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Original Research Article

Anti-Diarrhoeal Properties of the Ethanol Extract of *Terminalia glaucescens* Roots on Castor Oil-Induced Diarrhoea in Wistar Rats

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ABSTRACT

Terminalia glaucescens (family *Combretaceae*) is an indigenous tropical plant in Africa whose leaves are used in ethnomedicine for the management of dysentery, diabetes and microbial infections. The study evaluated the potentials of *Terminalia glaucescens* root extract for the management of diarrhoea using standard experimental animal model. The extract was evaluated for its acute toxicity (with 18 mice), phytochemical compositions and anti-diarrhoeal properties. Diarrhoea was induced with castor oil (1 mL) orally. Anti-diarrhoeal property of the extract was carried out in four (4) study protocols: Curative, Preventive, gastrointestinal motility test and enteropooling test. No death was recorded in animals that received extract up to 5000 mg/kg body weight. Phytochemical analyses recorded the presence of saponins, tannins, flavonoids, alkaloids, terpenoids, phenols and steroids. Preventive and curative studies showed significant reduction ($p < 0.05$) in number and frequency of wet stool in animals treated with the extract at all doses, except at 100 mg/kg body weight for curative studies (non-significant reduction; $p > 0.05$), relative to untreated controls. Also, administration of the extract to experimental animals significantly reduced ($p < 0.05$) distance travelled by charcoal meal (gastrointestinal motility) and volume of intestinal content at all doses relative to the untreated control. The extract at 400 mg/kg body weight compared favorably with the standard drug (Loperamide). The study highlighted the ethanol extract of the roots of *Terminalia glaucescens* as a promising and potent remedy for the ethnomedicinal management of diarrhoea.

Keywords: Anti-diarrhoeal, *Terminalia glaucescens*, Gastric motility, Enteropooling, phytochemical screening.

Introduction

The use of plants in various regions of the world, traditionally, as dependable sources of treatment for various diseases has been documented.[1] Such use has considerably contributed to primary healthcare delivery in the light of their knowledge as fundamental sources of products with therapeutic properties.[2] Diarrhoea is a disorder associated with the gastrointestinal tract which is characterized by increased frequency of stooling, alterations in stool consistency and bowel movement leading to an uncontrolled loss of fluid, nutrients and electrolytes.[3] It is a common public health challenge and a major cause of mortality and morbidity across various age cadres in non-industrialized countries with poor standard of hygiene.[3]

Terminalia glaucescens is an African indigenous plant belonging to the *Combretaceae* family. Its application in the treatment of diabetes has been reported in Cameroon.[4] In Nigeria, it is widely employed in the production of chewing stick due to its documented properties as an antimicrobial agent against oral disease-causing microbes.[5] Rahman and Choudhary reported the use of *Terminalia* species (leaves) for the management of dysentery; the leaf extracts has also been proven to be useful in the management of acquired immune deficiency syndrome at the later stage.[6,7] Its anti-plasmodial activity has also been reported.[8]

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However, there is no report, yet, on the use of the roots extract of *Terminalia glaucescens* as a remedy in the management of diarrhoea amidst the claim of its use in traditional medicine for management of diarrhoea. The study evaluated the antidiarrhoeal effect of the ethanol extract of the roots of *Terminalia glaucescens* on castor oil-induced diarrhoea in Wistar rats to provide a scientific basis for its traditional use. The toxicity and phytochemical profile are reported in tandem.

Materials and Methods

Plant sample and extraction procedure

Fresh roots of *Terminalia glaucescens* were collected from Agbo-okoh in Okpuje, Nsukka Local Government Area of Enugu State, Nigeria on 14th May, 2019. The plant was identified by a taxonomist at Bioresources Development and Conservation Programme (BDCP) Research Centre, Nsukka, Enugu State of Nigeria. Voucher specimen (#Interceed/0112) was deposited at the herbarium for references purposes. A laboratory miller was used to pulverize sun-dried root samples. The pulverized roots (500 g) was macerated in 1500 mL of absolute ethanol (BDH) with occasional shaking at consistent intervals for 72 h at room temperature. Filtration was done using muslin cloth and Whatman No. 1 filter paper and concentrated using rotary evaporator at a temperature of 30°C to obtain the ethanol extract of *Terminalia glaucescens* root (EETGR). EETGR was stored in the refrigerator and used for the study.

