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Antidiarrhoeal activity of ethanol and aqueous extracts of *Carum copticum* seeds in experimental rats

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ABSTRACT

Objective: To investigate the antidiarrhoeal activity of 95% total alcoholic extract (TAE) and total aqueous extract (TAQ) of *Carum copticum* (*C. copticum*) seeds. **Methods:** Antidiarrhoeal activity of *C. copticum* seed extracts at a dose of 100 mg/kg BW was evaluated using experimentally induced castor oil diarrhoea, gastrointestinal transit of charcoal meal and enteropooling activity in male wistar rats and compared to standard drugs. **Results:** At a dose of 100 mg/Kg BW (TAQ and TAE) significantly decreased the diarrhoeal droppings in castor oil induced diarrhoea, the mean distance travelled by charcoal meal showed a significant reduction in the secretion of gastrointestinal fluid accumulation by 39.90% to 50.70%. *C. copticum* extracts on castor oil induced fluid accumulation showed a greater inhibitory effect on Na^+ levels than on K^+ concentrations. **Conclusions:** These results suggest that *C. copticum* seed extracts could be used for the treatment of diarrhoea.

1. Introduction

Diarrhea, an important health problem worldwide, especially in developing countries, accounts for more than 5-8 million deaths in infants and children under 5 years of age, each year[1]. Since antiquity many plants are used as folk medicine to treat infectious diseases such as urinary tract infections, diarrhoea, cutaneous abscesses, bronchitis and parasitic diseases[2]. According to World health organization (WHO) about 80% of the world's population mainly depend on traditional medicine and the use of plant extracts is mainly involved in the traditional treatment[3]. Medicinal plants constitute the major component of the traditional medicine practiced worldwide due to the economical viability, accessibility and ancestral experience[4]. Therefore, the search for safe and more effective agents from plant origin has continued to be an important area of active research. Hence, the World Health Organization encouraged studies for the treatment and prevention of diarrhoeal diseases depending on traditional medical practices[5].

The ajowain (*Carum copticum* (*C. copticum*) L.) is an aromatic, grassy, annual plant belonging to Umbelliferae family which grows in the east of India, Pakistan, Iran, and Egypt with white flowers and small brownish fruits. The ajowain (*C. copticum* L.) is a popular spice and traditionally used in Indian system of medicine[6].

The phytochemical studies on *C. copticum* seeds have revealed the presence of alkaloids, steroids, carbohydrates, fixed oils, glycosides, tannins, proteins, saponin and flavonoids, cumene, thymene, aminoacids like lysine and threonine, calcium, iron, starch and dietary fiber essential oils like thymol, c-terpinene, pcymene[7,8].

Several studies on antitussive effect[9], inhibitory effect on histamine (H_1) receptors[10], antihypertensive, antispasmodic, bronchodilator and hepatoprotective activities[11], analgesic effect[12], Anti-inflammation activity[13], antioxidant and anti mutagenic activities[6] of *C. copticum* seed extracts have been reported in literature. The antidiarrhoeal activity of *C. copticum* is mentioned in Indian system of traditional medicine but there is no scientific evidence to prove its activity. Hence, we investigated the antidiarrhoeal activity of *C.*

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copticum seed extracts in experimental rats and is compared with the standard drugs such as loperamide and atropine sulphate.

2. Materials and methods

2.1. Plant Material and drugs

Seeds of *C. copticu*m were procured from a local market and authenticated by Assistant professor K. Madhava chetty of the Department of Botany, S.V. University, Tirupati, Andhra Pradesh, India. A voucher specimen (no. 5009) was deposited at Botany Department of S.V. University. Loperamide was obtained from Waksmanselsman Pharma Limited, Anantapur, Andhra Pradesh, India. Atropine sulphate obtained from High Media, Mumbai, India and all other chemicals used in this work were of analytical grade.

2.2. Animals

Male albino rats of body weight 150-250 g were procured from Sri Venkateswara Enterprises, Bangalore, India. Animals were acclimatized for 10 days to our animal house and housed three animals per cage. Animals were provided with standard rodent pellet diet and maintained in a temperature of (22±2) ℃ and humidity-controlled environment on a 12-h dark/light cycle. Animals were handled according to the rules and regulations of Institutional Animal Ethical Committee (IAEC), Sri Krishnadevaraya University, Anantapur, India.

2.3. Preparation of seed extract and dose

The TAE (Total alcoholic extract) was prepared by soaking 500 g seeds of *C. copticum* in 750 mL of 95% ethanol for 15 d. The clear extract obtained after filtration was concentrated in a water bath maintained at 55 ℃ to obtain a semisolid mass (yield weight 28.50 g). The seeds were extracted with 750 mL of double distilled water for 6 h at 55 ℃ to prepare the Total aqueous extract (TAQ). The extract thus obtained was concentrated to a semi solid mass (yield weight 35.87 g). TAE and TAQ were stored in a desicator until used for further experiments. The dose (100 mg/kg BW) of *C. copticum* was selected based on the previous study[13].

