



Evaluation of Anti-Ulcer Activity of the Seeds of *Oroxylum Indicum* in Swiss Albino Mice Against Ethanol-Induced Gastric Ulcer Model.

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KEYWORDS

Peptic ulcer, *Oroxylum indicum* seeds, Ulcer Index, Anti-oxidant.

ABSTRACT:

Objectives: The objective of this study is to assess the antiulcer effect of drug *Oroxylum indicum* in mice with ethanol-induced gastric ulcers. This study aims to evaluate ulcer healing by multiple parameters, including its ability to reduce oxidative stress, inhibit gastric acid secretion, offering a comprehensive understanding of its therapeutic potential.

Methods: The *Oroxylum indicum* seeds extract was extracted by maceration process, and further phytochemical screening was performed to detect phytochemicals in it. Some in-vitro tests of DPPH and Alkaline DMSO were performed to study the presence of antioxidants in the extract. Also, ethanol-induced gastric ulcer model has been selected for the study using *Oroxylum indicum* seeds extract at dose of 50 mg/kg, 100 mg/kg and 200 mg/kg. pH of the stomach tissue was measured and also Malondialdehyde and nitric oxide tests were performed.

Results: The results obtained from the data revealed that *Oroxylum indicum* seeds extract have an antioxidant effect and at a dose of 200 mg/kg 49.53% have the highest healing score as compared to standard pantoprazole 40 mg/kg 57.84%. Histopathological examination shows more ulcers in the disease control group and fewer ulcers were observed in *Oroxylum indicum* seeds extract group.

Conclusions: The present study concluded that *Oroxylum indicum* seed extract shows a good anti-ulcer effect at 200 mg/kg dose with its gastroprotective mechanism and antioxidant properties.

1. Introduction

Peptic ulcers are open sores that occur inside the lining of the stomach, esophagus and upper portion of small intestine. It mainly occurs due to an imbalance between aggressive (acid, pepsin) factor and defensive (mucus, bicarbonate secretions, prostaglandins) factor[1].

It mainly causes epigastric pain which is a primary symptom characterized by stomach discomfort that is frequently elevated by meals or other harmful substances, in addition to causing patients great suffering and also affecting patients' mental health[2], [3]. Medicinal plants play a major role in human health care because of their better compatibility and lesser side effects. Though many medicines have been developed for the treatment of peptic ulcers and their management, in this study an attempt has been made to show how *Oroxylum indicum* seeds that belongs to family

Bignoniaceae help in treating ulcers in mice with all focus on safety and efficacy. *Oroxylum indicum* plant is grown in many Asian countries and it contains many chemical components that have been proven to show different pharmacological activities, among which are the gastroprotective effect of *Oroxylum indicum* seed extract and investigate the mechanism underlying this effect[4], [5], [6]. A variety of experimental studies has been performed both in-vitro and in-vivo to determine the various uses of the plant. Also, various important chemical constituents have been isolated, which act as a source of medicinally important compounds. Flavonoids, the most abundant and important constituents, are found in almost all parts of the plant. Several studies have been performed to reveal information on *O. indicum*.



Fig 1. *Oroxyllum indicum* Seeds

2. Materials and Methods:

Collection and Authentication of plant:

The seeds of *Oroxyllum indicum* were collected from Pune and their Authentication was done by Mr. Mahesh Atale, MSc. Botany, Alarsin Pioneers in Ayurveda Research, Mumbai.

Selection of Animal:

Male healthy Swiss albino mice of weight 25-30 gm were selected for the use of the study. A total number of 36 mice were obtained from the central animal facility of the institute by Institutional Animal Ethics Committee and were acclimatized for seven days. Mice were kept separately in a clean propylene cage, at room temperature 22 ± 2 °C with 12 hours of day and night cycle. Mice were provided with a standard feed and water ad libitum.

