

Full Length Research Paper

Gastro-protective and ulcer-healing effects of *Erythrina sacleuxii* root extract on ethanol induced gastric ulcer in Wistar albino rats

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Peptic ulcer disease, characterized by erosion of the mucosal barrier, remains a common health issue in the 21st century, with an annual incidence of 0.1 to 0.3%. Challenges in management, including recurrence, drug interactions, side effects of conventional therapies, and *Helicobacter pylori* resistance, highlight the need for further research in identifying effective, safe, and economical treatments. Medicinal plants and their constituents are currently being explored for their gastro-protective and anti-ulcerogenic potential. *Erythrina sacleuxii*, a tree native to the coastal regions of Kenya and Tanzania, has been traditionally used to manage peptic ulcers, but its therapeutic effects lack scientific validation. This study evaluated the in vivo anti-ulcer activity of *E. sacleuxii* root extract (ESRE) on induced gastric ulcer in male Wistar albino rats. The study also determined the possible mechanism of action of ESRE through assessing oxidative stress markers. ESRE demonstrated significant ($p < 0.05$) anti-ulcer activity, with $80.82 \pm 5.13\%$ protection and $91.04 \pm 0.24\%$ healing in the 400 mg/kg treatment group. ESRE also countered ethanol-induced oxidative stress by increasing levels of Glutathione (GSH), Superoxide dismutase (SOD), and Catalase (CAT) while reducing lipid peroxidation. These antioxidant effects, along with mucosal protection and ulcer healing, suggest that ESRE could be further investigated for managing gastric ulcers.

Key words: *Erythrina sacleuxii*, root extract, gastroprotective and antiulcer properties.

INTRODUCTION

Peptic ulcer disease (PUD) is characterized by erosion of the protective lining in the epithelial mucosa of the

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esophagus, stomach, or duodenum due to acid-peptic factors resulting from acid-peptic factors (Alsinnari et al., 2022; Lanan and Chan, 2017). PUD induces morbidity across all age groups, with rising incidences and mortality observed in older individuals, making it a significant global health concern that demands attention. Ulcers, open sores that form on the inner lining of the stomach (gastric ulcer) or the small intestine (duodenal ulcer), exhibit inflamed lesions or excavations in the mucosa and protective tissue of the gastrointestinal tract, collectively referred to as PUD (Firehun and Nedi, 2023). The etiology of this condition is considered complex and multifactorial, involving an imbalance between the protective and aggressive agents of the gastric mucosa (Dimaline and Varro, 2007). Key mucosal defense factors include the production of cytoprotective prostaglandins (PGs), mucus, bicarbonate (HCO_3^-), nitric oxide (NO), an endogenous antioxidant system, and sufficient blood flow (Yandrapu and Sarosiek, 2015). Risk factors contributing to PUD development are categorized into endogenous factors such as hydrochloric acid (HCl) secretion, and pepsin activity, bile reflux, abnormal motility, decreased blood flow, and infection with *Helicobacter pylori* as well as exogenous factors such as poor eating habits, stress, and chemical agents such as alcohol, smoking, and long-term and excessive use of nonsteroidal anti-inflammatory drugs (NSAIDs) (Serafim et al., 2020).

PUD is a widespread global disease, ranking among the most common diseases of the 21st Century, impacting approximately 5 to 10% of the world population at least once in their lifetime (Farzaei et al., 2015), with an incidence of 0.1 to 0.3% per year. Common symptoms include epigastric abdominal pain, burning, nocturnal pain, or early satiety, nausea, vomiting, weight loss, and post-prandial abdominal pain, bloating, and hematemesis. The primary objectives of peptic ulcer therapy are to relieve pain, heal the ulcer, and prevent complications and recurrence (Serafim et al., 2020). The mainstay treatments for PUD are anti-secretory medications, such as histamine H_2 receptor antagonists and proton pump inhibitors (PPIs) and cytoprotectors (sucralfate and salts bismuth), antacids, demulcents, anti-*h. pylori* agents, and anticholinergics (Fossmark et al., 2019). While the development of PPIs marked a therapeutic breakthrough for peptic ulcers, there are limitations to pharmacological intervention, including side effects like osteoporosis and hypergastrinemia especially with prolonged usage (Yu et al., 2017). Furthermore, they exhibit limited efficacy in ulcer healing and recurrence of ulcerations, with associated drug interactions leading to substantial economic implications for users and healthcare systems (Varcus et al., 2018).

Consequently, managing PUD and its complications remain a significant medical challenge. Research into the development of gastroprotective agents that are not only

effective, inexpensive, and safe for long-term use but also protect the gastric mucosa without affecting acid secretion is highly recommended (Kuna et al., 2019). In response to the challenges associated with modern medicine, the exploration of medicinal plants and their isolated compounds have gained attention. The appeal of these natural remedies lies in their availability, better protection, lower cost, and reduced toxicity (Kuna et al., 2019). *Erythrina saculeuxii*, known for its traditional medicinal application, has a historical background in the treatment of trachoma, malaria, and elephantiasis using its stem, and syphilis with its roots (Quattrocchi et al., 2012). Scientifically, it has demonstrated moderate antifungal activity against *Piricularia oryzae* and *Rhizomucor miehei* (Ombito et al., 2018), weak cytotoxicity against human cancer cell lines, HeLa-S3 (Ombito et al., 2020), antiparasmodial activities against the chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum*, antimicrobial activity against *Staphylococcus aureus*, *Shigella* species, and *Candida albicans* (George et al., 2013). Traditionally used by the people of the coastal region of Kenya in the management of PUD, along with its recognized anti-inflammatory and antimicrobial properties, *E. saculeuxii* holds potential as an antiulcer agent in the treatment of gastric and duodenal ulcers (Ombito et al., 2020).

To contribute to the broader understanding of the gastroprotective and antiulcer properties of *Erythrina* species, this study investigated the gastro-protective and anti-ulcer activity of *E. saculeuxii* root extract (ESRE) on ethanol induced gastric ulcer in Wistar albino rats. This study also elucidated the mechanism of action employed by the plant extract. The research aimed to expand the knowledge base on the potential therapeutic applications of *E. saculeuxii* in addressing gastric and duodenal ulcers.

MATERIALS AND METHODS

Sample collection and preparation

The roots and leaves of *E. saculeuxii* were gathered from Mkongani ward, Kwale County, Kenya, near Kirewe secondary school (-4.18333, 39.45) in May of 2021. The collection was conducted with the assistance of a local herbalist, and the plant materials were carefully transported to the laboratory in sterile bags. To ensure accurate identification, a taxonomist at Jomo Kenyatta University Agriculture and Technology (JKUAT, GoK laboratories) verified the plant. For future reference, a voucher number MOM-JKUATBH 001A-2021 was assigned and stored in the herbarium, documenting the plant's botanical details. Prior to extraction, the roots of the plant were well cleaned by washing them under running tap water, then rinsing with distilled water and subsequently cutting them into tiny pieces. The pieces were dried under the shade, turned regularly to prevent fermentation and rotting. Once completely dried, the roots were pulverized using a plant mill. The resulting powder was weighed using an analytical scale and subsequently stored in an air-tight container, shielded from light, heat, and moisture, at room

temperature for future use. Approximately 1 kg of dried root powder was processed to yield a usable amount of the final product.

