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## EVALUATION OF ANTIULCER ACTIVITY OF ETHANOLIC EXTRACT OF *EUPHORBIA MILII* DES MOUL. LEAVES

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### ABSTRACT

Peptic ulcers are a common gastrointestinal disorder affecting millions of people worldwide. The main cause of peptic ulcers is the imbalance between the aggressive factors and the defensive factors in the stomach lining. Although there are several drugs available to treat peptic ulcers, prolonged use of these drugs can lead to several complications. Therefore, the use of plant-based drugs is gaining popularity as a safer alternative for treating peptic ulcers. This study employed the ethanol-induced method to evaluate the antiulcer activity of the ethanolic extract of *Euphorbia milii* Des Moul. Leaves in male Wistar albino rats. *Euphorbia milii* is a plant with several phytoconstituents that possess antioxidant, anti-inflammatory and mucus stimulating properties. *In-vitro* study was conducted using Fordtran's titration model and the results showed that the plant extract possess acid neutralizing capacity. For the *in-vivo* study, absolute ethanol was used for the induction of ulcer. The parameters studied include volume of gastric juice secretion, total acidity, pH of gastric juice, ulcer index and percentage protection, the results of which showed that the

plant extract could reduce the gastric juice secretion, total acidity, ulcer index and increase the pH of the gastric juice and percentage protection in a dose dependent manner. The histopathological studies were in support with these findings. Thus, the findings of this study showed that the ethanolic extract of *Euphorbia milii* leaves has significant antiulcer and antacid activity, making it a promising candidate for further investigation as a potential treatment for peptic ulcers.

**Keywords:** peptic ulcer, *Euphorbia milii*, Antiulcer, Fordtran's model, Absolute Ethanol

## INTRODUCTION

Peptic ulcers are the areas of degeneration and necrosis of gastrointestinal mucosa exposed to acid-peptic secretions. The incidence varies with the age, gender, geographical location and is associated with severe complications including hemorrhages, perforations, gastrointestinal obstruction, and malignancy. The normal stomach mucosa maintains a balance between protective and aggressive factors. Some of the main aggressive factors are gastric acid, abnormal motility, pepsin, bile salts, use of alcohol and nonsteroidal anti-inflammatory drugs (NSAID), as well as infection with microorganisms (*Helicobacter pylori* and others). On the other hand, mucus secretion, bicarbonate production, gastroprotective prostaglandin synthesis and normal tissue microcirculation protect against ulcer formation [1, 5-8].

The etiology of peptic ulcers may not be explained on the basis of a single factor but is multifactorial. It includes the following: [2, 3]

- *Helicobacter pylori* gastritis
- NSAIDs-induced mucosal injury

- Acid-Pepsin secretions
- Gastritis
- Dietary Factors
- Psychological factors
- Hormonal factors
- Alcohol

The alcohol absorption into the bloodstream occurs throughout the gastrointestinal tract and its direct contact with the mucosa can induce numerous metabolic and functional changes. These alterations may lead to marked mucosal damage, which can result in a broad spectrum of acute and chronic diseases, such as gastrointestinal bleeding and ulcers [2, 3]. Alcohol may interact directly with the gastric mucosa or it may act through a more general mechanism affecting the release of hormones and the regulation of nerve functions involved in acid secretion. Alcohol has constrictive effect on veins and arteries of the gastric mucosa, thus producing congestion, inflammation and tissue injury [3, 9-11].

*Euphorbia milii* Des Moul is a flowering plant that is indigenous to the Inselberg region of

Madagascar's Central Plateau, Africa. Recent research indicates that over 5% of *Euphorbia* species are utilised medicinally. *Euphorbia milii* Des Moul is frequently used in folk medicine to treat warts (in southern Brazil), cancer, hepatitis (in China), and trichiasis. Numerous researches on the chemical composition of *Euphorbia* species have been done. The results indicated the presence of several chemical compounds. The most frequently encountered phytochemicals in *Euphorbia milii* Des Moul are  $\beta$ -amyryn acetate,  $\beta$ -sitosterol, cycloartenol, lupeol, euphol, alkaloids, phenolic compounds, carbohydrates, anthocyanin,  $\beta$ -cyanin, proteins, amino acid, cardiac glycosides, steroids, anthraquinone, tannins, phlobatannins, reducing sugar, saponins, coumarin, triterpenes, and flavonoids [4, 15]. The present study was carried out to investigate the antiulcer activity of the ethanolic extract of *Euphorbia milii* Des Moul leaves in male Wistar albino rats using the ethanol-induced ulcer model.

