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(RESEARCH ARTICLE)

Protective action of ethanol extract of *Rinorea subintegrifolia* leaves and roots on gastric ulceration in Wistar rats

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Abstract

Medicinal plants rich in bioactive compounds have gained attention for their gastroprotective potential. This study evaluated the protective functions of ethanol extracts of *Rinorea subintegrifolia* leaves and roots against experimentally induced gastric ulceration in Wistar rats. Gastric ulcers were induced using ethanol and pylori ligation models of ulcer induction. Wistar rats were randomly divided into five groups, each comprising five rats. Group I received 5 ml/kg of normal saline (control), Group II was pretreated with 30 mg/kg of Ranitidine, Group III received 250 mg/kg of ELRS pretreatment, Group IV received 250 mg/kg of HRRS pretreatment and Group V received 250 mg/kg of ELRS +250 mg/kg of HRRS pretreatment. Following 14 days of pretreatment, gastric ulcers were induced and the stomachs excised for evaluation. Gastric tissues were assessed for ulcer index, gastric secretory parameters, and histopathological alterations. Pretreatment with *Rinorea. subintegrifolia* leaf and root extracts significantly reduced ulcer index and gastric acidity. Histopathological examination revealed marked preservation of gastric mucosal architecture in extract-treated groups, with reduced inflammatory cell infiltration and epithelial erosion. In conclusion, these findings suggest that ethanol extracts of *Rinorea subintegrifolia* leaves and roots possess notable gastroprotective activity, likely mediated through antisecretory, and cytoprotective mechanisms.

Keywords: Gastric Ulcers; *Rinorea Subintegrifolia*; Gastroprotection; Pyloric Ligation; Ulcer Index

1. Introduction

Peptic ulcer disease (PUD) is a prevalent gastrointestinal disorder which is characterized by localized injury to the gastric or duodenal mucosa arising from an imbalance between aggressive factors, such as gastric acid, pepsin, non-steroidal anti-inflammatory drugs (NSAIDs), alcohol, and oxidative stress [1,2], and protective mechanisms which include mucus secretion, bicarbonate production, and adequate mucosal blood flow [3,4]. Despite substantial progress in pharmacological management through proton pump inhibitors, H_2-receptor antagonists, and eradication therapies for Helicobacter pylori, PUD remains a major cause of morbidity worldwide, particularly in low- and middle-income countries where long-term treatment adherence, cost, adverse drug reactions, and relapse following therapy withdrawal remain significant challenges [5, 6].

Experimental animal models play a significant role in understanding the pathophysiology of gastric ulcers and in the preclinical evaluation of antiulcer and gastroprotective agents. The ethanol-induced gastric ulcer model is known to mimic acute mucosal injury caused by direct cytotoxic effects of ethanol, including disruption of the gastric mucus barrier, increased vascular permeability, excessive generation of reactive oxygen species, and lipid peroxidation leading to mucosal necrosis [7, 8]. Pyloric ligation model on the other hand induces gastric ulceration mostly through hypersecretion of gastric acid and pepsin, prolonged retention of gastric contents, and increased intragastric pressure [9], making it particularly useful for assessing anti-secretory and acid-neutralizing properties of test substances [10].

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The combined use of these models provides a comprehensive assessment of both cytoprotective and antisecretory mechanisms involved in ulcer prevention.

Plants are a key source of medicines, and they are frequently utilized to cure a variety of ailments by humans [11] and in recent years, there has been renewed interest in medicinal plants as alternative and complementary therapies for gastric ulcer management [12]. Numerous plant-derived phytochemicals, including flavonoids, tannins, saponins, alkaloids, and phenolic compounds, have been shown to exert antiulcer effects through antioxidant, anti-inflammatory, cytoprotective, and gastric acid inhibitory mechanisms [13]. These natural products are particularly attractive due to their availability, lower cost, and comparative safety profiles when compared with conventional synthetic drugs.

Rinorea subintegrifolia (family: Violaceae) is a tropical plant native to West and Central Africa and is traditionally used in folk medicine for the treatment of various ailments [14]. While phytochemical and antioxidant studies on related *Rinorea* species have demonstrated the presence of bioactive constituents capable of mitigating oxidative stress and inflammation [15, 16], there is a paucity of studies validating the gastroprotective potential of *Rinorea subintegrifolia*. Moreso, no study to date has systematically evaluated the protective effects of its leaf and root extracts using both ethanol-induced and pyloric ligation models of gastric ulceration and compared their effects.