Experimental animals

Eighty (80) Wistar rats (male and female) used for the study were obtained from the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. The animals were acclimatized for seven days and maintained under standard laboratory environment. The animals were kept in well-ventilated cages and fed with standard animal feed (Supreme finisher feeds) and clean water.

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The animals were handled according to the guidelines of the National Institute of Health on the care and use of laboratory animals (NIH, 1985). The research was approved by the Ethical Committee on the Use of Animals, Faculty of Biological Sciences, University of Nigeria, Nsukka, with the approval number: UNN/FBS/EC/1031.

Acute toxicity test of EETGR
Acute toxicity study was carried out using the Lorke's method.[9] The study was carried out in two phases with eighteen (18) mice. For each phase, nine (9) mice divided into three groups of three (3) animals each were employed. For phase I, groups 1, 2 and 3 received 10, 100 and 1000 mg/kg body weight of EETGR suspended in 3% (v/v) Tween 80, respectively and observed for behavioral changes and/or death for 24 h. For phase II, groups 1, 2 and 3 were administered 1600, 2900 and 5000 mg/kg body weight of EETGR suspended in 3% (v/v) Tween 80, respectively and monitored for changes in behavior or death for 24 h.

Phytochemical analysis of EETGR
Qualitative phytochemical analysis of EETGR followed the methods of Sofowora,[10] Trease and Evans,[11] Wallis,[12] Brindha *et al*.[13] and Harborne[14] for alkaloids, saponins, tannins, steroids, flavonoids, phenols and terpenoids tests accordingly. Quantitative phytochemical analysis followed the methods of Harborne,[14] Swain,[15] Brunner[16] for alkaloids, flavonoids, tannins, phenols and saponins accordingly.

Experimental design
Of the eighty (80) rats used for the study, twenty (20) rats were used for each of the four (4) different study protocols. For each study protocol, there were five (5) groups of four (4) animals per group. The four study protocols included: Curative, Preventive, enteropooling test and gastrointestinal motility test. The animals were grouped and administered 1 mL/kg, 3% (v/v) Tween 80 (group 1), 3 mg/kg of loperamide (group 2), extract 100, 200 and 400 mg/kg (groups 3, 4 and 5, respectively). Diarrhoea was induced with castor oil (1 mL) orally.

Anti-diarrhoeal studies
Curative effect of EETGR on castor oil-induced diarrhoea in rats
Studies on the curative effect of EETGR in animals with castor oil-induced diarrhoea followed the method of Awouters *et al*.[17] as modified by Mukherjee *et al*.[18] The animals were starved for 12 h prior to the experiment. The animals received 1 mL castor oil through oral route. After 1 h, group I animals received 1 mL/kg (3% v/v) Tween 80. Group II received 3 mg/kg loperamide and groups III, IV and V received 100, 200 and 400 mg/kg of EETGR suspended in 3% (v/v) Tween 80. After treatment, animals were placed in separately prepared cages and observed for the presence and frequency of wet stool per hour for 4 h. The mean number of stool recorded was used to calculate the percentage inhibition of diarrhoea using the values from control as baseline (100%), according to the equation:

$$\text{Stool Inhibition (\%)} = \frac{Mean\ No\ of\ Feaces\ of\ Control - Mean\ No\ of\ Feaces\ of\ Treated\ Group}{Mean\ No\ of\ Feaces\ of\ Control}\ x\ 100\%$$

