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Full Length Research Paper

Ethanolic leaf extract of *Verbena hastata* produces antidiarrhoeal and gastrointestinal motility slowing effects in albino rats

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The antidiarrhoeal effects of ethanol leaf extract of *Verbena hastata* were evaluated in rat .Studies were investigated on castor oil- induced diarrhoea and gastrointestinal motility activity in rat. The extract (200 and 400 mg/kg) elicited a greater anti-motility than 5 mg/kg atropine ($P < 0.05$) and significantly ($P < 0.05$) protected rats against castor oil- induced diarrhoea. The frequency of defaecation as well as the wetness of faecal droppings was significantly ($P < 0.05$) reduced. The oral LD_{50} of the extract was found to be greater than 5000mg/kg in mice. The result obtained shows that the ethanol leaf extract of *V. hastata* may contain some biologically active principles that are active against diarrhoea and this may be the basis for management of gastrointestinal disorders.

Key words: *Verbena hastate*, anti-diarrhoeal, gastrointestinal tract, castor oil.

INTRODUCTION

Diarrhoea is an important health problem especially in developing countries and it is the cause of 5 - 8 million deaths through out the world annually. To combat the problems of diarrhoea, the World Health Organization (WHO) initiated a diarrhoea disease control program to study traditional medicine practices and other related aspects, together with the evaluation of health education and prevention approaches (Syder and Merson, 1982; Mukherjee, 1995). The use of herbal drugs in the treatment of diarrhoea disease is a common practice in many countries of Africa. These plants, which abound in the environment, enjoy wide acceptability by the population and serve as cheaper alternatives to Orthodox medicine (Sofowora, 1993; Akah and Nwabie, 1994).

Verbena hastata (Vervain), family Verbenaceae, have broadly ovate leaves, about 2 m high. The plant is used traditionally in the treatment of various medical conditions. The leaves and roots are used as tonic, expectorant, emetic, diaphoretic and vermifuge; its leaves are used in the treatment of depression, tension and pain. The plant is used in parts of southern Nigeria for the treatment of fever, dysentery and diarrhoea.

However, the plant has not been experimentally tested for its antidiarrhoeal activity. Hence, an effort was made to investigate the plant extract in experimentally- induced diarrhoea in rats adopting modifications of the reported method of Awouters et al. (1978).

MATERIALS AND METHODS

Plant materials and extraction

Fresh leaves of *V. hastata* were collected by Mr Joseph L. Akpan from Nnug Ita, Akwa Ibom State, Nigeria. The plant was identified and authenticated by Prof. J. C. Okafor of Coal City University, Enugu, Nigeria. The leaves were cleaned, air-dried at room temperature for 7 days and crushed into coarse power using pestle and mortar. 220 g of the powdered materials was macerated

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with ethanol for 24 h with constant shaking. The liquid extract obtained was concentrated to dryness in vacuum at 40°C. The yield was (8.2% w/w). This ethanolic extract was used in all the studies with doses expressed in grams per kilogram body weight of the animal.

Experimental animals

Albino rats of either sex weighing between 200 - 250 g were used for the experiments. The animals were maintained at the Animal facility center, National Institute for Pharmaceutical Research and Development Idu, Abuja, Nigeria. They were fed with standard diet and had free access to water *ad libitum*. The animals were maintained under standard conditions of humidity, temperature and 12 h light/ 12 h darkness cycle. A standard protocol was drawn up in accordance with the good laboratory practice (G.L.P.) principles of the OECD as adopted by the WHO (WHO Document, 1998). The "Principles of laboratory animals care" (NIH publication No 85-23, 1985) were also followed in this study.

Acute toxicity test

The acute toxicity LD_{50} was estimated *p.o* in Swiss albino mice (20 - 25 g) following Lorke's method (1983). Dose levels used ranged from 100 - 5000 mg/kg of the ethanolic extract. The acute toxicity LD_{50} was calculated as geometric mean of the dose that resulted in 100% lethality and that which caused no lethality at all. Toxicity signs such as death, changes in physical appearance, behaviourial changes were observed for 72 h after administration of ethanol extract of the plant material.