Therefore, this study was designed to investigate the protective action of the ethanol extract of *Rinorea subintegrifolia* leaf and root against experimentally induced gastric ulcers in Wistar rats using ethanol and pyloric ligation models, exploring its potential as a source of novel, plant-based anti-ulcer agents.

2. Materials and method

2.1. Animals

Male Wistar rats weighing between 140-180 g were used in this study. The animals were purchased from the Department of Physiology Animal House, College of Health Sciences, Abia State University Uturu, Abia state, Nigeria and were housed in the animal house of the Department of Physiology, Edo State University, Edo State Nigeria where the experiment was carried out.

2.2. Drugs/Chemicals

Normal saline, Distilled water,10 % formalin, Ketamine, Xylazine,0.1M NAOH, Phenolphthalein, N- Hexane, Methanol, phenolphtaline, NaOH, Na2HPO4 (BDH Chemical Limited, England), NaH2PO4, CuSO4. 5H2O (BDH Chemicals, England), potassium iodide, KI (BDH Chemicals, England) Ethanol (Sigma Chemicals, Perth,Western Australia), Ranitidine (Zantac-GSK) was obtained from a local pharmarcy. All chemicals used in buffers and other solutions were of analytical grade.

2.3. Collection and preparation of plant material

Whole plants of *Rinorea subintegrifolia* including the roots were collected from Ibhiolulu in Edo state, Nigeria. The plant was taken to the Department of Botany, Ambrose Alli University Ekpoma, Edo state, where they were identified and authenticated by a Botanist at the University Herbarium. The leaves and cut pieces of roots were washed and air dried and thereafter grinded into powder and stored for further use.

2.4. Preparation of Extract

The extraction was done separately for the roots and the leaves. Three hundred and thirty-five grams of coarsely powdered root material of *Rinorea subintegrifolia* was extracted with 60 % ethanol using soxhlet apparatus. The extraction was carried out until the extractive becomes colorless. The extract was filtered through a cotton plug, followed by whattman filter paper (no.1). The extract was evaporated under reduced pressure using rotary vaccum evaporator The filterate was concentrated using rotary evaporator and was further dried in the oven set at 40°C for 24hours giving a yield of 10.44 % of a sticky gel like product. The extract was then packed into an amber-coloured bottle and stored at 4°C until required for animal experiments. 105 grams of the coarsely powdered leaves was used for the extraction using the same procedure as described in the root extraction, producing a 33.33% yield.

2.5. Qualitative phytochemical screening

The extract obtained from *Rinorea Subintegrifolia* plant was subjected to phytochemical screening using standard procedures as described by Trease and Evans [17], and Vinay et al. [18].

2.6. Antiulcer experiment

2.6.1. Ethanol-induced gastric ulcer.

Wistar rats were randomly divided into two groups of five animals each. Rats in group one served as the control group and received Normal saline (10 ml/kg). Rats in group two served as pretreatment group and received 250mg/kg of ELRS. Preatment were for a period of fourteen days. After pretreatment, the rats were fasted for 24 hours but had free access to drinking water. Rats in all groups received 1 ml/200 g body weight of absolute ethanol via gastric intubation for the induction of gastric ulcers, 60minuites after the last pretreatment. One hour later, the animals were sacrificed and their stomachs excised. Each stomach was opened along the greater curvature and rinsed with normal saline to remove gastric contents and blood clots. The lengths of the lesions were measured using a vernier caliper and the ulcer index taken as the sum of length of the lesions [19, 20]. The percentage of ulcer inhibition was calculated using the following formula: [(ulcer length (control) − ulcer length (treated))/ulcer length (control)] ×100 % [21, 20].

2.6.2. Pylorus-ligated ulcer technique

This procedure is as described by Gilbert et al[22]. This method was used to investigate if the ethanol extract of *Rinorea subintegrifolia* (ELRS) leaves has neutralizing properties and or antisecretory effects. Eighteen male rats were used in this study and were divided into 3 groups of 6 rats each. Group 1 served as control and was given normal saline (5 ml/kg), group 2 received ranitidine (30 mg/kg)[20] and group 3 received 250 mg/kg of ELRS. The pretreatment lasted for a period of fourteen days. The rats were then fasted for 24 hours but had free access to drinking water. After fasting, rats were anesthetized, the abdomen was incised, and the pylorus was ligated and the abdominal incision was closed. Four hours after the ligation, the animals were sacrificed using cervical dislocation and, the abdomen was opened and another ligature was placed around the esophagus in close proximity to the diaphragm. The stomachs were removed, and the gastric content was collected for determination of the gastric juice volume, pH and total acidity. The stomachs were also assessed for gastric ulcers and percentage inhibition of gastric ulcer was calculated.

