Yemen Journal of Medicine

https://yemenjmed.com

Article information

DOI: 10.63475/yjm.v4i2.0153

Article history:
Received: 19 June 2025
Accepted: 10 July 2025
Published: 22 September 2025

Correspondence to:
Hina Imran
Email: dr_hinaimran@yahoo.com
ORCID: 0009-0001-3279-2087

How to cite this article
Imran H, Sohail T, Ali Khan R, Iqbal W, Rahman M. Assessment of ethanolic extract of Ocimum sanctum whole plant (OSWP) in ethanol-induced gastric mucosal damage in rats. Yemen J Med. 2025:4(2): 399-404

Original Article

Assessment of Ethanolic Extract of *Ocimum sanctum* Whole Plant (OSWP) in Ethanol-Induced Gastric Mucosal Damage in Rats

Hina Imran[1], Tehmina Sohail[2], Rashid Ali Khan[3], Wasif Iqbal[4], Maimoona Rahman[5]

1 Senior Medical Officer, PCSIR Labs Complex, Karachi, Pakistan
2 Senior Scientific Officer, PCSIR Labs Complex, Karachi, Pakistan
3 Chief Scientific Officer, HOC-PRC, PCSIR Labs Complex, Karachi, Pakistan
4 Associate Professor, Vice Principal, Sindh Institute of Oral Health Sciences, Jinnah Sindh Medical University, Karachi, Pakistan
5 Staff, PCSIR Labs Complex, Karachi, Pakistan

ABSTRACT

Background: Previously, *Ocimum sanctum* (Lamiaceae), commonly known as holy basil, has been noted for its anti-ulcer properties, primarily attributed to its oil and leaf extracts. However, the anti-ulcer activity of an ethanolic extract derived from the whole plant has not been reported. This study aimed to evaluate the antiulcer effect of the ethanolic extract of *O. sanctum* whole plant (OSWP) in an experimental ulcer model.

Methods: The study, conducted at PCSIR Labs Complex in Karachi in August 2024, was approved by the Ethical Use of Experimental Animals Committee (IEC/OSWP-05). This study investigates the anti-ulcer activity of an ethanolic extract of OSWP in a rat model of ethanol-induced gastric mucosal damage at 200, 300, and 400 mg/kg body weight, with ranitidine (50 mg/kg) used as a positive control. Outcome measures included ulcer index (UI), percentage protection, and gastric pH.

Results: The ethanolic extract of OSWP demonstrated dose-dependent gastroprotective effects. At 200, 300, and 400 mg/kg, the UIs were 9.49 ± 2.40, 4.55 ± 1.44, and 2.5 ±2.25, respectively, with corresponding protection ranging from 48.9% to 86.54% for the 200 to 400 mg/kg doses ($p < 0.05$). Gastric pH increased from 5.6 to 7.6. In comparison, ranitidine (50 mg/kg) resulted in a UI of 4.0 ± 0.88, 78.47% protection ($p < 0.05$), and a pH of 7.0.

Conclusions: The study demonstrates the dose-dependent anti-ulcer activity in the ethanolic extract of OSWP, which may be further developed as a potential anti-ulcer agent. A graphical presentation of the whole experimental process is shown in Figure 1.

Key words: *O. sanctum* whole plant (OSWP), ethanol-induced ulcer model, ulcer index, % protection

INTRODUCTION

There are many forms of ulcer, such as mouth ulcer, esophagus ulcer, peptic ulcer, and genital ulcer. Among these, peptic ulcer is more common and considered the world's foremost gastrointestinal disorder that affects 10% of the world population. [1] Peptic ulcers are sores or lesions in the gastrointestinal mucosa. Their treatment typically involves the use of various chemically produced medications aimed at reducing stomach acid secretion, protecting the mucosa lining the stomach and upper small intestine, or

eradicating *H. pylori* infection. Before conducting clinical trials of new formulations, it is recommended to perform in vivo or in vitro studies. [2]

For antiulcer studies, a wide variety of in vivo methods are available. Among these, the induced ulcer model is broadly used because it closely resembles an acute ulcer in humans. [3]

Due to several adverse effects associated with existing synthetic drugs, there is always a need for a better drug with much less or probably no side effects from other sources. [4] Plant-based extracts have been one of the attractive sources of new drugs, with some of them demonstrated to possess promising anti-ulcer effects. In this modern era, 75% to 80% of the world's population still uses natural medicine, especially in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body, and fewer side effects. [5] With this concern, this study was planned for the anti-ulcer activity of the ethanolic crude extract of the *Ocimum sanctum* whole plant.

