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Evaluation of the Antidiarrhoeal Activity of Aqueous Stem Bark Extract of *Terminalia catappa* in Castor oil-induced Diarrhoeal Albino Rats

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Abstract

Diarrhoeal illness remains a key global health problem causing 15% of deaths among children under five years of age. This study evaluated the antidiarrhoeal activity of *Terminalia catappa* aqueous stem bark extract on albino rats. Antidiarrhoeal activity of *T. catappa* was assessed by measuring the stool inhibition, gastrointestinal motility and castor oil-induced enteropooling. Thirty (30) albino rats were divided into 6 groups of 5 rats each, where groups I and II remained the normal and negative control groups respectively; group III was treated with standard drug, groups IV, V and VI were treated with 100, 200 and 300 mg/kg b. wt. of aqueous stem bark extract of *T. catappa,* respectively. Percentage stool inhibition of treated groups was significantly greater than that of the negative control. However, group II treated with standard drug (loperamide) showed higher percentage inhibition. The volume of intestinal fluid decreased with increase in dose of the extract. The volume of intestinal fluid of the group treated with standard drug significantly decreased when compared to group treated with extract. Distance travelled by the charcoal significantly decreased at higher dose of extract. Aqueous stem bark extract of *T. catappa* has a dose-dependent antidiarrhoeal activity.

Keywords: Stool inhibition, diarrhoea, enteropooling, gastrointestinal motility, *Terminalia catappa*

Introduction

Diarrhoea, described as an increase in frequency of bowel movements, watery stool and abdominal pain, is a symptom common to a wide variety of gastrointestinal illnesses, and is an important public health challenge among children, especially of age below 5 years in developing regions of the world (Getachew *et al*., 2018; Mallick *et al*., 2020; Shine *et al*., 2020). It accounts for 9% of deaths among children (Rakotomanana *et al.,* 2020). It is most common in developing countries due to improper hygiene and lack of good water (Bitew *et* al., 2017; Yaya *et* al., 2018). The major factor in morbidity and mortality as a result of diarrhoea is caused by enteric infections by pathogenic microorganisms which includes *Rotavirus*, *E. coli*, *Salmonella* spp., *Campylobacter*, *Vibrio cholerae*, and *Shigella*. Diarrhoea may occur as a result of other diseases such as intestinal inflammation, irritable bowel syndrome (IBS), HIV/AIDS and ulcerative colitis (Vitali *et al*., 2006; Yongsi, 2008; Baldi and Grego-

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rio, 2009). Diarrhoea is usually managed with oral rehydration therapy. This treatment reduces dehydration caused by diarrhoea but does not treat the causative agent. The available antidiarrhoeal drugs have side effects such as constipation, nausea, and respiratory depression. Thus, there is a need to seek for newer antidiarrhoeal drugs with little or no side effects.

Terminalia catappa Linn, often referred to as tropical almond, is a large combrataceae plant which grows most commonly in tropical and subtropical countries distributed throughout the coastal environments (Marler, 2018). The almond is tolerant to strong winds, salt spray, and moderately high salinity in the root zone (Vadwale and Kola, 2017). It thrives mostly in sandy soils that have good air circulation and drainage. The fruit is elliptical in shape with a bluntly pointed apex, and it is about 7.1 cm long and 5.05 cm thick. When the fruit ripens, it turns from green to purple yellow and contains a hard shell or nut that protects a delicate edible seed (Dwevedi *et al*., 2016). The tree grows to a height of 3 to 8 m. *T. catappa* is a well-known medicinal plant that has been used to treat a number of diseases. Its fresh leaf juice is a well-known herb, used to make a lotion that treats leprosy and lice and is also taken orally for headaches and stomach aches (Prayitno *et al*., 2022). It is used as a traditional medicine to treat diabetes (Divya *et al*., 2019), wounds (Nugroho *et al*., 2019 a, b), and hepatitis (Chen *et al*., 2000). A number of phytochemicals which includes alkaloids, fatty acids, phenolics, terpenes/terpenoids and alkaloidswere identified to be present in extracts of *T. catappa* (Inheagwam et al., 2019; Nugroho *et al*., 2019b) and the extracts have been reported to possess antioxidant and anticancer properties (Poongulai and Sundararaman, 2016; Venkatalakshmi *et al*., 2016), hepatoprotective effects (Abdul Vahab and Harindran, 2016), anti-HIV reverse transcriptase (Tan *et al*., 1991), antifungal properties (Bognan *et al*., 2016) and aphrodisiac effect (Sharma and Rana, 2017). The plant extract is relatively safe and non-toxic for its LD_{50} is greater than 5000 mg/kg b.wt. (Enadeghe *et al*., 2022)

Materials and Methods

Collection and preparation of plant

Fresh stems and leaves of *T. catappa* were collected in September, 2021 and authenticated at the Dept. of Botany, Adamawa State University, Mubi and given a voucher number adsu/2022/101. The stem bark was washed and shredded into smaller pieces. It was air dried under shade at room temperature. The dried, shredded pieces were pulverized into powder using mortar and pestle. The powdered sample was stored in a well tight container and kept at room temperature until required.

