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Toxicological Evaluation and LD_{50} of Ethanolic Extracts of *Chromolaena odorata* and *Zingiber officinale* in Wistar Rats: Effects on Body Weight and Haematology in Indomethacin-Induced Ulceration

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Abstract

This study evaluated the acute toxicity, LD_{50}, effects on body weight, and haematological parameters of ethanolic extracts of *Chromolaena odorata* and *Zingiber officinale* in Wistar rats. Acute toxicity testing revealed no mortality at doses up to 5000 mg/kg for either extract; only slight weakness was observed in rats receiving 5000 mg/kg of *C. odorata*, indicating high safety margins (LD_{50} > 5000 mg/kg). Body weight monitoring over four weeks showed that indomethacin-induced ulcer groups experienced significant reductions in weight gain ($p < 0.05$), whereas extract-treated groups, particularly the combination extract at 100 mg/kg post-treatment (Group N), maintained body weight comparable to controls ($p > 0.05$). Haematological analysis demonstrated that ulceration caused significant declines in haemoglobin (Hb), packed cell volume (PCV), and red blood cell (RBC) counts ($p < 0.05$), alongside elevated white blood cell (WBC) counts. Pretreatment with *C. odorata* (100 mg/kg) improved Hb to 11.20 ± 0.20 g/dl and PCV to 33.67 ± 0.66%, while combination extract posttreatment at 100–200 mg/kg maintained Hb (13.30 ± 0.13 g/dl) and PCV (40.00 ± 0.44%) near normal levels and stabilized WBC counts (4.32–5.35 × 10^3/mm^3; $p > 0.05$ versus control). Post-treatment with the combination extract also significantly ameliorated haematological disruptions ($p < 0.05$). These findings suggest that both extracts are acutely safe and exert protective effects against indomethacin-induced haematological and physiological alterations. Moreover, combination therapy at moderate doses exhibited slightly superior efficacy, indicating potential synergistic interactions. Overall, ethanolic extracts of *C. odorata* and *Z. officinale*, particularly in combination, may offer a safe and effective prophylactic or therapeutic option for mitigating ulcer-associated haematological disturbances, warranting further studies on chronic safety and underlying molecular mechanisms.

Keywords: *Toxicology, LD50, Chromolaena odorata, Zingiber officinale, body weight, haematology*

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1. Introduction

Plant-based extracts are increasingly investigated as alternatives to synthetic drugs due to their safety, affordability, and therapeutic potential (Nwaka et al., 2015; Egbuna et al., 2020; Abiodun et al., 2024a; Abiodun et al., 2024b; Akwas et al., 2025; Ekesiobi & Onebunne, 2025). The therapeutic relevance of plant-based formulations is further supported by recent patented innovations, such as the cocktail derived from *Pteridium aquilinum*, *Mucuna pruriens*, and *Newboldia laevis* for diabetes management (Unegbu *et al*., 2025). *Chromolaena odorata* and *Zingiber officinale* are medicinal plants widely used in traditional medicine, with reported antimicrobial, anti-inflammatory, and antioxidant properties (Ekesiobi et al., 2025). Despite their widespread use, systematic toxicological studies are necessary to establish safety profiles. This study evaluates the acute toxicity (LD_{50}), effects on body weight, and

haematological parameters of ethanolic extracts of *C. odorata* and *Z. officinale* in Wistar rats.

In the quest for safer and more effective therapeutic agents, medicinal plants have garnered significant attention due to their natural origin, affordability, and broad pharmacological potential (Asomugha *et al*., 2013; Adam et al., 2022; Riaz et al., 2023; Egbuna et al., 2025; Noreen et al., 2025). Among these, *Chromolaena odorata* (Siam weed) and *Zingiber officinale* (ginger) stand out for their traditional usage across diverse cultures and an expanding scientific interest in their bioactive properties (Achara et al., 2025; Iheukwumere et al., 2025; Ilechukwu et al., 2025). *C. odorata*, widely used in ethnomedicine, is applied topically for wound healing and treatment of soft tissue infections; its leaves and extracts are also utilized as purgatives and for treating coughs and skin diseases (Asomugha *et al*., 2013). Such widespread use often proceeds under the assumption of safety, yet the presence of potent phytochemicals and phytotoxins (Akwas et al., 2025) necessitates formal toxicological validation. Aqueous and ethanolic extracts of *C. odorata* have been studied in sub-chronic and acute settings. Asomugha *et al*. (2013) reported that doses below 538.5 mg/kg caused no observable sub-chronic toxicity, though higher doses were associated with biochemical disturbances. Similarly, Asomugha *et al*. (2015) determined an LD_{50} of approximately 2154 mg/kg for the aqueous extract and >5000 mg/kg for the ethanolic extract, indicating relatively low acute toxicity in Wistar rats.

