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Research Article

Antidiarrhoeal Activity of Aqeous Leaves Extract of *Vitex doniana*

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ABSTRACT

Aim: The objective of this study was to investigate the aqueous leaves extract of *Vitex doniana* properties against experimental diarrhoea induced by castor oil in albino rats. The aqueous leaves extract of *Vitex doniana* (100, 200, and 400 mg/kg body weight) was administered orally to three groups of rats (five animals per group) in order to evaluate the activity of the extract against castor oil-induced diarrhea model in rat. Two other groups received normal saline (5mg/kg) and Loperamide (5mg/kg) as positive control. The effect of the extract on castor oil-induced diarrhoea, gastrointestinal transit and intestinal fluid accumulation (enteropooling) was assessed respectively. In this study, the phytochemical analysis of aqueous leaves extract of *Vitex doniana* revealed the presence of alkaloids, terpenoids, flavonoids, saponins, tannins and phenols. At oral doses of 100, 200, and 400 mg/kg body weight, the plant extract showed pronounced significant ($p<0.05$) antidiarrhoeal activity compared to the control group. No mortality and visible signs of general weakness were observed in the rats following the extract administration of up to a dose of 3000 mg/kg. The results showed that the aqueous leaves extract of *Vitex doniana* has a significant antidiarrhoeal activity which supports its use in traditional herbal medicine practice.

Keywords: Antidiarrhoeal activity, Castor oil, Intestinal transit, enteropooling, *Vitex doniana*

INTRODUCTION

The use of traditional medicines in West Africa is probably as old as the duration of human settlement in the region[1]. A medicinal plant provides an important source of new chemical substances with potential therapeutic effects. These have been used in traditional medicine for the treatment of several diseases and aliments[2]. It is already important to the global economy with demand steadily increasing not only in developing countries but also in industrialized countries[3]. *Vitex doniana* (Verbenaceae), commonly called black plum, is widely distributed in different parts of Nigeria. Various parts of the plant are used by traditional medicine practitioners in Nigeria for the management and treatment of several disorders which include rheumatism, hypertension, cancer, and inflammatory diseases[3]. In Sokoto (North-West Nigeria), *Vitex doniana* has been widely used for the treatment of various stomach disorder of which diarrhea is the most common.

Diarrhea is still one of the major health threats to population in tropical and subtropical countries[4]. In Nigeria and other African countries, it remains the number one killer disease among children under five years, while babies between the ages of 7-12months remain susceptible[5]. Dependency on plants as medicine in controlling diseases is common among rural population in Nigeria because of its relative safety and affordability compared to the cost of modern medicines. Therefore, there is the need to provide scientific basis of justification on the therapeutic uses of medicinal plants against infectious diseases. The present study was therefore design to validate this folkloric claim of *Vitex doniana* in the treatment of diarrhea by the Hausa Fulani community of Kebbi State, Northwest-Nigeria.

MATERIALS AND METHODS

Collection and identification of plant material: The fresh leaves of *Vitex doniana* was collected at the Biological Garden of Kebbi State University of Science and Technology, Aliero (KSUSTA), Nigeria. The plant was identified taxonomically and authenticated by Dr D. Singh at the Department of Biological Science, KSUST, Aliero, Nigeria.

Preparation of plant material: The leaves of *Vitex doniana* was air-dried and powered using a clean mortar and pestle. The powered leaves were cold-extracted in a container using distilled water as solvent. 100g of the powdered leaves were packed in a conical flask and macerated for 48hours, filtered and dried to obtain a dark-green solid residue. The resulting dry powder was taken as the aqueous leaves extract of *Vitex doniana*. The percentage yield of the aqueous leaves extract of *Vitex doniana* was 15.2%.

Animal: Albino rats of both sexes weighing 100-200g were used for the study. They were purchased from the Animal House of University of Jos, Plateau. All they animals were kept in the cage and allowed to acclimatized for two weeks in Biochemistry Laboratory of Kebbi State University of Science and Technology, Aliero, before the experiment started. The animals were fed with standard pelleted diet (Guinness Foods Nig. Ltd.) and water *ad libitum*. The container for the food and

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water were washed daily, as the food and water are renewed everyday, to ensure hygiene and maximum comfort for the animal.

Phytochemical Analysis: The phytochemical examination of the aqueous leaves extract of *Vitex doniana* was performed by the standard methods[6].

