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# Exploring the Gastroprotective and Antioxidant Potential of *Ocimum tenuiflorum* Ethanol Extract in an Ethanol-Induced Gastric Ulcer Model

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**KEYWORDS**

*Ocimum tenuiflorum*,

Malondialdehyde,

DPPH,

Gastric ulcers

**ABSTRACT:**

**Purpose:** This study aimed to provide pharmacological evidence of *Ocimum tenuiflorum* in preventing or healing peptic ulcers

**Methods:** The leaves *of Ocimum tenuiflorum* were collected from the western ghats of India and dried in a shed followed by extraction using the Soxhlet apparatus. Following extraction, Phytochemical tests were performed to check the presence of phytoconstituents. To demonstrate the antiulcer effect Ethanol-induced ulceration method was used. The extract's antioxidant activity and free radicle scavenging activity were studied using DPPH assay & DMSO test. The healing effect was Investigated after 7 days of dosing with extract at the doses of 125 mg/kg, 250mg/kg & 500 mg/kg. The ulcer index and pH of excised tissue were measured and the sample was analysed for histopathological changes. Malondialdehyde and nitric oxide tests were also performed to test the mucoprotective properties.

**Results**: The extract of *Ocimum tenuiflorum* at 250mg/kg & 500mg/kg reduced ulcer index and showed mucosal protection in histopathological evaluation against ethanol-induced gastric ulcers in mice. The plant extract had $IC_{50}$ of 226 (µg/ml) and showed strong antioxidant and free radicle scavenging properties.

**Conclusion**: *Ocimum tenuiflorum* plant showed positive results in gastric cytoprotection and ulcer healing. Further investigations are needed to highlight mechanism-based effects.

## 1. Introduction

A peptic ulcer is characterised by damage to a particular section of the gastrointestinal system produced by gastric acid or pepsin[1]. It might appear as a gastric, duodenal, or oesophageal ulcer. Symptoms of a peptic ulcer include nausea, vomiting, bloating, appetite changes, and burning[1]. Duodenal and gastric ulcers (GU) have a complex aetiology that is most likely caused by a mix of pathophysiologic abnormalities, and environmental, and hereditary factors[1]. Peptic ulcers are typically caused by *Helicobacter pylori* (HP), nonsteroidal anti-inflammatory medications, or other factors that affect mucosal defence and healing systems[1] . Current antiulcer treatments focus on stomach acid secretion, neutralise acid, prevent ulcers, and target *H. pylori*[2]. Gastric acid secretion-reducing drugs are known as H2 antihistamines or anticholinergics[2]. However, they can cause adverse effects such as blurred vision, dry mouth, constipation, gynecomastia, and galactorrhoea. Proton pump inhibitors, another kind, have been related to serious renal problems[3]. Prolonged usage of prostaglandin analogues may cause gastrointestinal discomfort and diarrhoea[3]. The pathogenesis of ethanol-induced peptic ulcers in rats involves a chain of events including disruption of the mucosal barrier due to decreased mucus secretion, increased gastric acid secretion leading to increased acidity, inhibition of prostaglandin synthesis disturbing mucosal integrity and blood flow regulation, oxidative stress-mediated by reactive oxygen species generation during ethanol metabolism, and subsequent inflammatory response characterised by release of inflammatory mediators and immune cell infiltration[4]. The literature showed that a variety of Ayurveda physicians and conventional medical practitioners treat gastric ulcers using a wide range of medicinal plants and polyherbal compositions[5].

