

Gastroprotective and Haematological Effects of *Chromolaena Odorata* Leaf Extract Against Ethanol-Induced Gastric Injury in Albino Rats

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ABSTRACT

Peptic ulcer disease remains an important gastrointestinal disorder, and acute ethanol exposure is widely used experimentally to produce gastric mucosal injury. This study evaluated the gastroprotective and haematological effects of *Chromolaena odorata* leaf extract against ethanol-induced gastric injury in adult albino rats. Thirty rats were allocated to six groups (n = 5): normal control, ulcer control, omeprazole (20 mg/kg), and *C. odorata* extract at 200, 400, or 800 mg/kg. The extract or omeprazole was administered 30 minutes before oral administration of 96% ethanol (10 ml/kg) to Groups 2-6; animals were sacrificed 2 hours after ethanol administration. Haematological, gastric biochemical, macroscopic, and histopathological outcomes were assessed. The ulcer control showed higher RBC ($7.61 \pm 0.31 \times 10^6/\text{mm}^3$), PCV ($46.80 \pm 1.30\%$), Hb ($17.14 \pm 0.62 \text{ g/dl}$), WBC ($13.08 \pm 1.65 \times 10^3/\text{mm}^3$), and platelet count ($253.0 \pm 6.58 \times 10^3/\text{mm}^3$) than the normal control. Pretreatment with *C. odorata* generally shifted these indices toward normal values, with the 800 mg/kg group recording RBC $6.96 \pm 0.22 \times 10^6/\text{mm}^3$, PCV $44.20 \pm 0.84\%$, WBC $9.81 \pm 0.43 \times 10^3/\text{mm}^3$, and platelets $239.80 \pm 3.11 \times 10^3/\text{mm}^3$. The extract also increased gastric mucus, reduced pepsin activity and total acidity, and preserved gastric histoarchitecture compared with the ulcer control. One-way ANOVA followed by Tukey post-hoc testing was used, with significance set at $p < 0.05$. These findings indicate an acute gastroprotective effect of *C. odorata* pretreatment against ethanol-induced gastric injury; they do not establish therapeutic efficacy in an established ulcer or long-term safety.

Keywords: *Chromolaena odorata*; gastroprotection; ethanol-induced gastric injury; haematology; gastric mucosa

1.0 INTRODUCTION

1.1 Statement of Problem

1.2 Aim and Objectives of the Study

The study aimed to evaluate the gastroprotective and haematological effects of *Chromolaena odorata* leaf extract against acute ethanol-induced gastric injury in albino rats. The specific objectives were to:

1. Determine the effect of *C. odorata* leaf extract pretreatment on haematological parameters following ethanol-induced gastric injury.
2. Assess gastric defensive and aggressive factors, including extractible mucus weight, pepsin activity, total acidity and gastric pH, following pretreatment with graded doses of the extract.
3. Evaluate the histopathological changes in the gastric mucosa following *C. odorata* pretreatment and ethanol-induced gastric injury.

1.3 Hypothesis

H0: There is no statistically significant difference in the measured outcomes among the normal control, ulcer control, omeprazole-treated group and the graded *C. odorata* pretreatment groups.

H1: At least one experimental group differs significantly in one or more measured outcomes.

2. METHODOLOGY

2.1 Research Design

The experimental research design was used for this study. The leaves of *Chromolaena odorata* for this study was collected around Okigwe township stadium Okigwe, Imo State, Nigeria.

2.2 Experimental Grouping and Allocation

A total of thirty adult albino rats were used for the study and assigned to six experimental groups of five animals each. The three extract doses (200, 400 and 800 mg/kg body weight) were included to permit assessment of graded dose-related responses alongside normal, ulcer and omeprazole controls.

2.3 Variables

2.3.1 Haematological Assay

Haematological analysis of the blood samples was performed in an automated haematology analyzer (BC-2300 model, Mindray Medical Co., China) with the procedure carried as specified by the producer. The parameters which were evaluated included: red blood cells count(RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV); mean corpuscular haemoglobin (MCH); mean corpuscular haemoglobin concentration (MCHC); platelets (PLT) and differential leukocytes count(WBC) counts were obtained at once for each blood sample.

Procedure for use of the 5 part Haematological Analyzer

To analyze a whole blood sample, the sample was presented to the diluent dispenser and the [DILUENT] key was pressed to aspirate 20 μ L of the sample into the dispenser. A diluted sample (about 1:300 unit?) was dispensed when the [DILUENT] key was pressed again. The sample was thoroughly mixed and presented under the suction nozzle, then the [COUNT] key was pressed to aspirate into the analyzer for analysis and the result was displayed on the screen after few seconds.

2.3.2 Determination of Enzymatic Anti-Oxidants

Superoxide Dismutase (SOD): Superoxide dismutase activity was assayed by method described by Arthur and Boyne (1985) as contained in Randox kit.

Catalase: Activity of catalase was assayed by the method of Sinha (1972)

Glutathione peroxidase: This was done according to the method of Paglia and Valentine (1967).

Reduced Glutathione: The reduced glutathione concentration was determined by the method of Exner et al (2000).

2.3.3 Liver Function Test

Alanin aminotransferase (ALT): This was determined using Randox commercial test kits, following the principles and procedures outlined by the commercial kit producer, Randox Laboratories Limited, UK.

Aspartate Amino transferase (AST): This was estimated using AST commercial kit and following standard produces prescribed by the producer, Randox Laboratories Limited, UK.

