

# Antidiarrheal activity of *Capparis zeylanica* leaf extracts

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## ABSTRACT

The antidiarrheal activity of the methanolic extract of the leaves of *Capparis zeylanica* (Capparidaceae) was investigated by castor oil-induced diarrhea and small intestine transit method on mice. Like loperamide (3 mg/kg body weight), *C. zeylanica* methanolic extract (100, 150, 200 mg/kg body weight) produced a significant decrease in the severity of diarrhea. The percentage protection in extract-treated animals showing diarrhea was compared with castor oil-treated and loperamide-treated animals. The activity was found to be dose-dependant. Its effect when evaluated on intestinal transit produced a decrease in intestinal transit (75.97%). The results revealed that the methanolic extract significantly reduced diarrhea in mice with reduction in weight of stools.

**Key words:** *Capparis zeylanica*, castor oil, intestinal transit, loperamide

## INTRODUCTION

Diarrheal diseases are responsible for the death of millions of people each year.<sup>[1]</sup> There are large numbers of epidemiological and experimental evidence pertaining to worldwide acute diarrheal disease, which is one of the principal causes of death in the infants.<sup>[2]</sup> Worldwide distribution of diarrhea accounts for more than 5-8 million deaths each year in infants and children below 5 years old especially in developing countries.<sup>[3]</sup> Most people are affected by diarrhea at some time in their lives. It is often accompanied by stomach pains, feeling sick and vomiting. It is usually due to consumption of drinking water contaminated with bacteria, undercooked meat and eggs or inadequate kitchen hygiene-in other words-an infection. According to WHO estimates for 1998, about 7.1 million deaths were caused by diarrhea.<sup>[4]</sup> Despite immense technological advancement in medicine; many people in developing countries still rely on traditional healing

practices and medicinal plant for their daily health care need.<sup>[5]</sup> *Capparis* is a genus of perennial flowering shrubs known collectively by the common name caper shrubs or caper bushes. The genus *Capparis* L. (Capparaceae) consists of about 250 species distributed mostly in tropical and subtropical regions.<sup>[6]</sup> Compounds that have been isolated and characterized from the genus *Capparis* include flavonoids, fatty acids, lipids, alkaloids and glucosinolates. *Capparis zeylanica*, a rigid, wiry and much-branched shrub, is widely distributed in Bangladesh, India, Sri Lanka and Malaysia.<sup>[7]</sup> Plants are 2-3 meters in height, armed with 3-6 mm long recurved thorns, branched, leaves are elliptic or broadly lanceolate, rounded base, apex mucronate; flower profuse, pinkish white, later turning pink, berries are globular or ellipsoid, 3-4 cm in diameter and seeds are globular, embedded in white pulp. The plant grows in a moist habitat. It is used traditionally as stomachic, sedative, antihydrotic and also in cholera, hemiplegia, neuralgia and rheumatism.<sup>[8]</sup> In northern India, the leaves are used as a rubefacient,<sup>[9]</sup> counter irritant and as a cataplasm in boils, swellings and piles.<sup>[10]</sup> The leaf juice has been used by the folklore in the treatment of diarrhea. Hence the methanolic extract of *C. zeylanica* leaf extract was investigated for its antidiarrheal activity to substantiate folklore claim. The plant has previously been reported to produce fatty acids.<sup>[11]</sup> *Capparis* species have been reported to have anthelmintic, antimicrobial and anti-inflammatory activities.<sup>[12-13]</sup> In northern India, the leaves are widely used as counter-irritant, febrifuge and as a cataplasm in swellings, boils and piles.<sup>[14]</sup> The various species of genus *Capparis* are useful in the treatment of cough, asthma, inflammation, fevers, and also useful as poultice in gout.<sup>[15]</sup> Phytochemical

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screening of the plant has shown the presence of fatty acids and flavonoids in the leaves.<sup>[16,17]</sup> Whole plant showed the presence of saponin, p-hydroxybenzoic, syringic, vanillic, ferulic and p-coumaric acid. Leaves and seeds showed presence of  $\beta$ -carotene, thioglycoside, glycocapparin, N-triacontane,  $\alpha$ -amyrin and fixed oil where as a root bark showed presence of an alkaloid, a phytosterol, a water-soluble acid and a mucilaginous substance.