Journal of Chemical Health Risks

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The Lamiaceae family includes the Ocimum species (*O. americanum, O. basilicum, O. gratissimum, and O. tenuiflorum*). Currently, it is employed as a traditional medicinal herb in Africa, India, and other nations. It is used to treat a variety of illnesses and conditions in Ayurveda and traditional Chinese medicine[6]. *Ocimum tenuiflorum* - Holy basil has upright, many-branched stems that are 30 to 60 cm tall and hairy. The leaves can be green or purple, simple, and have an oval blade that can reach up to 5 cm (2.0 in) in length. The blade commonly has a slightly serrated edge[6]. The leaves also have a desiccate phyllotaxy and a strong aroma to it[6]. On lengthy racemes, the purple blossoms are arranged in tight whorls[6]. In India and Nepal, two primary morphotypes are cultivated: green leaves (Sri or Lakshmi Tulasi) and purple leaves (Krishna Tulasi)[6]. The whole plant contains phytoconstituents such as β caryophyllene and methyl eugenol which are responsible for its pharmacological action[6,7]. As per the literature review, this plant has Antioxidant, Anti-inflammatory, wound-healing, and antimicrobial activity[8]. Ursolic acid, eugenol, carvacrol, linalool, caryophyllene, estragole, Rosmarinic acid, apigenin and cirsimaritin are the important compounds present in the leaves *O. tenuiflorum*[8]. According to previous research Ursolic acid has anti-inflammatory, wound-healing properties Eugenol has wound-healing properties Rosmarinic acid has free radicle scavenging properties[8]. Based on the fact that gastric ulcer induction involves oxidative stress and inflammation, the current study examines the antiulcer potential of this plant extract against the gastric pathophysiological alterations induced by ethanol in Swiss albino mice.

## 2. Material and Methods

### Collection and Authentication of Plant

The leaves of *Ocimum tenuiflorum* were collected from the western ghats of Maharashtra and dried under shade. The plant was authenticated by Mr. Mahesh Atale, MSc. Botany, Alarsin Pioneers in Ayurvedic Research, Andheri (E), Mumbai – 400 093 and specimen were submitted to Pharmacology department, Konkan Gyanpeeth Rahul Dharkar College of Pharmacy, Karjat, Maharashtra- 410201.

### Selection of Animals

Healthy male Swiss albino mice, 8-9 weeks old were used for the study. Mice were kept at the animal house in polypropylene cages, at 22 ± 20C, with a 12:12 hrs dark: light cycle. They were fed commercial feed and water and given *ad libitum*. Experimental protocols (HRFT/IAEC/2023-2024/10) were subjected to the scrutinization of the Institutional Animal Ethical Committee and were cleared by the same. All experiments were performed in the morning according to current guidelines (CCSEA) for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals[9].

### Chemicals and Reagents

All reagents, chemicals & Instruments were procured from Konkan Gyanpeeth Rahul Dharkar College of Pharmacy, Karjat, and Maharashtra. The pantoprazole tablet was procured from a local pharmacy shop.

### Preparation of extract

The dried leaves were coarsely powdered and extracted with 60% ethanol using a Soxhlet apparatus. The extracts were concentrated under a vacuum to obtain a dry residue. The percentage of yield was calculated[10,11].

### Preliminary Phytochemical Screening

The concentrated extracts were subjected to chemical tests as per the methods mentioned below for the identification of the various constituents such as Alkaloids, glycosides, tannins etc[12]

### Acute oral toxicity ($LD_{50}$) & Dose selection

An acute toxicity study was performed for the extracts to ascertain a safe dose by the acute oral toxic class method of the Organization of Economic Co-operation and Development (OECD), as per 423 guidelines[13]. In the literature survey, it was confirmed that the Ethanolic extract of *Ocimum tenuiflorum* leaves was safe and the $LD_{50}$ was reported to be 5000mg/kg[13]. Thus, for the research study, the doses of *Ocimum tenuiflorum* leaf extract were finalized to be 125mg/kg (low dose), 250mg/kg (medium dose), and 500mg/kg (high dose)[13].

Journal of Chemical Health Risks

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JCHR (2024) 14(5), 483-491 | ISSN:2251-6727

**In-Vitro Anti-Oxidant Studies**

**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 (George et al., 1996). 1 ml of test compound water was taken in the test tube. 1 ml of 0.1% ethanolic DPPH was added to the samples and incubated for 30 minutes in dark conditions. The samples were then observed for discolouration; from purple to yellow and pale pink were considered strong and weak positive respectively and, the absorbance of the mixture was measured at 518 nm[14].

