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Evaluation Antiulcer Activity of *Hemidesmus Indicus* Ethanol Extracts in Rats

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Abstract

It is suggested that *Hemidesmus Indicus* extracts can suppress gastric damage induced by aggressive factors. It is generally accepted that gastric ulcers result from an imbalance between aggressive factors and the maintenance of the mucosal integrity through endogenous defence mechanisms. The excess gastric acid formation by prostaglandin (PG) includes both increase in mucosal resistance as well as a decrease in aggressive factors, mainly acid and pepsin. Inhibitions of PG synthesis by aspirin coincide with the earlier stages of damage to the cell membrane of mucosal, parietal and endothelial cells. The preliminary phytochemical studies revealed the presence of flavonoids in aqueous and alcoholic extracts of Hemidesmus Indicus various flavonoids have been reported for its anti-ulcerogenic activity with good level of gastric protection. So the possible mechanism of antiulcer action of Hemidesmus Indicus may be due to its flavonoid content. In this study we observed that Hemidesmus Indicus provides significant anti-ulcer activity against gastric ulcers in rats.

Keywords: *Hemidesmus Indicus,Anti Ulcer activity,Toxicity,Gatsric Mucosa.*

METHODOLOGY

Experimental animals

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% and12:12 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.

Earlier work done:

- ✓ The ethanol extract of H. indicus significantly prevented rifampicin and isoniazid induced hepatotoxicity in rats.
- ✓ Antipyretic use of this plant has also been reported
- ✓ Reported the use of H. indicus against menstrual problems.

Marketed products:

Ananthmool /Sarvi powder 200gm.

SBL Hemidesmus Indicus Mother Tincture Q 30ml

Plant Material Collection

The Roots of *Hemidesmus Indicus* was collected from the Botanical garden and was identified and authenticated from 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.

PREPARATION OF PLANT EXTRACTS

Preparation of Alcoholic Extract:

Fresh Roots of *Hemidesmus Indicus* were collected and washed under tap water. The Roots extract used was prepared by taking 20gms of finely cut Roots into 250ml beaker containing 200ml of alcohol. The contents were mixed well and then the mixture was boiled upto 50-60^{0}C for 4-5hrs. Further the extract was filtered with whatmann filter paper. The filtrate was boiled until the concentrated residue is formed. The concentrated product was sealed in sample covers and stored under room temperature and used for further experiment to check the activities.

Selection of dose for animal study

The dose considered for the experiment on rats was obtained from conversion of human dose of *Hemidesmus Indicus* (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 2000 mg/kg body weight.

PHARMACOLOGICAL EVALUATION

Preparation of extracts:

The aqueous and alcoholic extracts of *Hemidesmus Indicus* suspended in water in presence of 3%v/v Tween-80 solution.

All the drugs were administered orally for experimental purpose. Each time preparations of the extracts were prepared when required. The drugs were administered at a constant volume of 10ml/kg for each animal.

ACUTE ORAL TOXICITY:

The acute oral toxicity of aqueous and alcoholic extracts of *Hemidesmus Indicus* was determined by using rats which were maintained under standard conditions. The animals were fasted 12 hour prior to the experiment, up and down procedure OECD guideline no. 425 were adopted for toxicity studies. Animals were administered with single dose of individual extract upto 2000mg/kg and observed for its mortality during 7days and 21days study period (long term) toxicity and observed upto 7days for their mortality, behavioral and neurological profiles.

SCREENING FOR ANTI-ULCER ACTIVITY

The aqueous and alcoholic extracts of *Hemidesmus Indicus* Roots were tested for antiulcer activity using various methods like Acetic acid induced, alcohol induced, paracetamol induced and pyloric ligation method.

Acute stress-induced ulcer

The rats were deprived of food for 24 h, although water was allowed. Albino rats weighing between 160 - 180 g were divided into 12 groups consisting of six animals each. Experimental design and dosing schedule was as follows.

Animals were divided into four (I-V) groups.

Group I - Control group received distilled water (1ml, p.o).

Group II- Ulcer control

Group III - Standard group received Cimetidine (32mg/kg i.p).

Group IV - Test group received alcoholic extract of *Hemidesmus Indicus* (250mg/kg p.o).

