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Research Article

Antiulcer activity of freeze-dried leaf extracts of *Rhus natalensis* in ethanol-induced ulcer rat models

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This study aims to investigate the ulcer healing effects of freeze-dried leaf extracts of *Rhus natalensis* on Ethanol-induced gastric ulcer model in Sprague Dawley rats. Male Sprague Dawley rats (40), 250-300g were randomly assigned into 5 groups each with 8 animals as follows: negative control (1 ml / 200 g ethanol), positive control (20 mg / kg Esomeprazole + 1 ml /200 g ethanol), normal control (normal saline), low dose (150 mg / kg + 1 ml / 200 g ethanol), and high dose (300 mg / kg + 1 ml/ 200 g ethanol). To induce ulcers, absolute ethanol was administered on the first day at a dose of 1 ml / 200 g body weight by oral gavage to all groups except the normal control. Treatments were administered 1 hour after ethanol administration and once daily up to the 6th day. The ulcer healing effects were assessed by determining the total ulcer area, pH, and total acidity of stomach contents, volume of stomach secretions, levels of GSH, and levels of malondialdehyde in gastric tissue, catalase activity, and superoxide dismutase activity in gastric tissue. The data was reported as the mean ± SEM. There was a significant reduction in the total ulcer area in the treatment groups. The results showed a significant increase in the pH of gastric secretions in the treatment groups and also a dose dependent reduction in the total acidity of gastric secretions of the same groups. In this study, there was a decrease in the volume of gastric secretions in the treatment groups as compared to the negative control which showed an increase in the volume of secretions. The study showed an increase in cellular antioxidant activity in the treatment groups by increased catalase activity, superoxide dismutase (SOD) activity, and levels of reduced glutathione (GSH). The study shows that *Rhus natalensis* possesses ulcer healing effects. The effects are potentially due to reduced acidity of gastric contents, increased pH of gastric contents, reduced volume of secretions, and increased activity of cellular antioxidant systems; catalase, superoxide dismutase, and reduced glutathione.

Keywords: *Rhus natalensis, ethanol-induced gastric ulcer, peptic ulcer disease*

Introduction

Peptic ulcer disease (PUD) is one of the most common gastrointestinal (GI) tract disorders with a global prevalence of about 10% (Beiranvand & Bahramikia, 2020). It's caused by an imbalance between the aggressive injurious factors e.g., Hydrochloric acid and pepsin, and defensive mucosal-protective factors e.g., prostaglandins, mucus, bicarbonate, epithelial regenerative capacity, and mucosal blood flow (Gou et al., 2025). *Helicobacter pylori* and chronic Non-steroidal anti-inflammatory drugs (NSAIDs) use are the most common causes of the imbalance between aggressive and defensive mucosal-protective factors that are associated with Peptic ulcer disease (Satoh et al., 2025). The conventional drugs used to treat PUD include proton pump inhibitors (1st line treatment), histamine H2 receptor antagonists, antacids, prostaglandin analogues (misoprostol), sucralfate, bismuth salts, and potassium-competitive acid blockers (Kuna et al., 2019). Chronic utilisation of the drugs used to manage PUD is associated with various adverse effects for example., PPIs cause hypergastrinemia, increased risk of bone fractures, Vitamin B12 deficiency, hypomagnesemia, and abdominal pains (Sánchez-Mendoza et al., 2024). Cimetidine causes gynecomastia (Iqbal et al., 2025),and impotence in men and galactorrhoea in women (Jenkins et al., 2017); hence need for alternative therapy with fewer side effects. They

are also associated with drug interactions when co-administered with other drugs; Omeprazole for example, increases toxicity of Diazepam, Warfarin, and Phenytoin by inhibiting their metabolism since they are all metabolised by Cytochrome P450 polymorphisms (Strand et al., 2017).

Medicinal plants are considered reservoirs for potential new PUD drugs because of their promising results in PUD treatment with limited or no adverse effects compared to the conventional drugs used (Mohammed et al., 2025). Some of the plants whose healing effects on PUD have been studied include; *Vernonia condensata* (Boeing et al., 2016), *Anacardium humile* (Luiz-Ferreira et al., 2008) and *Centella asiatica* (Zheng et al., 2016). WHO (2022) estimates that about 80% of the world population uses traditional medicine and that approximately 40% of approved pharmaceutical products in use today are derived from plants and natural substances for example aspirin from the Willow bark, birth control pills from wild yam, and artemisinin compounds from ancient Chinese medicine. Screening of various plant extracts for their pharmacological active agents has led to discovery of more effective and safer drugs (Palle et al., 2018). *Rhus natalensis* has been used in traditional African medicine to treat various diseases such as diarrhoea, colds, coughs, stomach-ache, gonorrhoea, hookworm infestation, syphilis (Bussmann et al., 2006), and heartburn (Jeruto et al., 2008). Studies on its antinociceptive effects have also been published (Kariuki et al., 2012). Scientific research on its use to treat peptic ulcers has not been documented despite its widespread use among communities in Kenya (Kigen et al., 2017). There's therefore need to scientifically ascertain its use in treating peptic ulcer disease. The aim of this study was therefore to investigate the ulcer healing effects of freeze-dried leaf extracts of *Rhus natalensis* on ethanol-induced gastric ulcer model in rats.

