

Full Length Research Paper

Evaluation of the gastrointestinal activity of the aqueous root extracts of *Talinum triangulare*

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The effect of the aqueous root extract of *Talinum triangulare* on the gastrointestinal system was evaluated in mice and rats using normal intestinal transit. Castor oil induced diarrhea and castor oil induced enteropooling models. The result obtained showed that, the effects of the aqueous root extract were dose dependent and biphasic in all the models used. The extract (500, 1000 and 2000 mg/kg) produced a significant ($p < 0.05$) dose dependent decrease in propulsion in normal intestinal transit. The extract at 2000 mg/kg produced a significant ($p < 0.05$) decrease in the frequency of defecation, severity of diarrhea and, afforded protection from diarrhea in rats treated with castor oil. Unlike atropine, the aqueous extract (500, 1000 and 2000 mg/kg) significantly ($p < 0.05$) inhibited castor oil induced enteropooling. The extract was found to contain alkaloids, saponins, tannins, combined anthraquinones, cardiac glycosides and phenol when subjected to phytochemical analysis. The extract gave an LD₅₀ of 5514 mg/kg when administered orally and 403 mg/kg when given intraperitoneally.

Key words: *Talinum triangulare*, enteropooling, biphasic, LD₅₀, phytochemical analysis.

INTRODUCTION

Herbal medicines are naturally occurring therapeutic compounds in biological organisms. The use of natural plant substances (botanicals) to treat and prevent illness has existed since prehistoric times and still flourishes today in many societies and cultures with many plants still in common use (Duke, 1998). Herbal medicine, which is also known as folk medicine, is known from every continent, essentially every tribe and, the world health organization estimates that up to 80% of the world's population relies mainly on herbal medicine for primary health care either in part or entirely (BGCI fact sheet).

It is therefore no surprise that they have become very important all over the world. Those plants used by people medicinally may be used as crude extracts which are relatively unpurified products; or purified extracts which are chemically modified yielding a semi-synthetic substance; or to produce a new totally synthetic drug by using a plant derivative as a model (Duke, 1998).

In industrialized countries, plants have contributed to more than 7,000 compounds produced by the

pharmaceutical industry (BGCI fact sheet) and over 25% of common medicines contain at least some compounds obtained from plants. Also, approximately 120 plant-based prescription drugs are in the market, and these drugs come from only 95 different species of plants (Duke, 1998).

The importance of medicinal plants in pharmacology is very crucial because they contain active constituents (that are used in the management of various disease conditions) such as; quinine for malaria, opioid analgesics for cancer pain, NSAIDs for pyrexia, laxatives for constipation, etc which have side effects both on acute and chronic administration. Hence, their study is important to the development of new and safer drugs.

Talinum triangulare (jacq.) Willd. is a herbaceous perennial plant widely grown in tropical regions as a leaf vegetable. It is probably native to tropical America and the crop is grown in West Africa, Southeast Asia and warmer parts of North America and South America. Along with *celosia* species, it is one of the most important leaf vegetable of Nigeria. Common names include; water leaf, leaf ginseng, American ginseng, Surinam purslane, Surinam spinach, Phillipine spinach, Ceylon spinach, Florida spinach, potherb fameflower, Lagos bogi,

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sweetheart, poslen, biala, espinafre de ceilao galaghati grasse, krokot belanda, and kumu manus (Pain, 2001). In Nigeria, *T. triangulare* is known as 'gbure' in Yoraba land and 'nte oka', or ofe bekee in Ibo land (Adodo, 2004).

This research work is designed to investigate the pharmacological effects of *T. triangulare* on the gastrointestinal system. The effect of the aqueous root extract of *T. triangulare* was determined on normal intestinal transit, and its antidiarrhoeal effect was also studied. The acute toxicity test and the phytochemical analysis of the plant extract were also carried out.

MATERIALS AND METHODS

Plant materials

The fresh roots of *T. triangulare* were collected from Abule-ado town in Lagos. The plant was identified by Mr. T. K Odewo of the Forestry Research institute of Nigeria (FRIN) Ibadan, where a voucher specimen is preserved (voucher No FHI 107620).