Immediately after each procedure, the animals were killed and their stomachs removed, opened, and the inner lining examined. The gastric lesions were counted, and an ulcerative index (UI) was calculated for each animal as follows:

UI = (n lesion I) + (n lesion II) 2 + (n lesion III) 3

Where:

I = presence of edema, hyperemia and single, submucosal, punctiform hemorrages; II = presence of submucosal, hemorrhagic lesions with small erosions;
III = presence of deep ulcer with erosions and invasive lesions.

Acute, gastric lesions were induced by stress according to the model. After oral

administration of 0.9% NaCl, Cimetidine and different doses of Hemidesmus Indicus extract, each rat was immobilized in a cylindrical cage and vertically immersed in water to the level of the xiphoid process for 17 h at 23°-25°C. After this, the animals were immediately killed, their stomachs removed, and the gastric lesions were counted.

Alcohol Induced Ulcers in Rats

Alcohol induced ulcer model, in rats was studied for all extractives of both plants to determine the ulcer index and ulcer inhibition. Albino rats weighing between 160 - 180 g were divided into 12 groups consisting of six animals each. Experimental design and dosing schedule was as follows.

Animals were divided into four (I-V) groups.

Group I - Control group received distilled water (1ml, p.o).

Group II- Ulcer control

Group III - Standard group received Omeoprazole for seven days (2mg/kg i.p).

Group IV - Test group received alcoholic extract of Hemidesmus Indicus (250mg/kg p.o) for seven days.

On the final day of dosing, the animals also received extractives and the standard drug thirty minutes before administration of 1ml of ethanol. Animals were sacrificed after one hour and the contents of the gastric juice in the stomach were aspirated. Later the stomachs were removed and kept immersed in saline for 5 min. Incisions of the stomach were performed along the greater curvature and linear haemorrhagic lesions in the glandular regions were observed. A pair of dividers was used to measure the length of all the lesions in the stomachs. The length (mm) of each lesion was determined at 10 x magnification and summed up per stomach. Ulcer index was the sum of length of all lesions for each stomach.

Stomachs were immersed in 10% formalin for 24 h to study the histopathological changes in treated and ulcerated rats. Photographs of the opened stomachs were taken. The percentage ulcer inhibition was calculated by the following formula and the results were tabulated.

$$\% \text{ Ulcer protection} = \frac{\text{Ulcer Index in Control} - \text{Ulcer index in Test}}{\text{Ulcer Index in Control}} \times 100$$

Histopathological Evaluation of Alcohol induced Ulcers

The stomachs of the all groups of animals were immersed in 10% formalin to study the histopathological changes. After the standard processing the wet ulcerated tissues were embedded in paraffin and cut into thick sections. Parameters used to study histopathological changes included shedding of gastric epithelium, gastric erosions, infiltration of neutrophils, oedema and inflammation.

Alcohol induced ulcer model was carried out with the different extractives of *Hemidesmus Indicus* based on the previous protocol to select the extractives with anti ulcer activity for further evaluation on other anti ulcer models.

Aspirin Induced Modified Pylorus Ligated Model

The selected extractives of both plants were subjected to anti ulcer studies using Aspirin induced model. Adult Wistar albino rats of either sex weighing 180-250 g were fasted for 48h with free access to water and divided into six groups of six animals each. They were placed in cages with grating floor to avoid coprophagy. The experimental design and dosing schedule was carried out as follows.

Group I: Normal control

Group II: Ulcer control (Solvent) (10 ml/kg) + Aspirin(200 mg/kg)

Group III: Ranitidine (50 mg/kg)

Group IV: ALHI (250 mg/kg)

In Aspirin induced ulcer model, one hour before pyloric ligation, Aspirin at a dose of 200 mg/kg was administered orally as a suspension in 0.1% CMC. The animals were orally treated with the extractives at doses of 100 and 200 mg/kg once daily for seven days and 1 hour before administration of Aspirin. The standard group of animals was also treated in the same way.

Pyloric ligations were performed under ether anaesthesia taking care to avoid damage to the pylorus and the blood vessels. After ligation the stomachs were replaced and abdominal wall sutured. Food and water was restricted during the post-operative period of 4 h. The animals were sacrificed at the

end of four hours using excess ether anaesthesia. Thereafter the stomachs were opened and the contents of the gastric juice were collected. The contents were centrifuged and various biochemical estimations were carried out in the collected samples of control and treated groups of animals. The stomach samples were soaked in saline for five minutes and fixed to boards for morphological examinations of ulcer indices. Photographs were taken for further reference.

