



Antiulcer Activity of the Ethanolic Extract from *Citrus Limetta* peels in the Ethanol Induced Ulcer Model in Swiss Albino Mice

Amol R. Chandekar¹, Sanjana Kapse¹, Mohan K. Kale¹, Atul Tripathi^{1*}

Department of Pharmacology, Konkan Gyanpeeth Rahul Dharkar College Of Pharmacy & Research Institute, Karjat, Maharashtra, India- 410201.

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ABSTRACT:

Peptic ulcer disease (PUD) is a common gastrointestinal disorder marked by ulcers in the stomach or duodenum. While numerous pharmacological treatments exist, there is growing interest in natural products and their bioactive compounds for their potential therapeutic advantages. This investigation examines the use of *Citrus limetta* peels extract in managing peptic ulcers, focusing on research conducted with Ethanol Induced Ulcer Model using Swiss Albino Mice. The standard of reference was Pantoprazole. Ethanol was used to induce ulcer in Mice. Peel extract of this plant may be a natural product useful in prevention and treatment of gastric lesions, acting by different mechanism. *Citrus Limetta* contains compounds like limonene, bergamol, linalool. These constituents show the necessary for Anti-Ulcer effect. The monoterpene limonene are widely used flavoring agents that are found in some common food items also. This is also used medicinally throughout the world to treat gastritis and gastric disorders. The Limonene provided effective gastroprotection against lesions induced by absolute ethanol and NSAID (non-steroidal anti-inflammatory drug). Limonene does not interfere with gastric H⁺ secretion, serum gastrin or glutathione (GSH) level in gastric mucosa.

1. Introduction

The healthcare professionals manage million cases of stomach ulcers annually. Gastric acid or pepsin-induced damage to the inner lining of the gastrointestinal tract, which frequently extends into the muscular layer of the gastric epithelium, is known as Peptic Ulcer Disease (PUD).^[1] It can also affect the lower esophagus, distal duodenum, or jejunum. Usually, it affects the stomach and the proximal duodenum.^[2] Duodenal ulcer pain normally appears two to three hours after a meal, but gastric ulcer pain usually manifests 15 to 30 minutes after eating. For all PUD patients, testing for *Helicobacter pylori* is now advised, and endoscopy may be required for diagnosis, especially in individuals with concerning symptoms.^[3] Ulcers are prevalent due to the commonality of their primary causes, which include the use of over-the-counter pain medications and a widespread bacterial infection known as *H. pylori*. Traditional peptic ulcer treatments have focused on neutralizing stomach acid, but recent understanding emphasizes that ulcers can occur despite acid presence due to the gastric mucosa's defense mechanisms.^[4] Research now highlights the importance of

"cytoprotection" and the role of *Helicobacter pylori* in ulcer development.^[5]

Citrus Limetta

Citrus limetta, the scientific name for the sweet lime, or "Mosambi," is native to Asia and is widely grown in China, India, and Thailand.^[6] Enjoy this fruit raw or juiced for a vitamin C-rich energy boost. Sweet lime juice and other citrus juices are major products in the worldwide juice and food sector.^[7] As a byproduct of making juice, the peel has valuable components like pectin, flavonoids, and essential oils. These powerfully scented essential oils are utilized as flavorings in foods, drinks, and medications. The high terpene content of citrus essential oils contributes to their diverse biological actions, which include antibacterial, antioxidant, anti-inflammatory, and anxiolytic properties. D-limonene, is especially major terpene in these oils.^[8]



2. Objectives

The objective of this research article is to investigate the anti-ulcer activity of ethanolic extract from *Citrus limetta* peels in an ethanol-induced ulcer model using Swiss albino mice. Peptic ulcers are a significant health concern, often resulting from increased gastric acid secretion and mucosal damage. *Citrus limetta*, commonly known as sweet lime, is rich in bioactive compounds, including flavonoids and ascorbic acid, which have shown in promoting gastric mucosal protection and enhancing healing processes. This study aims to delve into the potential therapeutic benefits of *Citrus limetta* peels, focusing on their efficacy in mitigating ulcer formation and promoting gastric mucosal integrity.