The pharmacological potential of *Z. officinale* is well documented, especially its anti-inflammatory, antioxidant, and digestive-supporting effects. Safety assessments generally support its tolerability. For instance, oral administration of ginger extract at doses up to 5000 mg/kg in mice elicited no acute toxicity (Peneme *et al*., 2023), and cosmetic safety panels continue to regard ginger-derived ingredients as safe within typical usage levels (Cosmetic Ingredient Review Panel, 2022). Sub-chronic experiments, such as a 90-day study in female Wistar rats, similarly reported no significant adverse effects on body weight or general health indicators (Yakubu *et al*., 2024). These findings emphasize the relative safety of ginger extracts, though detailed haematological evaluations remain limited.

Peptic ulcer disease, a chronic condition characterized by mucosal erosion of the stomach or duodenum, remains a major public health concern worldwide. Its etiology is multifactorial, involving *Helicobacter pylori* infection, excessive use of non-steroidal anti-inflammatory drugs (NSAIDs), stress, smoking, and dietary factors. Ulceration often leads to complications such as bleeding, perforation, and in some cases malignant transformation, contributing significantly to morbidity and mortality (Egbe, 2021). Conventional management strategies include the use of proton pump inhibitors, H2 receptor antagonists, and antibiotics for *H. pylori* eradication, but these treatments are associated with side effects, recurrence, and increasing antimicrobial resistance. As a result, there is a growing interest in plant-based alternatives that possess gastroprotective, anti-inflammatory, and antioxidant properties. Both *C. odorata* and *Z. officinale* have demonstrated promising protective effects against gastric mucosal injury, further justifying the need for detailed toxicological and haematological investigations of their extracts in preclinical models.

Despite promising safety signals, differences in extraction method (ethanolic extract), dosage, species, administration route, and treatment duration call for tailored toxicological assessments. Acute LD_{50} estimation remains a fundamental requirement in toxicology to establish safety thresholds for novel extracts (Lorke, 1983). Another vital but often overlooked parameter in such studies is body weight change, which serves as an integrative marker of metabolic and physiological response over time. Similarly, haematological indices—which include haemoglobin concentration, packed cell volume, red and white blood cell counts, and differential leukocyte percentages—offer critical insight into systemic impacts such as anaemia, immunomodulation, or bone marrow response. Given that *C. odorata* and *Z. officinale* are sometimes combined in ethnomedicinal formulations, investigating their combined toxicity and safety profile holds particular relevance, as combined effects may yield additive, synergistic, or antagonistic interactions.

Previous studies have provided foundational LD_{50} values for *C. odorata*, while observational studies in sub-chronic setups suggested emerging biochemical toxicity at higher doses (Asomugha *et al*., 2013; Asomugha *et al*., 2015). For *Z. officinale*, acute safety up to 5000 mg/kg has been noted (Peneme *et al*., 2023; Yakubu *et al*., 2024), although haematological outcomes were not deeply explored. Thus, there remains a gap in understanding the combined effects and haematological consequences of ethanolic extracts of these plants. The present study, therefore, aims to determine the acute toxicity and LD_{50} values of ethanolic extracts of *C. odorata* and *Z. officinale*, monitor their impact on body weight, and evaluate haematological parameters in Wistar rats, thereby contributing valuable insights to their safety profiles and therapeutic potential. Importantly, these findings may also provide a basis for exploring their usefulness in the prevention and management of peptic ulcer disease, where oxidative stress, inflammation, and altered haematological status play critical roles.

