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Full Length Research Paper

Antisecretory and Antimotility Effects of *Azima tetracantha* Leaf Extract in Experimental Diarrhoea

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The aqueous crude extract of the leaves of *Azima tetracantha* was studied for its phytochemical constituents and antidiarrhoeal activity using castor oil-induced diarrhoea and castor oil-induced enteropooling in rats. The phytochemical studies of the aqueous extract revealed the presence of alkaloids, flavonoids, tannins and saponins. The extract showed significant ($p < 0.001$) protection against castor oil-induced diarrhoea and castor oil-induced enteropooling at (100 mg/kg). The presence of some of the phytochemicals in the root extract may be responsible for the observed effects, and also the basis for its use in traditional medicine as antidiarrhoeal drug.

Key words: *Azima tetracantha,* enteropooling, anti-diarrhoeal.

INTRODUCTION

Diarrhoea is characterized by increased frequency of bowel movement, wet stool and abdominal pain (Ezekwesili et al., 2004). It is a leading cause of malnutrition and death among children in the developing countries of the world today (Victoria et al., 2000). Many governments and international organizations are trying to control this disease but the rate of incidence is still high, about 7.1 million per year (Park, 2000). Many synthetic chemicals like diphenoxylate, loperamide and antibiotics are available for the treatment of diarrhoea but they have some side effects. The natural drugs are used as antidiarrhoeal drugs, which are not always free from adverse effects (Hardman and Limberd, 1992). Therefore, the search for safe and more effective agents has continued to be an important area of active research. Since ancient times, diarrhoea has been treated orally with several medicinal plants or their extracts based on folklore medicine.

Azima tetracantha (Salvadoraceae) is known as 'Mulsangu' in Tamil and 'Kundali' in Sanskrit, respectively. Its root, root bark and leaves are used with food as a remedy for rheumatism (Kirtikar and Basu, 1984). It is a powerful diuretic given in rheumatism, dropsy, dyspepsia and chronic diarrhoea and as a stimulant tonic after confinement (Nadkarni, 1976). *A. tetracantha* as efficient acute phase anti-inflammatory drug is traditionally used by Indian medical practitioners (Ismail et al., 1997). *A. tetracantha* is used to treat cough, phthisis, asthma, smallpox and diarrhoea. The decoction of the stem bark is considered astringent, expectorant and used for fevers (Reddy et al., 1991). The present study was undertaken to evaluate the antidiarrhoeal potential of aqueous extract of leaves of *A. tetracantha* in normal and castor oil induced diarrhoeal rats.

MATERIALS AND METHODS

Plant

The fresh leaves of *A. tetracantha* (Salvadoraceae) were collected from the thanjavur. The collected leaves were identified and authenticated by a Botanist, Prof. Dr. M. Jegadesan, Department of Herbal and Environmental science, Tamil University, Thanjavur, Tamil Nadu. A Voucher B specimen (Specimen no: 26) was been

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Table 1. Preliminary phytochemical analysis of *Azima tetracantha* leaves crude extract.

Phytoconstituent	Tannin	Alkaloid	Flavonoid	Saponin	Glycoside
Aqueous extract	+	+	+	+	-

deposited at the Herbarium of the department. 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 leaves were air-dried and ground into powder. A total of 200 g of the powdered leaves were macerated in 500 ml of distilled water for 8 h and filtered through cheese cloth, glasswool and Whatman No. 1 filter paper. The filtrate was evaporated to dryness by heating in a water bath at a temperature of 100°C. The dry aqueous extract was stored in the refridgerator and used as the crude extract.

Animal

Healthy male albino rats (*Rattus norvegicus*) of weight 150 to 200 g, were obtained from the Animal House of the Tamil University. All the animals were housed in a clean and well-ventilated house conditions [temperature 23 ± 1°C; photoperiod (the interval in a 24- h period during which an organism is exposed to light)]: 12 h natural light and 12 h dark: humidity: 45 to 50%]. They were also allowed free access to Balanced Trusty Chunks (Sai Durga Foods Ltd, Banglore) and tap water. The cleaning of the cages was done daily.