Alkaline phosphates(ALP) activity: This was determined using ALP commercial kit, and following standard procedures as outlined by the producer Teco diagnostic, U.S.A

Serum Bilirubin content: Serum bilirubin content was determined using commercial test kits and following standard protocols prescribed by the producer Randox Laboratories limited, UK.

Serum Total protein content: This was determined using commercial test kits and following standard protocols prescribed by the producer Randox Laboratories, U.K.

2.3.4 Determination of Renal Function Parameters

Renal function parameters which include Urea, Creatinine, Sodium, Chloride, Potassium and Bicarbonate were determined using commercial test kits and following standard procedures as outlined by the producer Randox Laboratories Limited, U.K.

2.3.5 In-Vitro Antioxidant Assays

Nitric Oxide inhibition activity was determined by the method of Marcocci et al.,1994; Green et al., 1982.

Photometric assay of 2,2-Diphenyl-1-picrylhydrazyl (DPHY)

DPPH was analyzed by the DPPH Assay using spectrophotometer as done by Mensor et al., 2001.

Ferric reducing antioxidant power: The ferric reducing anti-oxidant power was carried out as used by Benzie and strain (1999).

2.3.6 Determination of Free Acidity and Total Acidity:

1ml of Gastric juice was taken into 100ml conical flask ,2-3 drops of Topfer's reagent was added and titrated with 0.01N NaOH until all traces of red colour disappears and the colour of the solution turns yellow orange (end point). The volume of Alkali added was noted. This volume corresponds to free acidity. 2-3 drops of phenolphthalein solution was added and titration was continued until a definite red tinge re-appears. The volume of alkali was recorded which corresponds to total acidity

Acidity was calculated using the formula:

$$\text{Acidity} \left(\frac{\text{mEq}}{\text{litre}} \right) = \frac{\text{Volume to NaOH} \times \text{Normality of NaOH}}{0.1} \times 100 \quad \text{Equ. 1}$$

2.3.7 Estimation of Pepsin Activity

Four place test tubes 1 and 2 containing 5ml of 1% bovin albumin in 0.01M HCl, 3 and 4 containing 10ml of 0.35 M trichloroacetic acid. The gastric juice was mixed with an equal volume of 0.01 M HCl warmed to 35°C. 1ml of this mixture was added to each of the test tubes of 1 and 4 incubated for 15 minutes. At the end the mixed content tube 1 and 3 were allowed to stand for about 4 minutes. 1 + 3 give test and 2 +4 give blank. The mixture was filtered. To 2ml of filtrate,10ml of NaOH was added. Then 1ml of phenol reagent was added and mixed by gentle rotation. After 30 minutes, the absorbance was measured at 680nm. The difference between test and blank gives the measures of peptic acidity.

2.3.8 pH of Gastric Juice

pH of gastric juice was determined by end point filtration at pH 7.0 using as titrant of sodium hydroxide solution of 0.71 equivalent litre (Ghosh et al., 2011)

2.3.9 Macroscopic Evaluation of Stomach Ulcer

The stomach was excised and carefully opened along the line of greater curvature to expose the walls for microscopic evaluation. The stomach content was washed off and the stomach viewed with the aid of a light microscope (x100) to determine the ulcer scores using the method of Raju et al., (2009).

The ulcerative lesions were counted and scored as follows: Normal stomach 0, Pinhole 1.0, Spot ulceration 1.5, Hemorrhage streak 2.0, Small erosion 2.5, Large erosion 3.0, Perforation 3.5

Mean ulcer score for each animal was used to express the ulcer index. The ulcer index (U.I) was calculated by using the formula:

$$U: I = \frac{(1 \times (\text{No of lesions of grade 1}) + 2 \times (\text{No. of lesions of grade 2}) + 3 \times (\text{No. of lesions of grade 3}))}{10} \quad \text{Equ. 2}$$

The percentage ulcer protection was determined using the formula of (SUZIK et al., 1976).

$$\text{Protection index} = 1 - \frac{\text{ulcer index with extract}}{\text{ulcer index with distilled water}} \times 100 \quad \text{Equ. 3}$$

2.4 The Experimental Method

Fresh leaves of *Chromolaena odorata* were washed with clean water and air dried under a shade for twenty-one days (21). The leaves were pulverized into fine powder, using a grinding machine.

500g of the powdered leaves of *C. odorata* was blended in 1.5 liters of methanol (AR) for 48 hours). The solution was filtered with whatman No1 filter paper and the filtrate concentrated to a solid residue using rotary evaporator.

Adult albino rats weighing 150-200 (both sexes) was obtained from animal house, Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture Umudike. The animals were housed under standard condition 12/12 light dark cycle starting 7:00am at temperature of 25± 20c. The rats were fed with standard grower pellets (Grand cereals ltd, Abia State) with access to clean drinking water for seven days (7) of acclimatization.

30 mature adult albino rats were used for acute study, the method used by Michael et al. (2013) was adopted. The albino rats were assigned into six group of 5 rats each and were treated as follows: Group 1: Normal control, No Treatment, Group 2: Ulcer control, No treatment, Group 3: received 20mg/kg Omeprazole, Group 4: received 200mg/kg *Chromolaena odorata*, Group 5: received 400mg/kg *Chromolaena odorata*, Group 6: received 800mg/kg *Chromolaena odorata*.