Evaluation of Ulcer Index and Inhibition

The ulcer index was calculated by counting the lesions with the aid of hand lens (10 X) and graded as follows.

0 = Normal coloured stomach

0.5 = Red colouration 1 = Spot ulcer

1.5 = Haemorrhagic streaks

2.0 = ulcers > 3 but < 5

3.0 = ulcers > 5

Mean ulcer score for each animal was expressed as ulcer index. Ulcer protection was calculated according to the standard formula.

$$\% \text{ Ulcer protection} = \frac{\text{Ulcer Index in Control} - \text{Ulcer index in Test}}{\text{Ulcer Index in Control}} \times 100$$

The volume and pH of the collected gastric juice was recorded. Free acidity and total acidity was calculated. Various bio-chemical estimations like total proteins, total hexoses, hexosamine, fucose, sialic acid, total carbohydrate and carbohydrate/protein ratio of the gastric juice were performed using standard methods.

Statistical analysis

The values were expressed as mean ± SEM data was analyzed using one-way ANOVA followed by T-test. Two sets of comparision had made. i.e. Normal control Vs All treated groups. Differences between groups were considered significant at $P<0.001$ and $P < 0.05$ levels

RESULTS

ACUTE TOXICITY STUDY

Administration of the *Hemidesmus Indicus* extracts *in* rats at doses of 200 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.

Effect on alcohol induced gastric ulcers

Oral administration of 80% alcohol produced haemorrhagic gastric lesions in glandular portion of stomach. Pretreatment with ALHI at the dose of 200 mg/kg and omeprazole (10 mg/ kg) significantly ($p<0.001$) protected the gastric mucosa as shown by reduced values of lesion index (20.33± 0.32 and 25.46± 0.72 respectively) against alcohol challenge as compared to solvent control (27.15 ± 0.21).

Table 1: Effect of *Hemidesmus Indicus* at various doses on alcohol induced gastric ulcer in rats.

Treatment (n=6)	**Dose mg/kg (p.o.)**	**Lesion index**	**% Inhibition of ulcer**	**Mucus content (μg Alcian blue/g wet tissue)**
1% CMC	-	27.15 ± 0.21	-	0.51 ± 0.01
Ulcer control	-	34.92±0.36	-	0.58±0.02
Omeprazole	10	25.46± 0.72	20.13	0.67 ± 0.01
ALHI	200	20.33± 0.32	45.09	0.89 ± 0.01

Values are mean ± S.E.M. n=number of animals in each group. Significant differences with respect to solvent control group were evaluated by Student's *t* – test. ($p<0.05$, $p<0.01$ and $p<0.001$).

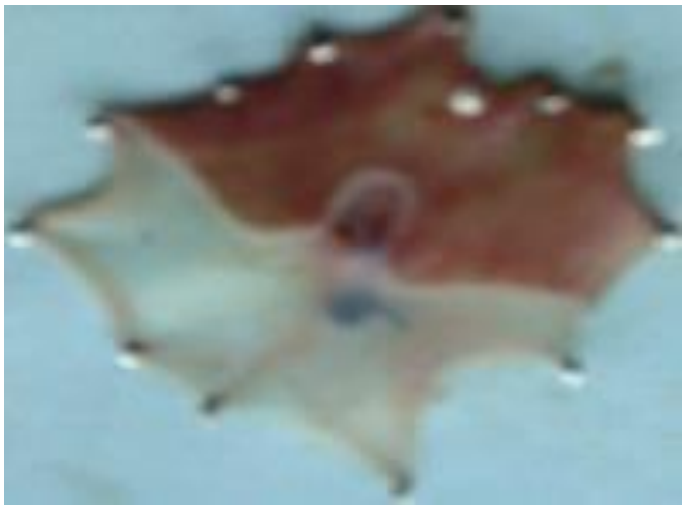

Fig-1: Effect of *Hemidesmus Indicus* on alcohol induced ulcers in the rats in the study- Normal Control

