nikon Trinocular Microscope, Model E-200, Japan[23].

Antibacterial activity—The study was performed on four reference bacterial strains i.e. *Escherichia coli* (ATCC 25922), *Shigella flexneri* (ATCC 12022), *Pseudomonas aeruginosa* (ATCC 27893), *Staphylococcus aureus* (ATCC 25323) and seven clinical bacterial isolates- *Salmonella typhi*, *Shigella dysenteriae*, *Proteus vulgaris, Klebsiella pneumonia, Shigella boydii*, *Bacillus cereus* and *Enterococcus faecalis*. All the cultures used for the study were obtained from the American Type Culture Collection (ATCC), MTCC, clinical strains preserved at Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

Antibacterial potential of the extract was determined using disc diffusion method where Muller Hinton Agar (MHA) plates were used as a nutrient medium. The suspensions of the bacterial strains were spread on the surface of MHA agar plates which were then allowed to dry for 5 min. The extract (ECS) to be tested was then applied on 6 mm sterile disc of Whatman flter paper no. 1 in different concentrations (100 and 200 mg/mL). The discs were placed on the surface of the nutrient medium and the extract was allowed to diffuse for 5 min. These plates were then incubated for 24 h at 37 °C and inhibition zones around the discs were examined in triplicate. For determining the MIC, the extract was first diluted with equal volume of nutrient broth which was further mixed in wells of microtiter plate. Briefly, 0.1 mL of standardized inoculums was added in each tube and the plates were incubated aerobically at 37 °C for 18-24 h. The minimum concentration showing no visible bacterial growth was considered as MIC[24,25].

Statistical analysis—The experimental results are expressed as mean±SE from 6 animals in each group followed by one-way analysis of variance (ANOVA). Newman–Keuls Multiple Comparision Test was applied for determining the statistical significance between different groups. However, two-way ANOVA followed by Bonferroni post test was performed for determining the faecal excretion rate. GraphPad Prism (version 4) software was used for all statistical analysis. P value <0.05 was considered significant.

Results

All the quantitative estimations of phytoconstituents are represented in Table 1. The acute oral toxicity study of the extract showed no signs of toxic effects on rats up to 5 g/kg. The observation also depicted a normal behaviour pattern of rats. Results of the faecal excretion study (Table 2) demonstrated a significant interaction between groups and time. Compared to the control group, rats treated with ECS at 500 mg/kg, po showed a significant ($P < 0.05$) reduction in excretion rate only after 7th h of treatment, whereas at 750 mg/kg, po of ECS, a significant reduction in discharge of pellets after 5th and 7th h ($P < 0.05$) of treatment was observed. Standard loperamide almost blocked the discharge of pellets and also showed a significant reduction in water content of faeces. The results obtained from the castor oil-induced gastrointestinal transit test using charcoal meal showed a significant ($P < 0.05$) effect only at 750 mg/kg, po of ECS with a peristaltic index (PI) of 53.175 ±3.137 compared to control group (PI: 71.225 ±2.982) (Fig. 1A).

Administration of castor oil to the rats produced copious diarrhoea. However, treatment with ECS at 750 mg/kg, po showed a delay in onset of diarrhoea which was quite comparable with loperamide and showed a significant reduction in frequency of purging i.e. decrease in total number and weight of wet as well as overall stools. ECS at 750 mg/kg, po,

Table 1—Quantitative estimations of *C. spiralis*

Phytoconstituents	Quantity
Total alkaloid content (% w/w in plant material)	0.108
Total carbohydrate content (mg/g d-fructose equivalent)	128.422
Total phenolic content (mg/g gallic acid equivalent)	18.610
Total tannin content (mg/g tannic acid equivalent)	14.700
Total flavonoid content (mg/g rutin equivalent)	6.578
Total flavonol content (mg/g rutin equivalent)	3.272
Total saponin content (mg/g diosgenin equivalent)	2.790

Table 2—Effect of *C. spiralis* on faecal excretion

[Values are mean ± SE from 6 animals in each group]

