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EVALUATION OF THE ANTIULCER ACTIVITY OF METHANOLIC EXTRACT OF THE LEAVES OF SAPONARIA OFFICINALIS IN RATS

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Abstract:

The present study evaluates the antiulcer activity of the methanolic extract of Saponaria officinalis leaves in rats. The plant material was extracted using methanol and subjected to preliminary phytochemical screening, which indicated the presence of saponins, flavonoids, tannins, and phenolic compounds. Antiulcer activity was assessed using standard experimental models such as ethanol-induced and pylorus ligation–induced gastric ulcers in Wistar albino rats. Parameters including ulcer index, gastric pH, acidity, and mucosal protection were evaluated. The extract showed a significant dose-dependent reduction in ulcer index and gastric acidity, along with enhanced gastric mucosal protection when compared to the control group. The findings suggest that Saponaria officinalis possesses promising gastroprotective and antiulcer potential.

Keywords: *Saponaria officinalis, Antiulcer activity, Methanolic extract, Rats, Phytochemicals, Gastroprotection.*

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1. INTRODUCTION:

On the other hand, the biological properties of many plant species traditionally utilized together with their bioactive components have been elucidated until now. The more classical bioassay-guided natural drug discovery process and the modern processes, including high-throughput screening, and even the new reverse Pharmacognosy approach, allowed the identification of a great number of bioactive phytochemicals.

Nevertheless, medicinal plants still have a hopeful future, as the phytochemical composition and the potential health benefits of many species have not yet been studied or still need to be more deeply investigated.

Peptic ulcer and other acidic symptom affect up to ten percentages of the humans with sufficient severity to prompt victims to seek medical attention. The more significant disease condition requiring medical fuscous is ulcer and gastro esophagealdisease1. In the US, approximately 4 million people have peptic ulcer (duodenal and gastric types), and 350 thousand new patient are diagnosed in each year, around 180 thousand peoples are admitted to hospital and treated with drugs yearly, and about five thousand patient from this case die each year as a result of ulcer condition. The lifetime of human being developing a peptic ulcer is about 10 percentages for Americans males and four percentages for female population2.

Peptic ulcers is wound in the lesions that are most often affected in younger to older adults population, but this may diagnosed in young adult life. They often appear without obvious sign and symptom, after a period of days to months of active phase of disease, it may heal with or without drug treatment. It also affect because of bacterial infections with H. Pylori.

Danger of ulcer 5:

Bleeding: Upper gastrointestinal (UGI) bleeding is the secondary common medical condition that effect high mortality in peptic ulcer. UGI bleeding commonly present along with hematemesis (vomiting with digested food and blood or coffee-ground like substance) and black, tarry stools (melana). Clinical diagnosis of UGI done by nasogastric tube lavage shows blood or coffee-ground like material presence. However this diagnosis may be negative when the bleeding arises beyond a closed pylorus region. Most of the patient's having bleeding ulcers can be treated with fluid and blood resuscitation, drug therapy, and endoscopic surgery.

Perforation: This ulcer may be spread to small intestine, oesophagus and large intestine ulcers account for 60, 20 and 20 percent of perforations.

Penetration: Ulcer penetration called due to the permeation of the ulcer among the bowel part without free perforation and filtration of whole contents inside the peritoneal cavity. Surgical treatment regimen recommended that permeation affect in twenty percentage of ulcers, but little proportion of penetrating ulcers become clinically important. The common symptom these complications include acidic irritation, weight reduction and diarrhea: watery vomiting is an uncommon, but diagnostic symptom. No evident clinical data is available in the treatment regimen and guidance for the curing of penetrating ulcers.

Obstruction: Gastric wall obstruction among the frequent ulcer symptoms. Most of the cases are related with duodenal or pyloric part ulceration is 5 percent of the patient populations. Changes in lifestyle and dietary:

Aspirin and related drugs (non-steroidal anti-inflammatory drugs),6 alcohol,7 coffee 8 (even decaf) and tea10 can interfere with the curing of the peptic ulcers. Smoking may also lowthe ulcer healing process11. People with ulcer symptom have been evaluated to had more carbohydrate than people with no ulcers,12 from this route may occur with a genetic susceptibility for the ulcer pathogenis13 .