Maceration method of extraction was employed to prepare ESRE. Briefly, 250 g of the plant root powder was immersed in 1000 ml of 80% ethanol and left to soak for 72 h. Throughout this period, the mixture was agitated at 360 rpm facilitating the dissolution of soluble components. Whatman's filter paper No.1 was then used for filtering to obtain the crude extract solution. This was followed by concentrating the crude extract to a minimum volume using a rotary evaporator at 45°C and reduced pressure. The concentrated solution was then subjected to oven drying overnight at 60°C. The mass of the resulting dry plant powder was used to calculate the yield of the plant extract.

Experimental animals

Male Wistar albino rats (n=48), aged 6 to 8 weeks and weighing 210 ± 10 g, were sourced from Kenyatta University's Biochemistry Department. The rats were housed in standard living conditions with normal bedding in plastic cages, as per Organisation for Economic Co-operation and Development (OECD) guidelines (OECD, 2008) within the Kenyatta University animal house. A one-week acclimatization period preceded the experimentation, during which the animals were provided with a standard diet (50 g/day) water and libitum. All procedures involving the animals adhere to the guide for the care and use of laboratory animals of the National Institutes of Health (NIH, 2011). The protocols were approved by the animal review committee at Kenyatta University, under the reference KU/ACUC/PROVAL/VOL.1/02, ensuring compliance with ethical standards and the welfare of the experimented animals.

Determination of radical scavenging activities of 1,1, diphenyl-2-picrylhydrazyl (DPPH)

Following the method described by Okawa et al. (2001), eight different concentrations of the studied plant extracts (3.906, 7.8125, 15.625, 31.25, 62.5, 125, 250, and 500 µg/ml) were prepared in methanol. Similar concentrations of L-ascorbic acid, a standard antioxidant, were also prepared. 2 ml of each studied extract was transferred into a clean test tube into which 1 ml of 0.3 mM DPPH in methanol was added. The reaction mixture was vortexed thoroughly and kept in the dark for 30 min at room temperature. The mixture's absorbance was quantified using spectrophotometry at a wavelength of 517 nm following incubation in the dark. The negative control was the 2.5 mL DPPH solution in 1 mL methanol. To calculate the percentage of DPPH radical scavenging activity, the following equation was used:

$$\% \text{ DPPH radical scavenging activity} = \frac{A_0 - A_1}{A_0} \times 100$$

where A₀ is the absorbance of the control, and A₁ is the absorbance of the extractives/standard. Subsequently, inhibition percentages were plotted against concentration and IC₅₀ was determined on a graph. The experiments were performed in triplicate at each concentration.

In vitro assay on the gastro protective effect of ESRE

For preliminary screening of ESRE to determine its ability to reduce gastric acidity, thereby having a gastro-protective effect, the following *in vitro* methods were conducted to test for the same. A stock solution

(100 mg/mL) was initially prepared by dissolving ESRE powder in 1% ethanol to prepare the sample. In triplicate, from the stock solution, a final diluted concentration of 1 mg/mL was prepared using 1% ethanol as the solvent of dissolution. The positive control was sodium bicarbonate (1 mg/mL in 1% ethanol), and the negative control was 1% ethanol. For each experiment, the setup was held at 37°C.

Antacid potency test of ESRE

The preliminary antacid test followed the method specified in the United States Pharmacopeia (USP) National Formulary (Yu et al., 1997). A continuous stirring of 40 mL in each test solution was performed over 1 min. Following that, standardized 0.5 M HCl (10 mL) was added. The mixture's pH was measured after being stirred continuously for 10 min.

Neutralizing effects of ESRE on artificial gastric acid

Artificial gastric juices were prepared as follows: 3.2 mg of pepsin and 2 g of NaCl were dissolved in 500 mL of distilled water. Followed by adding 7 mL hydrochloric acid, and then water was topped to a 1000 mL solution, and the pH of the solution was adjusted to pH 1.2. Next, 90 mL of prepared ESRE, sodium bicarbonate, and 1% ethanol were added separately to 100 mL of artificial gastric juice maintained at pH 1.2. The pH values were immediately determined to examine the neutralizing effects of ESRE on artificial gastric juice compared to the reference drug.

Acid-buffering capacity assay of ESRE

Buffering capacity was assessed using the Association of Official Analytical Chemists' Official Methods of Analysis. Each test solution contained 40 mL and was stirred continuously at 60 rpm. Forward titration was carried out by gradually adding 0.5 mL of standard 0.099207 M Hydrochloric acid (HCl) until the pH fell to 1.5, that is, Normal stomach pH. After that, the samples were back titrated with 0.5 mL of standard 0.100995 M Sodium hydroxide (NaOH) until the pH reached 10. After a 1-min equilibration time following the administration of acid or base, the initial pH level and all subsequent measurements were recorded. Based on Van Slyke's equation, the capacity to buffer acid was calculated: For each sample the total volume of acids added multiplied by the acid molarity divided by the overall change in pH (Watanabe et al., 2001).

Gastro protective effect on ethanol-induced gastric ulceration

To determine the gastroprotective effect of ESRE 6 groups of 4 animals per group were formed as follows:

- 1) Group (I), representing the normal control, was administered distilled water.
- 2) Group (II), serving as the ulcer control, was administered with distilled water followed by ethanol administration.
- 3) Group (III), serving as the positive control, was administered with the standard drug omeprazole at a dose of 20 mg/kg, followed by ethanol administration.
- 4) Group (IV) was administered with 400 mg/kg ESRE followed by ethanol administration.
- 5) Group (V) was administered with 200 mg/kg ESRE followed by ethanol administration.
- 6) Group (VI) was administered with 100 mg/kg ESRE followed by

ethanol administration.

