

Full Length Research Paper

Anti-diarrhoeal potential of the ethanol extract of *Gongronema latifolium* leaves in rats

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The leaves of *Gongronema latifolium* is used in Nigeria for the treatment of diarrhoea and thus, the phytochemical constituents as well as the acute toxicity of the ethanol extract of the leaves of *G. latifolium* and its effects [at graded doses of 200, 400 and 600 mg/kg body weight (b.w)] against castor oil-induced diarrhoea models in rats were evaluated. The parameters used for the evaluation of the castor oil-induced diarrhoea were: reductions in the wetness of faeces and rate of defaecation. To further understand the probable mechanisms of its anti-diarrhoeal action, its effects were evaluated on gastro-intestinal motility and castor oil-induced enteropooling. The phytochemical screening of the ethanol extract of the leaves of *G. latifolium* revealed the presence of saponins, tannins, alkaloids, flavonoids, glycosides and cardiac glycosides. The ethanol extract of the leaves of *G. latifolium* at the tested doses caused significant ($p < 0.05$) dose-dependent reductions of castor oil-induced diarrhoea, gastro-intestinal motility and castor oil-induced enteropooling in the treated rats. The results were comparable with those of the standard anti-diarrhoeal drug, atropine sulphate (2.5 mg/kg b.w). The extract was found to be non-toxic even at a dose as high as 5000 mg/kg b.w. The results indicate that the ethanol extract of the leaves of *G. latifolium* contains compounds with anti-diarrhoeal effect and may possibly originate an anti-diarrhoeal drug in time to come.

Key words: *Gongronema latifolium*, diarrhoea, castor oil and atropine sulphate.

INTRODUCTION

Diarrhoea is defined by the World Health Organisation as having three or more watery or loose bowel movements in a 24 h period (WHO, 2009). However, it is the consistency of the stools rather than the number that is most important. Frequent passing of formed stools is not diarrhoea. Diarrhoea is the frequent passage of loose, watery or soft stools with or without abdominal bloating, pressure and cramps commonly referred to as gas. It can come on suddenly, run its course and be helped with home care to prevent complications such as dehydration. Diarrhoeal infections are a leading cause of death worldwide and continue to take a high public health toll

especially on children. It is estimated that 3.3 million deaths occur each year among children under 5 years old. In Nigeria, diarrhoeal infection remains the number one killer disease among children under 5 years while 7 to 12 month old babies continue to be the most susceptible (Adu et al., 2000). Nigeria, the fourth largest economy in Africa with an estimated per capita income of \$350 has over half of its population living in poverty (WHO, 2009). This implies that very few people can afford orthodox medicine as panacea to their problems. In light of the above, there lies the need to search for plants with anti-diarrhoeal potentials so they could be

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Figure 1. *Gongronema latifolium* (Benth).

used to abate the death rate orchestrated by diarrhoea.

Gongronema latifolium (Benth) (Figure 1) (Asclepiadaceae) is a herbaceous shrub with yellow flowers and stem that yields characteristic milky exudates when cut. It is a climber with tuberous base; it is also found in deciduous forests from Guinea Bissau to Western Cameroon and widely dispersed elsewhere in tropical Africa. The plant is perennial, edible and has stems which are soft and pliable. Various parts of this plant particularly the stems and leaves are used as chewing sticks or liquor in places such as Sierra Leone (Akuodor et al., 2010). It is widely used in West African sub-region for a number of medicinal and nutritional purposes (Dalziel, 1937). It has been widely used in folk medicine as a spice and as a vegetable (Morebise et al., 2002). In Sierra Leone, a decoction or cold infusion of the pounded stem is used for colic and intestinal symptoms usually associated with worms (Deighton, 1957). In eastern states of Nigeria, the plant locally known as *Utazi* is a popular spice. The leaves are used to prepare food for mothers that have recently put to bed as it is believed to stimulate appetite, reduce post-partum contraction and quicken the return of menstrual cycle (Nwanjo et al., 2006). Previous scientific investigation has shown that *G. latifolium* possesses hypoglycaemic effect (Ugochukwu and Badaby, 2003). Anti-microbial activities of the leaf extracts had also been reported (Eleyinmi et al., 2006; Eleyinmi, 2007; Nwinyi et al., 2008). Morebise et al. (2002) showed that the leaf extract had anti-inflammatory property while its potential anti-ulcer, analgesic and anti-pyretic properties were investigated by Akuodor et al. (2010). According to Iwu (1993) as cited by Etta et al. (2012), the plant leaves (*G. latifolium*) have been found