30 minutes after the pretreatment with extract, all the animals were administered with ethanol via oral route except the normal which served as the control. The animals were sacrifice after 2 hours following the ethanol induction. The ulcer scores were read with magnified hand lens from harvested stomach which were opened longitudinally along the greater curvature with scissors and spread on a dissection board. Blood samples were collected for haematological analyses. Fresh stomach and jejunum samples were also collected and immediately transferred into cold chain, and afterwards, homogenized and centrifuged to obtain supernatant which were used for biochemical analysis.

2.5 Statistical Analysis

Data obtained from the six experimental groups were expressed as mean ± standard deviation (SD), with five animals per group (n = 5). For each measured parameter, differences among the treatment groups were evaluated using one-way analysis of variance (ANOVA). Where the ANOVA showed a significant overall difference, Tukey's multiple-comparison post-hoc test was used to determine the specific group means that differed. Statistical significance was set at p < 0.05. In the tables, mean values bearing different superscript letters are significantly different, whereas means sharing at least one superscript letter are not significantly different at p < 0.05.

3. RESULTS AND DISCUSSION

3.1: Haematological Parameters

Table 7 shows that acute ethanol-induced gastric injury was accompanied by significant alterations in several haematological indices. Compared with the normal control, the ulcer control recorded higher RBC (7.61 ± 0.31 versus $6.93 \pm 0.29 \times 10^6/\text{mm}^3$), PCV (46.80 ± 1.30 versus $44.20 \pm 0.84\%$), Hb (17.14 ± 0.62 versus 15.30 ± 0.80 g/dl), WBC (13.08 ± 1.65 versus $8.83 \pm 0.32 \times 10^3/\text{mm}^3$) and platelet counts (253.0 ± 6.58 versus $237.80 \pm 7.12 \times 10^3/\text{mm}^3$). The rise in WBC is consistent with an acute inflammatory response to ethanol-mediated mucosal injury, while the simultaneous increases in RBC, PCV and Hb are more plausibly interpreted as acute haemoconcentration than as new erythrocyte production, given the very short experimental interval.

Pretreatment with *C. odorata* produced a generally dose-related movement of these indices toward normal values. At 800 mg/kg, RBC and PCV were essentially restored to the normal-control values, WBC fell to $9.81 \pm 0.43 \times 10^3/\text{mm}^3$, and platelet count was not significantly different from the normal control. The 400 mg/kg dose also produced marked improvement, although some indices remained intermediate. MCHC was significantly elevated in the ulcer control but was brought closer to the normal range by extract treatment. The MCV and MCH rows show an unusual numerical identity across all groups; these values should therefore be checked against the original analyser printout before publication. Overall, the haematological pattern supports attenuation of the acute systemic and inflammatory consequences of ethanol injury rather than a direct erythropoietic effect of the extract.

Table 7: Result of Haematological Parameters

Treatments	Normal control	Ulcer control	Omeprazole, 20 mg/kg	Extract, 200 mg/kg	Extract, 400 mg/kg	Extract, 800 mg/kg
RBC ($\times 10^6/\text{mm}^3$)	6.93 ± 0.29^a	7.61 ± 0.31^c	$7.34 \pm 0.28^{b,c}$	$7.26 \pm 0.14^{a,b}$	$7.04 \pm 0.23^{a,b}$	6.96 ± 0.22^a
PCV (%)	44.20 ± 0.84^a	46.80 ± 1.30^c	$45.60 \pm 0.89^{b,c}$	$45.80 \pm 0.84^{b,c}$	$44.60 \pm 0.55^{a,b}$	44.20 ± 0.84^a
Hb (g/dl)	15.30 ± 0.80^a	17.14 ± 0.62^c	$15.98 \pm 0.79^{a,b}$	$16.38 \pm 0.26^{b,c}$	$16.08 \pm 0.40^{a,b}$	$15.54 \pm 0.57^{a,b}$
WBC ($\times 10^3/\text{mm}^3$)	8.83 ± 0.32^a	13.08 ± 1.65^d	$10.51 \pm 0.47^{b,c}$	11.38 ± 0.90^c	$10.28 \pm 0.78^{b,c}$	$9.81 \pm 0.43^{a,b}$
PLT ($\times 10^3/\text{mm}^3$)	237.80 ± 7.12^a	253.0 ± 6.58^b	243.20 ± 5.89^a	243.20 ± 6.72^a	237.40 ± 6.54^a	239.80 ± 3.11^a
MCV (fl)	$22.07 \pm 0.51^{a,b}$	$22.53 \pm 0.19^{b,c,d}$	21.76 ± 0.53^a	$22.56 \pm 0.13^{c,d}$	22.85 ± 0.26^d	$22.34 \pm 0.22^{b,c}$
MCH (pg)	$22.07 \pm 0.51^{a,b}$	$22.53 \pm 0.19^{b,c,d}$	21.76 ± 0.54^a	$22.56 \pm 0.13^{c,d}$	22.85 ± 0.26^d	$22.34 \pm 0.22^{b,c}$
MCHC (g/dl)	34.60 ± 1.21^a	36.62 ± 0.51^c	$35.04 \pm 1.35^{a,b}$	$35.77 \pm 0.29^{a,b,c}$	$36.05 \pm 0.62^{b,c}$	$35.15 \pm 0.84^{a,b}$

Values are presented as mean $\pm$ standard deviation ($n = 5$), and values with unlike superscripts are significantly ($P < 0.05$) different from any paired means across the row.