2. Materials and Methods

2.1 Plant Materials and Preparation

Fresh *Chromolaena odorata* leaves were harvested from Mgbakwu Farm in Anambra State, Nigeria, and rhizomes of *Zingiber officinale* were purchased from Eke Awka Market. Identification and authentication of the plants were carried out by a botanist at Chukwuemeka Odumegwu Ojukwu University, Uli. The collected samples were thoroughly washed, air-dried at ambient temperature, ground into fine powder, and preserved in airtight containers until required for analysis.

2.2 Extraction of Leaves of *Chromolaena odorata* and Rhizomes of *Zingiber officinale*

The collected plant materials were dried at room temperature for two weeks and subsequently pulverized using a corona manual grinder. The ethanolic extracts of *C. odorata* and *Z. officinale* were obtained following standard phytochemical extraction approaches, consistent with innovative methods

outlined in recent extraction patents (Okei & Uwakwe, 2025). A total of 200 g each of *C. odorata* leaf powder and *Z. officinale* rhizome powder was soaked separately in 2 L of 70% ethanol and allowed to extract for 24 hours. During this period, the mixtures were stirred at two-hour intervals to facilitate complete extraction. After 24 hours, the extracts were sieved separately using muslin cloth and further filtered through Whatman filter paper. The filtrates were concentrated using a water bath maintained at 50 °C. The final yield after concentration was 34.16 g for *C. odorata* and 99.48 g for *Z. officinale*. The concentrated extracts were transferred into airtight containers, stoppered, and stored in a refrigerator until required for use.

2.3 Experimental Animals

Wistar rats weighing between 120–150 g were procured from Chris Experimental Animal Farm and Research Laboratory, Mgbakwu, Awka. The animals were housed under standard laboratory conditions, with controlled temperature, adequate ventilation, and a 12-hour light/dark cycle. They were provided with commercial pelleted rat chow obtained from Eke Awka, Anambra State, and given free access to clean water *ad libitum* under strict hygienic conditions. All experimental procedures involving animals were conducted in accordance with the guidelines for the care and use of laboratory animals as approved by the Institutional Animal Ethics Committee and consistent with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. Ethical clearance for this study was obtained from the Chukwuemeka Odumegwu Ojukwu University Ethical Committee on Animal Use in Research.

2.4 Experimental Induction of Ulcer

Standard indomethacin was procured from Pax Pharmaceutical, Onitsha, Anambra State. Ulcer induction was carried out by a single oral administration of indomethacin at a dose of 20 mg/kg body weight, given orally at three-day intervals. To confirm induction, one rat from each group was sacrificed and the stomach examined for lesions as well as other macroscopic signs and symptoms of ulceration.

2.5 Experimental Design

A total of one hundred and forty-four (144) albino rats was employed in this study. Thirty-nine (39) male albino rats was used for acute toxicity assessment, while one hundred and five (105) rats was randomized into fifteen (15) groups of seven animals each, which span forty-four (44) days (one month and two weeks). Acute toxicity was monitored for 24 hours, with both phase I and phase II carried out accordingly. Baseline haematological parameters was evaluated prior to extract administration. The experimental groups and treatments are outlined as follows:

Group A – Normal control
Group B – Indomethacin (ulcer untreated)
Group C – Standard drug (20 mg/kg Omeprazole)
Group D – Indomethacin + 50 mg/kg *Chromolaena odorata* (Pretreatment)
Group E – Indomethacin + 100 mg/kg *Chromolaena odorata* (Pretreatment)
Group F – Indomethacin + 200 mg/kg *Chromolaena odorata* (Pretreatment)
Group G – Indomethacin + 50 mg/kg *Chromolaena odorata* (Post-treatment)
Group H – Indomethacin + 100 mg/kg *Chromolaena odorata* (Post-treatment)
Group I – Indomethacin + 200 mg/kg *Chromolaena odorata* (Post-treatment)
Group J – Indomethacin + 50 mg/kg combination extract (Pretreatment)
Group K – Indomethacin + 100 mg/kg combination extract (Pretreatment)
Group L – Indomethacin + 200 mg/kg combination extract (Pretreatment)
Group M – Indomethacin + 50 mg/kg combination extract (Post-treatment)
Group N – Indomethacin + 100 mg/kg combination extract (Post-treatment)
Group O – Indomethacin + 200 mg/kg combination extract (Post-treatment)