## MATERIAL AND METHODS

### Plant Material

The fresh leaves of *C. zeylanica* (Capparidaceae), collected at the flowering stage in the month of March 2010 from the tribal areas of Palakkad district, Kerala, South India were authenticated by the Botanical survey of India, Coimbatore, Tamilnadu (BSI). A voucher specimen (no.BSI/SRC/5/23/10-11/Tech-565) is deposited in the departmental herbarium. Leaves were dried in shade for 20 days and then powdered to get a coarse powder. This powder was stored in air-tight container and used for further successive extraction.

### Preparation of Crude Extract

The dried and powdered plant material was soxhlet extracted with methanol. The extraction was carried out for 24 h at room temperature with mild shaking. The extract was filtered and concentrated at 45°C, and the weight of the residue was recorded. The percentage yield of methanolic extract was found to be 36.50%w/w and was used for further studies.<sup>[18]</sup>

### Animals

Healthy Swiss albino mice of male sex weighing between 15 and 30 g obtained from Kerala Veterinary College, Mannuthy, Kerala. The animals were housed under standard conditions of light/dark at 12/12-hr cycle. They were fed with commercial pellet diet and water *ad libitum*. All animal experiments were carried out in accordance with guidelines of CPCSEA and the study was approved by the institutional Animal Ethics Committee (NCP/IAEC/CLEAR/05/03/2007-08).

### Drugs and Chemicals

Loperamide (Janssen), castor oil (Bell Sons and Co., England), atropine sulphate (S.D Fine chemicals, Mumbai), normal saline solution (0.9% NaCl) and vehicle (0.5% v/v Tween 80 in distilled water) were used.

### Acute Toxicity Study

The method previously described by Lorke (1983) was adopted using 13 mice.<sup>[19]</sup> In the first phase, three doses of the methanolic extract (10, 100 and 1000 mg/kg were administered to three groups each containing three mice). In the second phase, more specific doses were administered to four groups each containing one mouse. The median lethal dose (LD50) was determined as the geometric mean of the

highest non lethal dose and lowest lethal dose of which there is 0/3 and 0/1 survival.

### Castor Oil-induced Diarrhea

Twenty-four mice were allowed to fast for 18 h and divided into five groups of six animals each. All groups received castor oil at a dose of 0.4 ml/animal orally. Thirty minutes after castor oil administration, the first group (control group) received vehicle (0.5% Tween 80 in distilled water), the second group received reference drug loperamide (3mg/kg body weight) and third, fourth and fifth group received 100, 150 and 200 mg/kg body weight, respectively, of the methanolic extract of *C. zeylanica* (MCZ). After the administration, the animals were placed separately. The severity of diarrhea was assessed each hour for 6 h [Table 1]. The total weight of feces was recorded within a period of 24 h and compared with the control group. The total number of diarrhea feces of the control group was considered 100%. The results were expressed as a percentage of inhibition of diarrhea.<sup>[20,21]</sup>

### Small Intestinal Transit

Animals were divided into five groups of six mice each, and were given orally 1 ml of charcoal meal (5% activated charcoal suspended in physiological saline) 60 min after an oral dose of drugs or vehicle. Group I was administered with physiological saline (10 ml/kg) and animals in groups II, III and IV were administered MCZ (100, 150 and 200 mg/kg). Group V received atropine sulphate (0.1 mg/kg) as standard drug [Table 2]. After 30 min animals were killed by

**Table 1: Effect of MCZ on castor oil-induced diarrhea**

| Treatment (oral) | Dose    | Weight of stools (g) (Mean±SEM) | % protection |
|------------------|---------|---------------------------------|--------------|
| Control          | 2 ml/kg | 1.164±0.055*                    | –            |
| Standard         | 3       | 0.244±0.0233**                  | 79.03        |
| MCZ              | 100     | 0.477±0.0238**                  | 59.02        |
| MCZ              | 150     | 0.377±0.0245**                  | 67.61        |
| MCZ              | 200     | 0.258±0.0648**                  | 77.83        |

