



RESEARCH ARTICLE

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Toxicity study and ameliorative effects of the aqueous leaf extract of *Lecanoidiscus cupanioides* Planch (ex. Benth) on the stress-induced ulcer

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ARTICLE INFO

Article History:

Received: 27 October 2023
Revised: 27 March 2024
Accepted: 19 April 2024
Available online: 22 April 2024

Edited by: B. Tepe

Keywords:

Lecanoidiscus cupanioides
Toxicity studies
Stress induced ulcer
Water immersion

ABSTRACT

Lecanoidiscus cupanioides Planch (ex. Benth) is effective in treating burns, fevers, and inflammatory conditions. This study investigates the toxicity effects of the aqueous leaf extract (ALE) of *L. cupanioides* and its effect on stress-induced ulcers in animal models. The plant was collected and a 1:30 (g/ml) plant powder/solvent ratio was extracted using an ultrasonic bath at 50 °C for 45 min. Up-and-down procedure was used for acute toxicity. During subacute toxicity testing, a total of 20 mice were divided into four groups of five animals each. While group 1 served as control, groups 2, 3, and 4 received 250, 500, and 1000 mg/kg of the extract daily for 21 days. On day 22nd, animals were sacrificed and samples were collected for hematology, biochemical, and histological analyses. In the stress-induced ulcer activity, male albino mice were randomly separated into 5 groups of 5 animals, treated with the test drug, and then dissected after being stressed using the water immersion model. LD₅₀ was > 5000 mg/kg, and in biochemical examination, there was a significant decrease in the ALP level at medium and high doses (p value < 0.05) and non-significant alterations in the values of urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), body weight and hematology parameters (p > 0.05). The histology results showed no evidence of liver and kidney toxicity. In conclusion, aqueous leaf extract of *L. cupanioides* was safe following a single dose (at 5000 mg/kg) and following repeated doses for 21 days (at 250, 500, and 1000 mg/kg). This study demonstrated that the extract had a significant effect on stress-induced ulcers at various dose levels when compared to the control (native, negative control, and omeprazole). The animals treated with 200mg/kg and 400mg/kg of the extract showed a significant increase in ulcer score, ulcer index, ulcer severity, and total acidity (p < 0.05). The ulcer protection ability of the aqueous plant extract was dose-dependent.

1. Introduction

Medicinal plants have been employed across the world to treat health problems and diseases. These plants are becoming more commonly used in health care delivery, particularly in resource-constrained situations. Approximately 80% of the global population relies on traditional medicine for basic health care (Ekor, 2014; Ugwah et al., 2013). Although the employment of these plants has demonstrated significant potential with considerable worldwide demand, there are still questions regarding not just their use but also their safety (Ifeoma & Oluwakanyinsola, 2013). It is generally known that using medicinal herbs without first assessing their efficiency and safety profile can lead to harmful consequences for several organs. The liver and kidneys are the primary targets since they are involved in the metabolism

Reviewed by:

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e-ISSN: 2791-7509
doi: <https://doi.org/10.62313/ijbbp.2024.159>

and excretion of toxic substances (Hodges & Minich, 2015). Before being used on humans, newly created medications must undergo extensive toxicity testing (Arome & Chinedu, 2013), safety testing of these chemicals, pharmaceuticals, food and food additives, cosmetics, and industrial items is essential (Erhirhie et al., 2018). Since safety remains a key concern with the use of medicinal plants, it is imperative to undertake toxicity studies on them to determine their risk profile.

Peptic ulcer disease involves both gastric and duodenal ulcers, which cause death over the years (Malfertheiner et al., 2009). According to studies, peptic ulcer remains one of the main pathologies in about 10% of the human population (Sonnenberg, 2013). About 80–90% of patients with duodenal ulcers have *H. pylori* infection, and the same goes for 70–90% of patients with gastric ulcers (Thomas et al., 2005).

Major causes of peptic ulcers include *H. pylori* bacteria, emotional stress, NSAIDs, alcohol abuse, and smoking. Associated signs and symptoms include abdominal discomfort and nausea, bloating and abdominal fullness, dark or black stools, water brash, hematemesis, melena, and rarely acute peritonitis, among others (Iqbal et al., 2012; Ramasubramaniraja & Babu, 2011; Roy, 2016). The majority of peptic ulcer cases are due to *H. pylori* infection. Antiulcer drugs include antagonists of the histamine H₂ receptor antagonists (such as ranitidine), and inhibitors of proton pump (such as omeprazole), among others (Jain, 2016).