2.6 Acute Toxicity (LD_{50}) Studies

The acute toxicity of the extracts was evaluated using the method described by Lorke (1983). In the first phase, animals received graded doses of 10, 100, and 1000 mg/kg body weight. This phase served to indicate whether the extracts could be classified as highly toxic, moderately toxic, slightly toxic, or relatively safe. Since no mortality was observed in the first phase, the second phase involved administration of higher doses at 1600, 2900, and 5000 mg/kg. In cases where mortality occurs during the first phase, subsequent testing would typically employ lower doses based on the number of deaths recorded. Following administration, the animals were closely monitored for 24 hours and subsequently observed for a period of 14 days for clinical signs and symptoms of toxicity.

2.7 Body Weight Monitoring

Rats were weighed weekly for four weeks to assess changes in body weight across treatment and control groups.

2.8 Haematological Analysis

2.8.1 Determination of Packed Cell Volume (PCV)

Principle: When a whole blood sample is subjected to centrifugal force, the red blood cells (RBCs) are packed maximally, and the proportion of packed cells relative to the total blood volume is expressed as a percentage (Bain *et al*., 2011).

Procedure: Using the microhaematocrit method, well-mixed anticoagulated blood was drawn into capillary haematocrit tubes until approximately two-thirds full. Each tube was sealed at one end with a Bunsen flame and placed in the haematocrit centrifuge head in opposite pairs, with the open ends facing inward. The tubes were centrifuged for 5 minutes at 11,000 rpm, removed after centrifugation, and the packed cell volume was read using a microhaematocrit reader (Bain *et al*., 2011).

2.8.2 Determination of Haemoglobin Concentration (Hb)

Principle: When whole blood is added to Drabkin's reagent, potassium ferricyanide oxidizes haemoglobin ($Hb\text{-}Fe^{2+}$) to methaemoglobin ($Hb\text{-}Fe^{3+}$), which subsequently reacts with potassium cyanide to form cyanmethemoglobin, a stable compound. The colour intensity of this complex is directly proportional to haemoglobin concentration and is measured spectrophotometrically at 540 nm (Kumar & Kangle, 2022).

Procedure: Using the cyanmethemoglobin method, 5.0 ml of Drabkin's reagent was pipetted into two test tubes. A well-mixed EDTA blood sample (0.02 ml) was added, and the pipette was rinsed several times with the reagent to ensure complete transfer. The mixtures were allowed to stand at 25 ℃ for 10 minutes to allow full conversion. Samples were transferred into cuvettes and absorbance measured at 540 nm against a reagent blank. Haemoglobin concentrations (g/dl) were obtained from a pre-calibrated standard chart (Kumar & Kangle, 2022).

2.8.3 Determination of White Blood Cell (WBC) Count

Principle: Whole blood diluted with weak acid solutions such as glacial acetic acid leads to haemolysis of RBCs, leaving WBCs intact for enumeration (Bain *et al*., 2011).

Procedure: Using a WBC pipette, blood was drawn to the 0.5 mark and diluted with WBC diluting fluid to the 11 mark. After thorough mixing, the counting chamber and cover glass were cleaned, and the chamber was charged with the diluted blood. Under the microscope, the four large corner squares of the haemocytometer were examined under low power (10×), and WBCs were counted.

2.8.4 Determination of Red Blood Cell (RBC) Count

Principle: Whole blood is diluted with Gower's solution, which lyses WBCs while preserving RBCs for counting (Bain *et al*., 2011).

Procedure: A 1:200 dilution was prepared by mixing 20 µl of EDTA-anticoagulated blood with 3.98 ml of Gower's solution for 3 minutes. After cleaning the haemocytometer and cover glass, 10 µl of the diluted suspension was introduced into both chambers, avoiding air bubbles. The sample was allowed to stand for 3 minutes before examination. The haemocytometer was then viewed under 10× objective to locate the grid, and RBCs were enumerated using a 40× objective lens.