very efficacious as an anti-diarrhoea and anti-tussive. Presently, there are minutiae of information on the scientific justification for the use of *G. latifolium* leaves in the treatment of diarrhea traditionally. Therefore, the objectives of this research work were to evaluate the phytochemicals, acute toxicity and anti-diarrhoeal effect of the ethanol extract of the leaves of *G. latifolium* in normal and castor oil-induced diarrhoeal rats so as to provide the scientific basis for its application in folk medicine and for possible drug development.

MATERIALS AND METHODS

Plant

Fresh and apparently uninfected *G. latifolium* leaves were purchased from Ogige market, Nsukka, Enugu State, Nigeria. The botanical identification of the leaves was done at the Department of Botany, University of Nigeria, Nsukka.

Preparation of the extract

Fresh leaves of *G. latifolium* were washed with distilled water and spread on a clean mat in a well-ventilated room with regular turning to enhance even drying and avoid decaying. The leaves were shade-dried for three weeks and homogenised into fine particles using an electric blender. A known weight (500 g) of the ground leaves was macerated in 70% ethanol for 72 h at room temperature with constant shaking. The mixture was thereafter, filtered, concentrated in a rotary evaporator, dried in a boiling water bath and weighed.

Phytochemical analyses

Qualitative phytochemical analyses were carried out on the ethanol extract of the leaves of *G. latifolium* according to the procedures outlined by Harborne (1998) and Trease and Evans (1989).

Animals

A total of 90 adult male Wistar rats of (6 to 10) weeks old with average weight of 110 ± 30 g and 24 albino mice weighing 30 ± 5 g were obtained from the Animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The rats were acclimatised for one week under a standard environmental condition with a 12 h light and dark cycle and maintained on a regular feed and water *ad libitum*. The principles of laboratory animal care were followed. The University Animal Research Ethical Committee approved the experimental protocol used.

Chemicals and reagents

The chemicals used for this study were of analytical grade and procured from reputable scientific shops at Nsukka. They included the following: atropine sulphate [standard anti-diarrhoeal drug (Sigma-Aldrich, Inc., St. Louis, USA)], 45% (v/v) ethanol (BDH Chemicals Ltd., Poole, England), dilute tetraoxosulphate (vi) acid, 2% (v/v) hydrochloric acid, 1% (w/v) picric acid, methyl orange, activated charcoal, gum acacia, castor oil (laxative), normal saline (vehicle for dissolving the extract), Dragendorff's reagent, Mayer's

reagent, Wagner's reagent, Fehling's solution, 5% (w/v) ferric chloride solution, aluminium chloride solution, lead sub acetate solution and ammonium solution.

Acute toxicity study

The acute toxicity and lethality (LD_{50}) of the ethanol extract of the leaves of *G. latifolium* was determined using mice according to the modified method of Lorke (1983).

Castor oil-induced diarrhoea test

Castor oil-induced diarrhoea was evaluated using the method of Awouters et al. (1978) with slight modification.

Gastro-intestinal motility test

Gastro-intestinal motility was evaluated using the method of Mascolo et al. (1994).

Castor oil-induced enteropooling test

Castor oil-induced enteropooling was determined by the method of Robert et al. (1976).

Statistical analysis

The data obtained from the laboratory tests were subjected to one way analysis of variance (ANOVA). Significant differences were observed at $p \leq 0.05$. The results were expressed as means of five replicates \pm standard deviations (SD). This analysis was done using the computer software known as Statistical Package for Social Sciences (SPSS), version 18.

RESULTS

Qualitative phytochemical composition of the ethanol extract of the leaves of *Gongronema latifolium*

The qualitative phytochemical analyses showed the presence of tannins and flavonoids in very high concentration (Table 1). Saponins, alkaloids and cardiac glycosides were found to be present in moderately high concentration. Glycosides were found to be present in low concentration. Resins and acidic compounds were not detected.