2.7. Gastric ulcer determination

The stomach was opened along the greater curvature and ulcers produced were graded according to the method by Kulkarni [23]. The ulcer index for each rat was taken as the mean ulcer score using the following severity scores and multiplying the scores by the number of ulcers developed in each rat: 0.0 = normal colored stomach, 0.5 = red coloration, 1.0 = spot ulcers, 1.5 = hemorrhagic streaks, 2.0 = ulcers with area >3 but ≤5 mm^2, 3.0 = ulcers > 5 mm^2.

2.8. Percentage inhibition of ulcer.

This was determined using the formula below.

$$PI = \frac{UI_c - UI_T}{UI_c} \times 100$$

Where PI is percentage inhibition, UI_C is ulcer index for control group and UI_T is ulcer index for treatment group.

2.9. Determination of gastric content and gastric acidity

The contents of each stomach were collected in centrifuge tubes. The tubes were centrifuged at 8000 rpm for 10 min. The volume of supernatant fluid was measured and the pH measured using a pH meter The supernatant of gastric contents from each pylorus ligated rat was used. 0.2 ml of the supernatant was analyzed for hydrogen ion concentration by titrating against a 0.01 N solution of NaOH using phenolpthlein indicator. The experiment was done in triplicate. Acidity was expressed according to the formular described by Omayone et al. [24].

$$\text{Acidity} = \frac{\text{Vol.of NaOH x Normality x 100}}{\text{0.1 mEq/L}}$$

2.10. Histopathological study

Small sections of stomach tissues were taken from each stomach obtained from the pylorus ligation model were placed in 10% formalin for histological examination using standard procedure [25]

2.10.1. Procedure

The tissues were allowed to fix in 10% formal saline for 48hours. The tissues were grossed and cut into smaller pieces of 3mm thick in prelabelled tissue cassette. Water was removed from the tissue by putting it in ascending concentration of alcohol (70 %, 80 %, 90 %, 95 %, and 100 %) and processed using automatic tissue processor (LEICA TP1020). The tissues were made to go through two changes of xylene and three changes of molten paraffin at 65 degree centigrade. The processing time was 12 hours. The tissues were trimmed to expose tissue surface with microtome and was cooled on ice. The tissues were sectioned at 4microns using rotary microtome (LEICA RT2115). The sections were dried on hot plate and ready for staining using haematoxylin and eosin stain.

2.10.2. Staining

The section was deparaffinized in 2 changes of xylene for 4minutes each so that the stains can permeate. The slides were then immersed in a descending concentration of alcohol (100 %, 95 %, 90 %, 80 % and 70 %) for 1 minute in each alcohol solution so as to dehydrate it. The slides were rinsed in water and placed in Harris Haematoxylin for about 5 minutes. The slides were dipped in 1% acid-alcohol (2 dips) and rinsed in slow running water for about 10minutes to make the colour of the section to become blue. The slides were counterstained in Eosin for about 3minutes and briefly rinsed in water. The slides were again immersed in ascending concentration of alcohol (70 %, 80 %, 90 %, 95 % and 100 %) for about 30 seconds so as to dehydrate the preparation. The preparation was cleared of alcohol by dipping it in xylene for 1 minute. After this, the slides were blotted and mounted under cover slips using dibutylphthalatexylene (DPX) and air bubbles were prevented from entering. Each slide was then observed and analyzed under the microscope using x100 magnification. Also, photomicrograph of the slide preparations was taken.

2.11. Statistical analysis

Data obtained in this study were analyzed using SPSS (version 22) software package. The results were expressed as Mean± SEM and analyzed using one-way analysis of variance, followed by LSD post-hoc test. Results with $P< 0.05$, were considered significant.

3. Results

3.1. Phytochemical constituents of ethanol extract of *Rinorea Subintegrifolia* root and leaf

The result of the qualitative phytochemical screening of both root and leaf extracts of the plant is as shown in Table 1. The root extract of *Rinorea Subintegrifolia* showed the presence of Steroid, Terpenoid and Flavonoid which are present in small amount while reducing sugar and Cardiac Glycoside are present in high amount in the extract. The leaf extract showed the presence of trace amount of Steroid, Terpenoid, Starch and Tanins, moderate amount of flavonoid while Saponin and reducing sugar were in appreciable amount.