Experimental animals

Swiss mice (15 to 20 g) and Wistar rats (50 to 150 g) of either sex kept at the Laboratory Animal Centre of the College of Medicine, University of Lagos, and the Nigeria Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria, were used. The animals were maintained under standard environmental conditions and had free access to standard diet (Pfizer feeds Plc. Lagos, Nigeria) and water ad libitum

Other materials

These include glass and rubber funnels, cotton wool, beaker, measuring cylinders conical flask (Pyrex, England), syringes and needles, oral cannula, cages, feeding bottle, hot plate (Binatone Ind. Ltd, China) water bath (Techne (Cambridge limited, England), oven (Griffin and George Ltd. London) weighing Balance (Mettler PM 480 Delta range Blotting paper, knife, scissors, razor blade.

Drugs

Castor oil (Bell sons and Co Ltd, South Port, England); liquid paraffin (New health way Co. limited Lagos); atropine (Sigma chemical Co, St Louis MO, USA).

Chemicals

One percent aqueous hydrochloric acid, ferric chloride, benzene, 10% ammonia solution, aqueous sulphuric acid, pyridine, 2% sodium nitroprusside, 20% sodium hydroxide, acetic anhydride concentrated sulphuric acid, chloroform, glacial acetic acid, 5% ferric cyanide.

Reagents

Distilled water, Dragendorff's reagent, Mayer's reagent, Wagner's reagent, Fehling's solution A and B, Benedict's reagent, and ice

(Teaching Laboratories, Department of Pharmacology, Pharmacognosy and Biochemistry, University of Lagos).

Method

Extraction procedure

The fresh roots of *T. triangulare* were washed with distilled water, air dried and then chopped into smaller bits. The chopped roots (200 g) were boiled in 2 L of distilled water for 45 min. It was left for some hours to cool at room temperature. The solution was filtered and the filtrate was evaporated to dryness in an oven set at a temperature of 40°C. The dried extract was weighed and it gave a yield of 8.5% with reference to the chopped roots. It was then dissolved in distilled water to a concentration of 200 mg/ml and stored at 4°C until required.

Acute toxicity test

Five groups of mice consisting of 5 animals per group were each administered orally with doses of 1250 to 10000 mg/kg of *T. triangulare* extract. In the same manner, the extract (125 to 1000 mg/kg) was administered to another group of mice intraperitoneally. The control mice were given distilled water (1 ml/kg) the mice were closely observed for toxic symptoms and behavioural changes for the first 2 h of administration and mortality recorded within 24 h. The animals were observed for a further 7 days for any signs of delayed toxicity. LD₅₀ was estimated by log dose-probit analysis (Miller and Tainter, 1944).

Normal intestinal transit

Mice that have been fasted overnight but with free access to water were allotted to different treatment groups. The animals were treated orally with extract of *T. triangulare* (50 to 2000 mg/kg). Distilled water (10 ml/kg) was used as control. 30 min after treatment with the extract, the mice were administered a standard charcoal meal (0.2 ml per mouse of a 10% charcoal suspension in 5% gum acacia) intragastrically.

All the animals in each treatment group were sacrificed 30 min after administration of the charcoal meal, and the small intestine immediately isolated. Peristaltic index for each mouse was then expressed as a percentage of the distance traveled by the charcoal meal relative to the total length of the small intestine (Aye-Than et al., 1989).

Castor oil –induced diarrhea

Rats were divided into different groups and then treated with the extract (50 to 2000 mg/kg), Atropine (2 mg/kg) and liquid paraffin (10 ml/kg), one hour before the administration of castor oil (10 ml/kg). The control group was given distilled water (10 ml/kg). All the rats were administered orally. Each rat was kept in a glass funnel the floor of which was lined with blotting paper and observed for 4 h. The parameters observed were: the onset diarrhea, number of wet faeces, total number of faecal output and the total weight of wet stools. A numerical score based on stool consistency was assigned as follows; normal stool = 1; semi solid stool = 2 and watery stool = 3. Calculations were made for the respondent percentages and purging index, the latter by comparison with the control group (Awouters et al., 1978).

