Nigerian Journal of Pharmaceutical and Applied Science Research,
Vol.14 (1): 63-70; March 2025 ISSN: 2971-737X (Print); ISSN: 2971-7388.
Available at www.nijophasr.net https://doi.org/10.60787/nijophasr-v14-i1-584

Acute Toxicity and Antidiarrhoeal Potential of *Ipomoea triloba* and *Glyphaea brevis*: A Mouse Protection Study Against Clinical Bacterial Isolates

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ABSTRACT

Background: Acute infectious diarrhoea remains a significant global health issue and is the second leading cause of mortality and morbidity in Nigeria and worldwide among children under five and the elderly often caused by bacterial, viral, protozoal and helminthic infections that lead to dehydration and other complications. The present upsurge in the prevalence of microbial resistance to available antimicrobials necessitates the need for the search for alternative remedies for the treatment of acute infectious diarrhoea. Hence, this study investigated the safety profile and antidiarrhoeal efficacy of the plant extracts, both individually and in combination.

Methods: The acute toxicity profiles and antidiarrhoeal (mouse protection tests) properties of aqueous extracts of *Ipomoea triloba* (L.) (IA) and *Glyphaea brevis* (GA) against selected clinical bacterial isolates (*Escherichia coli*, *Salmonella typhii*, *Shigella spp*, *Klebsiella pneumoniae,* Staphylococcus aureus, *Proteus spp* and *Morganella morganii*.) were evaluated individually and in combination. These extracts were prepared using standardized methods, and their acute toxicity profiles were evaluated using improved Lorke's method.

Results: Results indicated LD_{50} values of 2449.0 mg/kg (IA) and 2449.4 mg/kg (GA) respectively. Mouse protection tests showed 94.00 ±10.50% protection when the extracts were administered in combination whereas the protection was reduced (54.76 ± 12.60 %) IA and (57.14 ± 21.21%) GA when the extracts were administered singly to the animals thus validating the use of the combination of the aqueous extracts of both plants folklorically.

Conclusion: Conclusively, these findings suggest that these plants possess potential antidiarrhoeal properties with relative non-toxic profiles thus, supporting their use locally in treating diarrhoea-related ailments. Further investigation into the active compounds responsible for these effects is required and ongoing.

Keywords*:* Antidiarrhoeal, alternative treatment, *Ipomoea triloba, Glyphaea brevis,* microbial resistance, acute toxicity, mouse protection tests.

1. INTRODUCTION

There has been a rise in microbial resistance to available antimicrobials due to their misuse and overuse. Thus, the World Health Organization (WHO) stressed the need for treatment remedies from alternative sources that would bring lasting solutions to this menace. Acute infectious diarrhoea is one of the leading causes of morbidity and mortality especially in paediatrics, the frail, immunocompromised and geriatrics. Diarrhoeal diseases have also been affected by antimicrobial resistance, particularly in underserved regions with limited access to healthcare. Diarrhoea prevalence in Nigeria remains a significant concern, particularly for children under five. According to a study published in the Journal of Global Health Reports (2022), diarrhoea was responsible for approximately 16% of all child deaths in Nigeria [1]. This represents a higher mortality rate compared to many other low- and middle-income countries. Data from the 2021 Nigerian Demographic and Health Survey (NDHS) indicate that the prevalence of diarrhoea in children under five was approximately 10% nationwide, with higher rates in the northern regions of the country due to poor access to clean water and sanitation [2]. Moreover, regional differences in diarrhoea prevalence have been noted in several studies. For example, the northern regions of Nigeria, particularly