After grouping the animals, they were fasted for 24 h (to empty stomachs), with free access to water before treatment began. Treatments with 1ml omeprazole and ESRE lasted 14 days, given once a day before ulcer induction with 96% ethanol. On the 15th day, after the rats had fasted for 24 h, 96% ethanol (5 ml/kg bwt) was administered by oral gavage to induce gastric ulceration in all groups, except the normal control group (Al-Sayed and El-Naga, 2015). The animals were humanely euthanized 1-h post-ethanol administration by cervical dislocation, and their stomachs were quickly removed, cut along the greater curvature, and rinsed with sterile saline. The stomach linings were photographed using a digital camera (10 megapixels 5 × zoom); while stretched on a flat plate to analyze the gastric lesions. The gastric samples were divided into two; one portion was dried in liquid nitrogen and then stored at -80°C until further analysis. The other portion for histopathology analysis was stored in 10% buffered formalin. Using imageJ analysis software, the area of the ulcerated lesions was calculated, and the ulcerated area was calculated as a proportion of the total stomach area using the formula described by Al-Sayed and El-Naga (2015):

$$\text{Gastric lesion index} = \frac{\text{Gastric lesion area (mm}^2\text{)}}{\text{Total gastric area (mm}^2\text{)}} \times 100$$

The inhibition % was calculated using the following formula:

$$\text{Inhibition percentage} = \frac{((\text{ulcerated area (ulcer control)} - \text{ulcerated area (treated)}))}{\text{Ulcerated area (ulcer control)}} \times 100$$

Healing effect on ethanol-induced acute gastric lesions

The study evaluated and compared the therapeutic impacts of ESRE on gastric lesions induced by ethanol, in comparison to omeprazole, a proton pump inhibitor. Six animal groups were established for this investigation, including a negative control group (n = 4), an ulcer control group (n = 4), a group treated with omeprazole 20 mg/kg (n = 4), and groups treated with ESRE at doses of 100 mg/kg (n = 4), 200 mg/kg (n = 4) and 400 mg/kg (n = 4). Following a 24 h fasting period, all groups, excluding the negative control group, were orally administered 96% ethanol (5 ml/kg bwt) via oral gavage to induce gastric ulceration, which occurred after 1 h of ethanol administration. After 24 h observation, the test groups received daily oral gavage doses of 1ml omeprazole (20 mg/kg) and ESRE (100, 200 and 400 mg/kg) for a duration of 14 days while the ulcer and negative control groups were given 1ml of the vehicle (normal saline). After the treatment period, the animals were humanely euthanized by cervical dislocation, and their stomachs were quickly removed, incised along the greater curvature, and rinsed with sterile saline. The stomachs were then photographed while stretched on a flat plate for subsequent analysis of gastric lesions. The gastric samples were then snap-frozen in liquid nitrogen and stored at -80°C until further analysis. A digital camera (10 megapixels 5 × zoom) was employed for photographing the gastric lining, and the ulcerated area was calculated as a proportion of the total stomach area using the formula described by Al-Sayed and El-Naga (2015).

$$\text{Gastric lesion index} = \frac{\text{Gastric lesion area (mm}^2\text{)}}{\text{Total gastric area (mm}^2\text{)}} \times 100$$

To determine the healing potential of ESRE the curative ratio was calculated using the formula:

$$\text{Curative ratio} = \frac{[\text{UI control}] - [\text{UI treated}]}{\text{UI Control}} \times 100$$

Histopathological examination

The assessment of pathological effects resulting from ulcer induction followed the methodology outlined in the sixth edition of Hill et al. (1996). Upon sacrificing the animals, stomach specimens were collected from the ulcer control group and the treated groups. These specimens were fixed in 10% formalin at room temperature for 24 h. The process was followed by washing with distilled water then dehydration using serial dilutions of alcohol (70%, 90% and absolute ethyl). Subsequently, the specimens were cleared in xylene and embedded in paraffin at 56°C in a hot air oven for 24 h. Microtome blades were then used to slice the sections into 4 µm thick sections. The tissue sections were collected on glass slides, deparaffinized, stained with hematoxylin and eosin dye then examined using a light electric microscope. Histological changes between the treated groups and the control group were examined under a light microscope. After examination, photomicrographs of stomach sections for all groups were captured using PixelPro software on a general-purpose laptop at a magnification of ×10.

Measurement of superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA) and glutathione (GSH) level activity

Frozen glandular stomach tissues were crushed in liquid nitrogen using a mortar and pestle. Approximately 100 mg of the resulting ground tissues were then homogenized using a 1 mL of extraction reagent (provided in assay kits) using a homogenizer. The homogenates were then centrifuged for 10 min at 4°C at 8000 rpm, and the resulting supernatant was used for assessing SOD, CAT and MDA activity. The activities of SOD, CAT and MDA were determined using (SOD Catalog no: BC0175, CAT Catalog no: BC0205, and MDA Catalog no: BC0025, all from Solar bio Company), following the manufacturer's protocols. To assay for GSH activity, ground gastric mucosa was washed twice in PBS. The washed tissues were then homogenized in 1 mL of Extraction reagent, followed by centrifugation for 10 min at 4°C at 8000 rpm. The resulting supernatant used to assay for GSH activity using GSH assay (Catalog no.: BC1175, Solar bio Company), following the manufacturer's instructions. A microplate spectrophotometer was used to measure absorbance at specific wavelengths: 560nm for SOD, 240 nm for CAT, 600 nm for MDA and 412 nm for GSH. The antioxidant enzyme activities were then compared to the weight of wet gastric tissue.

Statistical analysis

Statistical analysis for numerical data was done using one-way ANOVA in R-program 4.0.4. Mean comparison were conducted using Tukey HSD multiple-comparison test, where p-values (p≤0.05) were utilized to define the confidence interval. Graphical representation of the data was conducted using Origin Pro software, and all data are presented as mean ± standard error mean (SEM).

RESULTS

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

ESRE demonstrated *in vitro* DPPH radical scavenging

Table 1. *In vitro* antioxidant activity of ESRE.

Concentration (µg/ml)	Treatments		P-value
	<i>E. saculeuxii</i>	L-Ascorbic acid	
3.906	48.54 ± 0.51	59.148 ± 0.82	0.07
7.8125	62.321 ± 0.6	66.602 ± 0.37	-
15.625	63.855 ± 3.52	70.048 ± 1.83	-
31.25	65.231 ± 5.04	74.814 ± 0.71	-
62.5	68.904 ± 1.56	78.289 ± 0.45	-
125	72.468 ± 2.06	83.769 ± 0.04	-
250	78.745 ± 0.38	86.917 ± 0.04	-
500	79.982 ± 1.4	89.813 ± 0.03	-
IC ₅₀	260.4	207.4	-

Every experiment was run three times. Data are expressed as mean ± S.E.M (n = 3, p < 0.05) for all tested concentrations.

activities in a dose-dependent trend (Table 1). When compared to the DPPH radical scavenging activities of the plant extract, the standard (L-Ascorbic acid) performed better. The IC₅₀ of ESRE was 260.374 while the IC₅₀ of L-Ascorbic acid (standard) was 207.398 µg/mL.

***In vitro* gastro protective effect of ESRE**

Preliminary antacid test

Higher pH values of 1.21 ± 0.01 were detected in preliminary antacid tests of plant extracts compared to the negative control (1.14 ± 0.02) however these values were lower when compared to the standard (1.29 ± 0.03) even though not statistically significant (Table 2).