Materials and Methods

Ethical approval was sought from the Biosafety, Animal Use, Care and Ethics Committee, Faculty of Veterinary Medicine, University of Nairobi (reference number: FVM BAUEC/2023/444). The Guidelines for Ethical Conduct in the Care and Use of Animals were adhered to (APA Council of Representatives, 2012)

Animals

40 adult male Sprague Dawley rats weighing 250-300 g were used. The rats were acclimatised for two weeks before the start of the experiments. The rats were kept in cages under a 12-hour light/dark cycle under standard room temperature and humidity. Food (standard chow diet) and water were given *ad libitum.*

Study design

They were randomized into 5 groups of 8 rats each.

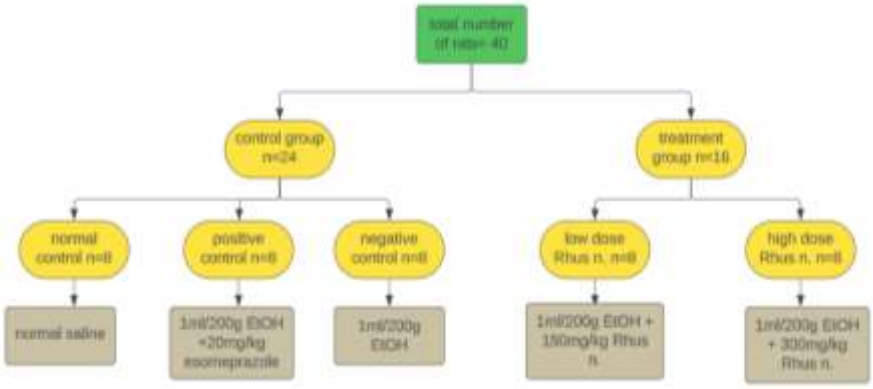


Figure 1. Image showing grouping and dosing of study animals

Collection and preparation of plant extracts

Fresh leaves of *Rhus natalensis* were obtained from Ngong forest in Nairobi, Kenya. A specimen was deposited at the University of Nairobi herbarium for identification and a voucher specimen was given. The leaves were dried under a shade for about one week. They were then be pulverized into powder by a milling machine. The powder was then stored in an air-tight container in a dark place. The powder was mixed with water in a ratio of 1:2 and left to stand overnight. The solvent obtained after decanting was freeze-dried using a freeze-drier machine and the end product was put in an air-tight sample container and stored in a desiccator.

Ulcer induction and administration of treatment

The animals were fasted for 12 hours before experimentation. Absolute ethanol was administered on the first day at a dose of 1 ml/200 g body weight by oral gavage to all groups except the normal control. The treatments were administered 1 hour after ethanol administration and once daily up to the 6th day as follows; 20 mg/kg of Esomeprazole to the positive control group, low (150 mg/kg), and high dose (300mg/kg) of *Rhus natalensis* to the low and high dose treatment groups respectively (the dosages were determined from previous studies). On the 6th day the rats were euthanized by cervical dislocation 4 hours after the treatment was given. A midline abdominal incision was made to obtain the stomach.

Measurement of pH and volume of gastric secretions

The stomachs were opened along the greater curvature. Stomach contents were then poured into a centrifuge tube and centrifuged at 3000 rpm for 10 minutes. The volume of the supernatant was measured using a measuring cylinder and its pH was measured using a digital pH meter.

Determination of total acidity

0.01 N sodium hydroxide (NaOH) solution was prepared by dissolving 0.4 g of NaOH in 1000 ml of distilled water. An aliquot of 1ml gastric juice was diluted with 1ml of distilled water and put in a 50 ml conical flask, two drops of phenolphthalein indicator were added and titrated with 0.01 N NaOH until a permanent pink colour was formed (pink colour for more than seconds). The volume of 0.01 N NaOH consumed was noted. Total acidity was expressed as mEq/L by the following formula:

$$\text{Acidity} = \text{Vol. of NaOH} \times \text{N} \times 100\text{mEq/L}/0.1$$

Determination of the total ulcer area

The stomachs were washed with sterile saline to remove gastric content and blood clots and pinned to a corkboard as described by Takagi & Okabe (1968). Photos of the stomach were taken, and the total ulcer area was determined using the Image J software. The area was expressed in mm^2.

Histological investigation

Sample of gastric tissue from all groups were fixed in 10% buffered formalin for 24 hours. The tissues were then processed and embedded in paraffin blocks, five- micrometre thick following which, they were then cut and stained using Haematoxylin and Eosin. The slides were then examined microscopically for histopathological changes such as sub-mucosal oedema, erosions, and neutrophil infiltration.

Determination of levels of malondialdehyde in gastric tissue

The level of malondialdehyde in gastric juice was determined as described by Ohkawa et al., 1979. Gastric tissue was homogenized in ice cold phosphate buffer (50 mM, pH 7.0). 2 ml of 10% Trichloroacetic acid was added to 1 ml of the sample. The mixture was then centrifuged at 5000 rpm for 5 minutes. 2 ml of the supernatant and 0.5 ml of 1% Thiobarbituric acid (TBA) were added, and the mixture was heated at 95 degrees in a water bath for 30 minutes. The solution was then cooled under running tap water. TBA-MDA complex was extracted by adding 3ml of n-butanol. The absorbance of the resultant pink extract was measured at 532 nm wavelength using a spectrophotometer. Levels of MDA were calculated and expressed as nanomoles per gram of wet tissue.