O. sanctum, commonly known as Tulsi, belongs to the family *Labiatae*. It contains several chemical constituents highly effective in a wide spectrum of diseases, like anticarcinogenic, anthelmintic, antiseptic, antirheumatic, antistress, and antibacterial. Clinical trials have reported the usefulness of *O. sanctum* in heart diseases, diabetes, anti-inflammatories, and immunomodulatory. [6] Previous studies have reported significant antiulcer activity by different parts of *O. sanctum*. Surender Singh [7] reported anti-ulcer activity of *O. sanctum* oil against ulcer-induced models. [8] A study reported the distillate of its leaves, while another study reported the ethanolic extract of leaves in gastric ulcer induced by multiple methods. [9,10] Scientifically, it is suggested that the plant should be studied as a whole instead of only examining the parts in isolation. However, there was no scientific investigation made on the antiulcer activity of the whole plant. Therefore, the present study was undertaken to evaluate the antiulcer effect of the ethanolic extract of *O. sanctum* whole plant (OSWP) in an experimental ulcer model.

MATERIALS AND METHODS

Plant material

The whole plant of *O. sanctum* (OSWP) was purchased from the local market and identified by its macromorphological features and botanical profile. A total of 500 g of cleaned, dried, powdered plant material was extracted with 2 L of ethanol. The extract was filtered through Whatman No. 1 filter paper, and the soaking and filtration process was repeated twice on the residue. The filtrates from each extraction were pooled together and concentrated using a rotary evaporator at 50°C to 60°C under reduced pressure. The resulting dark-colored extract was stored in an airtight container for later use.

Animals

Healthy male and female albino rats, weighing between 150 and 200 g, were used in this study. These animals were bred in the animal house at KLC PCSIR. They were housed in spacious plastic cages with sawdust bedding, and the animal room was maintained under standard experimental conditions (temperature 27 ± 2°C and a 12-hour light/dark cycle) throughout the study. All animals received a standard pellet diet and had access to water ad libitum.

Antiulcer activity and experimental design

Animals with 24-hour fasting with free access to water were employed and randomly divided into five groups (n = 6). The following treatments were given to these groups:

Group I: served as the control group, treated with distilled water orally.

Group II: treated with the ethanolic extract of OSWP at a 200 mg/kg dose orally.

Group III: treated with the ethanolic extract of OSWP at a 300 mg/kg dose orally.

Group IV: treated with the ethanolic extract of OSWP at a 400 mg/kg dose orally.

Group V: served as the standard group and received Ranitidine (50 mg/kg) orally.

One hour post-treatment with control, test, and standard drugs, ulcers were induced with absolute ethanol (1 mL/200 g b.w.) by oral route in all animal groups. [3] After 30 minutes of ethanol administration, all animals had been euthanized, and their stomachs were removed carefully.

Determination of ulcer index (UI)

The excised stomachs were thoroughly rinsed with distilled water and then opened along the greater curvature. The pH of the gastric contents was measured, and the mucosal surface was rinsed with cold distilled water to remove any blood or debris. Each stomach was carefully stretched and pinned to a white background on a wooden board for detailed examination and ulcer assessment. The open stomachs were inspected for hemorrhagic lesions. Ulcers in the gastric mucosa appeared as inflamed areas, marked by bands of hemorrhagic lesions. The severity of the lesions was scored based on their length, using the following scoring system:

No lesions ≥0

Lesions <1 mm, length = 1

Lesions 2–4 mm, length = 2

Lesions > 4 mm, length = 3

The UI for each rat was calculated by multiplying the number of lesions by their corresponding severity factor. The mean UI for each group was then determined.

Percentage protection of ulcer

The following formula was used for percentage protection:

$$\text{Percentage protection} = \frac{\text{Control mean UI} - \text{Test mean UI}}{\text{Control mean UT}} \times 100$$

pH determination

The pH of the gastric contents was measured using pH strips, with the color compared to the reference standard.