However, problems, such as nephrotoxicity, gynecostasia, thrombocytopenia, hepatotoxicity, and impotence (Chan & Leung, 2002; Sheen & Triadafilopoulos, 2011), which are associated with synthetic drugs have awakened the research on medicinal plants, which are natural and also described as an important source of modern medications (Albarri et al., 2017; AlMatar et al., 2018; Dharmani et al., 2005; Sharma et al., 2011).

Lecaniodiscus cupanioides Planch (ex Benth) is a tropical plant with a large geographic range in Asia and Africa. It is a member of the Sapindaceae family and is known in Nigeria by several names, including Ukpo (Igbo), Utantan (Edo), Kafi-nama-zaki (Hausa), and Akika (Yoruba). Traditionally, it is effective in treating burns, fevers, measles, abdominal swelling brought on by liver abscesses, and wounds and sores (Nafiu et al., 2013), as well as other ailments including cancer, measles, jaundice, diabetes, sexual dysfunction, typhoid, wounds, skin infections, and galactagogues (Ojo & Ndinteh, 2023).

Due to their higher cultural acceptance, lower side effects (Sam, 2019), lower costs, and lower availability (Alharbi et al., 2017), herbal and traditional medicine have gained ground as alternate remedies to several diseases.

As part of drug discovery, newly created medications must undergo extensive toxicity testing (Arome & Chinedu, 2013; Erhirhie et al., 2018). Though most medicinal plants are assumed to be safe, safety evaluation is not out of place (Olaniyan et al., 2016).

Interestingly, herbal remedies are becoming recognized for the management and treatment of peptic ulcers, as a viable alternative to synthetic pharmaceuticals that are sold commercially (Keshavarzi et al., 2014).

In this present study, *L. cupanioides* was investigated for its toxicity and anti-ulcer effects using the water immersion model in male albino mice.

2. Materials and methods

2.1. Plant material

The fresh healthy leaves of *L. cupanioides* were collected in July 2022 from Orba, which is situated in Nsukka, Enugu, Nigeria, and its geographical coordinates are 6° 51' 0" North, 7° 27' 0" East. Proper identification and authentication were done by a taxonomist, Mr. Felix Nwafor in the Department of Pharmacognosy and Environmental Science, University of Nigeria, Nsukka with voucher reference number of UNH/04/0330C. The fresh leaves weighing about 3 kg were cleaned and air/shade dried at room temperature. About 2 kg of dry sample was pulverized.

2.2. Preparation of the aqueous leaf extract

2.2.1. Ultrasonic-assisted extraction

Preliminary analysis to select the appropriate solvent for the extraction was done using ethyl acetate, dichloromethane, methanol, and water. These solvents were selected based on documented evidence of their ability to extract phenolic compounds. A 1:30 (g/ml) herbal powder/solvent mixture was extracted using an ultrasonic bath at 50 °C for 45 min. The extract was filtered and the filtrate was concentrated using a freeze dryer (Lyoquest – 55 plus, Germany). The total phenolic contents and yields of the extracts obtained were determined and the solvent with the highest ability to extract phenolic compounds (water) was selected for the next phase of extraction. The aqueous filtrates (extracts) were first frozen which resulted in the formation of ice crystals. The frozen filtrates in glass flasks (75 ml each) were connected through a manifold to the freeze dryer. The manifold allowed 8 samples to be freeze-dried at the same time. The manifold was opened to a headspace with a partial pressure of water vapor below the equilibrium vapor pressure of ice. This very low partial pressure was achieved using a condenser at -55°C – which caused the sublimation of the ice crystals (primary sublimation) and also the desorption of non-crystalline water present within the extract (secondary drying). The vacuum was maintained in the freeze-drying chamber using a vacuum pump to remove air from the chamber to increase the rate of transfer of water vapor from the extract to the condenser. The freeze-drying process continued uninterrupted till the samples were all dried which was indicated by an increase in the extract temperature to approach the value of the shelf temperature. Additional drying time (secondary drying) was allowed to ensure that non-crystalline water had been completely removed (Adami et al., 2020).

2.3. Phytochemical analysis procedure

The phytochemical analysis of the leaf extract and fractions was carried out using standard methods (Harborne, 1998; Sofowora, 1993; Trease, 2002).

