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Evaluation of anti-diarrhoeal activity of hydro alcoholic extract of *Chenopodium album* L.

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Chenopodium album L. (*Chenopodiaceae*), a wildly growing plant has been reported to possess number of medicinal properties including treatment of diarrhoea. The purpose of the present study was to evaluate scientifically the anti-diarrhoeal effects of *C. album* used traditionally in Indian system of medicine. The anti-diarrheal effect of hydro alcoholic (30:70) extract (HEMC) of aerial parts was studied against castor oil-induced-diarrhoea model in rats. The volume of intestinal content induced by PGE_2 was studied by enter pooling method. The gastrointestinal transit rate was expressed as the percentage of the longest distance traversed by the charcoal divided by the total length of the small intestine. Like Loperamide (3 mg/kg, orally) there were significant reductions in fecal out put and frequency of droppings when the hydro alcoholic extract of 200 and 400 mg/kg doses were administered orally compared with castor oil treated rats. The plant extracts also significantly retarded the PGE_2 induced entero pooling with reduction of volume by 40.34 and 59.88%, respectively. The extract at a dose of 200 and 400 mg/kg significantly inhibited (P<0.01) volume of intestinal content. HEMC and atropine sulphate (0.1 mg/kg) decreased the propulsion of the charcoal meal through the gastrointestinal tract when compared with the control. Distance travelled by the charcoal meal was reduced to 21.91 and 74.80% in the HEMC treated groups with the dose of 200 and 400 mg/kg, respectively, compared to control group. The remarkable anti-diarrhoeal effect of *C. album* extract against castor oil-induced diarrhoea model, PGE_2 enter pooling and intestinal transit by charcoal model attests to its utility in traditional medicine for diarrhoea.

Keywords: *Chenopodium album,* Antidiarrhoeal, Castor oil, PGE_2, Intestinal transit, Loperamide, Atropine sulphate.

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Introduction

Gastrointestinal diseases particularly constipation and diarrhoea are affecting 70% of the population worldwide[1]. In developing countries, the majority of people living in rural areas almost exclusively use traditional medicines in treating all sorts of disease including diarrhoea. There are large numbers of epidemiological and experimental evidence pertaining to worldwide acute-diarrhoeal disease, which is one of the principle causes of death in infants, particularly in malnourished in developing countries[2,3]. Thus it becomes important to identify and evaluate commonly available natural drugs as alternative to currently used anti-diarrhoeal drugs, which are not completely free from adverse effects[4]. Several studies have evaluated the effectiveness of some traditional medicines in treating diarrhoea, in different continents[5-7]. India has a great environmental and biological diversity compared with the rest of the world. A range of medicinal plants with anti-diarrhoeal properties has been widely used by the traditional healers, however, the effectiveness of many of these anti-diarrhoeal traditional medicines has not been scientifically evaluated. One such herb is *Chenopodium album* L. (Chenopodiaceae) which is found wild up to an altitude of 4700 m and cultivated throughout India particularly western Rajasthan, Kulu valley and Shimla. It is commonly known as Lamb's quarte, wild spinach, white goosefoot in English[8,9].

In traditional system of medicine, it is used as an anthelmintic, antidiarrhoeal, antiphlogistic, antirheumatic, contraceptive, odontalgic, laxative, cardiotonic, antiscorbutic, blood purifier, hepatic disorder, spleen enlargement, biliousness, intestinal ulcers, digestive, carminative, aphrodisiac, dyspepsia, flatulence, strangury, seminal weakness, pharyngopathy, splenopathy, hemorrhoids, ophthalamopathy, cardiac disorder and general debility[10-13]. The phytoconstituents

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isolated so far from the plant are ascorbic acid, β-carotene, catechin, gallocatechin, caffeic acid, p-coumaric acid, ferulic acid, β-sitosterol, campesterol, xanthotoxin, stigmasterol, n-triacontanol, imperatorin, ecdysteroid[14], cinnamic acid amide alkaloid[15], phenol, saponin, apocartenoids[16], crytomeridiol[17], n-trans-feruloyl-4-O-methyl dopamine, syringaresinol[18], lupeol and 3-hydroxy nonadecyl henicosanoate[19]. The pharmacological activities reported so far from this plant are antipruritic and antinociceptive[20], anthelmintic[21] and as vaginal contraceptive[22]. As there is no report on anti-diarrhoeal activity, this prompted us to investigate extracts of its aerial parts for validating this property.