## MATERIALS AND METHODS

### Experimental Animals

Male wistar albino rats weighing between 170-250g were used. They were maintained under standard laboratory conditions and were acclimatized for 7 days at a temperature of  $25\pm 2^\circ\text{C}$  with 12h light - dark cycle and relative

humidity [ $50\pm 10\%$ ] under hygienic condition. The animals were fed with standard food pellets and water ad libitum.

### Collection of the Plant and Preparation of Crude Extract

The leaves of *Euphorbia milii* were collected from Pathanamthitta district, Kerala, India on 29-06-2022. The plant was washed, cleaned to remove dirt, shade dried and grinded to get coarse powder. To prepare the extract, powder was extracted with 95% ethanol (20gm/300ml) by maceration for 7 days and then it was filtered through filter paper (whatman no.1). The filtrate was evaporated, the residue was collected, weighed and stored [12].

### Acute Toxicity Study

The acute toxicity study was carried out as per the procedure given in Organisation for Economic Co-Operation and Development [OECD]. Five albino rats (150-200g) were used in the study.

The animals were fasted overnight and provided only water. Test dose [2000mg/kg] was given for one animal. As the animal survived, four additional animals were dosed and were observed for 14 days for toxic symptoms like tremors, convulsions, behavioral changes, locomotion and mortality. Cage side observations including changes in skin, fur, eyes, mucous membrane,

respiratory, autonomic, central nervous system, somato motor activity, and behavioral patterns were also observed. Special attention was directed to tremors, convulsions, diarrhea, salivation, lethargy and coma. Body weight, food and water intake were recorded [13].

### ***In-Vitro* Method [14]**

#### **Acid Neutralizing Capacity Using Fordtran's Model**

For the preparation of artificial gastric juice, 2gm of NaCl and 3.2mg of pepsin were dissolved in 500ml distilled water. 7ml of 0.1M Hydrochloric acid and adequate water were added to make a 1000ml solution. The pH of the solution was adjusted to 1.2.

#### **Procedure**

Each freshly prepared 90ml of plant extract (200mg & 400mg), 90ml of distilled water and 90ml of reference drug (Sodium Bicarbonate

200mg & 400mg) were placed in a 250ml beaker and warmed to 37°C. A magnetic stirrer was continuously run at 30rpm to imitate the stomach movements. Each freshly prepared plant extracts, distilled water and reference drug was titrated with artificial gastric juice to the end point of pH 3. The consumed volume [v] of the artificial gastric juice was measured.

$$\text{Total consumed H}^+ \text{ ions [mmol]} = 0.063096[\text{mmol/ml}] \times v [\text{ml}].$$

### ***In-Vivo* Method [16]**

#### **Ethanol Induced Ulcer Model**

Male wistar albino rat weighing 170–250g were used for the study. Animals were divided into 5 groups each containing 6 animals. The ethanolic extract of *Euphorbia milii*, standard drug and normal saline were given orally for 7 days in the following manner:

**Table 1: Grouping & Procedure of *in-vivo* method**

GROUP NUMBER	GROUP NAME	TREATMENT
1	Vehicle Control	Normal Saline p.o 7 days
2	Disease Control	Normal Saline p.o 7 days + Absolute Ethanol 5ml/kg p.o on 8 <sup>th</sup> day
3	Standard	Lanzoprazole 30mg/kg p.o 7 days + Absolute Ethanol 5ml/kg p.o on 8 <sup>th</sup> day
4	Test Low Dose	E.E.Em 200mg/kg p.o 7 days + Absolute Ethanol 5ml/kg p.o on 8 <sup>th</sup> day
5	Test High Dose	E.E.Em 400 mg/kg p.o 7 days + Absolute Ethanol 5ml/Kg p.o on 8 <sup>th</sup> day

E.E.Em = Ethanolic Extract of *Euphorbia milii*

On 8<sup>th</sup> day, absolute ethanol (5ml/kg) was administered to all animals other than normal control group after overnight fasting. Animals were sacrificed 4h after administration of ethanol.

