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Evaluation of Anti-Diarrhoeal Activity of Capparis Zeylanica Leaf Methanolic Extract in Albino Wistar Rats

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ABSTRACT

India has a dense forest with plenty of medicinal plants which have been used as folklore medicines by the local people for many years. They used different plant parts of Capparisspecies to treat diarrhoea. Depending on the traditional use of some plants of Capparisgenus as antidiarrhoeal, this plant was selected to evaluate antidiarrhoeal activity in animal models. Wistar albino rats weighing 180 to 200 grams were used in this study. There were five groups in each experimental model with six animals in each group. The antidiarrhoeal activity was evaluated by different experimental models namely castor oil-induced diarrhoeaand magnesium sulphate induced diarrhoea. The methanolic extract of Capparis zeylanica leaves showed significant antidiarrhoeal activity against castor oil-induced diarrhoea and magnesium sulphate induced diarrhoea in rats. The methanolic extracts at 100, 200 and 400 mg/kg significantly inhibited diarrhoea. There was a significant dose-dependent anti diarrhoeal effect in both the animal models as compared to the standard drug ($P<0.01$). Based on the results in experimental models, the methanolic extract of Capparis zeylanica demonstrated significant reductions in faecal output when compared to the standard groups. In conclusion it can be said that tannins and flavonoids present in the plant extracts may be responsible for the antidiarrhoeal activity.

Keywords: anti-diarrhoeal, experimental models, Capparis zeylanica, methanolic, wistar albino rats.

1. INTRODUCTION

Diarrhoea is a very common ailment and national problem in many tropical countries and the cause of 4-5 million deaths throughout the world annually1,2. Children are more susceptible to this disease which is the second leading cause of death of children under 5 years old3. Diarrhoea results from an imbalance between the absorptive and secretory mechanism in the intestinal tract accompanied by intestinal hurry, resulting in an excess loss of fluid in the faeces4. Diarrhoea is characterized as rapid movement of faecal matter through intestine resulting in poor absorption of water, nutritive elements and electrolytes producing abnormal frequent evacuation of watery stools. According to world health organization, it is the one of the most common cause of morbidity and mortality in many developing countries affecting mainly the infants and children5. It is often caused by enterotoxins which are produced by bacteria such as Escherichia coli, Salmonella typhi, Salmonella typhimurium, Clostridium difficile, Clostridium freundii, Aeromonas hydrophila, Campylobacter jejuni and Vibrio cholera to name a few. These bacteria cause the influx of water and ions to the intestinal lumen and thus increase the intestinal motility, thereby causing watery stools. Such secretory diarrhoea is treated by the administration of oral rehydration salts in children or adults to reduce the loss of essential electrolytes and maintain the body fluids osmolality6. The major causative agents of diarrhoea in humans include Shigella flexneri, Staphylococcus aureus, Escherichia coli, Salmonella typhi and Candida albicans7,8. Alternatively, many opiod drugs like Diphenoxylate, Loperamide, Diloxanidefuroate for protozoal infections induced diarrhoea and dysentery, racecadotril, muscarinic receptor blockers like atropine sulphate 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 by racecadotril; intestinal obstruction and constipation by loperamide9. Combination of antidiarrhoeal drugs with different mechanisms of action are often used for synergistic action. The value of these combination have not been studied experimentally10. Capparisis a genus of about 850 species of woody trees, shrubs, vines, epiphytes, and hemi epiphytes in the family Moraceae. Capparis zeylanica is an umbrageous tree 9-12 meters having young branches at first, softly pubescent and afterwards glabrous. Flowering and fruiting period is from December-January. It is mostly found in dry and moist deciduous forest areas in South India. It is found mainly in Andhra Pradesh, Kerala, and Coimbatore, Dundigal, Namkkal, Niligiri, Salem, Theni, Tirunalveli and Vellore districts of Tamil Nadu state in India. The fruit is used as cardiotonic. The bark and leaves are used in liver and skin diseases. Bark is used in folklore practise11 for the treatment of cancer and hyperlipidemia. The leaf juice has antidysenteric activity. Roots possess antispasmodic activity. The leaves are reported to show antihyperglycemic12, gastroprotective13, invitro antioxidant14, antimicrobial, antihyperlipidemic15 activity. Inspite of all these pharmacological activities documented on the plant, antidiarrheaol activity has not been reported.