Materials and Methods

Plant material

The aerial parts of *C. album* were collected in the month of June 2008, identified and authenticated by Dr Shiddamallayya N, Asst. Director at National Ayurveda Dietetics Research Institute, Bengaluru, Karnataka. A voucher specimen (RRCBI/MCW/7) was deposited in the Herbarium of Department of Pharmacognosy, The Oxford College of Pharmacy, Bangalore. The aerial parts were dried in the shade and milled into coarse powder by a mechanical grinder then stored in closed vessel for further use.

Preparation of plant extract

Crude aerial parts of *C. album* were subjected to pulverizations and passed through sieve no. 40. The powder (300 g) was packed into a Soxhlet apparatus and extracted with petroleum ether (60-80ºC) for 18 h. The same marc was successively extracted with chloroform and afterwards with hydro alcohol (30:70) for 18 h. Each time the marc was dried and later extracted with other solvents. All the extracts were concentrated by rotary vacuum evaporator, evaporated to dryness and the percentage yield was found to be 2.3, 0.6 and 15.3% w/w. Only hydro alcoholic extract (HEMC) was used for further study on the basis of phytochemical study.

Phytochemical analysis

All the extracts of *C. album* were subjected to qualitative analysis for various phytoconstituents. Tests for common phytochemicals were carried out by standard methods[23].

Animals

Albino rats of either sex weighing 150-180 g and Swiss albino mice 25-30 g of either sex were obtained from the standard animal house, Bangalore, Karnataka, India. The animals were housed in micro nylon boxes in a control environment (temp 25±2°C) and relative humidity of 45-55% under 12 h light/dark cycles with standard laboratory diet and water *ad libitum*. The experimental protocol was approved by the Institutional animal ethics committee. CPCSEA guidelines were adhered during the maintenance and experiment.

Acute oral toxicity studies

Healthy adult albino mice of either sex were subjected to acute toxicity studies as per guidelines suggested by the Organization for Economic Cooperation and Development (OECD 2001). Healthy young adult albino mice of commonly used laboratory strains were employed. Each animal at the commencement of its dosing was between 8 and 12 weeks old and its weight was in an interval within ±20% of the mean weight of any previously dosed animals.The temperature in the experimental room was 22°C (±3°C). For feeding, conventional laboratory diets was used with an unlimited supply of drinking water. The animals are randomly selected marked enabling identification and kept in their cage for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. The test substance was administered in a single dose by gavage using a stomach tube or a suitable intubation canula. The animals were observed continuously for first 2 h for any gross change in behavioral, neurological and autonomic profiles or any other symptoms of toxicity and mortality if any, and intermittently for the next 6 h and then again after 24, 48 and 72 h for any lethality or death. The dose of the hydro alcoholic extract of 200 and 400 mg/kg were selected for the *in vivo* experiment on the basis of acute toxicity study (OECD guideline 425) as the dose of 1000 mg/kg does not produce any mortality and toxicity.

Castor oil induced diarrhoea

The antidiarrhoeal activity was evaluated as per the given procedure[24]. Rats were divided into four groups of six animals each, diarrhoea was induced by administering 1 mL of castor oil orally to rats. Group 1 served as control (1% aqueous tragacanth suspension, orally), group 2 received Loperamide (3 mg/kg) orally as suspension served as standard and group 3 and 4 received HEMC extract (200 and 400 mg/kg, orally) 1 h before castor oil

administration. The watery faecal material and number of defecation was noted up to 4 h in the transparent metabolic cages with filter paper at the base. Weight of paper before and after defecation was noted.