Table 1 Phytochemical constituents of ethanol extract of *Rinorea subintegrifolia* root and leaf

Phytochemical	Root extract	Leaf extract
Alkaloid	-	-
Phlobatanin	-	-
Reducing Sugar	+++	+++
Flavonoid	+	++
Cardiac Glycoside	++	-
Starch	-	+
Tannins	-	+
Terpenoid	+	+
Saponin	-	+++
Steroid	+	+

+ = Trace amount present, +++= Appreciable amount - = Absent

3.2. Effect of ethanol extract of R*inorea subintegrifolia* on relative stomach weight.

The result as shown in table 2 shows the effect of ethanol extract of *rinorea subintegrifolia* root and leaf on relative stomach weight. The result showed that ethanol leaf extract of *Rinorea subintegrifolia (ELRS)* significantly reduce relative stomach weight when compared with control (P<0.05)**.** The root extract also significantly reduced the relative stomach weight when compared with control. Coadministration of the root and leaf extracts also reduced the relative stomach weight when compared with the control (P>0.05).

Table 2 Effects of ethanol extracts of *Rinorea subintegrifolia* extracts on relative stomach weight (RSW)

Group	Treatment	RSW
1	Control (5 ml of Normal saline/kg)	0.0089±0.00089
2	Ranitidine(30 mg/kg)	0.0071±0.00036*
3	250 mg/kg ELRS	0.0063±0.00011*
4	250 mg/kg ERRS	0.0076±0.0011*
5	250 mg/kg ELRS + 250 mg/kg ERRS	0.0074±0.00039*

Results are expressed as mean ± standard error of mean. ** Means P<0.05 when compared with control*

3.3. Effects of *Rinorea subintegrifolia* extracts on ethanol- induced gastric ulcer.

The result (Table 3) showed that the ethanolic leaf extract of *Rinorea subintegrifolia s*ignificantly reduced ulcer index when compared with control (P<0.05) while the root extract had no significant effect on ulcer index when compared with control (P>0.05). The leaf extract offered an 87% gastric protection while the root extract offered a 14% protection against gastric ulcers induced by ethanol. Coadministration of the root and leaf extract offered a 91% protection

Table 3 Effects of *Rinorea subintegrifolia extracts* on ulcer index and percentage protection in ethanol induced model

Group	Treatment	Ulcer index	Percentage Protection (%)
1	Ulcer Control	2.29±0.09	
2	250 mg/kg ELRS	1.14±0.04*	87.72
3	250 mg/kg ERRS	1.96±0.06	14.41
4	250 mg/kg ELRS + 250 mg/kg ERRS	0.19±0.03*	91.70

Results are expressed as mean ± standard error of mean. ** Means P<0.05 when compared with control*

3.4. Effect of *Rinorea subintegrifolia* extracts on pyloric ligated - induced gastric ulcer.

The result as shown in table 4 showed that ethanol extract of *Rinorea subintegrifolia* significantly reduced ulcer indices when compared with control (P<0.05). The ethanol leaf extract (ELRS) offered 82.58 % protection. The percentage protection of the root extract (ERRS) against ulcer was 71 %. The combined leaf and root extract offered a 69 % protection against the ulcer. Ranitidine which serves as a standard drug (+) control also significantly reduce ulcer index when compared with control (P<0.05) with a 66% gastroprotection.

Table 4 Effects of *Rinorea subintegrifolia extracts* on ulcer indices and percentage protection in pyloric ligated- induced model

Group	Treatment	Ulcer index	Percentage protection
1	Control (5 ml of Normal saline/kg)	13.20±1.77	
2	Ranitidine(30 mg/kg)	4.40±1.07*	66.67
3	250 mg/kg ELRS	2.30±0.66*#	82.58
4	250 mg/kg ERRS	3.80±0.46*#	71.21
5	250 mg/kg ELRS + 250 mg/kg ERRS	4.00±1.21*	69.70

Results are expressed as mean ± standard error of mean. ** Means P<0.05 when compared with control, # means P<0.05 when compared with Ranitidine.*