2.4 Effect of solvent on total phenolic content of aqueous leaf extract

The effect of solvent on total phenolic content of aqueous leaf extract (ALE) of *L. cupanioides* was determined using the method described by Kim et al. (2003). One milliliter of the extracts (100 µg/ml) was mixed with 0.2 ml of Folin-Ciocalteu's phenol reagent. After 5 minutes, 1 ml of 7.6% Na₂CO₃ solution was added to the mixture followed by the addition of 2 ml of distilled water. The mixture (in duplicate) was incubated at 40 °C for 30 minutes, after which the absorbance was read at 760 nm using a UV-VIS spectrophotometer against a blank (containing every other

component of the mixture except the sample). The total phenolic content was estimated from the calibrated curve which was made by preparing gallic acid solution and expressed as milligrams of gallic acid equivalent (GAE) per gram of the extracts.

2.5. Ethical approval

The ethical review committee of the College of Pharmaceutical Sciences at Chukwuemeka Odumegwu Ojukwu University gave the animal study ethical clearance and issued an ethical approval number, PHACOOU/AREC/2023/019.

2.6. Source of experimental animals

2.6.1. Source of experimental animals for toxicity study

Adult mice (body weight:25-40 g) were procured in the Faculty of Pharmaceutical Sciences, Chukwuemeka Odumegwu Ojukwu University, Igbariam, Anambra state. These animals were used in the toxicity experiments and were housed in the animal house of the same institution, at approximately 12h/12h of light/dark cycle and at 25 °C. Vital grow pelleted feed, and free water were given ad libitum. Oral feeding was carried out using a stainless-steel gavage cannula coated at the tip to prevent lesions to the upper abdominal area.

2.6.2. Source of experimental animals for stress-induced ulcer

Male Swiss Albino mice (25 – 30 g) were used for this study. All the animals were obtained from the Animal House of the Department of Pharmacology, Enugu State University of Science and Technology, Enugu State, Nigeria. Animals were allowed to acclimatize for one week before the commencement of the study. Food and water were provided ad libitum. All animal experiments were conducted in compliance with the NIH guide for the care and use of laboratory animals [National Institute of Health (NIH) (2011) Pub No: 85-23].

2.7. Acute toxicity study

The acute toxicity test was carried out using the up-and-down procedure described by (Erhirhie et al., 2018). Healthy mice were divided into two main groups. The first group served as the untreated healthy control group of mice which received distilled water (10 ml/kg), while the other group received a single dose of 5000 mg/kg of the plant extract dissolved in 10 ml per body weight. After a single oral administration of the extract, mice were observed for the first 4 hours, 24 hours, and once daily for 7 days for signs of toxicity and mortality.

2.8. Sub-acute toxicity studies

A total of 20 mice were grouped into five categories of six animals each of both sexes.

Group 1 served as the untreated healthy control and received distilled water daily, whereas groups 2, 3, and 4 received a low dose (250 mg/kg body weight), a medium dose (500 mg/kg body weight), and a high dose (1000 mg/kg body weight) of *L. cupanioides* leaf extracts, respectively, daily for 21 days.

Group 1: Distilled water (control)

Group 2: 250 mg/kg of *L. cupanioides* leaf extract

Group 3: 500 mg/kg of *L. cupanioides* leaf extract

Group 4: 1000 mg/kg of *L. cupanioides* leaf extract

The mice were fasted in the evening of the 21st day and were euthanized on the 22nd day with an overdose of chloroform. Blood was collected from the inferior vena cava into plain tubes, and allowed to clot, then centrifuged at 3500 rpm for 10 minutes. The serum obtained was used for the estimation of biochemical parameters. The whole blood collected was placed in EDTA tubes for the determination of hematology parameters. The liver and kidney tissues were excised and placed in 10% formal saline for histological study (Erhirhie et al., 2023).

2.8.1. Serum biochemistry

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase activity (ALP), blood urea nitrogen (BUN), and creatinine ranges were determined using the commercial assay kits (Erhirhie et al., 2023).

2.9. Measurement of body weight

Mice body weights were taken before administration and every week to observe variation in body weight.

2.10. Hematology analysis

Assay of hematological parameters was carried out by using Midray BC-2800 Auto Hematology Analyzer (Udoudoh et al., 2020). A volume of 18 µl of the sample was presented to the analyzer for full blood count.