Determination of catalase activity

Stomach tissue catalase activity was determined using the Aebi kinetic method (Aebi, 1984). 1.9ml of 50mM phosphate buffer (pH 7.0) was added to 0.1ml of stomach tissue homogenate. Then, 1 ml of 30 mM H_2O_2 was added to the mixture. The initial and final absorbance in one minute was determined at 240 nm. The reagents were used as a blank. Concentrations were calculated using the molar extinction coefficient of 43.6 $Lmol^{-1}cm^{-1}$.

Estimation of reduced glutathione

Gastric tissue was homogenized in 0.3 M ice-cold phosphate buffer (pH 7.0). 0.9 ml of 0.1% EDTA and 1.5 ml of 20% Trichloroacetic acid were then added to 0.1 ml of the sample and mixed well. The mixture was allowed to stand for 5

minutes; it was then centrifuged at 3000 rpm for 5 minutes. 1.8 ml of Dithio-nitrobenzoic acid (DTNB) was added to 0.2 ml of supernatant and the solution mixed well. The absorbance of the solution was read at 412 nm, 3 minutes after the addition of DTNB.

Estimation of superoxide dismutase activity in gastric tissue

The estimation was done according to the method described by Marklund & Marklund, 1974. 3.0 ml of assay mixture was prepared as follows; 1 ml of 50 mM Tris-HCl buffer, 1.5 ml of 1mM diethylene triamine penta-acetic acid (DTPA), and 0.5 ml of tissue homogenate. The reaction was initiated by the addition of 0.5 ml of 10 mmol pyrogallol solution in 10 mmol HCl. Absorbance was read at 420 nm (a lag period of about 1 minute was given to allow the steady state of auto-oxidation of pyrogallol to be obtained). One unit of SOD was described as the amount of enzyme required to cause 50% inhibition of pyrogallol auto-oxidation per 3 ml of assay mixture. Results were expressed as units per mg of tissue.

Statistical analysis

Data was expressed as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) was used for comparison of means between the groups followed by Tukey's post-hoc test for significance. The level of significance was set at $P< 0.05$. Data analysis and graph production was done using SPSS.

Results

Effects of *Rhus natalensis* on the total gastric lesion area

There was a significant difference in the mean gastric lesion area between the treatment groups as determined by one-way ANOVA ($F [4; 25] = 345.478$, $P=0.000$). The test results showed that mean of the ulcer (Figure 2) areas of the negative control group (12.040 ± 0.6405) was significantly larger compared to the mean of positive control group (1.3840 ± 0.0563; P=0.000), low dose *Rhus natalensis* group (4.010 ± 0.1837; P=0.000), high dose *Rhus natalensis* (1.670 ±0.1255; P=0.000), and the normal group (0.8500 ± 0.2974; P=0.000). There were no significant differences between the mean ulcer area of the normal, positive, and high dose *Rhus natalensis* groups (Figure 3).

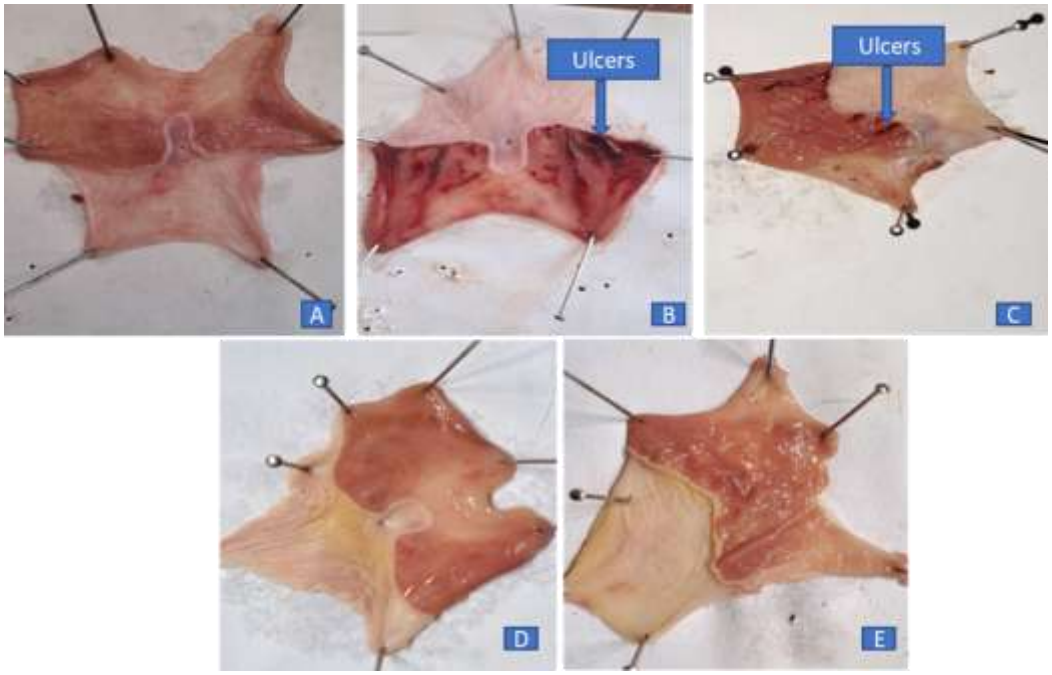


Figure 2. Images of samples of gastric tissues showing mucosal surfaces of the normal group (A), the negative control group (B), the low dose *Rhus natalensis* group (C), the high dose *Rhus natalensis* group (D), and the positive control (esomeprazole) group (E)

