Anti-diarrhoeal investigation from aqueous extract of *Cuminum cyminum* Linn. Seed in Albino rats

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ABSTRACT

Background: *Cuminum cyminum* Linn. (Umbelliferae), commonly known as Jeera. It is native from mediterranean region, but today widely cultivated in Asian countries. It has been reported to possess various medicinal properties and an important food ingredient. The seed of the plant are claimed for treatment of diarrhoea by various traditional practitioners. **Objectives:** Hence, the present investigation was undertaken to evaluate aq. extract of *C. cyminum* seeds (ACCS) against diarrhoea on albino rats. **Materials and Methods**: The animals were divided into five groups and the control group was applied with 2% acacia suspension, the standard group with loperamide (3 mg/kg) or atropine sulphate (5mg/kg) and three test groups administered orally with 100, 250 and 500 mg/kg of ACCS. The antidiarrhoeal effect was investigated by castor oil induce diarrhoea model, prostaglandin E_2 (PGE_2) induced enteropooling model, intestinal transit by charcoal meal test. **Results:** The ACCS showed significant ($P < 0.001$) inhibition in frequency of diarrhoea, defecation time delaying, secretion of intestinal fluid as well as intestinal propulsion as compared to control and the graded doses of tested extract followed dose dependent protection against diarrhoea. **Conclusions:** The study reveals that the ACCS is a potent antidiarrhoeal drug which supports the traditional claim.

Key words: Antidiarrhoeal effect, castor oil, enteropooling model, Intestinal transit, seed

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INTRODUCTION

Since ancient times, diarrhoea has been recognized as one of the most important health issue world widely, particularly afflicting those populations of socio-economically backward classes and from tribal areas to third-world countries.[1,2] Globally, about 2.2 million people have been killed annually by diarrhoea, majority of them are infants and children below the age of 5 years.[3,4] According to world health organization, it is the one of the most common cause of morbidity and mortality in many developing countries and became a leading cause of malnutrition in world to-day.[5] Diarrhoea is characterized by rapid movement of semisolid or watery feacal matter through intestine, three or more times in a day with severe or light abdominal pain and bowel sounds.[6-8] It was caused either by means of polluted water, consumption of contaminated food and by physical contact like handshake etc., The food or water contamination is mostly due to diarrhoeagenic microorganisms like Escheria coli, Campylobacter jejuni, Vibrio cholera, Giardia intestinalis, Cryptosporidium parvum, Entamoeba histolytica, Cyclospora cayetanensis, viruses like Rota or Adeno and some other species like Salmonella, Shigella, Clostridium etc., These microorganisms cause the influx of water and ions to the intestinal lumen by increasing intestinal motility, thereby causing dehydration of body.[9] Such type of symptom in diarrhoea is treated by the administration of oral rehydration salts in children or adults to maintain the body fluids osmolality.[10] Alternatively, some drugs like Diphenoxylate, Loperamide, Diloxanide furoate, Racecadotril, Atropine sulfate etc., are available in the market for treating diarrhoea. But all of the existing drugs suffer from adverse effects like the induction of bronchospasm, vomiting, intestinal obstruction, constipation etc.[11] In order to overcome these menace of anti-diarrhoeal drugs in world market, the World Health Organization (WHO) has introduced a programme, which encourages towards traditional herbal medicines.[12] Therefore, there has been

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great interest in herbal remedies for the treatment of such ailments.

Cuminum cyminum L. is commonly known as 'Jeera' in Hindi, belonging to Umbelliferae family. It is an annual shrub up to 10 to 50cm height, native from the Mediterranean region. The plant is indigenous to Turkistan or northern Egypt, but is today widely cultivated in Pakistan, India, Arabia, UAE, Israel, Iran, Iraq, Yugoslavia, Syria, Bulgaria, Malta, Sudan, Cyprus, China and Java.[13] Several studies on anti-diabetic,[14] antioxidant,[15] anti-bacterial,[16] anti-fungal,[17] bronchodilatory,[18] hepatoprotective and renoprotective,[19] chemopreventive,[20] anti-epileptic,[21] galactagogue,[22] Hypolipidemic,[23] male anti-fertility,[24] memory-enhancing and anti-stress[25] effect of *C. cyminum* seed extracts have been reported in literature. The antidiarrhoeal effect of *C. cyminum* is mentioned in different system of traditional medicine i.e. In Iranian folk medicine,[26] traditional medicine of Tunisia[27] and Ayurvedic medicinal system of India.[28] However, there is no scientific evidence on antidiarrhoeal activity of *C. cyminum* against above traditional claims so far. Hence, we investigated the antidiarrhoeal activity of *C. cyminum* seed extract in experimental rats [Figure 1].

