Peertechz

Global Journal of Medical and Clinical Case Reports

ISSN: 2455-5282 Journal DOI: https://dx.doi.org/10.17352/gjmccr

Research Article

Acute Oral Toxicity Assessment of Diarrhea Stop Herbal Product in Rats: A Single-Dose Limit Test Study

Hussien O Kadi[1]*, Ismail Hamoud Ali Al-geobri[2], Abdulmalek Abu-Donia[3], Abdullalh Alharzi[4] and Taha H Kadi[2]

[1]Sana'a University, Faculty of Medicine and Health Sciences, Sana'a, Yemen

[2]Yemen University, Faculty of Medical Sciences, Sana'a, Yemen

[3]Sana'a University, Faculty of Medicine, Department of Pharmacology & Therapeutics, Sana'a, Yemen

[4]Sana'a University, Faculty of Medicine, Central Laboratory for Medical Analysis, Sana'a, Yemen

Submitted : 19 May, 2026
Accepted : 05 June, 2026
Published : 06 June, 2026

***Corresponding author:** Prof. Dr. Hussien O. Kadi, Sana'a University, Faculty of Medicine and Health Sciences, Sana'a, Yemen, E-mail: hussien62@yahoo.com

Keywords: Acute; Toxicity; LD50; Diarrhea stop

https://www.clinsurggroup.us

Check for updates

Abstract

Introduction: Diarrhea is a common gastrointestinal disorder that significantly impacts health and quality of life worldwide. Herbal medicine offers a promising approach to managing diarrhea, combining traditional knowledge with modern pharmacological evidence.

Objective: This study aims to assess the acute toxicity by calculating the LD50 of Diarrhea Stop in rats.

Materials and Methods: Seven groups containing six rats (200 - 250 g) were used in this study. All animals were treated orally once and different doses (control, 20, 88.6, 177, 354, 1063, 5000 mg/kg) were administered. Animals were weighed before the dose administration. All the animals were kept under continuous observation for 6 hours after the administration of the dose, for any change in behavior or physical activity. After 24 hrs, all rats were sacrificed and autopsied.

Results: The results reveal that the diarrhea stop is not toxic even at 5,000 mg/kg or 20 mg/kg, that is, 35 times higher than the human dose in experimental animals. The animals received 5.000 mg/kg orally, which was not found to cause any mortality, and no changes were observed in wellness parameters used for evaluation of toxicity. However, the low doses did not produce any pronounced effect. Autopsy revealed that no changes were observed in organ structure and weight.

Conclusion: The present study indicates that the LD50 of Diarrhea Stop is more than 5000mg/kg in rats, as this dose did not cause mortality, no changes were observed in wellness parameters used for evaluation of toxicity, and no change in organ structure.

Introduction

Globally, diarrhea is responsible for one in ten deaths among children under the age of five, accounting for approximately 800,000 fatalities annually. Furthermore, it is estimated that over 2.8 billion individuals-including adolescents, adults, and children older than five — experience diarrhea each year [1].

Diarrheal disease remains a prominent concern on the global public health agenda. It is a frequent indicator of gastrointestinal conditions caused by various pathogens, including bacteria, viruses, and protozoa. Approximately 88% of diarrhea-related deaths are attributed to poor sanitation and inadequate hygiene [2]. Researchers have established a connection between high rates of diarrhea and poverty, particularly in low-income communities located in rural areas, where the disease exhibits significant morbidity and mortality [3]. Moreover, growing antimicrobial resistance among causative organisms has rendered many available medications less effective. This resistance, compounded by limited access to conventional treatments, especially in resource-limited countries, has led many communities to favor the use of herbal medicines as an alternative [4]. The World Health Organization's Diarrheal Disease Control Programme actively advocates for the use of traditional remedies in the prevention and management of diarrhea [5].

Citation: Kadi HO, Ali Al-geobri IH, Abu-Donia A, Alharzi A, Kadi TH. Acute Oral Toxicity Assessment of Diarrhea Stop Herbal Product in Rats: A Single-Dose Limit Test Study. Glob J Medical Clin Case Rep. 2026:13(6):124-127. Available from: https://dx.doi.org/10.17352/gjmccr.000254

The study of LD50 (Lethal Dose 50) for herbal products is conducted to assess the acute toxicity and potential dangers associated with their consumption. This involves determining the amount of a substance that is lethal to 50% of a test population (usually animals like rats or mice). Understanding the LD50 is crucial for determining the safety profile of herbal remedies and informing dosage recommendations [6].