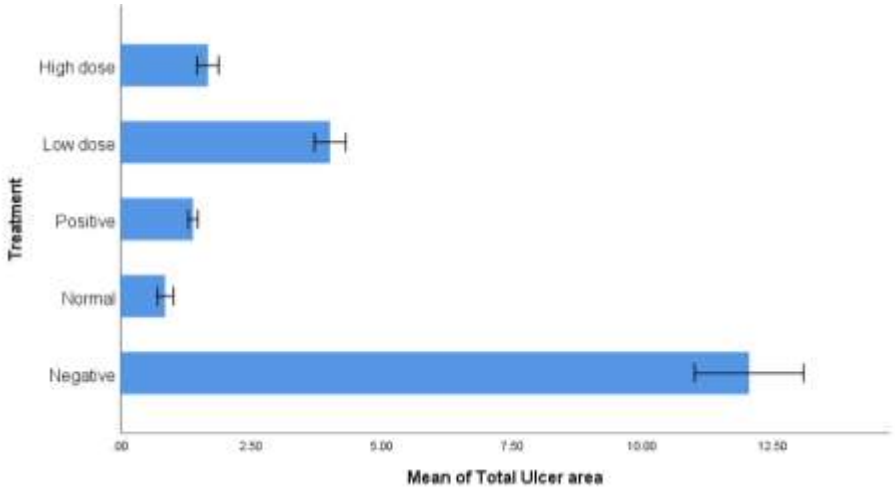


Figure 3. Effect of administration of normal saline (1.0 ml/kg) or Esomeprazole (20 mg/kg) or *Rhus natalensis* (150 or 300 mg/kg) on the mean of total ulcer area. N = 6 in each group and the arrow bars represents the standard error of the means

Effects of *Rhus natalensis* on gastric pH

There was a significant difference in the mean gastric PH between the treatment groups as determined by one-way ANOVA (F [4; 30] = 145.348, P=0.000). The test results showed that the mean of the gastric pH was significantly lower for the negative control group (2.524 ± 0.1027) compared to the positive control group (4.311 ± 0.0480; P=0.000), low dose *Rhus natalensis* (3.963 ± 0.0338; P=0.000), high dose *Rhus natalensis*, (4.239 ± 0.0463; P=0.000). There was no significant difference between the positive control, and high dose *Rhus natalensis* groups (Figure 4).

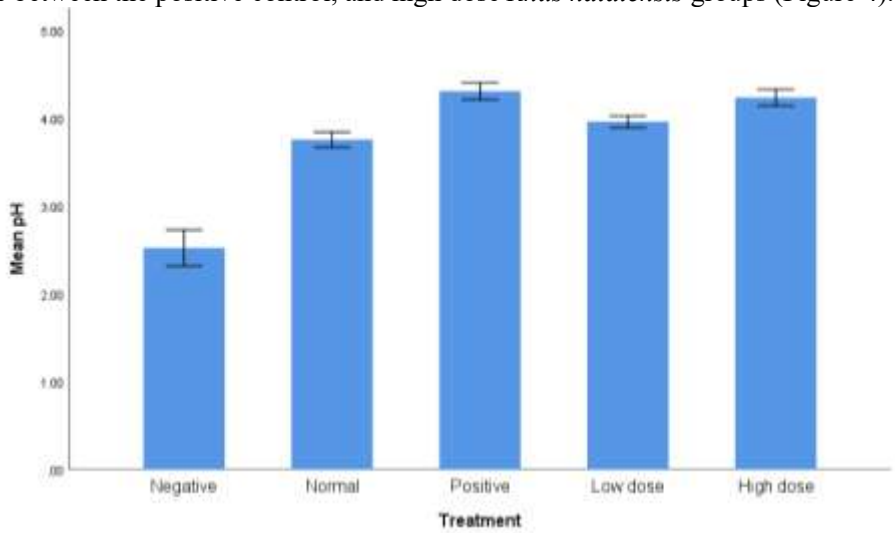


Figure 4. Effect of administration of normal saline (1.0 ml/kg) or Esomeprazole (20 mg/kg) or *Rhus natalensis* (150 or 300 mg/kg) on the mean pH. N = 7 in each group and the arrow bars represents the standard error of the means

Effects of *Rhus natalensis* on the total acidity of gastric secretions

There was a significant difference in the mean total acidity of gastric secretions between the treatment groups as determined by one-way ANOVA (F [4; 30] = 48.914, P=0.000). The total acidity of gastric secretions of the negative control (62.86 ± 2.641) was significantly higher compared to the normal control (27.14 ± 1.844; P=0.000), the positive control (30.00 ± 1.414; P=0.000), low dose (38.71 ± 2.032; P=0.000) and high dose *Rhus natalensis* (33.57 ± 2.103; P=0.000). The normal control, positive control, and high dose *Rhus natalensis* didn't show significant differences in the total acidity of their gastric secretions. However, the low dose *Rhus natalensis* group and the normal control showed a significant difference between their total acidity (P=0.0033) (Figure 5).