Sugar has also been reported to increase stomach pH14. Salt may cause the stomach and intestine irritation. Large uptakes of salt have been linked to higher risk of stomach ulcer15

One of the amino acid Known as Glutamine, is the important source in the energy in cells which coverthe stomach and intestine16. It is also prevent the stress ulcer related by large burns of the preliminary study about the pathogenesis of ulcers17.

MATERIALS AND METHOD:

The designing of methodology involves a series of steps taken in a systematic way in order to achieve the set goal(s) under the prescribed guidelines and recommendations. It includes in it all the steps from field trip to the observation including selection and collection of the medicinal plant, selection of dose value, standardization of protocol, usage of instruments, preparation of reagents, selection of specific solvents for extraction, formation of protocols and final execution of the standardized protocol. All this requires good build of mind and a good and soft technical hand to handle the materials and procedure in a true scientific manner.[91-92]

7.1 Drugs and Chemicals

Drugs and Chemicals used in this study were of analytical grade and of highest purity procured from standard commercial sources in India.

Table No: 7.1 Drugs and Chemicals

S.No	*Materials*	*Company Name*
1.	Ranitidine	Cipla
2.	Omeprazole	Cipla
3.	Alcohol	Merck

7.2. Instruments

Following instruments were required for the study:

Table No: 7.2- List of Instruments used for study

Name of the instrument	*Source*
Centrifuge	Dolphin
Digital weighing balance	Horizon
Heating mantle	ASGI®
Disection box	Camel
Refrigerator	Videocon
Actophotometer	Dolphin
Glass cylinder	ASGI®
Adhesive tape	YVR medivision Pvt Ltd
Thread	YVR medivision Pvt Ltd
Stop watch	ASGI®
Syringes	YVR medivision Pvt Ltd
Needles	YVR medivision Pvt Ltd
Soxhlet extractor	ASGI®
Condenser	ASGI®
Burette stand	Dolphin
Round bottom flask	ASGI® , Amar
Mixer	Videocon
Oven	ASGI®
Water bath	ASGI®
Stirrer/glass rod	ASGI®
Watch glass	ASGI®
Whatmann filter paper	Manipore microproducts, Ghaizabad.
Butter paper	ASGI®
Spatula	ASGI®
Rubber pipes	ASGI®

PRELIMINARY QUALITATIVE TEST

7.2. Preliminary Phytochemical Screening [93-96]

Preliminary phytochemical screening of the plant extract was carried out for the analysis of Alkaloids,

Carbohydrates, Tannins, Saponins, Steroids, Phenols, Flavonoids .as per the standard methods.

1. Detection of Alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide).Formation of a yellow coloured precipitate indicates the presence of alkaloids.

b).Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

c). Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

d). Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution).Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of Carbohydrates: Extracts were dissolved individually in 5ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.[97]

a). Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

b).Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

c).Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A&B solutions. Formation of red precipitate indicates the presence of reducing sugars.[98]

3. Detection of saponins

a). Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for15 minutes. Formation of 1cm layer off a am indicates the presence of saponins.

b). FoamTest: 0.5gm of extract was shaken with 2ml of water. If foam produced persists forten minutes it indicates the presence of saponins.[99]

4. Detection of steroids.

a). Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

b). Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.[100]

5. Detection of Phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of Tannins

Gelatin Test: To the extract,1 %gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

7. Detection of Flavonoids

Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Leadacetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

7.3. Experimental animals [101]

Wistar rats (150-200 g) and were procured from Animals were housed in appropriate cages in uniform hygienic conditions and fed with standard pellet diet (Amrul Laboratory Animal Diet) and water ad libitum. All the animals were maintained under standard conditions, that is room temperature 26 ± 1°C, relative humidity 45 - 55% and 12:12 h light – dark cycle. The animals were housed in large spacious hygienic cages during the course of the experimental period. Animal studies had approval of IAEC.

7.4. Plant Material Collection

The leaves of *Saponaria Officinalis* were collected from the Botanical garden and was identified and authenticated from Botanical Department. The plant material was cleaned, reduced to small fragments, air dried under shade at room temperature and coarsely powdered in a mixer. The powdered material was stored or taken up for extraction process.