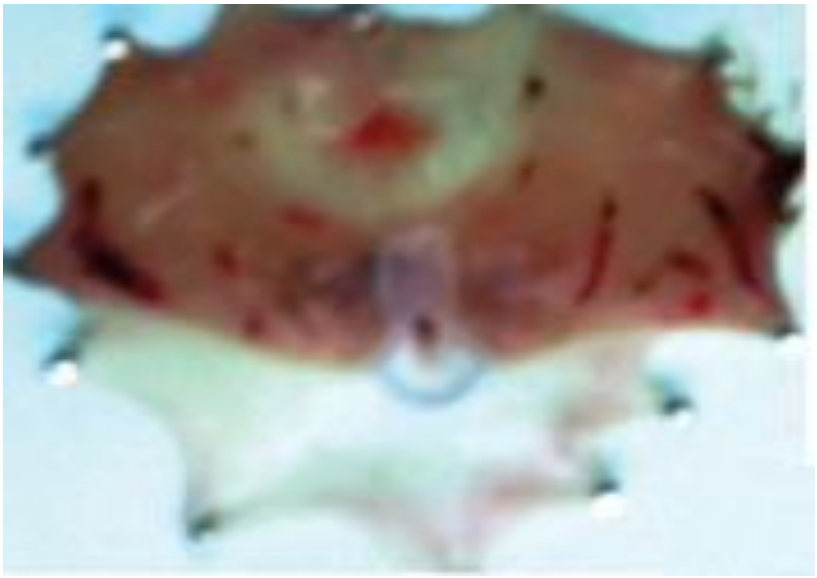

Fig-2: Effect of *Hemidesmus Indicus* on alcohol induced ulcers in the rats in the study Ulcer Control

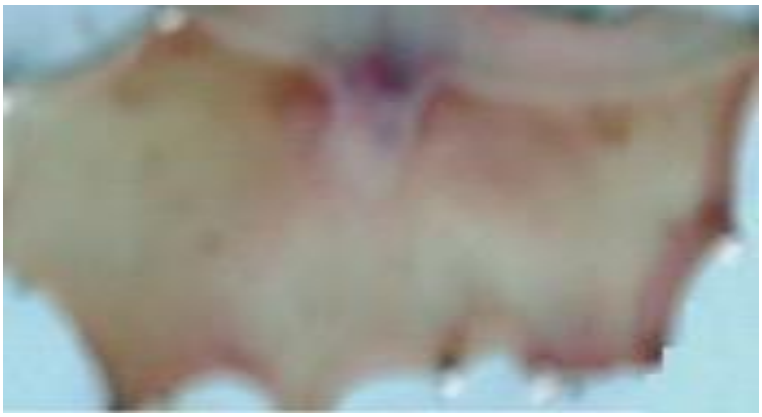

Fig-3: Effect of *Hemidesmus Indicus* on alcohol induced ulcers in the rats in the study ALHI (200 mg/kg) treated

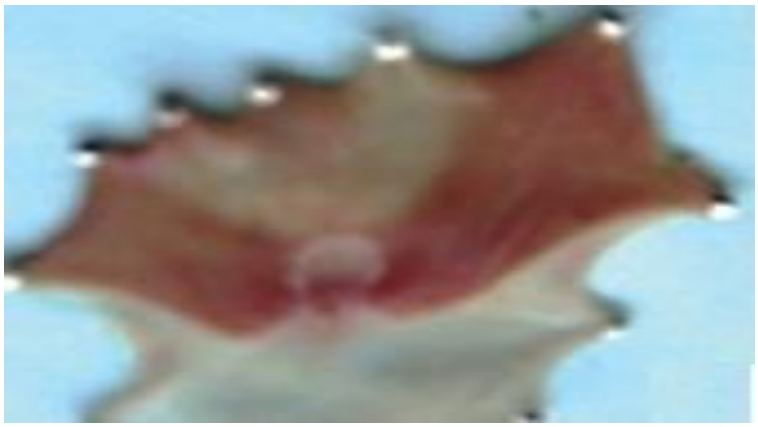

Fig-4: Effect of *Hemidesmus Indicus* on alcohol induced ulcers in the rats in the study Omeprazole (10 mg/kg treated)

Effect on Aspirin induced gastric ulcers

In *Hemidesmus Indicus* treated groups (200 mg/kg), the ulcer index values (0.33 ± 0.08 respectively) were significantly reduced ($p<0.001$) when compared to solvent control (0.69 ± 0.01), while the ulcer index for ranitidine treated group was 0.21 ± 0.01 ($p<0.001$). The % inhibition of ulcer showed by ALHI (200 mg/kg) and ranitidine was 50.9% and 54.8% respectively.