2.11. Histopathological examination

Following the conclusion of the examination, the kidney and liver of sacrificed mice were removed to look for lesions associated with toxicity such as hemorrhage, reduced glomerulus, and central vein. The removed organs were promptly preserved in 10% formalin solution and prepared for histological analysis (Luna, 1968).

2.12. Experimental design for anti-ulcer study

2.12.1. Animal grouping and treatment

Male albino mice were used for the study. They were randomly divided into 5 groups of 5 animals each.

Group 1: The native group was not subjected to ulcer induction.

Group 2: The group was subjected to ulcer induction and received 200 mg/kg of the extract

Group 3: The group was subjected to ulcer induction and received with 400 mg/kg of the extract

Group 4: The group was subjected to ulcer induction and received 20 mg/kg omeprazole as a standard antiulcer reference drug

Group 5: The group was subjected to ulcer induction and given distilled water as a vehicle control group

Treatments were done orally and experiments were performed at fixed times of the day between 9 a.m. and 1 p.m.

2.13. Water immersion stress-induced ulcer

Animals were pre-treated with test drugs for 7 days before the beginning of the experiment. The animals were fasted for 36 hours after the last day of pre-treatment with free access to water and were once more treated with the test drugs one hour before subjecting them to stress by free swimming. The animals were put into water (water depth 7 cm to avoid drowning) and allowed free

movement in the water for 6 h. After swimming, mice were removed, dried, sacrificed under ether anesthesia, dissected and their stomachs were removed (Parmar & Desai, 1993).

2.13.1 Measurement of gastric acid and pH

After dissection, the stomach of the mice was legated from its two ends; the pylorus and lower esophagus. A small incision was made for each fore stomach and the stomach contents were collected in centrifuge tubes. They were centrifuged at 3500 r/min for 15 min and the pH of the supernatant was determined using a digital pH meter. Subsequently, 1 ml of the gastric juice was withdrawn into a conical flask, and then 2 drops of phenolphthalein indicator were added and titrated against 0.01N NaOH until a permanent pink color was seen. The volume of 0.01N NaOH used was noted. The total acidity was expressed as mEq/l and calculated by the given formula (Abebaw et al., 2017).

2.14. Statistical analysis

Results were presented as mean \pm standard deviation ($n = 5$). The comparison of mean values among groups was carried out using

SPSS with one way analysis of variance (ANOVA) followed by post-hoc Dunnett's test. $p < 0.05$ was considered to be statistically significant.

3. Results and discussion

3.1. Phytochemical analysis

Phytochemical analysis of the aqueous leaf extract of *L. cupanioides* is shown in Table 1. *L. cupanioides* revealed the presence of all the tested phytochemicals. Phytochemical analysis of *L. cupanioides* revealed the presence of flavonoids as well as the phenolic constituents like tannins. Phenolic compounds have several clinical relevance. Polyphenols are beneficial against cancer, osteoporosis, cardiovascular diseases and diabetes, including peptic ulcers (Sumbul et al., 2011). Flavonoids are among the cytoprotective materials whose anti-ulcerogenic activity has been well established (Borrelli & Izzo, 2000). The presence of the aforementioned phytoconstituents could be attributed to the ability of *L. cupanioides* to suppress stress-induced ulcers.

Table 1. Preliminary phytochemical analysis of aqueous leaf extract (ALE)

Phytoconstituents	Methods	Aqueous extract
Alkaloids	Wagner test	+++
	Hager's test	+
	Drangendorff test	++
Flavonoids	Lead acetate test	++
	Zn.Hydrochloride test	+
Tannins & Phenols	FeCl ₃ test	+
Saponins	Foamingtest	+++
Steroids & Triterpenoids	Salkowski test	+
Glycosides	Killer killani	+
	Leiberman Buchard's test	+
Carbohydrates	Molish test	++
Proteins	Biuret	++

3.2. Total phenolic content

As shown in Table 2, the effect of various solvents for the extraction of polyphenols present in *L. cupanioides* revealed that water had higher total phenolic content (TPC) than methanol, ethyl acetate, and DCM. This suggests that water was the best solvent for extracting phenolic compounds in the leaf of *L. cupanioides*. This is substantiated by the presence of various phytochemicals in the leaf extract as reported in Table 1. In line with other studies, most medicinal plants with higher total phenolic content were extracted with polar solvents (Alara et al., 2021).