Treatment mg/kg, po	Faecal wet weight (g) at various times (h) after treatment				Wet/dry weight of faeces
	1	3	5	7	
Control	0.067 ±0.013	0.210 ±0.028	0.426 ±0.041	0.772 ±0.065	1.499 ±0.054
ECS 250	0.174 ±0.028	0.312 ±0.038	0.486 ±0.046	0.831 ±0.087	1.338 ±0.045
ECS 500	0.167 ±0.024	0.281 ±0.035	0.472 ±0.055	0.611 ±0.069^{ab}	1.278 ±0.041
ECS 750	0.081 ±0.020	0.199 ±0.026	0.232 ±0.035^{abc}	0.520 ±0.063^{ab}	1.122 ±0.026
Loperamide	0.042 ±0.019	0.073 ±0.027^{bc}	0.072 ±0.027^{abcd}	0.071 ±0.026^{abcd}	0.848 ±0.272^{a}

P values: < 0.05 vs. ^{a}Control, ^{b}ECS 250, ^{c}ECS 500 and ^{d}ECS 750

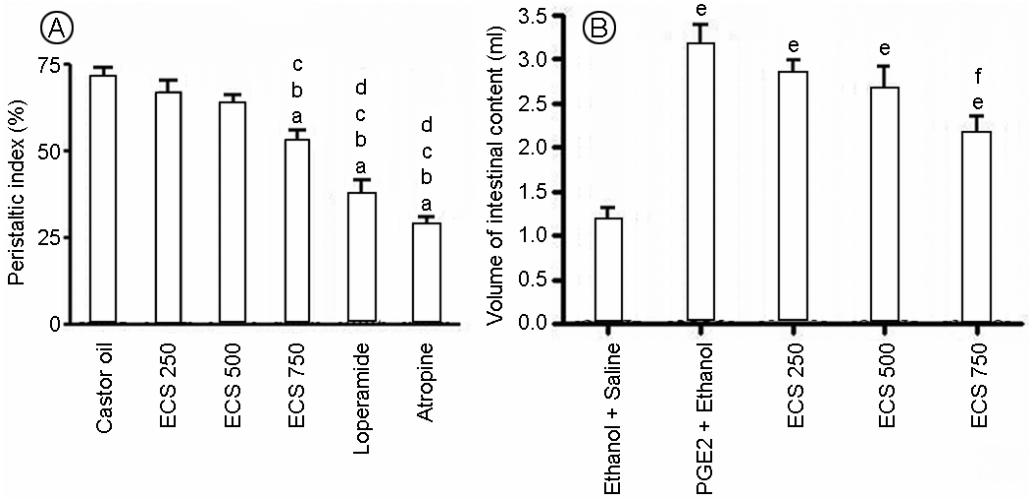

Fig. 1—Effect of *C. spiralis* on castor oil induced gastrointestinal transit (A) and PGE_2 induced enteropooling test (B). [Values are mean ± SE from 6 animals in each group] *P* values: < 0.05 vs. ^{a}Castor oil, ^{b}ECS 250, ^{c}ECS 500, ^{d}ECS 750, ^{e}Ethanol + Saline and $^{f}PGE_2$ + Ethanol.

also depicted a significant reduction in mean defecation of faeces, diarrhoea score and recovery from loss in body weight (Table 3). Inter hour effect depicted sever diarrhoea at the second and third hour of castor oil administration which was controlled quite considerably by treatment with ECS at the higher dose level. Castor oil produced a significant ($P < 0.05$) increase in the intestinal fluid volume compared to normal rats (Table 4). However, rats treated with 750 mg/kg, po of ECS showed a significant reduction in intestinal fluid accumulation which was found to be 2.383 ±0.162 mL compared to castor oil control group 3.566 ±0.296 mL. The intestinal fluid analyzed for Na^+ and K^+ concentration showed a marked increase in their secretion as observed in castor oil control group compared to normal rats. However, treatment with ECS at 750 mg/kg, po showed a significant reduction in the Na^+ and K^+ concentration which was more pronounced in case of Na^+ compared to K^+. PGE_2 significantly increased the intestinal fluid volume compared to the rats treated with ethanol in normal saline. However, treatment with ECS at 750 mg/kg, po showed a significant reduction in intestinal fluid volume (Fig. 1B). The extract at all dose level did not show any significant effect on gastric emptying as elicited through difference in weights of stomach content.

