



Article Info

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Antidiarrhoeal and Anti-emetic Activities of Aqueous Leaf Extract of *Ficus Thoningii* Blume in Young Chicks

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Treatments for diarrhoeal and vomiting with modern drugs have been associated with unwanted effects such as sedation and extrapyramidal effects. In this study, *Ficus thoningii* Blume leaf extract (FTE) was investigated for its anti-diarrhoeal and anti-emetic properties. Two study groups, namely anti-diarrhoeal and anti-emetic groups (n=36), were sorted into: *placebo*; drug-treatment, and 3 plant-extract treatment (*FT100*, *FT200* and *FT300*) subgroups (n = 6). In the *placebo* group, chicks were administered only saline solution (0.9 %) while chlorpromazine (150 mg/kg) and loperamide (2.5 mg/kg) served as positive control in the anti-emetic and antidiarrhoeal study groups, respectively. The plant-extract treatment groups (*FT100*, *FT200* and *FT300*) were treated with 100, 200, and 300 mg/kg doses of FTE, respectively. Vomiting was induced using oral copper sulphate solution (50 mg/kg) and chicks were observed for latency as-well-as number of retches. In the anti-diarrhoea study group, 30 mins after treatment with extracts, diarrhoea was induced with oral castor oil and latency as-well-as number of defecation was observed. Results revealed that treatment with 200 and 300 mg/kg of FTE, similar to chlorpromazine, inhibited the number of retching in chicks by 71% and 78%, respectively. Treatment with 100, 200 and 300 mg/kg of FTE, similar to loperamide, reduced the number of droppings in chicks by 45.4%, 42.9%, and 58.4%, respectively. Thus, the results showed that FTE possess anti-emetic and anti-diarrhoeal properties which qualifies it a potential research candidate in search of new drugs.

Keywords: Anti-diarrhoea, vomiting, *Ficus thoningii*.

1. Introduction

Vomiting is one of the protective mechanisms through which the body defends itself against toxins [1]. Also known as emesis, it is defined as the forceful expulsion of the contents of one's stomach through the mouth and sometimes the nose [1]. On the other hand, diarrhoea, which is known as the passage of more frequent watery stool than normal for an individual, is usually due to infection or irritation in the intestinal tract [2]. Diarrhoea and vomiting often constitute symptoms of a wide variety of conditions, which includes pregnancy, motion sickness, migraine, gastrointestinal obstruction, peptic ulcer, drug toxicity, renal failure, and hepatitis [2].

They are also known to accompany the administration of many drugs particularly cancer chemotherapeutic agents [3]. Drug induced nausea and vomiting may occur so regularly that, if not controlled, the discomfort associated may cause a patient to refuse further compliance to

pharmacotherapy [4]. Furthermore, the loss of water, electrolytes and minerals associated with vomiting and diarrhoea can cause dehydration and electrolyte abnormalities. Commonly seen in critically ill patient, these symptoms can complicate patients' condition, possibly extend the length of hospitalization, and even result to death [5]. Other clinical implications of diarrhoea and vomiting include unwillingness to eat or drink which result in nutritional deficits. They can also impair patient daily functioning as seen in the case of morning sickness [6]. In rare cases, excessive vomiting can tear the lining of the oesophagus causing Mallory-Weiss tear [7; 8]. Available antidiarrhoeal drugs such as diphenoxylate, loperamide, diloxanide furoate, racecadotril exert their effects by acting on the muscarinic parasympathetic system, which makes them carry varying degree of side effects such as bronchospasm, constipation, dry mouth,

dizziness and sleepiness. Similarly, commonly used anti-emetic agents such as chlorpromazine, promethazine, cetirizine, etc. are known to be associated with serious side effects such as sedation and extra pyramidal symptoms [9].

In view of these challenges, some patients have resulted to the use of herbs for the management of diarrhoea and vomiting. Thus, the need for further search for safe and efficacious drugs. Recently, there has been great interest in herbal remedies for the treatment of such ailments. Although several medicinal plants have gained importance for the treatment of diarrhoea and vomiting, many remain to be evaluated scientifically. *F. thonningii* is one of such plants that is widely used as antidiarrhoeal and anti-emetic and has been mentioned by several authors as effective in the treatment of wound, fever, diarrhoea, vomiting, gonorrhoea and diabetes [10]. Studies have shown that alkaloids were present in the leaves, roots and stem barks of *F. thonningii* while saponins and volatile oil were found in the stem bark, root bark, and fruit of the plant. Tannin was found in the fruit, root bark and leaves of *F. thonningii* while flavonoids were found in all the parts of *F. thonningii* [11; 12].