Statistical analysis

The data are presented as mean ± SD for each group. Statistical analysis was performed using the Student's *t*-test. The *p* values at <0.05 were considered significant (**) when compare with respective controls (https://www.graphpad.com/quickcalcs/ttest1/).

RESULTS

The dose-dependent gastroprotective effects of OSWP ethanolic extract were evaluated in a rat model of ethanol-induced gastric ulcers. OSWP demonstrated UIs of 9.49 ± 2.40, 4.55 ± 1.44, and 2.5 ± 2.25 at doses of 200, 300, and 400 mg/kg, respectively. Correspondingly, the percentage of protection observed ranged from 48.9% to 86.54% ($p < 0.05$) for the 200 to 400 mg/kg doses, with a pH increase from 5.6 to 7.6. In comparison, the standard drug ranitidine exhibited a UI of 4.0 ± 0.88 and provided 78.47% protection ($p < 0.05$) with a pH of 7.0 (**Table 1**; **Figures 1 & 3**).

The values are expressed as the mean ± SEM, $n = 6$. **$p < 0.05$ and *** $p < 0.05$ when compared with the control group.

DISCUSSION

Pre-fed test drug presented a significant decrease in the sore area (UI) and accelerated % protection in a dose-dependent manner. The test drug showed a marked reduction in mean UI 2.5 ± 2.25 with a significant increase in protection 86.54% with pH 7.6 at a 400mg/kg dose ($p < 0.05$). Standard drug exhibited 4.0 ± 0.88 UI with 78.47% protection ($p < 0.05$) with pH 7.0.

O. sanctum, commonly known for its wide range of medicinal properties, including anticarcinogenic, anthelmintic, antiseptic, antirheumatic, antistress, antibacterial, anti-inflammatory, and immunomodulatory effects, was studied for its antiulcer activity. Specifically, we investigated the ethanolic extract of OSWP in a rat model of alcohol-induced gastric ulcers. Among various ulcer models, the ethanol-induced gastric

Table 1: Effects of *O. sanctum* whole plant (OSWP) extract on ulcer index and pH of gastric content.

Sr No.	Pre-treatment animal groups	Ulcer index (mm^2)	Gastric pH
1.	Group I control-distilled water	18.58 ± 4.69	2.8
2.	Group II OSWP-200 mg/kg	9.49 ± 2.40**	5.6
3.	Group III OSWP-300 mg/kg	4.55 ± 1.44***	6.8
4.	Group IV OSWP-400 mg/kg	2.5 ± 2.25***	7.6
5.	Group V renitidine-50 mg/kg	4.0 ± 0.88***	7.0

Figure 1: Graphical presentation of the whole experimental process.