Oral administration of castor oil caused a significant production of NO compared to normal rats. Treatment with ECS only at higher dose i.e. 750 mg/kg, po depicted a significant ($P < 0.01$) reduction in NO level. Also, there was a marked reduction in the total carbohydrate (reducing sugars), protein and DNA content caused by castor oil compared to normal rats. However, treatment with ECS (750 mg/kg, po), prevented the loss of carbohydrates, protein and DNA significantly compared to castor oil control group (Table 5). Histopathologically distinct, clear and

Table 3—Effect of *C. spiralis* on castor oil induced diarrhoea model

[Values are mean ± SE from 6 animals in each group]

Treatment mg/kg, po	Onset time (min)	Total no of faeces	Total no of wet faeces	Loss in body weight	Total wt of faeces	Mean defecation	Diarrhoea score	Prot ection (%)	*In vivo* antidiarrhoeal index (%)
Normal	-	2.667 ±0.334	-	0.116 ±0.030	0.329 ±0.034	0.667 ±0.083	-	-	-
Castor oil	44.667 ±2.043	9.50 ±1.087[a]	7.166 ±0.60	1.094 ±0.101[a]	1.783 ±0.190[a]	2.375 ±0.271[a]	16.833 ±1.376	-	-
ECS 250	52.50 ±2.045	8.166 ±0.703[a]	5.0 ±1.064[b]	0.891±0.060[a]	1.366 ±0.160[a]	2.041 ±0.175[a]	12.334 ±1.054[b]	26.732	13.630
ECS 500	60.50 ±1.945	7.334 ±0.843[a]	4.166 ±0.477[b]	0.721 ±0.068[ab]	1.575 ±0.088[a]	1.833 ±0.210[a]	11.166 ±1.167[b]	33.663	22.778
ECS 750	83.334 ±2.485	5.833 ±0.749[ab]	3.334 ±0.614[b]	0.607 ±0.078[ab]	0.958 ±0.121[abcd]	1.458 ±0.187[ab]	7.50 ±0.991[bcd]	55.445	39.633
Loperamide	125.667 ±5.829	4.0 ±0.632[bcd]	1.50 ±0.428[bcd]	0.407 ±0.138[abcd]	0.577 ±0.065[bcde]	1.0 ±0.158[bcd]	4.0 ±0.894[bcde]	76.237	61.675

P values: < 0.05 vs. [a]Normal, [b]Castor oil, [c]ECS 250, [d]ECS 500 and [e]ECS 750

Table 4—Effect of *C. spiralis* on castor oil induced intestinal fluid accumulation

[Values are mean ± SE from 6 animals in each group]

Treatment mg/kg, po	Weight of intestinal content (g)	Volume of intestinal content (mL)	% Inhibition	Na^+ (mmol/L)	K^+ (mmol/L)
Normal	1.646 ±0.125	1.416 ±0.289	-	94.166 ±5.134	6.10 ±0.350
Castor oil	3.625 ±0.258[a]	3.566 ±0.296[a]	-	142.50 ±6.291[a]	8.716 ±0.548[a]
ECS 250	3.075 ±0.294[a]	3.216 ±0.162[a]	9.813	133.334 ±5.885[a]	8.066 ±0.431[a]
ECS 500	2.879 ±0.160[a]	3.0 ±0.146[a]	15.887	122.167 ±6.405[a]	7.766 ±0.407[a]
ECS 750	2.533 ±0.287[ab]	2.383 ±0.162[ab]	33.177	113.50 ±6.190[b]	7.083 ±0.309[ab]
Loperamide	1.731 ±0.117[bcde]	1.516 ±0.334[ebcde]	57.476	107.0 ±6.110[bc]	7.033 ±0.277[ab]

P values: < 0.05 vs. [a]Normal, [b]Castor oil, [c]ECS 250, [d]ECS 500 and [e]ECS 750.

Table 5—Effect of *C. spiralis* on biochemical parameters.