The acute toxicity and lethality (LD_{50}) of the ethanol extract of the leaves of *G. latifolium*

The result of this investigation shows that there was no lethality or any sign of toxicity in the four groups of three mice each that received 10, 100, 1000 mg/kg body weight of the ethanol extract of the leaves of *G. latifolium*

and 5 ml/kg body weight of normal saline, respectively, at the end of the first phase of the study. At the end of the second phase of the study, there was neither death nor obvious sign of toxicity in the groups of mice that received 1900, 2600 and 5000 mg/kg body weight of the ethanol extract of the leaves of *G. latifolium*.

Effect of the ethanol extract of the leaves of *G. latifolium* on castor oil-induced diarrhoea in terms of the wetness of faeces

The result of the castor oil-induced diarrhoea experiment (wetness of faeces test), shows that the rats in the group that received normal saline only (group 1) had significantly ($p < 0.05$) decreased numbers of wet faeces (0.00 ± 0.00 , 0.00 ± 0.00 , 0.25 ± 0.50 and 0.00 ± 0.00) at the first, second, third and fourth hours of post-treatment, respectively, when compared to the values (1.50 ± 0.58 , 2.00 ± 0.63 , 2.50 ± 1.41 and 1.50 ± 1.00) obtained for rats in the castor oil-treated control group (group 2) as shown in Table 2. The extract at the tested doses (200, 400 and 600 mg/kg body weight) each in a similar manner as the standard anti-diarrhoeal agent (atropine sulphate), significantly ($p < 0.05$) inhibited the wetness of faeces of rats in groups 4, 5 and 6 as evidenced by the significant ($p < 0.05$) reductions in the numbers of wet faeces of rats in groups 4 (1.00 ± 0.00), 5 (1.00 ± 0.41) and 6 (0.75 ± 0.58), at the fourth hour of post treatment when compared to the value (1.50 ± 1.00) obtained for rats in the castor oil-treated control group (group 2). The extract at the tested doses decreased, in a dose-related manner the wetness of faeces of rats in groups 4, 5 and 6 at the first, second, third and fourth hours of post treatment when compared to those of the rats in group 2. There were however, no significant ($p > 0.05$) differences between the numbers of wet faeces of rats in groups 4, 5 and 6 and that of the rats in the castor oil-treated control group (group 2) at the first hour of post treatment as shown in Table 2.

Effect of the ethanol extract of the leaves of *G. latifolium* on castor oil-induced diarrhoea in terms of the rate of defaecation

Castor oil treatment caused significant ($p < 0.05$) increases in the numbers of stools of the rats in the castor oil-treated control group (group 2) [3.50 ± 0.58 , 3.00 ± 0.58 , 2.75 ± 0.96 and 2.50 ± 0.35] at the first, second, third and fourth hours of post treatment, respectively, when compared to the values (1.50 ± 0.25 , 1.00 ± 0.10 , 0.50 ± 0.33 and 1.00 ± 0.50) obtained for rats in group 1 (group treated with normal saline only) as shown in Table 3. The extract at the doses of 400 and 600 mg/kg body weight each in a similar manner as the standard anti-diarrhoeal

Table 1. Qualitative phytochemical constituents of the ethanol extract of the leaves of *G. latifolium*.

Phytochemical constituent	Inference
Saponins	++
Tannins	+++
Alkaloids	++
Flavonoids	+++
Glycosides	+
Resins	ND
Cardiac glycosides	++
Acidic compounds	ND

ND, Not detected; +, present in low concentration; ++, present in moderately high concentration; +++ = present in very high concentration.

Table 2. Effect of the ethanol extract of the leaves of *G. latifolium* on the wetness of faeces.