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in states like Kano and Borno, report higher prevalence rates, exceeding 20% among children under five. This is attributed to poor sanitation, unsafe drinking water, and lower maternal education levels. Conversely, the southern regions, which tend to have better infrastructure and higher literacy rates, report lower prevalence rates, though pockets of high diarrhoea incidence remain, particularly in peri-urban slums [2]. Ongoing research has brought to the limelight the two plants used in this research- *Ipomoea triloba* and *Glyphaea brevis* [3,4]. Hence, this study investigated the antimicrobial efficacy of the aqueous extracts of the plants used folklorically for diarrhoeal episodes by assessing their acute toxicity profiles and protective efficacy against selected clinical bacterial isolates (*Escherichia coli*, *Salmonella typhii*, *Shigella spp*, *Klebsiella pneumoniae, Proteus spp* and *Morganella morganii*) singly and in combination. The acute toxicity (LD_{50}) test is a standardized toxicity test where death is the intended endpoint. It refers to the lethal dose of a drug that can cause the death of 50% of the experimental animals used for the study. This test determines the concentration of a substance that causes death in 50% of the test population (LD_{50}/LC_{50}) during short-term exposure [4]. The main way to assess acute toxicity is to administer a single dose of the extract to a group of test animals and then observe the effect of the extract on the animals over a period of 14 days. Key indicators include mortality, changes in body weight, behaviour, and pathological signs observed in major organs during necropsy. It is usually an initial screening step in the assessment and evaluation of the toxic characteristics of all compounds present in the test substance (plant extract). The greater the value, the safer the product (test extract). There are different tests used to determine acute toxicity and they include improved Lorke's method, OECD method, graphical method of Miller and Tainter, arithmetical method of Karber etc. [5] Model Mouse protection test involves the use of albino mice to investigate the potential of plant extracts or fractions to protect the animals which had a prior exposure to the infectious agent- parasite, bacteria etc. using doses between the minimum inhibitory concentration (MIC) and the acute toxicity (LD_{50}) test value [6].

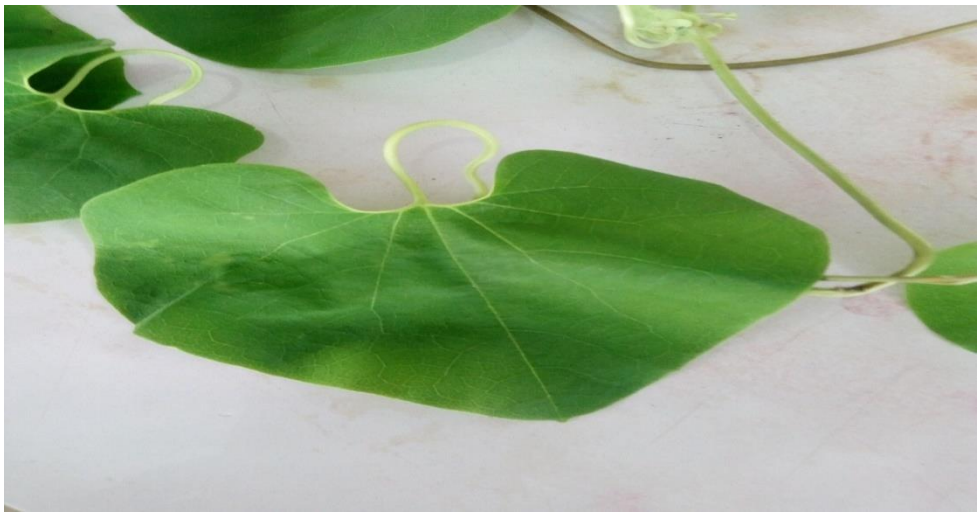

Figure 1: *Ipomoea triloba* in its Habitat (Source: Field data)

Fig 2: *Glyphaea brevis* in its natural habitat; Source: Field data

Glyphaea brevis is a medicinal plant whose leaves are used in a decoction for the treatment of palpitations, hepatitis, and poisoning in Cameroon. In Ivory Coast, it is used for the treatment of fever and female sterility [8] and for the relief of sleepiness, bacterial infections, convulsions, sexual impotency, and some age-related brain disorders in Nigeria [9,10].

2. Materials and Methods

2.1 Materials

Albino mice of both sexes, weighing between 15.0 and 21.0 g, were used in this study for acute toxicity of extracts

2.2 Methods

2.2.1 Acute toxicity (LD_{50}) of Extracts

Acute toxicity (LD_{50}) of the aqueous extracts of *Ipomoea triloba* and *Glyphaea brevis* was assayed in albino mice (15.0-21.0 g body weights) following the method of 'O'Callaghan [12].