Table 2.Effect of *Hemidesmus Indicus* at various dose levels on Aspirin induced gastric ulcer in rats.

Treatment (n=6)	**Dose mg/kg (p.o.)**	**Ulcer index**	**% Inhibition of ulcer**
1% CMC	-	0.69 ± 0.01	-
Ulcer control	-	0.85±0.03	--
Ranitidine	50	0.21 ± 0.07	50.9
ALHI	200	0.33 ± 0.08	54.8

Values are mean ± S.E.M. n=number of animals in each group; Significant differences with respect to solvent control group were evaluated by Student's *t* - test. ($p<0.001$).

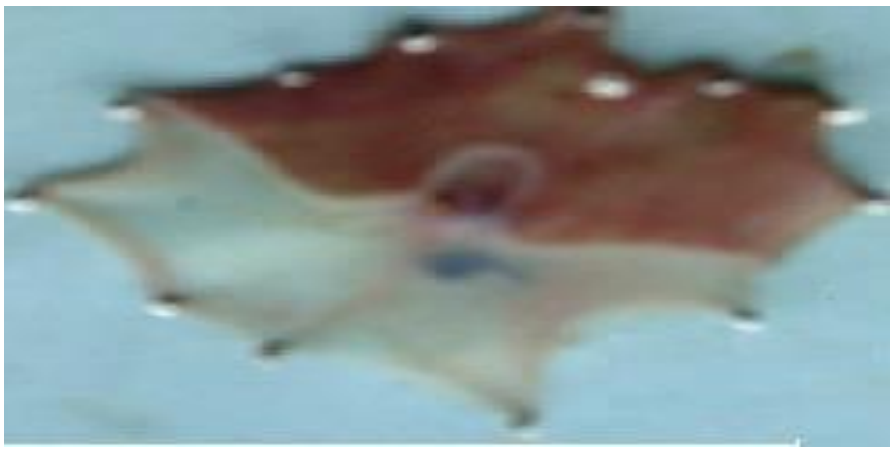

Fig-5: Effect of *Hemidesmus Indicus* on *Aspirin* induced ulcers in the rats in the study Normal Control

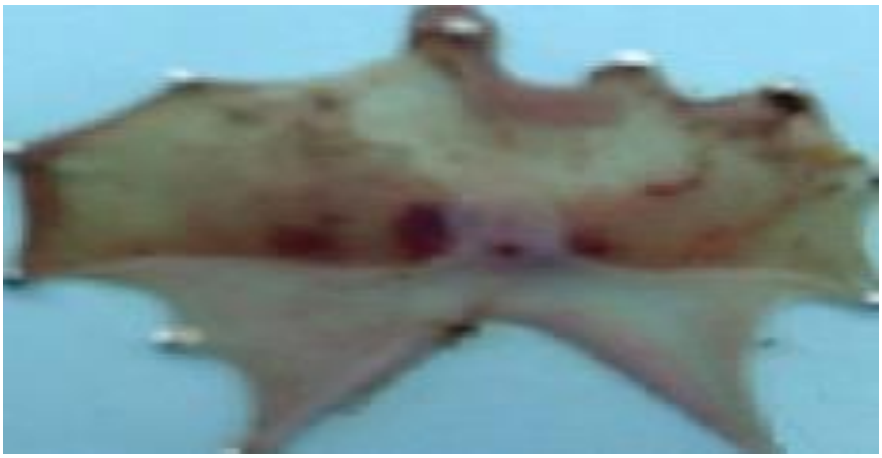

Fig-6: Effect of *Hemidesmus Indicus* on *Aspirin* induced ulcers in the rats in the study Ulcer Control