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

**Alkaline DMSO Method**

In this method, superoxide radical is generated by the addition of sodium hydroxide to air-saturated DMSO. The generated superoxide remains stable in solution and reduces nitro blue tetrazolium (NBT) into formazan dye at room temperature which can be measured at 560 nm. To the reaction mixture containing 1000 µL of alkaline DMSO, 300 µL of the drug samples in concentration 1000µg/ml and standard ascorbic acid 1000µg/ml was added in DMSO at various concentrations followed by 100 µL of NBT (0.1 mg). The absorbance was measured at 560 nm[14].

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

**Anti-ulcer activity (In-vivo Model)**

Animals were randomly assigned to 6 groups (n=06).

Group 01: Normal - Vehicle 10 ml/kg p.o.

Group 02: Ulcer Control: Vehicle 10 ml/kg + Ethanol (1 ml/kg) p.o.

Group 03: Standard: Pantoprazole 40 mg/kg + Ethanol (1 ml/kg) p.o.

Group 04: Test dose 1: OTEE 100 mg/kg + Ethanol (1 ml/kg) p.o.

Group 05: Test dose 2: OTEE 200 mg/kg + Ethanol (1 ml/kg) p.o.

Group 06: Test dose 3: OTEE 400 mg/kg + Ethanol (1 ml/kg) p.o.

Mice were fasted for 12 hrs. before the start of the experiment. Then the first group received an oral dose of the vehicle (1 ml/kg) and the third group pantoprazole (40 mg/kg), $4^{th}$, $5^{th}$ and $6^{th}$ groups received 125, 250, 500 mg/kg body weight of *Ocimum tenuiflorum* ethanolic extract (OTEE) respectively. This treatment was given for 7 days. On the $8^{th}$ day of the experiment ethanol was administered to the animals and euthanasia was carried out in a Co2 chamber followed by extraction of gastric tissue collection of gastric content and microscopical evaluation of tissue for abnormalities in the mucosal lining. Samples were then forwarded for histopathological evaluation[15].

The stomachs of each animal were extracted and opened along the greater curvature, and for examination of lesions. Lesions were counted by analysis in ImageJ software. The ulcers were scored according to the following scale:

0 = Normal, 0.5 = Red Coloration, 1 = Spot ulcer, 1.5 = Haemorrhagic streak, 2 = Deep lesions, 3 = Perforations

**Determination of ulcer index:** After scoring ulcers according to their severity, the mean ulcer score for each animal was expressed as ulcer index. The ulcer index was measured by using the following formula[15].

Ulcer Index (UI)= UN+US+UP ×10-1

Where, UI = Ulcer Index; UN = Average number of ulcers per animal; US = Average number of severities score UP = Percentage of animals with ulcer • % Inhibition of ulceration = (Ulcer index Control- Ulcer Index Test) ×100/ Ulcer Index Control

**Statistical Analysis:**

Statistical analysis was carried out using Graph Pad Prism5 software version 10.2.0 (Graph Pad Prism software Inc.) The values were expressed as mean ± SEM. The statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by DUNNET'S T-Test.

P values< 0.05 were considered significant.

Significance levels were as follows: * Indicates $p \leq 0.5$ as significant; ** indicates $p \leq 0.01$ as highly significant; *** indicates $p \leq 0.001$ as very significant.

Journal of Chemical Health Risks

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JCHR (2024) 14(5), 483-491 | ISSN:2251-6727

**Histopathological Evaluation**

Stomach tissues were stored in a 10% buffered formalin solution for histopathology assessment. Samples were analysed by "Unique Bio Diagnostics Enterprises" in Mumbai, India.