Table 2. Effect of solvent on total phenolic content of the aqueous leaf extract (ALE) of *L. cupanioides*

Solvent	GAE/g extract
Ethyl acetate	90.50
DCM	53.84
Methanol	145.22
Water	316.61

3.3. Acute oral toxicity study

The oral toxicity result in mice revealed the LD₅₀ value of *L. cupanioides* extract to be 5000 mg/kg or greater (LD₅₀ \geq 5,000 mg/kg body weight) as it showed no mortality or any adverse effect (Table 3). This suggests that the aqueous leaf extract of *L. cupanioides* is considered to be safe or non-toxic at a human

equivalent therapeutic dose. In line with this study, several medicinal plants, which are considered natural have estimated LD₅₀ above 5000 mg/kg (Ebbo et al., 2020; Erhirhie et al., 2023). Usually, pre-clinical toxicity studies are used in safety investigations to identify any potential hazardous effects of any medicine (Nurudeen et al., 2021).

Table 3. Acute oral toxicity of *L. cupanioides* aqueous leaf extract (ALE)

Group	Treatment	Result
A	Control	Absence of toxicity and death
B	5000 mg/kg	Absence of toxicity and death

3.4 Effect of the *L. cupanioides* aqueous extract on weight change

When assessing the hazardous effects of medications, toxic chemicals, and therapies, body weight is an important parameter. One of the earliest crucial indicators of toxicity can be variations in weight. Weight index evaluation of animal growth is common practice in toxicological studies since it aids in interpreting compound-related effects (Erhirhie et al., 2023). In this study, there was no significant change in the weights of animals administered different doses of extract for 21 days compared to the control ($p > 0.05$) (Table 4). This suggests that the extract did not distort feed intake, nutrient absorption, and metabolism (Oloyede et al., 2020).

Table 4. Effect of the *L. cupanioides* aqueous extract on weight change

	Body weight (g)				Weight change (%)
	Week 0	Week 1	Week 2	Week 3	
Control	29.40 ± 1.92	29.00 ± 1.41	29.80 ± 1.91	32.60 ± 1.48	10.09 ± 2.26
Low dose	30.50 ± 1.55 ^{ns}	29.00 ± 1.22 ^{ns}	27.25 ± 1.25 ^{ns}	27.75 ± 1.43 ^{ns}	1.23 ± 9.47 ^{ns}
Medium dose	30.25 ± 1.38 ^{ns}	30.00 ± 0.40 ^{ns}	33.00 ± 3.34 ^{ns}	30.75 ± 1.43 ^{ns}	1.34 ± 5.22 ^{ns}
High dose	30.00 ± 1.22 ^{ns}	29.80 ± 1.19 ^{ns}	30.20 ± 1.14 ^{ns}	31.60 ± 1.30 ^{ns}	4.67 ± 4.87 ^{ns}

Values are expressed as mean ± SEM, n = 5, ns: $p > 0.05$: Not statistically significantly different from the control group. * $p < 0.05$: Statistically significantly different from the control group.

3.5. Assessment of serum biochemical parameters

Safety studies are accomplished by the implementation of general pre-clinical toxicity experiments to uncover the potential poisonous effects of any drug majorly in the liver and kidney of animals (Farzamfar et al., 2008).

Biochemical analysis showed no significant variations in urea, creatinine, aspartate transaminase (AST), and alanine transaminase (ALT) values among the different treatment groups compared to the control ($p > 0.05$) (Table 5). On the other hand, oral administration of aqueous extract of *L. cupanioides*, resulted in a significant reduction in alkaline phosphate (ALP) level at medium and high doses when compared to the control group ($p < 0.05$). AST, ALP, and ALT are considered as indicators of liver function, according to El Hilaly et al. (2004). Elevated levels of these enzymes in the serum

act as a marker of hepatotoxicity (Harris, 2005). In this study, the level of AST at graded doses was not increased compared to that of the control; this is a clear indication that the extract may not be hepatotoxic. This is substantiated by a significant reduction in ALP levels in medium and high doses, which may serve as an indication of the extract's protection against liver damage. Similarly, urea and creatinine, which are biomarkers of kidney toxicity were not significantly altered when compared to the control group, suggesting that the extract is not toxic to the kidney. The absence of liver and kidney lesions in the histology results (Figures 1 and 2) strongly supports the absence of liver and kidney toxicity as revealed by the biochemical results (Table 5); this is an indication that repeated administration of the extract at concentrations of 250, 500 and 1000 mg/kg for three weeks may be safe.