Castor oil induced diarrhoea

The method described by Awouters et al. (1978) was followed. Healthy albino rats of either sex (200 - 250 g) were randomly selected and divided into five groups of five animals each. They were fasted for 18 h prior to the test, with free access to water. Group 1 received 20 ml/kg of normal saline (negative control), while those in group 2 receive loperamide (3 mg/kg) as positive control. Animals in group 3, 4 and 5 received *Verbena hastata* extract (100 - 400 mg/kg) respectively. Extract administration was by the oral route. The animals were housed singly in cages lined with transparent paper. One hour after pre-treatment with the extract, the animals were then administered with 1ml of castor oil orally. Thereafter, they were observed for 4 h for the presence of diarrhoea. Diarrhoea for the purpose of this study was taken to mean watery (wet), unformed stool

Gastrointestinal motility test (Charcoal meal)

Albino rats of either sex (200 - 250 g) were randomly divided into 5 groups of 5 rats each. They were fasted for 24 h prior to the test, but were allowed free access to water. Group 1 rats were treated with 20 ml/kg normal saline and served as control, while groups 2, 3 and 4 received different doses of the extract (100,200 and 400 mg/kg, *p.o)*. Group 5 rats received atropine sulphate (5 mg/kg, *p.o).* Thirty minutes after drug administration, 1 ml of charcoal meal (5% deactivated charcoal in 10% aqueous tragacanth) was administered orally to all animals in the study and 30 min later, all the rats were sacrificed and the abdomen opened. The small intestine was dissected out from the pylorus to the caecum and the total distance traveled by the charcoal plug along the small intestine was estimated for both the control and the treated groups. The percentage distance traveled by the charcoal meal from the pylorus to the caecum was noted.

Castor oil-induced enteropooling

In this method, rats were fasted for 18 h prior to the experiment. The rats were divided into five groups of five each. Normal saline (10 ml/kg *p.o)* was given to the first group. The second group received 1 ml of castor oil, while the last three groups received graded doses of *Verbena hastata* extract (100 - 400 mg/kg *p.o).*Thirty minutes later, all the rats were treated with 1 ml of castor oil. After thirty minutes, each rat was sacrificed and the whole length of the intestine from the pylorus to the caecum was dissected and the contents measured. Percentage reduction of the intestinal secretion (volume) was calculated

Phytochemical test

The ethanol leaf extract of *V .hastata* was subjected to quantitative phytochemical investigation according to standard methods (Trease and Evans, 1989).

Statistical analysis

Results were expressed as mean ± S.E.M. The significance of difference between mean was determined using one way analysis of variance

RESULTS

Phytochemical screening

Phytochemical analysis of the ethanolic extract gave a positive reaction for each of the following secondary metabolites: Saponins, Terpenes, Sterols, flavonoids and Carbohydrates.

Acute toxicity studies

There was no mortality observed in the mice upon oral administration, even at doses as high as 5000 mg/kg signifying that the LD_{50} was greater than 5000 mg/kg. Apart from sedation and mild weakness, *V. hastata* did not produce any major clinical signs of toxicity in mice during 4 day observation per

Effect of extract on castor oil induced diarrhoea

The ethanol leaf extract of *V. hastata* exhibited marked dose dependent anti diarrhoea activity in the study. The extract significantly inhibited both the frequency of defaecation as well as the wetness of the faecal dropping in rats. Four hundred milligrams per kilogram of the extract produced 100% inhibition of castor oil induced diarrhoea in rats and this result is similar to the effect of standard anti diarrhoea drug, loperamide 3 mg/kg (Table 1)

Table 1. Anti -diarrhoeal effect of the extract of *V. hastata* (100 - 400 mg/kg *p.o*) on castor oil- induced diarrhoea in rats.

Group	Dose (mg/kg)	Number of wet defaecation ± (SEM)	% maximal inhibition of diarrhea
Control	Normal saline	5.6 ± 0.25	0
Loperamide	3	0.0	100*
V. hastata	100	3.6 ± 0.24	35.71*
V. hastata	200	2.75 ± 0.43	50.89*
V. hastata	400	0.0	100*

Values are mean ± S E M, n = 5. *significant as compared to normal control P < 0.05.

Table 2. Effect of *V. hastata* (100 - 400 mg/kg *p.o)* on intestinal motility in rats (charcoal meal study).