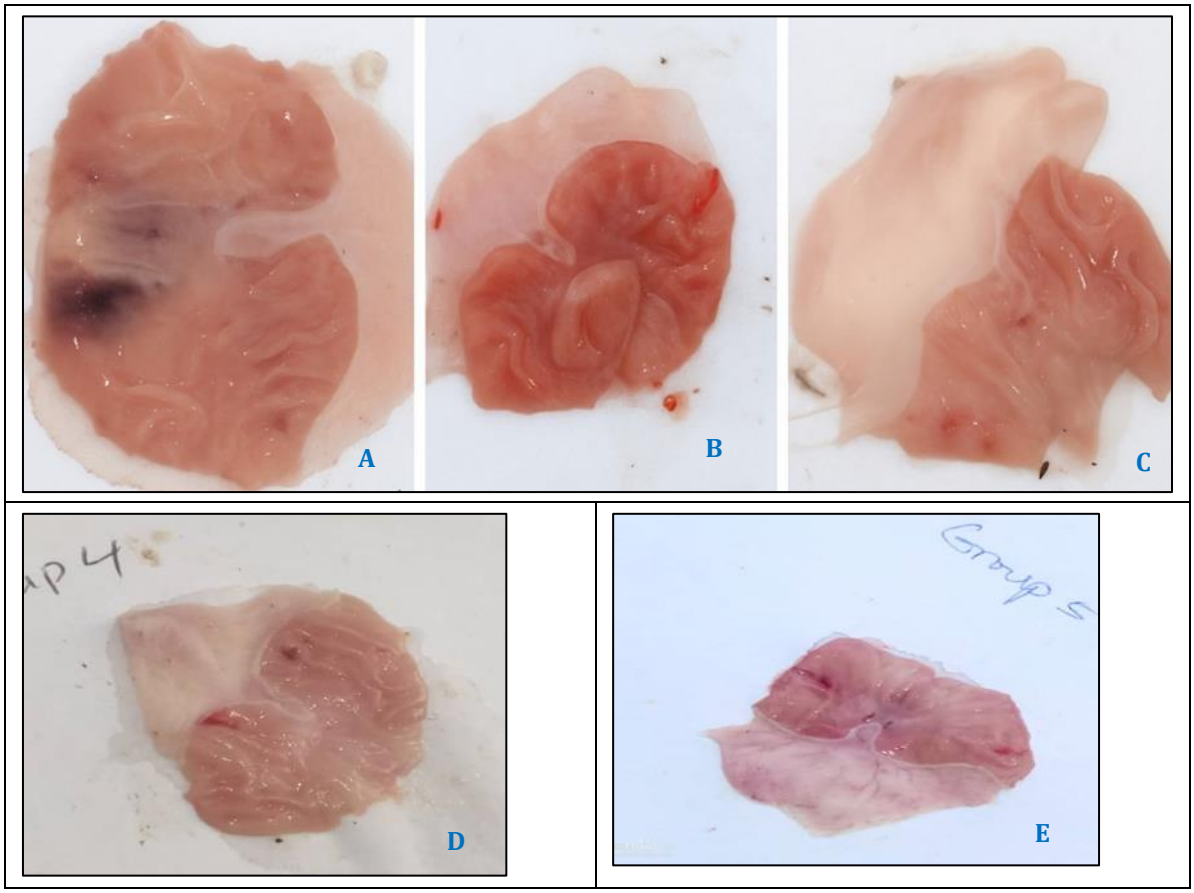

Figure 1 Photomacrograph of the stomach in all treatment groups showing severe ulceration in control (A) and mild ulceration in pretreated groups. A indicates control group, B= ranitidine pretreated group, C = ELRS pretreated group, D=ERRS pretreated group and E=ELRS+ERRS pretreated group

3.5. Effect of ethanol extract of *Rinorea subintegrifolia* on gastric juice volume

As shown in table 5, the ethanol leaf extract and root extract of *Rinorea subintegrifolia* significantly reduce gastric juice volume when compared with control. ($P<0.05$). The combination of both the leaf and root extract had no significant effect on gastric juice volume when compared with control ($P>0.05$). The effects of the various extracts was not significantly different from that of ranitidine ($P>0.05$).

Table 5 Effects of *Rinorea subintegrifolia* on gastric juice volume

Group	Treatment	Volume(ml)
1	Control (5 ml of Normal saline/kg)	1.69±0.64
2	Ranitidine(30 mg/kg)	0.54±0.06*
3	250 mg/kg ELRS	0.36±0.04*
4	250 mg/kg ERRS	0.74±0.16*
5	250 mg/kg ELRS + 250 mg/kg ERRS	0.92±0.12

Results are expressed as mean ± standard error of mean. ** Means P<0.05 when compared with control.*

3.6. Effect of ethanol extract of *Rinorea subintegrifolia* on gastric juice pH

The effect of ethanol extract of *Rinorea subintegrifolia* on gastric juice pH as shown in table 6 shows that ethanol leaf extract and the root extract of *Rinorea subintegrifolia* significantly increase gastric juice pH when compared with the control($P<0.05$). Combining the leaf and root extract also had a slightly significant effect in the pH when compared to the effect of the extracts when given separately. The effect of the standard drug, ranitidine was significantly higher than the effects of the extracts ($P<0.05$)

