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Research Article

EVALUATION OF ANTI-DIARRHOEAL ACTIVITY OF *AMARANTHUS TRICOLOR LINN* IN EXPERIMENTAL ANIMALS

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ABSTRACT

Objective: To evaluate the anti-diarrhoeal activity of different extracts of the leaves of Amaranthus tricolor Linn. using different animal models.

Methods: The shade dried leaves of Amaranthus tricolor.L was extracted with ethanol (95%) and then partitioned by petroleum ether, chloroform and ethyl acetate. The anti-diarrhoeal activity of various extracts of Amaranthus tricolor was evaluated using castor oil induced diarrheal model in rats, castor oil-induced enter pooling in rats and gastrointestinal motility test in rats.

Results: The results revealed that the ethanolic extract and ethyl acetate fractions significantly inhibited diarrhea and reduced the distance travelled by the activated charcoal in intestine in rats.

Conclusion: Our results showed that Amaranthus tricolor displayed potent anti-diarrhoeal properties, supporting the ethno-medical use given to this plant for treatment of diseases.

Key words: Amaranthus tricolor Linn, anti-diarrhoeal activity, castor oil, charcoal.

INTRODUCTION

Diarrheal is a global problem associated with gastrointestinal disturbances causing deaths worldwide and continues to be a challenge despite advancement in science. Acute infectious diarrhea contributes to significant morbidity and mortality worldwide with close to 70% of diarrhea being food borne disease. Herbal plants have been the basis of many traditional medicine systems throughout the world for thousands of years and continue to provide mankind with new remedies. About three quarter of the world's population relies on plants and plant extracts for their healthcare [1]. Plants having astringent properties, anti inflammatory property and Bulk forming agents are commonly used to treat diarrhea. Plants phenolics like phenolic acid, tannins and flavonoids are known to be potent anti-diarrheal and occur in vegetables, fruits, nuts, seeds, roots, barks and leaves. In addition to their anti-diarrheal properties, these compounds display a vast variety of pharmacological activities [2-4].

Amaranthus tricolor (Amaranthaceae) commonly known as ''Joseph's coat" or "Red amaranth" is cultivated mainly for its edible leaves throughout South-East Asia and many tropical countries. The plant (Amaranthus tricolor), especially the leaves has reported to have wide range of pharmacological activities, like anti-tumor effect [5], anti-ulcer activity [6], hepatoprotective activity [7] and inhibitory effect on cobra venom [8]. Betacyanins, the coloring pigments present in Amaranthus tricolor has reported to have antioxidant activity [9-10].The leaves of Amaranthus tricolor has been reported to have diuretic, anti-inflammatory activity and also for treating bladder distress. Phytochemical studies on Amaranthus tricolor resulted in the isolation of the antioxidant betacyanins and heteropolysacchrides [11]. Amaranthus tricolor is used in many folk claims as one of the traditional medicines and the plant has been extensively used in ayurveda and sidda for treating menorrhagia, haemorrhagic colitis, bowel hemorrhages, cough and bronchitis. Furthermore, the whole plant of A. tricolor L has been extensively used in ayurveda and sidda for treating diarrhea and dysentery. However, no study has been conducted to scientifically prove that leaves of Amaranthus tricolor possess anti-diarrhoeal activity. Hence, the present study was undertaken to evaluate the anti-diarrhoeal effect of the leaves of Amaranthus tricolor.

MATERIALS AND METHODS

Collection and Identification of the Plant material

Fresh leaves of Amaranthus tricolor were collected from Shantipura area of Anekal, India in the month of June. The taxonomical identification of the plant was done by Prof. Balakrishna gowda, GKVK, Bangalore.

Chemicals

Atropine sulfate, Loperamide, Sodium carboxy methylcellulose was purchased from Sigma Chemicals Co. (St. Louis, MO, USA), while castor oil was obtained from local vendor. All the other chemicals and reagents used for extraction process / phytochemical analysis were of analytical grade and procured from local firms.

Extraction and preliminary phytochemical investigation

The collected leaves of the plant were shade dried and reduced to coarse powder in a mechanical grinder and passed through sieve No. 40. The powdered material obtained was then subjected individually to extraction by cold maceration using rectified spirit (90%) for a total of seven days. The extracts were filtered and concentrated in rotary evaporator under reduced pressure to yield a thick green ethanolic extract. The crude extract thus obtained was partition-fractionated with 1:1 of petroleum ether and ethanol (50%), the mixture was shaken vigorously and kept for about 30 minutes to let the two layers separate. The upper layer consisted of petroleum ether, it was removed and concentrated in a rotary evaporator to obtain petroleum ether fraction (PEAT). The same procedure was repeated with the residue using equivalent volume of chloroform and ethyl acetate to obtain chloroform fraction (CAT) and ethyl acetate fraction (EAAT) respectively. The extracts thus obtained were subjected to phytochemical analysis [12].

Experimental animals

Albino Wistar rats of either sex weighing between 200 and 230 g were used. Institutional Animal Ethics Committee approved the experimental protocol; animals were maintained under standard conditions in an

animal house approved by Committee for the Purpose of Control, and Supervision on Experiments on Animals.

