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Anti-diarrhoeal Effect of Ethanolic Extract of *Cuminum cyminum* Linn. (ဇီယာစေ့) on Castor Oil-induced Diarrhoea in Mice Model

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This study was to determine phytochemical constituents, acute toxicity and anti-diarrhoeal activity of 80% ethanolic extract of cumin seeds. The phytochemical tests showed alkaloids, flavonoids, glycosides, tannins, steroids, phenols, saponins, and amino acids were present in the extract whereas resins, triterpine and cyanogenic glycoside were not. Acute toxicity test of the 80% ethanolic extract of cumin seeds showed no toxicity in albino mice, even with maximum permissible dose of 16 g/kg body weight. Anti-diarrhoeal activity was evaluated by castor oil-induced diarrhoeal test, enteropooling test and intestinal transit test on mice. Three doses (3, 6 and 9 g/kg body weight) of tested extracts showed significant anti-diarrhoeal effect (% inhibition of defecation) ($p<0.01$) when compared to control. The extracts of 6 g/kg, 9 g/kg and loperamide showed a significant decrease in the onset of defecation ($p<0.05$) when compared to control. All doses of tested extracts showed anti-diarrhoeal effects comparable to loperamide (6 mg/kg) as seen by a marked decrease in the number of diarrhoeal stools, from 21.17±4.71 to 5±3.41, 3.67±3.55, 0.5, respectively, and 4.17±0.71 in loperamide at 4 hour ($p<0.01$). There was also a significant percent reduction in the volume of intestinal content of the test extract (65.46±19.08, 64.93±16.78, 61.15±20.56) and the values were comparable to standard drug loperamide (60.13±12.21). The anti-diarrhoeal index of test extract and standard drug loperamide were 79.16%, 80.91%, 87.83% and 76.65%, respectively. Therefore, it was concluded that the 80% ethanolic extract of cumin seeds possesses significant anti-diarrhoeal activity.

INTRODUCTION

Myanmar is a tropical country with diarrhoea as a common health problem. In Myanmar, traditional medicines are widely used in the treatment of a variety of disorders. Medicinal herbs constitute an indispensable component of the traditional medicine practiced worldwide due to affordability, accessibility and ancestral experience. WHO has encouraged studies for treatment and prevention of diarrhoeal diseases depending on traditional medical practices.

Diarrhoea is defined as the production of stool of abnormally loose consistency, usually associated with excessive frequency of defecation and with excessive stool output. Normal stool output is approximately 100 to 200 g/day.[1] Diarrhoea disease is a leading cause of mortality and morbidity, especially among children in developing countries resulting in a major health care problem.[2] Diarrhoea can cause by a temporary problem, like an infection, or a chronic problem, like an intestinal disease. Diarrhoea commonly results from gastroenteritis caused by bacterial infection, viral infection, food intolerance, parasites, reaction to medicine, intestinal diseases, functional bowel disorders, such as irritable bowel syndrome, in which the intestines do not work normally.[3]

Many plants available in India are used in traditional folklore medicine for the treatment of diarrhoea and dysentery.[4] Ayurveda is an ancient form of Indian medicine, which deals with plants and plant products. This indigenous form of medicine uses the active ingredients present in plants for treating diseases.[5]

Cuminum cyminum Linn. (Family: Umbelliferae) is widely cultivated, used and consumed in fairly large quantities by Indians. Cumin is widely used in Ayurevedic medicine. Cumin is the seed of a small Umbelliferous plant. Cumin seeds are very useful in digestive disorders like biliousness, sickness, indigestion, atonic syspepsia, diarrhoea, malabsorption syndrome and flatulent colic. One spoon of cumin seeds is boiled in a glass of water and the concoction is mixed with one teaspoon of fresh coriander leaf juice and a pinch of salt. This decoction can be taken twice daily after meals as medicine for diarrhoea.[6] The present study was undertaken to investigate the phytochemical activity, the acute toxicity and anti-diarrhoeal activity of the 80% ethanolic extract of *C. cyminum* Linn. on castor oil-induced diarrhoea in albino mice.