Extraction Procedure

One hundred grams (100 g) of the powdered sample was soaked in 400 mL of distilled water for 48 h at room temperature with vigorous shaking at 3 h intervals. The crude extract was filtered using Whatman No. 1 filter paper and evaporated to dryness at 60ºC using water bath. The dried extract was stored in airtight bottles at room temperature until required. The dried powdered extract was thereafter reconstituted in water to obtain the administered doses of 100, 200 and 300 mg/kg b. wt.

Experimental Animals

Adult albino rats weighing between 120 – 150 g were purchased from Animal Resource Unit, National Veterinary Research Institute (NVRI) VOM, Plateau State, Nigeria. The rats were housed in well ventilated cages and were given standard laboratory diet with free access to drinking water. The rats were handled according to the guidelines for the protection and handling of laboratory animals by the Institute of Laboratory Animal Resource (1986) and approved by University Ethical Committee, with an approval number ADSU/IACEC/ANP-A045/2022. The animals were left in the laboratory environment for one week before the commencement of the experiment.

Stool inhibition

The method described by Gunakkuru *et al*. (2005) was adopted. A total of thirty (30) albino rats were divided into six groups of five rats each. After a 12 h period of fasting, diarrhoea was induced in rats in groups II - VI by administering 1.0 mL of castor oil. Thirty minutes after castor oil administration, rats of groups I and II received 1.0 mL distilled water, group III received 2 mg/kg b. wt. loperamide (standard drug), groups IV – VI

received 100, 200 and 300 mg/kg b.wt. of aqueous stem bark extract of *T. catappa*, p.o. respectively. Separate metabolic cages were used for each animal, which were lined with white cardboard paper that was replaced every hour. The number of diarrhoeal faeces were counted for 4 h. The total number of diarrhoeal faeces of the normal control group was considered 100%.

% inhibition = (Control - Test) × 100/Control.

Castor Oil-Induced Enteropooling

Castor oil-induced enteropooling was determined by the method describe by Mascolo *et al.* (1994). Another set of thirty (30) adult rats were fasted for 12 h and divided into six groups of five animals each. Diarrhoea was induced in rats in group II – VI by administering 1 mL castor oil to each rat. An hour after diarrhoea induction, rats in group III received 2 mg/kg b.wt. loperamide while 100, 200 and 300 mg/kg b.wt. of aqueous stem bark extract of *T. catappa*, p.o. were administered to rats in group IV, V and VI respectively. After an hour, the rats were sacrificed by ether anesthesia. The edges of the intestine from pylorus to ceacum were tied with thread and the intestine removed. The intestinal fluid was collected into a graduated tube and the volume taken.

Measurement of gastrointestinal transit time using charcoal

The method described by Robert *et* al. (1976) was adopted. Another set of thirty (30) adult rats were fasted for 12 h and randomly divided into six groups of five rats each. The rats in groups II –VI received castor oil (1 mL). One hour after castor oil administration, standard drug (2 mg/kg b. wt.) was administered to the rats in group III while 100, 200 and 300 mg/kg b.wt. of stem bark extract of *T. catappa,* p.o. were administered to rats in groups IV, V and VI respectively. All the rats received 1 mL of charcoal meal (10% suspension in 5% gum acacia) orally after 30 min of administration. The rats were sacrificed by ether (20% v/v) anesthesia and the small intestine was carefully separated from mesentery to avoid being stretched. For each animal, gastrointestinal transit was calculated as percentage distance travelled by charcoal meal to the total length of intestine.

Statistical analysis

Statistical package for social sciences software version 23.0 was used for computing the mean and analyzing data statistically. Data is presented as the mean ± SD of five replicates. It was statistically analyzed with one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT). The level of significance was taken at 5 % confidence interval ($p < 0.05$).

Results

Table 1 shows the stool inhibition of aqueous stem bark extract of *T. catappa* on castor oil-induced diarrhoea. The number of wet stools of the treated groups significantly ($p<0.05$) decreased when compared to the negative control. The percentage inhibition of all the treated groups significantly increased in a dose-dependent manner when compared with the negative control group, though the group that received the standard drug was significantly greater than the groups treated with the aqueous stem bark extract.

Table 1: Effect of aqueous stem bark extract of *T. catappa* on stool inhibition in castor oil-induced diarrhoeal rats

Groups	Number of wet stool	% Inhibition
Group I (Normal control)	0 ± 0.00^{a}	100 ± 0.00^{e}
Group II (Negative control)	13 ± 0.22^{e}	0 ± 0.00^{a}
Group III (2 mg/kg loperamide)	3 ± 0.09^{b}	77 ± 0.15^{d}
Group IV (100 mg/kg b.wt. extract)	9 ± 0.12^{d}	30 ± 0.17^{b}
Group V (200 mg/kg b.wt. extract)	5 ± 0.21^{c}	62 ± 1.01^{c}
Group VI (300 mg/kg b.wt. extract)	4 ± 0.08^{c}	69 ± 0.56^{c}

Values are presented as mean ± S.D of 5 rats. Values with different superscripts down the column are significantly different at ($p < 0.05$).