Neutralizing effect

The results obtained envisage that ESRE at 100mg demonstrated a remarkable neutralizing effect by increasing the artificial gastric juice's pH from pH 1.2 to 1.46 ± 0.061. This showed that ESRE has a better neutralizing effect than the negative control, which showed a final pH of 1.37 ± 0.005 and can also be considered as a better response, since it was almost comparable with that of Standard sodium bicarbonate (1.52 ± 0.088) (Table 3).

Acid buffering capacity

Table 4 shows the plant extracts and the standard solution of sodium bicarbonate and 1% ethanol's buffering capacity. As observed, ESRE exhibited higher acid buffering capacity of 0.121 ± 0.001 as compared to that of the standard (0.112 ± 0.004). This is in line with the

findings of the Tukey HSD post hoc test, which demonstrated that there was no significant difference between the ESRE buffering capacity and sodium bicarbonate. Thus, it can be inferred that the ethanol extract of ESRE has the same level of gastric acid buffering capacity as sodium bicarbonate.

ESRE gastro protective effect against ethanol-induced gastric lesions

Administration of ethanol led to hemorrhagic lesions that were visible as elongated bands running parallel to the glandular stomach's long axis as shown in Figure 1. The ulcer index and percentage protection exhibited by ESRE on ethanol induced ulceration is presented in Table 5. In the ulcer control group, ulcer induction resulted in 19.24 ± 4.35 mm² gastric lesion. In the animals treated with extracts, there was a noticeable inhibition in the amount of ulceration. The ESRE at the dose of 100 mg/kg showed less gastric protection (59.65 ± 13.89% inhibition) against ulceration, with an ulcer index of 9.17 ± 2.85 mm² as compared to Omeprazole (20 mg/kg) and 200 mg/kg ESRE which demonstrated gastric protection (63.86 ± 7.56 and 67.28 ± 15.08% inhibition) against ulceration with ulcer index of 8.62 ± 2.09 and 5.71 ± 2.22 mm², respectively. ESRE at the dose of 400 mg/kg demonstrated the highest gastric protection (80.82 ± 5.13% inhibition) with an ulcer index of 4.8 ± 1.55 mm² when compared to the ulcer control group as well as the other groups. There was a significant reduction in the ulcerated region in the groups treated with ESRE at the dosages of 200 and 400 mg/kg as compared to the ulcer control group. These results demonstrated strong antagastritis activity of the plant and the activity was dose dependent. There were no stomach ulcers in the health control group. Severe hemorrhagic lesions in the stomach

Table 2. Preliminary antacid test results of negative and positive controls, and ESRE.

Sample	Initial pH	Final pH
Plant extract	5.44	1.21±0.01
Sodium bicarbonate	8.84	1.29±0.03
1% Ethanol	6.35	1.14±0.02*

Values are presented in triplicates; Mean ± S.E.M (Standard Error of Mean); *= Significant different at $p>0.05$. Negative Control (1% Ethanol). At the 0.05 level, the mean difference is significant.

Table 3. Acid neutralizing effect of gastric juice of the negative and positive controls, and ESRE.

Sample	pH	% Acid Neutralized
Negative control	1.37 ± 0.005	14.17 ± 0.481
Sodium bicarbonate	1.52 ± 0.088	26.94 ± 7.365
Plant extract	1.46 ± 0.061	21.67 ± 5.092

Values are expressed as a mean ± SEM of triplicate set up ($p>0.05$).

Table 4. Acid buffering capacity of positive control and ESRE (1 mg/MI).

Sample	Initial pH	Volume of acid consumed	Acid buffering capacity	Time
Sodium bicarbonate	8.96 ± 0.06	24.83 ± 2.17	0.112 ± 0.004	49.67 ± 4.33
Plant extract	5.86 ± 0.15	25.67 ± 2.77	0.121 ± 0.001	51.33 ± 5.55

Values are presented in triplicates; Mean ± S.E.M (Standard Error of Mean); ($p>0.05$). By dividing the titratable alkalinity (mmol H+) by the overall change in pH units, the acid buffering capacity was calculated.

mucosa were seen in the ulcer control group. Pre-treatment with ESRE at doses of 100, 200, and 400 mg/kg resulted in protection against ethanol-induced ulceration. Pre-treatment with omeprazole (20 mg/kg) protected against ulceration due to ethanol administration.

Effect of ESRE on ethanol-induced gastric histopathological findings in gastro-protective model

Histopathological aberrations in gastric tissues of the different experimental groups are presented in Figure 2. Gastric tissues from normal control group:

A) showed normal histological structures of the mucosa (M) including intact glandular structures and intraglandular connective tissues, intact submucosa and muscularis externa. Severe focal ulceration in the mucosal layer was caused by ethanol administration, with hemorrhage and

severe necrosis in the submucosal layer. Furthermore, the submucosa of the ulcer group.

(B) has been shown to have edema, infiltration of IgE cells and congested blood vessels. Pretreatment with omeprazole (F) resulted in necrosis and moderate focal ulceration, with edema and inflammatory cells infiltration in the underlying submucosa. Pre-treatment with the lower dose.

(C) ESRE demonstrated a lower protection compared to the omeprazole treated group since severe focal ulceration was observed in the mucosal area as well as several necrotic cells and hemorrhagic patches in the submucosa and widespread edema. Pre-treatment with ESRE 200 mg/kg.

