

RESEARCH ARTICLE

# Antidiarrheal and antiulcer activity of *Amaranthus spinosus* in experimental animals

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## Abstract

The ethanol extract (50%) of the whole plant of *Amaranthus spinosus* Linn. (Amaranthaceae) (ASE) significantly inhibited travel of a charcoal meal at three different doses of ASE, but when 400 mg/kg of ASE was repeated in the presence of yohimbine, intestinal propulsive inhibition decreased, while morphine reversed the activity. The percentages related to controls for the onset of diarrhea were 16.58, 83.42, and 116.18% at doses of 100, 200, and 400 mg/kg of ASE, while with morphine this value was 123.93% compared to controls. The percentage purging frequency related to controls was 41.09, 64.38, 71.23, and 86.30% at three different doses of ASE and with morphine, respectively. The inhibitions in intestinal accumulation were 8.9, 48.16, and 68.06% at doses of 100, 200, and 400 mg/kg of ASE, respectively, compared to control, while inhibition with yohimbine was 50.78%. Antidiarrheal indices of ASE were 23.55, 49.16, and 76.53 at the three different doses of ASE, while morphine had a maximum index of 88.45. Protection in ethanol-induced ulcer was 51.07, 55.91, 77.95, and 60.75%, but in aspirin-induced ulcer it was 41.33, 61.77, 80.88, and 74.66% at doses of 100, 200, and 400 mg/kg of ASE and with cimetidine, respectively. Lipid peroxidation was also associated with a concomitant decrease in ulcer index, while protection was 56.96, 63.29, 81.01, and 52.32% at three different doses of ASE and with cimetidine in cold restraint-induced ulcer.

**Keywords:** *Amaranthus spinosus*; *Amaranthaceae*; antidiarrheal; antiulcer

## Introduction

Diarrhea is a major health problem, especially for children under the age of 5 years, and up to 17% of all deaths in pediatric in-patients are related to diarrhea. It continues to be one of the leading causes of mortality and morbidity, especially in children in developing countries, including India (Anonymous, 1979; Black et al., 1982). According to the World Health Organization (WHO) estimation for the year 1998, there were about 7.1 million deaths due to diarrhea (Park, 2000). The WHO has established a diarrheal diseases control program (CDD), which includes the study of traditional medicinal practices, together with the elevation of health education and prevention approaches (Rao et al., 2004). *Amaranthus spinosus* Linn. (Amaranthaceae) is commonly known as “Kate

Wali Chaulai (Kanatabhaji)” in Hindi, and is used as a vegetable and cultivated throughout India, Sri Lanka, and many other tropical countries. In Ayurveda (Indian traditional system of medicine) the plant is used as a digestive, laxative, diuretic, stomachic, and antipyretic, to improve appetite, biliousness, blood diseases, burning sensation, leprosy, bronchitis, rat bite, piles, and leucorrhoea, while the boiled leaves and root are given to children as a laxative, emollient, and poultice for abscesses, boils, and burns (Kirtikar & Basu, 2001; Mishra, 1986). The leaves are used to treat rheumatic pain, stomach-ache, eczema, gastroenteritis, gallbladder inflammation, boils, abscesses, snake bite, colic menorrhagia, and arthritis (Hema et al., 2006). The leaves of *A. spinosus* are reported to produce inhibition of prostaglandin biosynthesis *in vitro* (Ibewuik et al., 1997). In India the root extract is given as a vermicide