Preventive effect of EETGR on castor oil-induced diarrhoea in rats
Studies on the preventive effect of EETGR on animals with castor oil-induced diarrhoea followed the method of Awouters *et al*.[17] as modified by Mukherjee *et al*. [18] The animals were starved for 12 h prior to the experiment. Animals in groups III, IV and V received 100, 200 and 400 mg/kg of EETGR suspended in 3% (v/v) Tween 80 orally, group II received 3 mg/kg of loperamide, while group I animals received 1 mL/kg (3% v/v) Tween 80. One hour after receiving the different treatments, the animals received 1 mL of castor oil by oral route. Immediately after induction, animals were placed in separate cages and observed for the presence and frequency of wet stool per hour for 4 h. The mean number of stool of the treated groups was compared with the control. The mean number of stool recorded was used to calculate the percentage inhibition of diarrhoea using the values from control as baseline (100%), using the equation:

$$\text{Stool Inhibition (\%)} = \frac{Mean\ No\ of\ Feaces\ of\ Control - Mean\ No\ of\ Feaces\ of\ Treated\ Group}{Mean\ No\ of\ Feaces\ of\ Control}\ x\ 100\%$$

Gastrointestinal motility test
Studies on the effect of EETGR on gastrointestinal motility followed the method of Mascolo *et al*.[19] The animals were starved for 12 h prior to the experiment. The animals received 1 mL castor oil through oral route. After 1 h, group I animals received 1 mL/kg (3% v/v) Tween 80. Group II received 3 mg/kg of loperamide and groups III, IV and V received 100, 200 and 400 mg/kg of EETGR suspended in 3% (v/v) Tween 80. One hour after, all the animals received 1 mL charcoal meal (0.5 mL 10% charcoal suspended in 10% gum acacia) through oral route. Animals were sacrificed following chloroform anesthesia 1 h after. The intestine was excised and placed on moist filter paper. The length of intestine from pylorus to the caecum and distance travelled by charcoal meal were measured using meter rule. Gastrointestinal transit was calculated using the distance travelled by charcoal meal relative to the length of the intestine.

Enteropooling test analysis
Studies on the effect of EETGR on volume of intestinal content followed the method of Robert *et al*..[20] The animals were starved for 12 h prior to the experiment. The animals received 1 mL castor oil through oral route. After 1 h, group I animals received 1 mL/kg (3% v/v) Tween 80. Group II received 3 mg/kg of loperamide and groups III, IV and V received100, 200 and 400 mg/kg of EETGR suspended in 3% (v/v) Tween 80. One hour after treatment, animals were sacrificed following chloroform anesthesia. The intestine was removed, both ends tied and weight determined. The content was milked into calibrated tube and the volume read and recorded. After milking, the weight of the empty intestine was again determined and the difference calculated. The change in volume in treated animals was evaluated relative to control. The value for control was taken as 100%.

Statistical analysis
The data obtained were analyzed using IBM Statistical Product and Service Solution (SPSS) version 20.0 for windows. One-way analysis of variance (ANOVA) was used to establish and compare mean values. Differences were accepted significant at $p < 0.05$. Results are expressed as mean ± standard error of mean (SEM).

Results and Discussion

Acute toxicity (LD_{50}) of the EETGR
Table 1 shows the results of the acute toxicity test of EETGR. Neither death nor behavioral changes were recorded in mice up to 5000 mg/kg body weight of the extract within 24 hours. The results suggest that the extract is relatively safe at the doses studied. Its use in livestock feed formulation is therefore justified.

Phytochemical constituents of EETGR
Qualitative phytochemical screening showed that alkaloids, tannins, terpenoids, saponins, phenols, flavonoids and steroids were present. Quantitative phytochemical analysis showed that EETGR contain considerable levels of phenols, tannins and flavonoids (Table 2). Alkaloids and terpenoids were present in moderate quantities while saponins and steroids were available in low quantities. According to Hill,[21] the most important compounds include tannins, flavonoids, alkaloids and phenolics due to their known pharmacological activities. Flavonoids act in the gastrointestinal tract exerting anti-secretory, anti-ulcer and antidiarrhoeal activities.[22,23] The Antidiarrhoeal properties of tannins and phenolics has been attributed to their antioxidant activities.[18] The obtained results therefore suggest that EETGR possess some bioactives which could serve as potential remedy for diarrhoea and other related conditions.