Despite these folkloric claims, there is currently limited scientific research to support the traditional use of *F. thonningii*. This study aimed to investigate the anti-diarrheal and antiemetic properties of *Ficus thonningii* as well as its acute toxicity profile.

2. Materials and Methods

2.1 Plant Collection, Identification and Extraction

The leaves of *Ficus thonningii* Blume (Moraceae) were collected from Wamakko local government area of Sokoto state in August, 2015. The plant was authenticated at the herbarium of the Department of Pharmacognosy and Ethnopharmacy, Usmanu DanFodiyo University Sokoto, Nigeria. A voucher specimen was prepared and deposited in the herbarium (voucher no. PCG/UDUS/MORA/0005). The plant leaves were then dried under the shade and size-reduced into powder with a milling machine (Innotex Intl. Ltd, Nigeria). Using cold maceration technique, for 72 hours, 6 kg of the powdered leaves was extracted in 10 litres of distilled water. The aqueous leaf extract was then filtered and evaporated in a water bath at

40°C to yield a dry extract that was stored at -20 °C.

2.2 Experimental Animals

Seventy-two young chicks (3 days old, weighing 120-140 g) obtained from the animal house of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University (ABU), Zaria. The animals, kept in plastic cages fortified with saw dust, then maintained under standard environmental condition and liberally fed with standard chick feed and distilled water. The chicks were transferred to the laboratory environment to acclimatize for 24 hours prior to commencement of the experiment.

The animal study was approved by the Animal Ethics Committee, Usmanu Danfodiyo University, Sokoto, where ethical clearance number UDUS/UREC/2015/011 was issued.

2.3 Phytochemical Screening

The preliminary phytochemical screening of FTE was conducted on the basis of qualitative colour reaction with specific reagents as described by [13] and demonstrated by [14].

2.4 Acute Toxicity Studies (LD₅₀)

The LD₅₀ of FTE was determined in chicks through oral and intraperitoneal routes using the Lorke's method [15]. The method was carried out in two phases. In phase 1, 3 groups of 3 chicks each received the extract of 10, 100, and 1000 mg/kg body weight and observed (for a period of 24 hours) for signs of toxicity and death. The second phase of the test involves the use of 3 and 4 groups (n=1) drawn from the outcome of the first phase. For instance, where zero mortality of chicks was observed in phase I, oral of doses of 1600, 2900 and 5000 mg/kg of the extract were recommended in the second phase of the test. Where a single mortality was observed at 1000 mg/kg via the intraperitoneal route, *i.p.* doses of 200, 400, 800 and 1600 mg/kg were administered to four groups of rats (n=1). Similarly, in the second phase, chicks were observed for signs of toxicity and mortality for 24 hours. The LD₅₀ was determined by calculating the geometric mean of the lowest lethal dose and highest non-lethal dose (1/1 and 0/1).

2.5 Anti-emetic activity

The protocol demonstrated by Sadia *et. al.* [16] was used to evaluate the antiemetic activity of FTE in young chicks. Thirty-six young chicks (3

days old, weighing 120-140 g) were divided into placebo, chlorpromazine (CPZ) and 3 plant extract (FTE100, FTE200 & FTE300) treatment groups (n=6). The placebo group was given normal saline (10 ml/kg), the CPZ group received 150 mg/kg oral chlorpromazine dissolved in 0.9 % saline containing 5 % DMSO and 1 % tweens 80 as solvent while the plant extract treatment groups received 100, 200, and 300 mg/kg of FTE as oral gavages. Each chick was allowed to stabilize in a beaker for 10 minutes. At 50 mg/kg dose, copper sulphate was administered orally to each chick. The onset and number of retching was observed for a duration of 1 hr. The percentage inhibition of retching was calculated as follows;

$$\text{Inhibition (\%)} = [(A-B)/A] \times 100$$

Where A = Frequency of retching in control group; B = Frequency of retching in treatment groups.