Preparation of extract:

The obtained seeds were washed and dried in the sunshine. A coarse powder was created by crushing the dried seeds. These powdered seeds were soaked in ethanol for 24 hours at 4°C. The extract was then filtered using Whatman filter paper. The resulting solution was subjected to a rotary vacuum evaporator. The sample was dried in a lyophilizer and the solid residue was obtained. The resulting brownish extract was stored in a container and kept in a refrigerator (0-4°C) until use[7].

Preliminary Phytochemical Screening:

The phytochemical analysis of ethanolic seeds extract of *Oroxyllum indicum* was carried out to reveal bioactive

compounds such as alkaloids, steroids, glycosides, phytosterols, phenols, saponin, terpenoids and flavonoids[8].

Acute Oral Toxicity Studies:

The safety of *Oroxyllum indicum* seed extract was determined by a literature review, where the acute oral toxicity was carried out as outlined in OECD guidelines, it stated that the ethanolic extract has an LD₅₀ of 2000 mg/kg. Therefore, the doses of *Oroxyllum indicum* seed extract were determined to be 50 mg/kg, 100 mg/kg and 200 mg/kg[9].

In-Vitro Anti-Oxidant Studies:

Determination of Antioxidant Activity by DPPH radical scavenging assay:

The antioxidant activity of the sample compound or extract was evaluated by free radical scavenging activity using DPPH (1,1-diphenyl-2, picryl-Hydrazyl) free radical. 1 ml of extract of different concentrations (50,100,150,200,250 µg) was added to the test tube along with 1 ml of 0.1% ethanol DPPH solution. The final mixtures were then incubated in the dark for 30 mins. Then, the colour change of the sample was observed. The colour change from purple to yellow and pale pink was considered strong positive and weak positive, responsibility, and the absorbance of the mixture was measured at 517 nm. Ascorbic acid was used as standard. DPPH radical scavenging activity (%) = [(control absorbance – test sample absorbance) / (control absorbance)] × 100

The IC₅₀ value of the crude extract was compared with that of ascorbic acid, which was used as standard. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity[10].

Superoxide radical scavenging activity by Alkaline DMSO method:

To the reaction mixture containing 100 µL of alkaline DMSO, 30 µL of the drug samples in concentration (50 µg/ml to 250 µg/ml) and standard ascorbic acid (50 µg/ml to 250 µg/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)] × 100[11].



Grouping of animals:

All mice were divided into 6 groups (n=6), Each group included six animals divided into normal control, disease control, standard control and three test doses (low, medium and high dose). Animals were housed separately in propylene cages at 22°C and (12:12) light-dark cycle. All animals were acclimated for 7 days before the experiment. Animals were given food and water regularly. Food was removed 24 hrs before the experiment but water was still available. (add a grouping of animals)

Group 1: Normal Control- Vehicle 10 ml/kg p.o.

Group 2: Ulcer Control- Vehicle 10 ml/kg + Ethanol (1 ml/kg) p.o.

Group 3: Standard Pantoprazole 40 mg/kg + Ethanol (1 ml/kg) p.o.

Group 4: Test dose 1- OISE 50 mg/kg + Ethanol (1 mg/kg) p.o.

Group 5: Test dose 2- OISE 100 mg/kg + Ethanol (1 mg/kg) p.o.

Group 6: Test dose 3- OISE 200 mg/kg + Ethanol (1 mg/kg) p.o.

Dosing of animals:

During the study period, animals in the normal control group were administered saline only and those in the disease control group were administered Vehicle and ethanol. The standard group was administered with 40 mg/kg of Pantoprazole and Test groups 4, 5, and 6 were administered with 50 mg/kg, 100 mg/kg and 200 mg/kg of *Oroxylum indicum* seed extract. All groups were orally treated for 7 days.

Induction of ulcer:

As all the mice received their respective doses, after 60 mins all groups were orally treated with 1 ml of ethanol solution for gastric ulcer induction except the normal control group. Animals were euthanised after 1 hour of ethanol administration and stomachs were excised to determine gastric damage. The stomach content obtained was centrifuged at speed of 1000 revolutions per minute for 20 mins to evaluate gastric pH[12].