Castor oil induced diarrhea

Animals were divided into three groups (n = 6). The first group of animals, which served as control was administered with castor oil (1 ml/rats). The second group castor oil (1 ml/rats) + crude powder (100 mg/100 g body weight). Third group received standard drug, loperamide (3 mg/kg) orally as suspension.

Phytochemical screening

The aqueous crude leaves extract of the plant was subjected to qualitative chemical screening for the identification of the tannins, alkaloids, flavonoids, saponin and glycosides using standard procedures (Trease and Evans, 1996).

Castor oil-induced diarrhoea in rats

A total of 18 albino rats (*R. norvegicus*) were divided into three groups of six animals in each. All rats were fasted for 18 h and received castor oil at a dose of 1 ml/animal orally (p.o.) using orogastric cannula for induction of diarrhea (Doherty, 1981). Thirty minutes after castor oil administration, rats of group I (control) received 1.0 ml/100 g of 0.9% NaCl in distilled water (normal saline) and rats of groups II, received 100 mg/kg of *A. tetracantha* leaves crude powder extract p.o. and group III received standard drug, loperamide (3 mg/kg p.o.), respectively. The animals were placed separately in metabolic cages over white clean Whatman filter paper which was changed every hour. The severity of diarrhoea was assessed each hour for 4 h. The total number of diarrhoea feces of the control group was considered 100%.

% Inhibition = (Control - Test) × 100/Control

Castor oil-induced enteropooling

Castor oil-induced enteropooling was determined by the method of Robert et al. (1976). The adult rats (*R. norvegicus*) selected without sex discrimination were fasted for 18 h and divided into three groups of six animals each. Castor oil (1 ml) was administered orally to these animals. One hour later, Group I received 1 ml/100 g of normal saline solution and rats of groups II received 100 mg/kg
A. tetracantha leaves crude powder p.o and group III received standard drug, loperamide (3 mg/kg orally), respectively. After 2 h of treatment, the rats were sacrificed by ether anesthesia. The edges of the intestine from pylorus to ceacum were tied with thread and the intestine was removed and weighed. Intestinal content was collected by measuring cylinder, and volume measured.

Statistical analysis

Results are presented as means ± standard deviation (SD) and simple percentages. The student 't' test' was used to determine the significant difference between two groups ($p < 0.001$).

RESULTS

Phytochemical analysis

The phytochemical results confirm the presence of alkaloids, flavonoids, tannins and saponins in extracts but in variable quantities (Table 1). These are the phyto-chemicals which are essential in many medicinal plants responsible for the antidiarrhoeal (Patricia et al., 2005). The reported medicinal property of the plant might be due to the presence of these bioactive components in *A. tetracantha*. Phytochemical such as glycosides, were found to be absent in the extract.

In the castor oil-induced diarrhoea experiment, aqueous extract of *A. tetracantha* produced a markedly antidiarrhoeal effect in the rats, as shown in Table 2 (Figure 1). At dose of 100 mg/kg, the extract significantly decreased ($p < 0.01$) the total number of watery stool produced upon administratin of castor oil (4.84±0.31 at 100 mg/kg) compare to the control group (22.00 ± 0.90). The effect of the dose of the extract was similar to that of the standard drug, loperamide (3 mg/kg). *A. tetracantha* leaves crude extract significantly ($p < 0.001$) inhibited castor oil-induced enteropooling in rats at oral dose 100 mg/kg (Table 3 and Figure 2). The intestinal fluid in control animals was 3.29 ± 0.06 ml .The inhibition of intestional accumulation was 78% ($p < 0.001$) at dose 100 mg/kg of the drug. The standard drug, loperamide (3 mg/kg) also significantly inhibited ($p < 0.001$) intestinal

Table 2. Effect of *Azima tetracantha* leaves crude extract on castor oil induced diarrhea.