Note: Gower's solution contains 12.5 g sodium sulphate, 33.3 ml glacial acetic acid, and 100 ml distilled water.

2.8.5 Determination of Differential Leukocyte Count

Principle: A peripheral blood smear stained with Romanowsky dyes (e.g., Leishman stain) enables morphological examination of RBCs, WBCs, and platelets, as well as identification of leukocyte subtypes (Bain *et al*., 2011).

Procedure: A drop of well-mixed anticoagulated blood was placed on a clean glass slide and spread to form a thin smear, which was air-dried. The smear was flooded with Leishman stain for 2 minutes, followed by dilution with twice its volume of buffered distilled water. The mixture was gently aerated to ensure uniform staining and allowed to stand for 8 minutes. Excess stain was rinsed off with buffered water, and the slide was air-dried in an upright position. Microscopic examination was performed under oil immersion using the 100× objective lens to identify and count neutrophils, lymphocytes, eosinophils, basophils, and monocytes.

2.9 Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using the SPSS version 23. ANOVA was done to find out whether the readings were significant or not. P values <0.05 were considered as significant and P>0.05 were considered as not significant.

3. Results

3.1 Acute Toxicity (LD50)

The acute toxicity evaluation of the ethanolic extracts of *Chromolaena odorata* and *Zingiber officinale* showed no mortality at doses up to 5000 mg/kg (Tables 1 and 2). In the first phase (10–1000 mg/kg), all animals appeared normal with no signs of distress. In the second phase (1600–5000 mg/kg), the extracts were well tolerated; however, mild weakness was observed at the highest dose (5000 mg/kg) of *C. odorata*, while *Z. officinale* produced no observable adverse effects. These findings indicate that both extracts possess a high margin of safety, with oral LD_{50} values greater than 5000 mg/kg in Wistar rats.

Table 1: Acute toxicity studies of *Chromolaena odorata* (LD50)

Dose (mg/kg)	Weight (g)	Observation	Phase
10	126	Normal	Phase I
10	123	Normal	Phase I
10	125	Normal	Phase I
100	127	Normal	Phase I
100	121	Normal	Phase I
100	123	Normal	Phase I
1000	125	Normal	Phase I
1000	125	Normal	Phase I
1000	124	Normal	Phase I
1600	123	Normal	Phase II
2900	130	Normal	Phase II
5000	136	Slightly weak	Phase II

Table 2: Acute toxicity studies of Ginger (LD50)

Dose (mg/kg)	Weight (g)	Observation	Phase
10	121	Normal	Phase I
10	130	Normal	Phase I
10	127	Normal	Phase I
100	121	Normal	Phase I
100	122	Normal	Phase I
100	123	Normal	Phase I
1000	129	Normal	Phase I
1000	127	Normal	Phase I
1000	132	Normal	Phase I
1600	131	Normal	Phase II
2900	130	Normal	Phase II
5000	135	Normal	Phase II

3.2 Body Weight

Figure 1 shows the weekly body weight changes. Control rats maintained steady growth, while indomethacin-induced groups had reduced weight gain. Extract-treated groups exhibited improved weight maintenance compared to ulcer-untreated groups.