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Effect of EETGR in castor oil-induced diarrhoea in Wistar rats

Table 3 presents the curative effect of EETGR on castor oil-induced diarrhoea in Wistar rats. There was a significant ($p < 0.05$) reduction in diarrhoea in the treated groups (groups 2, 4 and 5) relative to untreated group (group 1). The reduction in diarrhoea in group 3 relative to group 1 was non-significant ($p > 0.05$). The extract compared favorably with the standard drug in reducing diarrhoea at 400 mg/kg body weight.

Table 4 presents the preventive effect of EETGR on castor oil-induced diarrhoea in the experimental animals. The number of droppings at different hours of the study showed significant reduction ($p < 0.05$) at all doses of the extract relative to the untreated control. At 200 mg/kg (96% inhibition) and 400 mg/kg (98% inhibition), EETGR showed inhibition of diarrhoea that compared favorably with the standard drug, loperamide (100% inhibition).

It is therefore evident from these results that administration of EEGTR 1 h before (preventive) or 1 h after (curative) induction of diarrhoea significantly ($p < 0.05$) mediated reduction in diarrhoea in experimental animals compared to untreated controls. From our findings, the preventive study showed better anti-diarrhoeal potentials than the curative studies as the percentage inhibition of diarrhoea observed in preventive study is higher than that for curative studies at the same doses. This observation agrees with the report of Majumdar *et al*.[24] According to, Majumdar *et al*.,[24] prior treatment with some plant extracts conferred protection to the gastrointestinal tract from ricinoleic acid irritation and hence hyper-secretion and hyper-motility are minimized.

The use of Castor oil as an induction agent in diarrhoea models is an appropriate model since it introduces changes in parameters that are measurable in terms of the number of stools, volume of intestinal content and gut motility. The mechanism of induction of diarrhoea involves the hydrolysis of oil by intestinal lipases to liberate ricinoleic acid; this causes the irritation that encourages inflammation of gastric mucosa, resulting in the production and release of prostaglandins. Inhibition of the reabsorption of electrolytes and water and consequent elevation of the weight and volume of intestinal content is another suggested mechanism of diarrhoea induction by castor oil. Prostaglandin stimulates epithelial permeability, redness of the intestinal mucosa, hyper-motility and gastrointestinal secretion thus inhibiting the reabsorption of water and electrolytes.[25,26] These two effects synergistically account for the observed diarrhoea following the administration of castor oil. As a result, molecules that inhibit prostaglandin production will ultimately undermine diarrhoea induction by castor oil.[17] Based on this, it could be postulated that EETGR achieved diarrhoea inhibition by altering or interfering with prostaglandin synthesis or activity.

The mechanism of action of loperamide in inhibiting gastric motility and gastric secretion is documented.[27] The use of loperamide in the management of diarrhoea induced by prostaglandin and castor oil is also known.[28] The phytochemical screening showed high amount of flavonoids in the extract. Flavonoids are known to alter the synthesis of enzymes in prostaglandin pathway,[29,30] and as a result, inhibiting the synthesis of prostaglandin. The flavonoids, individually or in synergy with other phytochemicals, account for the observed effects.

Effect of EETGR on gastro-intestinal motility

Figure 1 presents the effect of EETGR on gastrointestinal motility of the experimental animals. A significant reduction ($p < 0.05$) in gut motility was recorded in groups 3, 4 and 5 relative to the untreated group (group 1). The administration of the extract produced significantly lower ($p < 0.05$) intestinal transit of charcoal meal in groups 4 and 5 relative to the standard drug, loperamide. The ability of the EETGR to reduce the movement of charcoal meal depicts its ability to reduce stooling frequency. Gastric movement suppression encourages absorption of water and electrolytes; this ultimately adds to reducing stool watery nature.[31] Hyper-motility of the GIT induced by castor oil has been reported to be associated indirectly with cholinergic systems as evidenced by atropine.[32]

Effect of EETGR on volume of intestinal content (enteropooling test) in Wistar rats.