Table 1. Results of acute toxicity of *Talinum triangulare* root extract (oral) in mice.

Group	No. of animals	Dose (mg/kg)	Log dose	Mortality	% Mortality	Probit
1	5	Control	-	0/5	0	-
2	5	1250	3.10	0/5	0	-
3	5	2500	3.40	1/5	20	4.16
4	5	5000	3.70	2/5	40	4.75
5	5	10000	4.00	4/5	80	5.84

Table 2. Results of acute toxicity of *Talinum triangulare* root extract (intraperitoneal) in mice.

Group	No. of animals	Dose (mg/kg)	Log dose	Mortality	% Mortality	Probit
1	5	Control	-	0/5	0	-
2	5	125	2.10	0/5	0	-
3	5	250	2.40	1/5	20	4.16
4	5	500	2.70	3/5	60	5.25
5	5	1000	3.00	5/5	100	-

Castor oil induced enteropooling

Rats were divided into different treatment groups. One hour after treatment with extract or distilled water (1 ml/kg), rats received castor oil (2 ml per rat) intragastrically. All drugs were administered orally. The animals were sacrificed one hour later. The entire small intestine was removed after ligation at the pyloric end and ileocaecal junction respectively and weighed. The intestinal contents were expelled into a graduated tube and the volume was measured. The intestine was reweighed and the difference between full and empty intestines was calculated (Robert et al., 1976).

Phytochemical examination of the aqueous extract of *T. triangulare*

Phytochemical analysis was carried out on the aqueous extracts (Itarborne, 1973; Trease and Evans, 1989).

Statistical analysis

Results are expressed as mean \pm standard error of mean (S.E.M.). Student's t- test and analysis of variance (ANOVA) were used to analyze the significance of the results.

RESULTS

Acute toxicity test in mice

The summary of the acute toxicity study carried out on the aqueous root extract of *T. triangulare* in mice using the method of Tainter and Miller (1944) is shown in Tables 1 and 2. There was no death recorded, following oral administration of the aqueous extract at a dose of 1250 mg/kg. However, oral doses of 2500, 5,000 and 10,000 mg/kg caused 20, 40 and 80% mortality respectively (Table 1). Intraperitoneal administration of the extract at a dose of 125 mg/kg caused no death,

while the doses of 250,500 and 1000 mg/kg caused 20, 60 and 100% death, respectively (Table 2). Using SPSS Version 17, probit analysis was performed to determine the LD₅₀ at probit value of 0.5 (Figures 1 and 2). The value of LD₅₀ obtained (for oral route was 5514 mg/kg and for intraperitoneal route was 403 mg/kg).

The behavioural changes observed in the animals after 2 h of extract administration (oral and intraperitoneal routes) were; mouse writhing, mild diarrhea and anorexia. These were observed for all doses used.

Phytochemical analysis

The result obtained after subjecting the aqueous root extract of the *T. triangulare* to phytochemical analysis showed that it contains alkaloids, saponins, tannins, combined anthraquinones cardiac glycosides and phenols. The colour of the extract is dark brown.

Normal intestinal transit

In control animals, the charcoal meal traversed 68.49% of the total length of the small intestine. Low doses of the extract; 50, 100 and 200 mg/kg produced little or no significant ($p < 0.05$) increase in intestinal propulsion relative to control (6.0, 2.69 and 0.07%).

High doses of the extract, 500, 1000 and 2000 mg/kg produced 7.55, 12.26 and 45.07% significant ($p < 0.05$) inhibition of intestinal propulsion relative control.