2.2.2 In vivo Antidiarrhoeal Activity Assay by Model Infection and Protection Test (in singles and in combination)

To assess the efficacy of these extracts in protecting experimental animals from infection, the study followed the animal protection method previously described by Ekong et al. [13]. The mice were divided into six groups, each consisting of six animals. Each group received an intraperitoneal injection of 0.5 mL of fresh bacterial suspensions previously determined to be sensitive to the extracts, in order to establish infection. The aqueous extracts of *Ipomoea triloba* and *Glyphaea brevis* were then administered individually and in combination at one-hour and five-hour intervals respectively. The dose used corresponded to the minimum inhibitory concentration (MIC) of the targeted bacteria but was lower than the LD_{50} for mice [6]. Throughout the five-day study period, the animals had unrestricted access to food and water (*ad libitum*), and mortality rates and other clinical observations were recorded.

The control groups were structured as follows:

- **Negative control:** Six groups of six mice each, to which distilled water only was administered
- **Positive control:** Two groups received standard antibiotics—metronidazole (5.7 mg/kg) and cephalexin (3.57 mg/kg), with six animals per group.

Each group was administered 0.5 mL of a standardized bacterial suspension intraperitoneally. The extracts were given at one-hour and five-hour intervals on the first day (that is only 2 doses were administered). The animals had *ad libitum* access to food and water, and daily observations, including mortality, were recorded over the five-day period.

For combination treatments, the aqueous extracts of *Ipomoea triloba* and *Glyphaea brevis* were administered in the following ratios:

- $100LD_{50}$:$100LD_{50}$ – 2449.0 mg/kg : 2449.4 mg/kg
- $50LD_{50}$:$50LD_{50}$ – 1224.5 mg/kg : 1224.7 mg/kg
- $25LD_{50}$:$25LD_{50}$ – 612.25 mg/kg : 612.35 mg/kg
- $12.5LD_{50}$:$12.5LD_{50}$ – 306.13 mg/kg : 306.18 mg/kg

2.2.3 Ethical Issues

All necessary ethical considerations as regards the use of animals and humans in research were satisfactorily met. The principle of beneficence and nonmaleficence was employed and the identity of subjects from whose stools the bacterial isolates were obtained was kept confidential. The care and use of animals were conducted in accordance with the National Institute of Health Guide for the Use of Laboratory Animals [14]. Moreover, ethical approval for animal use was obtained from the Health Research Ethics Committee on Animal Use (HREC), University of Uyo, Nigeria.

2.3 Statistical Analysis

Statistical analysis was done using an electronic calculator to determine the percentage protection conferred on the experimental animals by the extracts when administered individually and in combination.

3. RESULTS

Tables 1 and 2 present the results of acute toxicity of the crude aqueous extracts of *Ipomoea triloba* and *Glyphaea brevis* respectively. The results indicate that the crude extracts of both plants are relatively non-toxic, with LD50 values of 2449.0 mg/kg for *Ipomoea triloba* and 2449.4 mg/kg for *Glyphaea brevis.* The Hodge and Sterner scale (Table 3) was used to interpret the safety levels of both plants in aqueous state.

Table 1: Acute toxicity (LD_{50}) of *I. triloba* aqueous extract

Test groups	Dose (mg/kg)	No. of animals	No. of deaths within 24 h	% mortality in 24 h
1	5000	3	3	100
2	3000	3	1	33.3
3	1000	3	0	0
4	950	3	0	0
5	900	3	0	0
6	850	3	0	0
7	800	3	0	0
8	500	3	0	0

Route of administration-intraperitoneally n=24; LD_{50} (mg/kg) = $\sqrt{D_a} \times D_b$ = 2449.0 mg/kg

Table 2: Acute toxicity (LD_{50}) of *G. brevis* aqueous extract

Test groups	Dose (mg/kg)	No. of animals	No. of deaths within 24 h	% mortality in 24 h
1	5000	3	3	100
2	3000	3	1	33.3
3	1000	3	0	0
4	950	3	0	0
5	900	3	0	0
6	850	3	0	0
7	800	3	0	0
8	500	3	0	0

Route of administration-intraperitoneally n=24; LD_{50} (mg/kg)= 2449.4 mg/kg

For each mouse, the observation was made for 24 hr and symptoms of toxicity and rate of mortality in each group were noted. At the end of the study period, expired animals were counted for the calculation of LD_{50}.