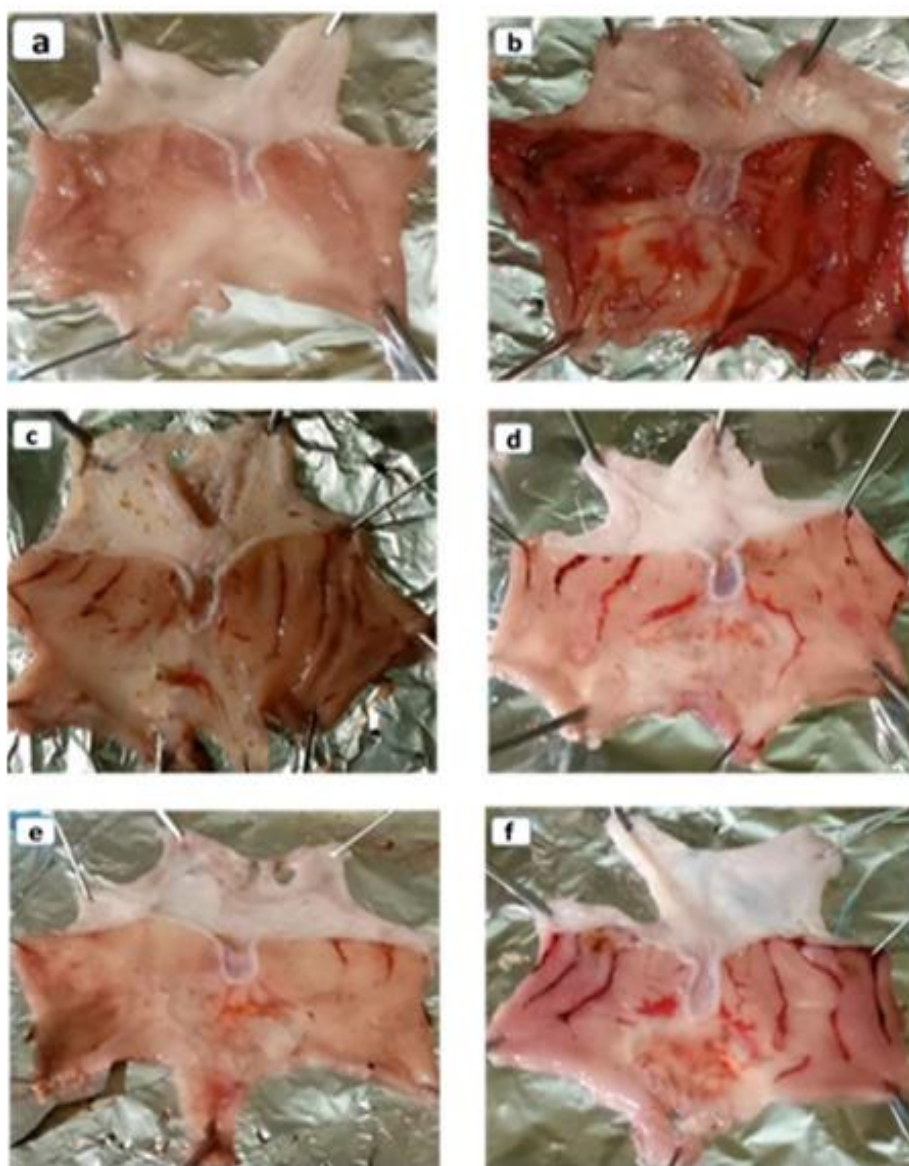
(D) resulted in comparable results as those for omeprazole pre-treated group. Notably, ESRE 400 mg/kg .

(E) pre-treatment demonstrated significant protection from ethanol-induction as evidenced by the intact mucosal layer and glandular elements, absence of necrosis and

Table 5. Gastro protective effect of ESRE on ethanol-induced gastric ulcers.

Treated Groups	Total stomach area (mm ²)	Ulcerated area (mm ²)	Ulcer Index	Protection ratio (%)
Ulcer control	3063.76 ± 147	573.71 ± 4.35	19.24 ± 4.35	-
OMP (20 mg/kg)	2571.88 ± 253.5	207.35 ± 2.09	8.62 ± 2.09	63.86 ± 7.56
ESRE (100 mg/kg)	2437.18 ± 134.1	231.5 ± 2.84	9.17 ± 2.85	59.65 ± 13.89
ESRE (200 mg/kg)	3088.63 ± 191.93	187.73 ± 2.22	5.71 ± 2.22*	67.28 ± 15.08
ESRE (400 mg/kg)	2434.91 ± 154.78	110.04 ± 1.55	4.8 ± 1.55*	80.82 ± 5.13
P-value	-	-	0.0168	0.589

Values are presented as Mean ± SEM. n=4 rats/group, p>0.05. *Significant different from the ulcer control group. After ulcer induction, rats were treated once a day for 14 days with ESRE 100 mg/kg, ESRE 200 mg/kg, ESRE 400 mg/kg and Omp 20 mg/kg.

**Figure 1.** Macroscopic images of gross gastric mucosa evaluation; (a) normal control; (b) ulcer control; (c) 100 mg/kg; (d) 200 mg/kg; (e) 400 mg/kg; (f) omeprazole 20 mg/kg.

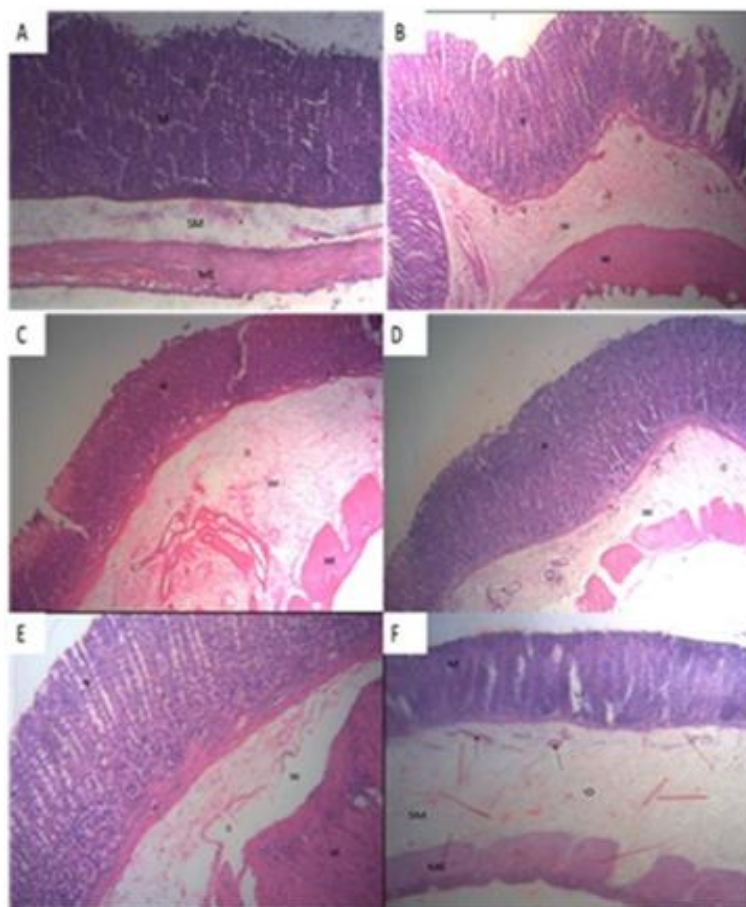


Figure 1. Histological evaluation of stomach sections of rats (A) normal control; (B) ulcer control; (C) 100 mg/kg; (D) 200 mg/kg; (E) 400 mg/kg; (F) omeprazole 20 mg/kg at (magnification $\times 10$). O- Edema; Black arrow- hemorrhagic patches; U- Ulceration; Red arrow- necrotic cells; M-Mucosa; SM-submucosa; ME-muscularis externa

hemorrhage. Furthermore, there was less submucosal edema and inflammatory cell infiltration.