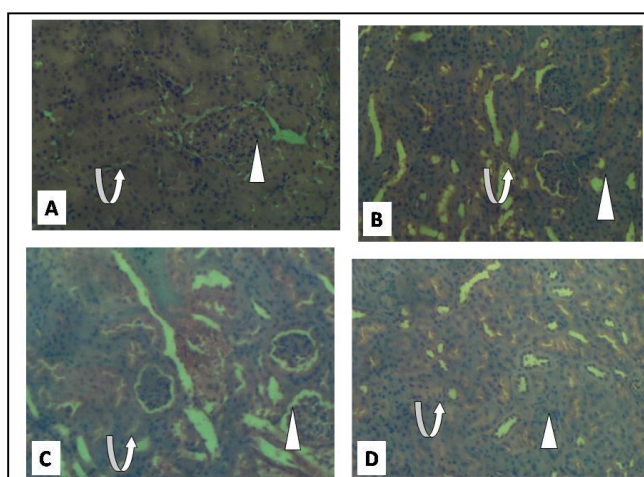


Figure 1. A, B, C, and D: Photomicrograph shows kidney tissue of albino rat with kidney histology consistent with normal morphology. The Renal capsules (arrowhead) and the tubules (curved arrow) are normal with no sign of injury (H&E, x400)

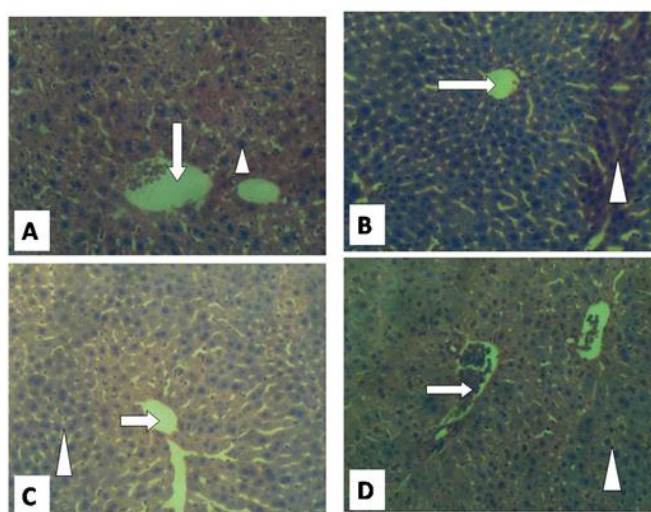


Figure 2. A, B, C, and D: Photomicrograph of liver tissue shows morphology consistent with normal liver histology. The hepatocytes (arrowhead) and central vein (arrow) are normal with no obvious sign of injury (H&E X 400).

Table 5. Effect of *L. cupanioides* aqueous leaf extract on serum biochemical parameters

	ALT (U/l)	AST (U/l)	ALP (IU/l)	UREA (mg/dl)	CREATININE (mg/dl)
Control	4.77 ± 1.19	131.74 ± 1.20	49.56 ± 11.67	37.67 ± 0.13	0.98 ± 0.13
Low dose	4.64 ± 0.66 ^{ns}	129.94 ± 1.31 ^{ns}	33.81 ± 10.28 ^{ns}	33.82 ± 5.60 ^{ns}	1.00 ± 0.20 ^{ns}
Medium dose	5.92 ± 1.37 ^{ns}	126.61 ± 2.06 ^{ns}	16.46 ± 4.87*	25.64 ± 5.20 ^{ns}	1.28 ± 0.19 ^{ns}
High dose	4.77 ± 0.74 ^{ns}	128.03 ± 1.07 ^{ns}	17.01 ± 4.72*	30.10 ± 5.99 ^{ns}	1.2 ± 0.19 ^{ns}

Values are expressed as mean ± SEM; n = 5, ns: $p > 0.05$: Not statistically significantly different from the control group. * $p < 0.05$: Statistically significantly different from the control group.