2.4. Castor oil induced diarrohea

Rats fasted for 24 h were randomly allocated to four groups of six animals each. Group I received 1% Carboxy methyl cellulose (CMC) (10 mL/kg BW), group II and III received TAE and TAQ extracts (100 mg /kg BW) of *C. copticum* seeds, respectively, and IV group was given loperamide (3 mg/kg P.O)[14]. After 60 min each animal was given with 2 mL of castor oil by gastric intubation, and placed in a separate cage and observed for 4 h defecation. The characteristic diarrhoeal droppings were noted following the method of Venkateswara Rao *et al*[15].

2.5. Small intestinal transit

This test is done by following the method reported by Teke *et al*[16]. The rats were divided randomly into three major groups noted as 30, 45, and 60 min. Rats were fasted 18 hours and placed in a cages. Each animal received 1 mL of charcoal meal (5 % activated charcoal suspended in CMC). For each major group, one test group received the TAE and TAQ extracts (100 mg/kg BW) orally.

The fourth group received atrophine sulphate at 0.1 mg/kg BW i. P route as standard drug, while the fifth group received 1 % CMC (10 mL /kg). Charcol meal administered 60 min after the vehical or extract. After 30, 45, and 60 min of charcoal meal treatment respectively, animals of each group were sacrificed by cervical dislocation, and intestine was removed without stretching and placed length wise on moist filter paper and its total length measured. The distance moved by charcoal from the pyloric sphincter to caecum was equally measured. This distance was expressed as percentage of the length of the small intestine.

2.6. Castor oil induced enteropooling

The rats fasted for 24 h (water ad libitum) were randomized and divided into five groups of six rats each. Group I was administered physiological saline (10 mL/kg BW), group II was administered castor oil only (2 mL). Groups III, IV, and V were administered TAE and TAQ extract of *C. copticum*, and loperamide, respectively, 1h prior to castor oil administration. After 30 min the rats were killed by cervical dislocation and exsanguinated; the small intestine was ligated both at the pyrolic sphincter and at the ileocaecal junctions. The small intestine was weighed (w_1) and reweighed (w_2) and the length (L) measured.

The difference in weight divided by the length, shows the enteropooling in mg of fluid per centimeter of small intestine[4]. The entire small intestine contents were expelled into a graduated measuring cylinder, and volume of contents was recorded. The intraluminal fluid was centrifuged at 3 000 rpm for 15 min and the Na^+ and K^+ concentrations in the supernatant were measured by flame photometry[15].

2.7. Statistical analysis

The results were expressed as mean±SD. Data were analyzed for significant difference using Duncan's Multiple Range (DMR) test. Significance is presented at level of $P<0.001$, $P<0.01$ and $P<0.05$.

3. Results

3.1. Castor oil induced diarrhoea

In the castor oil induced diarrhoeal method, the TAE and TAQ extract of *C. copticum* produced a marked anti diarrhoeal effect in the rats, as shown in table 1. At a dose of 100 mg/kg, TAQ and TAE extracts caused extract dependent decrease the total number of wet faeces produced upon

administration of castor oil (28.11±1.38 and 22.36±1.69) compared to the castor oil control group (62.16±2.85). The effect of the TAE dose of the extract was similar to that of the standard drug, loperamide (3 mg/kg). The corresponding reduction percentages of faecal matter were 51.55% and 60.52%, respectively for TAQ and TAE extracts. Loperamide (3mg/kg), a standard anti diarrhoeal drug inhibited the diarrhoea by 66.83% (Table 1).

3.2. Gastro intestinal transit

TAE and TAQ extracts at a dose of 100 mg/kg decreased the intestine propulsion. The greater inhibitory effect of the *C. copticum* extract on charcoal meal propulsion (transit percent) was observed for TAE at 100 mg/kg BW for different transit periods (30, 45 and 60 min) in comparison with that of the control group (Figure 1). These effects were comparable to those of atropine sulphate at a dose of 0.1 mg/kg BW.

3.3. Enteropooling method

TAQ and TAE extracts at a dose of 100 mg/kg showed

Table 1
Effect of *C. copticum* extracts on castor oil-induced diarrhoea in experimental rats (n=6).