Subject	Dose (mg/kg)	No. of watery stool diarrhea	% Inhibition
Control (castor oil)	1 ml	22.00±0.90	--
Azima tetracantha leaves crude extract	100	4.84±0.31***	78
Loperamide	3	2.00±0.26***	90

Values are expressed as mean ± SD. n = 6, *** P< 0.001 compared with control.

Table 3. Effect of extract of *Azima tetracantha Leaves* crude extract on castor oil-induced enteropooling.

Subject	Dose (mg/kg)	Fluid volume (ml)	% Inhibition
Control (castor oil)	1 ml	3.29±0.06	--
Azima tetracantha leaves crude extract	100	0.74±0.02***	78
Loperamide	3	0.43±0.01***	87

Values are expressed as mean ± S.D. n = 6, *** P< 0.001 compared with Control

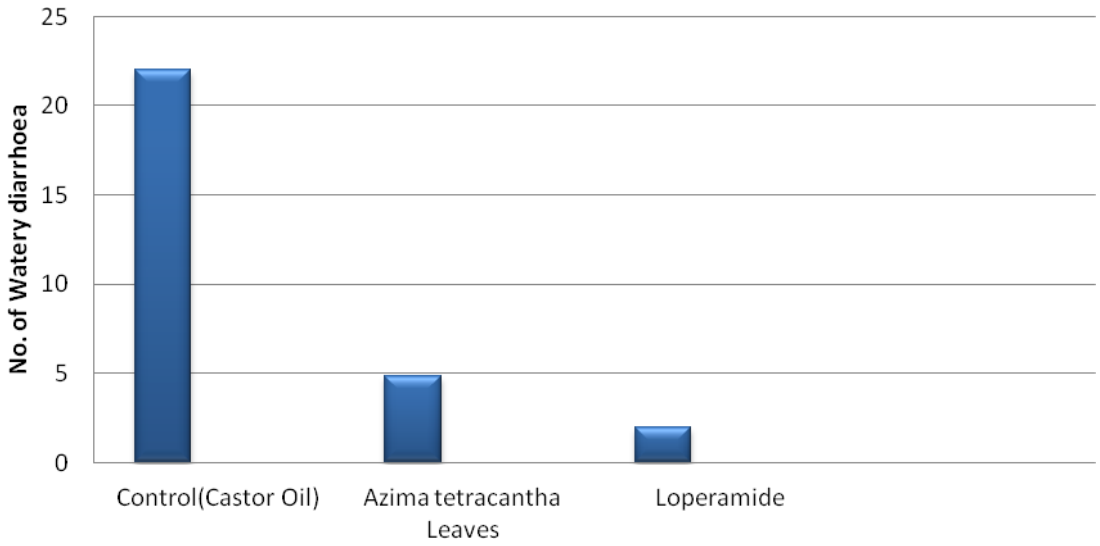

Figure 1. A graph of the effect of *Azima tetracantha* leaves crude extract on castor oil induced diarrhea.

fluid accumulation (87%).

DISCUSSION

Diarrhoea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract accompanied by rush resulting in an excess loss of fluid in the faeces. In some diarrhea, the secretory component predominates while other diarrhoea is characterized by hypermotility (Chitme et al., 2004). Castor oil causes diarrhoea due to its active metabolite, ricinoleic acid (Ammon et al., 1974; Watson and Gordon, 1962) 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 (Galvez et al., 1993). Castor oil reported to induce diarrhoea by increasing the volume of intestinal contents by preventing the re-absorption of water. The liberation of ricinoleic acid results in irritation and inflammation of intestinal mucosa leading to release of prostaglandin (Pierce et al., 1971).