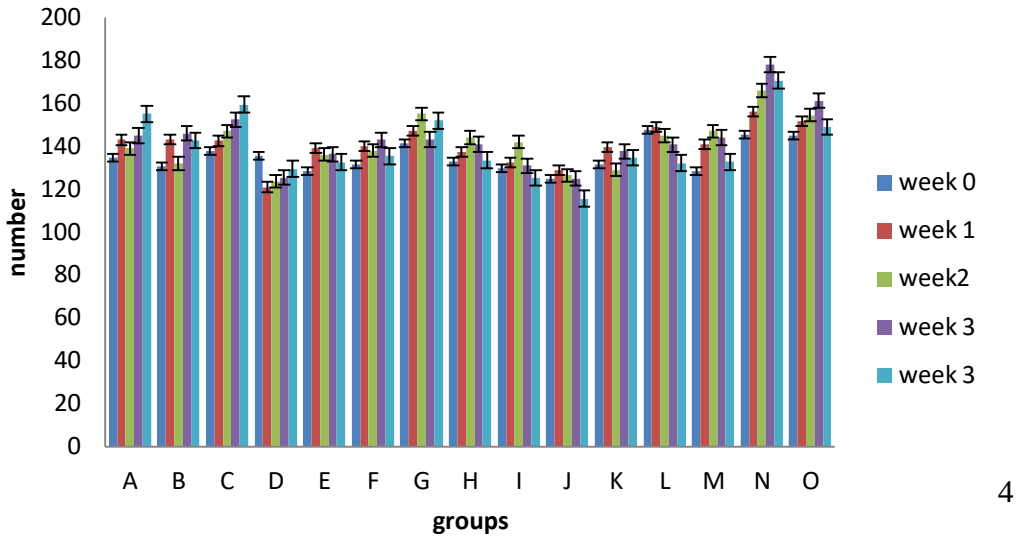

Figure 1: Body weight of each group for a period of 4 weeks

3.3 Haematological Parameters

The effects of *Chromolaena odorata*, *Zingiber officinale*, and their combination on haematological parameters in indomethacin-induced ulcerated rats are presented in Tables 3 and 4.

The normal control group (Group A) maintained the highest haemoglobin (Hb) and packed cell volume (PCV) values (14.78 ± 0.15 g/dl; 44.20 ± 0.42%), with balanced leukocyte distribution. In contrast, the ulcer untreated group (Group B) and particularly the indomethacin post-treatment groups (e.g., Group H) exhibited pronounced declines in Hb and PCV (as low as 8.30 ± 0.00 g/dl and 25.00 ± 0.00%, respectively), consistent with ulcer-induced anaemia.

The standard drug (Group C, omeprazole 20 mg/kg) moderately improved Hb (12.00 ± 0.00 g/dl) and PCV (36.00 ± 0.00%), though not to control levels. Pretreatment with *C. odorata* (Groups D–F) showed dose-dependent protective effects, with 100 mg/kg (Group E) improving Hb to 11.20 ± 0.20 g/dl and PCV to 33.67 ± 0.66%. Post-treatment groups (G–I) showed less recovery, indicating stronger prophylactic than therapeutic benefit.

Combination extract groups (J–O) demonstrated notable improvements compared to single extracts. The 100 mg/kg pretreatment group (Group N) produced near-normal values (Hb 13.30 ± 0.13 g/dl; PCV 40.00 ± 0.44%), closely aligning with controls. Post-treatment groups (M–O) also showed improvements but to a lesser degree than their pretreatment counterparts.

White blood cell (WBC) counts were markedly elevated in the ulcer untreated group (Group H: 8.50 × 10^3/mm^3), with a parallel decrease in red blood cell (RBC) count (1.10 × 10^{12}/L), reflecting systemic inflammation and anaemia. Extract-treated groups displayed significant amelioration. Notably, Groups K and L (100–200 mg/kg combination pretreatment) maintained WBC counts around 5.35 × 10^3/mm^3 and RBC counts above 4.17 × 10^{12}/L, approaching the normal control values (4.20 × 10^3/mm^3; 4.79 × 10^{12}/L).

Overall, the results indicate that both *C. odorata* and *Z. officinale* extracts mitigated indomethacin-induced haematological alterations. Combination pretreatment at moderate doses (100–200 mg/kg) showed slightly improved protective effects compared with single extracts, particularly in red cell indices, though values did not fully return to normal. Post-treatment with combination extracts (100 mg/kg) more effectively maintained WBC counts near control levels, suggesting potential additive benefits in stabilizing haematological parameters.