MATERIALS AND METHODS

Phytochemical constituents of 80% ethanolic extract of C. cyminum Linn.

Investigation for phytochemical constituents of *C. cyminum* Linn. was carried out by using the method of Harborne,[7] Linsted,[8] and Central Council for Research in Union Medicine.[9]

Acute toxicity test

The 80% ethanolic extract of cumin seeds was tested by the method of Litchfield and Wilcoxon.[10] Mice were randomly divided into 4 groups (I to IV), 10 in each group. They were fasted for 18 hours. The group I mice served as the control orally given with distilled water only. The mice in groups II to IV were orally given with 4 g/kg, 8 g/kg and 16 g/kg body weight of the 80% ethanolic extract of cumin seeds suspended in distilled water, respectively. The animals were observed for the first six hours continuously for the mortality if any and then every 24 hours for two weeks.

Anti-diarrhoeal activity of 80% ethanolic extract of C. cyminum Linn.

The anti-diarrhoeal effect of 80% ethanolic extract was studied with castor oil-induced diarrhoea test, castor oil-induced enteropooling test and castor oil-induced small intestinal trasit test.

(i) Castor oil-induced diarrhoeal test

Mice were fasted overnight with water *ad lib* and were randomly divided into five groups (I to V) (six animals, each). The mice in group I served as the control and they were orally given only 10 ml/kg b.w of distilled water. Group II received loperamide 6 mg/kg b.w and served as the standard. Group III, IV, and V were respectively treated orally with 3, 6 and 9 g/kg b.w of the 80% ethanolic extract of cumin seeds. Diarrhoea was induced by administration of 10 ml/kg of castor oil orally to mice. The animals were then placed in individual cages on a clean paper. The number of the diarrhoeal dropping was counted every hour for 4 hours and the mean number of the stool, passed by the treatment groups was compared with control group.[11]

(ii) Castor oil-induced enteropooling test

The accumulation of the intraluminal fluid was determined by the method of Robert, *et al*.[12] Mice were fasted overnight with water *ad lib* and divided into 5 groups (I to V) (six animals, each). Group I which received distilled water (10 ml/kg) orally served as the control. Group II which received loperamide (6 mg/kg b.w) served as the standard. Group III, IV and V received 3, 6 and 9 g/kg b.w, respectively of the 80% ethanolic extract of the dried cumin seed, one hour before the oral administration of castor oil. After one hour, the mice were killed. The small intestines were removed after tying both ends with threads and weighed. The intestine was squeezed

out slowly and its content was collected into a measuring test tube. The volume was measured. Then, the intestine was reweighed and the difference between full and empty intestine was determined. The content of the fecal matter in the intestine was calculated.

(iii) Castor oil-induced small intestinal transit test

Mice were divided into five groups (I to V) (six animals, each). They were fasted for the period of 18 hrs. The mice of group I (10 ml/kg) which received distilled water orally served as the control. Group II received loperamide 6 mg/kg b.w served as the standard. Group III, IV and V received the 80% ethanolic extract of cumin seeds (3, 6, 9 g/kg b.w), respectively, 1 hour before administration of castor oil (10 ml/kg b.w). Marker 10 ml/kg (10% charcoal suspension in 5% gum acacia) was administered orally 1 hr after castor oil treatment. The mice were sacrificed after thirty minutes. The distance travelled by the charcoal plug from pylorus to caecum was determined and measured (cm) and expressed as a percentage of the total length of the small intestine.[11]

RESULTS

Phytochemical tests on the 80% ethanolic extract of *C. cyminum* Linn. showed that alkaloids, flavonoids, glycosides, tannins, steroids, phenols, saponins and amino acid were present whereas resin, triterpine and cyanogenic glycoside were absent. Acute toxicity study on the mice treated with 4, 8 and 16 g/kg doses of the 80% ethanolic extract of cumin seeds were kept under observation for two weeks. It was observed that there was no lethality at this dose level.