Table 6 Effect of *Rinorea subintegrifolia* on gastric juice pH

Group	Treatment	pH
1	Control (5 ml of Normal saline/kg)	2.33±0.54
2	Ranitidine(30 mg/kg)	6.18±0.24*
3	250 mg/kg ELRS	4.82±0.02*#
4	250 mg/kg ERRS	4.43±0.35*#
5	250 mg/kg ELRS + 250 mg/kg ERRS	4.23±0.04*#

Results are expressed as mean ± standard error of mean. * Means P<0.05 when compared with control, # means P<0.05 when compared with Ranitidine.

3.7. Effects of ethanol extract of *rinorea subintegrifolia* on total titrable acidity of gastric juice.

The result (table 7) shows that the ethanolic leaf extract of *Rinorea subintegrifolia* significantly reduce total acidity of gastric juice when compared with ulcer control(P<0.05). This was also the trend in the ethanolic root extract of Rinorea subintegrifolia as it also significantly reduces total acidity of gastric juice when compared with control. (P< 0.05). The combined extract of the leaf and root also significantly reduce total acidity of gastric juice (P<0.05). The effect of the standard drug(ranitidine) was not significantly different from that of ELRS.

Table 7 Effects of *Rinorea subintegrifolia* on total acidity of gastric juice

Group	Treatment	Total acidity(mEq/l)
1	Control (5 ml of Normal saline/kg)	12.33±1.24
2	Ranitidine(30 mg/kg)	4.92±1.06*
3	250 mg/kg ELRS	5.84±0.59*
4	250 mg/kg ERRS	7.10±0.13*#
5	250 mg/kg ELRS + 250 mg/kg ERRS	8.99±0.26*#

Results are expressed as mean ± standard error of mean. * Means P<0.05 when compared with control, # means P<0.05 when compared with Ranitidine.

3.8. Histopathological findings

The histopathological findings as shown in the photomicrographs (Figure 2) showed different alterations to the histology of the stomach tissues. In the control group, the stomach tissue of the animals showed degeneration of the stomach lining with severe ulceration (U), moderate granulated tissue slough (GTS) and chronic inflammatory cell(CIC). In the ELRS group, the stomach tissue of the animals exhibited moderate aggregate of inflammatory cells (IC) within the sub mucosa. The ranitidine group showed mild ulceration (U).