3.6. Assessment of hematological parameters

3.6.1 Effect of *L. cupanioides* aqueous leaf extract on hemoglobin, white blood cell, mean platelet volume, platelet distribution width, platelet crit, and platelet count

Hematology assessment serves as information regarding the safety profile of a substance on blood physiology (Nurudeen et al., 2021). Changes in blood parameters could be a result of alterations in cellular integrity, membrane permeability, metabolism, or even exposure to harmful compounds (Oloyede et al., 2020). The effect

of the aqueous leaf extract on all the hematological parameters showed no significant effect in extract treated group ($p > 0.05$) when compared to the control (Figure 3). This suggests that the extract may not alter the homeostatic process of the blood cells by distorting the transport of oxygen, alteration of immune defense mechanisms, and alteration in platelet function. Consistent with previous studies, aqueous extract of *L. cupanioides* roots produced insignificant changes in PCV and Hb levels (Joshua & Timothy, 2011), which also suggested that the extract has no potential to cause both macrocytic and microcytic anemia (Nurudeen et al., 2021).

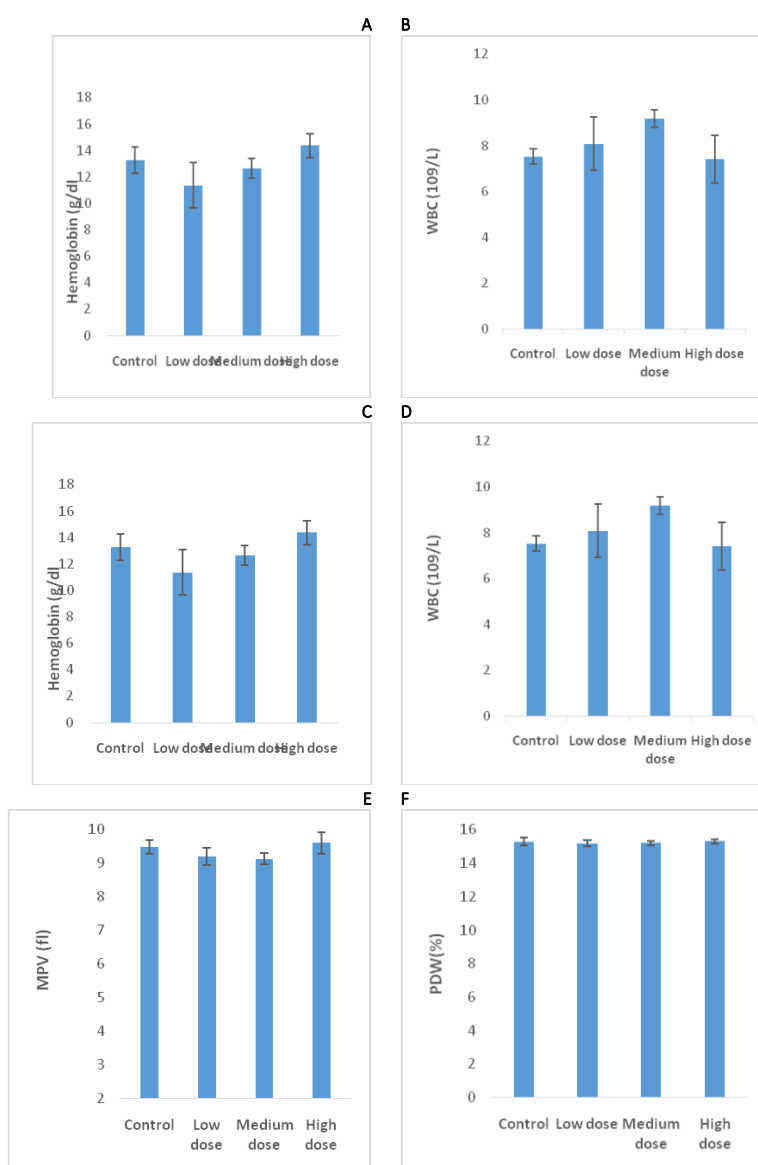


Figure 3. Effect of *L. cupanioides* aqueous leaf extract on hematological parameters of Wistar mice Haemoglobin (A): White blood cell, (B): Mean platelet volume, (C): Mean cellular volume, (D): Platelet distribution width, (E): Plateletcrit, and (F): Platelet

3.7. Histology results

3.7.1. Kidney histology

Figure 1 shows photomicrograph of kidney tissue from control albino rats (plate A) and kidney histology results showing tests consistent with normal morphology and architecture (plates B, C, and D). The renal capsules (arrowhead) and the tubules (curved arrow) are normal with no sign of injury (H&E, x400).

3.7.2. Liver histology

From Figure 2, plate A shows the control group showing morphology consistent with normal liver histology architecture. Tissue shows congestion of the central vein (arrow), but the hepatocytes are

normal (H&E X 400). The same applies to the test groups (plates B, C, and D).