Therefore, the medium lethal dose (LD_{50}) was more than 16 g/kg b.w. The anti-diarrhoeal activity was investigated using 80% ethanolic extract of cumin seeds in castor oil-induced diarrhoea mice models. Anti-diarrhoeal activity was evaluated by castor oil-induced diarrhoea test, entero-pooling test and intestinal transit test. Three doses (3, 6, 9 g/kg b.w) of tested extracts showed significant anti-diarrhoeal effect (% inhibition of defecation) ($p<0.01$) when compared to control. The extracts of 6 g/kg, 9 g/kg and loperamide showed significantly decreased onset of defecation ($p<0.05$) when compared to control. All doses of tested extracts showed anti-diarrhoeal effects comparable to loperamide (6 mg/kg) as seen by a marked decrease in the number of diarrhoeal stools (21.17±4.71 to 5±3.41, 3.67±3.55, 0.67±0.82, and 4.17± 0.71 at 4 hour ($p<0.01$) (Table 1).

Table 1. Antidiarrhoeal effect of ethanolic extract of *C. cyminum* Linn. and loperamide on castor oil-induced dropping in mice

Group	Treatment	Mean number of defecation in four hours	% Inhibition of defecation
I	Castor oil+D/W 10 mg/kg (control)	21.17±4.71	-
II	Castor oil+Ethanolic extract 3 g/kg	5±3.41**	76.38
III	Castor oil+Ethanolic extract 6 g/kg	3.67±3.55**	82.66
IV	Castor oil+Ethanolic extract 9 g/kg	0.67±0.82**	96.83
V	Castor oil+Standard loperamide 6 mg/kg	4.17±0.71**	80.30

**=$p<0.01$, comparison between different types of treatment and control (castor-oil only)

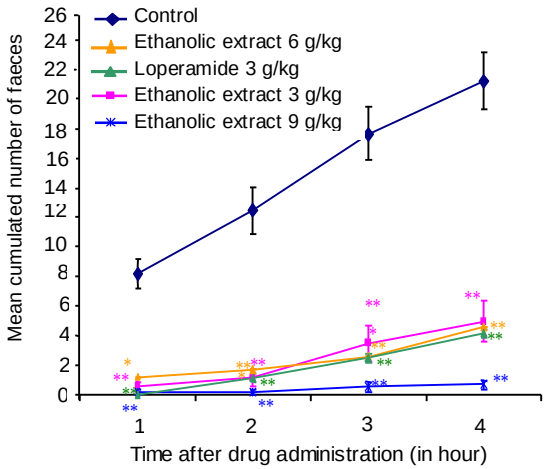

Fig. 1. Comparative effects of 80% ethanolic extract of *C. cyminum* Linn. 3 g/kg, 6 g/kg, 9 g/kg, and standard drug loperamide on mice with castor oil-induced diarrhea at various time intervals (Each point represents as mean±SD from the experiments ,**=$p<0.01$)

Comparative effects of 80% ethanolic extract of *C. cyminum* Linn. 3 g/kg, 6 g/kg, 9 g/kg, and standard drug loperamide on mice with castor oil-induced diarrhea at various time intervals are shown in Fig. 1.

Table 2. Anti-diarrhoeal effects of ethanolic extract of *Cuminum cyminum* Linn. and loperamide on castor oil-induced enteropooling in mice

Gp	Enteropooling			
	Weight of intestinal content (g)		Volume of Intestinal content (ml)	
	Normal	%Reduction	Normal	%Reduction
I Control(Castor oil only)				
	0.73±0.27	-	0.98±0.09	-
II Ethanolic extract (3 g/kg b.w)				
	0.35±0.08**	46.95±23.45	0.33±0.17**	65.46±19.08
III Ethanolic extract (6 g/ kg b.w)				
	0.40±0.16*	34.6±48.03	0.34±0.15**	64.93±16.78
IV Ethanolic extract (9 g/kg b.w)				
	0.43±0.16*	30.27±43.62	0.37±0.18**	61.15±20.56
V Castor oil+Loperamide (6 mg/kg b.w)				
	0.46±0.09*	31.49±25.08	0.39±1.07**	60.13±12.21

Gp=Group, *=p<0.05, **=p<0.01, comparison between different types of treatment and control (castor-oil only)