3.2 Liver Function Parameters

As shown in Table 8, ethanol-induced ulceration was associated with higher serum ALT, AST, ALP and bilirubin values than those of the normal control, indicating that the acute gastric insult was accompanied by biochemical evidence of systemic/hepatic stress. Pretreatment with *C. odorata* reduced ALT and AST in a dose-related manner. At 400 and 800 mg/kg, ALT and AST were not significantly different from the normal control, while

total protein also returned to values comparable with normal. ALP was partially corrected, because the extract-treated groups shared statistical similarity with the normal control but did not show a clear monotonic decline across doses.

Bilirubin deserves a more cautious interpretation: although all extract doses lowered bilirubin relative to the ulcer control, the treated groups remained significantly different from the normal control. Thus, the data support partial rather than complete normalization of hepatic biochemical indices. This pattern agrees with earlier work showing that *C. odorata* can attenuate rises in hepatic enzymes under experimental toxic stress (Ikewuchi et al., 2021) and with Agu Francis Uchenna et al. (2023), who reported ALT, AST and ALP values in *C. odorata*-treated ulcerated rats that approached those of normal controls. The present findings therefore suggest a protective effect, but they do not justify concluding that the extract is entirely free of hepatotoxic potential.

Table 8: Liver Function parameters

Treatments	Total protein (g/dl)	ALT (u/l)	AST (u/l)	ALP (u/l)	Bilirubin (mg/dl)
Normal control	5.88±0.28 ^a	24.60±3.51 ^a	39.80±1.48 ^a	79.20±7.46 ^a	0.70±0.05 ^a
Ulcer control	6.80±0.30 ^{b,c}	39.60±4.72 ^c	52.40±3.21 ^b	89.20±2.86 ^b	0.98±0.09 ^c
Omeprazole, 20 mg/kg	6.98±0.11 ^c	35.40±3.85 ^{b,c}	43.40±3.29 ^a	86.80±7.60 ^b	0.86±0.06 ^b
Extract, 200 mg/kg	6.59±0.42 ^b	35.20±3.96 ^{b,c}	42.80±2.78 ^a	84.80±2.59 ^{a,b}	0.84±0.06 ^b
Extract, 400 mg/kg	6.17±0.30 ^a	32.40±2.30 ^a	43.60±4.34 ^a	85.00±3.61 ^{a,b}	0.85±0.05 ^b
Extract, 800 mg/kg	5.89±0.17 ^a	31.20±3.63 ^a	41.60±3.29 ^a	85.20±3.27 ^{a,b}	0.81±0.08 ^b

Values are presented as mean ± standard deviation (n = 5), and values with unlike superscripts are significantly (P < 0.05) different from any paired means within the column.

3.3 Extractible Mucus Weight, Total Acidity, Pepsin Activity and pH

Table 9 demonstrates the expected disruption of gastric defensive and aggressive factors after ethanol administration. The ulcer control had a marked reduction in extractible mucus weight (0.012±0.002 g), together with increased pepsin activity (28.88±2.74 µmol tyrosine/ml), increased total acidity (117.02±3.17 mmol/L) and a lower gastric pH (2.37±0.11) compared with the normal control. These changes indicate weakening of the mucosal barrier and a more aggressive luminal environment.

C. odorata pretreatment improved all four indices. Mucus weight increased progressively from 0.026±0.003 g at 200 mg/kg to 0.034±0.009 g at 800 mg/kg, although it did not fully reach the normal-control value. In contrast, the 800 mg/kg dose reduced pepsin activity and total acidity to values statistically comparable with the normal control and restored pH to the normal range. The effect was at least comparable with omeprazole for several of these parameters. Earlier experimental studies similarly reported gastroprotection by *C. odorata*: Mahmood et al. (2006) observed substantial protection of ethanol/HCl-damaged gastric mucosa, while Peter et al. (2020) reported significant reduction of ulcer indices in absolute-ethanol and other ulcer models. These earlier findings support the interpretation that the extract may act through reinforcement of mucosal defence together with suppression of acid-peptic aggression.

Table 9: Extractible mucus weight, total acidity, Pepsin activity, pH

Treatments	Extractible mucus weight (g)	Pepsin activity (μmol tyrosine/ml)	Total acidity (mmol/L)	pH
Normal control	0.039 $\pm$ 0.003d	18.54 $\pm$ 2.16a	89.88 $\pm$ 3.08a	2.93 $\pm$ 0.11b
Ulcer control	0.012 $\pm$ 0.002a	28.88 $\pm$ 2.74c	117.02 $\pm$ 3.17c	2.37 $\pm$ 0.11a
Omeprazole, 20 mg/kg	0.025 $\pm$ 0.003b	23.76 $\pm$ 1.12b	95.96 $\pm$ 3.10b	2.93 $\pm$ 0.16b
Extract, 200 mg/kg	0.026 $\pm$ 0.003b	23.70 $\pm$ 2.44b	96.34 $\pm$ 3.76b	3.03 $\pm$ 0.13b
Extract, 400 mg/kg	0.033 $\pm$ 0.003c	23.82 $\pm$ 0.77b	92.00 $\pm$ 3.03a,b	3.06 $\pm$ 0.13b
Extract, 800 mg/kg	0.034 $\pm$ 0.009c	20.66 $\pm$ 0.52a	90.46 $\pm$ 3.16a	3.08 $\pm$ 0.09b

Values are presented as mean $\pm$ standard deviation (n = 5), and values with unlike superscripts are significantly (P < 0.05) different from any paired means within the column.