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among the Santhali and Paharia in eastern Bihar, while an aqueous decoction of the plant is given to check chronic diarrhea in Southern Orissa (Parrotta, 2001). The plant is used in the treatment of diarrhea in traditional medicine systems in tropical countries, and it is routinely prescribed as an antidiarrheal drug in Thai traditional medicine (Sawangjaroen & Sawangjaroen, 2005). The juice of *Amaranthus spinosus* is used by tribal people of Kerala, India to prevent swelling around the stomach, while the leaves are boiled without salt and consumed for 2–3 days to cure jaundice (Hema et al., 2006). The plant has a high concentration of antioxidant components, high nutritive value due to the presence of fiber and proteins, and a high concentration of essential amino acids, especially lysine (Cao et al., 1996; Odhav et al., 2007; Teutonico & Knorr, 1985; Vinson et al., 1998). *Amaranthus spinosus* is also used as an anti-inflammatory, antimalarial, antibacterial, antimicrobial, antidiuretic, and antiviral agent and in hepatic disorders (Olajide et al., 2004; Stintzing et al., 2004; Van Dunen, 1985). The water extract of the plant showed significant immunostimulating activity (Lin et al., 2005), and a stem extract showed antimalarial activities (Hilou et al., 2006). *Amaranthus spinosus* has several active constituents including alkaloids, flavonoids, glycosides, phenolic acids, steroids, amino acids, terpenoids, lipids, saponins, anthraquinone derivatives, volatile oils, organic acids, betalains,  $\beta$ -sitosterol, stigmasterol, linoleic acid, rutin, catechuic tannins, polyuronides, and carotenoids. The betalains in the stem bark of *A. spinosus* were identified as amaranthine, isoamaranthine, hydroxycinnamates, quercetin, and kaempferol glycosides (Hilou et al., 2006; Ibewuik et al., 1997; Rastogi & Mehrotra, 1999; Srinivasan et al., 2005; Stintzing et al., 2004). It also contains amaranthoside, a lignan glycoside, amaricin, a coumaroyl adenosine, along with stigmasterol glycoside and betaines such as glycine betaine and trigonelline (Azhar-ul-Haq et al., 2006; Blunden et al., 1999).

Whole plants of *Amaranthus spinosus* are used as food additives and for the treatment of diarrhea and ulcer in traditional systems of medicine. However, the plant has already been validated scientifically as an antidiarrheal agent *in vitro* against *Blastocystis hominis* (Sawangjaroen & Sawangjaroen, 2005), but the *in vivo* antidiarrheal and antiulcer activity of the plant has not been investigated. In our study, the 50% ethanol extract of the whole plant of *Amaranthus spinosus* (ASE) was evaluated for antidiarrheal and antiulcer activity in experimental animals, along with the likely mode of action.

## Materials and methods

### *Plant material and preparation of extract*

Whole plants of *Amaranthus spinosus* were freshly collected from the botanical garden of the National

Botanical Research Institute, India in October 2006. The plant material was identified and authenticated taxonomically by Dr. R. L. S. Shikarwar at the National Botanical Research Institute, Lucknow. A voucher specimen (NAB 75006) of the collected sample was deposited in the departmental herbarium for future reference. The plants were washed with distilled water to remove dirt and shade dried. The dried materials were powdered and passed through a 10-mesh sieve. The coarsely powdered material (1 kg) was extracted with petroleum ether three times to remove the fatty material and the marc was further macerated three times with ethanol (50%, v/v). The extracts were filtered, pooled, and concentrated at reduced temperature (5°C) on a rotary evaporator (Buchi, USA) and then freeze-dried (Freezone® 4.5; Labconco, USA) at high vacuum ( $133 \times 10^{-3}$  mbar) and a temperature of  $-40 \pm 2^\circ\text{C}$  (ASE: yield 6.12%, w/w). For pharmacological tests the extract was suspended with carboxymethyl cellulose (1%, w/v) in double distilled water.

### *Animals*

Male Swiss albino mice weighing 20–25 g and Sprague-Dawley rats weighing 140–160 g were procured from the National Laboratory Animal Center (NLAC), Central Drug Research Institute, Lucknow. They were kept in the departmental animal house in a temperature controlled room at  $25 \pm 2^\circ\text{C}$ , relative humidity 44–56%, light and dark cycles of 10 and 14 h, respectively, for 1 week before and during the experiments. Animals were provided with a standard rodent pellet diet (Amrut, India) and the food was withdrawn 18–24 h before the experiment, although water was allowed *ad libitum*. All studies were performed in accordance with the guide for the care and use of laboratory animals as adopted and promulgated by the Institutional Animal Care Committee, CPCSEA, India (Reg. No. 222/2000/CPCSEA). All the chemicals used were of analytical grade from standard companies and water was double distilled. A standard orogastric cannula was used for oral drug administration.