7.5. Preparation of plant extracts [102]

7.5.1 Preparation of ethanolic Extract:

Shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place. Defatted powdered of *Saponaria Officinalis* has been extracted with ethanol solvent using maceration process for 48 hrs., filtered and dried using vacuum evaporator at 40ºC (yield of extract was 9.40% with

respect to dry material). Just prior to use, the substance was dissolved in physiological saline solution.

7.6. Selection of dose for animal study [103]

The dose considered for the experiment on rats was obtained from conversion of human dose of *Saponaria Officinalis* (3-5 g/kg). The conversion factor of human dose (per 200 g body weight) is 0.018 for rats. Hence the calculated dose for the rats (considering human dose 3 and 5 g/kg) is 200 mg/kg. Acute toxicity was done at dose of 2000mg/kg body weight as per OECD guidelines No 423.

7.7. PHARMACOLOGICAL EVALUATION

ACUTE ORAL TOXICITY: [104]

The acute oral toxicity of ethanolic extracts of *Saponaria Officinalis* was determined by using rats which were maintained under standard conditions. The animals were fasted 12 hours prior to the experiment, up and down procedure OECD guideline no. 423 were adopted for toxicity studies. Animals were administered with single dose of individual extract up to 2000mg/kg and observed for its mortality during 14days and 21days study period (long term) toxicity and observed up to 14days for their mortality, behavioral and neurological profiles.

7.9 SCREENING FOR ANTI-ULCER ACTIVITY

The Ethanolic extracts of *Saponaria Officinalis* were tested for antiulcer activity using various methods like pyloric ligation induced gastric ulcer and Ethanol-induced gastric ulcer.

7.9.1 PYLORIC LIGATION IN RATS[105]

Animals were divided into four (I-V) groups.
Group I - Normal (Distilled water)
Group II- Control (Pyloric ligation)
Group III - EESO (150mg/kg) Single dose
Group IV - EESO (300mg/kg) Double dose
Group V - Ranitidine (20mg/kg)

The animals were divided into 5 groups, each consisting of six rats. Control group received distilled water only. Second group of rats are pyloric Ligated. Third and fourth groups received EESO in a dose of 150 and 300 mg/kg. The fifth group of animals received Ranitidine in the dose of 20mg/kg as a reference drug for ulcer protective studies. After 45 min of the treatment, pyloric ligation was done by legating the pyloric end of stomach of rats of respective groups under ether anesthesia at a dose of 35mg/kg of body weight. Ligation was done without causing any damage to the blood supply of the stomach. Animals were allowed to recover and stabilize in individual cages and were deprived of water during post-operative period. Rats were sacrificed after 4hr of surgery and ulcer scoring was done. Gastric juice was collected and gastric secretion studies were performed according to the standard procedure.

Ethanol induced ulcer model
Group I - Normal (Distilled water)
Group II- Control (Ethanol)
Group III - EESO (150mg/kg)
Group IV - EESO (300mg/kg)
Group V - Omeprazole (20mg/kg)

The ulcer was induced by administering absolute ethanol (1ml/200g). All the animals were fasted for 36 hours and then ethanol was administered to induce ulcer. The animals were divided into five groups, each consisting of six rats. The Group I - Normal group received Distilled water, second group received Ethanol. Third and fourth groups received EESO in a dose of 150 and 300 mg/kg. The fifth group of animals received Omeprazole in the dose of 20 mg/kg as a reference drug. They were kept in specially constructed cages to prevent coprophagia during and after the experiment. The animals were anaesthetized 1 hr later with anaesthetic ether and stomach was incised along the greater curvature and ulceration was scored. A score for the ulcer was studied to pyloric ligation induced ulcer model.