**Biochemical Tests**

**Determination of malondialdehyde (MDA):** Stomach ulcer tissue homogenate was prepared, a mixture of 0.4 ml of 10% stomach ulcer homogenate added 1.5 ml of 8.1% Sodium dodecyl sulphate. 1.5 ml of 20% acetate buffer (pH 3.5) and 1.5 ml of 0.8% TBA solution were added to the above mixture. The mixture was heated at 95℃ for 60 min and cooled to room temperature. After cooling add 5 ml of n-butanol-pyridine (15:1). Vortexed the mixture thoroughly and allowed it to stand until the organic and aqueous layers did not get separated. Recorded absorbance of the organic layer at 532 nm on a UV-visible spectrophotometer [16].

**Determination of total nitrite/nitrate contents (N.O.)**: Stomach ulcer tissue homogenate was prepared, taken a mixture of 0.4 ml of 10% stomach ulcer homogenate was added 50µl 30% ZnSO4 for protein precipitation. Then, the precipitated protein was removed by centrifugation for 15 minutes. 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 cooled. Recorded absorbance at 540 nm on a UV-visible spectrophotometer[17].

## 3. Results

**Preliminary Phytochemical Screening**

Table no. 01 represents the organoleptic characteristics of the extract similarly, Table no. 02 shows the results of basic phytochemical tests.

**Table 1 Organoleptic characteristics of *Ocimum tenuiflorum* extract**

| Sr. No | Organoleptic Properties | Observation |
|---|---|---|
| 1 | Colour | Green |
| 2 | Odour | Aromatic |
| 3 | Taste | Slightly pungent |
| 4 | Texture | Smooth |

**Table 2 Results of preliminary phytochemical tests of *Ocimum tenuiflorum* extract**

| Compounds | Test | Inference |
|---|---|---|
| Alkaloids | Mayer's Test | Present |
| | Wagner's Test | Present |
| Flavonoids | Shinoda Test | Present |
| | Ferric Chloride Test | Present |
| Glycosides | Keller-Kilani Test | Absent |
| Tannins | Ferric chloride test | Present |
| | Lead acetate | Present |
| Saponin | Foam test | Present |
| Carbohydrates | Molisch's test | Absent |
| | Benedict's test | Absent |

**In Vitro Antioxidant Activity**:

**DPPH Assay**: DPPH assay is used to assess the free radicle scavenging property of extract by comparing the Inhibitory concentration ($IC_{50}$) of the test (OTEE) and standard (Ascorbic acid) as shown in Table no. 03 it was observed that *Ocimum tenuiflorum* ethanolic extract demonstrated stronger antioxidant activity.

Ascorbic acid (Standard) $IC_{50}$ = 208 (µg/ml)

OTEE $IC_{50}$ (Test) = 226 (µg/ml)

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(5), 483-491 | ISSN:2251-6727

**Table 3 Observation of % inhibition of DPPH Ascorbic acid and OTEE (Test sample)**

| Sr no | Concentration (µg/ml) | % Inhibition Ascorbic Acid | % Inhibition OTEE |
|---|---|---|---|
| 1 | 12.5 | 11.29 | 22.58 |
| 2 | 25 | 16.20 | 31.91 |
| 3 | 50 | 25.18 | 26.31 |
| 4 | 100 | 44.90 | 31.40 |
| 5 | 200 | 66.29 | 47.53 |
| 6 | 400 | 67.5 | 71.30 |