### *Antidiarrheal activity*

#### **Gastrointestinal propulsion**

The mice were divided into six groups, each group including six mice. Group 1 served as the control group and received CMC (Sodium Carboxymethyl Cellulose 1%, w/v). Groups 2, 3, and 4 were pretreated with ASE at the doses of 100, 200, and 400 mg/kg, p.o., respectively, while group 5 received yohimbine (1 mg/kg, s.c.) 30 min before ASE 400 mg/kg, p.o., and group 6 was pretreated with morphine (10 mg/kg, s.c.).

Twenty minutes after the above treatments, each mouse received 0.2 mL standard charcoal meal (5%

activated charcoal suspended in CMC) orally. The mice were stunned and killed 30 min later by cervical dislocation, and the small intestine was rapidly dissected out and placed on a clean surface. The small intestine was carefully inspected and the distance traveled by the charcoal meal from the pylorus was measured (Ruwart et al., 1980). The percentage inhibition and peristaltic index were calculated according to the following formulae:

$$\text{Peristaltic index} = \frac{\text{Distance moved by charcoal (cm)}}{\text{Intestinal length (cm)}} \times 100$$

$$\% \text{Inhibition} = \frac{(A - B/A)}{A} \times 100$$

where A is the distance moved by the charcoal (cm) in the control group, and B is the distance moved by the charcoal (cm) in the treated group.

#### Castor oil induced diarrhea

The mice were divided into five groups, each including six mice. Diarrhea was induced by the oral administration of castor oil to the mice, 0.2 mL per animal, while ASE at doses of 100, 200, and 400 mg/kg, orally, and morphine (10 mg/kg, s.c.) were administered 30 min before castor oil administration. Control animals received CMC. Each animal was then kept under a glass funnel, the floor of which was lined with blotting paper, and observed for 4 h. The following parameters were observed: the time elapsed between administration of the cathartic agent and excretion of the first diarrheic feces (wet feces that left a halo on the filter paper), the total number of fecal outputs as well as the number of diarrheic feces excreted by the animals in 4 h, and the total weight of the diarrheal stools in that period of time. A numerical score based on stool consistency was assigned as follows: normal stool=1, semi-solid stool=2, and watery stool=3. The onset was measured as the time interval in minutes between the administration of castor oil and the appearance of the first diarrheal stool. Calculations were made for the corresponding percentage and purging index, the latter by comparison with the control group (Acounters et al., 1978; Mukherjee et al., 1998). The *in vivo* antidiarrheal index (ADI *in vivo*) was then expressed according to the formula (Aye-Tham et al., 1989):

$$\text{ADI } in \text{ vivo} = 3 \sqrt{D \text{ freq} \times G \text{ meq} \times P \text{ freq}}$$

where D freq is the delay in defecation time or diarrheal onset (as % of control), G meq is the gut meal travel reduction (as % of control), and P freq is the purging frequency, or reduction in the number of stools (as % of control).

#### Castor oil induced enteropooling

The rats were divided into six groups, each group including six rats. Group 1 served as controls and received

CMC. Groups 2, 3, and 4 were pretreated with the doses of 100, 200, and 400 mg/kg, p.o. of ASE, respectively, 1 h prior to castor oil (0.2 mL/animal) administration to all rats. Group 5 received yohimbine (1 mg/kg, s.c.) 30 min before administration of ASE 400 mg/kg, while group 6 was pretreated with morphine (10 mg/kg, s.c.). Thirty minutes later, the rats were sacrificed, exsanguinated after ligation at both the pyloric and the ileocecal junctions, and weighed. The intestinal contents were expelled into a graduated measuring cylinder and the volume was recorded. The intestine was reweighed, and the difference between full and empty intestines was calculated (Dicarlo et al., 1994; Robert et al., 1976).