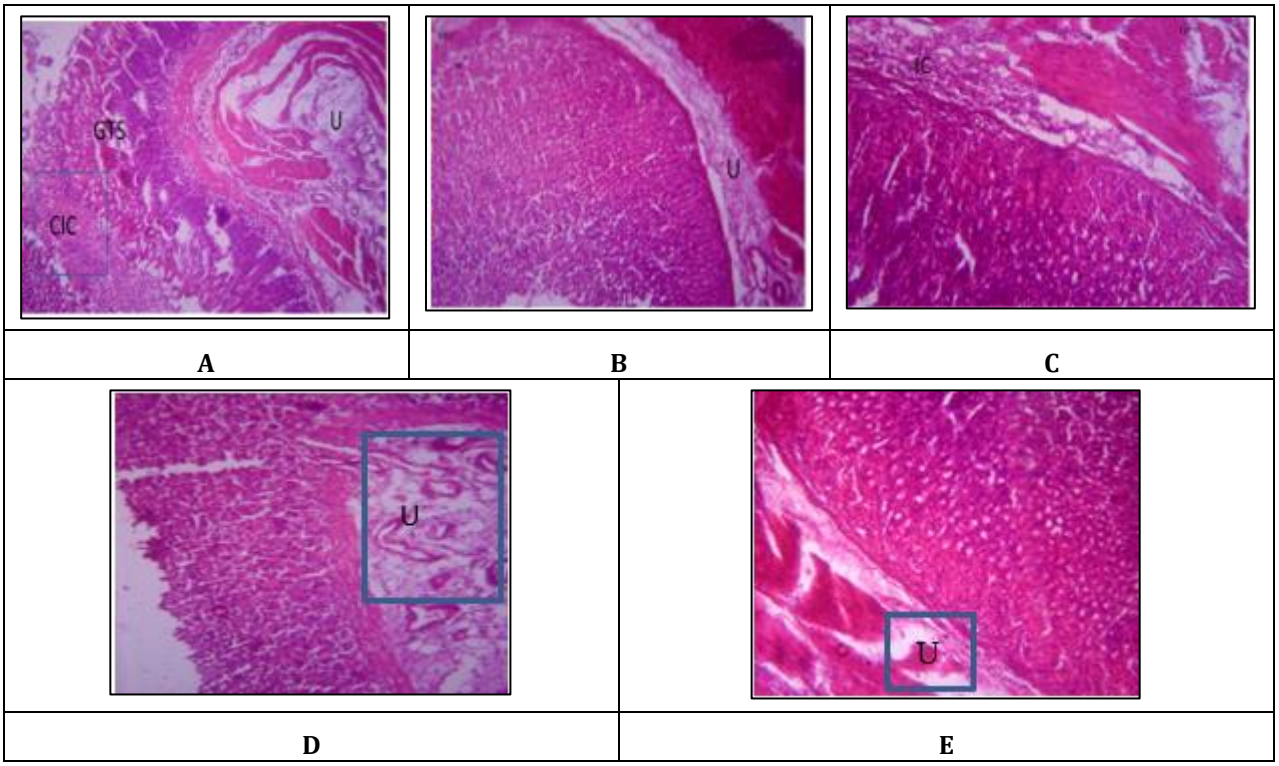

Figure 2 Photomicrograph of the stomach, showing the effect of ELRS on stomach tissue of rat in pyloric ligated ulcer model. Stomach histopathology showed micrographs of stomach sections (H and E; x150). A = Control group, B = Ranitidine pretreated group, C= ELRS pretreated group, D= ERRS, and E= ELRS+ERRS

4. Discussion

4.1. Phytochemical Constituents in *Rinorea subintegrifolia*

Phytochemical evaluation of *Rinorea subintegrifolia* revealed the presence of flavonoids, tannins, terpenoids, saponins, and steroids, with these bioactive constituents occurring in higher concentrations in the leaves than in the roots. These compounds have long been recognized for their therapeutic relevance due to their diverse biological and pharmacological activities. Phenolic compounds are known to exert cytoprotective and antiulcerogenic effects while flavonoids protect the gastric mucosa by enhancing prostaglandin synthesis, inhibiting histamine release, suppressing histidine decarboxylase activity, and scavenging free radicals [26]. Saponins and triterpenoids contribute to ulcer protection by promoting mucus formation on the gastric mucosa and reducing acid-induced injury through prostaglandin modulation, whereas tannins act by precipitating proteins at ulcer sites to form a protective barrier that limits the penetration of toxic substances and enzymatic degradation of the gastric lining [27, 28]. Thus, the phytochemical profile observed in this study corroborates previous reports and supports the medicinal potential of *Rinorea subintegrifolia* in gastric ulcer management.

4.2. Effects of *Rinorea subintegrifolia* on ulcer indices and percentage protection in ethanol induced model and relative stomach weight.

Relative stomach weight serves as a reliable marker of gastric inflammation, as increases often reflect edema, cellular infiltration, and tissue damage. The significantly elevated relative stomach weight observed in the control group indicates a severe inflammatory response following ulcer induction, whereas the comparatively lower values in the extract-treated groups suggest attenuation of inflammation. This reduction may be attributed to the anti-inflammatory and cytoprotective effects of the extract, limiting tissue swelling and pathological changes. These findings are in agreement with earlier studies reporting increased relative organ weight in pathological tissues due to inflammatory processes [29].

The damaging effects of ethanol have been exploited in developing the ethanol model of peptic ulcers. The model is independent of gastric acid secretion and resembles acute peptic ulcers in humans [30]. Ethanol induced ulcer model

is useful for studying the efficacy of potential drugs or testing agents that have cytoprotective and/or antioxidant activities.

Result of this study showed that ethanol leaves and the coadministration of the extract protected against gastric ulcer in ethanol model. This protection might be due to saponin, flavonoids and phenols present in the extract and hence the ability of the extract to reduce gastric acid parameters such as total acidity and gastric ulcer. Tannins which is present in the leaf extract are known to prevent ulcers because of their vasoconstricting and astringent effects. The latter facilitates the microproteins' precipitation on the ulcer site, forming a layer over the lining that prevents gastric secretions and protects the mucosa from harmful substances and elements [31]. This finding is in