Treatment	Dose (mg/kg)	Onset time (min)	Total number of faecal matter	No. of wet faecal matter	Reduction (%)
Castor oil control	–	29.16±1.16	64.16±1.47	62.16±2.85	–
TAE	100.0	69.33±0.04	25.33±1.63**	22.36±1.69***	60.52
TAQ	100.0	54.50±0.62	31.08±1.96*	28.11±1.38***	51.55
Loperamide	3.0	98.66±0.50	21.28±1.29***	16.56±1.09***	66.83

Values are presented as mean ± SD. *P < 0.05, **P < 0.01, ***P <0.001 when compared to castor oil control.

Table 2
Effect of *C. copticum* extracts on castor oil-induced fluid accumulation in experimental rats (n=6).

Treatment	Dose (mg/kg)	Intestinal fluid (mL)	Inhibition (%)	Na^+ (mmol/L)	K^+ (mmol/L)
Control	–	1.36±0.31	–	119.6±0.64	4.92±0.14
Castor oil	2 mL	4.26 ± 0.14^{y}	–	154.5 ± 1.30^{x}	6.34±0.68
TAE	100.0	2.10 ± 0.07^{c}	50.70	122.6 ± 1.36^{a}	5.64±0.71
TAQ	100.0	2.56 ± 0.12^{c}	39.90	127.3 ± 1.35^{b}	6.10±0.69
Loperamide	3.0	1.69 ± 0.14^{c}	60.32	121.1 ± 1.02^{a}	5.17±0.45

Values are mean ± SD. ^{x}P<0.001, ^{y}P<0.001 as compared to control. ^{a}P<0.05, ^{b}P<0.01 and ^{c}P<0.001 as compared to castor oil control.

significant reduction of the intestinal fluid accumulation of 39.90% and 50.70% (P < 0.001), respectively (Figure 2). Both the extracts showed similar the reduction of the intestinal fluid accumulation as loperamide, which reduced to a greater inhibition (60.32 %) of the intestinal fluid accumulation (P < 0.001). The sodium and potassium ion concentrations were estimated in the intestinal fluids of all the groups, and a greater inhibitory effect was observed on sodium ions level compared to potassium ions level (Table 2). The physiological K^+ concentration in the intestinal fluid of castor oil group, TAE group, TAQ group and standard drug group was not significantly different as compared to the control group.

The sodium ions concentration in the intestinal fluid of castor oil treated group significantly increased, whereas it was significantly reduced in TAQ and TAE extracts treated groups (P<0.05) as well as in control group; the sodium ions concentration inhibitions caused by TAQ and TAE extracts were 17.60% and 20.64%, while for potassium ions concentrations were 3.78% and 11.04%, respectively. Both the extracts (TAQ and TAE) showed similar reduction of sodium ions when compared to standard drug loperamide, which reduced to 21.61 % of sodium ions.

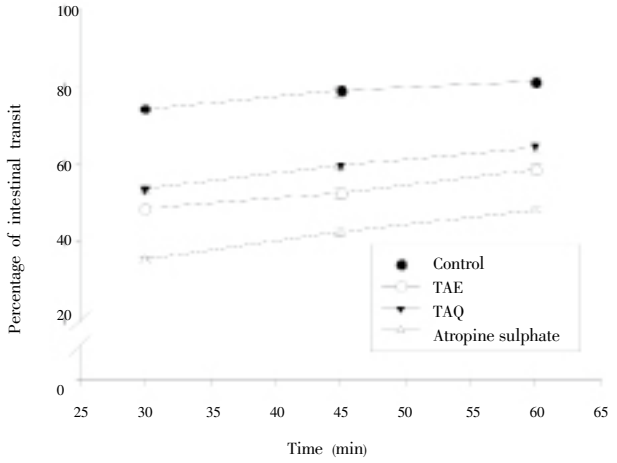

Figure 1

Figure 1
Effects of TAE and TAQ extracts of C. copticum on gastro intestinal transit of wistar rats administered with charcoal meal. Values are the mean of six determinations ± SD.

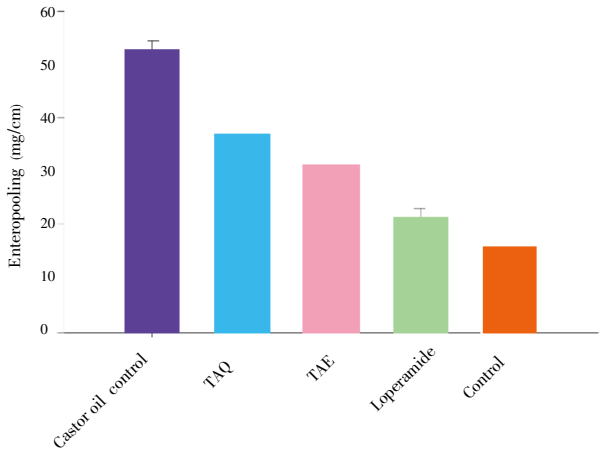

Figure 2

Figure 2
Results of effects of TAE and TAQ extracts of *C. copticum* on enteropooling induced by castor oil in wistar rats. Values are the mean of six determinations ± SD.