#### Induction of acute gastric mucosal damage

The antiulcerogenic activity of ASE was evaluated in rats using three different assay models for the induction of acute gastric mucosal lesions: 100% ethanol (1 mL/200 g, 1 h) (Hollander et al., 1985), aspirin at a dose of 200 mg/kg (20 mg/mL, 4 h) (Goel et al., 1985), and 2 h cold restraint stress (Sairam et al., 2002) induced gastric lesions. Test groups of rats were pretreated with 100, 200, and 400 mg/kg of ASE, p.o., respectively, cimetidine 50 mg/kg orally, and reduced glutathione 150 mg/kg intraperitoneally, 1 h prior to subjecting them to acute gastric mucosal damage. After each experiment, the animals were killed by cervical dislocation. The stomach of each rat was excised and cut along the greater curvature, washed carefully with 5.0 mL of 0.9% NaCl, and inflated on cork. The ulcers were examined and the severity of the ulcers was determined as described by Sairam et al. (2001).

The curative ratio (%C) was determined as follows:

$$\%C = 100 - \left( \frac{\text{Ulcer index}_{\text{treated}} \times 100}{\text{Ulcer index}_{\text{control}}} \right)$$

#### Estimation of lipid peroxidation

The fundic part of the stomach was homogenized (5%) in ice-cold 0.9% NaCl with a Potter-Elvehjem glass homogenizer for 30 s. The homogenate was centrifuged at 800g for 10 min followed by centrifugation of the supernatant at 12,000g for 15 min to obtain the mitochondrial fraction (Das & Banerjee, 1993). A volume of the homogenate (0.20 mL) was transferred to a vial and was mixed with 0.2 mL of an 8.1% (w/v) sodium dodecyl sulfate solution, 1.50 mL of a 20% acetic acid solution (adjusted to pH 3.5 with NaOH), and 1.50 mL of a 0.8% (w/v) solution of thiobarbituric acid (TBA), and the final volume was adjusted to 4.0 mL with distilled water. Each vial was tightly capped and heated in a boiling water bath for 60 min. The vials were then cooled under running water. Equal volumes of tissue, blank, or test samples and 10% trichloroacetic acid were transferred into a centrifuge

tube and centrifuged at 1000g for 10 min. The absorbance of the supernatant fraction was measured at 532 nm (Beckman DU 650 spectrometer). A control experiment was processed using the same experimental procedure except the TBA solution was replaced with distilled water. 1,1,3,3-Tetraethoxypropane was used as standard for calibration of the curve, and results are expressed as nmol/mg protein (Jamall & Smith, 1985).

### Statistical analysis

All data are presented as mean  $\pm$  SEM and were analyzed by SPSS for Windows, version 9 (SPSS, Chicago, IL, USA) for possible significant interrelation between the various groups using the independent samples *t* test. A value of  $p < 0.05$  was considered statistically significant.

## Results

### Gastrointestinal propulsion

In the control group, the charcoal meal traveled 92.1% of the total length of the small intestine, while at the doses of 100, 200, and 400 mg/kg, p.o. of the ASE treated groups, the small intestinal transit was significantly inhibited in a dose-related manner. Percentage inhibitions were 19.17, 22.12, and 54.17% ( $p < 0.001$ ), respectively, but when 400 mg/kg of ASE was repeated in the presence of yohimbine (1 mg/kg, s.c.), an  $\alpha_2$ -adrenergic receptor antagonist, the intestinal propulsive inhibition decreased from 54.17 ( $p < 0.001$ ) to 22.28% ( $p < 0.001$ ). Morphine (10 mg/kg, s.c.), an opioid agonist, caused an intestinal inhibition of 64.7% ( $p < 0.001$ ), which is significantly greater than that produced by the highest dose of ASE (400 mg/kg, 54.17%) (Figure 1).