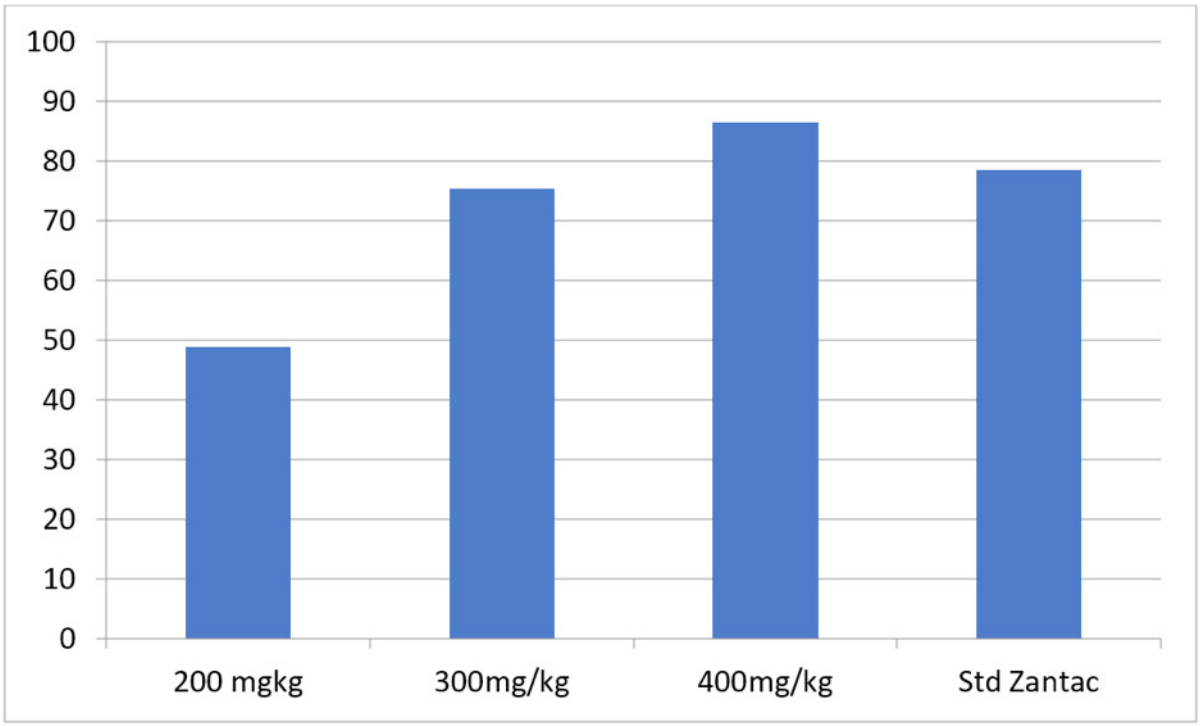

Figure 2: *O. sanctum* whole plant ulcer protection (% protection).

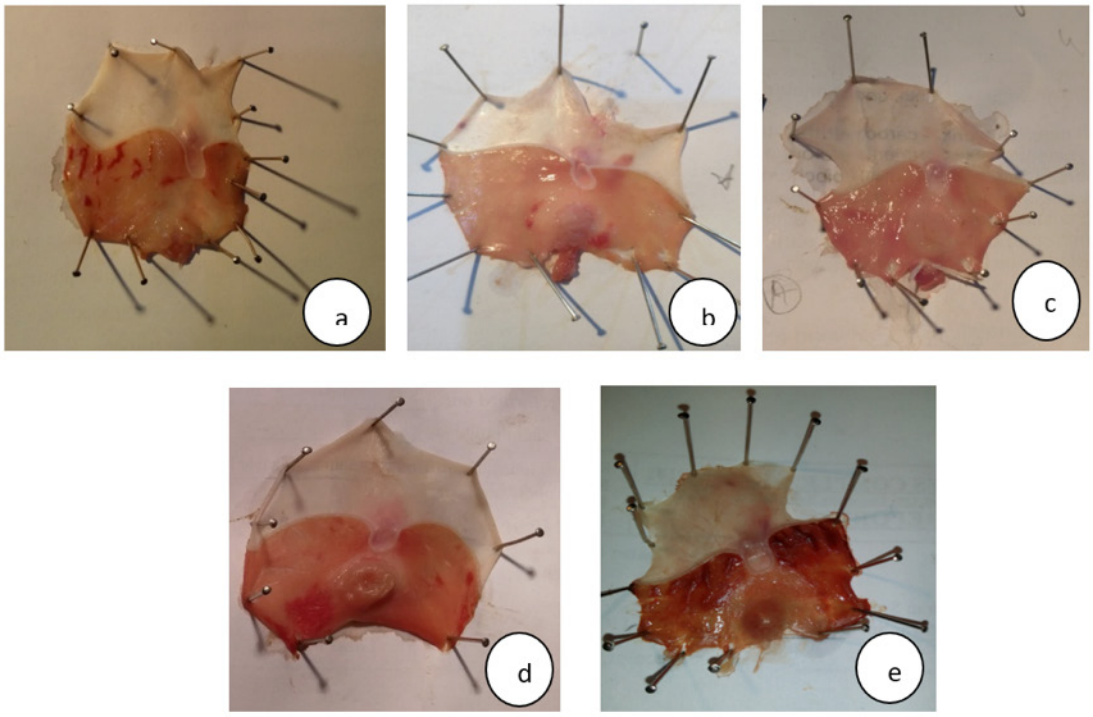

Figure 3. Effect of different doses of *O. sanctum* whole plant on the severity of gastric lesions. (a) 200 mg/kg, (b) 300 mg/kg, (c) 400 mg/kg, (d) standard, and (e) control.

ulcer model is preferred for preclinical evaluation of potential antiulcer agents, as it is a widely used experimental model. [11] Ethanol damages the gastric mucosa by increasing mucosal permeability, disrupting gastric secretion, causing microvascular changes, and potentially leading to bleeding and necrotic tissue injury within minutes of administration. [12] In our study, gross examination revealed significant gastric mucosal damage in rats of the control group following intragastric gavage of absolute alcohol. The damage was characterized by elongated macroscopic lesions of varying sizes, accompanied by intense hemorrhage and hyperemia.