The stomach was cut and stretched out at the greater curvature and the number of ulcers was determined microscopically with the use of the lens.

The ulcer score was calculated by following the system,

0 – No ulcers

1 – Superficial ulcer

2 – Deep ulcer

3 – Perforation

Ulcer index was calculated by the formula[13]:

$$UI = UN + US + UP*10^{-1}$$

Where, UI = Ulcer Index; UN = Average of number of ulcers per animal;

US = Average of severity score and UP = Percentage of animal with ulcer.

$$\text{Percentage ulcer inhibition} = \frac{UI(\text{Control}) - UI(\text{Test})}{UI(\text{Control})} \times 100$$

Histopathological Examination:

The gastric tissues from the mice were fixed with a 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 haematoxylin and eosin (H&E). Pathological observations were performed with a light microscope.

Biochemical Test:

Determination of Malondialdehyde:

In this assay stomach ulcer tissue homogenate was prepared, taken a mixture of 10% stomach ulcer homogenate added 8.1% Sodium dodecyl sulphate. And 1.5ml of 20% acetate buffer (pH 3.5) and 1.5 ml of 0.8% TBA (Thiobarbituric acid) solution were added to the above mixture. The mixture was heated at 95°C for 60 minutes and cooled to room temperature. After cooling added 5 ml of n-butanol-pyridine as described in the article, vortexed the mixture thoroughly and allowed to stand until the organic layer at 532 nm on a UV-visible spectrophotometer[14].

Determination of Nitrate Content:

Nitric oxide content was quantified by measuring nitrite/nitrate concentration using the Griess reagent. Briefly, the above homogenate was deproteinized by



absolute ethanol for 4hr at 4°C and it was centrifuged at 12,000 g for 15 mins. Vanadium trichloride was added to an aliquot of supernatant at 0.8% (w/v) of 1 ml HCL for reduction of nitrate to nitrite. Followed by the addition of Griess reagent. Absorbance was measured at 546 nm. Results are expressed as 1mol/gm tissue[15].

Statistical Analysis:

Statistical analysis was carried out using Graph Pad Prism5 software version 10.2.0 (Graph Pad Prism software Inc.) Data were expressed as mean \pm SEM. Results were analysed by Dunnett's t-test followed by a one-way analysis of variance (ANOVA). Each sample was compared with the control group. Differences were considered significant if $P < 0.05$.

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.

3. Results:

Phytochemical Screening:

Table 1. Results of Phytochemical screening of *Oroxylum indicum* seed Extract.

Phytochemical	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

As shown in above table.1, Phytochemical studies Qualitative phytochemical investigation discovered the presence of alkaloid compounds [Appearance of red colour]; flavonoids and tannins [The pink colour shows the presence of flavonoids and blackish precipitate indicated the presence of tannins] and carbohydrates were absent.

In-Vitro Anti-Oxidant Test on Extract:

Below Fig 2. shows antioxidant activity of Seeds of *Oroxylum indicum* extract was carried out by DPPH assay using Ascorbic acid as a standard reference Antioxidant..

IC₅₀ of Ascorbic acid(Standard) = 102.69 μ g/ml

IC₅₀ of seeds of *Oroxylum indicum* Extract = 122.58 μ g/ml.

Table 2. Observation of % Inhibition of DPPH Ascorbic acid and OISE Test sample.