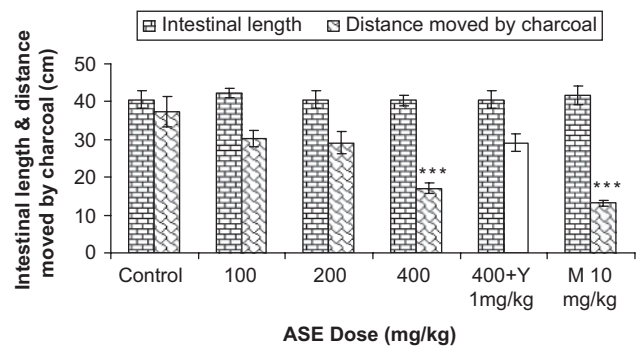
### Castor oil induced diarrhea

Four hours after castor oil administration, all mice in the control group produced copious diarrhea. Pretreatment of mice at the doses of 100, 200, and 400 mg/kg of ASE caused a dose-dependent and significant delay in the onset of diarrhea, decrease in the frequency of stooling (reduction in number of wet stools and total stools),

decrease in the weight of wet stools, and decrease in the general diarrhea score, including hard stool, mild, and copious diarrhea. The percentages related to controls for diarrhea onset were 16.58, 83.42 ( $p < 0.01$ ), and 116.18% ( $p < 0.001$ ) at doses of 100, 200, and 400 mg/kg of ASE, while with morphine (10 mg/kg) this value was 123.93% ( $p < 0.001$ ) compared to controls. The percentage purging frequency relative to controls (number of stools) was 41.09, 64.38 ( $p < 0.05$ ), 71.23 ( $p < 0.01$ ), and 86.30% ( $p < 0.01$ ) at three doses of ASE and with morphine, respectively. The standard antidiarrheal drug, morphine (10 mg/kg, s.c.), produced a significantly greater inhibitory effect on all the diarrhea parameters investigated compared with the highest dose of ASE (Table 1).

### Castor oil induced intestinal fluid accumulation

The intestinal fluid in control animals was  $1.91 \pm 0.06$  mL. Percentage inhibition in intestinal accumulation was 8.9 ( $p < 0.05$ ), 48.16 ( $p < 0.001$ ), and 68.06% ( $p < 0.001$ ) at the doses of 100, 200, and 400 mg/kg, p.o. of ASE, respectively, compared to the control group, while the inhibition with yohimbine (1 mg/kg, s.c.) was 50.78% ( $p < 0.001$ ), antagonizing the effect of 400 mg/kg of ASE. The standard drug, morphine (10 mg/kg, s.c.), also inhibited the intestinal fluid accumulation with a percentage inhibition of 78.53% ( $p < 0.001$ ). Pretreatment with the different doses



**Figure 1.** Effect of 50% ethanol extract of *Amaranthus spinosus* (ASE) on gastrointestinal propulsion. 100, 200, 400 mg/kg, Y, and M represent doses of ASE, yohimbine (1 mg/kg, s.c.), and morphine (10 mg/kg, s.c.). Values are expressed as mean  $\pm$  SEM,  $n = 6$  mice. \*\*\* $p < 0.001$  compared to control group.

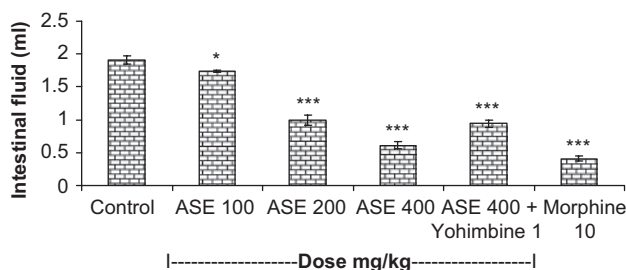
**Table 1.** Effect of 50% ethanol extract of *Amaranthus spinosus* (ASE) on castor oil induced diarrheal parameters.

Group	Dose (mg/kg)	Onset of diarrhea (min)	No. of wet stools	Total no. of stools	Wt. of wet stools (g)	Mean total diarrhea score
Control	1% CMC	100.7 $\pm$ 5.64	7.3 $\pm$ 1.51	9.3 $\pm$ 1.41	0.270 $\pm$ 0.04	13.0 $\pm$ 0.64
ASE	100	117.4 $\pm$ 7.73	4.3 $\pm$ 0.86	6.2 $\pm$ 0.77	0.196 $\pm$ 0.04	11.2 $\pm$ 2.06
ASE	200	184.7 $\pm$ 22.82**	2.6 $\pm$ 0.63*	4.5 $\pm$ 0.78*	0.121 $\pm$ 0.04*	08.6 $\pm$ 2.14
ASE	400	217.7 $\pm$ 13.81***	2.1 $\pm$ 0.44**	3.7 $\pm$ 0.56**	0.098 $\pm$ 0.03**	07.3 $\pm$ 0.92***
Morphine	10	225.6 $\pm$ 8.36***	1.0 $\pm$ 0.34**	2.6 $\pm$ 0.54**	0.710 $\pm$ 0.04***	05.7 $\pm$ 0.76***

100, 200, and 400 mg/kg represent doses of ASE; morphine, 10 mg/kg, s.c. Values are expressed as mean  $\pm$  SEM,  $n = 6$  mice.