Group	Treatment	Number of wet faeces after the			
		1 st hour	2 nd hour	3 rd hour	4 th hour
1	5 ml/kg of normal saline (vehicle) only	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.25 ± 0.50 ^a	0.00 ± 0.00 ^a
2	Vehicle + 1 ml per oral (p.o) of Castor oil (CO)	1.50 ± 0.58 ^b	2.00 ± 0.63 ^b	2.50 ± 1.41 ^b	1.50 ± 1.00 ^b
3	2.5 mg/kg of atropine sulphate + 1 ml p.o of CO	0.75 ± 0.50 ^c	0.75 ± 0.50 ^c	1.00 ± 0.00 ^c	0.50 ± 0.57 ^c
4	200 mg/kg of the ethanol leaf extract + 1 ml p.o of CO	1.50 ± 0.12 ^b	1.75 ± 0.51 ^b	2.00 ± 0.79 ^b	1.00 ± 0.00 ^d
5	400 mg/kg of the ethanol leaf extract + 1 ml p.o of CO	1.50 ± 1.00 ^b	1.75 ± 0.42 ^b	1.50 ± 0.29 ^d	1.00 ± 0.41 ^d
6	600 mg/kg of the ethanol leaf extract + 1 ml p.o of CO	1.00 ± 0.82 ^b	1.00 ± 0.00 ^c	1.25 ± 0.50 ^d	0.75 ± 0.58 ^d

Values carrying superscripts different from those of the controls for each hour are significantly different (p<0.05).

Table 3. Effect of the ethanol extract of the leaves of *G. latifolium* on the rate of defaecation.

Group	Treatment	Number of stools after the			
		1 st hour	2 nd hour	3 rd hour	4 th hour
1	5 ml/kg of normal saline (vehicle) only	1.50 ± 0.25 ^a	1.00 ± 0.10 ^a	0.50 ± 0.33 ^a	1.00 ± 0.50 ^a
2	Vehicle + 1 ml per oral (p.o) of Castor oil (CO)	3.50 ± 0.58 ^b	3.00 ± 0.58 ^b	2.75 ± 0.96 ^b	2.50 ± 0.35 ^b
3	2.5 mg/kg of atropine sulphate + 1 ml p.o of CO	1.50 ± 0.50 ^a	1.25 ± 0.82 ^a	1.00 ± 0.38 ^c	0.50 ± 0.58 ^c
4	200 mg/kg of the ethanol leaf extract + 1 ml p.o of CO	3.25 ± 0.96 ^b	2.75 ± 0.96 ^b	2.75 ± 0.50 ^b	2.25 ± 0.58 ^b
5	400 mg/kg of the ethanol leaf extract + 1 ml p.o of CO	3.00 ± 0.35 ^b	2.50 ± 0.58 ^b	1.50 ± 0.10 ^c	1.50 ± 0.35 ^c
6	600 mg/kg of the ethanol leaf extract + 1 ml p.o of CO	2.50 ± 0.29 ^b	1.50 ± 0.50 ^a	1.50 ± 0.27 ^c	1.00 ± 0.50 ^a

Values carrying superscripts different from those of the controls for each hour are significantly different (p<0.05).

agent (atropine sulphate), significantly (p<0.05) inhibited the rate of defaecation of rats in groups 5 and 6 as evidenced by the significant (p<0.05) reductions in the numbers of stools of rats in groups 5 (1.50 ± 0.35) and 6 (1.00 ± 0.50), at the fourth hour of post treatment when compared to the value (2.50 ± 0.35) obtained for rats in the castor oil-treated control group (group 2). The extract at the tested doses decreased, in a dose-dependent

manner, the rate of defaecation of rats in groups 4, 5 and 6 at the first, second, third and fourth hours of post treatment when compared to those of the rats in group 2. There were however, no significant (p>0.05) differences between the numbers of stools of rats in groups 4, 5 and 6 and that of the rats in the castor oil-treated control group (group 2) at the first hour of post treatment as shown in Table 3.