Castor oil induced diarrhea

Four hours after castor oil administration, the rats in the

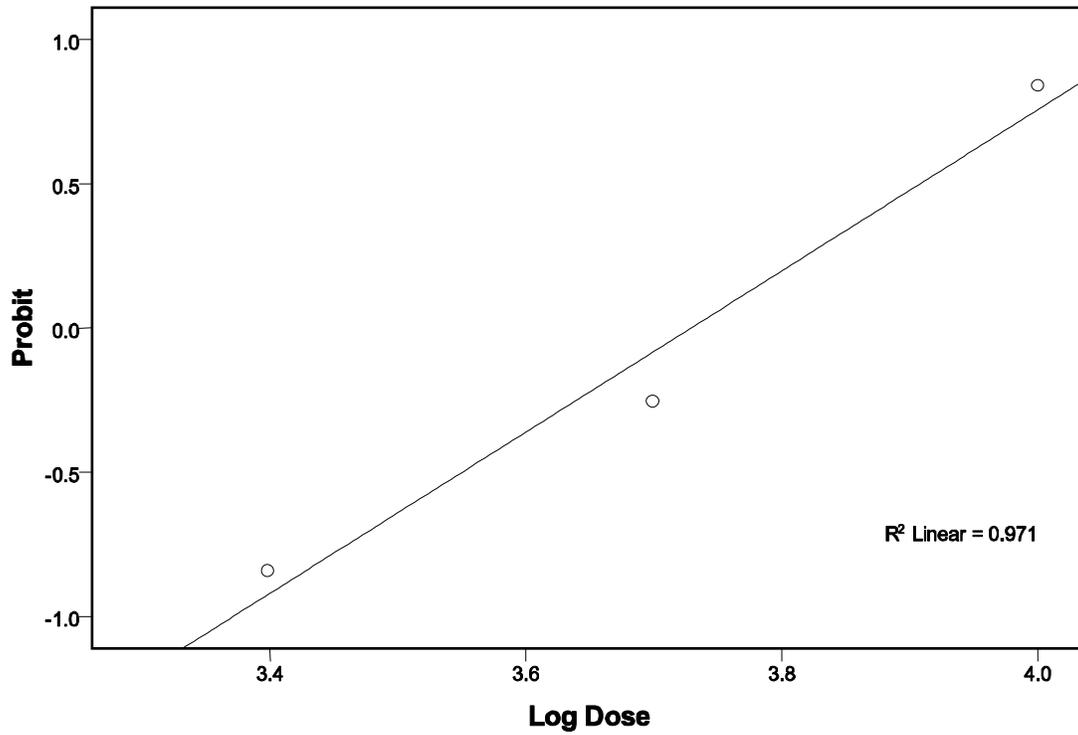


Figure 1. Graph of Probit vs. log dose using SPSS for determination of LD₅₀ (oral).