Administration of EETGR demonstrated a significant ($p < 0.05$) and dose-related reduction in the mean volume of intestinal content of groups that received 100, 200 and 400 mg/kg body weight relative to the untreated animals (group 1). The reduction in the mean volume of the intestinal contents caused by EETGR at doses of 400 mg/kg body weight compared favorably with that achieved by administering the standard drug (Figure 2). The observed reduction in volume of intestinal content is accounted for by a compensatory reduction in the amount of electrolyte and water fluxing into the intestinal lumen, suggesting that EETGR may enhance the absorption of electrolyte and water from the intestine and this is consistent with the inhibition of hyper-secretion as indicated earlier.[3] These observations suggest to a great extent that EETGR decreases gastrointestinal hyper-secretion and volume of intestinal content by encouraging water, electrolytes and solutes absorption from the lumen of the intestine.

Table 1: Acute toxicity (LD_{50}) of ethanol extract of *Terminalia glaucescens* root

Phase I	Dosage (mg/kg bwt)	Mortality
Group 1	10	0/3
Group 2	100	0/3
Group 3	1000	0/3
Phase II		
Group 1	1600	0/3
Group 2	2900	0/3
Group 3	5000	0/3

n = 3

Table 2: Phytochemical Constituents of ethanol extract of *Terminalia glaucescens* root

Phytochemical Constituent	Inference	Amount (mg/g)
Flavonoids	+	4.94 ± 0.0027
Alkaloids	+	2.74 ± 0.0045
Saponins	+	0.74 ± 0.0031
Tannins	+	5.94 ± 0.0031
Terpenoids	+	1.93 ± 0.0025
Steroids	+	0.84 ± 0.0055
Phenols	+	8.15 ± 0.0021

*Values are Mean ± SD, (n = 3) Key: + indicates present.

Table 3: Curative effect of the ethanol extract of *Terminalia glaucescens* root on castor oil-induced diarrhoea in Wistar rats

		Mean number of wet feaces for 4 hours					
Group	**Treatment**	**1 h**	**2 h**	**3 h**	**4 h**	**Mean ± SD**	**% Inhibition**
Group 1	(1 mL/kg) 3%(v/v) Tween80	5.50	5.25	1.75	1.50	3.50 ± 0.83^{a}	-
Group 2	3 (mg/kg) bwt Loperamide,	2.50	0.50	0.00	0.00	0.75 ± 0.47^{b}	79
Group 3	100 (mg/kg) bwt EETGR	4.25	2.00	1.75	1.00	2.25 ± 0.62^{a}	36
Group 4	200 (mg/kg) bwt EETGR	2.50	1.75	1.50	1.00	1.69 ± 0.33^{d}	52
Group 5	400 (mg/kg) bwt EETGR	2.00	0.50	0.25	0.00	0.69 ± 0.28^{b}	80

[a-d]Presence of similar superscripts indicates no significant difference ($p > 0.05$), while different superscripts in a column indicate significant difference ($p < 0.05$). n = 4. EETGR = ethanol extract of *Terminalia glaucescens* root

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Table 4: Preventive effect of ethanol extract of *Terminalia glaucescens* root in castor oil-induced diarrhoea in Wistar rats

		Mean number of wet feaces for 4 hours					
Group	**Treatment**	**1 h**	**2 h**	**3 h**	**4 h**	**Mean ± SD**	**% Inhibition**
Group 1	(1ml/kg) 3%(v/v) Tween80	5.50	5.25	1.75	1.50	3.50 ± 0.83^{a}	-
Group 2	3 (mg/kg) bwt Loperamide,	0.00	0.00	0.00	0.00	0.00	100
Group 3	100 (mg/kg) bwt EETGR	1.00	0.75	0.75	0.00	0.63 ± 0.34^{b}	82
Group 4	200 (mg/kg) bwt EETGR	0.25	0.25	0.00	0.00	0.13 ± 0.09^{c}	96
Group 5	400 (mg/kg) bwt EETGR	0.25	0.00	0.00	0.00	0.06 ± 0.34^{d}	98

[a-d]Presence of similar superscripts indicates no significant difference ($p > 0.05$), while different superscripts in a column indicate significant difference ($p < 0.05$). n = 4. EETGR = ethanol extract of *Terminalia glaucescens* root