Thirty mature adult albino rats were used for the acute gastroprotective study, adopting the method described by Michael et al. (2013). The animals were assigned to six groups of five rats each: Group 1, normal control (no ulcer induction and no treatment); Group 2, ulcer control (ethanol only); Group 3, omeprazole 20 mg/kg; Group 4, *C. odorata* 200 mg/kg; Group 5, *C. odorata* 400 mg/kg; and Group 6, *C. odorata* 800 mg/kg.

The study was designed as a pretreatment model. Omeprazole or *C. odorata* extract was administered 30 minutes before ethanol exposure. Thereafter, all groups except the normal control received ethanol orally to induce acute gastric mucosal injury. The animals were sacrificed 2 hours after ethanol administration. Stomachs were harvested, opened longitudinally along the greater curvature and examined for gross lesions. Blood samples were collected for haematological analyses, while fresh stomach and jejunum samples were collected, maintained under cold conditions, homogenized and centrifuged to obtain supernatants for biochemical analyses.

Treatments	Urea (mg/dl)	Creatinine (mg/dl)	Na ⁺ (mEq/L)	K ⁺ (mEq/L)	Cl ⁻ (mEq/L)	HCO ₃ ⁻ (mmol/L)
Normal control	20.63 $\pm$ 1.46 ^a	0.68 $\pm$ 0.06 ^a	131.76 $\pm$ 2.14 ^a	4.47 $\pm$ 0.09 ^a	89.44 $\pm$ 1.57 ^a	20.02 $\pm$ 0.40 ^a
Ulcer control	24.85 $\pm$ 1.56 ^b	0.86 $\pm$ 0.07 ^c	141.00 $\pm$ 4.18 ^c	4.62 $\pm$ 0.12 ^a	92.94 $\pm$ 1.48 ^b	20.98 $\pm$ 0.41 ^b
Omeprazole, 20 mg/kg	21.91 $\pm$ 1.43 ^a	0.75 $\pm$ 0.08 ^{a,b}	137.54 $\pm$ 4.42 ^{b,c}	4.46 $\pm$ 0.12 ^a	91.16 $\pm$ 1.31 ^a	20.34 $\pm$ 0.39 ^a
Extract, 200 mg/kg	21.09 $\pm$ 1.55 ^a	0.74 $\pm$ 0.04 ^{a,b}	136.84 $\pm$ 2.69 ^{b,c}	4.45 $\pm$ 0.09 ^a	90.50 $\pm$ 1.40 ^a	20.16 $\pm$ 0.36 ^a
Extract, 400 mg/kg	20.67 $\pm$ 1.99 ^a	0.78 $\pm$ 0.07 ^{b,c}	135.32 $\pm$ 1.60 ^{a,b}	4.54 $\pm$ 0.16 ^a	89.94 $\pm$ 0.89 ^a	20.16 $\pm$ 0.50 ^a
Extract, 800 mg/kg	20.32 $\pm$ 1.19 ^a	0.71 $\pm$ 0.06 ^{a,b}	134.98 $\pm$ 2.65 ^{a,b}	4.50 $\pm$ 0.25 ^a	90.56 $\pm$ 1.19 ^a	19.94 $\pm$ 0.38 ^a

3.5 Histopathological Analysis

2.5 Statistical Analysis

Data from the six experimental groups were expressed as mean $\pm$ standard deviation (SD), with five animals per group ($n = 5$). Each outcome was analysed separately using one-way analysis of variance (ANOVA) to test for an overall group effect. Where the omnibus ANOVA was significant, Tukey's multiple-comparison post-hoc test was applied to identify specific pairwise group differences while controlling for multiple comparisons within that outcome. Statistical significance was set at $p < 0.05$. In the tables, means bearing different superscript letters are significantly different, whereas means sharing at least one superscript letter are not significantly different at $p < 0.05$. Given the small group size, findings were interpreted together with the magnitude and direction of the observed group differences rather than on statistical significance alone.