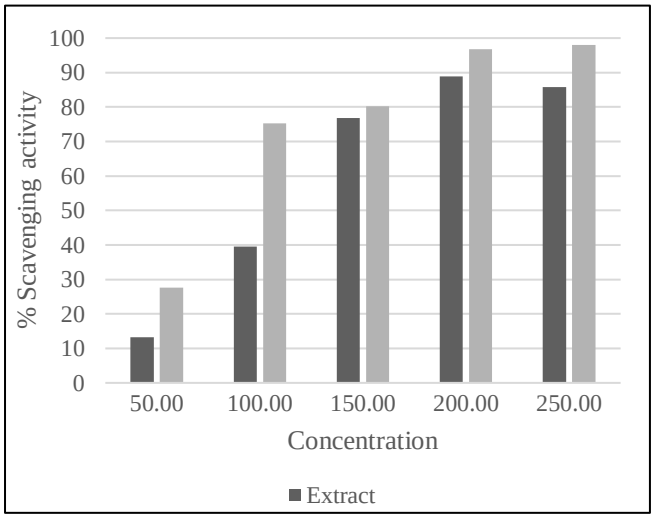


**Figure 1 Superoxide Radical Scavenging Activity of *Ocimum tenuiflorum* ethanolic Extract by Alkaline DMSO**

**Anti-oxidant activity by alkaline DMSO assay**

The Alkaline DMSO method is a technique used to assess the antioxidant potential of various compounds. As shown in Figure no 03 superoxide radical scavenging activity of *Ocimum tenuiflorum* extract was assessed by the alkaline DMSO method. The extract sample strongly inhibited the superoxide radical generation.

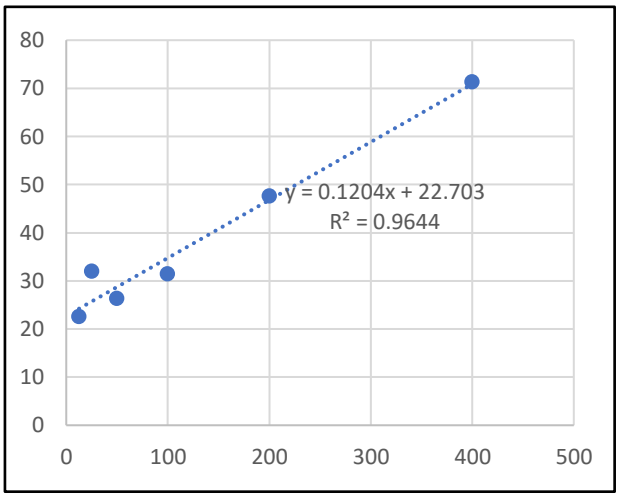


**Figure 2 : Standard curve of the Test sample (OTEE)**

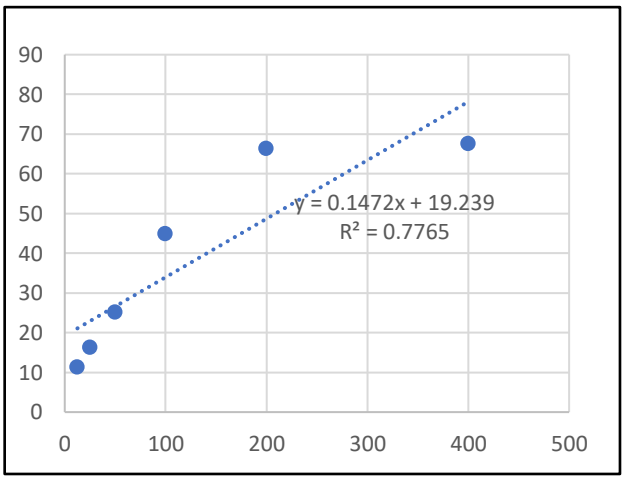


**Figure 3: Standard curve of ascorbic acid**

**In Vivo Antiulcer Activity:**

**I. Ulcer Index & % Inhibition of Ulcer:**

It was found that 500 mg/kg of *Ocimum tenuiflorum* extract dose shows significant inhibition of ulcers compared with the standard and ulcer control group.

Table no 04 shows that the % of the occurrence of ulcers in the stomach is significantly inhibited by the standard drug pantoprazole and 125 mg/kg, 250 mg/kg, and 500 mg/kg of OTEE. [F (5,29) = 9.67; P < 0.05, Fig no. 04].