2. METHODOLOGY

2.1 Plant Material

The plant material was collected from Tirupathi and was authenticated by Osmania University, Hyderabad and was given the voucher number 0949.

2.2 Extraction Process

The plant material was dried under shade, pulverized by a mechanical grinder passed through a #40 sieve and stored in tightly closed container for further use. The coarse powder of leaves is extracted in a soxhlet apparatus by using methanol as a solvent. After extraction, the solvent is removed by vacuum distillation. The semi solid mass was stored in a desiccator for further use. Preliminary phytochemical screening was done to identify the presence of alkaloids, steroids, flavonoids and tannins in leaf extract.

2.3 Phytochemical Screening

The preliminary phytochemical screening of the extracts was performed by the method described by Khandelwal16.

2.4 Experimental Animals

Wistar Albino rats weighing around 150 to 200 grams of either sex were maintained at uniform laboratory conditions in standard steel cages, provided with food and water and were acclimatized for a week before the experiment in the animal house of the college certified by Institutional animal ethics committee.

2.5 Experimental Methods

2.5.1 Acute Toxicity studies

Acute oral toxicity study was performed as per OECD-423 guidelines category IV (acute toxic class method,). Albino mice (n = 3) of either sex selected by random sampling technique were employed in this study. The animals were kept fasting for 4 hours with free access to water only. The plant extract was administered orally with maximum dose of 2,000 mg /kg body weight by gastric intubation. The mortality was observed for three days. If mortality was observed in 2 out of 3 animals or 3 out of 3 animals then the dose administered was assigned as toxic dose. If mortality was observed in 1 animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose such as 3,000 mg/kg of body weight (OECD 2006).

2.5.2 Castor oil induced diarrhoea

Induction of secretory diarrhea was done according to the method described by Karthik17with slight modifications. The rats weighing around 150 to 200 grams were fasted for 18 hours. They were divided into five groups with six rats in each group (n=6). The first group of animals were served with castor oil (2 ml). The second group received standard drug, Loperamide (3 mg/kg) orally as suspension. The third, fourth and fifth group were treated with CZME 100, 200 and 400 mg/kg respectively. After 60 minutes, the animals of each group received 2 ml of castor oil orally. Watery faecal material and frequency of defecation were noted upto 4 hours in transparent metabolic cages with plastic dishes weighed and placed at the base of the cages. The weight of plastic dish before and after the defecation were noted and compared with the control.

2.5.3 Magnesium sulfate induced diarrhoea

Diarrhoea was induced using the same method as that described in the previous experimental model with the only difference that magnesium sulfate was used at the dose of 3 mg/kg instead of castor oil. Again in this model, the faecal matter and the frequency of defecation were noted up to 4 hours in the transparent metabolic cages with pre weighed plastic dishes placed at the base of the cages. Weight of the plastic dishes were recorded before and after defecation and compared with the control group.

3. RESULTS

3.1 Phytochemical screening

Preliminary phytochemical screening of methanolic extract of Capparis zeylanicashowed the presence of alkaloids, steroids, tannins, saponins, terpenoids and flavonoids.

3.2 Results of Acute toxicity studies

The results of acute toxicity studies showed that the methanolic extract of leaf did not show any mortality upto a dose of 1000 mg/kg .

3.3 Effect of CZME on castor oil induced diarrhoea

In this experimental model the test group CZME 400 mg showed maximum inhibitory effect with 76% with number of diarrhoel faeces noted as (3.83 ± 0.70) as compared with the standard drug, Loperamide with 86.5% with the number of faeces (2.16 ± 0.47). Other test groups CZME 100 mg and CZME 200 mg showed significant inhibitory effect with 27.1% and 59.3% respectively and the number of diarrhoeal faeces were recorded as (11.66 ± 0.56) and (6.5 ± 0.76) respectively as shown in Table 1 and Figure 1

Table 1: Number of Diarrhoeal Faeces Observed in Treated Groups

Treatment	Dose(mg/kg)	Number of Diarrhoeal faeces	Inhibition of Diarrhoea
Negative control	Castor oil(2ml)	16.3 ± 30.66	--
Loperamide	3mg	2.16 ± 0.47	86.5%
CZME (100mg/kg) + castor oil (2ml)	100mg	11.66 ± 0.56	27.1%
CZME (200mg/kg) + castor oil (2ml)	200mg	6.5 ± 0.76	59.3%
CZME (400mg/kg) + castor oil (2ml)	400mg	3.83 ± 0.70	76%

Values are expressed as ± S.E.M (n=6). P<0.01,when compared to the control.