GROUP 1

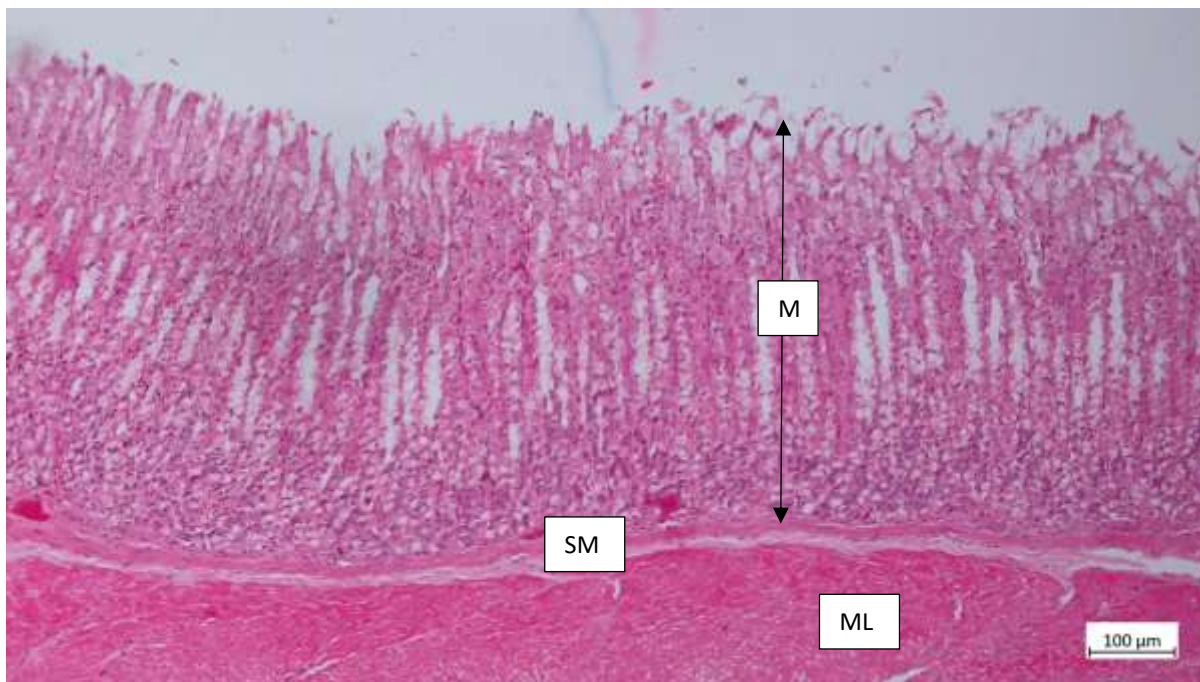


Plate 1: The sections of the stomach presented in this group showed the normal gastric histomorphology. The normal histo-architecture of the mucosa, sub-mucosa and muscularis were observed. Mucosa (M), Sub-mucosa (SM), Muscular layer (ML), E x100.

GROUP 2

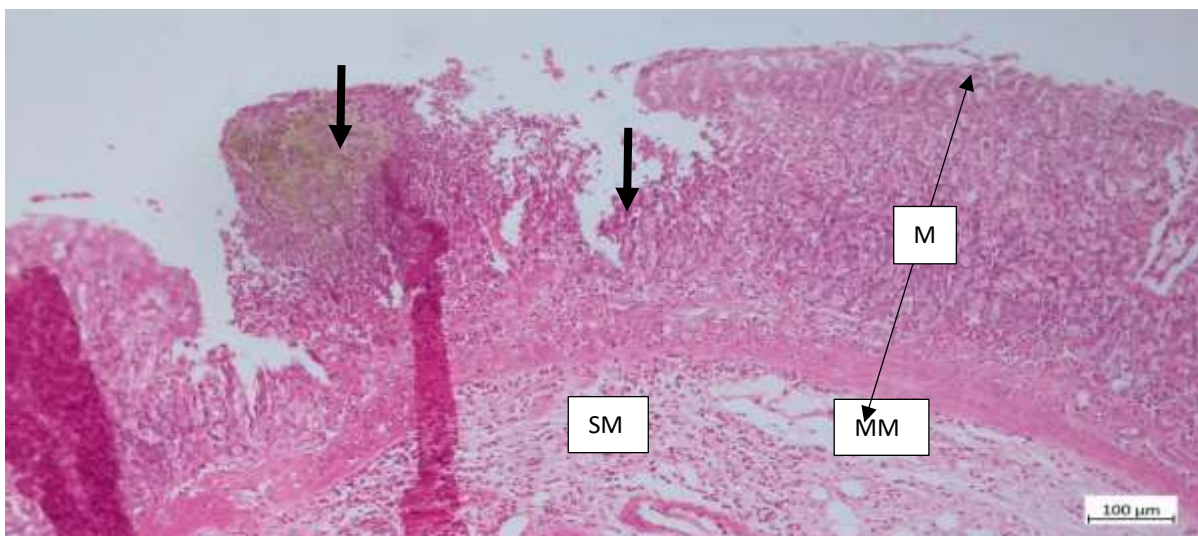


Plate 2: The sections of the stomach presented in this group showed multifocal areas of mucosal necrosis (arrow) with edema and infiltration of inflammatory leukocytes into the sub mucosa (SM). Mucosa (M), Muscularis mucosa (MM), HE x100.