Stomachs were excised, cut along greater curvature, washed with 0.9% NaCl and ulcer areas on stomach's surface were examined macroscopically. The sum of area was expressed as UI and percentage inhibition was

calculated. Luminal contents were removed, gastric contents were collected in a test tube and centrifuged. The samples were analysed for gastric juice volume, pH and total acidity.

**Measurement of Gastric Juice Volume and pH**

The contents were drained into tubes and centrifuged at 1000 rpm for 10 mins, and the volume was noted. Then an aliquot of of 1ml gastric juice was diluted with 1ml of distilled water, and pH of the solution was measured

using a pH meter.

**Determination of Total Acidity**

An aliquot of 1ml of gastric juice diluted with 1ml distilled water was taken into a 50 ml conical flask, and 2 drops of phenolphthalein indicator was added to it and titrated with 0.01N NaOH until a permanent pink colour was observed. The volume of 0.01N NaOH consumed was noted. The acidity was expressed as mEq/L by the following formulae:

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH} \times 100}{\text{mEq/L} \cdot 0.1}$$

Where V=volume and N= normality

**Ulcer Index (UI)**

The mucosa was flushed with saline and stomach pinned on a frog board. The lesion in glandular portion was examined under a 10x

magnifying glass and length was measured using a divider and scale and gastric ulcer was scored. Severity of each ulcer was recorded in the following manner:

Table 2: Evaluation of Ulcer index

SCORING OF ULCER	OBSERVATION
0	Normal Stomach
0.5	Red Colouration
1	Spot Ulcer
1.5	Haemorrhagic Streaks
2	Deep Ulcer
3	Perforations

**Calculation of ulcer index**  $UI = UN + US + UP \times 10^{-1}$

UI = Ulcer index

UN = Average no.of ulcer per animal  
US= Average of severity score

UP = Percentage of animals with ulcer

**Percentage of Ulcer Inhibition**

$$\text{Percentage of ulcer inhibition} = \frac{UI \text{ of Ulcer Control} - UI \text{ of Treated group}}{UI \text{ of Ulcer Control}} \times 100$$

**Histopathology**

Stomach tissues were immersed in 10%

UI of Ulcer Control

formalin filled bottles for 24 hours for histopathological examination. The central

part of ulcerated tissue was cut in half along the longest diameter. In the case of whole stomach protected from ulcer damage, the section was taken from the basal part of the tissue. After processing, the sections were stained with haematoxylin - eosin dye for histopathological examination.

**Statistical Analysis**

Values are expressed as mean ± standard error mean [SEM] for group of 6 animals. The results were analysed by one way analysis of variance [ANOVA] followed by Dunnett’s multiple comparison test. Difference between

groups were considered significant at p < 0.05. statistical analysis was carried out using Graph Pad Prism 9.5.1 and Graph Pad Instat software version 3.1.

**RESULTS**

**Crude Extract & Percentage Yield**

The powdered plant material was extracted with ethanol by maceration. The extract was filtered, evaporated and stored in refrigerator. The percentage yield was found to be 12% w/w.

**Evaluation of Acute Toxicity**

Table 3: Results of Acute Toxicity Study

S. No.	PARAMETERS	RESULTS
1	Motor Activity	Normal
2	Tremors	Absent
3	Convulsion	Absent
4	Straub Reaction	Absent
5	Pilo Erection	Absent
6	Loss of Light Reflex	Absent
7	Sedation	Absent
8	Muscle Relaxation	Absent
9	Hypnosis	Absent
10	Analgesia	Absent
11	Ptosis	Absent
12	Lacrimation	Absent
13	Diarrhoea	Absent
14	Changes in Skin Colour	No Change