Scoring of ulcers

Normal stomach - 0
Red coloration - 0.5
Spot ulcer - 1
Hemorrhagic streak - 1.5
Ulcers (< 2mm) - 2
Ulcers (>2 < 4 mm) perforation -3 Ulcers (< 4mm) -4

Mean ulcer score for each animal was expressed as ulcer index. The percentage of ulcer protection was determined by

$$\% \text{ of ulcer protection} = \frac{\text{Control mean ulcer index} - \text{Test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

Determination of free acidity

$$\text{Acidity} = \frac{\text{Volume of sodium hydroxide} \times \text{Normality} \times 100\text{mEq/L}/100\text{g}}{0.1}$$

Statistical analysis

The values are represented as mean ± S.E.M, and Statistical significance between treated and control groups was analyzed using of one-way ANOVA, followed by Dennett's test where $P<0.05$ was considered statically significant

RESULTS:

Phytochemical screening test

The freshly prepared extract of the leaves of *Saponaria Officinalis* was subjected to phytochemical screening tests for the detection of various active constituents. The extract showed the presence of alkaloids, tannins, steroids, phenolic and flavonoids, carbohydrates, saponins and glycosides in crude extract of *Saponaria Officinalis* leaves as depicted in Table 8.1.

Table 8.1: Result of chemical group tests of the ethanolic Extract of *Saponaria Officinalis* leaves

Test	Ethanolic Extract
Carbohydrates	-
Tannins	+
Flavonoids	++
Saponins	+
Phenols	++
Steroids	+
Alkaloids	++
Glycosides	+

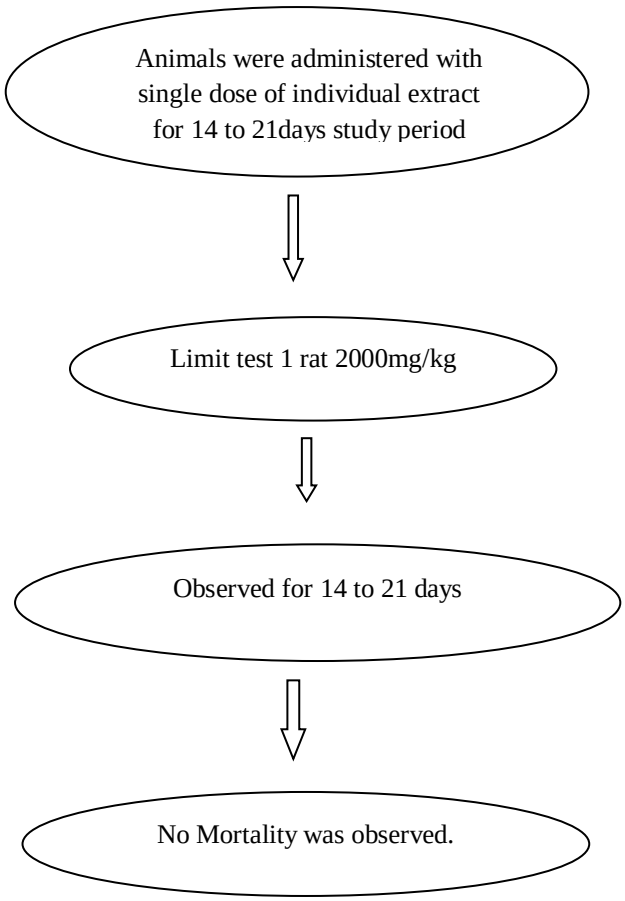
Alcoholic extract; (+): Present; (-): Absent; (+++); Reaction intensity is high; (++): Reaction intensity is medium; (+): Reaction intensity is normal;

ACUTE TOXICITY STUDY

Administration of the *Saponaria Officinalis* extracts *in* rats at doses of 2000 mg/kg by oral gavage did not reveal any adverse effects or signs of toxicity.

Observations twice daily for fourteen days also did not reveal any drug related observable changes or mortality. Accordingly, the acute oral LD50 of the extractives was concluded to exceed 2000 mg/kg body weight, the highest dose tested in the study.