MATERIALS AND METHODS

Chemicals

Gum acacia, castor oil, charcoal meal (SD Fine chemicals, Mumbai); Lopermaide (Micro labs, Bangalore), Ethanol (Merck, Mumbai); PGE_2 (Astrazeneca, Bangalore) and Ketamine, Atropine Sulphate (Hi Media, Mumbai) were procured. All other reagents and chemicals were of analytical grade.

Plant material

The dried seeds of *Cuminum cyminum* were collected from local region of Bhopal, Madhyapradesh, India.

Figure 1: The seed of *Cuminum cyminum*

Further taxonomic identification and authentification was conducted at the Department of Botany, Dr. H. S. Gour Central University, Sagar, MP, India, where a plant specimen was deposited in the herbarium for further reference.

Preparation of extract

The collected seeds were allowed to shade-dry and powdered to course consistently in a grinder mill. Then the coarse powder was passed through 40 mesh size and stored in an airtight container at room temperature. Accurately 250gm of coarse powder was weighed and extracted in water by adopting a simple maceration procedure at room temperature, for seven days, in conical flasks with occasional shaking and stirring. Then the extract was filtered and concentrated to dryness at room temperature to avoid decomposition. The yield of the extract was found to be 21.49% w/w and preserved in refrigerator for further use. The ACCS was subjected to qualitative analysis for various phytoconstituents.[29] A known volume of extract was suspended in distilled water and was orally administered to the animals by gastric intubation using a feeding needle during the experimental period.

Experimental animals

Adult albino rats (200-250 g) of either sex were procured from the animal house of Vedica College of Pharmacy, Bhopal, India. The study protocol was approved from the Institutional Animal Ethics Committee (IAEC) under the reference no. 1693/PO/a/13/CPCSEA and CPCSEA guidelines were adhered during the maintenance and experiment. All animals were maintained under standard husbandry conditions with food and water *ad libitum*.

Acute oral toxicity study

The animals were fasted overnight prior to the experiment. Different doses (50-2000 mg/kg, P.O) of the aqueous extract were administered to groups of rats and they were observed continuously for 1 h and then at half-hourly intervals for 4 h, for any gross behavioural changes and further up to 72 h, followed 14 days for any mortality as per the OECD Guideline 425. The seed extract of *Cuminum cyminum* was found to be non-toxic up to the maximum dose of 2000 mg/kg body weight. Dose selected for antidiarrhoeal evaluation was 100, 250 and 500 mg/kg respectively.

Evaluation of *in-vivo* antidiarrhoeal activity

Castor oil induced diarrhoea model

Albino wistar strain rats of either sex (150-200 gm) were fasted overnight and divided into five groups ($n = 6$).[30] Group I served as control (2% acacia suspension, orally), group II served as standard (Loperamide at a dose of 3 mg/kg orally as suspension) and group III, IV and V were administered the graded dose of ACCS extract (100,

250 and 500 mg/kg respectively). After 60 min of drug treatment, the animals of each group received 1 ml of castor oil orally. The frequency of diarrhoea, wt of faecal material and delay of defecation time was noted up to 4 h in the transparent metabolic cages with pre weighed plastic dishes placed at the base. Weight of plastic dish before and after defecation was noted and compared to control.

Effect on charcoal meal test

Albino wistar rats were treated as described earlier except that Atropine sulfate (5 mg/kg IM) was used as a standard drug.[31] One hour later after drug treatments, each of these animals were given 1mL of charcoal meal (3% charcoal suspension in 5% suspension of acacia) by oral route to induce diarrhoea. All animals were sacrificed after 30 min; the stomach and small intestine were removed and extended on a clean glass surface. The distance travelled by the charcoal meal from the pylorus to caecum was measured and expressed as a percentage of distance travelled by charcoal plug for each animal.

Prostaglandin E_2 (PGE_2) induced enteropooling model

Overnight fasted rats of either sex were divided into six groups; each group carries six animals.[32] Group I served as negative control (2% acacia suspension orally); group II served as positive control (PGE2 100 μg/kg p.o.); group III, IV and V were received ACCS of 100, 250 and 500 mg/kg P.O. respectively; and group VI served as standard (loperamide 3 mg/kg orally as suspension). After 30 min of extract administration, PGE2 was administered to induce diarrhoea. After two hours of administration of PGE2, each rat was sacrificed by administering excessive dose of ketamine and the small intestine was removed after tying the end with thread and weighed. The intestinal contents were collected by milking into a graduated tube and their volume was measured by measuring cylinder. The intestine was reweighed and the difference between full and empty intestine was calculated.