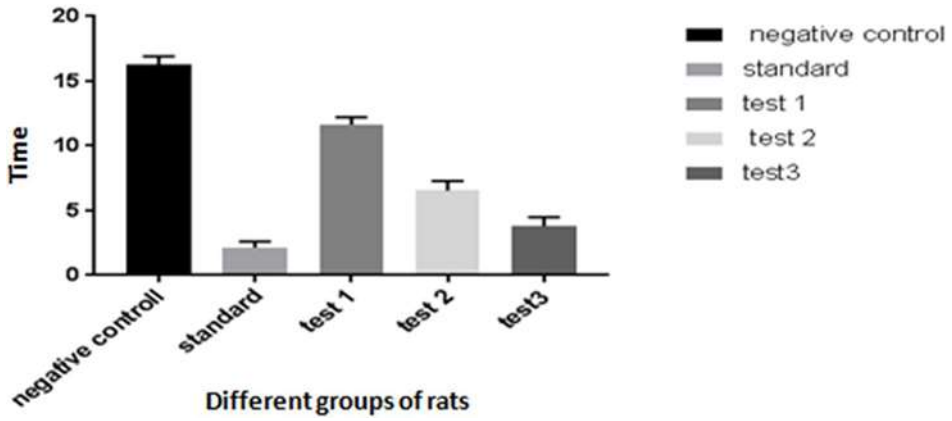

Figure 1: Number Of Diarrhoeal Faeces Observed In Treated Groups

3.4 Effect of CZME on magnesium sulfate induced diarrhoea

In this experimental model the test group CZME 100 mg/kg showed 57% inhibition to diarrhoea with number of diarrhoeal faeces as (13.16 ± 0.60). The test groups CZME 200 mg/kg showed a significant diarrhoeal inhibition with 77% where gradual decrease in the number of diarrhoeal faeces (8.66 ±0.88) was observed. The test group CZME 400 mg/kg showed a maximum diarrhoeal inhibition at 84% with minimum number of diarrhoeal faeces noted as (6.66 ± 1.38) as shown in table 2 and Figure 2.

Table 2: Number Of Diarrhoeal Faeces Observed In Treated Groups

Treatment	Dose(mg/kg)	Number of Diarrhoel faeces	Inhibition of	Diarrhoea
Negative control	Castor oil (2ml)	20 ± 1.06		-----
Loperamide	3mg	2.16 ± 0.47	89 %	

CZME (100mg/kg) + castor oil (2ml)	100mg	13.16 ± 0.60	57%
CZME(200mg/kg) + castor oil (2ml)	200mg	8.66 ±0.88	77%
CZME(400mg/kg) + castor oil (2ml)	400mg	6.66 ± 1.38	84%