The test group pretreated with OSWP showed minimal signs of mucosal injury, with a lower percentage of damage compared to the control group. Our study demonstrated a dose-dependent antiulcer effect, with 400 mg/kg body weight being the most effective dose. This dose significantly protected the gastric mucosa against ethanol-induced damage, as evidenced by a reduction in the UI, increased percentage of protection, and a rise in pH in a dose-dependent manner. At doses of 200, 300, and 400 mg/kg, the test drug resulted in a significant reduction in the mean UI 9.49 ± 2.40, 4.55 ± 1.44, and 2.5 ± 2.25, respectively, and achieved ulcer protection of 48.9%, 75.5% and 86.54% with corresponding increases in pH to 5.6, 6.8, and 7.6. The standard drug, ranitidine, produced a UI of 4.0 ± 0.88 with 78.47% protection and a pH of 7.0. In contrast, the control group, which received intragastric administration of absolute ethanol (1 mL/200 g for 30 minutes), developed severe hemorrhagic gastritis with a high UI score of 18.58 ± 4.69 and a pH of 2.8, indicating multiple linear hemorrhagic ulcers and multifocal erosions (Table 1; Figure 1 and 2). The highest dose of the test drug was found to be slightly more potent than the standard ranitidine.

It is recommended that the plant be studied as a whole, rather than focusing solely on its individual parts in isolation. [4] Scientific data on various parts of *O. sanctum* are reported for its antiulcer effects. Surender Singh [7] reported *O. sanctum* fixed oil for antiulcer effect against ulcer induced by various methods, and they concluded that *O. sanctum,* due to its anti-inflammatory property, significantly reduces UI in a dose-dependent manner. Our work on the ethanolic extract of the same plant also showed a significant reduction in UI in a dose-dependent manner. A three-day study [13] conducted on different doses of aqueous and ethanolic leaf extracts of O. *sanctum* reported 6.50 UI at a 100 mg/kg ethanolic extract dose against cold restraint stress. Another study [10] on aqueous extract of *O. sanctum* reported 17.2 UI with 52.52% inhibition at a 200 mg/kg dose against ethanol-induced gastric ulceration. The researchers [14] worked on aqueous extract of *O. sanctum* leaves reported its effects against indomethacin-induced gastric ulcer with 27 UI and 49.22% inhibition at 200mg/kg dose, while 22 UI and 58.63% inhibition at 400 mg/kg dose. All this reported work correlates with our study.

O. sanctum contains a variety of chemical constituents that have biological activity, including saponins, flavonoids, triterpenoids, and tannins, which exhibit antioxidant, anti-inflammatory, and adaptogenic activities. It is well accepted that natural antioxidants are vital for restoring gastric tissue. Along with protecting the gastric mucosa against cell damage, they also enhance the defense systems against degenerative diseases. [15–20] The principle constituent of *O. sanctum* is eugenol. Eugenol is reported to have antiulcer, antiseptic, analgesic, antibacterial, and anti-inflammatory properties. The therapeutic potential effect of *O. sanctum* L. has been found to be largely due to eugenol. [15]

Many studies reported that the ulcer-healing property of *O. sanctum* seems to be based on its mucoprotective activity and its antisecretory effect. *O. sanctum* affects neural pathways controlling acid secretion, thereby strengthening the animal's physiological capabilities to decrease stress and hence ulcers. [15,21] Nowadays, few anti-inflammatory drugs are also ulcerogenic. [22,23] *O. sanctum* possesses both anti-inflammatory and antiulcer activities. [7] Therefore, it would be an additional benefit to use *O. sanctum* in such cases.