Dose of 2000mg/kg neither produced mortality nor any signs of morbidity

**Effect of Ethanolic Extract of *Euphorbia milii* on *In-Vitro* Acid Neutralizing Capacity**

Table 4: Results of *in-vitro* Study

DRUG	CONSUMED VOLUME OF ARTIFICIAL GASTRIC JUICE [ml]	H <sup>+</sup> IONS CONSUMED [mmol]
Control (Distilled Water)	4.7166 ± 0.05426	0.2967 ± 0.003419
Standard (Sodium Bicarbonate) 200mg	25.5325 ± 0.04216**	1.611 ± 0.002656**
Standard (Sodium Bicarbonate) 400mg	44.0439 ± 0.0428**	2.779 ± 0.002697**
Test Low Dose ( <i>Euphorbia milii</i> ) 200mg	17.8616 ± 0.05578***	1.127 ± 0.003524***
Test High Dose ( <i>Euphorbia milii</i> ) 400mg	38.0531 ± 0.04282***	2.401 ± 0.002697***

Data are represented as the mean ± S.E.M [n=6]. \*\*P<0.01 versus Control, ##p<0.01 versus Standard

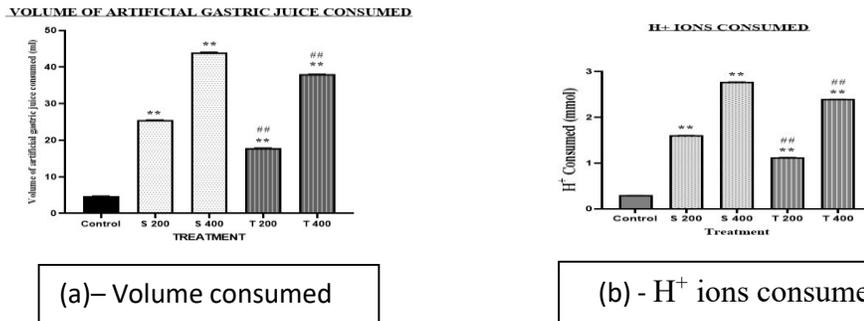


Figure 1: Data are represented as the mean ± S.E.M [n=6]. \*\*P<0.01 versus Control, ##p<0.01 versus Standard