[Values are mean ± SE from 6 animals in each group]

Treatment mg/kg, po	NO (Units in mole/ mg of protein)	Total carbohydrates (Conc mg/g of tissue)	Total protein (Units in mg/ 100 mg of tissue)	Total DNA (Units in mg/ 100 mg of tissue)
Normal	0.845 ±0.080	1.101 ±0.068	1.812 ±0.126	0.143 ±0.0112
Castor oil	3.716 ±0.255[a]	0.505 ±0.049[a]	0.759 ±0.031[a]	0.091 ±0.0056[a]
ECS 250	3.242 ±0.229[a]	0.577 ±0.065[a]	0.936 ±0.049[a]	0.103 ±0.0056[a]
ECS 500	2.957 ±0.257[a]	0.699 ±0.075[a]	1.202 ±0.094[ab]	0.111 ±0.0043[a]
ECS 750	2.448 ±0.248[abc]	0.813 ±0.085[ab]	1.347 ±0.141[abc]	0.122 ±0.0059[b]
Loperamide	2.358 ±0.230[abc]	0.776 ±0.051[ab]	1.444 ±0.153[abcd]	0.134 ±0.0051[bc]

P values: < 0.05 vs. [a]Normal, [b]Castor oil, [c]ECS 250 and [d]ECS 500.

intact epithelia with normal glands was observed in normal rats while in castor oil treated groups, denaturation and necrosis was observed in epithelia. Treatment with standard loperamide and ECS prevented the damage to quite an extent which was evident through a proliferative effect on fibrous tissues (Fig. 2).

The results also demonstrated a potential antibacterial activity of ECS against majority of bacterial strains where maximum inhibition was achieved at 200 mg/mL as evident through diameter of zone of inhibition around extract. The extract was more effective against Gram positive bacteria compared to Gram negative bacteria showing MIC values ranging from 1.562 to 6.25 mg/mL. However, ECS was found to be ineffective against *K. pneumonia*, whereas, *P. vulgaris* was found to be less affected (Table 6).

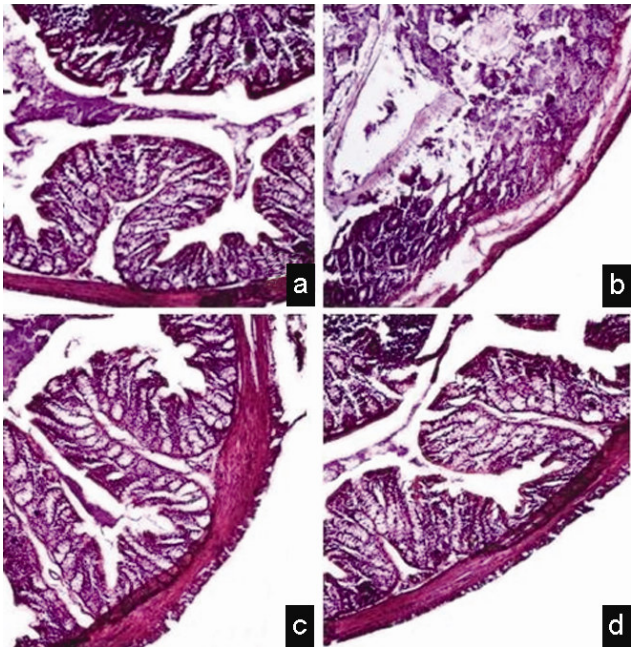

Fig. 2—Effect of *C. spiralis* rhizomes extract on rat colons [a: Normal control rat colon, b: Castor oil control rat colon, c: ECS 750 mg/kg, po treated rat colon and d: Loperamide (2 mg/kg, po) treated rat colon. H & E, 40X]

Discussion

In the present study, an attempt was made to scientifically validate the use of rhizomes of *C. spiralis* in treatment of diarrhoea. The results depicted a significant antidiarrhoeal activity of ECS at 750 mg/kg, po which was evident through different animal models, biochemical parameters, histopathological and antibacterial studies. The quantitative estimations performed in the present study showed the presence of carbohydrate and alkaloids as a major component while tannins, phenolic, flavonoids, flavonols and saponins were found to be in less quantity. The acute oral toxicity study showed that the extract was safe up to 5 g/kg with no signs of toxicity. From the faecal excretion study, it was presumed that the extract at higher dose significantly decreased the frequency of defecation and wetness of the faeces which may be attributed to an antimotility and antisecretory property similar to loperamide[26].