Journal of Chemical Health Risks

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JCHR (2024) 14(5), 483-491 | ISSN:2251-6727

**Table 4 Observation Table for Ulcer Index & % Inhibition of Ulcer**

| Treatment | Dose(mg/kg) | Ulcer Index | % Inhibition of Ulcer |
|---|---|---|---|
| Ulcer Control | 1 ml/kg | 46.63 ± 5.712 | - |
| Standard (Pantoprazole) | 40 ml/kg | 19.63 ± 3.687** | 57.90 |
| OTEE | 125 mg/kg | 27.89 ± 5.617 | 40.18 |
| OTEE | 250 mg/kg | 26.90 ± 6.999 | 42.31 |
| OTEE | 500 mg/kg | 24.52 ± 6.082* | 47.41 |

All values represent Mean ± SEM, n=6 in each group.

P < 0.05. The control group is compared with standard and extract doses, and * represents the significance.

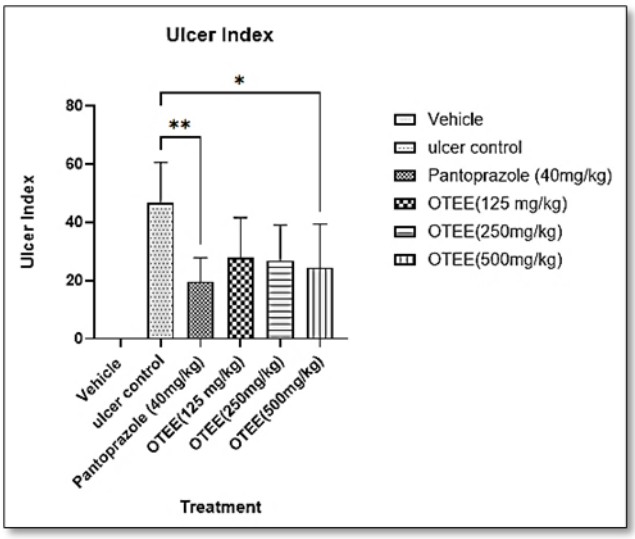


**Figure 4 Effect of OTEE on ulcer index in Ethanol induced ulcer**

## II. pH of Gastric Juice:

Low pH is responsible for more damage and abrasion in the mucosal lining.

In this study, pH is increased by standard (Pantoprazole) and test (*Ocimum tenuiflorum* ethanolic extract 125mg/kg, 250mg/kg, 500mg/kg) group when compared with the ulcer control group. [F (4, 25) = 8.295; p < 0.05, Fig no. 05]

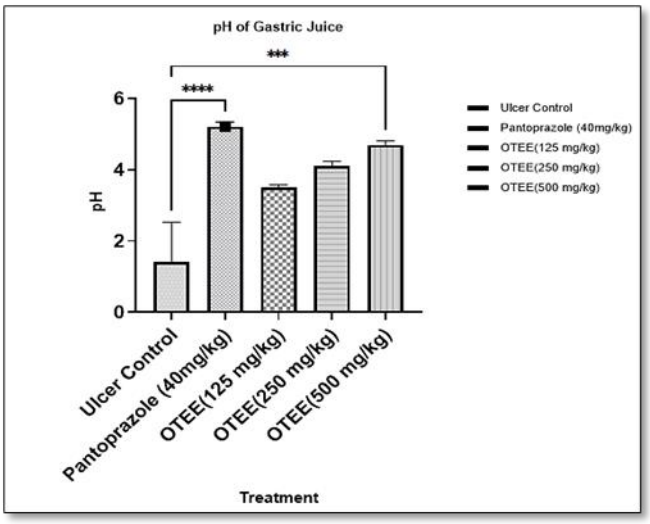


**Figure 5 Effect of OTEE on pH of gastric juice**

## III. Morphological and histopathological images of gastric tissue

Ethanol increases vascular permeability and exposes gastric mucosa to the proteolytic and hydrolytic actions of pepsin and HCl, causing blood flow stasis and microvascular disruption.