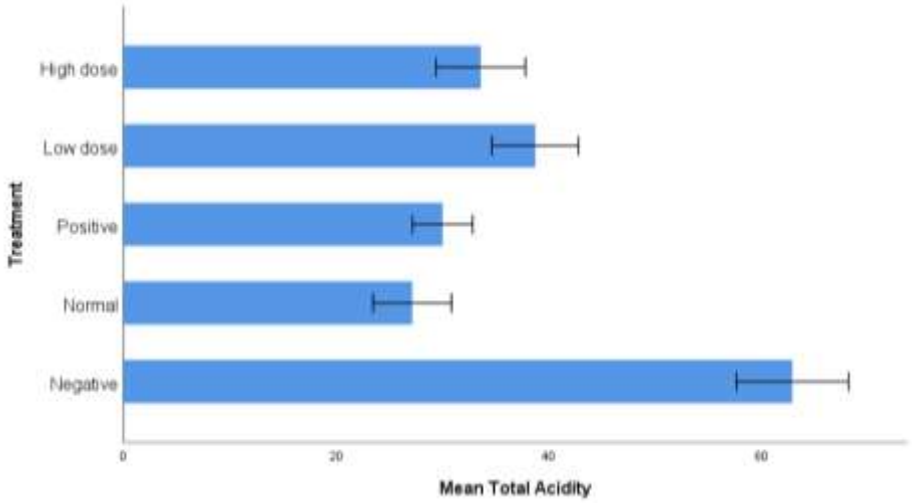


Figure 5. Effect of administration of normal saline (1.0 ml/kg) or Esomeprazole (20 mg/kg) or *Rhus natalensis* (150 or 300 mg/kg) on the mean of total acidity. N = 7 in each group and the arrow bars represents the standard error of the means

Effects of *Rhus natalensis* on the volume of gastric secretions

There was a significant difference in the mean volume of gastric secretions between the treatment groups as determined by one-way ANOVA (F [4; 30] = 20.046, P=0.000). The mean of the volume of gastric secretions was significantly reduced in the normal control (1.271 ± 0.0865; P=0.000), positive control (1.014 ± 0.0857; P=0.000), low dose (1.457 ± 0.0896; P=0.002), and high dose (1.100 ± 0.1024; P=0.000) *Rhus natalensis* group compared to the negative control (1.971 ± 0.0522). There was no significant difference in the mean volume of gastric secretions between the positive control and the high dose *Rhus natalensis* group (P=0.952) (Figure 6).

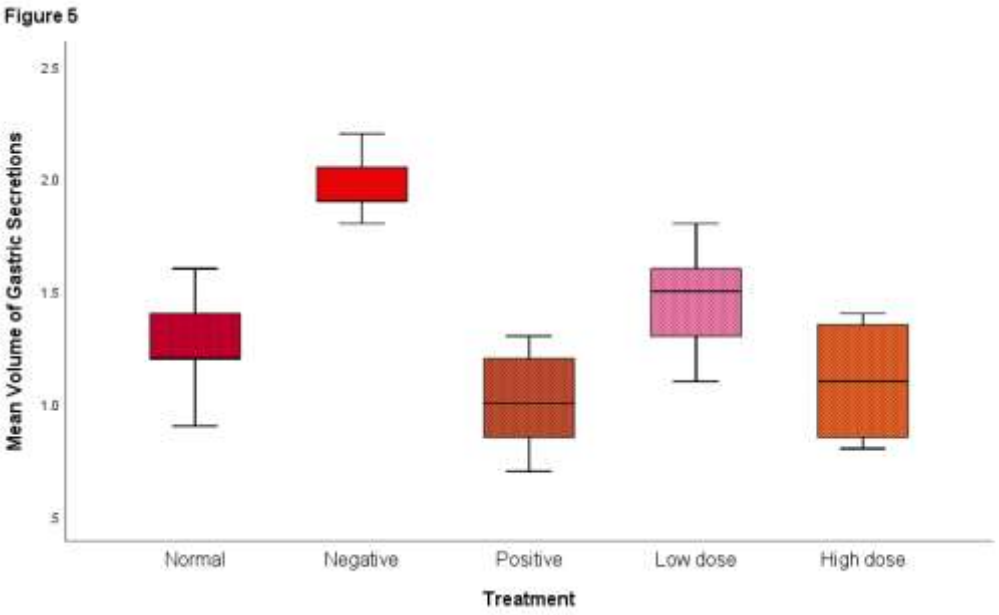


Figure 6. Effect of administration of normal saline (1.0 ml/kg) or Esomeprazole (20 mg/kg) or *Rhus natalensis* (150 or 300 mg/kg) on the mean of volume of gastric secretions. N = 7 in each group and the arrow bars represent the standard error of the means

Effects of *Rhus natalensis* on gastric tissue catalase activity

Catalase activity (micromoles of H_2O_2/minute) in gastric tissue was significantly increased in the normal control (6.193 ± 0.3077), positive control (5.318 ± 0.6329), low dose (4.817 ± 0.2177), and high dose (5.505 ± 0.2176) *Rhus natalensis* groups compared to the negative control (3.027 ± 0.4128). Post-hoc statistical analysis using Tukey's multiple comparisons test showed no significant differences in catalase activity between the normal control, positive control, low dose, and high dose *Rhus natalensis* groups (Table 1).