Significant results obtained from the castor oil induced gastrointestinal transit test at 750 mg/kg, po of ECS is indicative of an antisecretory like action contributing to an reduction in intestinal propulsive movement[3]. The effect of ECS on castor oil-induced diarrhoea significantly reduced the frequency and severity of diarrhoea, which was justified through calculated *in vivo* antidiarrhoeal index (ADI). Here it was found that, the higher the ADI values the more was the extract effective in curing diarrhoea. The effect of ECS on castor oil induced intestinal fluid accumulation was reported to be highly significant at

Table 6—Antibacterial activity of *C. spiralis.*

Bacterial strains	Zone of inhibition (in mm) [Values are mean ± SE from 3 individual readings]			MIC (mg/mL)
	100 mg/mL	200 mg/mL	Ciprofloxacin	
B. cereus	7.766 ±0.348	9.833 ±0.284	25.933 ±0.392	1.562
S. aureus	7.766 ±0.218	10.33 ±0.317	27.833 ±0.317	1.562
E. faecalis	6.766 ±0.272	10.3 ±0.321	30.3 ±0.360	3.125
S. flexneri	7.366 ±0.669	8.4 ±0.264	20.8 ±0.321	3.125
S. typhi	6.166 ±0.317	7.8 ±0.321	25.866 ±0.260	6.25
S. dysenteriae	7.233 ±0.260	8.966 ±0.338	24.334 ±0.260	6.25
P.. vulgaris	7.133 ±0.384	9.533 ±0.366	24.033 ±0.409	6.25
E. coli	7.633 ±0.240	12.166 ±0.448	27.966 ±0.233	3.125
K. pneumoniae	-	-	28.8 ±0.360	-
P. aeruginosa	8.166 ±0.240	12.1 ±0.230	26.166 ±0.433	6.25
S. boydii	9.266 ±0.290	10.334 ±0.317	25.766 ±0.328	6.25

750 mg/kg, po compared to control group. The diarrhoea inducing property of castor oil is due to its active metabolite i.e. ricinolic acid, which reduces active Na^+ and K^+ absorption and decrease Na^+, K^+ ATPase activity resulting in altered electrolyte permeability of intestinal mucosa[27]. The results obtained from the Na^+ and K^+ analysis showed a significant reduction in their loss in group treated with 750 mg/kg, po of ECS which may be attributed to an inhibition in active secretion of ricinolic acid resulting in activation of Na^+, K^+ ATPase activity[28]. The retardation in castor oil induced fluid accumulation was well supported by PGE_2 induced enteropooling study which demonstrated an antienteropooling effect of ECS at 750 mg/kg, po Mascolo *et al*.[29] have reported that castor oil induces the release of nitric oxide (NO), which in turn provokes the generation of prostaglandins by colonic cells. In the present study, a decline in NO level was observed which may prevent nitrosative and oxidative stress induced mucosal damage[30]. The combined effect can be attributed to either a decrease in mucosal secretion or increase in mucosal absorption thus, delaying the passage of gastrointestinal contents and allowing the faeces to become desiccated which retards its movement through the colon[3]. The increased level of protein and DNA in the tissue may attribute to a cellular proliferative action of ECS. In addition, the histopathological studies revealed a proliferative action of ECS on colonic cells showing minimum degeneration of colonic fibrous tissues seldom to that of untreated group.

The results obtained from antibacterial evaluation demonstrated a potential efficacy of ECS, which was characterized by inhibition in growth of bacteria including those responsible for diarrhoea. Pathogenic microorganisms play a vital role in causing diarrhoea[25]. The most common causative agents include *Escherichia coli*, which effects around 2–5% in developed and 14–17% in developing countries. The other important causative microorganisms include *Campylobacter spp, Salmonella spp* and *Shigella spp*[1]. Thus, from the outcome of the present study, the rhizomes of the weed may be used as a potential antibacterial agent.

Among the phytoconstituents quantified, alkaloids have been previously reported to posses antidiarrhoeal activity[3,31,32]. The rhizomes have shown the presence of very high amount of carbohydrates (reducing sugars) which acts as a means of storing and transporting energy. Sugars have been shown to posses ecological role in protection from wounding, infections and detoxification from foreign substances[32]. Tannins present in the extract act as a protein denaturating agent producing protein tannates which makes intestinal mucosa more resistant and reduces secretion[27]. Phenols and flavonoids, however present in less quantity, have been reported to show their effect through their antioxidant and free radical scavenging activities[30]. Thus, the conjugation of these phytoconstituents has enhanced the antidiarrhoeal activity of ECS.