As shown in Figure 6, the Pantoprazole (Std.) group had fewer haemorrhages, damaged epithelial structure and cellular infiltration showing nearly normal gastric mucosa. OTEE (125mg/kg) and OTEE (250mg/kg) showed moderate haemorrhages, and structural damage whereas, OTEE (500mg/kg) showed mild haemorrhages and cellular infiltrations as well as less structural damage.

Journal of Chemical Health Risks

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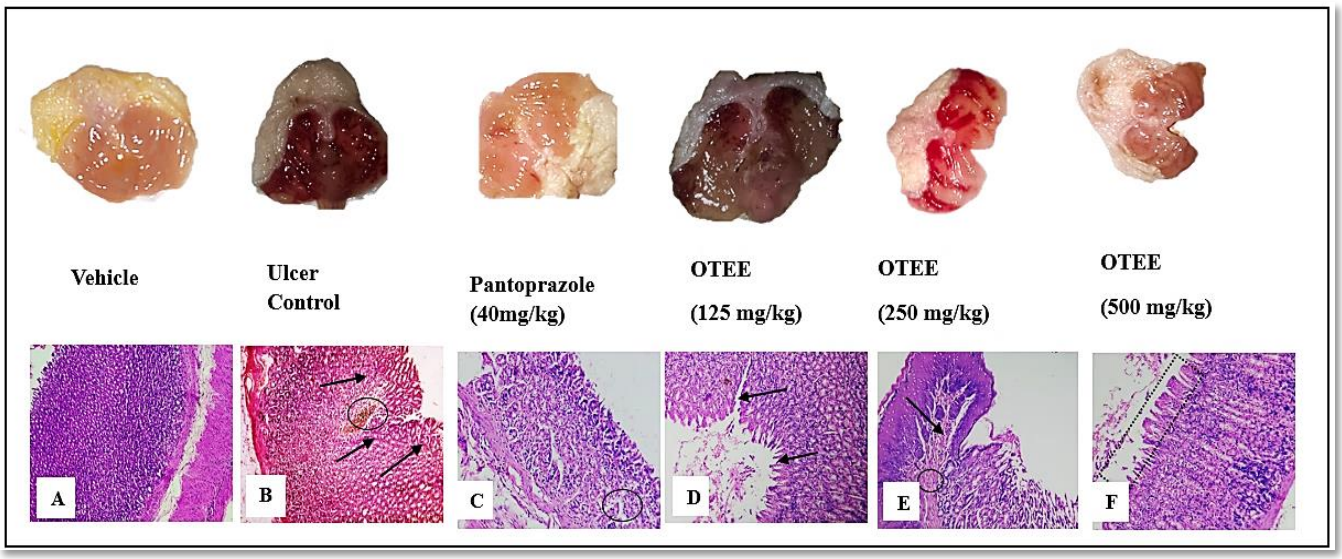


***Figure 6*** **Histopathological samples illustrating stomach tissue from various experimental groups: Vehicle (A); Ulcer control (B); Standard (Pantoprazole) (C); OTEE 125 mg/kg (D); OTEE 250 mg/kg (E); and OTEE 500mg/kg (F). Note: Structural loss of tissue (arrows), hyperaemia (circles)**