Table 1. Effects of *Rhus natalensis* on gastric tissue catalase activity

Dependent variable	I (Group)	J (Group)	Mean	Sig
Effect on catalase activity (U/g)	Negative (μ= 3.027 ± 0.4128)	Normal	6.193 ± 0.3077	0.0001
		Low dose	4.817 ± 0.2177	0.0001
		High dose	5.505 ± 0.2176	0.0001
		Positive control	5.318 ± 0.6329	0.0001
Effect on malondialdehyde levels (nmol/g)	Negative (μ= 7.524 ± 0.0989)	Low dose	4.526 ± 0.1043	0.0001
		High dose	4.172 ± 0.1115	0.0001
		Positive control	4.048 ± 0.0997	0.0001
	Normal (μ=3.933 ± 0.0596)	Low dose	4.526 ± 0.1043	0.0026
Effect on glutathione levels (nmol/g)	Low dose (μ=66.2 ± 0.8831)	Normal	78.75 ± 1.375	0.0001
		High dose	74.22 ±1.653	0.0023
		Positive control	76.34 ± 0.8831	0.0002
	Negative (μ=43.09 ± 1.420)	High dose	74.22 ±1.653	0.001
		Low dose	66.2 ± 0.8831	0.001
		Positive control	76.34 ± 0.8831	0.001
Effect on superoxide dismutase activity (units/mg)	Negative (μ=2.084 ± 0.0791)	Normal	4.949 ± 0.0945	0.001
		Low dose	3.591 ± 0.1068	0.001
		High dose	4.789 ± 0.0491	0.001
		Positive control	4.638± 0.0576	0.001

Effects of *Rhus natalensis* on levels of malondialdehyde in gastric tissue

The negative control group showed the highest levels of Malondialdehyde (7.524 ± 0.0989) nmoles/g of tissue, while the normal control showed the lowest levels (3.933 ± 0.0596) nmoles/g of tissue. Treatment with Esomeprazole, low dose, and high dose *Rhus natalensis* significantly reduced the levels of malondialdehyde when compared to the negative control: esomeprazole (4.048 ± 0.0997) vs low dose *Rhus natalensis* (4.526 ± 0.1043) vs high dose *Rhus natalensis* (4.172 ± 0.1115) vs the negative control (7.524 ± 0.0989) nmoles/g of tissue.

Post hoc statistical analysis using the Tukey's multiple comparisons test revealed significant differences between the normal control group and the low dose group (P=0.0026). However, there was no significant differences between the normal control, positive control and the high dose *Rhus natalensis* group (Table 1).

Effects of *Rhus natalensis* on reduced glutathione (gsh) levels in gastric tissue

The negative control group had the lowest levels of reduced glutathione (43.09 ± 1.420), while the normal control group had the highest levels (78.75 ± 1.375) micromoles/mg tissue in gastric tissue. Administration of 20mg/kg Esomeprazole, 150mg/kg, and 300mg/kg of *Rhus natalensis* significantly raised the levels of reduced glutathione when compared to the negative control group: (76.34 ± 0.8831), (66.2 ± 0.8831), (74.22 ±1.653), and (43.09 ± 1.420) respectively. There were significant differences between the low dose *Rhus natalensis* group and the normal control P<0.0001, the low dose *Rhus natalensis* and the positive control group P=0.0002, and the Low dose *Rhus natalensis* group and the high dose *Rhus natalensis* group P=0.0023. There were no significant differences between the normal control group, the positive control group, and the high dose *Rhus natalensis* group. The effect of *Rhus natalensis* was in a dose- dependent manner (Table 1).

Effects of *Rhus natalensis* on superoxide dismutase activity in gastric tissue

The negative control group had the lowest activity of Superoxide dismutase (2.084 ± 0.0791) while the normal control group had the highest SOD activity (4.949 ± 0.0945). Administration of 20mg/kg esomeprazole, 150mg/kg *Rhus natalensis*, and 300mg/kg *Rhus natalensis* significantly raised SOD activity when compared to the negative control(ethanol) group; esomeprazole (4.638± 0.0576), 150mg/kg *Rhus natalensis* (3.591 ± 0.1068), and 300mg/kg *Rhus natalensis* (4.789 ± 0.0491).

The Rhus natalensis groups showed a dose dependent increase in SOD activity. However, there was no significant differences between the normal control, the positive control, the low dose, and high dose Rhus natalensis groups.

