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EVALUATION OF GASTRIC ANTI-ULCER ACTIVITY OF HYDRO-ALCOHOLIC EXTRACT OF *SAPINDUS EMARGINATUS* LEAVES IN ALBINO WISTAR RATS

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Abstract:

Aim: To evaluate the gastric anti-ulcer activity of hydroalcoholic extract of *Sapindus emarginatus* leaves in experimental animals.

Materials and Methods: HAESE was investigated in pylorus ligation and ethanol induced ulcer models in Wistar rats. In both models, the common parameter determined was ulcer index. HAESE at doses of 250, 500 mg/kg (p.o.) was used to determine whether it could produce significant inhibition of the gastric lesions induced by pylorus ligation and ethanol.

Results: The extract (250 and 500 mg/kg) showed significant ($P < 0.05$) reduction in gastric volume and ulcer index as compared to the control in both of the two models.

Conclusions: It can be concluded that HAESE possesses antiulcerogenic as well as ulcer healing properties, which might be due to its antisecretory activity.

Key-words: *Sapindus emarginatus*, Omeprazole, pylorus ligation, gastric ulcer

Abbreviations: HAESE (hydroalcoholic extract of *Sapindus emarginatus*), UI (ulcer index).

1. Introduction:

Peptic ulcer disease is a serious gastrointestinal disorder¹. The formation of peptic ulcers depends on the presence of acid and peptic activity in gastric juice plus a breakdown in mucosal defenses. There are two major factors that can disrupt the mucosal resistance to injury: non-steroidal anti-inflammatory drugs (NSAIDs) e.g. aspirin and *Helicobacter pylori* (*H. pylori*) infection². But in practically, many drugs were used to treat this disease but many of

them cause adverse effects and recurrent infections frequently occur within a few weeks because of difficulty in eradication of *H. pylori*³. This has been rationale for the development of new antiulcer drugs and search for novel molecule. Drugs of plants origin are gaining popularity and investigating for the various disorders including peptic ulcer. The objective of the present study was to evaluate the effectiveness of leaves extract in preventing the formation of gastric ulcer experimentally by ethanol-induced gastric damage and pylorus ligation induced ulcers in rats. Drug treatment of peptic ulcers is targeted at either counteracting aggressive factors [acid, pepsin, active oxidants, platelet aggravating factor (PAF), leukotrienes, endothelins, bile or exogenous factors including NSAIDs] or stimulating the mucosal defences [(mucus, bicarbonate, normal blood flow, prostaglandins (PG), nitric oxide⁴). The goals of treating peptic ulcer disease are to relieve pain, heal the ulcer and prevent ulcer recurrence. Currently there is no cost-effective treatment that meets all these goals. Hence, efforts are on to find a suitable treatment from natural product sources.

The members of genus *Sapindus* are well known for their medicinal values. For thousands of years it has been used for various purposes. *Sapindus emarginatus* is known as Ritha, reetha, aritha, kanma etc. Due to the presence of saponins, they are known for their surfactant & detergent properties. The fruits of *Sapindus emarginatus* are commonly used for hair problems and also in preparation of shampoos⁵. Traditionally it used as anti-inflammatory and antipyretic. The seed is intoxicant and the fruit rind has oxytropic action. Nut powder is used as Nasal Insufflations. Seeds of *Sapindus emarginatus* contain anti-inflammatory oil which is traditionally used to purify the blood⁶. Historically it has been used in folk remedies as a mucolytic agent, emetic, paralysis of limbs, treatment of chlorosis. Soapnuts are also used as effective aid for the treatment of skin problems like eczema, itching and psoriasis. Its fruits are natural substitute for chemical soaps and hair dyes. Effect on reproductive organs also has been reported^{7,8}.

Pericarp contains triterpene saponins, commonly used as antifertility, antipruritic and anti-inflammatory agents in traditional Indian and Thai medicines. The roots are used as expectorant and demulcent and also are used for cure of hysteria and epilepsy^{9,10}.