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  compared to control group.





**Figure 2.** Effect of 50% ethanol extract of *Amaranthus spinosus* (ASE) on intestinal fluid accumulation. Values are expressed as mean  $\pm$  SEM,  $n=6$  rats. \* $p<0.05$ , \*\*\* $p<0.001$  compared to control group.

of ASE showed a dose-dependent inhibition in castor oil induced fluid accumulation (Figure 2).

### In vivo antidiarrheal index

The antidiarrheal index (ADI) is a measure of the combined effects of these different components of diarrhea such as purging frequency, onset of diarrheal stools, and intestinal fluid accumulation. Results for the *in vivo* antidiarrheal index were 23.55, 49.16, and 76.53 at the doses of 100, 200, and 400 mg/kg, p.o. of ASE, while morphine gave a maximum index of 88.45. ASE produced a dose-dependent antidiarrheal index, although its greatest effect was lower than that produced by morphine.

### Induction of acute gastric mucosal damage

ASE showed significant dose-dependent protection in ethanol- and aspirin-induced ulcer. The percentage protection values were 51.07, 55.91 ( $p<0.05$ ), 77.95 ( $p<0.01$ ), and 60.75% ( $p<0.05$ ) in ethanol-induced ulcer; however, in aspirin-induced ulcer the corresponding values were 41.33 ( $p<0.05$ ), 61.77 ( $p<0.01$ ), and 80.88% ( $p<0.001$ ) at doses of 100, 200, and 400 mg/kg, p.o. of ASE, while with cimetidine (50 mg/kg, p.o.) this was 74.66% ( $p<0.01$ ) (Table 2).

### Estimation of lipid peroxidation activity

Cold restraint stress significantly increased the ulcer index, with a concomitant increase in lipid peroxidation, compared with the control group. ASE, at dose levels of 100, 200, and 400 mg/kg, p.o., significantly reduced lipid peroxidation, with a concomitant decrease in the ulcer index, and the percentage protection values were 56.96 ( $p<0.01$ ), 63.29 ( $p<0.01$ ), and 81.01 ( $p<0.001$ ) with respect to the compounds, compared with cimetidine, 52.32%, and reduced glutathione, 77.21% ( $p<0.001$ ) (Table 3).

**Table 2.** Effect of 50% ethanol extract of *Amaranthus spinosus* (ASE) on ethanol- and aspirin-induced changes in gastric ulcers.

Group	Dose (mg/kg)	Ethanol-induced ulcer		Aspirin-induced ulcer	
		Ulcer index ratio	% Curative	Ulcer index ratio	% Curative
Control	1% CMC	18.6 $\pm$ 4.5	—	22.5 $\pm$ 3.4	—
ASE	100	09.1 $\pm$ 1.2	51.07	13.2 $\pm$ 2.2*	41.33
ASE	200	08.2 $\pm$ 1.2*	55.91	08.6 $\pm$ 2.7**	61.77
ASE	400	04.1 $\pm$ 1.7**	77.95	04.3 $\pm$ 1.3***	80.88
Cimetidine	50	07.3 $\pm$ 1.6*	60.75	05.7 $\pm$ 2.8**	74.66

100, 200, and 400 mg/kg represent doses of ASE; cimetidine, 50 mg/kg, p.o. Values are expressed as mean  $\pm$  SEM,  $n=6$  rats.

\* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$  compared to control group.

**Table 3.** Effect of 50% ethanol extract of *Amaranthus spinosus* (ASE) on ulcer index and lipid peroxidation against cold restraint stress-induced gastric ulcers.