Acute toxicity studies

Acute oral toxicity was determined by using female, nulliparous and non pregnant mice weighing 18-22 g. The animals were fasted for 3 hrs prior to the experiment. Up and down procedure OECD guideline no. 425 was adopted for toxicity studies [13] http://www.epa.gov/ oppfead1/harmonization/). Animals were administered with single dose of extract and observed for their mortality during 48 hours study period (short term) toxicity.

Anti-diarrheal activity

Castor oil-induced diarrhea [14]

Wistar rats were fasted for 18hours and divided into six groups of five animals each. Various treatments were given, Group I (control) animals were treated with 0.5 % sodium carboxymethyl cellulose, Group II (standard) animals were treated with loperamide (3 mg/kg, p.o.), a positive control. Group III-VI was treated with different leaf extracts of Amaranthus tricolor (200 mg/kg, p.o.). Animals were placed separately in individual cages lined with filter paper. One hour after pre-treatment with the different extracts, the animals were challenged with 1 ml of castor oil orally. Thereafter, they were observed for 4h for the presence of diarrhea defined as watery (wet), unformed stool, the filter papers were changed every hour.

Gastrointestinal Motility Test [15]

Wistar rats were fasted for 18 h and divided into six groups of five animals each, group I animals served as control and were treated orally with 0.5 % w/v sodium carboxymethyl cellulose in distilled water. Group II animals served as standard and treated with atropine (5 mg/kg, i.p.) a positive control. Animals of Group III-VI were treated with different leaf extracts of Amaranthus tricolor (200 mg/kg, p.o.). After 1 h, each animal was administered orally with charcoal meal 0.25 ml (10% charcoal in 0.5 % w/v Sodium carboxymethy cellulose). Thirty minutes later, the animals were sacrificed. Total small intestine from pylorus to caceum was isolated and the total length and the length traveled by the charcoal meal were measured. This distance was expressed as a percentage of the length of the small intestine.

% Inhibition = (Mc-Md/ Mc) × 100

Where Mc: mean number of defecation travelled by charcoal meal;

Md: mean number of defecation travelled by drug or extract.

Castor Oil Induced Enteropooling [16-17]:

Wistar rats were fasted for 18 h and divided into six groups of five animals each, group I animals served as control and were treated orally with 0.5 % w/v sodium carboxymethyl cellulose in distilled water. Group II animals served as standard and received loperamide (3 mg/kg, p.o). Animals of Group III-VI were treated with different leaf extracts of Amaranthus tricolor (200 mg/kg, p.o.) one hour before the oral administration of castor oil (2 ml). One hour later, the rats were sacrificed and the small intestine was removed after tying the ends with threads and weighed. The intestinal content was collected into a graduated cylinder and their volume was measured. The intestine was reweighed and the difference between the full and empty was calculated.

Statistical analysis

Data were presented as mean ± standard error mean (SEM) of three determinations. Statistical analyses were performed using a one-way analysis of variance, P<0.05 were considered statistically significant. Results were calculated by employing the statistical software (SPSS 13.0, SPSS Inc., USA).

RESULTS

Preliminary Phytochemical Investigation

Preliminary phytochemical screening of the leaf extracts revealed the presence of steroids, triterpinoids and saponins in PEAT and CAT. While EAT and EAAT showed the presence of alkaloids, carbohydrates, proteins, saponins, flavonoids, tannins and glycosides.

Acute oral toxicity

In acute oral toxicity study, no mortality was observed after treatment with the highest tested dose (2000 mg/kg p.o) of all the extracts of leaves.

Castor oil-induced diarrhea

As shown in Table-1 the EAT and EAAT leaf extracts significantly decreased the total number of wet feces produced upon administration of castor oil and this result is similar to the effect of the standard anti-diarrheal drug, loperamide (3mg/kg). While the PEAT and CAT didn't show any significant anti-diarrheal effect in the rats.

Effect on intestinal transit time

As shown in Table-2 the EAT and EAAT leaf extracts significantly slowed down the propulsion of charcoal meal through the gastrointestinal tract when compared to the control group. Atropine part produced a marked decrease in the propulsive movement and the intestinal length travelled by charcoal meal. While the PEAT and CAT didn't show any significant effect on the intestinal transit time.

Table 1: Effect of ethanolic extract, petroleum ether, chloroform, and ethyl acetate fractions of *Amaranthus tricolor* leaves on castor oil induced diarrhea.

Group	Number of wet feaces in 4h	% inhibition
Vehicle Control	8.00 ±1.12	-
Loperamide (3mg/kg, p.o)	1.0 ± 0.18***	87.5
EAT (200mg/kg, p.o)	1.1 ± 1.48***	86.3
PEAT (200mg/kg, p.o)	5.6 ± 0.24	30.0
CAT (200mg/kg, p.o)	6.01 ± 0.91	25.1
EAAT (200mg/kg, p.o)	0.8 ± 0.29***	90.0

AT: *A.tricolor*. L, EAT: Ethanolic extract of AT, PEAT: Petroleum ether extract of AT, CAT: Chloroform extract of AT, EAAT: Ethyl acetate extract of AT.