The effect of the aqueous stem bark extract of *T. catappa* on the volume of intestinal fluid is shown in Table 2. The volume of intestinal fluid of the treatment groups significantly decreased when compared to the negative control group with increase in dose of the extract. The volume of intestinal fluid of groups that received 200 and 300 mg/kg b.wt. of *T. catappa* were not significantly

(p>0.05) different from each other, likewise when compared with the normal control group.

Table 2: Effect of aqueous stem bark extract of *T. catappa* on castor oil-induced enteropooling in rats

Groups	Volume of intestinal fluid
Group I (Normal control)	1.40 ± 0.09^{ab}
Group II (Negative control)	2.47 ± 0.34^{c}
Group III (2 mg/kg loperamide)	1.07 ± 0.03^{a}
Group IV (100 mg/kg b.wt. extract)	1.75 ± 0.16^{b}
Group V (200 mg/kg b.wt. extract)	1.55 ± 0.07^{ab}
Group VI (300 mg/kg b.wt. extract)	1.37 ± 0.10^{ab}

Values are presented as mean ± S.D of 5 rats. Values with different superscripts down the column are significantly different at ($p < 0.05$).

Table 3 shows the percentage distance travelled by the charcoal in the intestine of castor oil-induced diarrhoeal rats. The percentage distance travelled by charcoal of groups V and VI showed significant decrease when compared with the negative control group. However, group IV treated with the extract showed no significant difference when compared with the negative control group. The percentage travelled by charcoal of rats in groups V and VI were not significantly different from rats in group III (rats administered loperamide).

Table 3: Effect of aqueous stem bark extract of *T. catappa* on gastrointestinal motility in castor oil-induced diarrhoeal rats

Groups	% Distance travelled by the charcoal
Group I (Normal control)	28.50 ± 3.10^{a}
Group II (Negative control)	48.50 ± 2.72^{c}
Group III (2 mg/kg loperamide)	32.50 ± 2.75^{b}
Group IV (100 mg/kg b.wt. extract)	49.00 ± 2.70^{c}
Group V (200 mg/kg b.wt. extract)	39.25 ± 3.57^{b}
Group VI (300 mg/kg b.wt. extract)	34.25 ± 4.30^{b}

Values are presented as mean ± S.D of 5 rats. Values with different superscript down the column are significantly different at ($p < 0.05$).

Discussion

Diarrhoea occurs as a result of an imbalance between the secretory and absorptive mechanisms in the intestinal tract accompanied by increase in frequency leading to loss of excess fluid in the faeces (Das *et al*., 2018). Clinically, the secretory action predominates in some cases of diarrhoea, whereas others are characterized by hypermotility (Yadav and Tangpu, 2007). The reduction in wet stool by the extract may indicate that the extract possesses antidiarrhoeal potential by inhibiting the hyper-secretion of fluids and electrolytes. Castor oil has an active metabolite, ricinoleic acid, produced in the small bowel when lipases hydrolyze castor oil, which is poorly absorbed from the lumen of the intestine (Asrie *et al*., 2016). This active metabolite causes diarrhoea by stimulating the peristaltic activity in the small intestine causing changes in the electrolyte permeability of the intestinal mucosa and also stimulates the release of endogenous prostaglandins (Owolabi *et al*., 2016). The reduction of intestinal fluid observed may indicate that the extracts have inhibitory potential against ricinoleic acid action and peristalsis or it increased the water and electrolyte reabsorption and reduced mucosal secretion thereby reducing castor oil-induced enteropooling. Ahmed *et al*. (2022) reported that steroids are one of the major phytochemicals responsible for the anti-enteropooling activity of plants. In remediation of diarrhoea, antisecretory agents and antimotility agents remain the major factors necessary for depleting the diarrhoeal pathophysiologic changes (Scalbert, 1991). Intestinal transit was significantly reduced as observed in the distance travelled by the charcoal which may indicate that the extract has inhibitory potential against the intestinal transit motility and thus allows for better fluid reabsorption. Report by Scalbert (1991) and Tripathi (1994) revealed that tannates reduce the mucosal motility and make the intestinal mucosa more resistant. This suggests that tannins present in the aqueous stem bark extract of *T. catappa* (Nugroho *et al*., 2019b) mediated the antidiarrhoeal activity of the aqueous stem bark extract of the plant.

Conclusion

These results indicate that the aqueous stem bark extract of *T. catappa* has a significant dose-dependent antidiarrhoeal activity. The reduction in the gastrointestinal transit prompted by the extract

provides a rationale for the usage of the stem bark of *T. catappa* in the management of diarrhoea as it delays gastrointestinal motility and allows for proper water absorption.

Conflict of interest

Authors declare that there are no conflicts of interests.

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