The current study is justified by the fact that peptic ulcers have a significant incidence in Pakistan and around the world. Current treatments are expensive and have limited effects on chronic pathologies, impairing immune function, and there is no pharmaceutical product on the market that is 100% effective. [24,25] Nowadays, herbal medicine is becoming a viable alternative treatment to the commercially available synthetic drugs for peptic ulcer management or treatment. Results of the present study clearly indicate that the ethanolic extract of *O. sanctum* had significant gastroprotective effects when compared with the control. Our data suggest that *O. sanctum* treatment has a significant percentage of ulcer protection in a dose-dependent manner. Antiulcerogenic activity of *O. sanctum* at the highest dose, 400 mg/kg, was comparable and slightly higher than the standard drug ranitidine. However, further study is required to know the exact mechanism of action and to isolate the active molecule responsible for the anti-ulcer activity.

Strengths of the study

This study offers several strengths, including the novel use of a whole plant extract of *Ocimum sanctum*, which may provide a broader spectrum of bioactive compounds compared to extracts from only leaves or oil. The use of multiple doses (200, 300, and 400 mg/kg) enables a clear evaluation of dose-dependent effects and helps identify an optimal therapeutic range. Including ranitidine as a standard control strengthens the study by allowing direct comparison with an established anti-ulcer drug. Additionally, the assessment of UI, percentage protection, and gastric pH offers a comprehensive evaluation of the extract's gastroprotective potential.

Limitations of the study

It lacks phytochemical characterization, making it unclear which specific compounds contribute to the anti-ulcer effects. Histopathological analysis is lacking, which could have further strengthened and supported the study's findings.

CONCLUSIONS

Based on the foregoing observations, it could be concluded that *O. sanctum* acts as an antiulcer agent and possesses significant gastroprotective effect against alcohol induced gastric damage, and its effect was comparable to that of the standard drug ranitidine. However, more experimentation and detailed molecular analysis of active constituents of *O. sanctum* are required for a definite conclusion.

AUTHORS' CONTRIBUTION

Each author has made a substantial contribution to the present work in one or more areas, including conception, study design, conduct, data collection, analysis, and interpretation. All authors have given final approval of the version to be published, agreed on the journal to which the article has been submitted, and agree to be accountable for all aspects of the work.

SOURCE OF FUNDING

None.

CONFLICT OF INTEREST

None.