It is generally estimated that, out of 300,000 species of higher plants, approximately 10,000 of them have been documented for medicinal use. Out of these only 150-200 plants have been preceded beyond initial preclinical evaluations. Various factors attributing to the above fact includes very less yield of phytoconstituents, improper system of production, non economical production and less availability of precursor of the natural product that can be converted economically by semi-synthesis to the final bulk active product[33]. In the present study, it can be concluded that, the antidiarrhoeal activity of ECS may be due to antimotility and antisecretory type effect mediated through nitric oxide path way. The overall effect was found to be highly significant at 750 mg/kg, po compared to other treated groups. The present results have scientifically validates the traditional claims of the rhizomes in the treatment of diarrhoea and have also justified the cost effective substitution of *C. spiralis* in place of *A. heterophyllum* in curing diarrhoea. However, further research is on to isolate pure phytoconstituents(s) mainly responsible for the antidiarrhoeal activity of rhizomes that may lead to discovery of new antidiarrhoeal lead molecule.

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Disclosure statement

No competing financial interests exist.

References

1 Thapar N & Sanderson, I R, Diarrhoea in children: an interface between developing and developed countries, *Lancet,* 363 (2004) 641.

2 DeWeta H, Nkwanyanaa M N & Van Vuurenb S F, Medicinal plants used for the treatment of diarrhoea in northern Maputaland, KwaZulu-Natal Province, South Africa, *J Ethnopharmacol,* 130 (2010) 284.

3 Akindele A J & Adeyemi O O, Evaluation of the antidiarrhoeal activity of *Byrsocarpus coccineus*, *J Ethnopharmacol,* 108 (2006) 20.

4 Kirtikar K R & Basu B D, *Indian medicinal plants* (M/s Bishen Singh Mahendra Pal Singh. Dehradun, India) 1975.

5 Anandakumar A, Rajendran V, Thirugnanasambantham M P, Balasubramanian M, Muralidharan R, The pharmacognosy of Nattu Atividayam – the corms of *Cryptocoryne spiralis* Fisch, *Anc Sci Life,* 1 (1982) 200.

6 Mudaliar M K S, *Siddha Meteria Medica,* Vol. 1, 2nd edition (Medicinal Plants Division, Indian Medicine, Chennai, India) 2008.

7 Prasad S K, Sahu A N & Hemalatha S, Cytomorphological and physicochemical evaluations of *Cryptocoryne spiralis*, *J Herbs, Spices Med Plants,* 18 (2012) 304.

8 Wagner H & Bladt S. *Plant drug analysis,* 2nd edition. (Springer-Verlog Berlin Heidelberg, Germany) 1996.

9 Yemm E W & Willis A J, The estimation of carbohydrates in plant extracts by anthrone*, Biochem J,* 57 (1954) 508.

10 Hagerman A, Harvey-Muller I & Makkar H P S, Quantification of tannins in tree foliage- a laboratory manual,. Vienna: *FAO/IAEA,* 537 (2000) 4.

11 Kumaran A & Karunakaran J, *In vitro* antioxidant activities of methanol extracts of five Phyllanthus species from India, *LWT - Food Sci Technol,* 40 (2007) 344.

12 Baccou J C, Lambert F & Sauvaire Y, Spectrophotometric method for the determination of total steroidal sapogenin, *Analyst,* 102 (1977) 458.

13 Organization for Economic Co-operation and Development (OECD). OECD guidelines for the testing of chemicals- 425 (Acute oral toxicity – up and down procedure). Available from: http://www.oecd.org [last accessed on 2006 Mar 23], 2006.

14 Croci T & Bianchetti A, Stimulation of faecal excretion in rats by a-adrenergic antagonist, *J Pharm Pharmacol,* 121 (1992) 1557.

15 Rogge M C & Taft D R, *Preclinical drug development* (Taylor & Francis Group. Boca Raton, FL) 2005.

16 Adeyemi O O, Akindele A J & Ogunleye E A, Evaluation of the antidiarrhoeal effect of *Sanseviera liberica* Gerome & Labroy (Agavaceae) root extract, *J Ethnopharmacol,* 123 (2009) 459.