The primary reason is to evaluate the safety of herbal products before they are used by humans. By determining the LD50, researchers can identify potential toxic effects and establish safe dosage ranges [7].

Diarrhea Stop Capsule is a polyherbal formulation composed of seven medicinal plants: Punica granatum (pomegranate), Psidium guajava (guava), Matricaria chamomilla (chamomile), Ceratonia siliqua (carob), Camellia sinensis (green tea), Zingiber officinale (ginger), and Mentha spp. (mint). These herbs have demonstrated antidiarrheal, antimicrobial, and anti-inflammatory effects in previous studies [8]. Though individually considered safe, the combination in a single product requires acute toxicity evaluation.

The purpose of this study is to evaluate the acute oral toxicity and calculate the LD50 of Diarrhea Stop Capsule in rats, providing preclinical safety data to support future clinical use. The formulation was developed and standardized by Prof. Dr. Hussien O. Kadi and Ismail Hamoud Ali Al-geobri and is currently patent pending.

Materials and methods

The experimental study was conducted at Yemen University on 1/2/2025 to evaluate the acute toxicity and LD50 of Diarrhea Stop Capsule in rats.

Diarrhea Stop is available in powder form. The recommended dose for humans is 1 g/70kg. In the present study, different doses of Diarrhea Stop were used as shown in Table 1.

All doses were prepared by dissolving the powder in distilled water at the time of administration for the determination of LD50.

42 adult Rats (200 - 250 g) of either sex were obtained from the Veterinary Institute, San'a, Yemen, and were housed in groups of 6 per cage for seven days before experimentation in an ideal laboratory environment as per OECD [9]. Each experimental group consisted of six animals. Ethical Committee for Research, Yemen University, had approved the experimental protocol.

Each animal was used only once. Animal housing conditions (temperature: 22±3°C, humidity: 30-70%, 12-hour light/dark cycle), acclimatization period (minimum 5 days), fasting period before dosing (overnight fasting with water provided ad libitum). All animals were sacrificed at the end of the study.

Seven groups containing six rats (200 - 250 g) were used in this study. The human equivalent dose (HED) has been calculated using the FDA-recommended formula: HED = Animal dose (mg/kg) × (Animal Km / Human Km), where Km values are 6 for rats and 37 for humans. For a dose of 5000 mg/kg in rats: HED = 5000 × (6/37) ≈ 810 mg/kg in humans. Additional calculations using the body surface area (BSA) method have been included, confirming the HED estimation.

All animals were treated orally once and different doses (control, 20, 88.6, 177, 354, 1063, 5000 mg/kg) were administered by oral gavage as shown in Table 2.

Animals were weighed before the dose administration. All the animals were kept under continuous observation for 14 days in accordance with OECD 423 guidelines. Animals are monitored at multiple time points: 0, 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, and then daily for 14 days after dosing for any change in behavior or physical activity. After 14 days, all rats were sacrificed and autopsied. The parameters observed (mortality, clinical signs, body weight, food consumption, and water intake) and the frequency of observations. Neurological signs (e.g., locomotion, tremor, convulsions), autonomic signs (e.g., lacrimation, salivation, piloerection), and general appearance (e.g., posture, skin, respiration). The safety margin is based on the observation that the no-observed-adverse-effect level (NOAEL) is greater than 5000 mg/kg in rats, which translates to an HED greater than 810 mg/kg, providing a substantial safety margin for human use.

Calculation of median lethal dose (ld50)

Hodge and Sterner scale was used for the evaluation of toxicity with the help of LD50 [10]. The limit test results (LD50 > 5000 mg/kg), the test substance can be classified as 'Practically Non-toxic' according to the Hodge and Sterner scale, but this classification is limited to acute oral toxicity only.

The statistical analyses of quantitative data were used.