Lethal Dose (LD_{50}) Determination: The up and down procedure as described by Dixon[7] was used to evaluate the oral acute toxicity of aqueous leaves extract of *Vitex doniana*. Five non-pregnant adult albino rats were randomly selected from the pull of acclimatized rats were used for this experiment. The animals were weighed individually, marked and housed individually in cages prior to treatment. The rats to be treated were fasted overnight but allowed free access to water. Freshly prepared aqueous extract of *Vitex doniana* leaves was administered orally at a limited dose of 3000mg/kg. The first animal was dosed and observed for sign of toxicity or death. If the animal survives, the same procedure was adopted until all the five rats were dosed and observed for 48hours for signs of acute toxicity, morbidity and mortality for the first 48hours and up to 14 days. The behavioral changes and other changes observed in animals were recorded according to Organisation for Economic and Cultural Development (OECO) 425 guidelines [7].

Antidiarrhoea Studies: Castor oil induced diarrhoea: Diarrhoea was induced by method of Diurno *et al.* [8]. Animals were fasted for 24 hours but allowed free access to water. Rats were divided into five groups of five animals each, diarrhoea was induced by administering 2 ml of castor oil orally to rats. Group I treated as control (2 ml/kg, p.o. saline), group II received Loperamide (5 mg/kg p.o) served as standard and group III - V received aqueous leaves extract of *Vitex doniana* (100, 200 and 400 mg/kg, p.o) 1 hour before castor oil administration respectively. The animals were placed in individual cages over clean filter papers. One hour after the administration of the castor oil, the presence of characteristic diarrhoea droppings and the dry stools were observed and counted for 4 hours. The absence of which was considered as protection from diarrhoea[8] and the percentage protection calculated[9].

Gastrointestinal motility test: Rats were fasted for 18 h divided into five groups of five animals each, Group I received 2 ml/kg normal saline orally, group II received Loperimide (5 mg/kg, o.p.), group III - V received aqueous leaves extract of *Vitex doniana* (100, 200 and 400 mg/kg, p.o) respectively, 1 hour before administration of castor oil. One ml of marker (10% charcoal suspension in 5% gum acacia) was administered orally 1 hour after castor oil treatment. The rats were sacrificed after 1hour and the distance traveled by charcoal meal from the pylorus was measured and expressed as percentage of the total length of the intestine from the pylorus to caecum[10].

Castor oil-induced enteropooling: Intraluminal fluid accumulation was determined by the method of Robert *et al.* [11] and Dicarlo *et al.* [12] respectively. Fasted rats were divided into five groups of five animals each one hour before oral administration of castor oil (2ml/rats) for induction of diarrhea. Group I received (2ml/kg p.o.) of normal saline and serve as control. Group II received Loperamide (5 mg/kg p.o.) and served as the standard. Groups III, IV and V received aqueous leaves extract of *Vitex doniana* (100, 200, and 400 mg/kg p.o.) respectively. After one hour, the rats were sacrificed, and the small intestine ligated at both the pyloric and the ileocaecal junction. The small intestine was weighed and its contents collected by milking into a graduated tube allowing the volume to be measured. The intestine was then reweighed and the difference between full and empty weights calculated.

STATISTICAL ANALYSIS

Data were analyzed by one-way ANOVA followed by Dennett's t-test using Instat® (Graph Pad software, U.S.A). At 95% confidence interval $p<0.05$ was considered statistically significant.

RESULTS

Preliminary phytochemical analysis and acute toxicity test: The phytochemical analysis of aqueous leaves extract of *V. doniana* revealed the presence of alkaloids, terpenoids, flavonoids, saponins, tannins, phenols and absence of resins, steroids and glycosides. Oral administration of the extract to the rats up to 3000 mg/kg neither showed mortality nor any apparent signs of weakness in the animals.

Effect of castor oil induced diarrhea: In the castor oil-induced diarrhoea experiment, the aqueous extract of *V. doniana* produced a marked antidiarrhoeal effect in the rats (Table 1). Diarrhoea was clinically apparent in all the animals of control group 45 min after administration of castor oil for the next 4 hour. A significant ($p<0.05$) reduction in the number of defecations over four hours was achieved with aqueous leaves extract of *V. doniana* at a dose dependent fashion when compared to the control. Highest percentage inhibition of defecation in the extract treated groups was observed at 400mg/kg of the extract (42.31%) while the Loperamide treated group retained the maximum percentage inhibition of defecation (57.69%).