PGE_2 induced enter pooling

The enter pooling method was evaluated as per the given method[25]. Overnight fasted rats were divided in to four groups of six animals each. Group 1 received 1% aqueous tragacanth suspension orally served as a control, group 2 received PGE2 control, was administered with PGE_2 (100 µg/kg p.o.) and groups 3 and 4 received the HEMC extract of 200 and 400 mg/kg orally, respectively. Immediately after extract administration, PGE_2 was administered. After 30 min following administration of PGE_2, each rat was sacrificed and whole length of the intestine from pylorus to caecum was dissected out, its content collected in measuring cylinder and volume measured.

Small intestinal transit

The small intestinal transit was evaluated as per the method[26]. Rats were fasted for 18 h divided into four groups of six animals each, Group1 received 1 % aqueous tragacanth suspension orally served as a control, group 2 received atropine (0.1 mg/kg, s.c.), group 3 and 4 received 200 and 400 mg/kg orally of the HEMC extract, respectively. One ml of marker (10% charcoal suspension in 10% aqueous tragacanth) was administered orally 30 minutes after treatment. The distance traveled by charcoal plug from pylorus to caecum was measured and expressed as percentage of the distance traveled by charcoal plug for each of animal for the total length of the intestine from the pylorus to caecum.

Statistical Analysis

The experimental results were expressed as mean ±SEM of six animals. Analysis of variance was performed by ONE WAY ANOVA followed by Newman–Keul's multiple range tests. Probability values less than (P<0.01) were considered significant.

Results

The phytochemical analysis of the hydro alcoholic extract HEMC revealed the presence of alkaloids, terpenes, steroids, tannins, flavonoids and carbohydrates. Petroleum ether extract showed the presence of steroids and terpenes and chloroform extract showed the presence of alkaloids and steroids.

Castor oil induced diarrhoea

Castor oil produced watery diarrhoea, which lasted up to 24 h in the vehicle treated control group. The HEMC exhibited pronounced anti-diarrhoeal effect in a dose dependent manner following oral pretreatment on castor oil-induced diarrhoea compared with the control. The extract prolonged the onset time of diarrhoea, 70.83 min and 132.33 min, at the dose of 200 and 400 mg/kg, respectively. Although the effect is significant but comparatively it is lesser than the Loperamide 3 mg/kg (200 min). The extract significantly (P<0.01) inhibited both the frequency of defaecation as well as the wetness of the faecal droppings of rat (Tables 1a & b and Fig. 1). Treatment with HEMC in the dose of 200 and 400 mg/kg reduced the weight of defecate as well as reduced the frequency of defecation compared with the control group. The inhibition was 39.15 and 56.8%, with the dose of 200 and 400 mg/kg, respectively. The standard drug Loperamide (3 mg/kg) produced an inhibition of 81.19%.

PGE_2 induced enter pooling

PGE_2 induced a significant increase in the fluid volume of rat intestine in the control group (Table 2 and Fig. 2). The extract inhibited PGE_2-induced enteropooling significantly (P<0.01) in rats by both the doses. The percentage of reduction of

Table 1b—Percent protection against castor oil induced diarrhoea

Treatment	% Protection
Loperamide (3 mg/kg)	81.19
HEMC (200 mg/kg)	39.15
HEMC (400 mg/kg)	56.80

Table 1a—Evaluation of anti-diarrhoeal activity of hydro alcoholic extract of *C. album* by castor oil induced diarrhoea

Treatment	Mean wet defecation	Mean increase in weight of paper (g)	Delay in defecation time (min)
Control	9.167 ± 0.87	3.023 ± 0.49	32.167±7.305
Loperamide (3 mg/kg)	1.833 ± 1.05**	0.533 ± 0.29**	200.00±22.509**
HEMC (200 mg/kg)	4.667 ± 0.99**	2.140 ± 0.25^{ns}	70.833±2.845^{ns}
HEMC (400 mg/kg)	3.667 ± 0.80**	1.407 ± 0.51*	132.33±29.261**