2. Materials and Methods:

2.1. Preparation of the extract

The Leaves of *Sapindus emarginatus* were obtained from Surrounding areas of mangalagiri mandal and guntur Dist, Andhra Pradesh and authenticated by Dr. S. M. Khasim, Department of Botany, Acharya Nagarjuna University, guntur. Voucher specimen was deposited to herbarium of nirmala college of pharmacy After authentication, Fresh leaves were collected, washed under running tap water and dried in shade for two weeks. Dried leaves were powdered, sieved and stored in an air tight container at room temperature. Dried powder (400 g) was extracted sequentially with petroleum ether and hydro-alcohol (30:70) by using soxhlation method. The extracts were concentrated to dryness using rotary evaporator. The extracts were preserved.

2.2. Experimental Animal

Wister albino rats of weighting between 160 to 200 gm were used for the study. The animals were house in standard condition (temperature 24±2 with 50 to 60% relative humidity and a 12 hours light dark cycle). The entire animal had free access to water and normal diet (Hindustan lever). The study was approved by Institutional Animal Ethical Committee (IAEC) and was in accordance with the guideline of the Committee for the Purpose of Control and Supervision of Experimental Animal (CPCSEA).

2.3. Acute toxicity study of the extract

Adult albino rats (150-200 g) were divided into three groups each containing 6 rats. The rats were fasted for 6 h with only access to water ad libitum before experimental study. Group I, II, and III animals were administered various doses of methanolic extract of *Sapindus emarginatus* (S.emarginatus hydro-alcoholic extract) i.e. 500, 1000 and 2000mg/kg. All the doses were administered by oral route. No mortality was reported even after 72 hours. This indicates that the hydro alcoholic extract is safe up to a single dose of 3g/kg body weight.

2.4. Pyloric ligation in rats

Animals were divided into four groups, each consisting of six rats. Rats in group I, served as control group, received distilled water (1 mL) orally. Rats in group II received omeprazole (20 mg/kg) which was used as a reference drug for ulcer protective studies. Rats in group III and IV received *Sapindus emarginatus* hydro-alcoholic extract at doses of 250 and 500 mg/kg, respectively. After 45 min of *Sapindus emarginatus* hydro-alcoholic extract and omeprazole

treatment, pyloric ligation was done by ligating the pyloric end of stomach of rats of respective groups under ether anaesthesia at a dose of 35 mg/kg bw. 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 postoperative period. After 4 h of surgery, rats were sacrificed and ulcer scoring was done. Gastric juice was collected and gastric secretion studies were performed^{12,13}.

2.5. Ethanol induced ulcer model

The ulcer was induced by administering ethanol. All the animals were fasted for 36 h before administration of ethanol. The animals were divided into four groups, each consisting of six rats. Rats in group I, served as control group, received distilled water (1 mL) orally. Rats in group II were administered with omeprazole (20 mg/kg) as a standard reference drug. Rats in group III and IV received *Sapindus emarginatus* hydro-alcoholic extract at doses of 250 and 500 mg/kg, respectively. The gastric ulcers were induced in rats by administering absolute ethanol (90%) (1 mL/200 g) orally, after 45 min of *Sapindus emarginatus* hydro-alcoholic extract and omeprazole treatment. They were kept in specially constructed cages to prevent coprophagia during and after the experiment. The animals were anaesthetized 1 h later with anaesthetic ether and stomach was incised along the greater curvature and ulceration will be scored. A score for the ulcer was study similar to pyloric ligation induced ulcer model¹⁴.

2.7. Scoring of ulcer will be made as follows

0 = Normal coloured stomach

0.5 = Red colouration

1 = Spot ulcer

1.5 = Haemorrhagic streaks

2 = Ulcers ≥ 3 but ≤ 5

3 = Ulcers >5

Calculation of ulcer Index¹⁵:

$U1 = UN + US + UP \times 10^{-1}$

U1 = Ulcer Index

UN = Average of number of ulcer per animal

US = Average of severity score

UP = Percentage of animal with ulcer

Mean ulcer score for each animal is expressed as ulcer index.

The percentage protection was calculated using the formula,

$$\text{Percentage protection} = 100 - U_t \div U_c \times 100$$

Where

U_t = Ulcer index of treated group

U_c = Ulcer index of control group

3. Results

Table-1: Effect of *Sapindus emarginatus* leaf extract on various parameters in pyloric ligation induced gastric ulcer.