Effect of aqueous leaves extract of *V. doniana* on intestinal transit of charcoal meal: The aqueous leaves extract of *V. doniana* significantly ($p<0.05$) decreased propulsion of charcoal meal in the rat gastrointestinal tract at oral doses of 100 - 400 mg/kg compared with the control group that receiving normal saline (2ml/kg) (Table 2). However the effect is not comparable to that of the standard drug loperimide (5 mg/kg) which was more markedly reduced (44.0%).

$$\% \text{ Inhibition} = \frac{\text{Distance travelled by charcoal meal in control group} - \text{Treated Group}}{\text{Distance travelled by charcoal meal in control group}} \times 100$$

Table 1: Effect of aqueous leaves extract of *V. doniana* on castor oil induced diarrhoea in rats.

Groups	Treatment	Mean defecation in 4 hours	%inhibition of defecation
I	Castor oil (2ml p.o) + 2ml/kg Normal saline	5.20±0.49	__
II	Castor oil (2ml p.o)+5mg/kg Loperamide	2.20±0.20[a]	57.69%[a]
III	Castor oil (2ml p.o) + 100mg/kg *V. Doniana*	3.80±0.37[a]	26.92%[a]
IV	Castor oil (2ml p.o) + 200mg/kg *V. doniana*	3.40±0.24[a]	34.62%[a]
V	Castor oil (2ml p.o) + 400mg/kg *V. doniana*	3.00±0.72[a]	42.31%[a]

Values are expressed as mean ± SEM. [a](p< 0.05) significant different when compared with the control.

Table 2: Effect of aqueous leaves extract of *V. doniana* on GIT motility

Group	Treatment	Length of Intestine(Cm)	Distance Traveled by Charcoal Meal (Cm)	% Intestinal Transit
I	2ml/kg Normal saline (p.o) and charcoal meal (1ml, p.o)	90.48 ± 1.69	84.00 ± 5.34	0
II	5mg/kg Loperamide drug (p.o) and charcoal meal (1ml, p.o)	94.90 ± 1.58	46.98 ± 0.65[a]	44.00[a]
III	100mg/kg *Vitex doniana* (p.o), and charcoal meal (1 ml, p.o)	83.62 ± 5.83	53.60 ± s3.56[a]	36.19[a]
IV	200mg/kg *V.doniana* (p.o) and charcoal meal (1ml, p.o).	73.74 ± 2.79	46.80 ± 2.08[a]	39.80[a]
V	400mg/kg *V.doniana* (p.o) and charcoal meal (1ml, p.o)	92.4 ± 2.16	60.60 ± 4.69[a]	27.86[a]

Values are expressed as mean ± SEM. From the experiment [a](p <0.05) significantly different when compared with control group.

Table 3: Effect of aqueous leave extract of *V. Doniana on* enteropooling in albino rats.

Group	Treatment	Mean weight of intestine (g)	Mean weight of intestine (g)	Volume of intestinal content (ml)	% Inhibition
I	Normal saline (2ml/kg p.o)	5.64 ± 0.27	4.32 ± 0.21	1.32 ±0.07[b]	--
II	Loperamide (5mg/kg p.o)	5.36 ± 0.25	4.54 ± 0.27	0.48 ± 0.04[a]	63.63%[a]
III	*Vitex doniana* (100mg/kg p.o),	6.18 ± 0.60	4.88 ± 0.80	1.28 ± 0.43[b]	3.03%
IV	*Vitex doniana* (200mg/kg p.o),	8.36 ± 1.33	6.48 ± 0.88	0.98 ± 0.10[a]	25.75%[a]
V	*Vitex doniana* (400mg/kg p.o),	5.28 ± 0.27	4.46 ± 0.27	0.82 ± 2.30[a]	37.88%[a]

Values are expressed as mean ± SEM. [a](p<0.05) significantly different from the control. [b](p<0.05) significantly different when compared with the standard drug (Loperamide).

Effect of aqueous leave extract of *V. Doniana* on castor oil-induced enteropooling: Castor oil caused accumulation of water and electrolytes in intestinal loop. *V. Doniana* extract significantly (P < 0.05) inhibited castor oil-induced enteropooling in rats at oral doses of 200mg/kg (25.75%) and 400 mg/kg (37.88%) in a dose dependent manner compare to the control (Table 3) while there was no significant difference (p<0.05) at oral dose of 100mg/kg (3.03%). The standard drug, loperamide (5 mg/kg), also significantly inhibited (P < 0.05) intestinal fluid accumulation (63.63%).