The research will employ a controlled experimental design where Swiss albino mice will be divided into groups receiving varying doses of the ethanolic extract, alongside a control group. The objective is to assess parameters such as ulcer index, histopathological changes in gastric tissues, and levels of gastric acid secretion. By measuring these outcomes, the study seeks to provide a comprehensive understanding of the extract's protective mechanisms against ethanol-induced ulcers. Ultimately, this research aims to contribute to the growing body of evidence supporting the use of natural products in gastroprotective therapies, with the hope of identifying a viable, plant-based alternative for ulcer treatment. The findings may pave the way for further exploration of *Citrus limetta* as a functional food or nutraceutical, with implications for public health and dietary recommendations in ulcer management.

3. Methods

Experimental Animals:

Healthy Swiss Albino Mice (20-35gm) were used for the study. The animals were procured from the central animal facility of the institute. The use of these animals and the study protocols were approved by the Institutional Animal Ethics Committee of college. Mice were kept at the animal house in polypropylene cage, at 22±2°C, with 12:12 hrs. dark:light cycle. They were provided with mice feed and water *ad libitum*.

Plant materials and Extraction:

The *Citrus limetta* peels were shade-dried, chopped into small pieces, and coarsely powdered by using a chopper. Then these coarse powders were subjected for the successive extraction by using ethanol by Soxhlet method. Approximately 25-50 g sample were placed in filter paper.^[9] Close the filter paper and keep in siphon tube. Prior to setting up the apparatus an empty flask was weighed to ensure accuracy, in the experiment. Water was passed through the condenser, with the inlet at the bottom and the outlet at the top, to prevent air bubbles. Ethanol (70-80°C) was then added to the siphon tube, filling it halfway before starting the heating process. The water bath was set to 70-75°C, and the system was allowed to run for 7 hours. When the ethanol cleared in the siphon tube, extraction was complete. The sample was removed from the siphon tube, ethanol was recovered, and the filter paper with the sample was placed in a drying oven for 3 hours.^[10] Once the sample was free of the ether smell, it was considered dried. The filter paper and flask were weighed, and the extracted substance was collected in the flask. The extracts were then dried and stored at 4°C.^[11]

Phytochemical Screening :

Ethanolic extract of *Citrus limetta* peels were subjected to phytochemical screening, which revealed the presence of flavonoids, phenols, tannins, glycosides, terpenoids, steroids and amino acids. Peels contain flavonoids having antiulcer effects, especially limonene and bergamol.^[12-13]

Acute Oral Toxicity:

The safety of *Citrus limetta* peel extract was determined by the literature review. It is stated that the ethanolic extract has an LD50 of 2000mg/kg dose. Animal consumption of the plant is another indication that it is less poisonous. Therefore, the dosages of *Citrus limetta* were determined to be 200 mg high dose, 100 mg median dose, 50 mg low dose for purpose of the Research Investigation.^[14]

Experimental Design :

Swiss Albino Mice will be divided into six groups of six animals per group and animals were fasted for 24



hrs prior to the experiment in cages to avoid coprophagy . six groups were grouped as

Table no : 1 Grouping of animals

Sr.no	Group	Test substance	Animals required per group	Dose
1	GROUP 1 (vehicle)	Vehicle	6	10ml/kg
2	GROUP 2 (Ulcer control)	Ethanol	6	1ml/kg
3	GROUP 3 (Standard)	Pantoprazole	6	40mg/kg
4	GROUP 4 (Test 2)	CLPE + Ethanol (Low Dose)	6	50mg/kg + 1ml/kg
5	GROUP 5 (Test 3)	CLPE + Ethanol (Intermediate Dose)	6	100mg/kg + 1ml/kg
6	GROUP 6 (Standard)	CLPE + Ethanol (High Dose)	6	200mg/kg +1ml/kg

Group I – received Vehicle (10 ml/Kg) as normal control.