**Evaluation of Antiulcer Activity of Ethanolic Extract of *Euphorbia milii***

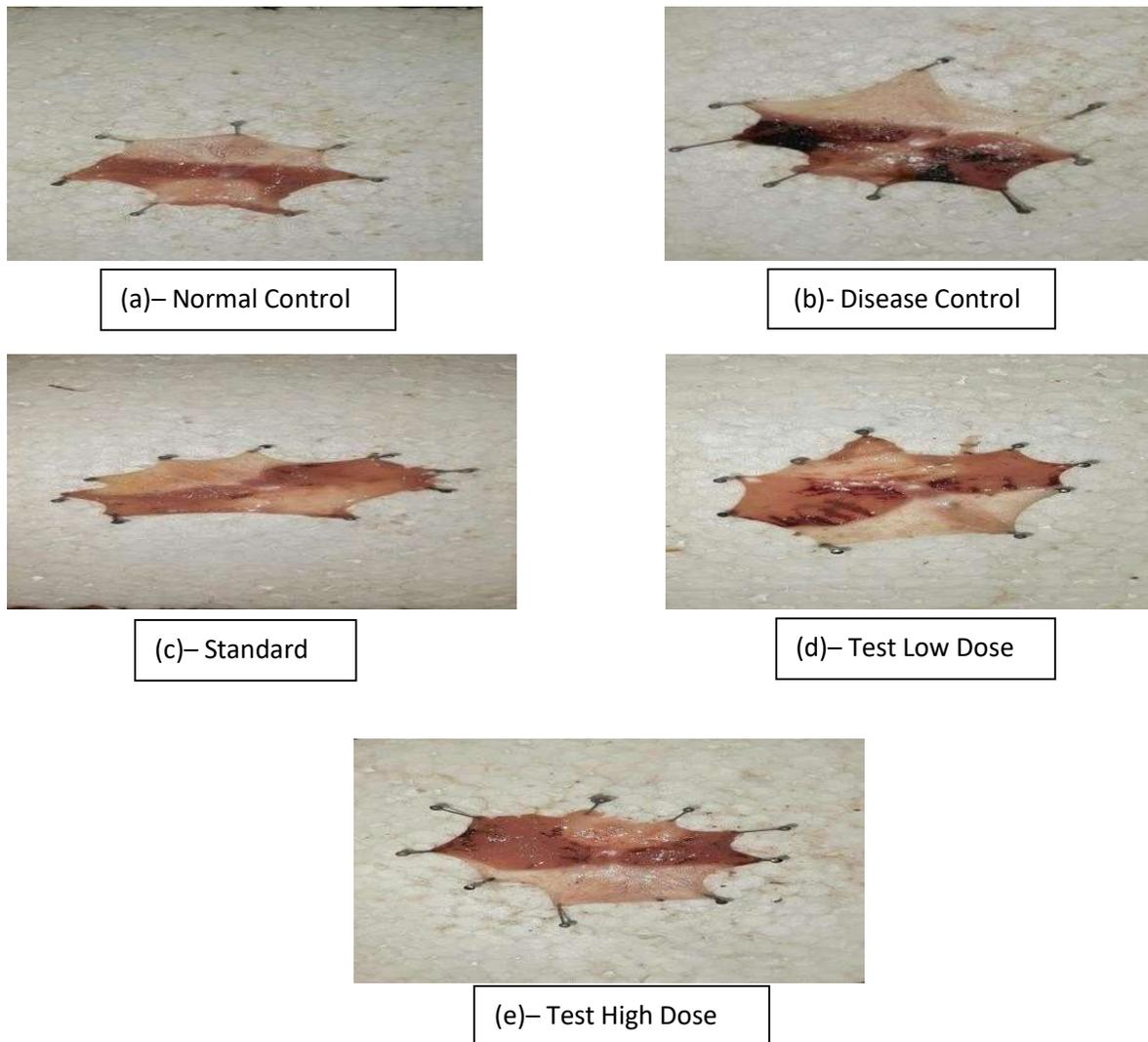


Figure 2: Macroscopical view of dissected stomachs

Effect of Ethanolic Extract of *Euphorbia milii* on Gastric Scretion, Total Acidity, pH, Ulcer Index and Percentage Protection

Table 5: Effect on Gastric secretion, Total acidity, pH, Ulcer index, Percentage protection

GROUP	VOLUME OF GASTRIC ACID SECRETION (ml/kg)	TOTAL ACIDITY	pH	ULCER INDEX	PERCENTAGE PROTECTION
Normal control	1.513 ± 0.02333	26.895 ± 0.2635	3.112 ± 0.02007	-	-
Disease control	4.565 ± 0.03905**	70.133 ± 0.2987**	1.477 ± 0.03026**	20.830 ± 0.1489**	-
Standard	0.893 ± 0.03621**###	22.120 ± 0.2535**###	2.878 ± 0.03978**###	5.622 ± 0.1570**###	71.885 ± 0.3061**###
Test low dose	2.348 ± 0.02845**###	35.230 ± 0.3186**###	1.903 ± 0.02201**###	9.245 ± 0.1583**###	52.980 ± 0.3079**###
Test high dose	1.130 ± 0.04091**###	30.170 ± 0.2936**###	2.375 ± 0.03170**###	7.493 ± 0.1656**###	63.938 ± 0.2899**###

Data are represented as the mean ± S.E.M [n=6]. \*\*P<0.01 versus Normal Control, ##p<0.01 versus Disease Control