Table 1: Haemoglobin, PCV, Neutrophils, Lymphocytes, Eosinophils, Basophils

Group	Hb (g/dl)	PCV (%)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Basophils (%)
A	14.78±0.15	44.20±0.42	53.00±0.78	39.70±0.89	-	1.00±0.00
B	12.70±0.21	38.20±0.66	53.20±0.48	40.00±1.04	-	1.00±0.00
C	12.00±0.00	36.00±0.00	57.50±1.50	42.00±1.15	-	-
D	12.30±0.00	37.00±0.00	56.00±0.00	38.50±0.50	-	1.00±0.00
E	11.20±0.20	33.67±0.66	57.00±0.00	38.33±1.66	-	1.00±0.00
F	11.30±0.00	34.00±0.00	54.33±1.33	39.33±2.60	-	-
G	10.60±0.00	32.00±0.00	57.50±1.50	42.00±2.00	-	-
H	8.30±0.00	25.00±0.00	59.00±0.00	40.00±0.00	-	-
I	10.60±0.00	32.00±0.00	57.5±1.50	42.00±0.00	-	-
J	10.60±0.00	32.00±0.00	57.5±1.50	42.00±0.00	-	-
K	12.07±0.08	36.25±0.25	53.75±0.47	40.25±1.31	-	1.00±0.00
L	12.07±0.08	36.25±0.25	53.75±0.47	40.25±1.31	-	1.00±0.00
M	12.70±0.00	38.20±0.66	53.40±0.510	39.80±1.11	-	1.00±0.00
N	13.30±0.13	40.00±0.44	53.40±0.50	39.80±1.12	-	-
O	11.30±0.00	34.00±0.00	53.67±0.67	41.00±0.00	-	-

Table 2: White Blood Cells and Red Blood Cells

Group	WBC (×10^3/mm3)	RBC (×10^12/L)
A	4.20±0.03	4.79±0.15
B	4.34±0.04	4.16±0.04
C	6.40±0.10	4.10±0.00
D	5.75±0.05	4.10±0.00
E	5.10±0.07	3.22±0.17
F	6.36±0.03	3.30±0.12
G	7.40±0.01	2.10±0.00
H	8.50±0.00	1.10±0.00
I	6.40±0.01	2.10±0.00
J	6.40±0.00	2.10±0.00
K	5.35±0.03	4.17±0.05
L	5.35±0.03	4.17±0.05
M	4.32±0.04	4.16±0.04
N	4.32±0.04	4.16±0.04
O	5.36±0.03	3.30±0.00

4. Discussion

The current study found that ethanolic extracts of *Chromolaena odorata* (C. odorata) leaves and *Zingiber officinale* (ginger) rhizomes exhibited excellent safety profiles in the acute oral toxicity assay, with LD_{50} values exceeding 5,000 mg/kg. These findings are consistent with current literature on the acute toxicity of these plant extracts.

4.1 Acute Toxicity (LD_{50})

The absence of mortality and only minimal clinical signs—such as slight weakness for *C. odorata* at 5,000 mg/kg—indicate a high safety margin for both extracts. This aligns with a prior report indicating LD_{50} > 5,000 mg/kg for ethanolic *C. odorata* in Wistar rats, while the aqueous extract was less safe, with an LD_{50} of approximately 2,154 mg/kg (Asomugha *et al*., 2015).

For ginger, a comprehensive safety assessment by the Cosmetic Ingredient Review Expert Panel reported no toxicity or mortality in Wistar or Sprague-Dawley rats at single oral doses up to 5,000 mg/kg (Cosmetic Ingredient Review Expert Panel, 2022). Moreover, an independent study calculated the oral LD_{50} of ethanol extract of *Z. officinale* to be ~3,807 mg/kg in rats, somewhat lower yet still demonstrating low acute toxicity (Hamman *et al*., 2022). These data confirm that while ginger extract is generally safe in acute settings, its LD_{50} may be nearer to 3,800 mg/kg in some formulations, offering a point of comparison.

4.2 Body Weight Effects

In this study, indomethacin-induced ulcerative rats (Group B) lost weight over four weeks, while extract-treated groups—particularly the combination extract post-treatment (Group N, 100 mg/kg)—not only maintained but modestly increased body weight, closely mirroring control rats. This protective effect could be due to improved general health and reduced metabolic stress from ulceration.