Group II - received ethanol (v/v)(in distilled water) at 1ml/kg as Ulcer control.

Group III – received standard oral dose of pantoprazole(40mg/kg).

Group IV – received Low dose of CLPE(50mg/kg).

Group V- received Intermediate dose of CLPE(100mg/kg)

Group VI-received High dose of CLPEGroup(200mg/kg)

Here, CLPE is *citrus limetta* peel extract

IV, V, VI will be administered extract for 1 hr. after the drug treatment the animals will be Administered with

Ethanol 1ml/kg p.o. induce ulcers for 4 hrs. The animals will be sacrificed after 4 hr. in CO₂ chamber and stomach is opened and percentage inhibition of ulcer will be determined.^[15-17]

In Vitro :

DPPH Radical Scavenging Assay:

Antioxidant activity in the sample compounds was estimated for their free radical scavenging Activity by using DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) free radicals .1 ml of test compounds water were taken in the test tube. 1 ml of 0.1% ethanolic DPPH was added over the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively and , the absorbance of the mixture was measured at 518 nm.

DPPH radical scavenging activity (%) = [(Absorbance of control - Absorbance of test sample) / (Absorbance of control)] x 100^[18]

DMSO :

To the reaction mixture containing 100 µL of alkaline DMSO, 30 µL of the drug samples in concentration (20 µg/ ml to100µg/ml) and standard ascorbic acid (20 µg/ ml to100µg/ml) was added in DMSO at various concentrations followed by 10 µL of NBT(Nitroblue tetrazolium) (0.1 mg).The absorbance was measured at 560 nm.

Scavenging of Superoxide radical by Alkaline DMSO Method (%) = [(Absorbance of control - Absorbance of test sample) / (Absorbance of control)] x 100^[19]

Biochemical Analysis :

Malondialdehyde (MDA)

Stomachulcertissuehomogenatewas prepared, taken a mixture of 0.4 ml of 10% stomach ulcer homogenate added 1.5ml of 8.1% Sodium dodecyl sulphate.1.5 ml of 20% acetate buffer (pH 3.5) and 1.5 ml of 0.8% TBA(Thiobarbituric acid) solution were added to above mixture. The mixture was heated at 95°C for 60 min and cooled to room temperature.After cooling added 5 ml of n-butanol-pyridine



(15:1). Vortexed the mixture thoroughly and allowed to stand until the organic and aqueous layers will not get separated. Recorded absorbance of organic layer at 532 nm on UV-visible spectrophotometer.^[20]

Nitric Oxide Test (NO)

Stomachulcertissuehomogenate was prepared, taken a mixture of 0.4 ml of 10% stomach ulcer homogenate added 50µl 30% ZnSO₄ for protein precipitation. Then, precipitated protein was removed by centrifugation for 15min. 100µl of the resulting supernatant was diluted to 300µl with water and treated with 300µl vanadium trichloride (0.8 g % in 1 M HCl), followed by rapid addition of 150µl sulphanilamide (2% in 5% HCl) followed by 150µl N-1-(naphthyl) ethylenediamine dihydrochloride (0.1%). The mixtures were then incubated at 37°C for 30 min. and then cooled. Recorded absorbance at 540 nm on UV-visible spectrophotometer.^[21]

Histopathological Examination

The Gastric tissues from mice were fixed with 10% buffered formalin solution . the formalin-fixed stomach sections were embedded in paraffin wax .then the gastric samples were sectioned at a thickness of 5µm and stained with hematoxylin and eosin (H&E) .Pathological observation were performed with a light microscope .^[22]

Statistical Analysis

The data obtained from the animal experiment were analysed using Graph Pad Prism .results for each category expressed as the mean+SEM . An one-way Analysis of various (ANOVA) was used to analyse statistical difference , followed byDunnetts test . testwith a p-value <0.05 were considered statistically significant.