Sr no.	Concentration (μ g/ml)	% Inhibition of DPPH Ascorbic Acid	% Inhibition of DPPH OISE
1	50	40.4	31.36
2	100	47.2	38.29
3	150	57.8	65.55
4	200	76.1	69.38
5	250	80.9	81.55

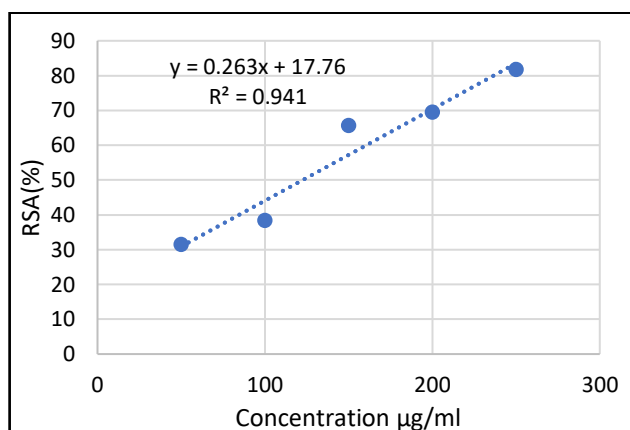


Fig 2. Standard curve of DPPH radical scavenging activity of seeds of *O. indicum* extract

Alkaline DMSO Method:

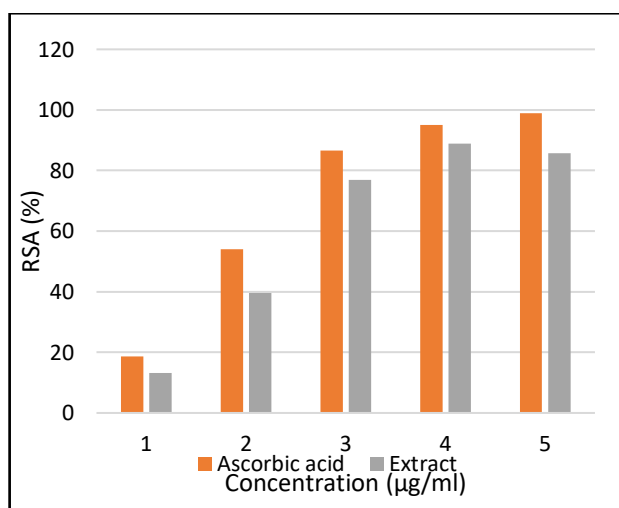


Fig 3. Radical Scavenging Activity of Seeds of *Oroxyllum indicum* Extract.

Above Fig 3. Shows the efficacy of *Oroxyllum indicum* seed extract in eliminating superoxide radicals which was evaluated through the alkaline DMSO method. The results indicated a notable decrease in the production of superoxide radicals by the extract.

Anti-Ulcer Evaluation:

Ethanol-induced Gastric Ulcers.

Ethanol-induced gastric damage showed gross mucosal lesions, including long haemorrhage bands and petechial lesions in ulcer control group. Animals pre-treated with ethanol extract of *Oroxyllum indicum* seeds and standard drug pantoprazole showed very mild lesions and sometimes no lesion at all. *Oroxyllum indicum* seeds

showed a dose-dependent curative ratio compared to ulcer control groups as shown in Table 4. The extract exhibited an inhibition percentage of 39.21%, 41.06% and 49.53% at doses of 50, 100 and 200 mg/kg doses respectively. The ulcer protective action of extracts at different doses was better than that of the standard drug, pantoprazole which exhibited an inhibition percentage of 57.84%.

Table 3. Effect of *Oroxyllum indicum* seeds on Ulcer index in ethanol-induced gastric ulcer.

Group	Ulcer index (UI)	Percentage inhibition (%)
Ulcer Control (1ml/kg)	45.64 ± 5.692	-
Pantoprazole (40mg/kg)	19.24 ± 3.136**	57.84%
OISE (50mg/kg)	27.74 ± 5.418	39.21%
OISE (100mg/kg)	26.90 ± 5.012	41.06%
OISE (200mg/kg)	22.03 ± 5.655**	49.53%

NOTE: OISE – *Oroxyllum indicum* seed extract.