STATISTICAL ANALYSIS

The values were expressed as Mean ± SEM of six animals. For statistical analysis of the data, group means were compared by one-way Analysis of variance followed by Dunnett's t'test. Probability values with $P < 0.05$ were considered as significant. It was carried out with graph pad in Stat 3 software.

RESULTS

Qualitative phytochemical screening

Phytochemical analysis of the crude eve positive reaction for each of the following secondary metabolites: Alkaloids, Glycosides, Flavonoids, Coumarins, Lignins, Tannins, Terpenoids, Carbohydrates, Protein, fatty acids, phenolic compounds and amino acids.

Effect on castor oil-induced diarrhoea

After administration of castor oil, the diarrhoea was clinically apparent in all the treated groups as compared to control for next 4 h. This was markedly reduction of number of wet faeces as well as frequency of diarrhoea and also observed delay of defecation time. The dose of 500 mg/kg is closer resemblance to the effect of the standard antidiarrhoeal drug as shown in Table 1.

Effect on charcoal meal test

All the doses of ACCS and atropine sulphate decreased the propulsion of the charcoal meal as compared to control group. The distance travelled by the charcoal meal was found to be 37.81, 39.91 and 44.88% in the ACCS treated groups with the dose of 100, 250, 500 mg/kg respectively; where as the standard showed 50.52%, compared to control group. All these observations were significant ($P < 0.001$) reduction with dosed dependency in intestinal transit as compared to control. The activity of test extract at dose of 500 mg/kg on charcoal meal test was found to be more effective when compared to atropine, an anticholinergic drug as shown in Table 2.

Effect on prostaglandin E_2 induced enteropooling model

The ACCS (100, 250, 500 mg/kg) slowed down the intestinal fluid accumulation by 36.27% to 76.27% in respect to PGE2 treated control group. All the doses of the extract produced a significant ($P < 0.001$) decrease of intestinal propulsive movement and also dose dependency

Table 1: Effect of aqueous extract of *cuminum cyminum* seed on castor oil induced diarrhoea in rats

Groups	Treatment	Mean frequency of diarrhoea	Mean wt of fecal drops	Mean wt of faeces after 4hr	Delay in defecation time (min.)
I	Control	3.8±0.011	11.8±0.32	1.85±0.07***	38.94±2.31
II	Loperamide (3 mg/kg)	0.5±0.4***	2.4±0.45***	0.25±0.1***	195.42±4.21
III	ACCS (100 mg/kg)	2.7±0.6*	7.8±0.69***	1.13±0.4***	84.32±3.54
IV	ACCS (250 mg/kg)	1.9±0.5**	7.0±0.52***	0.72±0.07***	134.32±5.98
V	ACCS (500 mg/kg)	1.3±0.8**	5±0.05***	0.39±0.05***	179.23±6.23

The values were expressed as mean±SEM (n=6) *P<0.05, **P<0.01 and ***P<0.001 vs control. ACCS=Aqueous extract of *cuminum cyminum* seed

Table 2: Effect of aqueous extract of *cuminum cyminum* seed on charcoal meal induced peristalsis

Groups	Treatment	Mean length of intestine (cm)	Mean distance travelled by charcoal meal (cm)	Mean % movement of charcoal after 30 min	% inhibition
I	Control	46.18±1.04	39.67±2.80	84.94±2.78	15.06
II	Atropine SO_4 (5 mg/kg)	43.65±1.64	21.12±2.47***	49.48±0.65***	50.52
III	ACCS (100 mg/kg)	44.11±1.24	27.65±1.21***	62.19±3.28***	37.81
IV	ACCS (250 mg/kg)	46.5±1.25	27.45±1.06***	60.09±2.48***	39.91
V	ACCS (500 mg/kg)	46.8±1.324	25.59±1.07***	55.12±2.66***	44.88

The values were expressed as mean±SEM., *n*=6. ***P*<0.01 and ****P*<0.001 vs control. ACCS=Aqueous extract of *cuminum cyminum* seed

Table 3: Effect of aqueous extract of *cuminum cyminum* seed on PGE_2 induced enteropooling in rats

Groups	Treatment	Mean volume of intestinal fluid (ml)	% protection
I	Control	1.94±0.067	-
II	PGE_2 (100µg/kg p.o.)	2.95±0.10	100
III	Loperamide (3 mg/kg)	0.41±0.08***	86.10
IV	ACCS (100 mg/kg)	1.88±0.079***	36.27
V	ACCS (250 mg/kg)	1.15±0.103***	61.01
V1	ACCS (500 mg/kg)	0.7±0.055***	76.27

The values were expressed as mean±SEM (*n*=6) **P*<0.05, ***P*<0.01 and ****P*<0.001 vs control. ACCS=Aqueous extract of *cuminum cyminum* seed

was observed between the doses of ACCS as shown in Table 3.