4. Results

Phytochemical screening

Table no 2: Result of phytochemical screening analysis of *Citrus limetta* peel extract.

Sr.no	Phytoconstituents	Test	<i>Citrus limetta</i> peel
1	Carbohydrates	Molish's test	Absent
2	Flavonoids	Shinoda	Present
		NaOH	Present
3	Alkaloids	dragendorff	Present
		Mayer	Present
		Wagner	Present
4	Quinines	Borntager	Present
5	Tannins	Benedict	Present
6	Saponins	Foam test	Absent

			extract
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4	Quinines	Borntager	Present
5	Tannins	Benedict	Present
6	Saponins	Foam test	Absent

Anti Ulcer Evaluation :

Ethanol induced gastric ulcers

Table no 3 : Mean Ulcer Score in ethanol induced ulcer

Treatment	Mean Ulcer Score	% Inhibition
Vehicle	0.00±0.00	0
Ulcer control	48.67±7.109	0
Standard	19.47±3.919	61.43
CLPE(50mg/kg)	44.39±7.229	40.91
CLPE(100mg/kg)	32.30±6.532	44.37
CLPE(200mg/kg)	22.82±4.121	58.48

Effects of ethanolic extract of *Citrus limetta*peels ulcerinduced by ethanol in mice are shown in table no 3 .Ethanol induced ulcer shows gastric damage such as gross mucosal lesion,including long haemorrhage bands and petechial lesions.animal are pre-treated with extract of *Citrus limetta* peels and pantoprazole showed very mild lessions and sometimes no lesions at all , when compared to ulcer control group .*Citrus limetta* peels showed a dose dependent curative ratio compared to ulcer control groups. The extract exhibited inhibition percentage of 40.91, 44.37 and 58.48 at dose of 50,100,200 mg/kg respectively .theulcerprotective action of extracts at different doses was better than that of standard drug, pantoprazole which exhibited inhibition percentage of 61.43% .

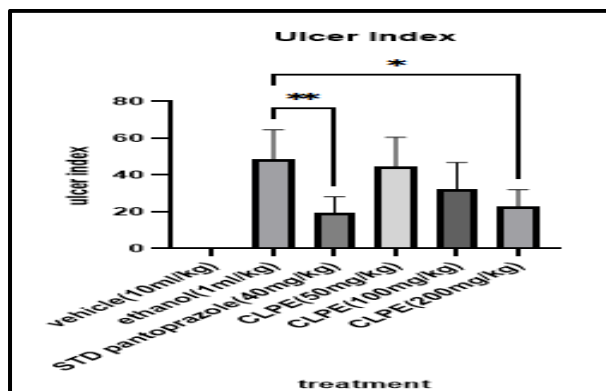


Fig no: 1graph of ulcer index

All the three test doses of the *Citrus limetta* peel Extract showed dependent decrease in Ulcer Index when it was compared against Pantoprazole which was used as Standard. Ulcer index was calculated by Ulcer Score given in Dasputre et al. (2011). Also, the percent Ulcer, and Uler inhibition was determined by use if ImageJ software. Ulcer control group was compared with Normal control group. Standard and Extract treated groups were compared. Values are the mean ± SEM of n=6 mice/treatment. Significance **p<0.01

Gastric PH:

Table no 4: Evaluation of gastric pH value

Treatment	pH
Ulcer Control	1.410±1.120
Standard	5.210±0.1300
CLPE (50mg/kg)	3.250±0.1900
CLPE (100mg/kg)	3.750±0.1700
CLPE (200mg/kg)	4.110±0.1587

pH of gastric juice was increased compared to ulcer control group. The effect of Extract on pH showed significant (p<0.01) effect at 200mg/kg and 100mg/kg dose compared to ulcer control animals.