Values are expressed as ± S.E.M (n=6). P<0.01, when compared to the control

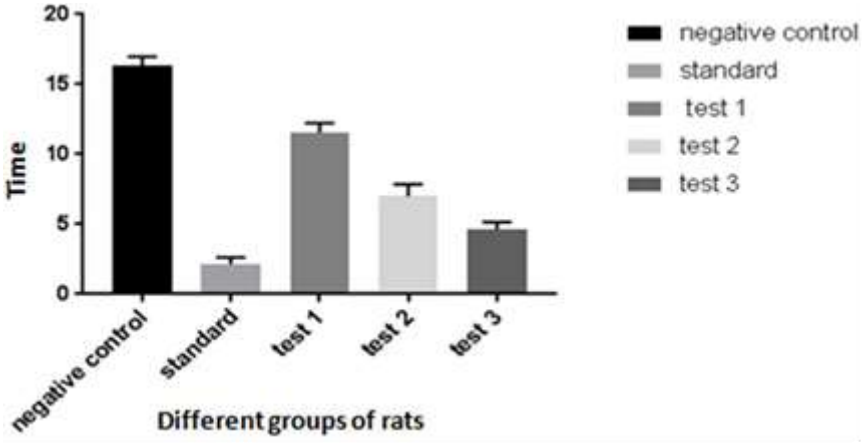

Figure 2: Number Of Diarrhoeal Faeces Observed In Treated Groups

4. DISCUSSION

The use of medicines derived from plants has been commonly used as folk medication in the treatment of diarrhoea. People in the developing countries still rely on this type of treatment system18. Among the various medicinal plants, antidiarrhoeal activity was found in plants which possess phytoconstituents like alkaloids, tannins19, flavonoids20 and triterpenes21. From the earlier studies, it has been reported that Capparisbengalensis containing tannins and flavonoids possessed antidiarrhoeal activity and the underlying mechanism appears to be spasmolytic and anti-enteropooling property by which it produced relief from diarrhoea22. Antidiarrhoeal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydroelectrolytic secretion23. The inhibitory effect of flavonoids on intestinal motility in a dose related manner was earlier reported24,25. Triterpenes have also been reported to show considerable antidiarrhoeal activity26. Tannins present in many plants denature protein to form the protein tannate, which makes the intestinal mucosa more resistant and reduces secretion27. Previous studies have shown that antidysenteric and antidiarrhoeal properties of medicinal plants are due to the presence of tannins, alkaloids, flavonoids, saponins, reducing sugars, sterols and triterpenes28. Diarrhoea can be induced by castor oil through the production of active metabolite ricinolic acid29. Castor-oil induced diarrhoea is described to be an appropriate model that characterizes secretorydiarrhoea30.Though several mechanisms have been proposed to explain the diarrhoeal effect of castor oil, it is well known that diarrhoea is initiated by its metabolite ricinoleic acid through a hypersecretory response which activates intestinal smooth muscles through EP3 prostaglandin receptors31. Ricinoleic acid also reduces active Na+ and K+ absorption and decrease Na+K+ ATPase activity in the small intestine and colon. The liberation of ricinoleic acid results in irritation and inflammation of intestinal mucosa, leading to release of prostaglandins, which results in stimulation of secretion and thereby prevents the reabsorption of Nacl and H2O and resulting in diarrhoea. More precisely, castor oil elevates the biosynthesis of prostaglandin which results in irritation and inflammation of the intestinal mucosa to stimulate the motility and secretion. Several other mechanisms had been earlier proposed to explain the diarrhoeal effect of castor oil which includes inhibition of intestinal Na+K+ATPase activity, thus reducing normal fluid absorption.

Suppression of the intestinal fluid accumulation by the extract might also suggest the inhibition of gastrointestinal function.Castor oil model, therefore, incorporates both secretory and motility diarrhoea. Magnesium sulphate is known to increase the permeability of electrolytes at the level of the intestinal mucosa, alongside the secretion of cholecystokinin in the duodenum leading to hyper secretion which inhibits fluid reabsorption. Loperamide used in this study as reference drug acts by inhibiting the peristaltic activity, through indirect effect on circular and longitudinal muscle of the intestinal wall, also by stimulating the absorption of water and electrolytes by enterocytes by increasing the intestinal transit time of the bowel content. Capparis zeylanicamethanolic leaf extract showed dose dependent inhibition of frequency of defecation in both experimental models, castor oil induced diarrhoea and magnesium sulphate induced diarrhoea. There was a maximum diarrhoeal inhibition observed with the test group CZME 400 mg/kg which showed minimum number of faeces. Although Capparis zeylanicaleaf methanolic extract showed significant inhibition in all test groups (100 mg, 200 mg and 400 mg).

5. CONCLUSION

Capparis zeylanica leaf methanolic extract has shown a significant antidiarrhoeal effect in albino wistar rats in both, castor oil induced and magnesium sulphate induced diarrhoea model. It is evident from our study that CZME contains pharmacologically active ingredients that are responsible to show antidiarrhoeal activity.

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