3.8. Anti-ulcer activity

3.8.1. Effect of *L. cupanioides* aqueous leaf extract on gastric pH, ulcer severity, ulcer index, total acidity, and ulcer protection

Generally, *H. pylori* infection is the main pathogenic cause of peptic ulcers. It has been noted that even after *H. pylori* has been eliminated, ulcer recurrence still occurs. Psychological stress may be related to ulcer recurrence in hp-negative ulcer patients (Moriya et al., 2011). Usually, emotional stress results in a decline in mucosal defense which could alter the elements that preserve the mucosa intact (Jain, 2016; Périco et al., 2015).

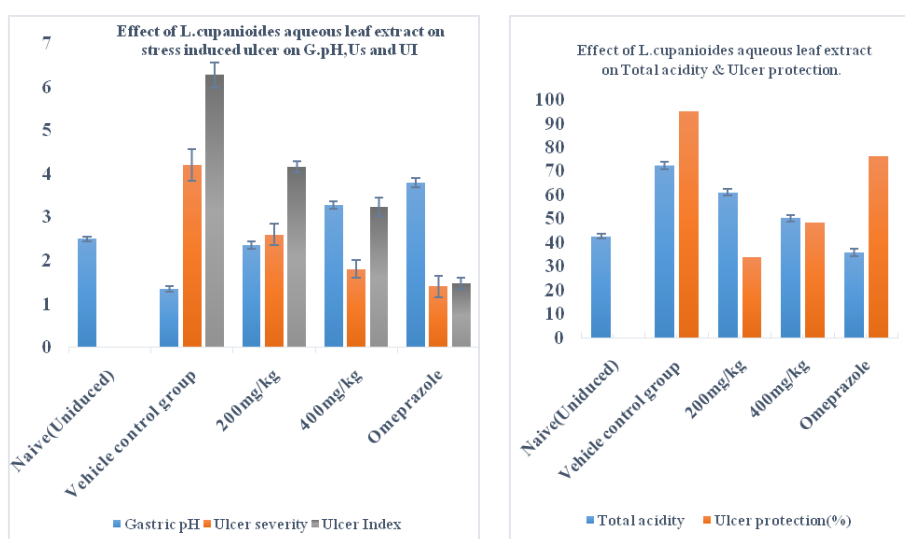


Figure 4. Effect of *L. cupanioides* aqueous leaf extract on gastric pH, ulcer severity, ulcer index total acidity, and ulcer protection

In an attempt to discover an ideal antiulcer agent with limited side effects, we also assessed the leaf extract of *L. cupanioides* for its possible antiulcer activity. The study revealed a significant influence of the extract on stress-induced ulcers at different dose levels administered when compared to the control (native, negative control, and omeprazole). When compared to both native and omeprazole, the animals treated with 200mg/kg and 400mg/kg of the extract showed a significant increase ($p < 0.05$) in ulcer score, ulcer index, ulcer severity, and total acidity, but a dose-dependent increase in ulcer protection ability of the aqueous plant extract was observed (Figure 4).

4. Conclusions

This study demonstrated that acute exposure of animals to a single dose of the aqueous leaf extract of *L. cupanioides* was safe at 5000mg/kg body weight, and did not produce obvious signs of toxicity and death. Repeated administration of 250, 500, and 1000 mg/kg doses of the aqueous leaf extract of *L. cupanioides* over 21 days revealed no deleterious effect on body weight, hematological, liver, and kidney biomarkers, and histology.

On the other hand, the aqueous leaf extract (ALE) of *L. cupanioides* significantly increased ($p < 0.05$) ulcer score, ulcer index, ulcer severity, and overall acidity, although with a dosage-dependent increase in ulcer prevention ability.

Acknowledgments

The authors wish to thank the management of the Faculty of Pharmaceutical Sciences, Chukwuemeka Odumegwu Ojukwu University Igbariam, Anambra State, for giving us access to their animal facility, where part of this study was carried out.

Conflict of interest

The authors confirm that there are no known conflicts of interest.

Statement of ethics

All animal experiments were conducted in compliance with the 2010/63/EU Directive on the protection of animals used for scientific purposes and approved by the institution's animal ethical committee (PHACOOU/AREC/2023/019).

Availability of data and materials

All data generated or analyzed during this study are included in this published article. On request, the associated author can provide more information.

Funding

None.

CRedit authorship contribution statement

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Supplementary File

None.

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