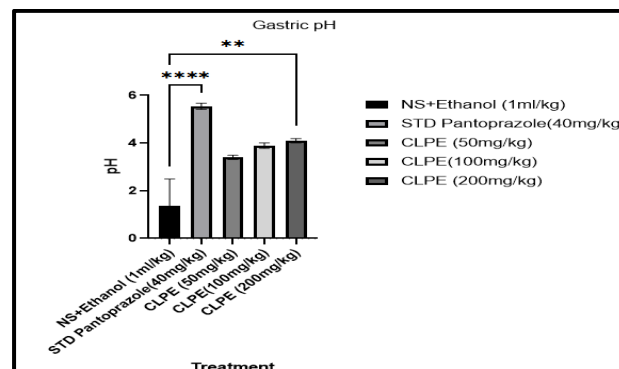


Fig no 2 : Gastric pH level

In-Vitro Tests On Extract:

DPPH Standard : The Antioxidant Activity of peels of *Citrus limetta* extract was carried out by DPPH Assay using ascorbic acid as standard Antioxidant DPPH Scavenging Activity of peels of *Citrus limetta* at different concentration.^[23]

Table no 5: IC₅₀ determination of extract

Sr.no	Sample	IC ₅₀
1	Ascorbic acid	115.61
2	<i>Citrus limetta</i>	127.21

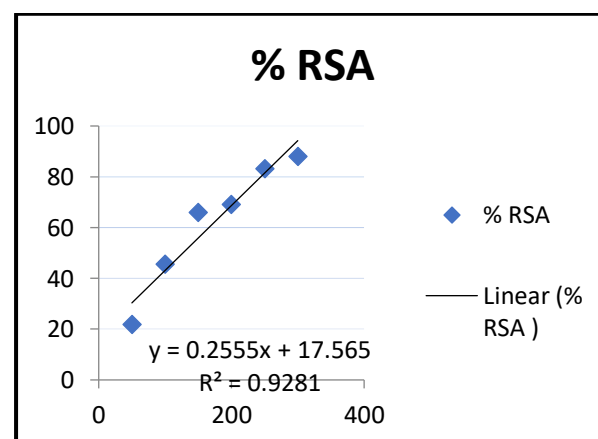


Fig no :3 DPPH Radical scavenging activity of *Citrus limetta* peels extract

Results represented in above table all Extracts exhibited dose dependent increase in anti-oxidant activity.



AlkalineDMSO Test

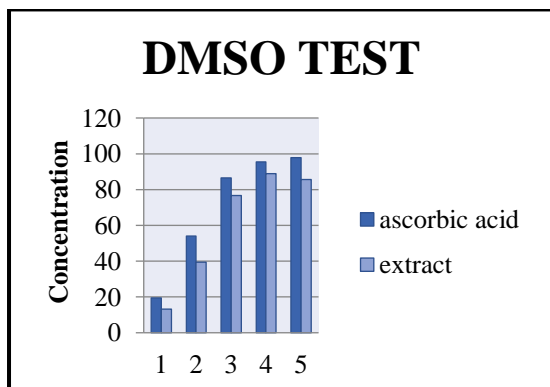


Fig no : 4 Radical scavenging activity of peels of *Citrus limetta* extract

Antioxidants serve as a defence for cells by neutralizing free radicals ,thus shielding vital biological elements from harm and warding illnesses. The efficacy of *Citrus limetta* peels extract in elimination superoxide radical was evaluated through the alkaline DMSO method. The results indicated a notable decrease in production of superoxide radicals by the extract.

BiochemicalTests :

Malonyldialdehye (MDA)

All the 3 test doses of ethanolic extract of peels of *Citrus limetta* showed dose dependant decrease in MDA level activity when it was compared against the negative control group.Effect of ethanolic extract of *Citrus limetta* peels on MDA level in ethanol induced ulcer in swiss albino mice .