Group	Dose (mg/kg)	Ulcer index ratio	% Curative	Lipid peroxidation
Control	1% CMC	00.0 $\pm$ 0.0	—	0.41 $\pm$ 0.01
Cold restraint stress	—	23.7 $\pm$ 3.1***	—	0.51 $\pm$ 0.02**
ASE	100	10.2 $\pm$ 1.8**	56.96	0.38 $\pm$ 0.02***
ASE	200	08.7 $\pm$ 1.9**	63.29	0.34 $\pm$ 0.02***
ASE	400	04.5 $\pm$ 1.7***	81.01	0.25 $\pm$ 0.01***
Cimetidine	50	11.3 $\pm$ 2.3**	52.32	0.42 $\pm$ 0.01**
Reduced glutathione	150	05.4 $\pm$ 1.4***	77.21	0.22 $\pm$ 0.01***

100, 200, and 400 mg/kg represent doses of ASE; cimetidine, 50 mg/kg, p.o.; reduced glutathione, 150 mg/kg, i.p. Values are expressed as mean  $\pm$  SEM,  $n=6$  rats.

\* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$ , cold restraint stress group compared to control group while treated group compared to cold restraint stress group.

## Discussion and conclusion

The 50% ethanol extract of the whole plant of *Amaranthus spinosus* (ASE) significantly inhibited small-gastrointestinal propulsive movement in mice. Inhibition by the highest dose (400 mg/kg) (54.17%) was, however, lower than that of the standard drug, morphine (10 mg/kg, s.c.) (64.70%). The data suggest that the effect on gastrointestinal propulsive movement is mediated via the  $\alpha_2$ -adrenergic receptor, since yohimbine (1 mg/kg, s.c.) significantly reduced (22.28%) the ASE induced transit delay in the animals. It is known that stimulation of the  $\alpha_2$ -adrenergic receptor causes transit delay (Hsu, 1982; Ruwart et al., 1980). The action of castor oil as a diarrhea inducer has been widely studied (Izzo, 1996), and it is known that the most active component is ricinoleic acid, which produces permeability changes in the intestinal mucosal membranes to water and electrolytes, resulting in watery luminal content that flows rapidly through the small and large intestines (Gaginella et al., 1975; Shobha & Thomas, 2001). ASE also showed a dose-related inhibition in all

diarrheal parameters: onset of diarrhea, total number of stools, number of wet stools, and measured frequency and severity of diarrhea. The percentage inhibition by the standard drug, morphine, was greater than that of the highest dose of ASE. Drugs affecting motility, frequency, and consistency of diarrhea also affect secretion (Amresh et al., 2004; Hsu, 1982). The intraluminal fluid accumulation induced by castor oil was blocked by ASE in a dose-related manner. ASE inhibited the castor oil induced intestinal fluid accumulation without affecting the weight of the intestinal content. The intestinal content was, however, more viscous in ASE-treated than in control rats. Involvement of the  $\alpha_2$ -adrenoceptor mechanism was further confirmed by the antagonistic action of yohimbine in the enteropooling test. Clinically, diarrhea may result from disturbed bowel function, in which case there is impaired intestinal absorption, excessive intestinal secretion of water and electrolytes, and rapid bowel transit (Gurgel et al., 2001). The antidiarrheal index (ADI) is a measure of the combined effects of these different components of diarrhea such as purging frequency and onset of diarrheal stools, as well as intestinal frequency. ASE produced a dose-dependent antidiarrheal index, although its greatest effect was lower than that produced by morphine. Activation of the sympathetic innervation of the intestines results in the inhibition of peristaltic activity and a reduction in tone. This inhibitory effect is mediated mainly by  $\alpha_2$ -adrenoceptors. Activation of the pre-junctional  $\alpha_2$ -adrenoceptors on the parasympathetic terminals may also play an important role in the inhibitory action of sympathetic nerve stimulation of gastrointestinal motility by inhibiting acetylcholine release (Berthelsen & Pettinger, 1977). The sympathetic nervous system also controls the balance between absorption and secretion in the ileum through activation of the mucosal  $\alpha_2$ -adrenoceptors. Stimulation of these receptors in the ileum results in a decrease in ion fluxes, consistent with the ability of  $\alpha_2$ -adrenoceptor agonists to inhibit intestinal fluid secretion. ASE in the gastrointestinal propulsive movement and enteropooling tests exhibited effects similar to those of  $\alpha_2$ -selective agonists, and the attenuation of these effects in the presence of yohimbine, an  $\alpha_2$ -adrenoceptor antagonist, suggests a role for  $\alpha_2$ -receptors in ASE antidiarrheal effects. The presence of phenolics, tannins, and flavonoids could contribute to the antidiarrheal activity of ASE. Tannins have been reported to be responsible for the use of many plants in the treatment of diarrhea (Tripathi, 1994; Uddin et al., 2004). All these results clearly demonstrate that ASE possesses antidiarrheal activity due to inhibitory effects on both gastrointestinal propulsion and fluid secretion.