17 Gunakkunru A, Padmanaban K, Thirumal P, Pritila J, Parimala G, Vengatesan N, Gnanasekar N, Perianayagam J B, Sharma S K & Pillai K K, Anti-diarrhoeal activity of *Butea monosperma* in experimental animals, *J Ethnopharmacol,* 98 (2005) 241.

18 Rao C V, Vijayakumar M, Sairam K & Kumar V, Antidiarrhoeal activity of the standardised extract of *Cinnamomum tamala* in experimental rats, *J Nat Med,* 62 (2008) 396.

19 Bell F K, O'Neill J J & Burgison R M, Determination of the oil/water distribution coefficients of glyceryl trinitrate and two similar nitrate esters, *J Pharm Sci,* 52 (1963) 637.

20 Krystal G & Graham A F, A sensitive method for estimating the carbohydrate content of glycoproteins*, Anal Biochem,* 70 (1976) 336.

21 Burton K, A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid, *Biochem J,* 62 (1956) 315.

22 Lowry O H, Rosebrough N J, Farr A L & Randall R T, Protein measurement with the Folin phenol reagent, *J Biol Chem,* 193 (1951) 265.

23 Qin Y, Wang J B, Kong W J, Zhao Y L, Yang H Y, Dai C M, Fang F, Zhang L, Li B C, Jin C & Xiao X H, The diarrhoeogenic and antidiarrhoeal bidirectional effects of rhubarb and its potential mechanism, *J Ethnopharmacol,* 133 (2011) 1096.

24 Teke G N, Kuiate J R, Ngouateu O B & Gatsing D, Antidiarrhoeal and antimicrobial activities of *Emilia coccinea* (Sims) G. Don extracts, *J Ethnopharmacol,* 112 (2007) 278.

25 Lumpu S N, Lutete G T, Kabangu O K, Kanyanga R C, Apers S, Pieters L & Vlietinck A J, Assessment of the antidiarrhoeal properties of the aqueous extract, the 80% methanol extract and its soluble fractions of the leaves of *Alstonia congensis* Engl. (Apocynaceae) in Wistar rats, *J Ethnopharmacol,* 142 (2012) 620.

26 Tangpu V & Yadav A K, Antidiarrhoeal activity of *Rhus javanica* ripen fruit extract in albino mice, *Fitoterapia,* 75 (2004) 39.

27 Sairam K, Hemalatha S, Kumar A, Srinivasan T, Jai Ganesh, Shankar M & Venkataraman S, Evaluation of anti-diarrhoeal activity in seed extracts of *Mangifera indica*, *J Ethnopharmacol,* 84 (2003) 11.

28 Akter R, Hasan R S M, Hossain M, Jamila M, Mazumder E H & Rahman S, *In vitro* antioxidant and *in vivo* antidiarrhoeal activity of hydromethanolic extract of *Xanthium Indicum* Koenig. Leaves, *Eur J Sci Res,* 33 (2009) 305.

29 Mascolo N, Angelo A I, Autore G, Barbato F & Capasso F, Nitric oxide and castor oil induced diarrhoea, *J Pharmacol Exp Ther,* 263 (1994) 291.

30 Hu J, Gaob W, Ling N & Liu C, Antidiarrhoeal and intestinalmodulatory activities of Wei-Chang-An-Wan extract, *J Ethnopharmacol,* 125 (2009) 450.

31 Biswas S, Murugesan T, Sinha S, Maiti K, Gayen J R, Pal M & Saha B P, Antidiarrhoeal activity of *Strychnos potatorum* seed extract in rats, *Fitoterapia,* 73 (2002) 43.

32 Otshudi A L, Vercruysse A & Foriers A, Contribution to the ethnobotanical, phytochemical and pharmacological studies of traditionally used medicinal plants in the treatment of dysentery and diarrhea in Lomela area, Democratic Republic of Congo (DRC), *J Ethnopharmacol,* 71 (2000) 411.

33 James D. McChesney, Venkataraman S K, Henri J T, Plant natural products: Back to the future or into extinction?, *Phytochemistry*, 68 (2007) 2015.