All values are mean ± SEM, n=5. *p < 0.05, **p < 0.01 when compared to control group.

Table 2: Effect of ethanolic extract, petroleum ether, chloroform, and ethyl acetate fractions of *Amaranthus tricolor* leaves on intestinal transit

Group	Total length of intestine	Mean distance travelled by charcoal	% intestinal Transit
Vehicle Control	90.1 ±1.81	80.0 ±1.01	88.8
Atropine (5mg/kg, i.p)	92.1 ±1.98	35.0 ± 0.14**	38.0
EAT (200mg/kg, p.o)	93.4 ±2.83	40.1 ± 0.71**	42.9
PEAT (200mg/kg, p.o)	93.6 ±3.12	76.1 ± 0.42	81.3
CAT (200mg/kg, p.o)	89.1 ±4.81	63.1 ± 0.31	70.8
EAAT (200mg/kg, p.o)	92.6 ±5.31	42.1 ± 0.19**	45.5

All values are mean ± SEM, n=5. *p < 0.05, **p < 0.01 when compared to control group.

Table 3: Effect of ethanolic extract, petroleum ether, chloroform, and ethyl acetate fractions of *Amaranthus tricolor* leaves on castor oil induced enteropooling.

Group	Volume of intestinal content (mL)	Weight of intestinal content (g)	% inhibition
Vehicle Control	2.15 ±0.38	2.91 ±2.09	-
Loperamide (3mg/kg, p.o)	0.68 ±0.91**	1.01 ±0.89**	65.2
EAT (200mg/kg, p.o)	0.82 ± 0.78**	0.98 ± 3.10**	66.3
PEAT (200mg/kg, p.o)	1.85 ±1.21	2.35 ± 3.64	19.2
CAT (200mg/kg, p.o)	1.62 ±0.97	2.88 ±1.08	1.03
EAAT (200mg/kg, p.o)	0.95 ±1.27**	1.39 ±2.17**	52.2

All values are mean ± SEM, n=5. *p < 0.05, **p < 0.01 when compared to control group

Effect on castor oil-induced enteropooling

As shown in Table-3 the EAT and EAAT leaf extracts leaf extracts was found to possess anti-enteropooling activity .The extracts significantly decreased intestinal fluid volume in rats. The effect of the extract was comparable to that of the standard drug (Loperamide). While the PEAT and CAT didn't show any significant effect on castor oil-induced enteropooling.

DISCUSSION

Diarrhea is usually considered a result of altered motility and fluid accumulation within the intestinal tract. Castor oil causes diarrhea due to its active metabolite, recinolic acid [18] which increases peristaltic activity in the small intestine leading to changes in the electrolyte permeability of the intestinal mucosal membrane. The precise mechanism of action of castor oil is through elevated prostaglandin biosynthesis [19-20]. Prostaglandin contributes to the pathophysiological functions in gastrointestinal tract [21]. Inhibitors of prostaglandin biosynthesis delay castor oil induced diarrhea [22]. Many anti-diarrheal agents act by reducing the gastrointestinal motility and / or the secretions. Phytochemical screening of the extracts revealed the presence of tannins, saponins, steroids, terpenes, alkaloids and flavonoids which have all been reported to have anti-diarrheal activity [23-24]. In addition, its anti-diarrheal action may also be due to the presence of denatured proteins, which form protein tannates. It has been previously demonstrated that protein tannates make the intestinal mucosa more resistant and hence, reduce secretion and peristaltic movement [25-26]. Flavonoids are reported to inhibit contractions induced by spasmogenics [27-28]. The anti-diarrheal activity of the EAT and EAAT was comparable to that of standard drug (Loperamide), which is the most efficacious and widely employed anti-diarrheal drug. Loperamide antagonizes the diarrheal activity induced by castor oil [29]. Loperamide, apart from regulating the gastrointestinal tract, is also reported to slow down transit in the intestine, reduce colon flow rates and consequently any effect on colonic motility [30-31]. The anti-muscarinic drug, atropine decreased the propulsive movement in the charcoal meal study. This is possible due to its anti-cholinergic effect [32]. The significant inhibition of the castor oil-induced enteropooling in rats suggests that ethanolic and ethylacetate leaf extracts of Amaranthus tricolor produces relief in diarrhea by spasmolytic activity in-vivo and also anti-enteropooling effects. In conclusion, the present study revealed that Amaranthus tricolor contains pharmacologically active substances effective for management of diarrhea. Further studies are required to fully investigate the mechanisms responsible for this observed anti-diarrheal activity.

CONCLUSION

The data presented here indicate that the marked anti- diarrheal activity of leaf extracts of Amaranthus tricolor seems to be due to presence of flavonoids like flavones, flavanes and flavonols which act in similar fashion as redutones by donating the electrons and reacting with free radicals to convert them into more stable product and terminate free radical chain reaction. In addition, these results form a good basis for selection of the plant for further pharmacological investigation. The present study supports the folkloric usage of this plant.

Conflict of interest statement

We declare that we have no conflict of interest.

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