2.6 Anti-diarrhoea activity

Method described by Unigwe *et al.*, [17] was adopted. Thirty-six young chicks (3 days old, weighing 120-140 g) were divided into placebo, loperamide (LMD) and 3 plant extract (FTE100, FTE200 & FTE300) treatment groups (n=6). The placebo group was given normal saline (10 ml/kg), the LMD group received 2.5 mg/kg oral loperamide while the FTE treatment groups received 100, 200, and 300 mg/kg of FTE as oral gavages. Thirty minutes later, chicks were placed in separate cages with white paper-sheet floor. Diarrhoea was induced in chicks by oral administration of 1ml castor oil. The onset and number of defaecation spots on the white plain sheet paper that was replaced every hour were observed for 4 hours. Percentage inhibition of diarrhoea was calculated and compared with between the experimental groups.

2.7 Statistical analysis

Data obtained in this study was expressed as mean \pm SEM. Using statistical package for social science software (SPSS, version 20), data obtained was first tested for normality of distribution using Shapiro-Wilk test. After testing for distribution, data was analysed for statistical variance using One-way ANOVA and Tukey's post hoc test. At $p < 0.05$, results were considered significant.

3. Results and Discussion

3.1 Extraction

The extraction yielded 4.0% extract of the total weight of the crude plant. The crude extract was sticky dark brownish substance that was odourless and tasteless in nature.

3.2 Phytochemical screening

The preliminary phytochemical screening of the crude extract of *FT* leaves revealed the presence of carbohydrate, alkaloids, saponins, steroids, flavonoids and phenolics. However, tannins and anthraquinones were found to be absent (Table 1).

Table1. Phytochemical constituents of the aqueous leaf extract of *FT*.

Test	Constituents	Inference
Molisch's	Carbohydrate	+
Frothing	Saponin	+
Lead acetate/Ferric Chloride	Tannins	-
Lieberman Buchard	Steroids	+
Meyer's and Wagner's	Alkaloids	+
Borntrager's	Anthraquinone	-
Sodium hydroxide	Flavonoids	+

Key: + present, - absent.

3.3 Median lethal dose (LD₅₀)

The acute toxicity test of the leaf extract of *Ficus thonningii* in young chicks (Table 2) revealed no mortality following, up to 5000 mg/kg, oral administration of the leaf extract. However, at higher doses (in phase 2), other signs of toxicity such as decreased food intake, drowsiness, loss of activity were observed. Via the intraperitoneal route, LD₅₀ was found to be lower.

Table 2: Median lethal dose (LD₅₀) of the leaf extract of *FT* in young chicks.

Administration Route	Animal Specie	LD ₅₀ (mg/kg)
Oral	Chicks	>5000
IP	Chicks	1265

Substances with LD₅₀ value > 5000mg/kg are considered orally non-toxic (Lorke, 1983).

3.4 Anti-emetic Activity

Chicks in the *placebo* group recorded the highest number of retching (74.4 \pm 5.6). Treatment with the anti-emetic drug, chlorpromazine, was found to significantly reduce the number of retches seen in the placebo group to about 25.6 \pm 4.6 ($p < 0.05$) thus accounting for 65.6% inhibition of retching in chicks. Treatment with 200 and 300 mg/kg doses of FTE, to a similar extent as

chlorpromazine treatment, was found to significantly reduce retching in chicks to 25.4 ± 0.9 and 15.8 ± 2.7 ($p < 0.05$), thus accounting for 65.9% and 78.8% inhibition, respectively. On the other hand, the latency of retching, which was also recorded during this study, was found to be significantly higher in the CPZ as well as all FTE treatment groups ($p < 0.05$) compared to the *placebo* group (Table 3).

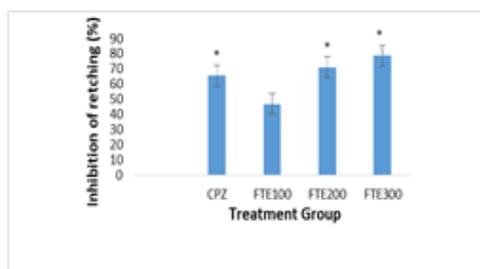


Figure 1: Percentage inhibition of retching in chicks. Data represent mean \pm SEM. * indicates significance compared to placebo ($p < 0.05$, one-way ANOVA). CPZ = Chlorpromazine treatment group; FTE = *Ficus thonningii* leaf extract treatment group.