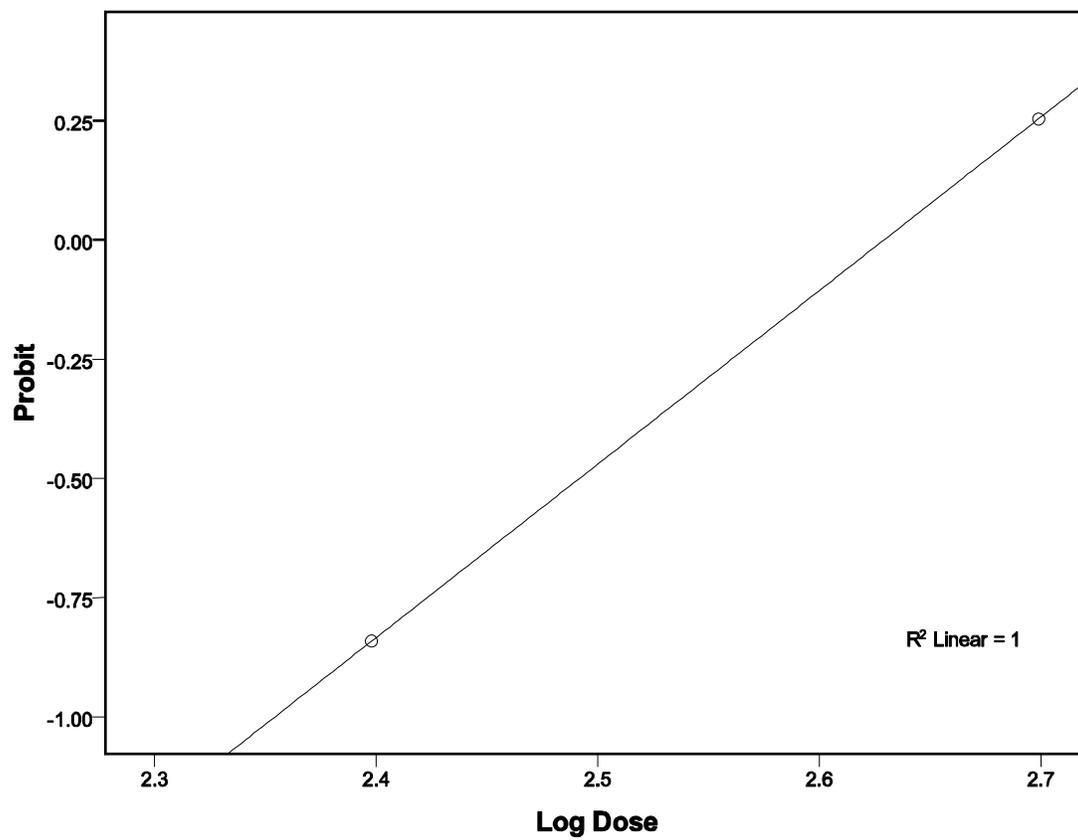


Figure 2. Graph of Probit vs. log dose using SPSS for determination of LD₅₀ (intraperitoneal).

Table 3. Effect of low doses of *Talinum triangulare* root extract on normal intestinal transit.

Group	Dose (mg/kg)	Peristaltic index (%)	Propulsion (%)
Control	-	68.49±1.96	-
Extract	50	72.65±4.76	6.07
	100	70.33±1.93	2.69
	200	68.44±3.68	0.07

Values are mean ± SEM of 6 experiments.

Table 4. Effect of high doses of *Talinum triangulare* root extract on normal intestinal transit.

Group	Dose (mg/kg)	Peristaltic index (%)	Propulsion (%)
Control	-	68.49±1.96	-
Extract	500	63.32±18*	7.55
	1000	60.09±2.85*	12.26
	2000	37.62±5.13*	0.07

Values are mean ± SEM of 6 experiments *P < 0.05 vs. control.

control group had copious diarrhea. Pretreatment of rats with low doses of the aqueous extract (50 to 200 mg/kg) score but significantly ($p < 0.05$) less than the effect produced by liquid paraffin. However, the differences in other diarrhoeal parameters were not dose dependent and not significant. High doses of the aqueous extract (500 to 2000 mg/kg) caused a dose dependent delay in onset of diarrhea, frequency of stooling (reduction in number of stools and total number of wet stools), and total diarrhoeal score. However, only 2000 mg/kg gave a significant ($P < 0.05$) effect compared to control.

Low doses of the aqueous root extract produced no significant ($p < 0.05$) effect on normal intestinal transit (Table 3). High doses of 500, 1000 and 2000 mg/kg of the aqueous root extract produced significant inhibition of intestinal transit (Table 4). Low doses of the aqueous root extract produced no significant ($P > 0.05$) effect on castor oil induced diarrhoeal (Table 5). Only the 2000 mg/kg dose of the aqueous root extract produced a significant inhibition of castor oil induced diarrhoeal but was significantly ($P < 0.05$) less than the effect produced by atropine (Table 6).