Table 3. Hodge and Sterner toxicity scale.

S/no	Term	LD_{50}
1	Extremely toxic	Less than 1 mg/kg
2	Highly toxic	1-50 mg/kg
3	Moderately toxic	50-500 mg/kg
4	Slightly toxic	500-5000 mg/kg
5	Practically non-toxic	5000-15000 mg/kg

The Hodge and Sterner toxicity scale (Table 3) was used to interpret the acute toxicity results obtained at the end of the tests indicating toxicity levels and subsequent safety for human consumption.

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Tables 4 and 5 present the results for *in vivo* antidiarrhoeal activity and protection efficacy of the aqueous extracts of *Ipomoea triloba* and *Glyphaea brevis* against the diarrhoeal isolates when both extracts were administered individually to the animals.

Table 4: Table showing model infection, in vivo antidiarrheal activity and protection efficacy of aqueous extracts of I. triloba against establishment of infections by diarrhoeal bacterial isolates

Test isolates	Number of animals used	No of deaths within five days												No. of survivor within five days		Percentage death per group		Percentage protection per group	
		Test						Positive Controls						Test	Control	Test	Control	Test	Control
		1	2	3	4	5	Total	1	2	3	4	5	Total						
EC	6	-	1	1	-	-	2	-	-	-	-	-	0	4	6	33.33	0.00	66.67	100.00
SA	6	2	2	-	-	-	4	-	-	-	-	-	0	2	6	66.67	0.00	33.33	100.00
KP	6	2	-	-	-	-	2	-	-	-	-	-	0	4	6	33.33	0.00	66.67	100.00
SD	6	-	2	1	-	-	3	-	-	-	-	-	0	3	6	50.00	0.00	50.00	100.00
ST	6	2	1	-	-	-	3	-	-	-	-	-	0	6	6	50.00	0.00	50.00	100.00
PM	6	-	2	-	-	-	2	-	-	-	-	-	0	4	6	33.33	0.00	66.67	100.00
MM	6	-	2	1	-	-	3	-	-	-	-	-	0	3	6	50.00	0.00	50.00	100.00

Key: EC- *Escherichia coli*, SA- *Staphylococcus aureus*, KP- *Klebsiella pneumoniae,* SD- *Shigella dysenteriae,* ST- *Salmonella typhi,* PM – *Proteus mirabilis,* MM- *Morganella momorsidica*, -= No death; Positive controls- metronidazole and cephalexin

Table 5: Table showing model infection, *in vivo* antidiarrheal activity and protection efficacy of the aqueous extracts of *G. brevis* against establishment of infections by diarrhoeal bacterial isolates

Test isolates	Number of animals used	No of deaths within five days												No. of survivor within five days		Percentage death per group		Percentage protection per group	
		Test						Positive Controls						Test	Control	Test	Control	Test	Control
		1	2	3	4	5	Total	1	2	3	4	5	Total						
EC	6	-	1	1	-	-	2	-	-	-	-	-	0	4	6	33.33	0.00	66.67	100.00
SA	6	-	-	-	-	-	0	-	-	-	-	-	0	6	6	0.00	0.00	100.00	100.00
KP	6	-	3	-	-	-	3	-	-	-	-	-	0	3	6	50.00	0.00	50.00	100.00
SD	6	-	2	1	-	-	3	-	-	-	-	-	0	3	6	50.00	0.00	50.00	100.00
ST	6	-	2	1	-	-	3	-	-	-	-	-	0	3	6	50.00	0.00	50.00	100.00
PM	6	1	2	-	-	-	3	-	-	-	-	-	0	3	6	50.00	0.00	50.00	100.00
MM	6	-	2	2	-	-	4	-	-	-	-	-	0	2	6	66.67	0.00	33.33	100.00

Key: EC- *Escherichia coli*, SA- *Staphylococcus aureus*, KP- *Klebsiella pneumoniae,* SD- *Shigella dysenteriae,* ST- *Salmonella typhi,* PM – *Proteus mirabilis,* MM- *Morganella momorsidica*, -= No death; Positive controls- metronidazole and cephalexin

Table 6 displays the in vivo antidiarrhoeal activity and protective efficacy of the combined aqueous extracts of *Ipomoea triloba* and *Glyphaea brevis* against diarrhoeal isolates in the test animals.