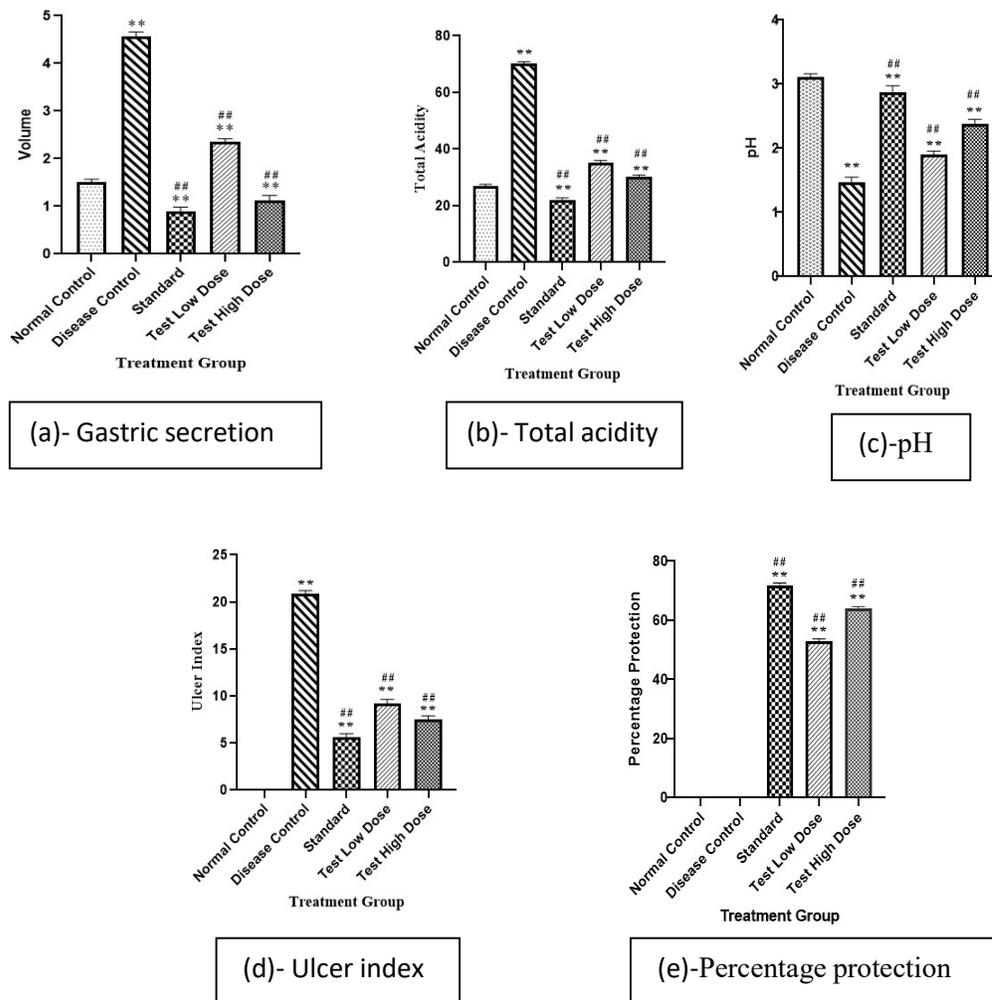
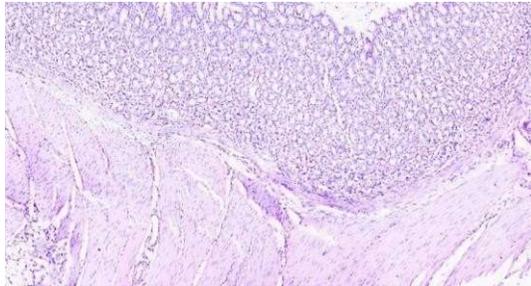
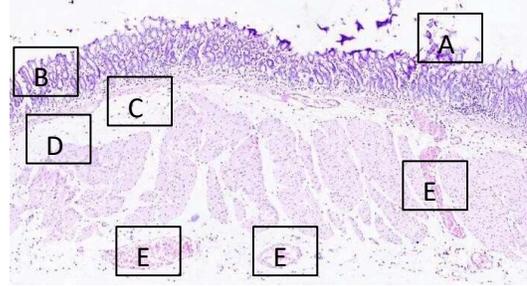


Figure 3: Data are represented as the mean ± S.E.M [n=6]. \*\*P<0.01 versus Normal Control, ##p<0.01 versus Disease Control

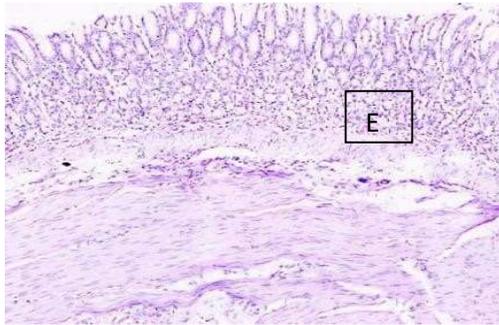
### Histopathological Examination of Gastric Mucosal Tissue Section of Rats in Ethanol Induced Model