GROUP 3

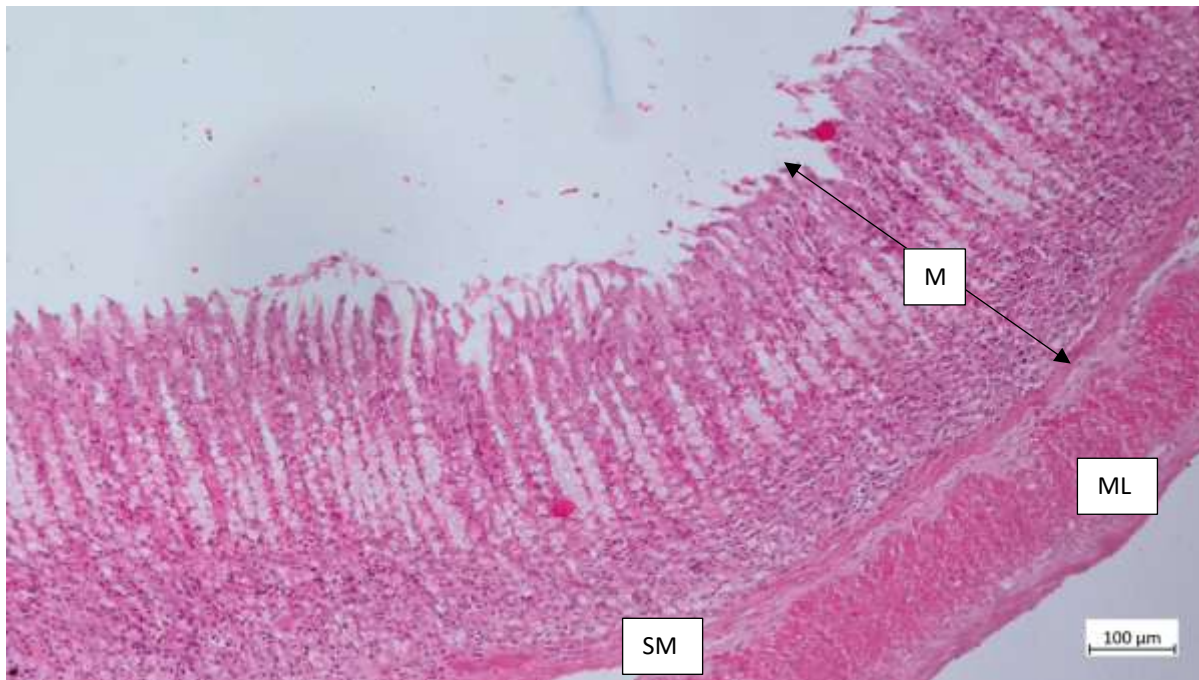


Plate 3: The sections of the stomach presented in this group showed the normal gastric histomorphology. The normal histo-architecture of the mucosa, sub-mucosa and muscularis were observed. Mucosa (M), Sub-mucosa (SM), Muscular layer (ML), HE x100.

GROUP 4

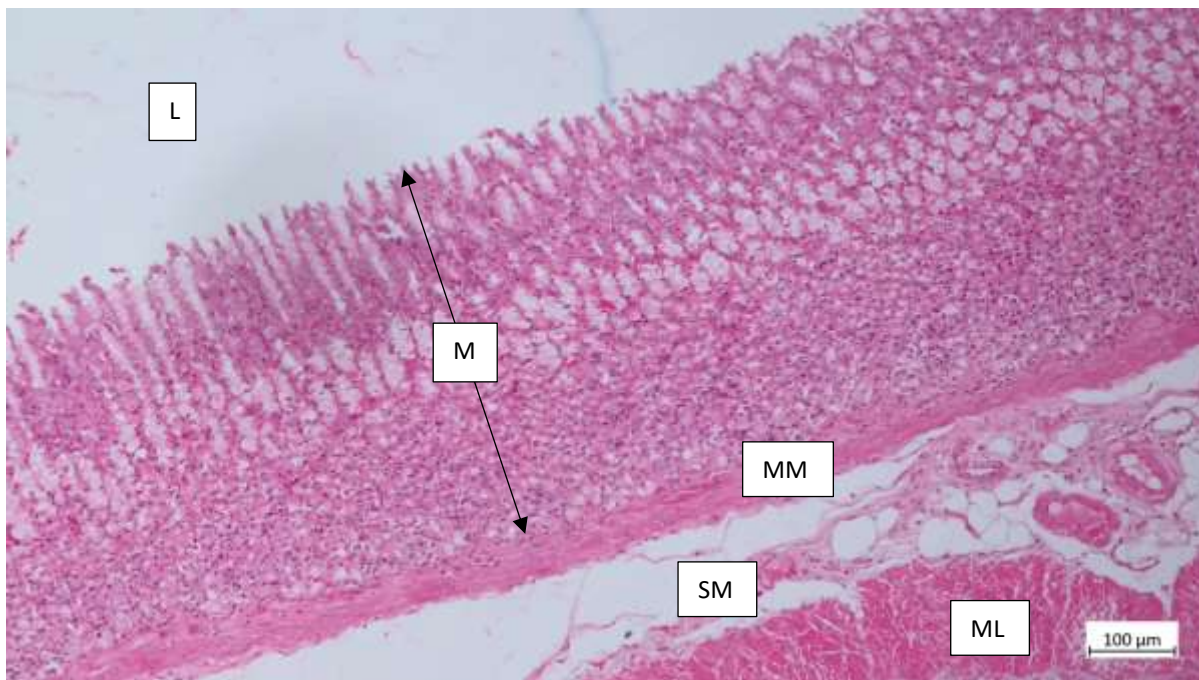


Plate 4: The sections of the stomach presented in this group showed the normal gastric histomorphology. The normal histo-architecture of the mucosa (M), sub-mucosa (SM) and muscularis (ML) were observed. Muscularis mucosa (MM), Lumen (L), HE x100.