Table 6: Table showing model infection, *in vivo* antidiarrheal activity and protection efficacy of the combination of aqueous extracts of *I. triloba* and *G. brevis* against establishment of infections by diarrhoeal bacterial isolates

Test isolates	Number of animals used	No of deaths within five days												No. of survivor within five days		Percentage death per group		Percentage protection per group	
		Test						Positive Control						Test	Control	Test	Control	Test	Control
		1	2	3	4	5	Total	1	2	3	4	5	Total						
EC	6	-	-	-	-	-	0	-	-	-	-	-	0	6	6	0.00	0.00	100.00	100.00
SA	6	-	-	-	2	-	2	-	-	-	-	-	0	4	6	25.00	0.00	75.00	100.00
KP	6	-	1	-	-	-	1	-	-	-	-	-	0	5	6	16.67	0.00	83.33	100.00
SD	6	-	-	-	-	-	0	-	-	-	-	-	0	6	6	0.00	0.00	100.00	100.00
ST	6	-	-	-	-	-	0	-	-	-	-	-	0	6	6	0.00	0.00	100.00	100.00
PM	6	-	-	-	-	-	0	-	-	-	-	-	0	6	6	0.00	0.00	100.00	100.00
MM	6	-	-	-	-	-	0	-	-	-	-	-	0	6	6	0.00	0.00	100.00	100.00

Key: EC- *Escherichia coli*, SA- *Staphylococcus aureus*, KP- *Klebsiella pneumoniae,* SD- *Shigella dysenteriae,* ST- *Salmonella typhi,* PM – *Proteus mirabilis,* MM- *Morganella momorsidica*, -= No death; Positive controls- metronidazole and cephalexin

4. DISCUSSION

The acute toxicity results (2449 mg/kg for *Ipomoea triloba* and 2449.4 mg/kg for *Glyphaea brevis*) suggest that these plant extracts are relatively non-toxic, as classified by the Hodge and Sterner scale (Table 3). This indicates their potential safety for human use as therapeutic agents. The Mouse Protection Test results demonstrated a protective effect when the aqueous extracts were administered individually. For *Ipomoea triloba*, the overall protection rate was 54.76 ± 12.60%, with the lowest protection observed against *Staphylococcus aureus* (33.33%) and the highest protection against *Escherichia coli, Klebsiella pneumoniae,* and *Proteus mirabilis* (66.67%). *Shigella dysenteriae* and *Salmonella typhi* showed intermediate protection levels (50%). Similarly, *Glyphaea brevis* conferred an overall protection of 57.14 ± 21.21%, with the least protection against *Morganella morganii* (33.33%) and the highest against *Staphylococcus aureus* (100%). However, when the aqueous extracts of both plants were combined, the protection rate significantly increased to 94.00 ± 10.50%. For comparison, the positive control groups treated with standard antibiotics—metronidazole and cephalexin—both achieved 100% protection. These findings are consistent with those of Alozie et al. [4], who reported acute toxicity and mouse protection effects of the ethanol extract of *Ipomoea triloba* (50–83.3%). Given that the combined extract demonstrated a protective efficacy comparable to standard drugs, it may be reasonable to propose the inclusion of these plants in the treatment regimen for acute hypersecretory diarrhea.

5. CONCLUSION

In conclusion, the therapeutic outcomes observed in both in vitro and in vivo studies support the traditional use of these plants, particularly in combination, for the treatment of acute infectious hypersecretory diarrhoea.

Acknowledgement

Authors acknowledge the assistance of the laboratory personnel in the animal house, Department of Pharmacology, Faculty of Pharmacy for their dedication to the research work.

Authors' Contributions

The research work was carried out in collaboration between Authors. Author MFA carried out the laboratory investigations and wrote the first draft of the manuscript, USE designed and supervised the work, UUA and TYM managed the literature search and analyses of the study. All authors read and approved the final manuscript for submission.

Conflict of interest

All authors attest to the absence of existing conflict of interest regarding the research work.

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