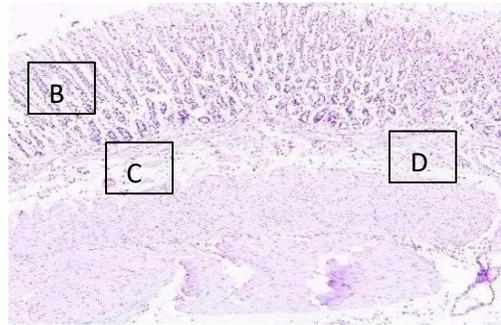
(a)- Normal control



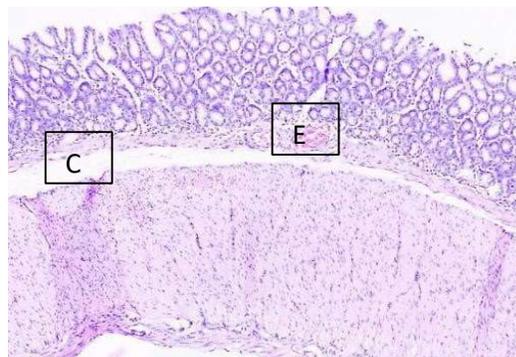
(b)- Disease control



(c)- Standard



(d)- Test low dose



(e)- Test high dose

A-surface mucosal epithelial destruction, B – mucosal edema, C – submucosal edema, D- Infiltration and inflammation, E – blood vessel congestion

Figure 4: Histopathology of Gastric Mucosal Section

## DISCUSSION

The results obtained from this study provide valuable insights into the potential therapeutic benefits of *Euphorbia milli* in the treatment of ethanol-induced gastric ulcers. *Euphorbia milli* is a rich source of diverse phytochemicals, including flavonoids such as quercetin, polyphenols such as anthocyanins and tannins, phytosterols like beta-sitosterol, and triterpenoids like lupeol, euphol, and betacyanin, which have been well-documented in the literature.

Various scientific literatures have convincingly demonstrated that quercetin have antioxidant activity. So it prevents oxidant injury and it also decreases gastric acidity through anti-apoptotic mechanism. The gastroprotective effect of  $\beta$ -sitosterol have been well documented in various experimental models. Tannins are known to reduce ulcer formation by forming a protective layer over the gastric mucosa. Lupeol prevent ulcers by its ability to stimulate the secretion of gastric mucus. This promising evidences from previous studies has paved the way for the present investigation of the anti-ulcer effect of *Euphorbia milii*.

Acute toxicity study was performed using OECD guideline 425 [Acute Oral Toxicity:Up and Down Procedure (UDP)] using 5 female

rats. LD50 was found to be greater than 2000mg/kg. Hence 1/10<sup>th</sup> (200mg/kg) and 1/5<sup>th</sup> (400mg/kg) of this dose was selected for the study. *In-vitro* study was conducted using Fordtran's titration model. The consumed volume of artificial gastric juice and H<sup>+</sup> ions for water, standard (200 & 400mg) and plant extract(200&400mg) shows that plant extract have significant antacid property lesser than that of sodium bicarbonate but higher than water. *In-vivo* study was conducted using ethanol induced method. From the macroscopic observations, pretreatment with ethanolic extract of *Euphorbia milii* effectively alleviated the ethanol-induced gastric ulcers in rats. All treatment groups exhibited significant dose-dependent reduction of gastric lesion area in gastric mucosa as well as increment of gastric acid pH, reduction in gastric juice volume, reduction in total acidity, reduction in ulcer index and increase in percentage protection. It is also supported by the pathological changes such as decrement of epithelial destruction, reduction in mucosal and submucosal edema, and reduction in infiltration and inflammation. Thus, this study provides compelling evidence that *Euphorbia milii* has potent antacid and anti-ulcer properties. These effects may be attributed to the presence of multiple phytoconstituents, including quercetin,

betasitosterol, lupeol, and tannins, which possess gastroprotective, antioxidant, and anti-inflammatory properties.

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