Groups	Treatments	Doses (mg/kg) mL / animal	Ulcer index	% Protection	pH of gastric juice	Gastric juice (mL)
I	Control	1	15.60±1.50	–	2.40±0.20	9.20±0.20
II	Omeprazole	2	2.20±0.50*	86	4.70±0.15*	2.20±0.18*
III	Extract	250	3.50±0.50	74	3.40±0.20	4.60±0.12
IV	extract	500	2.50±0.60*	82	4.20±0.18*	3.90±0.15*

*: P < 0.05 as compared to control group.

Figure 1: Effect of *Sapindus emarginatus* leaves extract on various parameters in pylorus ligation induced gastric ulcer.

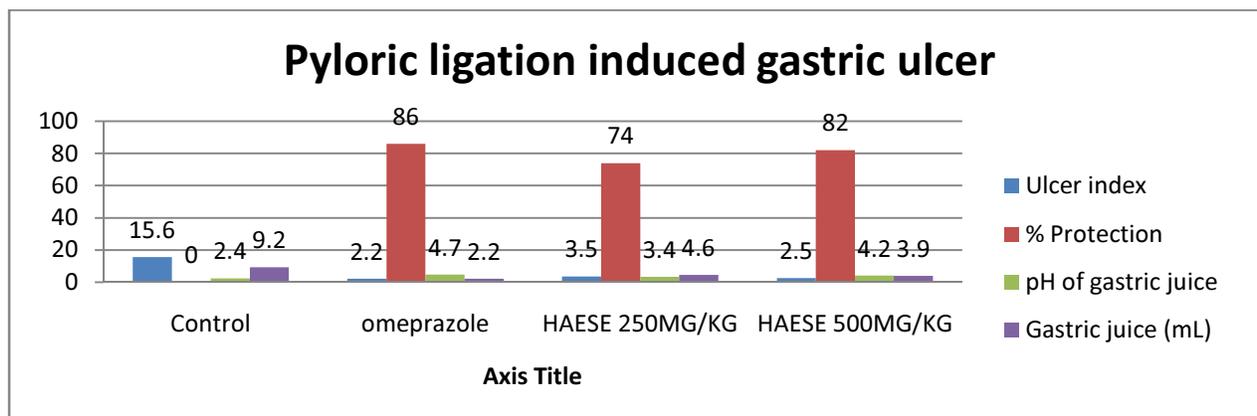
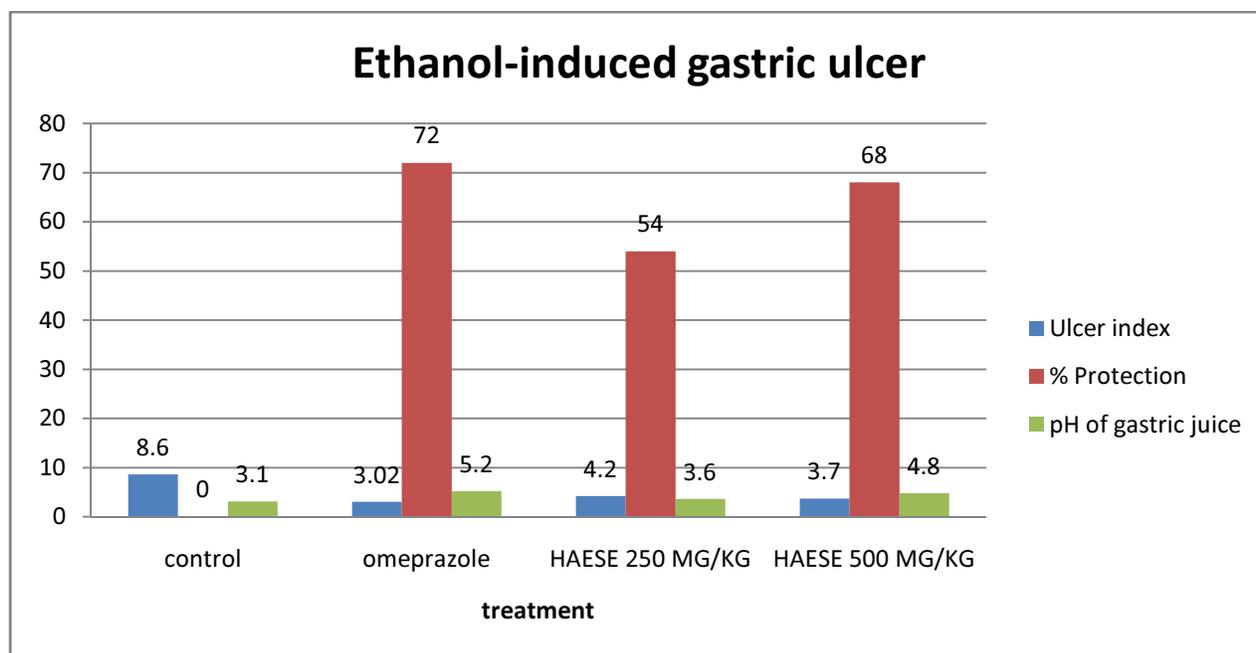