GROUP 5

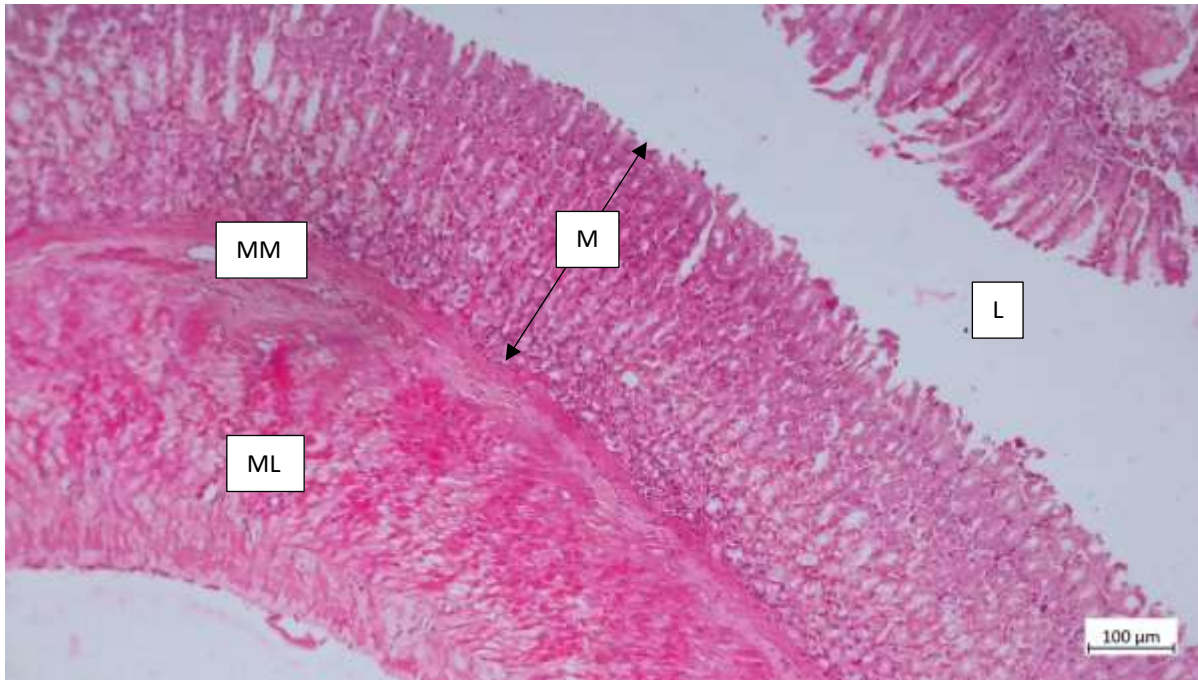


Plate 5: The sections of the stomach presented in this group showed the normal gastric histomorphology. The normal histo-architecture of the mucosa (M), sub-mucosa and muscularis (ML) were observed. Muscularis mucosa (MM), Lumen (L), HE x100.

GROUP 6

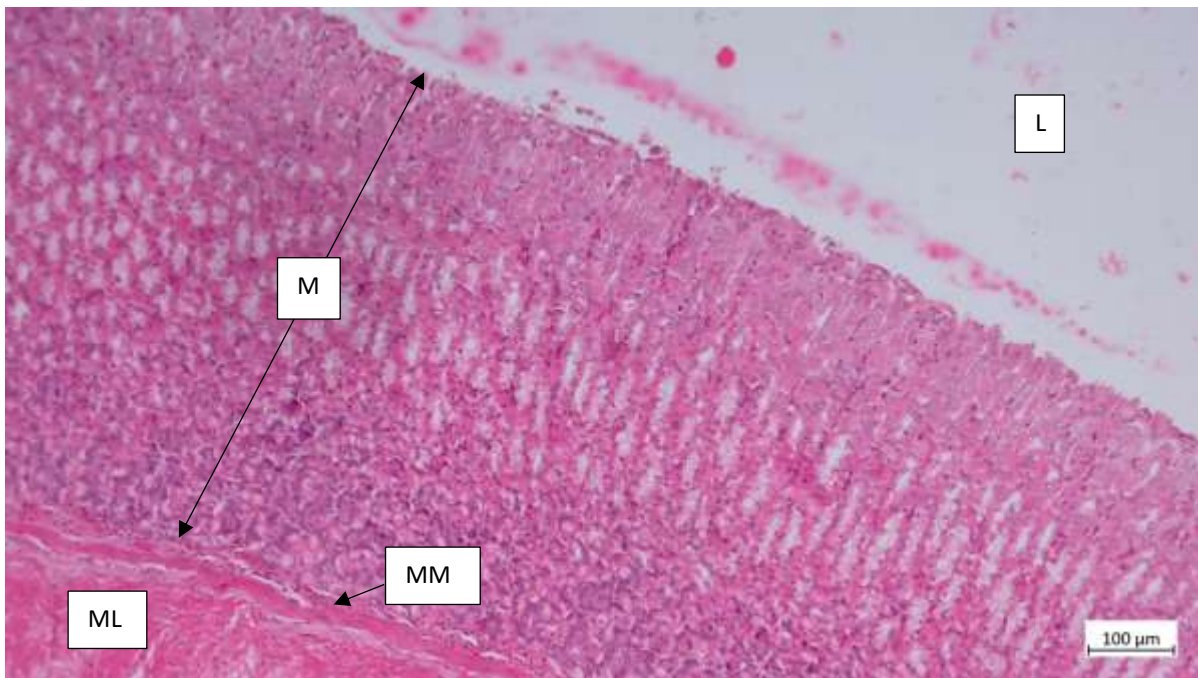


Plate 6: The sections of the stomach presented in this group showed the normal gastric histomorphology. The normal histo-architecture of the mucosa (M), sub-mucosa and muscularis (ML) were observed. Muscularis mucosa (MM)

Lumen (L), HE x100.

4 CONCLUSION AND RECOMMENDATION

This acute pretreatment study demonstrates that *Chromolaena odorata* leaf extract has gastroprotective activity against ethanol-induced gastric injury in albino rats. Pretreatment with the extract attenuated ethanol-associated alterations in haematological indices, improved gastric mucus and acid-peptic parameters, and preserved gastric histo-architecture, with several outcomes showing greater normalization at the higher doses. Because the extract was administered before ethanol exposure, the findings support a preventive/gastroprotective effect rather than treatment or healing of an already established ulcer. The short experimental duration also does not establish long-term safety. Further studies should include dedicated acute and repeated-dose toxicity testing, standardized phytochemical characterization of the extract, quantitative histological scoring, and therapeutic designs in which treatment begins after ulcer establishment.

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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