CHART FOR ACUTE ORAL TOXICITY

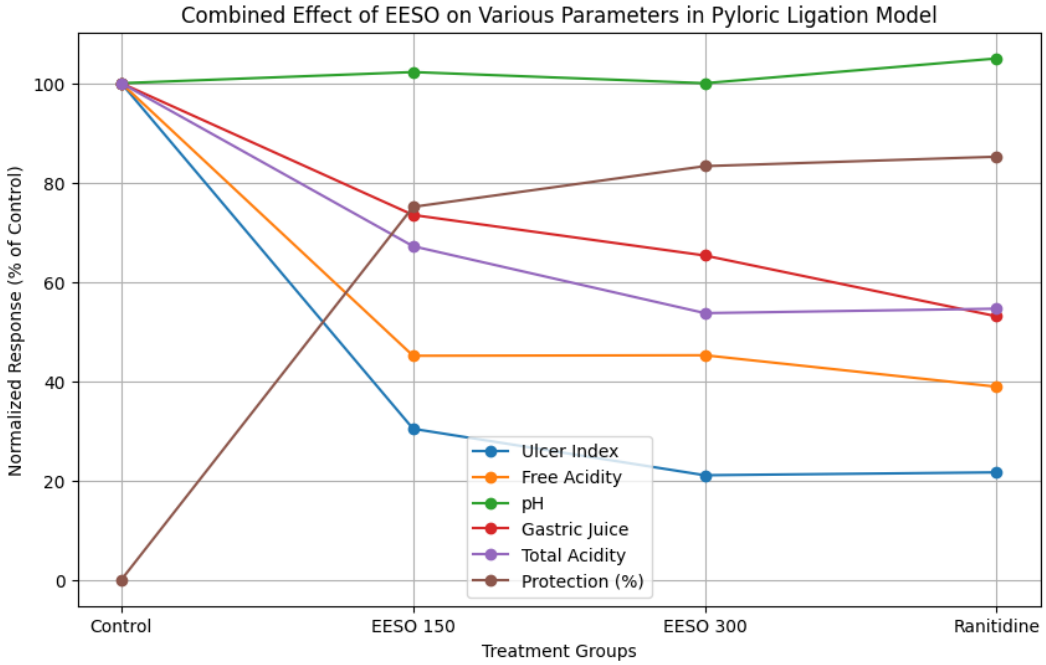


PYLORIC LIGATION IN RATS

Pyloric ligation induced gastric ulcer in pyloric ligation induced ulcer model, oral administration of EESO in two different doses showed significant reduction in ulcer index, gastric volume, free acidity, total acidity compared to the central group. EESO exhibited a protection index of 75.1% and 85.2% at the dose of 150 and 300 mg/kg respectively, whereas Ranitidine as reference standard exhibited a protection index of 86.2% (Table 8.2).

Table 8.2: Effect of EESO on various parameters in pyloric ligation induced gastric ulcers

Group	Treatment	Ulcer index	Free acidity meq/ltr	PH of gastric juice	Gastric Juice	Total acidity meq/ltr	Protection (%)
I	Normal (distilled water)	---	40.2 ± 0.1	2.11 ± 0.2	4.5 ± 0.1	62.0 ± 0.2	---
II	Control (pyloric ligation)	17.1 ± 2.1	93.1 ± 2.3	4.01 ± 0.1	4.9 ± 0.2	112.1 ± 0.3	---
III	EESO (150mg/kg)	5.2 ± 0.3	42.0 ± 0.1	4.10 ± 0.2*	3.6 ±2.1	75.3 ± 0.5	75.1 %
IV	EESO (300mg/kg)	3.6 ± 0.2*	42.1 ± 0.2*	4.01 ± 0.1*	3.2±0.2*	60.2 ± 0.2*	83.3%
V	Ranitidine (20mg/kg)	3.7 ± 0.1*	36.2 ± 0.2*	4.21 ± 0.3*	2.6± 0.3*	61.2 ± 1.4*	85.2%