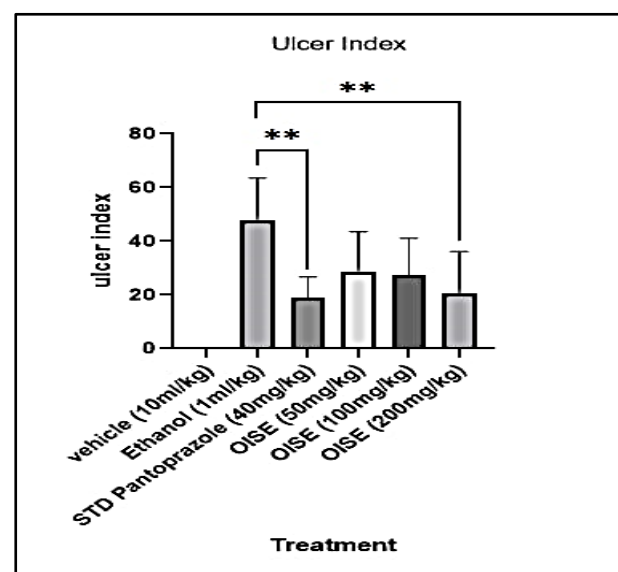


Fig 4. Effect of OISE on ulcer index in Ethanol-induced ulcer.

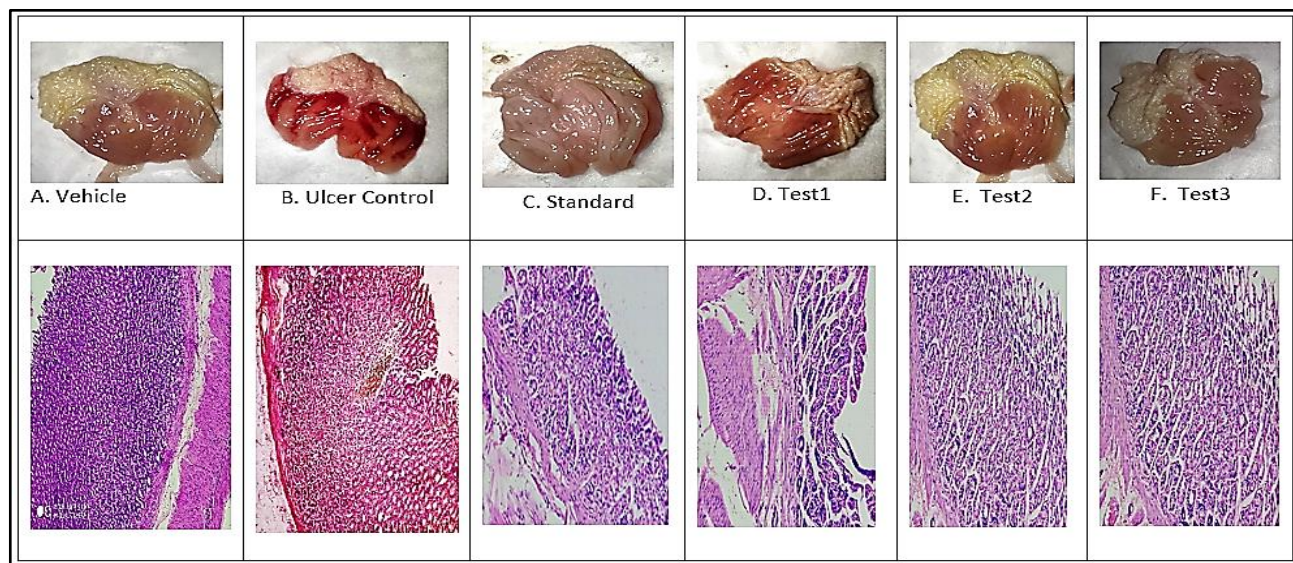


Fig.4 shows each group consist of 6 animals, Data is presented in mean \pm SEM** means significant difference ($p \leq 0.05$) in compare to ulcer control group.