Group	Dose (mg/kg)	Mean distance traveled (%)
Control	Normal saline	62.4 ± 6.25
V. hastata	100	48.4 ± 4.73*
V. hastate	200	40.8 ± 3.71*
V. hastate	400	31. 2 ± 2.70*
Atropine	5	45. 2±3.19*

Values are mean ± S E M, n = 5. *Significant as compared to normal control P < 0.05.

Table 3. Effect of *V. hastata* extract (100 - 400 mg/kg, *p.o*) on castor oil- induced enteropooling in rats.

Group	Dose	Volume of intestinal content (ml).
Control	Normal saline	0.72±0.20
Castor oil	1 ml	3.84±0.25
V. hastate	100 mg/kg	2.32±0.19*
V. hastate	200 mg/kg	2.2±0.14*
V. hastate	400 mg/kg	1.42±0.13*

Values are mean ±SEM, n = 5. *significant as compared to normal control P < 0.05.

Effects on intestinal transit time

The effects of extract on gastrointestinal motility revealed that the ethanolic leaf extract of *V. hastate* caused a significant decreased in gut motility in a dose dependent manner. This observation was significantly different from what was seen in the control group. Two hundred and four hundred milligram per kilogram body weight of the extract exerted greater anti-motility effects than 5 mg/k g of atropine sulphate (Table 2).

Effect on castor oil induced enteropooling

V. hastate was found to possess an anti-enteropooling activity. Oral administration of castor oil produced a significant increase in the intestinal fluid when compared to control rats that received normal saline. *V. hastata* extract exerted a significant inhibitory effect against castor oil-induced activity in a dose dependent manner (Table 3).

DISCUSSION

In this study, preliminary phytochemical screening and evaluation of anti-diarrhoea activity of ethanolic leaf extract of *V. hastate* were carried out. Flavonoids, saponins, sterols and terpenes, which have been reported for their antidiarroeal activity (Galvez et al., 1991; Longanga et al., 2000), are also found in the extract studied.

The inhibition of experimental diarrhoea and the reduction in fecal output by a substance are the basis of the

pharmacological evaluation of a potential anti-diarrhoeal agent.Castor oil causes diarrhoea due to its active metabolite, recinolic 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. The precise mechanism of action of castor oil is through elevated prostaglandin biosynthesis (Bruton, 1985; Galvez et al., 1993). Prostaglandin contributes to the pathophysiological functions in gastrointestinal tract (Sanders, 1984). Inhibitors of prostaglandin biosynthesis delay castor oil induced diarrhoea (Awouter et al., 1978). It has been shown that E type of prostaglandins cause diarrhoea in experimental animals as well as in human beings. The mechanism has been associated with dual effects on gastrointestinal motility as well as water and electrolyte transport (Beubler and Juan, 1979). The maximal effect produced by the extract was similar to that produced by loperamide, which is at present one of the most efficacious and widely employed antidiarrhoea drugs; loperamide effectively antagonized diarrhoea induced by castor oil (Niemegeers et al., 1974). Loperamide, apart from regulating the gastrointestinal tract, is also reported to slow down transit in the intestine, reduce colon flow rate, and consequently any effect on colonic motility (Theoderau et al., 1991). The therapeutic effect of lopermide is believed to be due to its antimotility and antisecretory properties (Couper, 1987).

Atropine and different doses of the extract significantly decreased intestinal transit time. This is possible due to its anticholinergic effect (Brown and Taylor, 1996), atropine being less potent than the leaf extract at 200 and 400mg/kg body weight. The significant inhibition of the castor oil- induced enteropooling in rats suggests that the extract produced relief in diarrhoea by spasmolytic in vivo and anti-enteropooling effects. It is also possible that flavonoids present in the ethanolic leaf extract may be responsible for the antidiarrhoeal activity .Flavonoids has been ascribed to their ability to inhibit intestinal motility and hydro-electrolytic secretion (DiCarlo et al., 1993). In addition, flavonoids possess antioxidant properties, which are presumed to be responsible for the inhibitory effects exerted upon several enzymes including those involved in the arachidonic acid metabolism.

The present study has shown that Verbena hastata contains pharmacologically active substance(s) with antidiarrhoea properties. Further study is to be carried out to fractionate and purify the extract to fully investigate the mechanisms responsible for the antidiarrhoeal activity observed.

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