Discussion

Rhus natalensis is used by traditional medical practitioners for management of various medical conditions. The roots of *Rhus natalensis* are pounded in water and the juice is drunk for treatment of flu, abdominal pain, gonorrhoea, hookworm infestation and diarrhoea management (Kokwaro, 2009). The leaves are used to treat coughs, and steam from boiled leaves is inhaled for colds (Kokwaro, 2009). Fruits are eaten to prevent chest problems (Bussmann et al., 2006). The plant is also an antimalarial (Obakiro et al., 2020), and also treats back pains, postpartum pains, and headache (Kigen et al., 2017). The plant is also used for the treatment of liver and spleen diseases and peptic ulcers (Kigen et al., 2017). Various phytochemical compounds have been isolated in *Rhus natalensis* e.g., flavonoids, bioflavonoids, tannins, and terpenes (Márquez-Flores et al., 2025). The aim of this study was to investigate the ulcer healing effects of freeze-dried extracts of *Rhus natalensis*. Ulcer healing was assessed based on the degree of ulceration of the gastric mucosa, total acidity of gastric secretions, gastric pH, volume of gastric secretions, levels of oxidative stress markers, and levels of both enzymatic and non-enzymatic cellular antioxidant systems. Alcohol concentrations of 10% or more disrupt the gastric mucosal barrier and increase mucosal permeability. Therefore, ethanol administration in the rats during this study disrupts the integrity of the mucus layer paving the way for hydrochloric acid to damage gastric mucosa epithelial cells causing ulceration. There was a significant reduction in the total ulcer area in the treatment groups. The total ulcer area is an indicator of the degree of gastric mucosal injury. The study shows a non-dose dependent reduction in the total ulcer area since there were no statistically significant differences between the low dose and high dose *Rhus natalensis* groups. A decrease in the total ulcer area is an indicator of ulcer healing. The healing might have been as a result of the decrease in the total acidity of gastric contents, a decrease in the volume of gastric secretions, and an increase in the cellular antioxidant activity (Lu et al., 2025). The ulcer healing effects could have also been due to increased prostaglandin synthesis. Prostaglandins I2 and E2 are mucosal protective factors as they reduce acid secretion, increase mucus, and bicarbonate production and also increase mucosal blood flow (Mohamed et al., 2021). The other possible ulcer healing effect of *Rhus natalensis* might have been due to anti-inflammatory effects since the inflammatory response is responsible for the progressive trigger and worsening of gastric ulcers (Gugliandolo et al., 2021). The results of this study showed a significant increase in the pH of gastric secretions in the treatment groups and also a dose dependent reduction in the total acidity of gastric secretions of the same groups. Hydrochloric acid is a major mucosal aggressive factor due to its proteolytic and hydrolytic effects (Satoh et al., 2025). Reduction in the total acidity of gastric contents as demonstrated in this study confers a protective function to the gastric mucosa by eliminating a major aggressive factor (Gupta et al., 2023). The rise in pH might have been caused by inhibition of hydrochloric acid secretion by the parietal cell proton pumps in the same way that proton pump inhibitors such as Omeprazole function. It might have also resulted from increased prostaglandin synthesis; prostaglandins reduce hydrochloric acid production by inhibiting histamine-stimulated increase in cyclic-AMP, they also increase mucosal blood flow which buffers and removes any protons that back-diffuse into the lamina propria (Cohen, 1987). Increased bicarbonate production could have also been a possible mechanism by which *Rhus natalensis* leaf extracts raised the gastric pH in the study. The findings from this study are similar to results from other studies investigating ulcer healing effects in other plant extracts such as *Clausena excavata* leaves (Albaayit et al., 2016). In this study, there was a decrease in the volume of gastric secretions in the treatment groups as compared to the negative control which showed an increase in the volume of secretions. This could be due to reduced gastric juice secretion. The major components of gastric juice; hydrochloric acid and pepsin are mucosal aggressive factors (Al-Qaisi et al., 2025) and therefore a reduction in the volume of gastric acid secretions reduces two major gastric mucosa injurious factors. The first-line therapy for gastric acid; the proton-pump inhibitors e.g., Omeprazole are antisecretory agents (Katayama et al., 2017).

The results showed an increase in cellular antioxidant activity in the treatment groups as indicated by increased catalase activity, superoxide dismutase (SOD) activity, and levels of reduced glutathione (GSH). Ethanol metabolism results in the production of reactive oxygen species through conversion of its metabolite acetaldehyde into reactive oxygen species by Xanthine oxidase (Sami et al., 2025). Superoxide dismutase catalyses conversion of superoxide anion to hydrogen peroxide which is more stable, hence less reactive, and less injurious (Paguigan et al., 2014). Catalase enzyme converts hydrogen peroxide to water and oxygen (Salehi et al., 2018). Reduced glutathione acts as a hydrogen donor in the detoxification of hydrogen peroxide (Weschawalit et al., 2017). An increase in the activity and/or levels of the cellular antioxidant system therefore confers a cytoprotective effect on gastric mucosal cells (Sugano et al., 2012). The increase in cellular activity could be due to the phytochemical compounds that have been isolated in *Rhus natalensis*. For example, flavonoids have a free hydroxyl group on their aromatic ring which is responsible for their antioxidant activity (Sharifi-Rad et al., 2018). The findings are similar to many studies showing the antioxidant effects of plant extracts as an ulcer healing or protecting mechanism. The study showed a decrease in the levels of malondialdehyde (a marker of lipid peroxidation) in the treatment groups when compared to the negative control. Ethanol metabolism produces reactive oxygen species that attack the double bonds in membrane polyunsaturated lipids resulting in the formation of peroxides which are also unstable and reactive (Ayala et al., 2014). Therefore, a reduction in the levels of