Table 3: Latency of retching in young chicks treated with leaf extract of *Ficus thonningii*.

Treatment Group	Mean Retches	Retching Latency/mins
Placebo	74.4 \pm 5.6	1.7 \pm 0.3
CPZ	25.6 \pm 4.63*	4.2 \pm 0.6*
FTE100	39.6 \pm 2.13*	3.8 \pm 0.7*
FTE200	21.4 \pm 0.93*	4.3 \pm 0.8*
FTE300	15.8 \pm 2.72**	4.7 \pm 1.0*

Data represent mean \pm SEM. * indicates significance compared to placebo ($p < 0.05$, one-way ANOVA). CPZ = Chlorpromazine treatment group; FTE = *Ficus thonningii* leaf extract treatment group.

3.5 Anti-diarrhoea Activity

The chicks in the *placebo* group recorded the highest mean number of droppings (7.7 ± 1.1) throughout the course of the study, after oral administration of castor oil. Treatment with loperamide was found to significantly reduce the mean number of droppings seen in the placebo group to 2.2 ± 0.72 ($p < 0.05$). Similarly, as shown in Table 4, treatment with all the three doses (100, 200 and 300 mg/kg) of FTE significantly reduced the number of droppings when compared with the placebo group. On the overall, the antidiarrheal activity of the leaf extract of *FT* was observed to increase with increasing doses (dose dependent) as the highest dose of 300 mg/kg showed the same degree of reduction in mean droppings as loperamide treatment (Table 4). On the latency of defaecation (commencement of droppings), except the chicks treated with loperamide, which recorded an onset of 38 mins, the placebo as well as all the FTE treatment groups recorded onset of less than 28 minutes (Figure 2).

Table 4: Mean droppings of chicks treated with leaf extract of *FT*.

Treatment group	Mean number of dropping				
	1 Hr	2 Hr	3 Hr	4 Hr	Mean
Placebo	7.20 \pm 2.85	14.2 \pm 3.02	7.00 \pm 1.30	2.40 \pm 1.44	7.7 \pm 1.10
LMD	1.40 \pm 0.98*	3.00 \pm 1.55*	2.20 \pm 1.36*	2.20 \pm 0.66	2.2 \pm 0.72*
FTE100	4.20 \pm 0.73	8.60 \pm 2.32	3.00 \pm 1.34*	1.00 \pm 0.63	4.2 \pm 0.98*
FTE200	6.20 \pm 2.03	5.40 \pm 1.50*	3.20 \pm 1.46*	2.60 \pm 1.17	4.4 \pm 1.20*
FTE300	3.40 \pm 1.33*	4.00 \pm 1.07*	3.40 \pm 1.01*	2.00 \pm 0.84	3.2 \pm 0.63*

Data represent mean \pm SEM. * indicate significance ($p < 0.05$, one-way ANOVA). LMD = Loperamide treatment group; FTE = *Ficus thonningii* leaf extract treatment group.

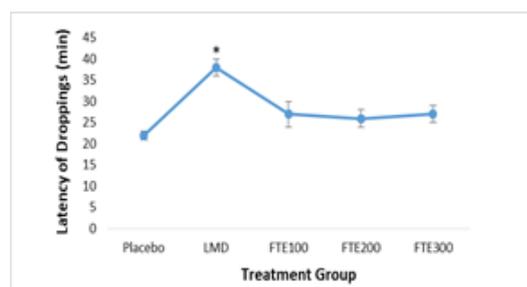


Figure 2: Latency of droppings of chicks treated with leaf extract of *FT*.

Data represent mean \pm SEM. * indicate significance compared to placebo ($p < 0.05$, one-way ANOVA). LMD = Loperamide treatment group; FTE = *Ficus thonningii* leaf extract treatment group.

3.6 Discussion

Preliminary phytochemical screening of *FT* leaf extract in current study revealed the presence of alkaloids, saponins, flavonoids, carbohydrate and steroids. These phytochemical constituents which have been reported by previous studies are known to possess anti-nociceptive, anti-diabetic, anti-inflammatory, anti-oxidant and many more activities [18; 19]. Flavonoid, a phytochemical constituent found in *FTE* leaf extract, has been reported to act against emesis in chicks [20]. In another study, flavonoids and polyphenols were reported to be responsible for the antidiarrheal properties of plant extracts by inhibiting intestinal motility, and water and electrolytes secretion [21; 22]. Thus, the antidiarrheal and anti-emetic activities of the leaf extract of *Ficus thonningii* could be attributed to the presence of flavonoids and phenols.