Gastric pH:

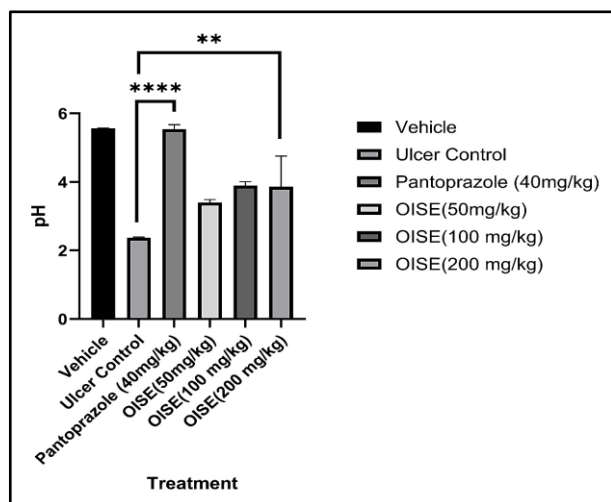


Fig 5. Effect of OISE on Gastric pH level

The above Fig 5. shows pH of gastric juice was increased in the OISE(200 mg/kg) group compared to the ulcer control group. The effect of Extract on pH showed a significant effect at 200mg/kg and 100mg/kg doses compared to the ulcer control group. As controlling pH is essential in ulcer healing by minimizing tissue damage, promoting an environment conducive to healing and supporting the effectiveness of *Oroxylum indicum* seed extract.

Fig. 6 Histopathological effect of Seeds of *Oroxylum indicum* extract on Ethanol-induced ulcers.

Morphological And Histopathological Image of Gastric Tissue:

Above Fig 6. shows Ulcer control group B. showing disruption of the epithelial structure of cells and interstitial oedema of submucosa and inflammation. Test group 3 shows more preserved gastric epithelium less oedema of submucosa and a gradual decrease in inflammation compared to ulcer control group B. Standard C group showing gastric epithelium preserved and discrete oedema of submucosa.

Biochemical Test:

Determination of Malondialdehyde (MDA):

MDA is a metabolite for oxidative stress which is generated by unsaturated fatty acids through ROS-activated lipid peroxidation. Thus, MDA is deemed as the biomarker of lipid peroxidation and used to quantify and identify oxidative stress.

As shown in below Fig 7. all three test doses of the ethanolic extract of seeds of *Oroxylum indicum* showed a dose-dependent decrease in MDA level activity when it was compared against the Ulcer control group.

Effect of ethanolic extract of *Oroxylum indicum* seeds Extracts on MDA level in ethanol-induced ulcer in Swiss albino mice.

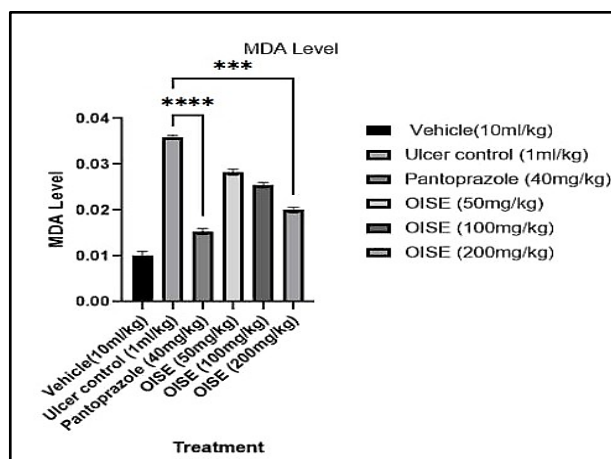


Fig 7. Effect of OISE on MDA levels in gastric tissue

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

Determination of Nitrate Content:

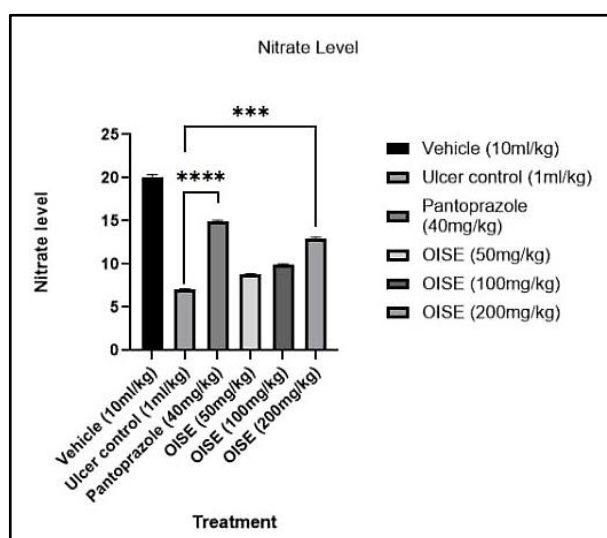


Fig 8. Effect of OISE on Nitrate levels of gastric tissue.

The result shown in the above Fig 8. shows that ethanol i.e. ulcer control group administration decreased the gastric nitric oxide level and all 3 OISE doses show a dose-dependent increase in nitric oxide level.

4. Discussion:

The present study was undertaken to screen anti-ulcer activities using experimental models in Swiss albino mice. Preliminary phytochemical tests were carried out on the ethanolic extract of *Oroxylum indicum* seeds

which showed presence of phytoconstituents like alkaloids, flavonoids, glycosides, tannins and saponin. The antioxidant activity was observed in DPPH radical scavenging assay. DPPH radical scavenging activity of *Oroxylum indicum* seed extract was compared with standard ascorbic acid. Although standard antioxidants had higher scavenging activity at all tested concentrations than the extract, the extract still showed good radical scavenging activity. Similarly, alkaline DMSO assay, the antioxidant activity of *Oroxylum indicum* seeds extract was assessed using ascorbic acid as the reference standard. The scavenging activity of the extract was found to be strongly comparable to that of the standard, reinforcing its potential as a significant antioxidant.

The anti-ulcer effect of ethanolic extract was evaluated using an ethanol-induced gastric ulcer model. The results revealed that the ethanol administration in the control group resulted in immense ulceration in comparison to with normal group. However, treatment with pantoprazole at a dose of 40 mg/kg and ethanolic extract of *Oroxylum indicum* seeds extract at doses of 50 mg/kg and 100 mg/kg prior to ethanol administration exhibited significant inhibition of ulcers in mice. Among the test samples, the best result was obtained at a dose of 200 mg/kg, which is potentially effective when compared with the standard drug.

Histopathological study indicates the ethanol-induced gastric damage showed gross mucosal lesions, including haemorrhage and structural damage. Ethanol increases permeability, and decreases gastric mucosa and inflammation. While the *Oroxylum indicum* extract shows a dose of 50 mg/kg and 100 mg/kg of mild preserved gastric epithelium compared to the ulcer control group. Extract at the high dose of 200 mg/kg shows the most preserved gastric epithelium and less gastric damage. Additionally, gastric levels of MDA and nitrate were found. The result showed an increase in NO that helps heal the ulcer by maintaining gastric mucosal integrity and regulation of acid, mucus secretion and gastric mucosal blood flow. This study also performed a malondialdehyde assay which results show extracts of *Oroxylum indicum* have decreased gastric MDA levels than the ulcer control group, which also signifies that it has higher antioxidant activity and can reduce oxidative stress caused by ulcers.



5. Conclusion:

It can be concluded by this study that seed extract of the *Oroxylum indicum* plant possesses a significant anti-ulcer property by its gastroprotective effect, it is evident from its significant reduction in a number of ulcers induced by acidified ethanol, reduction of inflammatory process, stimulation of cellular antioxidants mechanism and increase amount of mucus.

It treated animals significantly by inhibiting ulcer formation in mice decreasing acid secretion and increasing gastric pH, and by extracting antioxidant activity, thus extract of this plant can suppress gastric damage induced by aggressive factors.

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