Castor oil induced enteropooling

There was a dose dependent and non-significant increase in enteropooling, (that is, weight of intestinal content and volume of intestinal content) by low doses of the extract which was less than the effect of liquid paraffin, which produced a significant ($P < 0.05$ using ANOVA) increase in weight of intestinal content and volume of intestinal content (Table 7). High doses of the extract (500 to 200 mg/kg) produced a dose dependent and significant ($P < 0.05$ ANOVA) decrease in both weight and volume of intestinal content (Table 8). Their individual anti-enteropooling effects was significantly ($P <$

caused a dose dependent but not significant ($p < 0.05$) acceleration in onset of diarrhea and total diarrhoeal 0.05 using ANOVA) higher than that produced by atropine. Low doses of the aqueous root extract produced no significant ($p < 0.05$) effect on castor oil induced enteropooling (Table 7). High doses of 500, 1000 and 2000mg/kg the aqueous root extract produced no significant ($p < 0.05$) effect on castor oil induced enteropooling (Table 8).

DISCUSSION

The aim of the work was to investigate the claims made by traditional herbal practitioners on the effectiveness of the roots of *T. triangulare* on the gastrointestinal system. Several tests were employed in evaluating the effect of the aqueous extract of the plant on gastrointestinal motility.

In the normal intestinal transit as in other test models, the activity of the aqueous root extract was dose dependent and biphasic. Low doses produced a slight laxative effect and the greatest effect was shown by 50 mg/kg dose of the extract, which increased the propulsive movement of the standard charcoal meal in the small intestine by 6.07%. However, the result was not significant ($P < 0.05$) and it cannot be confidently said that, the extract at low doses possess laxative effects.

The result of high doses of the extract on normal intestinal transit showed that, the extract produced a decrease in propulsion movement of the standard charcoal meal in the small intestine, suggesting an antispasmodic activity. This effect was significant ($P < 0.05$) for all the high doses with the highest effect shown by 2000 mg/kg of the extract; which produced 45.07% inhibition of small intestinal propulsion.

Administration of castor oil causes the release of

Table 5. Effect of low doses *Talinum triangulare* root extract on castor oil induced diarrhoea.

Group	Dose (mg/kg)	Onset of diarrhoea (min)	No. of Wet stools	Total no. of stools	Weight of wet stools (g)	Total weight of wet stools (g)	Diarrhoeals core	Propulsion (%)
Control	-	92.0±18.65	5.33±0.9	8.17±1.49	2.67±0.48	3.03±0.47	17.17±1.82	-
Extract	50	87.6±10.5	5.80±1.07	8.00±1.14	2.49±0.17	2.76±0.27	17.60±2.77	2.50
	100	88.2±15.96	4.40±0.81	6.40±1.03	1.52±0.39	1.88±0.41	17.40±1.63	1.34
	200	90.2±12.22	4.80±0.37	8.20±1.02	1.80±0.53	2.28±0.55	17.20±1.07	0.17
Liquid paraffin	10 ml/kg	71.2±10.29	7.80±0.49*	9.80±1.39	2.72±0.18	3.19±0.4	23.40±1.6*	36.28

Values are mean ± SEM of 5-6 experiments *P < 0.05 vs. control.

Table 6. Effect of high doses *Talinum triangulare* root extract on castor oil induced diarrhoea.

Group	Dose (mg/kg)	Onset of diarrhoea (min)	No. of Wet stools	Total no. of stools	Weight of wet stools (g)	Total weight of wet stools (g)	Diarrhoeals core	Propulsion (%)
Control	-	92.0±18.65	5.33± 0.49	8.17±1.49	2.67±0.48	3.03±0.47	17.17±1.82	-
Extract	500	110.8 ±31.44	4.67±1.09	8.83±0.98	1.61±0.46	2.21±0.50	1.65±2.69	3.9
	1000	177.2±32.32	3.80±0.73	7.40±1.5	1.57±0.46	2.10±0.54	13.60±2.6	20.76
	2000	178.0.±28.32*	2.60±0.51*	6.80±1.24	1.42±0.33	1.82±0.46	9.00±1.7*	47.58
Atropine	2	237.6±2.4*	1.00 ±1.0*	1.20±0.97*	0.26±0.26*	0.39±0.26*	2.80±2.56*	83.69

Values are mean ± SEM of 6 experiments *P<0.05 vs control.

Table 7. Effect of low doses of *Talinum triangulare* root extract on castor oil induced enteropooling.