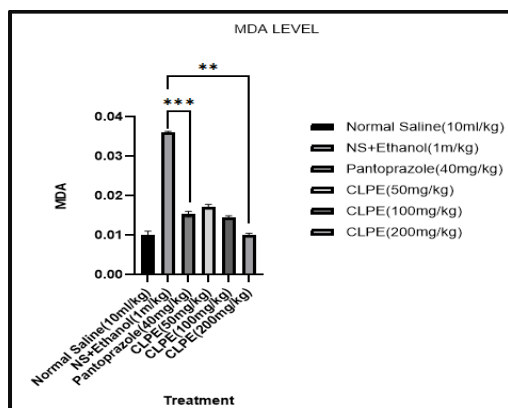


Fig no: 5 MDA Level

As shown in graph ethanol i.e Ulcer control group increased the gastric MDA level And administration of Extracts similar to standard group exhibit a significant reduction in MDA level in stomach tissue.

Table no 6 : MDA Level

Treatment	MDA Level
Vehicle	0.010±0.0010
Ulcer control	0.036±0.00030
Standard	0.015±0.00070
CLPE (50mg/kg)	0.017±0.0006
CLPE (100mg/kg)	0.014±0.0005
CLPE (200mg/kg)	0.010±0.0005

Determination Of Gastric Nitric Oxide

Table no 7 :TotalNitrate level

Treatment	NO Level
Vehicle	20.1±0.243
Ulcer control	7.11±0.021
Standard	14.95±0.128
CLPE(50mg/kg)	8.75±0.249
CLPE(100mg/kg)	9.95±0.487
CLPE (200mg/kg)	10.25±0.41

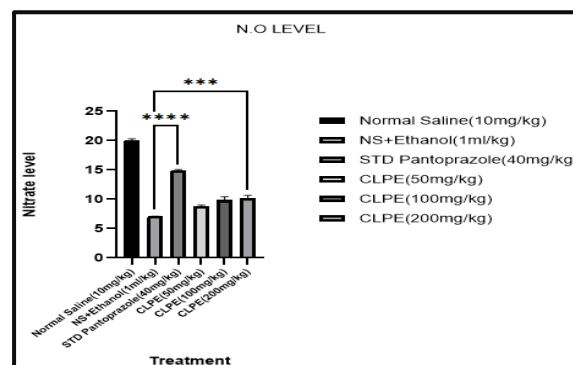


Fig no : 6 Nitrate Level



The result shown in graph shows that ethanol i.e ulcercontrol group administration decreased the gastric nitric oxide level and all 3 doses shows dose depend increase in nitric oxide level .

Histopathology

Ulcer control group B. showing disruption of epithelial structure of cells and interstitial oedema of submucosa and inflammation. While test group more preserved gastric epithelium and less oedema of submucosa and gradual decrease in inflammation compared to ulcercontrol group. Standard C. group showing gastric epithelium preserved and discrete oedema of submucosa

5. Discussion

In this study, the anti-ulcer activity of *Citrus limetta* peel extract (CLPE) was evaluated using Swiss albino mice through both in vitro and in vivo methods. The study utilized different dosages of the extract to explore its effect on ulcer inhibition, with comparisons to a standard drug, pantoprazole. The results indicate that the *Citrus limetta* peel extract possesses significant ulcer protective properties, comparable to conventional treatments.

Phytochemical Screening

The phytochemical analysis revealed that the *Citrus limetta* peel extract contains active compounds, including flavonoids, tannins, and alkaloids, all of which are known to contribute to medicinal properties such as antioxidant and anti-inflammatory effects. The presence of these compounds in the peel suggests that *Citrus limetta* has the potential to act as a bioactive agent in the treatment of ulcers. Notably, the presence of alkaloids was detected through Dragendorff's and Mayer's tests, and tannins were identified using Benedict's test. These components are known to play an essential role in providing gastroprotective effects by modulating gastric acid secretion and promoting the healing of mucosal tissues .