Table-2: Effect of *Sapindus emarginatus* leaf extract on various parameters in ethanol induced gastric ulcer.

Group	Treatments	Doses (mg/kg) mL/animal	Ulcer index	% Protection	pH of gastric juice
I	control	1	8.60±0.08	-	3.10±0.20
II	Omeprazole	20	3.02±0.26*	72	5.20±0.90*
III	Extract	250	4.20±0.04*	54	3.60±0.15
IV	extract	500	3.70±0.60*	68	4.80±0.17*

*: P<0.05 as compared to control group.

Figure 2: Effect of *Sapindus emarginatus* leaves extract on various parameters in ethanol induced gastric ulcer.



3.1. Pyloric ligation induced gastric ulcer.

In pyloric ligation induced ulcer model, oral administration of HAESE (Hydro-alcoholic extract of *Sapindus emarginatus* leaves) in two different doses showed significant reduction in ulcer index, gastric volume, as compared to the control group. It was showing protection index of 74% and 82% at the doses of 250 and 500 mg/kg,

respectively in comparison to control whereas omeprazole as reference standard drug showed reduction of ulcer 86%

(Table 1 and Figure 1)

3.2. 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, black and dark red lesions. HAESE showed significant protection index of 54% and 68% with the doses of 250 and 500 mg/kg, respectively in comparison to control.

Omeprazole as reference standard drug showed reduction of ulcer 72% (Table 2 and Figure 2)

4. Statistical analysis

The Dunnett's test was employed for statistical comparison. In all the cases, values of $P < 0.05$ were considered significant. All values were presented as mean \pm SE. In pyloric ligation induced ulcer model, oral administration of *Sapindus emarginatus* hydro-alcoholic extract in two different doses showed significant reduction in ulcer index, gastric volume, as compared to the control group. It was showing protection index of 74% and 82% at the doses of 250 and 500 mg/kg, respectively in comparison to control whereas Omeprazole as reference standard drug showed reduction of ulcer 86% (Table 1 and Figure 1)

5. Discussion

Peptic ulcers etiology is not clear in several cases but still it is generally accepted that the imbalance between the aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanism¹⁶. Pylorus ligation induced ulcer was used to study the effect of leaves extracts on gastric acid secretion and mucus secretion. The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach. This increase in the gastric acid secretion causes ulcers in the stomach. The original Shay rat model involves fasting of rats for 36 h followed by ligation of pyloric end of the stomach. The ulcer index is determined 5 h after pylorus ligation. The lesions produced by this method are located in the lumen region of the stomach. Many authors have modified the original model. In the present study, the Shay rat model described by Kulkarni was followed. Ethanol induced gastric ulcer was employed to study the cytoprotective effect of the extracts. Ethanol induced gastric lesion formation may be due to stasis in gastric blood flow which contributes to the development of the hemorrhage and necrotic aspects of tissue injury. Alcohol rapidly penetrates the gastric mucosa apparently causing cell and plasma membrane damage

leading to increased intracellular membrane permeability to sodium and water. The massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium^{17,18}.

6. Conclusion:

In conclusion, *Sapindus emarginatus* hydro-alcoholic extract possesses anti-ulcer activity which might be due to its antisecretory and cytoprotective activities.

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