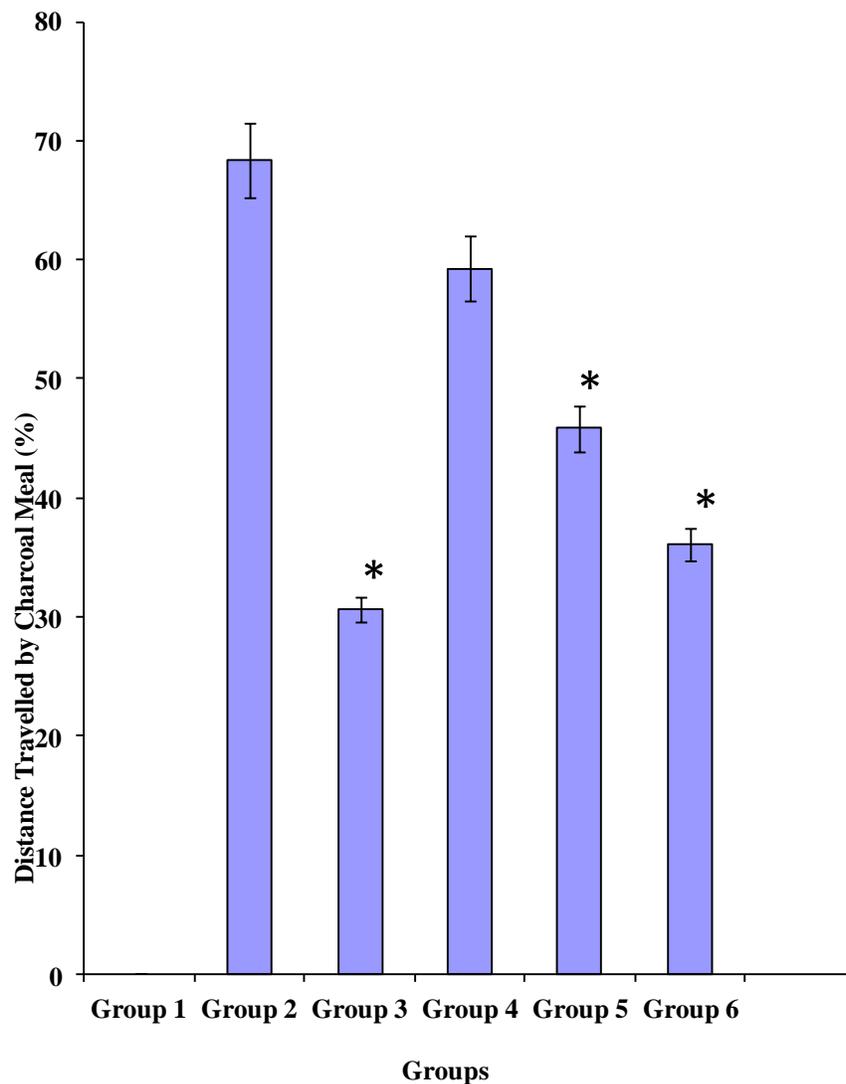


Figure 2. Effect of the ethanol extract of the leaves of *G. latifolium* on the gastro-intestinal motility. Data are represented as means \pm SD; * = significantly ($p < 0.05$) lower compared to group 2.

Effect of the ethanol extract of the leaves of *G. latifolium* on gastro-intestinal motility

The gastro-intestinal motility test was used to determine the propulsive movement along the gastro-intestinal tract (GIT) of rats. As shown in Figure 2, the extract at the doses of 400 and 600 mg/kg body weight, each significantly ($p < 0.05$) decreased the percentage distance travelled by the charcoal meal along the gastro-intestinal tract of rats in groups 5 and 6 when compared to the value obtained for rats in the charcoal meal-treated control group (group 2). The observed effects were dose-dependent with percentage distance travelled by charcoal meal as 59.25 ± 2.73 , 45.85 ± 1.96 and 36.10 ± 1.38 for

rats in groups 4, 5 and 6, respectively, when compared to the value (68.35 ± 3.13) obtained for rats in the charcoal meal-treated control group (group 2). The effects of the 400 and 600 mg/kg body weight of the extract were comparable to that of the standard anti-diarrhoeal agent (atropine sulphate) as shown in Figure 2.