**=P<0.01= Very significant; *=P<0.05= Significant; Not significant (ns) =P>0.05

Number of animals (N) =6; Values are expressed as mean ±SEM

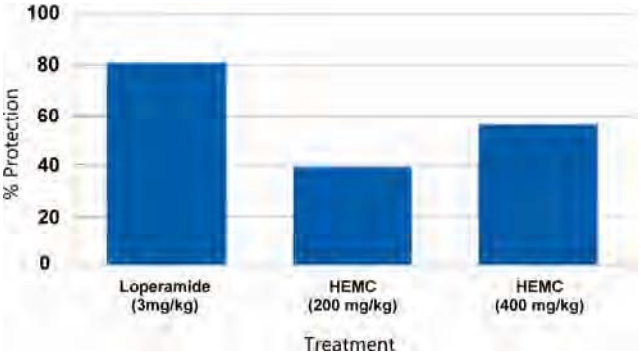

Figure. 1—Evaluation of anti-diarrhoeal activity of hydro alcoholic extract of *C. album* by castor oil induced diarrhoea

Table 2—Evaluation of anti-diarrhoeal activity of hydro alcoholic extract of *C. album* by PGE_2 induced enteropooling

Treatment	Volume of intestinal fluid (ml)
PGE_2 control	3.240 ± 0.0979
Vehicle control	3.080 ± 0.1020
HEMC (200 mg/Kg)	1.933 ± 0.1978**
HEMC (400 mg/Kg)	1.300 ± 0.1125**

**=P<0.01= very significant; Not significant (ns) =P>0.05; Number of animals (N) =6

Values are expressed as mean±SEM

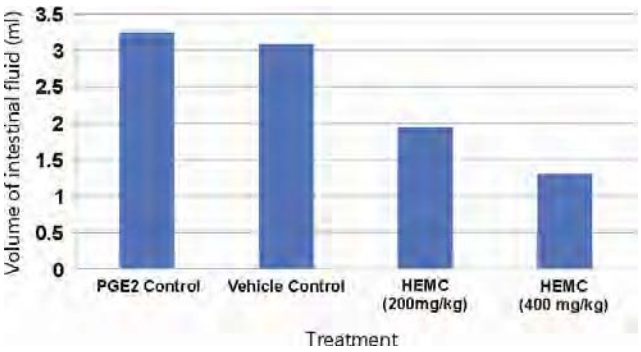

Figure. 2—Evaluation of anti-diarrhoeal activity of hydro alcoholic extract of *C. album* by PGE_2 induced enteropooling

enteropooling was 40.34 and 59.88 with the dose of 200 and 400 mg/kg of HEMC, respectively in comparison to PGE_2 control.

Small intestinal transit

HEMC and atropine sulphate (0.1 mg/kg) decreased the propulsion of the charcoal meal through the gastrointestinal tract when compared with the control (Table 3, Fig. 3). Distance traveled by the charcoal meal was reduced to 21.91 and 74.80% in the HEMC treated groups with the dose of 200 and 400 mg/kg, respectively, compared to control group.

Table 3—Evaluation of anti-diarrhoeal activity of hydro alcoholic extract of *C.album by* charcoal meal test

Treatment	% movement of charcoal meal
Control	98.440 ± 1.560
Atropine sulphate (0.1 mg/kg)	49.878 ± 8.043**
HEMC (200 mg/kg)	74.802 ± 2.946**
HEMC (400 mg/kg)	21.913 ± 5.188**

**=*P*<0.01= Very significant; Number of animals (N) =6; Values are expressed as mean ±SEM

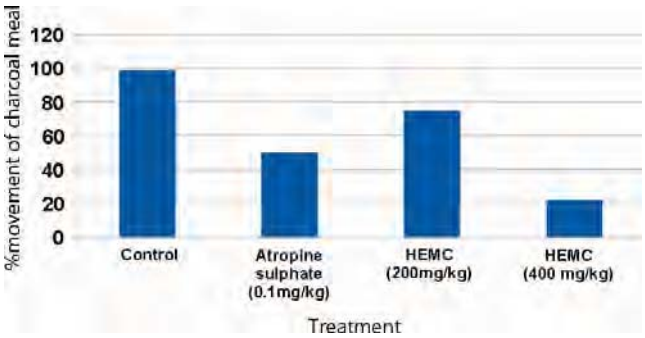

Figure. 3—Evaluation of anti-diarrhoeal activity of hydro alcoholic extract of *C. album* by charcoal meal test

Atropine on the other hand, produced a marked decrease in the propulsive movements and the intestinal length traversed by charcoal meal was 49.87%. These observations were significantly (*P*<0.01) different from what was seen in the control group. 400 mg/kg dose of the extract exerted greater anti-motility effects than 0.1 mg/kg of atropine.