Table 3. Anti-diarrhoeal activity of ethanolic extracts of *C. cyminum* Linn. and loperamide on castor oil-induced treatment test in mice

Gp	Delaying defecation time (min)	Gut meal travel distance (cm)	Purging frequency in number of stool	*In vivo* anti-diarrhoeal index (%)
I Control				
	15.83±17.06	52.00±6.47	21.17±4.71	0
II Ethanolic extract (3 g/kg)				
	106.7±105.24*	12.33±5**	5±3.4**	79.16
III Ethanolic extract (6 g/kg)				
	132.5±93.52*	14.17±5.84**	3.67±4.65**	80.91
IV Ethanolic extract (9 g/kg)				
	175±89.89**	12±3.03**	0.67±0.82**	87.83
V Loperamide (6 mg/kg)				
	135.83±75.19**	19.00±6**	4.17±0.71**	76.65

Gp=Group, *=p<0.05, **=p<0.01, comparison between different types of treatment and control (castor-oil only)

All these doses of extracts and standard loperamide showed significantly reduced number of faecus. There was significantly inhibited castor oil-induced enteropooling in 3, 6, 9 g/kg b.w dose levels. The percent reduction weights of intestinal content (gm) in these test groups were 46.95±23.45, 34.6±48.03, 30.27±43.62, respectively and standard drug loperamide was 31.49±25.08. The percent reduction volumes of intestinal content (ml) in treatment group with three doses of 80% ethanolic extract of cumin seeds (3, 6, and 9 g/kg) and standard drug loperamide (6 mg/kg b.w) were 65.46± 19.08, 64.93±16.78, 61.15±20.56 and 60.13 ±12.21, respectively (Table 2).

From these results, anti-diarrhoeal index of test extract and loperamide were calculated and were found to be 79.16%, 80.91%, 87.83%, and 76.65%, respectively (Table 3).

DISCUSSION

Preliminary phytochemical tests on some selected Myanmar medicinal plants revealed in some cases the presence of alkaloids and glycosides as the major components whereas in some other plant materials, steroids, tannin, flavonoids and phenolic compounds are mostly found. Anti-diarrhoeal properties of medicinal plants were found to be due to tannins, alkaloids, saponins and flavonoids.[13] In this study, the phytochemical constituents of 80% ethanolic extract of *C. cyminum* Linn. revealed the presence of anti-diarrhoeal properties compounds (alkaloids, tannins, saponins and flavonoids).

Preliminary studies of the acute toxicity test were carried out before experiments. The acute toxicity test of the 80% ethanolic extract of cumin seeds on albino mice was done. The mice were treated with three doses of (4, 8, 16 g/kg) of this extract. The mice were found to be alive and healthy with maximum permissible dose of 16 g/kg during the observation period of 2 weeks. Therefore, the median lethal dose (LD_{50}) was supposed to be more than 16 g/kg. Thus, cumin seeds were found to be less toxic and had no harmful effects.

The 80% ethanolic extract of cumin seeds has shown dose dependent anti-diarrhoeal activity in a castor oil-induced model in albino mice. The effect of 80% ethanolic extract of cumin seeds on castor oil-induced diarrhoeal test in mice was studied.

This indicated that three doses (3, 6, 9 g/kg b.w) of the extract had similar activity as standard drug loperamide (6 mg/kg b.w) in regard to the dose related effect and wetness of the fecal dropping when compared to control mice.

In enteropooling test, 3, 6 and 9 g/kg b.w of 80% ethanolic extract of cumin seeds significantly reduced the inhibited % reduction in volume of intestinal content compared with control. This indicated that all doses of test extract produced significant anti-secretory effect. In intestinal transist test, significant reduction in reduced motility was found with three doses of the extract and standard loperamide. This indicated that all dose of the extract and loperamide showed significant anti-motility.

The anti-diarrhoeal index of test extract and loperamide were 77.9% to 88% and 77%, respectively. Therefore, these results indicated that the 80% ethanolic extract of cumin seeds was effective as standard drug loperamide.

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