4. Discussion

Diarrhoea is characterized by increased frequency of bowel movement, wet stool and abdominal pain. The present study reported the protective effect of alcohol and water extracts of *Carum copticum* seeds on various diarrhoea induced models such as castor oil induced diarrhoea, gastro intestinal transit and enteropooling method in male rats.

In the present investigation, the TAE and TAQ extracts of seeds of *C. copticum* at a dose of 100 mg/kg exhibited a significant inhibition of castor oil induced diarrhoea in extract dependent manner in experimental rats. TAE and TAQ extracts significantly decreased the diarrhoeal droppings when compared to castor oil group. The results obtained for TAE and TAQ extracts were similar to that of the standard drug loperamide (3 mg/kg). Diarrhoea onset time was significantly increased by both the TAE and TAQ extracts of *C. copticum* as compared to the castor oil control group. Loperamide is an opioid receptor agonist and acts on the μ-opioid receptors in the mysenteric plexus large intestine but it does not affect the central nervous system like other opioids.

In the evaluation of gastrointestinal transit atrophine sulphate was used as standard drug. Atrophine sulphate is known to inhibit gut motility probably due to its anti-muscarinic effect[17]. The TAE and TAQ extracts also appeared to act on all parts of the intestine. Thus, they reduce the intestinal propulsion in the charcoal meal treated model for different transit periods (30, 45, and 60 min) at a test dose of 100 mg/kg BW. Activated charcoal prevents the absorption of drugs and chemicals into the system by avidly adsorbing them on the surfaces of the charcoal particles[16]. In this study activated charcoal was used in the gastrointestinal motility test to find out the effects of these extracts on the peristaltic movement.

TAE and TAQ extracts led to a marked reduction in the total volume of the intestinal contents. This indicates that TAE and TAQ extracts of Carum copticum reduced diarrhoea by increasing the reabsorption of electrolytes and water or by inhibiting induced intestinal accumulation of fluid just as loperamide[16]. Loperamide acts by decreasing the transit velocity and increasing the capacity of the intestines to retain their fluids[16].

The action of castor oil as a diarrhoea inducer is due to its active constituent ricinoleic acid which is liberated from the action of lipases on castor oil. This causes irritation to the intestinal mucosa and elicits inflammation, which releases prostaglandins and nitric oxide which in turn stimulate gastrointestinal secretions, motility, epithelial permeability[18] and edema of the intestinal mucosa, thereby preventing the re-absorption of Na^+, K^+ and water[19].

Likewise, TAE and TAQ extracts decreased the amount of faecal matter and slowed down the propulsion of charcoal meal through gastro intestinal tract, and also caused a marked reduction in the volume of the intestinal contents and increased the re-absorption of water, sodium and potassium ion concentration. The sodium and potassium transport in the intestine has been related to membrane bound enzyme sodium and potassium ATPase. In diarrhoeal conditions the decrease in Na^+ and K^+-ATPase occurs relating to an interruption in the normal water and electrolyte absorption, Therefore, the reduction of water together with Na^+ accumulation might have an effect on the activity of Na^+ and K^+-ATPase[15]. Hence, the *C. copticum* seed extracts stimulates the re-absorption of intestinal fluids in the small and large intestines.

Antidiarrhoeal and anti dysenteric properties of medicinal plants were found to be due to the presence of tannins, flavonoids, saponins, alkaloids, sterols, reducing sugars and triterpenes[20,21]. The phytochemical studies on *C. copticum* seeds have revealed that the presence of flavonoids, tannins, saponins and sterols. Thus, these chemical constituents may be responsible for the *in vivo* anti-diarrhoeal activity of *C. copticum* seed extracts. Flavonoids have anti-diarrhoeal activity, which have ability to inhibit intestinal motility and hydroelectrolytic secretions which are known to be altered in diarrhoeic conditions[22].

Tannins and tanic acid present in the anti diarrhoeal plants denature the proteins in intestinal mucosa by forming the protein tannates, which make the intestinal mucosa more resistant to chemical

alteration, and hence reduce secretion[23].

In conclusion, the results obtained in the present study suggest that *C. copticum* seed extracts have beneficial effect in controlling the diarrhoea in experimental rats. The antidiarrhoeal property of *C. copticum* is mediated through inhibition of hypersecretion, gastrointestinal motility and increase of gastric transit time. The *C. copticum* could be used in the treatment of diarrhoea.

Conflict of interest statement

We declare that we have no conflict of interest.

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