DISCUSSION

Diarrhoea is usually considered a result of altered motility and fluid accumulation within the intestinal tract. Many antidiarrhoeal agents act by reducing the gastrointestinal motility and its secretions. Castor oil causes diarrhea due to its active metabolite i.e. recinolic acid, which increases peristaltic activity in the small intestine leading to changes in the electrolyte permeability of the intestinal mucosal membrane. The precise mechanism of action of castor oil is through elevated level of prostaglandin biosynthesis. The aq. extract of *Cuminum cyminum* observed that statistically significant inhibition of the intestinal transit and secretion induced by castor oil in rats. These inhibition may be due to presence of flavonoidal and terpenoidal derivatives, by inhibiting the release of autocoids and prostaglandins in intestinal cells.[33,34]

Phytochemical screening revealed the presence of tannins, alkaloids, glycosides, sugars, terpenes and flavonoids in the aq. extract of *Cuminum cyminum*. Earlier studies have shown that the anti-dysenteric and anti-diarrhoeal properties of medicinal plants were due to the presence of tannins, alkaloids, saponins, flavonoids, sterols or triterpenes and reducing sugars.[35,36] Hence, tannins, reducing sugars, triterpenes and flavonoids may be responsible for the mechanism of action of the anti-diarrhoeal activity of *Cuminum cyminum*. This could be due to the fact that the aq. extract increased the re-absorption of water by decreasing the intestinal motility as well as intestinal transit in the charcoal meal test. In addition, its antidiarrhoeal action may also be due to the presence of denatured proteins and tannic acid or tannins, which form protein-tannate complex. These complexes coat over the intestinal mucosa and make the mucosa more resistant and hence, reduce secretion and peristaltic movement.[37-39] The secretory diarrhoea is associated with activation of Cl^- channel, results massive secretion of watery diarrhoea from the intestinal lumen. The aq. extract may inhibit the watery secretion from luminal wall by reverting this mechanism. The antidiarrhoeal effect of the aq. extract may be also due to an inhibition of muscle contraction, as observed by charcoal meal and consequently, in a reduction of intestinal propulsion. The inhibition of intestinal muscle contraction is due to presence of flavonoidal constituents.[40-42] The ACCS resulted in markedly reduction in frequency of diarrhoea, the weight and volume of intestinal contents as well as intestinal transit. The dose of 500 mg/kg of the extract exerted greater antidiarrhoeal effect as comparable to other doses. These results justify the usefulness of ACCS in antidiarrhoeal model as shown in Figure 2.

In summary, the present study revealed that the ACCS showed marked reduction in frequency of diaarrhoeal stool and the reduction in weight and volume of intestinal content, as well as a modest reduction in intestinal transit. The mechanism of antidiarrhoeal effect may be due to reduction of gastrointestinal motility, inhibition of the synthesis of prostaglandin and intestinal muscle contraction. These above mechanisms are due to presence of pharmacologically active substances i.e. tannins, terpenoids and flavonoids etc., Further studies are required to fully investigate the mechanisms responsible for this observed antidiarrhoeal activity.

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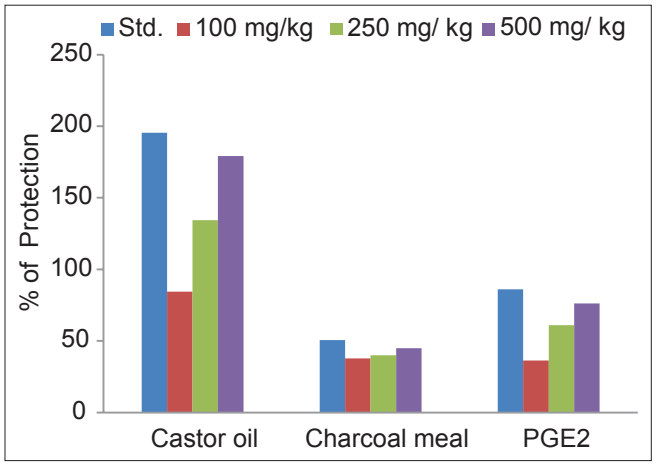

Figure 2: Comparative study of antidiarrhoeal potential of graded dose of ACCS Vs Std drug.

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