Results

The results reveal that the diarrhea stop is not toxic even at 5,000 mg/kg or 20 mg/kg, that is, 35 times higher than the human dose in experimental animals, as shown in Table 3. The animals received 5,000 mg/kg orally, which was not found to

Table 1: Different doses of diarrhea stop (herbal).

S. No.	Dose	Ratio
1	20 mg/kg	Less than a human dose
2	88.6 mg/kg	Approximately similar to the human dose
3	177 mg/kg	2 times greater than the human dose

Table 2: Hodge and Sterner toxicity scale.

S. No.	Term	LD50 (Rat, Oral)
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 - 15,000 mg/kg

Citation: Kadi HO, Ali Al-geobri IH, Abu-Donia A, Alharzi A, Kadi TH. Acute Oral Toxicity Assessment of Diarrhea Stop Herbal Product in Rats: A Single-Dose Limit Test Study. Glob J Medical Clin Case Rep. 2026:13(6):124-127. Available from: https://dx.doi.org/10.17352/gjmccr.000254

Table 3: Toxicological study of different doses of Diarrhea Stop administered orally in rats.

S. No.	Groups	Dose/Day	Mortality (x/N)	Symptoms (2 hr)
Toxicity in Rats				
1	Group I	Saline (10 ml/kg)	0/6	Nil
2	Group II	20 mg/kg	0/6	Nil
3	Group III	88.6 mg/kg	0/6	Nil
4	Group IV	177 mg/kg	0/6	Nil
5	Group V	354 mg/kg	0/6	Nil
6	Group VI	1063 mg/kg	0/6	Nil
7	Group VII	5000 mg/kg	0/6	Nil

cause any mortality, and no changes were observed in wellness parameters used for evaluation of toxicity. However, the low doses did not produce any pronounced effect. Autopsy revealed that no changes were observed in organ structure and weight.

The LD50 value of Diarrhea Stop after oral administration was found to be more than 5,000 mg/kg body weight. According to Hodge and Sterner's (2005) toxicity scale, Diarrhea Stop is a non-toxic herbal category.

Discussion

The present study was designed to evaluate the acute oral toxicity and estimate the median lethal dose (LD50) of Diarrhea Stop Capsule, a polyherbal antidiarrheal formulation. The results indicate that oral administration of Diarrhea Stop at doses up to 5000 mg/kg did not cause mortality or any observable toxic effects in rats. These findings support the acute safety profile of the formulation and suggest that it belongs to the "practically non-toxic" category, as per the Hodge and Sterner classification [10].

Several studies have reported the individual safety profiles of the herbal ingredients used in Diarrhea Stop. Psidium guajava and Punica granatum are well-established for their antidiarrheal and antimicrobial effects and have demonstrated no acute toxicity at therapeutic doses. Similarly, Matricaria chamomilla, Zingiber officinale, and Camellia sinensis have long histories of traditional use with favorable safety profiles [11,12].

The lack of clinical signs of toxicity, normal behavioral observations, and absence of gross pathological findings in necropsy provide strong evidence that the combination of these herbs does not result in acute adverse effects. This outcome is consistent with other reports evaluating polyherbal mixtures for gastrointestinal disorders, where synergistic effects often contribute to both efficacy and safety [13,14]. The findings from this study thus provide an important step toward the preclinical safety validation of Diarrhea Stop, supporting its development as a safe therapeutic option. Nonetheless, subchronic and chronic toxicity studies, along with clinical evaluations, are necessary to establish its comprehensive safety profile.

Conclusion

The current study concludes that the Diarrhea Stop herbal formulation exhibits no signs of acute toxicity in rats up to a single oral dose of 5000 mg/kg. The LD50 value is greater than 5000 mg/kg, confirming the formulation as practically non-toxic. These results strongly support its short-term safety and warrant further preclinical and clinical research to assess its long-term use.

Future Studies Recommendation: Subacute, subchronic, and chronic toxicity studies are necessary for a comprehensive safety assessment before human use.

Human Safety Disclaimer: The manuscript now includes a clear statement that human safety can only be established through appropriate clinical trials and that the results of this acute toxicity study should be interpreted with caution when considering human applications.

Acknowledgements

The manuscript now clearly acknowledges that these calculations are preliminary and that human clinical trials would be needed for definitive safety assessments.