REFERENCES

1. Zapata-Colindres JC, Zepeda-Gómez S, Montaño-Loza A, Vázquez-Ballesteros E, de Jesús Villalobos J, Valdovinos-Andraca F. The association of Helicobacter pylori infection and nonsteroidal anti-inflammatory drugs in peptic ulcer disease. Can J Gastroenterol. 2006;20(4):277-280.
2. Han M, Xu J, Lin Y. Approaches of formulation bridging in support of orally administered drug product development. Int J Pharma. 2022;629:122380.
3. Imran H, Sohail T, Rehman AU, Syed S. Dry ripe fruit of Aegle marmelos as an anti-ulcer agent against ethanol induced gastric mucosal injury. Pak J Pharm Sci. 2022;35(6):1677-1682.
4. Karimi A, Majlesi M, Rafieian-Kopaei M. Herbal versus synthetic drugs; beliefs and facts. J Nephropharmacol. 2015;4(1):27-30.
5. Vimala G, Gricilda Shoba F. A review on antiulcer activity of few Indian medicinal plants. Int J Microbiol. 2014;2014:519590.
6. Dharmani P, Palit G. Exploring Indian medicinal plants for antiulcer activity. Indian J Pharmacol. 2006;38(2):95-99.
7. Surender Singh DK. Majumdar evaluation of the gastric antiulcer activity of fixed oil of *Ocimum sanctum* (Holy Basil). J Ethnopharmacol. 1999;65(1):13-19.
8. Vaseem A, Ali M, Afshan K. Activity of tulsi leaves (*Ocimum sanctum* Linn.) in protecting gastric ulcer in rats by cold restrain method. Int J Basic Clin Pharmacol. 2017;6(10):2343-2347.
9. Tyagi L, Sharma N, Sharma A, Ahmad S. Evaluation of synergistic antiulcer activity of *Aegle marmelos* and *Ocimum sanctum* in ulcer induced Wistar rats. Panacea J Pharm Pharmaceut Sci. 2019;8(2):01-09.
10. Ghangale GR, Mahale Tushar, Jadhav ND. Evaluation of antiulcer activity of *Ocimum sanctum* in rats. Vet World. 2009;2(12):465-466.
11. Arab HH, Salama SA, Omar HA, Arafa EA, Maghrabi IA. Diosmin protects against ethanol-induced gastric injury in rats: Novel anti-ulcer actions. PLoS One. 2015;10(3):e0122417.
12. Moleiro FC, Andreo MA, Santos Rde C, Moraes Tde M, Rodrigues CM, Carli CB, et al. Mouririelliptica: Validation of gastroprotective, healing and anti-Helicobacter pylori effects. J. Ethnopharmacol. 2009;123(3):359-368.
13. Kaniganti S, Das MC, Bannaravuri R. Antiulcer activity of aqueous and ethanolic extracts of *Ocimum sanctum* leaves in albino rats. Int J Basic Clin Pharmacol. 2016;5(4):1353-1358.
14. Mirje MM, Sameer-uz-Zaman SZ. Evaluation of the anti-ulcer activity of *Ocimum sanctum* Linn (tulsi) in indomethacin-induced gastric ulcers in albino rats. Int J Life Sci Biotech Pharm Res. 2014;3(1):274-279.
15. Brito SA, de Almeida CLF, de Santana TT, Oliveira AR, Bezerra do Nascimento Figueiredo JC, Torres Souza I, et al. Antiulcer activity and potential mechanism of action of the leaves of *Spondias mombin* L. Oxid Med Cell Longev. 2018;2018:1731459.
16. Klein-Júnior LC, Santin JR, Niero R, de Andrade SF, Cechinel-Filho V. The therapeutic lead potential of metabolites obtained from natural sources for the treatment of peptic ulcer. Phytochem Rev. 2012;11:567-616.
17. Hussain L, Akash MSH, Naseem S, Rehman K, Ahmed KZ. Antiulcerogenic effects of *Salmalia malabarica* in gastric ulceration-pilot study. Adv Clin Exp Med. 2015;24:595-605.
18. Pan JS, He SZ, Xu HZ, Zhan XJ, Yang XN, Xiao HM, et al. Oxidative stress disturbs energy metabolism of mitochondria in ethanol-induced gastric mucosa injury. World J Gastroenterol. 2008;14(38):5857-5867.
19. Alirezaei M, Dezfoulian O, Neamati S, Rashidipour M, Tanideh N, Kheradmand A. Oleuropein prevents ethanol-induced gastric ulcers via elevation of antioxidant enzyme activities in rats. J Physiol Biochem. 2012;68(4):583-592.
20. Rishabha M, Ajay K, Anupama S, Kulkarni GT. Pharmacological screening, ayurvedic values and commercial utility of *Aegle marmelos*. Int J Drug Dev Res. 2012;4:28-37.
21. Vaseem A, Subhani G, Afshan K, Ali M, Khan MMA, Rumana MT. Effect of *Ocimum sanctum* Linn. in stress induced gastric ulcers in rats. Int J Med Res Health Sci. 2015;4(2):508-510.
22. Drini M. Peptic ulcer disease and non-steroidal anti-inflammatory drugs. Aust Prescr. 2017;40(3):91-93.
23. Bjarnason I, Scarpignato C, Holmgren E, Olszewski M, Rainsford KD, Lanas A. Mechanisms of damage to the gastrointestinal tract From nonsteroidal anti-inflammatory drugs. Gastroenterology. 2018;154:500-514.
24. Rafiq M, Rizwan M, Ahmad HI, Lashari MH, Shahzad KA. Incidence and risk factors associated with peptic ulcer in different cities of Punjab, Pakistan. Biomed Lett. 2024;10(1):51-58.
25. Venturini CL, Sabino Damazo A, José Dias Silva M, de Araujo Isaias Muller J, Maria Oliveira D, de Freitas Figueiredo F, et al. Antiulcer activity and mechanism of action of the hydroethanolic extract of leaves of *Terminalia argentea* Mart. In different in vivo and in vitro experimental models. J Ethnopharmacol. 2024;318(B):116972.