Effect of ESRE on oxidative stress markers

Compared to the normal control group, a significant decrease in GSH levels by six-folds was observed when treated with absolute ethanol. By comparison, the GSH ulcer group's level of GSH was significantly lower than that of the pre-treated groups with ESRE (100 and 200 mg/kg), in a dose-dependent manner (5.1 and 6.2 fold, respectively). The high dose group (400 mg/kg) increased the GSH levels higher than the normal group (Table 6). A no significant reduction in SOD and CAT levels of 19.79 and 54.97% respectively was recorded for the absolute ethanol treated group compared to the normal control

group. Notably, pre-treatment with ESRE (100 and 200 mg/kg) induced a marked increase in CAT levels by 4.79 and 66.39%, respectively, in comparison to the ethanol treated group. Pre-treatment with 400 mg/kg ESRE resulted in a significant increase in CAT levels of 177.16% when compared to the ethanol-treated group. Pre-treatment with ESRE at 100, 200 and 400 mg/kg caused a rise in the SOD levels of 7.3, 17.63 and 26% as compared to the ethanol treated group, however this rise was not statistically significant (Table 7).

In contrast to the other oxidative markers, the MDA level was found to be significantly higher in the group that received ethanol treatment by 80.37%, when compared with the normal control group. However, this rise in the level of MDA was attenuated by pre-treatment with ESRE. Notably, pre-treatment with ESRE at 100, 200 and 400 mg/kg resulted in a significant reduction in the MDA

Table 6. Effect of ESRE pre-treatment on oxidative markers in Wistar rats.

Experimental Groups	CAT (U/g tissue)	Increase (%)	GSH (ug/g tissue)	Increase (folds)	MDA (nmol/g tissue)	Decrease (%)	SOD (u/g weight)	Increase (%)
Normal control	105.8 ± 34.46	-	425.25 ± 33.18	-	12.53 ± 0.8	-	14 ± 2.65	-
Ulcer control	47.64 ± 20.89	-	70.18 ± 9.53 b	-	22.6 ± 0.86 ^b	-	11.23 ± 2.41	-
OMP (20mg/kg)	109.06 ± 11.54	129.01	427.15 ± 71.92 ^a	6.1	13.95 ± 1.67 ^a	38.27	12.75 ± 2.39	13.54
ESRE (100 mg/kg)	49.92 ± 9.39	4.79	360.66 ± 25.20 ^a	5.1	14 ± 1.34 ^a	38.05	12.05 ± 1.74	7.3
ESRE (200 mg/kg)	79.27 ± 0.48	66.39	437.23 ± 72.26 ^a	6.2	13.02 ± 1.19 ^a	42.39	13.21 ± 1.64	17.63
ESRE (400 mg/kg)	132.04 ± 9.62 ^{ad}	177.16	468.96 ± 43.19 ^a	6.7	10.43 ± 0.17 ^a	58.85	14.15 ± 1.07	26

The data are presented as means ± SEM (n = 4). CAT- Catalase; GSH- reduced glutathione; MDA- malondialdehyde; SOD- superoxide dismutase. Superscripts represent; a: Significantly different from the ethanol treated group at p < 0.05, b: Significantly different from the normal control group at p < 0.05.

Table 7. Healing effect of ESRE on ethanol-induced gastric ulcers.

Treated Groups	Total stomach area (mm ²)	Ulcerated area (mm ²)	Ulcer Index	Curative ratio (%)
Normal Control	-	0	0	0
Ulcer Control	3176.75 ± 170.95	823.69 ± 122.25	26.63 ± 5.07	0
OMP (20 mg/kg)	3459.6 ± 551.17	414.06 ± 15.44	13.18 ± 2.53 ^a	50.52 ± 9.52
ESRE (100 mg/kg)	2718.08 ± 121.55	584.91 ± 4.18	21.66 ± 1.03	18.68 ± 3.88 ^c
ESRE (200 mg/kg)	2216.981 ± 554.04	220.25 ± 73.71	9.16 ± 1.04 ^{ad}	65.62 ± 3.9 ^d
ESRE (400 mg/kg)	3271.58 ± 62.91	78.61 ± 3.61	3.61 ± 0.06 ^{ad}	91.04 ± 0.24 ^{cde}

Values are presented as Mean ± Standard Error of the mean (S.E.M). n=4 rats/group, p>0.05. Rats were treated once a day for 14 days with ESRE 100 mg/kg, ESRE 200 mg/kg, ESRE 400 mg/kg and OMP 20 mg/kg following ulcer induction with absolute ethanol. The superscript represents: (a) Significantly different from the ethanol treated group at p < 0.05, (b) Significantly different from the normal control group at p < 0.05, (c) Significantly different from the omeprazole group at p < 0.05, (d) Significantly different from the corresponding group treated with ESRE 100 mg/kg at p < 0.05, e: Significantly different from the corresponding group treated with ESRE 200 mg/kg at p < 0.05.

levels of 38.05, 42.39 and 58.85%, as compared to the ethanol treated group (Table 7). Pretreatment with omeprazole markedly elevated GSH by 6.1

folds, however it increased the levels of CAT and SOD by 129.01 and 13.54%, respectively, when compared to the ethanol treated group; although,

this increase was not statistically significant. While omeprazole (OMP) pretreatment significantly decreased MDA levels by 38.27% in comparison