4.3. Effects of Rinorea subintegrifolia on ulcer indices and percentage protection in pyloric ligated- induced model.

Peptic ulcers arise when excessive gastric acid overwhelms the protective mechanisms of the gastrointestinal mucosa, leading to erosion of the gastric lining. While gastric acid is essential for digestion, mineral absorption, and control of bacterial overgrowth, its hypersecretion predisposes the stomach to acid-related disorders such as peptic ulcer disease [32]. The pylorus ligation model, which reliably induces gastric ulceration through stress-mediated hypersecretion and prolonged stasis of acidic gastric contents, remains a valuable tool for evaluating agents that modulate gastric secretion and mucosal defense [33]. In the present study, pyloric ligation produced marked gastric ulceration, whereas pretreatment with ethanolic extracts of *Rinorea subintegrifolia* significantly reduced ulcer severity. Both the leaf and root extracts conferred notable gastroprotection, as reflected by reduced ulcer indices; however, the leaf extract demonstrated superior efficacy, providing 82% protection compared to 71% for the root extract and 66% for ranitidine. Although co-administration of the extracts did not enhance protection beyond that of the individual treatments, it still attenuated ulcer formation. Overall, the greater gastroprotective effect observed with *Rinorea subintegrifolia* extracts relative to ranitidine highlights their strong potential in protecting the gastric mucosa against acid-induced injury.

4.4. Effects of *Rinorea subintegrifolia* on gastric juice secretion(gastric juice volume, pH and total acidity)

The prevention and management of acid-related disorders largely depend on reducing gastric acidity and/or strengthening mucosal defense mechanisms. Suppression of gastric acid secretion through antisecretory agents therefore remains a central strategy in the treatment of acid-related diseases [34]. In the present study, pylorus-ligated control rats exhibited significantly higher gastric juice volume and total acidity, alongside a significantly lower gastric pH, compared with pretreated groups, confirming that pylorus ligation induces hypersecretion and hyperacidity. These findings are consistent with previous reports indicating that increased gastric juice volume during hypersecretion accelerates digestion of the gastric mucosa, thereby predisposing to ulcer formation [35].

Pretreatment with the ethanolic leaf and root extracts of *Rinorea subintegrifolia* significantly reduced gastric juice volume relative to the control group, indicating suppression of gastric secretory activity. This reduction suggests a concomitant decrease in gastric acid output, an effect comparable to that produced by ranitidine, as no significant difference was observed between the extract-treated groups and the positive control. In addition, both extracts significantly increased gastric juice pH and reduced total acidity, effects that are critical to mucosal protection, since total acidity reflects the concentration of hydrogen ions and elevated values are indicative of hyperacidity [36]. Previous studies have demonstrated that inhibition of gastric secretion protects the gastroduodenal mucosa against pylorus ligation-induced injury and that reductions in gastric juice volume are closely associated with decreases in total acidity and ulcer severity [31, 37].

Notably, the ethanolic leaf extract exerted a more pronounced antisecretory effect than the root extract, as evidenced by a greater reduction in gastric juice volume, a higher gastric pH, and a more significant decrease in total acidity. These findings suggest that the gastroprotective activity of *Rinorea subintegrifolia* is mediated, at least in part, through inhibition of gastric acid secretion, elevation of gastric pH, and attenuation of gastric hyperacidity, thereby limiting acid-induced mucosal injury.

4.5. Effects of *Rinorea subintegrifolia* on Histopathology of the stomach

Histopathological examination of the stomach revealed marked ulceration of the surface epithelium in the ulcer. The underlying stroma appeared thin and fibrocollagenous, with prominent infiltration by chronic mononuclear inflammatory cells, mainly lymphocytes. In contrast, these pathological changes were less pronounced in the extract-pretreated groups, which exhibited only mild to moderate ulceration of the mucosal surface epithelium. In these groups, the stromal tissue showed mild infiltration of mononuclear inflammatory cells, predominantly lymphocytes, with

histological features that were largely comparable to those observed in normal gastric tissue.These features are consistent with the results from the macroscopic study and supports the biochemical findings in this study.

5. Conclusion

This study shows that ethanol leaf and root extracts of *Rinorea subintegrifolia* exhibit gastroprotective effects against experimentally induced gastric ulceration in rats. These effects were evidenced by reductions in ulcer index, relative stomach weight, gastric juice volume, and total acidity, alongside increased gastric pH and improved histopathological features.The leaf extract showed greater protective activity than the root extract and produced effects comparable to ranitidine. These findings suggest a potential role for *Rinorea subintegrifolia* in gastric mucosal protection.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

We declare no conflict of interest.

Statement of ethical approval

Ethical approval was obtained for this study from the Institutional Animal Ethics Committee with clearance number EDSUREC25/130/131. Animals handling protocol conform to the guidelines of the Edo State University Iyamho Nigeria Animal Research Ethics Committee (EDSU-AREC) for laboratory animal care and use.

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