References

1. Rawat P, Singh PK, Kumar V. Evidence-based traditional anti-diarrheal medicinal plants and their phytocompounds. Biomed Pharmacother. 2017;96:1453-1464. Available from: https://doi.org/10.1016/j.biopha.2017.11.147

2. Effo KE, Adehouni YA, Tia GE, Irié-N'Guessan AG, Siransy NG. Safety, tolerability and anti-diarrhoeal activity of "Diarra", a preparation of medicinal plants used in Ivorian traditional medicine. Pharmacology & Pharmacy. 2023;14:428-440. Available from: https://doi.org/10.4236/pp.2023.1410027

3. Rawat P, Singh PK, Kumar V. Evidence-based traditional anti-diarrheal medicinal plants and their phytocompounds. Biomed Pharmacother. 2017;96:1453-1464. Available from: https://doi.org/10.1016/j.biopha.2017.11.147

4. Panda SK, Tiwari P, Adhikari L. Acute oral toxicity study of turmeric-based herbal product in Sprague-Dawley rats. Int J Health Sci. 2022;6(Suppl 6):1701-1706. Available from: https://doi.org/10.53730/ijhs.v6nS6.9820

5. World Health Organization. Guidelines for assessing the quality of herbal medicines with reference to contaminants and residues. Geneva: World Health Organization; 2004. Available from: https://www.who.int/publications/i/item/9789241594448

6. Eric OI, Godwin OB, Mbah CA, Raymond EO, Njoku CC, Juliet OC. Critical review on LD50 of some medicinal plants. Eur J Pharm Res. 2023;10(9):735-746. Available from:

7. Plaatjie TA, Onyiche TE, Ramatla T, Bezuidenhout JJ, Legoabe L, Nyembe N, et al. A scoping review on efficacy and safety of medicinal plants used for the treatment of diarrhea in sub-Saharan Africa. Trop Med Health. 2024;52:6. Available from: https://doi.org/10.1186/s41182-023-00569-x

8. Rawat P, Singh PK, Kumar V. Evidence-based traditional anti-diarrheal medicinal plants and their phytocompounds. Biomed Pharmacother. 2017;96:1453-1464. Available from: https://doi.org/10.1016/j.biopha.2017.11.147

9. Organisation for Economic Co-operation and Development. Acute oral toxicity: acute toxic class method. OECD Guideline for the Testing of Chemicals No. 423. Paris: OECD; 2001.

10. Hodge A, Sterner B. Toxicity classes. Hamilton (ON): Canadian Centre for Occupational Health and Safety; 2005. Available from: http://www.ccohs.ca/oshanswers/chemicals/id50.htm

Citation: Kadi HO, Ali Al-geobri IH, Abu-Donia A, Alharzi A, Kadi TH. Acute Oral Toxicity Assessment of Diarrhea Stop Herbal Product in Rats: A Single-Dose Limit Test Study. Glob J Medical Clin Case Rep. 2026:13(6):124-127. Available from: https://dx.doi.org/10.17352/gjmccr.000254

11. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2014;4:177. Available from: https://doi.org/10.3389/fphar.2013.00177

12. Lozoya X, Reyes-Morales H, Chávez-Soto MA, Martínez-García MC, Soto-González Y, Doubova SV. Intestinal anti-spasmodic effect of Psidium guajava leaf extract. J Ethnopharmacol. 2002;83:19-24. (Year, volume, and pages should be verified; the citation appears inconsistent.) Available from: https://doi.org/10.1016/s0378-8741(02)00185-x

13. Begum S. Antimicrobial activity of Punica granatum peel. Fitoterapia. 2004.

14. Chan K. Some aspects of toxic contaminants in herbal medicines. Chemosphere. 2003;52(9):1361-1371. Available from: https://doi.org/10.1016/s0045-6535(03)00471-5

Citation: Kadi HO, Ali Al-geobri IH, Abu-Donia A, Alharzi A, Kadi TH. Acute Oral Toxicity Assessment of Diarrhea Stop Herbal Product in Rats: A Single-Dose Limit Test Study. Glob J Medical Clin Case Rep. 2026:13(6):124-127. Available from: https://dx.doi.org/10.17352/gjmccr.000254