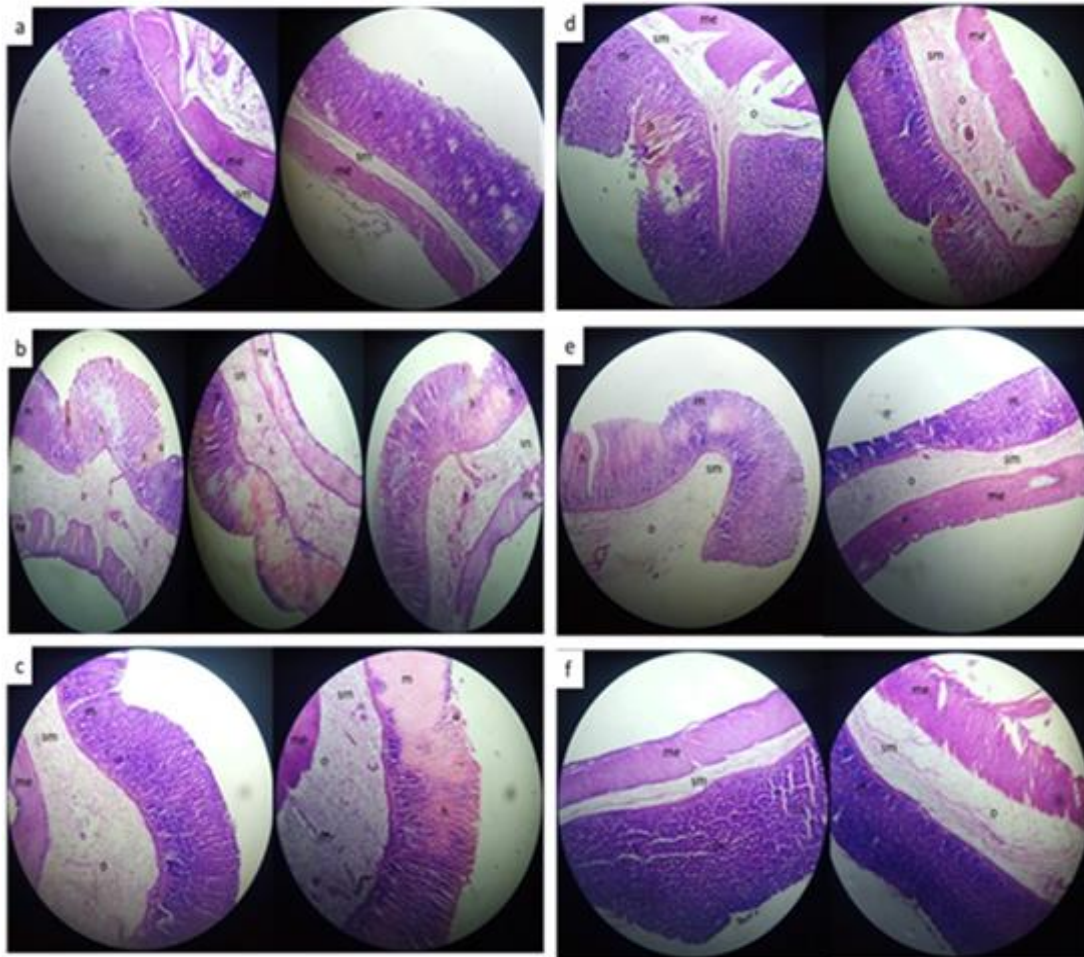


Figure 3. Histological evaluation of stomach sections of rats; (a) normal control; (b) ulcer control; (c) omeprazole 20 mg/kg; (d) 100 mg/kg; (e) 200 mg/kg; (f) 400 mg/kg at (magnification $\times 10$).

with the ethanol treated group (Table 6).

ESRE ulcer healing potential on ethanol-induced gastric lesions

Ethanol administration resulted in moderate ulceration in the glandular stomach which appeared as hemorrhagic patches comparable to inflammation. In the ulcer control group, ulcer induction resulted in $26.63 \pm 5.07 \text{ mm}^2$ gastric lesion. Treatment with ESRE and omeprazole resulted in improvement in the level of ulceration by recording good curative ratios. The ESRE at the dose of 100 mg/kg showed less gastric ulcer healing ($18.68 \pm 3.88\%$ curation), with an ulcer index of $21.66 \pm 1.03 \text{ mm}^2$. Treatment with ESRE 200 mg/kg recorded a $9.16 \pm 1.04 \text{ mm}^2$ ulcer index demonstrating marked gastric ulcer healing ($65.62 \pm 3.9\%$ curation) which was significantly

higher than ESRE 100 mg/kg and comparable to treatment with Omeprazole (Ulcer index- $13.18 \pm 2.53 \text{ mm}^2$ and % curation- 50.52 ± 9.52). ESRE at the dose of 400 mg/kg demonstrated significant ($p < 0.05$) gastric ulcer healing potential ($91.04 \pm 0.24\%$) as compared to all the treatment groups with the lowest ulcer index of $3.61 \pm 0.06 \text{ mm}^2$ when compared to the ulcer control group as well as the other treatment groups (Table 7).

Effect of ESRE on ethanol-induced gastric histopathological findings in ulcer-healing model

Histopathological aberrations in gastric tissues of the different experimental groups are presented in Figure 3. Gastric tissues from normal control group:

(1) Showed normal histological structures of the mucosa

(m) including intact glandular structures and intraglandular connective tissues, intact submucosa (sm) as well as the muscularis externa (me). The administration of absolute ethanol induced severe focal ulceration (u) in the mucosal layer, with severe necrosis and hemorrhage (h) in the submucosal layer. In addition, edema (o), inflammatory cells infiltration and congested blood vessels were observed in the submucosa of the ulcer group.

(2) Pre-treatment with omeprazole.

(3) Resulted in moderate focal ulceration and necrosis, with edema and inflammatory cells infiltration in the underlying submucosa. Pre-treatment with the lower dose ESRE 100 mg/kg.

(4) Demonstrated a lower protection compared to the omeprazole treated group since severe focal ulceration was observed in the mucosal area as well as several necrotic cells and hemorrhagic patches in the submucosa and widespread edema. Pre-treatment with ESRE 200 mg/kg.

(5) Resulted in comparable results as those for omeprazole pre-treated groups. Notably, the pre-treatment of rats with ESRE 400 mg/kg.

(6) Demonstrated significant protection from ethanol-induction as evidenced by the intact mucosal layer and glandular elements, absence of hemorrhage and necrosis. Furthermore, there was less submucosal edema and inflammatory cell infiltration.

Effect of ESRE on oxidative stress markers in the ulcer healing model

A significant reduction in the GSH levels by 8.87-folds was observed in the group treated with absolute ethanol in comparison with the normal control group (Table 8). This was in contrast the groups treated with ESRE which showed a significant elevation in the level of GSH, in a dose-dependent manner compared to the ulcer group. Treatment with ESRE 100 mg/kg resulted in elevation of GSH levels by 4.85-folds however when compared to the ulcer control group this elevation was not statistically significant. Treatment with ESRE 200 and 400mg/kg resulted in a statistically significant ($p < 0.05$) elevation of the GSH levels by 10.56 and 13.16-folds to levels which were even higher than the normal group. A no significant reduction in SOD and CAT levels by 5 and 43.21% respectively was recorded for the group treated with absolute ethanol in comparison with the normal control group. Notably, treatment with ESRE (100, 200 and 400 mg/kg) induced a marked increase in CAT levels by 29.67, 125.91 and 165.54% respectively in comparison to the ethanol treated group. Treatment with ESRE at 100 mg/kg had no effect on the SOD levels as compared to the ethanol treated group, but treatment with ESRE 200 and 400 mg/kg resulted in a rise in the SOD levels of 1.6 and

5.08%, respectively however this rise was not statistically significant.

In contrast to the other oxidative markers, a significant elevation in the MDA level was observed in the ethanol-treated group by 96.21% when compared with the normal control group. Treatment with ESRE, however, attenuated this elevation in the MDA level. As observed, treatment with ESRE at 100, 200 and 400mg/kg resulted in a significant reduction in the MDA levels of 15.44, 17.37 and 57.92% respectively as compared to the ethanol treated group. Treatment with omeprazole (20 mg/kg) significantly increased GSH levels by 13.78 folds. Also, it increased the levels of CAT and SOD by 59.86 and 14.39%, respectively, when compared to the ethanol treated group although this increase was not statistically significant. While OMP treatment none significantly decreased MDA levels by 31.66% in comparison with the ethanol treated group.