In Vitro Antioxidant Activity

The antioxidant activity of *Citrus limetta* peel extract was assessed using DPPH radical scavenging assays. The results showed that the extract exhibited a dose-dependent increase in antioxidant activity, with a close

comparison to ascorbic acid, a standard antioxidant. This indicates that CLPE has the potential to neutralize free radicals, which are known to damage the gastric mucosa and exacerbate ulcer conditions .

Additionally, the extract demonstrated significant efficacy in scavenging superoxide radicals via the alkaline DMSO method. This suggests that the extract can potentially reduce oxidative stress, which is a contributing factor to ulcer formation. Antioxidants play a crucial role in protecting gastric mucosa from damage by free radicals generated from ethanol-induced gastric ulcers .

In Vivo Anti-Ulcer Activity

The ethanol-induced ulcer model in Swiss albino mice revealed a notable decrease in ulcer scores following the administration of *Citrus limetta* peel extract. Three different dosages of the extract (50, 100, and 200 mg/kg) were tested, and all showed a reduction in the ulcer index in a dose-dependent manner. The highest dose (200 mg/kg) exhibited the greatest ulcer inhibition (58.48%) compared to the standard drug pantoprazole, which had an inhibition percentage of 61.43%. The ulcer protection was attributed to the anti-inflammatory and antioxidant properties of the bioactive compounds present in the extract .

Gastric pH and Biochemical Markers

The analysis of gastric pH showed a significant increase in pH levels in animals treated with CLPE, indicating reduced acidity in the stomach. This is an important factor in ulcer prevention as low gastric pH levels are associated with increased gastric acid secretion, which contributes to ulcer formation. The group treated with 200 mg/kg of CLPE exhibited a pH level comparable to the pantoprazole-treated group, further demonstrating its gastroprotective effect .

Biochemical markers, such as malondialdehyde (MDA) levels, were also evaluated to assess lipid peroxidation in the gastric tissues. CLPE treatment significantly reduced MDA levels, indicating a reduction in oxidative stress and lipid peroxidation. Moreover, nitric oxide (NO) levels were increased in the CLPE-treated groups, suggesting enhanced gastric mucosal defense mechanisms, as nitric oxide is known to promote vasodilation and protect the gastric mucosa .



Histopathological Observations

Histopathological examinations of gastric tissues further confirmed the gastroprotective effect of CLPE. The ethanol-treated control group showed severe mucosal damage, including hemorrhagic streaks and deep ulcers. However, the groups treated with *Citrus limetta* extract exhibited mild lesions, with the highest dose showing almost complete protection of the gastric mucosa. The results suggest that the extract not only inhibits ulcer formation but also promotes the healing of damaged gastric tissues.

Conclusion:

The results of this study demonstrate that *Citrus limetta* peel extract exhibits significant anti-ulcer activity, primarily through its antioxidant and anti-inflammatory properties. The extract effectively reduced the ulcer index, increased gastric pH, reduced oxidative stress markers such as MDA, and enhanced nitric oxide production. These findings suggest that *Citrus limetta* peel extract could be a potential natural alternative to conventional ulcer treatments such as pantoprazole.

The study also highlights the importance of further research to isolate and characterize the specific bioactive compounds responsible for the anti-ulcer activity. Understanding the mechanisms by which these compounds exert their gastroprotective effects can pave the way for developing new therapeutic agents derived from natural products.

In conclusion, *Citrus limetta* peel extract holds promise as a safe and effective treatment for peptic ulcer disease. Its antioxidant and anti-inflammatory properties, coupled with its ability to modulate gastric acid secretion and promote mucosal healing, make it a strong candidate for further pharmacological investigation. The findings of this study contribute to the growing body of evidence supporting the use of natural products in the treatment of gastrointestinal disorders.

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