Group	Dose (mg/kg)	Weight of intestinal content (g)	Volume of intestinal content (ml)
Control	-	2.07±0.1	2.02±0.08
Extract	50	2.07±0.16	2.43±0.17
	100	2.40±0.29	2.35±0.26
	200	2.14±0.09	2.00±0.08
Liquid paraffin	10 ml/kg	2.60±0.38	2.53±0.24

Values are mean ± SEM of 5 experiments.

ricinoleic acid. Ricinoleic acid induces changes in mucosal fluid and electrolyte transport that results in a hypersecretory response and diarrhoeal (Ammon et al., 1974; Gagarella et al., 1975). The aqueous root extract at low doses increase the castor oil in-duced intestinal fluid accumulation

(enteropooling) but none of the doses produced a significant (P < 0.05) effect. The standard drug, liquid paraffin, produced a significant (P < 0.05) increase in weight of intestinal content because it retards colonic absorption of water. At high doses, the aqueous extract produced a dose-dependent

and significant (P < 0.05) inhibition of the castor oil induced intestinal fluid accumulation (enteropooling).

However, the intestinal content was more viscous in high doses of the extract-treated rats than low doses of the extract-treated

Table 8. Effect of high doses of *Talinum triangulare* root extract on castor oil induced enteropooling.

Group	Dose (mg/kg)	Weight of intestinal content (g)	Volume of intestinal content (ml)
Control	-	2.07±0.1	2.02±0.08
Extract	500	1.72±0.42*	1.6±0.37*
	1000	1.65±0.37*	1.58±0.36*
	2000	1.40±0.45*	1.43±0.47*
Atropine	2	3.42±0.99*	3.13±0.66*

Values are mean ± SEM of 5 experiments *P < 0.05 using ANOVA.

and control rats. This increased viscosity could have resulted from the effects of the extract on intestinal transit. Atropine did not inhibit castor oil induced enteropooling and gain in weight of intestinal content suggesting that, mediators other than acetylcholine are involved in castor oil induced enteropooling.

Clinically, diarrhoea may result from disturbed bowel function, in which case there is impaired intestinal absorption, excessive intestinal secretion of water and electrolyte, and a rapid bowel transit (Gurgel et al., 2001). Also, drugs affecting motility, frequency and consistency of diarrhoea also affect secretion (Di Carlo et al., 1994). Low doses of the extract only accelerate the onset of diarrhoea and increase the total diarrhoea score in a dose-dependent manner non-significantly ($P < 0.05$) without any pattern of difference in other diarrhoea.

However, the high doses of the extract produced a dose dependent reduction in the frequency and severity of diarrhoea produced by castor oil.

The total number of stools, number of wet stools, weight of wet stools and total weight of stools, were all decreased in a dose-dependent manner with the highest and significant effects ($P < 0.05$) observed at 2000 mg/kg of the extract. However, the effects of the extract on these diarrhoea parameters were significantly ($P < 0.05$) less than those produced by atropine. The general diarrhoea score of the extract (2000 mg/kg) was 47.58% compared to atropine (2 mg/kg, 83.69%). In this study, atropine produced a significant reduction in the number of stools due to its anti cholinergic effects (Brown and Taylor, 2001). Liquid paraffin produced 36.20% propulsion in general diarrhoea score because; it retards intestinal absorption of faecal water.

Administration of the extract orally, produced toxic effects with an LD₅₀ of 5514 mg/kg. Also, the intraperitoneal route produced toxic effects with an LD₅₀ of 403 mg/kg. This result shows that, the aqueous extract is better tolerated when administered orally than through the intraperitoneal route. It is relatively safe through the oral route

Conclusion

In conclusion, the investigation carried out proved that, the aqueous root extract of *T. triangulare* does not

possess any significant laxative effect but has antidiarrhoeal effect in doses of 500 to 2000 mg/kg. This may account for its use in traditional medicine as an antidiarrhoeal agent. However, further tests need to be carried out to determine the mechanism of action of the extract and *in vitro* effect.

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