DISCUSSION

Numerous *Erythrina* species have been used in folk medicine to treat many diseases such as inflammation and gastrointestinal disorders. The traditional usage of *E. saculeuxii* suggests that it could possess antibacterial, analgesic, and anti-inflammatory activity (Holetz et al., 2002), suggesting its potential antiulcer activity. The ethanol-induced gastric-ulcer model in rats is commonly used in assessing the activity of antiulcer agents (Boutemine et al., 2018). It is the most commonly used model to induce gastric ulcer because this method is a rapid and easy way to evaluate anti-ulcer activity of any plant extract or active ingredient (Mahmood et al., 2011). It was applied in this study to explore the potential gastroprotective and ulcer healing effect of ESRE. In the gastro-protective model, absolute ethanol administration induced severe hemorrhagic lesions.

This finding was supported by the histopathological examination, where severe focal ulceration, hemorrhage, degeneration and necrosis were detected in the stomach mucosa of the ulcer control group. Also, severe submucosal edema and inflammatory cells infiltration were observed. ESRE pre-treatment significantly decreased the total ulcerated area, in a dose-dependent manner. At the highest dose, ESRE 400 mg/kg provided 80.88% gastroprotection which was significantly higher than that provided by omeprazole (67.28%). This gastro protection was also evidenced by ESRE ameliorating the previously described ethanol-induced alterations in stomach histopathology. Similarly, treatment with ESRE and the standard drug Omeprazole exhibited a remedial effect against ethanol-induced gastric ulcer. Notably, the group treated with the highest dose of ESRE showed no trace of gastric ulceration, indicating highest anti-ulcerogenic potential within the study. The dose-dependent decrease

Table 8. Effect of ESRE treatment on oxidative markers in Wistar rats.

Experimental Groups	CAT (U/g weight)	Increase (%)	GSH (μ g/g tissue)	Increase (folds)	MDA (nmol/g weight)	Decrease (%)	SOD (U/g weight)	Increase (%)
Normal Control	47.77 $\pm$ 12.33	43.21	249.12 $\pm$ 11.78	8.87	1.32 $\pm$ 0.47	96.21	11.19 $\pm$ 0.47	5
Ulcer Control	27.13 $\pm$ 5.36	-	28.07 $\pm$ 4 ^b	-	2.59 $\pm$ 1.12	-	10.63 $\pm$ 0.09	-
OMP 20 mg/kg	43.37 $\pm$ 10.5	59.86	386.76 $\pm$ 12.59 ^{ba}	13.78	1.77 $\pm$ 0.16	31.66	12.16 $\pm$ 0.26	14.39
ESRE 100 mg/kg	35.18 $\pm$ 9.28	29.67	136 $\pm$ 55.72 ^c	4.85	2.19 $\pm$ 0.87	15.44	10.37 $\pm$ 0.73	-
ESRE 200 mg/kg	61.29 $\pm$ 31.5	125.91	296.4 $\pm$ 36.19 ^{ad}	10.56	2.14 $\pm$ 0.84	17.37	10.8 $\pm$ 0.51	1.6
ESRE 400 mg/kg	72.04 $\pm$ 7.32	165.54	369.42 $\pm$ 18.59 ^{ad}	13.16	1.09 $\pm$ 0.54	57.92	11.17 $\pm$ 0.56	5.08

Data are expressed as means $\pm$ Standard Error of the Mean (S.E.M) (n = 4). CAT- Catalase; GSH- reduced glutathione; MDA- malondialdehyde; SOD- superoxide dismutase. Superscript represent; a: Significantly different from the ethanol treated group at p < 0.05., b: Significantly different from the normal control group at p < 0.05, c: Significantly different from the omeprazole group at p < 0.05, d: Significantly different from the corresponding group treated with ESRE 100 mg/kg at p < 0.05.

in the total ulcerated area observed in this study aligns with other studies on various plant extracts such as *Glycyrrhiza glabra* (Nugroho et al., 2016), *Aloe vera* (Yusuf et al., 2004), and *Curcumin* spp (Orona-Ortiz et al., 2019) which demonstrated similar dose-dependent effects in reducing gastric ulceration in animal models. The observed gastro-protection and ulcer healing effect is likely associated with the presence of antioxidant components in the plant extract. This finding highlights the promising antiulcer properties of ESRE and underscores its potential as a therapeutic agent for gastric ulcer treatment.

The pathogenesis of ethanol-induced gastric damage is closely linked to the generation of reactive oxygen species (ROS) (Mousa et al., 2019). However, the presence of antioxidants plays a crucial role in defending cells and tissues against oxidative damage (Stahl and Sies, 2003), enhancing cytoprotection in peptic ulcers and promoting the healing of gastric ulcers (Mota et al., 2009). In the current study, the administration of ethanol induced a marked oxidative stress, as

indicated by increased lipid peroxidation (MDA) in gastric mucosa, along with significant reduction of antioxidant capacity in the ulcer group. The levels of GSH, SOD and CAT were significantly depleted as compared to normal control values. Interestingly, pre- and post-treatment with ESRE attenuated the ethanol-induced oxidative stress and lipid peroxidation. At the highest dose of 400 mg/kg, ESRE not only restored GSH, SOD and CAT levels but exceeded their normal values. Additionally, it returned the increased MDA levels to normal, confirming the potent antioxidant effect of ESRE. In conclusion, the observed gastro-protection and ulcer-healing potential of ESRE could be linked, at least in part, to its phenols' inhibitory effect on lipid peroxidation and oxidative stress.

In the etiology of gastrointestinal disorders oxidative stress plays a role (Mota et al., 2009). The process of gastrointestinal ulceration is supported by an increase in oxidizing agents as well as the accumulation of endogenous antioxidants (Bhattacharyya et al., 2014). From the IC50 value described in the results, it was

observed that ESRE has radical scavenging activity for DPPH but not statistically significant when compared to ascorbic acid. ESRE lacks antacid potential but has profound neutralizing effects. A Tukey HSD post hoc test showed no significant difference in the buffering capacity of ESRE and sodium bicarbonate.

Conclusion

This study is the first one to provide evidence that ESRE exhibits strong gastro-protection and a potent ulcer-healing effect against ethanol-induced gastric ulcer. This effect was evident through a reduction in the ulcerated area, attenuation of ethanol-induced oxidative stress and the preservation of an intact mucosal layer, with the absence of hemorrhage and necrosis in the gastric tissues. The noteworthy ulcer-healing effect of ESRE is likely attributed partially by its capacity to alleviate ethanol-induced oxidative stress. ESRE demonstrated the ability to enhance the cellular

antioxidant defense mechanism, thereby reducing the elevated tissue levels of MDA induced by ethanol administration. These findings underscore the potential therapeutic value of ESRE in treating gastric ulcers and highlight its role in mitigating oxidative stress associated with this condition. *In vitro* antacids test demonstrated that ESRE lacks the potential of being an antacids however remarkable DPPH radical scavenging activity presented suggests that ESRE has potential to protect the gastric mucosa, supported by the oxidative enzyme assay. Artificial gastric juices were prepared in this study for *in vitro* antacids tests; it is recommended that gastric juices extracted from experimental animals be used to further expound on the neutralizing effect and acid buffering capacity of ESRE.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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