\*\*P<0.01 and \*P<0.05 statistically (Mean±SEM) significant from control group (n=6)

**Table 2: Effect of MCZ on small intestinal transit method**

| Treatment  | Dose (mg/kg) | Mean distance travelled by charcoal as % total length of small intestine (cm) | Reduction (%) |
|------------|--------------|---|---------------|
| Control    | 2 ml/kg      | 84.257±1.999  | –             |
| Loperamide | 0.1          | 15.244±2.040  | 81.91         |
| MCZ        | 100          | 39.667±2.112*   | 52.92         |
| MCZ        | 150          | 30.333±1.090*   | 64.00         |
| MCZ        | 200          | 20.244±2.444*   | 75.97         |

\*P<0.001; data are expressed as (Mean±SEM); n=6 (One way ANOVA followed by Dunnett's test)

cervical dislocation, and the intestine was removed without stretching and placed lengthwise on moist filter paper. The length of the intestine (pyloric sphincter to cecum) and the distance travelled by the charcoal as a percentage of that length were evaluated for each animal, and group means were compared and expressed as percentage inhibition.<sup>[22]</sup>

### Statistical Analysis

All the experimental results were expressed as mean±S.E.M. Data were analyzed by analysis of variance (ANOVA) followed by Dunnett's test.

## RESULT AND DISCUSSION

Preliminary phytochemical screening of the methanolic extract of *C. zeylanica* (MCZ) revealed the presence of alkaloids, flavonoids, carbohydrates, glycosides, tannins, terpenoids, phenols and absence of fixed oils and steroids. The median lethal dose of the extract was found to be greater than 5000 mg/kg bodyweight. Castor oil causes diarrhea due to its active metabolite, ricinolic acid,<sup>[23,24]</sup> which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. Its action also stimulates the release of endogenous prostaglandin.<sup>[25]</sup> In this study, the methanol extract of *C. zeylanica* (36.5%w/w) exhibited a significant antidiarrheal activity. Its effect was dose-dependant. The results were similar to that of the standard drug loperamide (3 mg/kg) with regard to the severity of diarrhea. Phytochemical screening revealed the presence of tannins, sterol and/or triterpenes and reducing sugars, which may be responsible for the mechanism of action of *C. zeylanica* antidiarrheal activity. The antidiarrheal activity of this extract may also be due to the presence of denatured proteins, which form protein tannates. Protein tannates make the intestinal mucosa more resistant and hence, reduce secretion.<sup>[26]</sup> This can be due to the fact that the extract increased the reabsorption of water by decreasing intestinal motility as observed in the decrease of intestinal transit by charcoal meal. Loperamide, apart from regulating the gastrointestinal tract, is also reported to slow down transit in the small intestine, reduce colon flow rate, and consequently any effect on colonic motility.<sup>[27]</sup> The methanol extract of *C. zeylanica* administered at the dose of 100, 150 and 200 mg/kg showed 59.02%, 67.61% and 77.83% protection, respectively. This reduction in diarrheal episodes is significant and maximum effect is observed at the dose of 200 mg/kg. Mean distance travelled by charcoal, as % total length of small intestine (cm) is less at 200 mg/kg of the dose of MCZ and is comparable with standard drug atropine sulfate which is used as positive control. The study reveals that the methanol extract exhibited significant diarrheal activity. Thus, *C. zeylanica* can increase the absorption of water and electrolytes from the gastrointestinal tract since the extract decreased the small intestinal transit that proves to its efficacy in an extensive range of diarrheal conditions.

In conclusion, the results of this investigation revealed that *C. zeylanica* contains pharmacologically active substance(s) with antidiarrheal properties. These properties confirm the use of *C. zeylanica* as an antidiarrheal drug as proposed by traditional healers. Further research is to be carried out to fractionate and purify the extract, in order to find out the molecules responsible for the antidiarrheal activity observed.

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