These observations correspond with literature reporting that ginger extract, even at high single doses (up to 5,000 mg/kg), did not affect body weight in rodents in acute or sub-chronic studies (Cosmetic Ingredient Review Expert Panel, 2022; White, 2007). Although direct data on body weight in *C. odorata* toxicology studies are limited, the absence of adverse effects up to very high doses implies metabolic tolerance.

4.3 Haematological Effects: Anaemia and Inflammation

The indomethacin-only ulcer untreated group (Group B) demonstrated significant haematological disruption, including declines in haemoglobin (Hb), packed cell volume (PCV), and red blood cell (RBC) counts, alongside elevated white blood cell (WBC) counts. These alterations indicate ulcer-induced anaemia and systemic inflammation—common consequences of NSAID-induced mucosal injury and gastrointestinal blood loss.

In contrast, rats pretreated with *C. odorata* (Groups D–F) or combination extracts (Groups J–L) showed improved haematological indices, while post-treatment groups (G–I and M–O) also exhibited recovery, though to a lesser degree. The 100 mg/kg combination post-treatment group (Group N) was particularly effective, with Hb, PCV, RBC, and WBC values closely matching those of the normal control (Group A). This indicates that the extracts, especially in combination, exert potent anti-inflammatory and haemoprotective effects in ulcerated rats.

While specific haematological data for *C. odorata* are sparse, similar studies evaluating ethanol extracts of other botanicals show modest increases in RBC and Hb with extract administration (Oladunmoye, 2006; Achara et al., 2025). Meanwhile, ginger extract has well-documented anti-inflammatory and antioxidant activities (Cosmetic Ingredient Review Expert Panel, 2022), which may explain its ability to moderate WBC elevation and preserve red cell integrity in the present study.

4.4 Synergistic Benefits of Combination Therapy

The superior performance of the combination–pretreatment groups over single extracts (i.e., Groups J–L vs. D–F) suggests potential synergistic interaction. The combination appears to better preserve haematological homeostasis, possibly by integrating distinct yet complementary phytochemical mechanisms—such as the wound-healing and anti-inflammatory compounds in *C. odorata* and the antioxidant and gastroprotective constituents of ginger.

Although direct studies on such combinations are still emerging, the principle of synergy in ethnomedicinal formulas is well established. Future studies might clarify which bioactive compounds mediate these effects and whether they include flavonoids, phenolics, or other phytochemicals.

4.5 Comparison with Prior Findings

The results presented here align well with previous investigations into the toxicity and therapeutic potential of these extracts:

For *C. odorata*, earlier acute toxicity assays established an LD_{50} for the ethanolic extract > 5,000 mg/kg in Wistar albino rats (Asomugha *et al*., 2015; Yakubu *et al*., 2023), corroborating the high margin of acute safety seen here.

Regarding ginger, CIR panel documents no adverse outcomes at up to 5,000 mg/kg; similarly, a study calculating an LD_{50} of

~3,807 mg/kg indicates an acceptable safety profile (Hamman *et al*., 2022; Cosmetic Ingredient Review Expert Panel, 2022). In addition, ginger extract has been shown to elicit only minor or transient biochemical alterations in sub-acute studies, with no significant effect on body weight or haematology at moderate doses (Cosmetic Ingredient Review Expert Panel, 2022; White, 2007).

5. Conclusion

The ethanolic extracts of *Chromolaena odorata* and *Zingiber officinale* demonstrated safety in acute toxicity studies, with LD_{50} values exceeding 5,000 mg/kg in Wistar rats. Both extracts, especially when combined at 100 mg/kg, showed significant gastroprotective and haemoprotective effects against indomethacin-induced ulceration. Notably, the 100 mg/kg combination post-treatment group (Group N) exhibited haematological parameters (Hb, PCV, RBC, and WBC) that closely approximated those of the normal control group, alongside preservation of body weight. These findings suggest a synergistic interaction between the two extracts, enhancing their therapeutic efficacy. Further investigations are warranted to evaluate chronic safety and to elucidate the molecular pathways underlying their protective actions.

Conflict of interests: Authors declare no conflict of interests.

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