Effect of the ethanol extract of the leaves of *G. latifolium* on castor oil-induced enteropooling in terms of the weight of intestinal contents

Figure 3 shows that castor oil induced significant ($p < 0.05$) increase in the weight of the intestinal contents of rats in

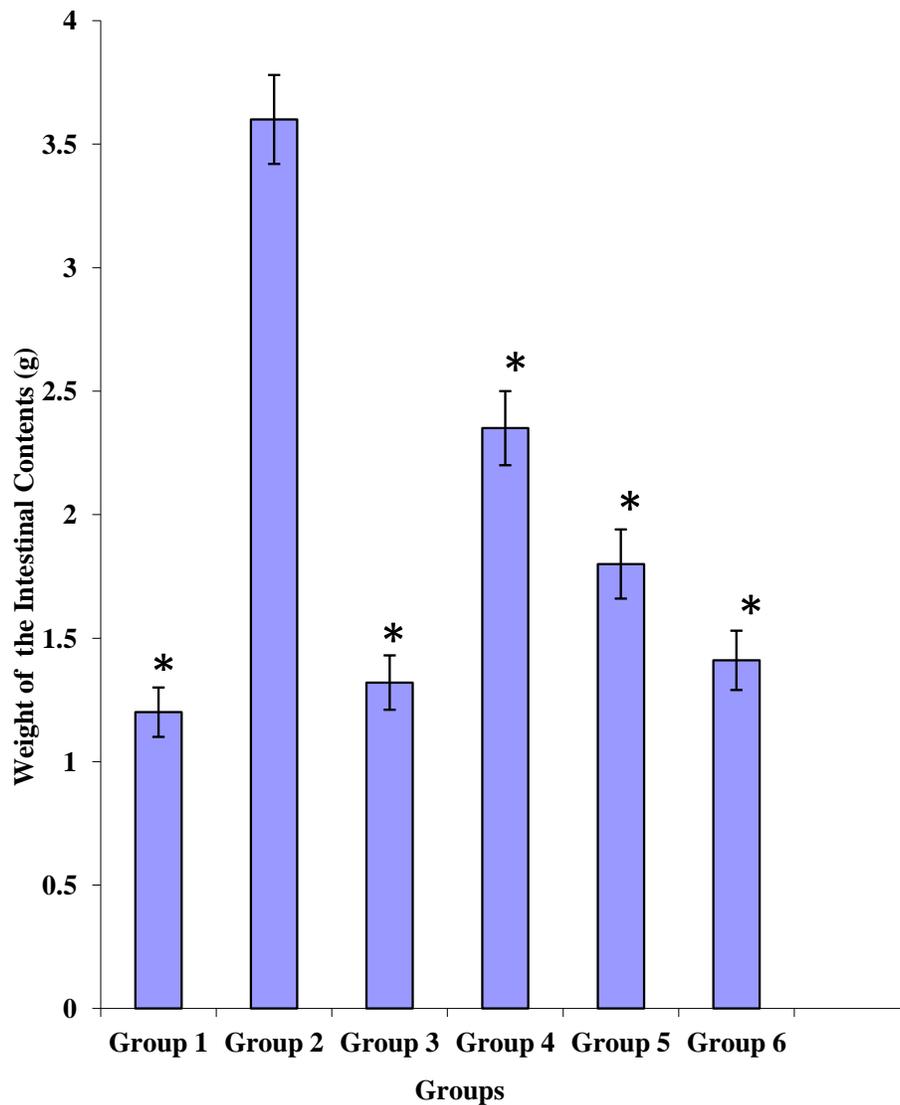


Figure 3. Effect of the ethanol extract of the leaves of *G. latifolium* on the weight of intestinal contents. Data are represented as means \pm SD; *significantly ($p < 0.05$) lower compared to group 2.

group 2 (3.60 ± 0.18) when compared to the value obtained for rats in group 1 (1.20 ± 0.10) which received normal saline only. The standard anti-muscarinic drug, atropine sulphate (2.5 mg/kg body weight) caused significant ($p < 0.05$) reduction in the weight of the intestinal contents of rats in group 3 (1.32 ± 0.11) when compared to the value (3.60 ± 0.18) obtained for rats in the castor oil-treated control group (group 2). The extract, at the tested doses, significantly ($p < 0.05$) and dose-dependently reduced the weight of the intestinal contents of rats in groups 4, 5 and 6 when compared to that of the rats in the castor oil-treated control group (group 2). The effects of the extract doses were comparable to that obtained with the anti-muscarinic drug (Figure 3).