The results of this study revealed that the aqueous leaves extract of *A. tetracantha* produced statistically significant protection against diarrhoea and was found to be comparable to loperamide; a drug widely employed against diarrhea disorders which effectively antagonizes

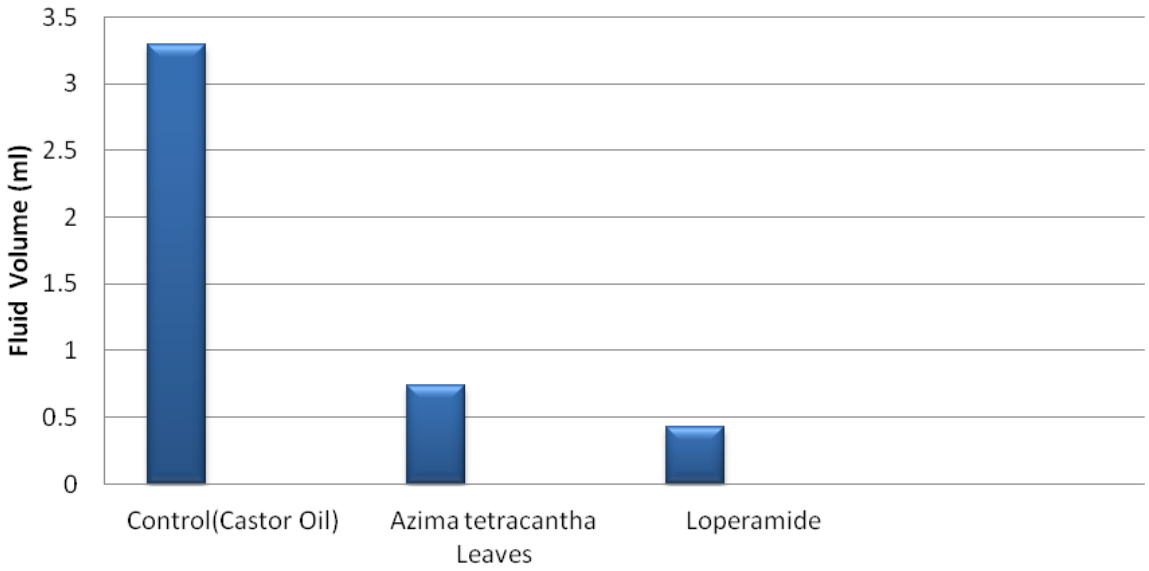

Figure 2. A graph of effect of *Azima tetracantha* Leaves crude extract on castor oil-induced enteropooling.

diarrhoea induced by castor oil, prostaglandin and cholera toxin (Niemegeers et al., 1974; Karim and Adeikam, 1977; Facack et al., 1981). The pharmacological effect of loperamide is due to its antimotility and antisecretory properties (Couper, 1987).

The antidiarrhoeal activities of medicinal plants have been attributed to the presence of bioactive agents such as tannins, alkaloids, saponins, flavonoids, steroids and terpenoids (Havagiray et al., 2004). While the flavonoids are known to inhibit intestinal motility and hydroelectrolytic secretion (Venkatesan et al., 2005), tannins denature proteins in the intestinal mucosa by forming protein tannates which make intestinal mucosa more resistant to chemical alteration and reduce secretion (Havagiray et al., 2004). Therefore, the anti- diarrhoeal activity of *A. tetracantha* leaves crude extract observed in this study may be attributed to the presence of tannins, flavonoids, alkaloids and saponins in the crude extract.

Conclusion

The antidiarrhoeal activity of *A. tetracantha* leaves crude extract observed in this study may be attributed to the presence of tannins, flavonoids, alkaloids and saponins in the crude extract. The prolonged onset of diarrhoea, inhibition of castor oil-induced enteropooling and the suppressed propulsive movement observed in this study are indications of antidiarrhoeal potential of *A. tetracantha* leaf crude extract. Further studies are how- ever needed to establish the safety of the extract and to possibly isolate the active principle responsible for the observed effects.

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