malondialdehyde indicates reduced lipid peroxidation hence reduced cell membrane damage (Chari et al., 1993). The pathophysiology of peptic ulcer disease is complex and multifactorial. It's caused by an imbalance between aggressive and defensive mucosal-protective factors(Sánchez-Mendoza et al., 2024). The aggressive factors of the mucosa include hydrochloric acid, pepsin, and bile (Lu et al., 2019). The defensive factors of the mucosa include; mucus-bicarbonate layer, mucosal blood flow, and prostaglandins (Colak et al., 2025). The imbalance between the aggressive and protective factors is caused by factors such as infection by *Helicobacter pylori*, chronic use of non-steroidal anti-inflammatory drugs, alcohol intake, smoking of tobacco and bile reflux (Mohammadi et al., 2024). *Helicobacter pylori* is a gram-negative motile bacteria that colonizes the gastric mucosa of approximately 50% of people in the world (Pan & Jiao, 2025). *H. pylori* bacteria cause PUD due to production of toxins: Vacuolating cytotoxin A, Cytotoxin associated gene A (CagA) and a Type IV secretion system (T4SS) (Salama et al., 2013). The bacteria have various virulence factors that enable it to survive in the acidic conditions in the stomach, cross the mucus barrier, and get to the mucosal cells for adherence (Oshima & Miwa, 2016). The virulence factors include; Urease enzyme, flagella, its helical shape, adherence factors, Cytotoxin associated gene A (CagA), and Vacuolating cytotoxin A (Salama et al., 2013). Urease enzyme produces ammonium ions that neutralize the stomach acid(Clyne & Ó Cróinín, 2025). The ammonium ions also facilitate the motility of the bacteria by changing the properties of the mucus from a gel at low pH into a solution through which the bacteria can freely swim at high pH (Pan & Jiao, 2025). NSAIDs are used indiscriminately to treat fever, osteoarthritis, joint pains, and cardiovascular disease because of their analgesic, antipyretic, and anti-inflammatory effects (Sharifi-Rad et al., 2018). However, their long-term use has been associated with PUD (Sabiu et al., 2015). They are the second most common cause of PUD after *Helicobacter pylori* (Lanas & Chan, 2017). NSAIDs inhibit cyclooxygenase-1 and cyclooxygenase-2 enzymes that catalyse synthesis of prostaglandins from arachidonic acid. Prostaglandins are mucosal protective factors that regulate gastric acid secretion, increase mucus and bicarbonate synthesis, and mucosal blood flow via prostaglandin receptors (EP) 1-4 (Kuna et al., 2019). Thus, inhibition of prostaglandin synthesis by NSAIDs reduces mucus and bicarbonate synthesis, impairs mucosal blood flow, and increases gastric acid secretion (Lu et al., 2025). NSAIDs disrupt mitochondrial transmembrane potential leading to the release of reactive oxygen species such as superoxide and hydrogen peroxide which cause cellular membrane damage by lipid peroxidation and eventually result in cell death (Matsui et al., 2011). Ethanol causes an imbalance in cellular antioxidant processes by increasing the activity of Xanthine oxidase (Hasanuzzaman et al., 2020). Xanthine oxidase catalyses the production of free radicals from acetaldehyde, a product of alcohol metabolism (Teshome et al., 2019). The free radicals increase lipid peroxidation, which damages the cell membranes of epithelial cells, causing epithelial erosion. Ethanol also decreases the synthesis of prostaglandins which are important mucosal protective factors, therefore decreasing bicarbonate and mucus secretion (Chari et al., 1993). Ethanol increases oedema and removal of epithelial cells by increasing microvascular and vascular permeability which can cause damage to the gastrointestinal mucosa. Ethanol- dependent production of leukotrienes – immune mediators that cause inflammation- might also contribute to ethanol-induced PUD (Katary & Salahuddin, 2017).

Conclusion

In conclusion, this study shows that *Rhus natalensis* possesses ulcer healing effects. The effects are potentially due to reduced acidity of Gastric contents, increased pH of gastric contents, reduced volume of secretions, and increased activity of cellular antioxidant systems; catalase, superoxide dismutase, and reduced glutathione. *Rhus natalensis*, therefore shows potential of being an alternative treatment for Peptic ulcer disease. The major limitation of the study is that it only shows the effects of *Rhus natalensis* on ethanol-induced gastric ulcer model, therefore further studies need to be done on the other gastric ulcer induction models to determine the effects of *Rhus natalensis* on them. Further studies also need to be done to determine the exact binding sites of *Rhus natalensis* on gastric parietal cells that result in its effects on gastric acid secretion, and to also determine its probable side effects.

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Author contributions

JGS, HNK, AWM, CGG designed the study. JGS, HNK, AWM, CGG did the experimental work. JGS, HNK, AWM, CGG analyzed the experimental data and wrote the paper. All authors reviewed the manuscript.

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Ethics approval

The consent to carry out the study was obtained from the Biosafety, Animal Care, and Use Committee of the Department of Veterinary Anatomy and Physiology at the University of Nairobi (FVM BAUEC/2023/444), adhering to the guidelines outlined in the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

Competing Interests

The author declares no conflict of interest. The manuscript has not been submitted for publication in other journal.

AI Tool Declaration

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

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