## I. Biochemical Tests

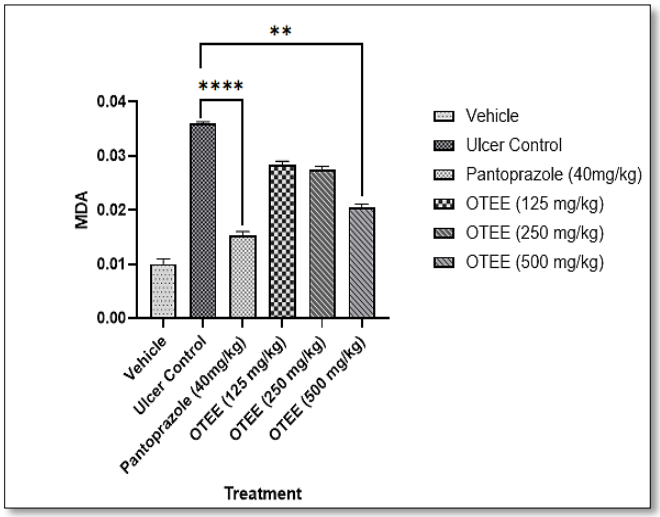


**Figure 7 Effect of OTEE (125, 250 & 500 mg/kg) on malondialdehyde (MDA) levels in gastric tissues. Values are expressed as mean ± S.E.M. (n = 6)**

As shown in Figure (07), the Ulcer control (ethanol) group increased the gastric MDA level compared to the vehicle group ($P < 0.05$). Administration of OTEE at doses of 125, 250 and 500 mg/kg, similar to the Pantoprazole group exhibited a dose-dependent reduction in MDA levels. [$F (5, 30) = 19.45$; $p < 0.05$, Fig no.07]

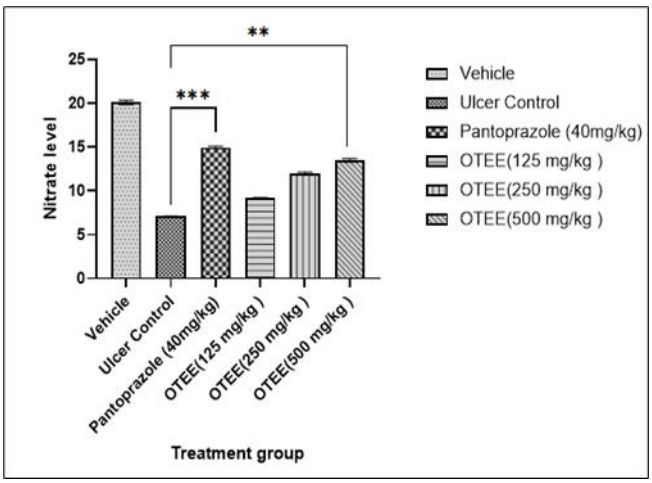


**Figure 8 Effect of OTEE (125, 250 & 500 mg/kg) on nitric oxide (N.O) levels in gastric tissues. Values are expressed as mean ± S.E.M.**

The results (Fig.08) showed that Ethanol significantly reduced the gastric nitric oxide levels in mice. Administration of test doses of the extract showed a dose-dependent increase in gastric nitric oxide levels Fig.08. [$F (5,30) = 12.8$; $p < 0.05$, Fig no.08] Values are expressed as mean ± S.E.M.; (n = 6)

### Discussion

Preliminary phytochemical analysis of Ethanolic extract of *Ocimum tenuiflorum* leaves showed the presence of alkaloids, flavonoids, phenols, tannins, fixed oils, and

Journal of Chemical Health Risks

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JCHR (2024) 14(5), 483-491 | ISSN:2251-6727

glycosides. The Study suggests that *Ocimum tenuiflorum* proves to exhibit Anti-Ulcer activity. It has been shown to improve mucosal protection and reduce gastric pH and mucosal abrasion and lesions, gastric levels of MDA were found to be decreased and nitrite levels were increased which shows the anti-inflammatory and muco-protective nature of test extract. The extract contains various flavonoid constituents (Caffeic acid, Rosmarinic acid) responsible for anti-oxidant & free radicle scavenging properties. This was analysed by performing DPPH and DMSO assays on the extract before animal testing. A further detailed study needs to be performed to understand the possible mechanism of action for Anti-Ulcer activity.

**Conclusion**:

This study demonstrated the protective and healing effect of the ethanolic extract of *Ocimum tenuiflorum* on induced gastric ulcers. These results partially justify the plant's traditional use based on active phytochemical compounds and highlights its potential use towards healing of ulcer while suggesting more need of research for its mechanism of action.

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