Acute toxicity test in animals is a very important tool in drug development. It provides valuable information on the toxicity of a substance that can be used in its risk assessment [23]. In current study, the LD_{50} of *FT* leaf extract was found to be greater than 5000 mg/kg. Any product with LD_{50} greater than 5 g/kg is of no practical interest in toxicological studies and hence considered safe [15]. Although, side effect such as dizziness, drowsiness and sedation were observed at higher doses, it can be deduced that the extract of *FT* leaves is relatively non-toxic when administered via oral route. This finding may account for the long term history of safety of *Ficus thonningii* use in traditional medicine practice as no case of toxicity following intake of this plant has been reported.

Results of antiemetic study revealed that the leaf extract of *Ficus thonningii* at 100, 200 and 300 mg/kg recorded 46.8%, 71.2% and 76.8% inhibition of retches, respectively, when compared to the placebo group that received normal saline. This outcome showed that treatment with *Ficus thonningii* leaf extract, similar to chlorpromazine treatment, was found to significantly reduce the number of retching induced by copper sulphate ($p < 0.05$) in a dose-dependent fashion [24].

Diarrhoea, as evidenced by increased number droppings, was apparent in the chicks within minutes of castor oil administration. The diarrheal episodes induced by castor oil were significantly reduced following oral administration of 2.5mg/kg loperamide. Similarly, treatment with *FT* leaf extract was observed to significantly reduce the diarrheal episodes induced by castor oil when compared with the *placebo* ($p < 0.05$). Thus, the outcome of anti-diarrhoeal study reveals that *FT* leaf extracts also possess antidiarrhoeal activity. However, a further probe into the efficacy of individual doses of FTE revealed their differential potencies. At the first hour after castor oil administration, only treatment with 300 mg/kg of the extract showed significant anti-diarrhoeal activity compared to the *placebo* ($p < 0.05$) while treatment with lower doses of 200 and 100 mg/kg only showed significant reduction in diarrhoeal episodes ($p < 0.05$) after 2 and 3 hours of castor oil administration, respectively, compared to the *placebo* group. Thus, FTE possess similar pattern of antidiarrheal activity as its anti-emetic activity where its pharmacologic activity increases with increasing dose [24].

The exact anti-diarrhoea and antiemetic mechanism of *FT* leaf extract is not clear. However, since it is able to inhibit copper sulphate-induced retching, which is elicited via peripheral mechanism, it is believed that *FT* leaf extract has peripheral anti-emetic action [25]. On the other hand, with respect to its anti-diarrhoea mechanism, *FT* leaf extract can be deduced to block the diarrhoeal responses elicited by the metabolites of castor oil, ricinoleic acid and perhaps nitric oxide. Ricinoleic acid has been shown to produce diarrhoea through production of inflammatory mediators (prostaglandins and histamines) that initiate a hypersecretory response: irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which stimulate motility and secretion. It's also reported to induce diarrhoea by increasing the volume of intestinal content by

prevention of the reabsorption of Water. Overall, the molecular mechanism may involve activation of Cl⁻ channels, Na⁺ K⁺ ATPase, and stimulation of prostaglandin formation and platelet activating factor [26]. Therefore, *Ficus thonningii* leaf extracts may exert its antidiarrhoeal effect by reverting these mechanisms.

4. Conclusion

Results of the study shows that *Ficus thonningii* leaf extract is relatively non-toxic and possess antidiarrheal and anti-emetic properties that may be attributed to its flavonoid and other phenolic contents. These findings provide convincing evidence that the aqueous leaf extract of *Ficus thonningii* possesses remarkable safety and usefulness in the management of diarrheal and vomiting, thus giving credence to its widespread traditional use as medicine by the local population of Northern Nigeria. However, further studies are required to isolate and characterize the pure bioactive compound(s) as well as to determine its precise mechanisms of action.

Conflict of interest

The authors declare no conflict of interest.

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