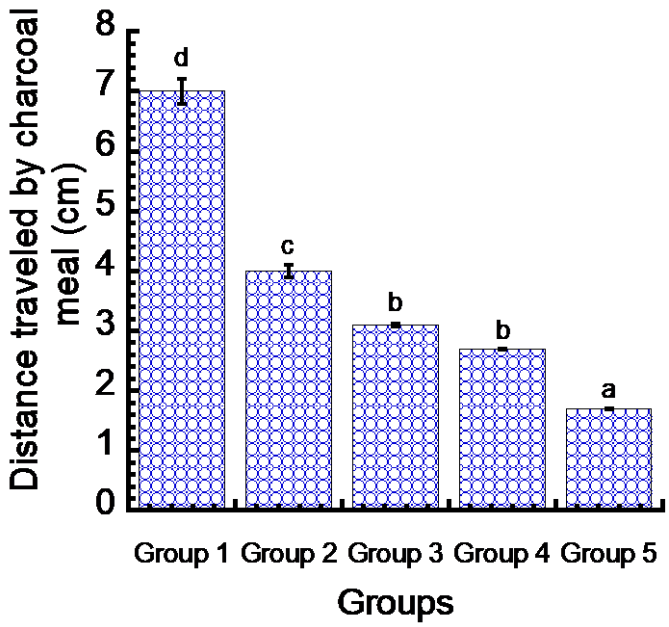

Figure 1: Effect of ethanol extract of *Terminalia glaucescens* root (EETGR) on distance travelled by charcoal meal in castor oil-induced diarrhoea in Wistar rats.
Group 1 = 1 mL/kg of 3% (v/v) Tween 80; Group 2 = 3 mg/kg bwt Loperamide; Group 3 = 100 mg/kg bwt EETGR; Group 4 = 200 mg/kg bwt EETGR; Group 5 = 400 mg/kg bwt EETGR.
[a-d]Presence of similar superscripts indicates no significant difference ($p > 0.05$), while different superscripts in a column indicate significant difference ($p < 0.05$).

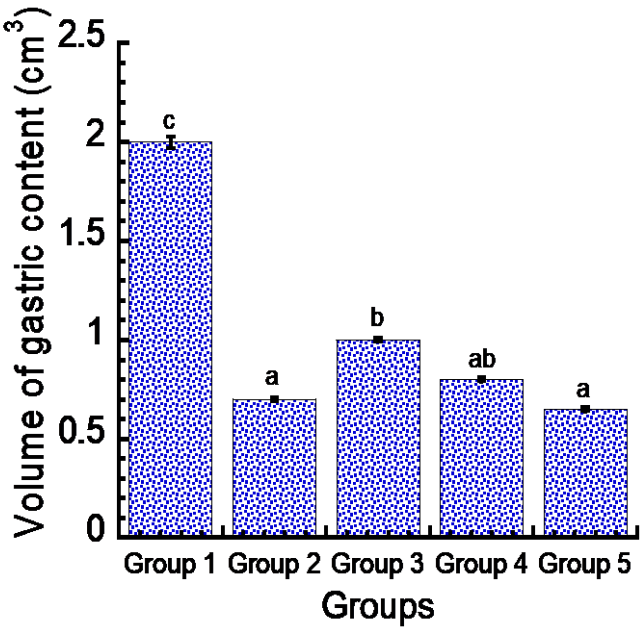

Figure 2: Effect of ethanol extract of *Terminalia glaucescens* root (EETGR) on volume of intestinal fluid content.
Group 1 = 1 mL/kg of 3% (v/v) Tween80; Group 2 = 3 mg/kg bwt Loperamide; Group 3 = 100 mg/kg bwt EETGR; Group 4 = 200 mg/kg bwt EETGR; Group 5 = 400 mg/kg bwt EETGR.
[a-c]Presence of similar superscripts indicates no significant difference ($p > 0.05$), while different superscripts in a column indicate significant difference ($p < 0.05$).

Conclusion

From the findings, it could be submitted that EETGR is relatively safe. EETGR possesses a potent diarrhoea healing effect. The study has established that *Terminalia glaucescens* root has potential as a remedy in the management of diarrhoea. There is need for further studies to isolate the bioactive compounds and to establish the mechanism of action as well as detail toxicity studies.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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