Effect of the ethanol extract of the leaves of *G. latifolium* on castor oil-induced enteropooling in terms of the volume of intestinal contents

As shown in Figure 4, castor oil induced significant ($p < 0.05$) increase in the volume of the intestinal contents of rats in group 2 (3.30 ± 0.20) when compared to the value obtained for rats in group 1 which received normal saline only (0.84 ± 0.08). The standard anti-diarrhoeal agent, atropine sulphate (2.5 mg/kg body weight) caused significant ($p < 0.05$) reduction in the volume of the intestinal contents of rats in group 3 (1.00 ± 0.09) when compared to the value (3.30 ± 0.20) obtained for rats in the castor oil-treated control group (group 2). The extract,

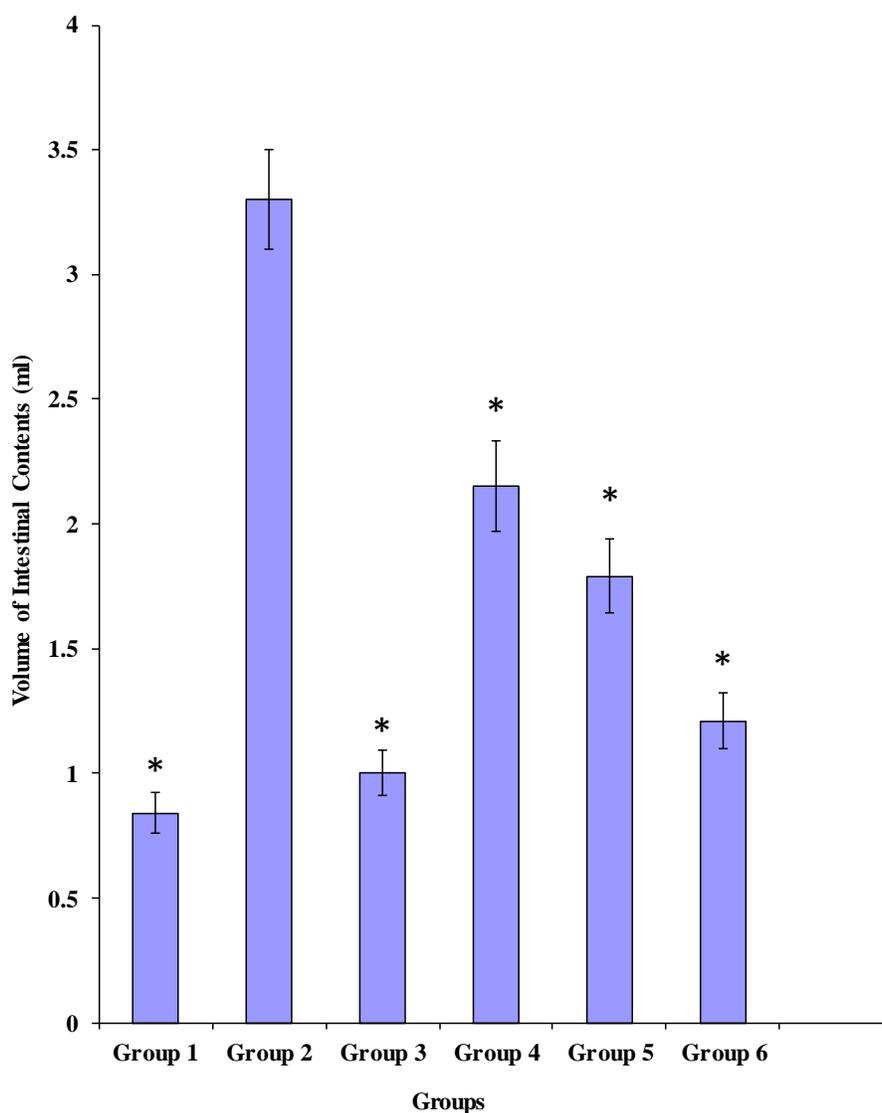


Figure 4. Effect of the ethanol extract of the leaves of *G. latifolium* on the weight of intestinal contents. Data are represented as means \pm SD; * = significantly ($p < 0.05$) lower compared to group 2.

at the tested doses, significantly ($p < 0.05$) and dose-dependently reduced the volume of the intestinal contents of rats in groups 4, 5 and 6 when compared to that of the rats in the castor oil-treated control group (group 2). The effects of the extract doses were also comparable to that obtained with the anti-diarrhoeal drug (Figure 4).