Ulcers are thought to be due to an imbalance between offensive factors such as acid and pepsin and

defensive factors such as mucin secretion, cell proliferation, prostaglandins, etc. (Rao et al., 2001). Lipid peroxidation has been postulated to be one of the important factors in ulcerogenesis (Sairam et al., 2002). ASE showed dose-dependent antiulcerogenic activity in different models of acute gastric ulcer induced by ethanol, aspirin (Table 2), and cold restraint stress (Table 3). The incidence of ethanol-induced ulcers, which is predominant in the glandular part of the stomach, has been reported to stimulate the formation of leukotriene  $C_4$  resulting in the damage of rat gastric mucosa (Peskar et al., 1986). Similarly, synthetic nonsteroidal anti-inflammatory drugs (NSAIDs) cause mucosal damage by interfering with prostaglandin synthesis, and the incidence of ulceration is observed on the glandular part of the stomach (Ferreira & Vane, 1974). Evidence also establishes the fact that prostaglandins normally have a protective function in the stomach by maintaining gastric microcirculation and causing gastric secretion of bicarbonate and mucus (Vane, 1971; Flemstrom & Turnberg, 1984). The mucosal protection induced by nonprostanoid compounds may be mediated through the mobilization of endogenous prostaglandins (Konturek et al., 1987). Thus, it is possible that one of the mechanisms of the antiulcerogenic effect of ASE may involve a close relationship between these predisposing factors. Several factors are known to participate in the maintenance of the physiological milieu of the organism, and stress plays an important role in the etiopathology of gastric duodenal ulceration with an increase in gastric motility, vagal overactivity (Cho et al., 1976), mast cell degranulation (Cho & Ogle, 1979), decreased gastric mucosal blood flow (Hase & Moss, 1973), and decreased prostaglandin synthesis (Rao et al., 1999). Recently it has been established that the generation of reactive oxygen species and complex neurotransmitter interactions are involved (Puri et al., 1994). Experimental data indicate that cold restraint stress aggravated ulcer severity and lipid peroxidation significantly as compared to unstressed rats (Table 3). Malondialdehyde (MDA), a stable lipid hydroperoxide, provides an index of lipid peroxidation in biological tissues (Ohkawa et al., 1979). In support, cold restraint stress causes a marked production of MDA in the gastric mucosa with increased production of  $O_2^-$  within the tissue, and an elevated  $O_2^-$  level is thought to increase the concentration of cellular radicals. These radicals appear to function in conjointly to induce cell degeneration via peroxidation of membrane lipids, breaking of DNA strands, and denaturing of cellular proteins (Fridovich, 1986). This effect was significantly reversed by prior administration of ASE, providing a close relationship between free radical scavenging and antiulcer activity. Evidence has been provided that reduced glutathione exerts its antioxidant defense mechanism

by metabolizing lipid peroxides and scavenging endogenous  $H_2O_2$  (Das & Banerjee, 1993). In conclusion, this study supports the antidiarrheal and antiulcer activity of *Amaranthus spinosus* in the pharmacological models used, and, thus, its use in traditional medicine is confirmed by its free radical scavenging activity.

**Declaration of interest:** The authors alone are responsible for the content of this paper.

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