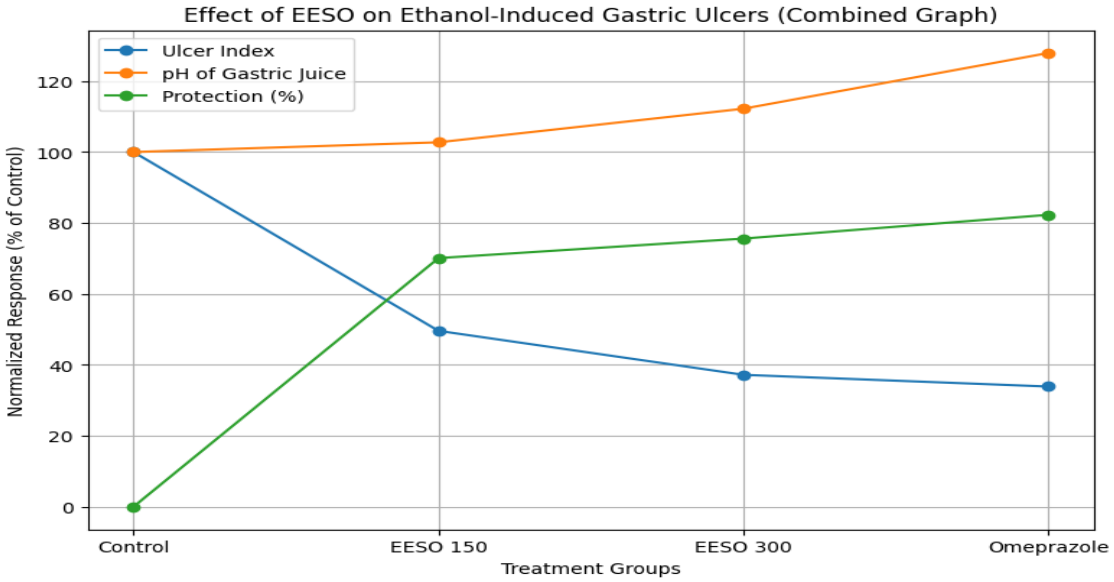
ETHANOL-INDUCED GASTRIC ULCER

In control animal, oral administration of absolute ethanol produced characteristic lesions in the glandular portion of rat stomach which appeared as elongated bands of thick, blackish red lesions. EESO has shown significant protection index of 70.1% and 75.6% with the dose of 150 and 300 mg/kg respectively whereas Ranitidine as reference standard showed protection index of 82.3% (Table 8.3).

Table 8.3: Effect of EESO on various parameters in Ethanol induced gastric ulcers

Group	Treatment	Ulcer index	P^H of gastric juice	Protection (%)
I	Normal (distilled water)	---	1.10 ± 0.1	---
II	Control (Ethanol)	12.1 ± 0.2	4.01 ± 0.3	---
III	EESO (150mg/kg)	6.0 ± 0.1	4.12 ± 0.2	70.1%
IV	EESO (300mg/kg)	4.5 ± 0.2*	4.50 ± 0.2*	75.6%
V	Omeprazole (20mg/kg)	4.1 ± 0.2*	5.13 ± 0.1*	82.3%

Values are expressed as mean ± SEM of observations, Statistical comparisons as follows: Significant *P <0.005 compared to control group.



CONCLUSION:

Peptic ulcer disease is one of the most common gastrointestinal disorders. It mainly involves an imbalance between the offensive and defensive factors. Many synthetic drugs such as H2 blockers, gastro protective and proton pump inhibitors are available in the market but they are showing many side effects. Medicinal plants and their products are considered to less side effects and more efficacy when compared to synthetic drugs. Many medicinal plants and natural analogues showed prominent anti-ulcer and gastro-protective activities. Based on this literature review the plant Saponaria Officinalis was selected for screening antiulcer activity.

NSAID's like aspirin and paracetamol causes gastric mucosal damage by decreasing prostaglandin levels through inhibition of PG synthesis. Alcohol and Aqueous extract of the plant of Saponaria Officinalis was significantly effective in protecting gastric mucosa against paracetamol induced ulcers at all the dose level studied.

Pyloric ligation induced gastric ulcer and Ethanol-induced gastric ulcer model ethanolic extract of Saponaria Officinalis at a dose of 150 and 300mg/kg body weight p.o was found to exhibit significant cytoprotective action when compared to control (pyloric ligation) group using Omeprazole 20mg/kg p.o as a standard drug

On the basis of the present results and available reports, it can be concluded that the anti-ulcer activity elucidated by Saponaria Officinalis could be mainly due to the modulation of defensive factors through an improvement of gastric cytoprotection and partly due to acid inhibition.

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