DISCUSSION

The phytochemical analyses of the leaves of *G. latifolium* in the present study show that they contain saponins,

tannins, alkaloids, flavonoids, glycosides and cardiac glycosides. Flavonoids are known to modify the production of cyclo-oxygenase 1 and 2 and lipo-oxygenase (Moroney et al., 1988) thereby inhibiting prostaglandin production. Earlier studies have also reported that the anti-diarrhoeal activity of medicinal plants may be due to alkaloids, saponins, tannins, sterols and reducing sugar (Galvez et al., 1993; Longanga et al., 2000). Tannins denature proteins in the intestinal mucosa by forming protein tannates which make intestinal mucosa more resistant to chemical alteration and reduce secretion (Sule et al., 2009). Therefore, it is most likely that these constituents either singly or in combination,

contributed to the anti-diarrhoeal effect of the extract.

Acute toxicity test on the ethanol extract of the leaves of *Gongronema latifolium* using mice showed an LD₅₀ value of greater than 5000 mg/kg body weight which indicates that the leaves of *G. latifolium* might be regarded as being safe with no risk of acute toxicity. Its high degree of safety is also consistent with its popular use as a local spice in food.

The ethanol extract of the leaves of *G. latifolium* significantly and dose-dependently, decreased the castor oil induced-diarrhoea with the 600 mg/kg body weight of the extract being the most effective. The extract might have exerted its anti-diarrhoeal action via anti-secretory mechanism which was evidenced by the reductions of total number of wet faeces and the rate of defaecation. This, in part, implies that the leaves of *G. latifolium* possess anti-diarrhoeal effect. It is possible that the extract was able to exert its anti-diarrhoeal effect through the inhibition of electrolyte permeability in the intestine and/or prostaglandins release. Prostaglandins are implicated in the patho-physiology of diarrhoea (Haruna et al., 1997). Diarrhoea induced by castor oil, occurs when there is hydrolysis of the oil by intestinal lipases resulting in the release of ricinoleic acid. The ricinoleic acid released produces an irritating reaction on the wall of the intestine thus enhancing the peristaltic activity of the small intestine. Also, ricinoleic acid like other anionic surfactants, reduce the net absorption of water and electrolytes (Almeida et al., 1995). The ricinoleic acid is also associated with endogenous stimulation of prostaglandins release (Zavala et al., 1998). No investigation thus far, had been carried out on the effect of *G. latifolium* on diarrhoea however, but a study on the anti-diarrhoeal activity of methanol extract of *Saccharum spontaneum* is consistent with our findings (Mynol et al., 2008).

Pre-treatment with the extract, reduced the movement or transit of the charcoal meal along the gastro-intestinal tract which apparently, indicates that, the extract may be capable of reducing the frequency of stooling in diarrhoeal conditions. Delay in gastro-intestinal motility might have caused further absorption of water from faeces and might have additionally, contributed to the reduction of its watery texture. It is likely, that the extract reduced gastro-intestinal motility through anti-cholinergic effect. Anti-cholinergic agents are known to inhibit gastro-intestinal hyper-motility (Ezenwali et al., 2009). This parallels the report of Anup et al. (2007).

The extract remarkably decreased the intestinal fluid accumulation (enteropooling) in terms of the weight and volume of the intestinal contents. These effects which are direct consequences of reduced water and electrolytes secretion into the small intestine imply that the extract may be capable of enhancing electrolyte absorption from the intestinal lumen. However, since electrolyte absorption determines the efficiency of nutrient absorption

(Duggan et al., 2002), it is likely that the enhanced electrolyte absorption by the extract may have encouraged the absorption of other intestinal contents. Our finding in this regard, is also not at variance with that of Anup et al. (2007).

In conclusion, the above observations are indicative of the fact that the leaves of *G. latifolium* as employed in traditional medicine, reduce diarrhoea by decreasing the wetness of faeces, rate of defaecation, castor oil induced enteropooling and gastro-intestinal motility. This study, therefore, lend pharmacological credence to the ethno-medicinal claim of the use of the leaves of *G. latifolium* in the management of diarrhoea.

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