Discussion

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. In some cases the secretory component predominates, while some are characterized by hyper motility. The use of castor oil induced diarrhoea model in our study is logical because the autocoids and prostaglandins are involved have been implicated in the causation of diarrhoea in man[27,28]. The results of the present study show that the extract of *C. album* produced a statistically significant reduction in the severity and frequency of diarrhoea produced by castor oil. It is also noted that the extract significantly inhibited PGE_2 induced intestinal fluid accumulation and the volume of intestinal content, dose dependently more than atropine. In this study, atropine produced an increased intestinal transit time possibly due to its anti-cholinergic effect[29]. An increase in intestinal transit time with atropine could

also result due to reduction in gastric emptying[30]. Both mechanisms may also be responsible for anti-diarrhoeal activity of *C. album* extract.

Castor oil is also reported to induce diarrhoea by increasing the volume of intestinal content by prevention of the reabsorption of water. 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[31]. Thereby prevents the reabsorption of NaCl and H_2O[32]. Probably extract increased the reabsorption of NaCl and water by decreasing intestinal motility as observed by the decrease in intestinal transit by charcoal meal. The anti-diarrhoeal activity of the extract may also be due to the presence of denature proteins forming protein tannates which make the intestinal mucosa more resistant and reduce secretion[33]. The secretory diarrhoea is associated with an activation of Cl-channels, causing Cl-efflux from the cell, which results in massive secretion of water into the intestinal lumen and profuse watery diarrhoea[34]. The extract may inhibit the secretion of water into the lumen by reverting this mechanism. Anti-dysenteric and anti-diarrhoeal properties of medicinal plants were found to be due to tannins, alkaloids, saponins, flavonoids, sterols and/or triterpenes and reducing sugars[35]. The phytochemical analysis of the HEMC extract revealed the presence of alkaloids, terpenes, steroids, tannins, flavonoids and carbohydrates. These constituents may mediate the anti-diarrhoeal property of the *C. album* extract. Sesquiterpenes, diterpenes, terpenes, flavonoids and terpenoid derivatives are known for inhibiting release of autocoids and prostaglandins; thereby inhibit the motility and secretion induced by castor oil[36-39].

Castor oil is a suitable model of diarrhoea in rats, since it allows the observation of measurable changes in the number of stools, enteropooling and intestinal transit. The extract resulted in a 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. This signifies the usefulness of this model and the clinical effect of the extract (Fig. 4).

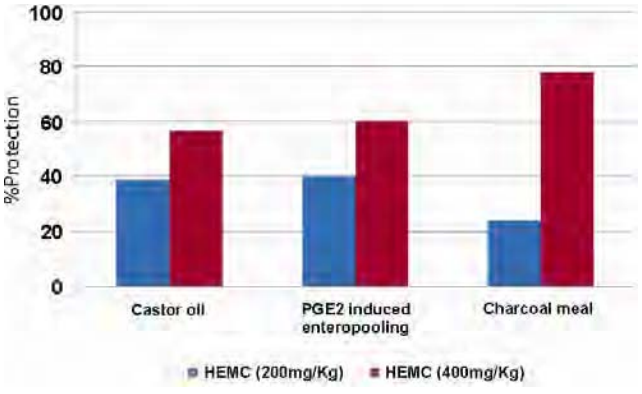

Figure. 4—Comparison of protection potential of *C. album* against diarrhoea

Conclusion

The remarkable anti-diarrhoeal effect of *C. album* extract against castor oil diarrhoea, model attest to wide range of utility in secretory and functional diarrhoeas. *C. album* extract may be useful in a wide range of diarrhoeal states, due to both disorders of transit e.g. functional diarrhoeas, radiation diarrhoea or due to abnormal secretory mechanisms like in cholera or *E. coli* enterotoxin induced diarrhoea. Further studies are needed to completely understand the mechanism of anti-diarrhoeal action of *C. album* extract.

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