DICUSSION

The result of the toxicological effects confirms that the aqueous leaves extract of *Vitex doniana* is non toxic at the dose rate of 3000mg/kg. In this study, therefore, LD50 is greater than 3000mg/kg. Plants have the capacity to synthesize divers array of chemicals, and understanding how these phytochemicals function in plants will enhance our understanding of the mechanism by which they benefit humans. These phytochemicals function to attract beneficial and repel harmful organisms, serves as protectants and respond to environmental changes in plants. In humans, they can have complementary and overlapping actions including antioxidants, modulation of detoxification enzymes, stimulation of the immune system, reduction of inflammation, modulation of steroid metabolism, antibacterial, antihelmintic and antiviral effects[13]. In this study, the phytochemical analysis of aqueous leaves extract of *Vitex doniana* revealed the presence of alkaloids, terpenoids, flavonoids, saponins, tannins and phenols. Flavonoids and reducing sugars obtained from selected traditional medicinal plants in Bangladesh and some parts of the world were reported by Rahman and Wilcock[14] and Palombo[15] respectively to exhibit antidiarrhoeal properties. Longanga *et al.*[16] screened a number of medicinal plants and showed that antidiarrhoeal activities of these plants were due to tannins, alkaloids, saponins, flavonoids, steroids, terpenes and glycosides contained in them. The presence of these phytochemical constituents in *Vitex doniana* may be responsible for its antidiarrhoeal effect.

Many plants conveniently available in Africa are used in traditional folklore medicine for the treatment of

diarrhea[17]. The aqueous extract of *Vitex doniana* exhibited significant anti-diarrheal activity against gastrointestinal motility. Studies show that activated charcoal avidly absorbs drugs and chemicals on the surface of charcoal meal particles thereby preventing absorption[18]. Thus gastrointestinal motility test with activated charcoal was carried out to find the effect of the aqueous extract of *Vitex doniana* on peristalsis movement. The result shows that the aqueous extract of the leaves suppressed the propulsion of charcoal meal thereby increased the absorption of water and electrolytes.

Castor oil is an effective laxative. It decreases fluid absorption, increases secretion in the small intestines and colon and affects smooth muscle contractibility in the intestine. Castor oil produces diarrhea due to its active component recinoleic acid. Several mechanisms have been supposed to be involve in the antidiarrheal effect of castor oil[19]. These include inhibition of intestinal Na^+,K^+-ATpase activity to reduce normal fluid absorption. Activation of adenylate cyclase or mucosal CAMP-mediated active secretion, stimulation of prostaglandins formation, platelets-activating factor and most recently nitric oxide has been claimed to contribute to diarrheal of castor oil[20]. Despite the fact that numerous mechanisms have been proposed for the diarrheal effect of castor oil, it has not been possible to define its correct mechanism of action[21]. Aqueous leaves extract of *Vitex doniana* may act on any of the above stated mechanism.

Diarrhoea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract accompanied by hurry, resulting in an excess loss of fluid in the faeces[22]. In some diarrhoea, the secretory component predominates while other diarrhoeas are characterized by hypermotility. The use of castor oil induced diarrhoea model in this study is because autocoids and prostaglandins are involved and these have been implicated in the causation of diarrhoea in man[23]. The liberation of ricinolieic acid from castor oil results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which stimulate motility and secretion[24]. The results of this study revealed that the leaves extract of *Vitex doniana* produced statistically significant protection against diarrhoea and was found to be comparable to loperamide; a drug widely employed against diarrhea disorders which effectively antagonizes diarrhoea induced by castor oil, prostaglandin and cholera toxin[25].

The secretory diarrhea is associated with an activation of Cl^- channels causing Cl^- efflux from the cells. The efflux of Cl^- results in massive secretion of water into the intestinal lumen and profuse watery diarrhea[26] (Brown and Taylor 2000). The involvement of muscarinic receptor effect was confirmed by increased production of both gastric secretion and intraluminal fluid accumulation induced by castor oil. The aqueous leaves extract of *Vitex doniana* may inhibit the secretion of water into the intestinal lumen and this effect maybe partly mediated by both α_2 adrenoceptor and muscarinic receptor systems. The significant inhibition (P<0.05) of castor oil induced enteropooling in rats suggests that the extract of *Vitex doniana* produced relief in diarrhea by spermolytic activity *invivo* and enteropooling effect[21].

In conclusion, the results of this investigation revealed that aqueous extract of *Vitex doniana* contains pharmacologically active substances with antidiarrhoeal properties, thus justifying its widespread use by the local population for these purposes. Further research is needed to fully investigate the mechanisms involved in the pharmacological activities, to isolate and characterize the active constituents of *Vitex doniana*. The isolated compound may serve as useful prototypes of antidiarrhoeal drugs of natural origin possessing the desired pharmacological activities while lacking certain untoward effects.

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