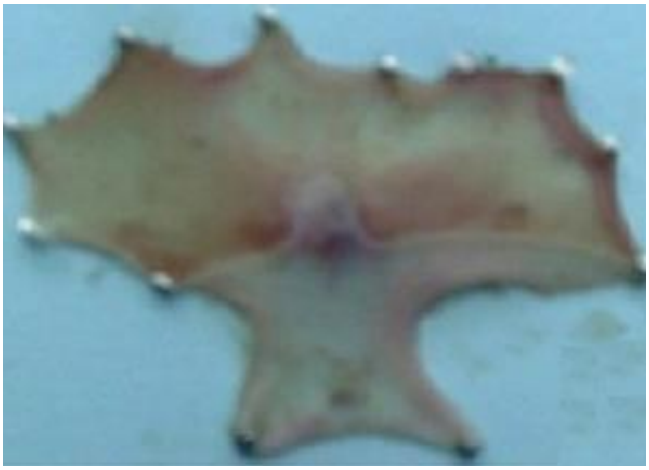

Fig-7: Effect of *Hemidesmus Indicus* on *Aspirin* induced ulcers in the rats in the study ALHI (200 mg/kg) treated

Fig-8: Effect of *Hemidesmus Indicus* on *Aspirin* induced ulcers in the rats in the study Ranitidine (50 mg/kg treated)

Stress-induced ulcers:

In water immersion stress induced ulcers, the mean score value of ulcer inhibition was found to be significant (P<0.001) for 200 mg/kg of the extract. The percentage ulcer inhibition
was 85.76 for 200 mg/kg for alcoholic extracts, and that of the standard was found to be 94.21.

Table 3. Effect of *Hemidesmus Indicus* at various dose levels on Stress induced gastric ulcer in rats.

Group	Ulcer index	Percentage inhibition
Normal Control	00.00±0.00	-----
Ulcer control	21.19±2.77	------
Standard	2.24±0.11	94.21
ALHI	4.42±2.75	85.76

Values are mean ± S.E.M. n=number of animals in each group; Significant differences with respect to solvent control group were evaluated by Student's *t* - test. (p<0.001).

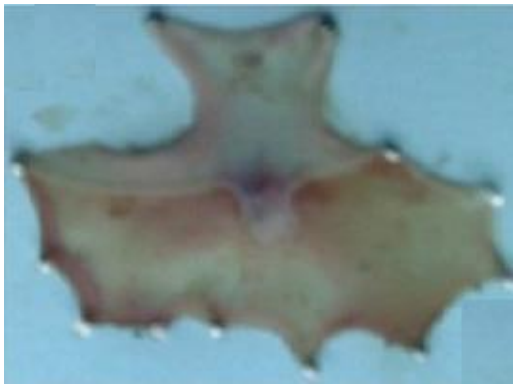

Fig-9: Effect of *Hemidesmus Indicus* on stress induced ulcers in the rats in the study Normal Control

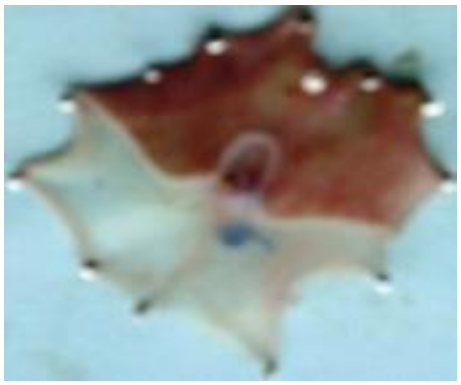

Fig-10: Effect of *Hemidesmus Indicus* on stress induced ulcers in the rats in the study

Ulcer Control

Fig-11: Effect of *Hemidesmus Indicus* on stress induced ulcers in the rats in the study ALHI (200 mg/kg) treated

Fig-12: Effect of *Hemidesmus Indicus* on stress induced ulcers in the rats in the study Omeprazole (10 mg/kg treated)

DISCUSSION

The anti-ulcer activity of Hemidesmus Indicus was evaluated by employing alcohol/paracetamol/acetic acid/stress induced gastric ulcers in rats. Alcohol and paracetamol induced ulcer models were used because they represent some of the most common causes of gastric ulcer in humans. Many factors and mechanisms are implicated in the ulcerogenesis and gastric mucosal damage induced by different models employed in the present study involving the increase of gastric acid output, vascular injury, depletion of gastric wall mucin, mucosal damage induced by non-steroidal anti-inflammatory drugs and free radical production.

Alcohol induced gastric injury is associated with significant production of oxygen free radicals leading to increased lipid peroxidation which causes damage to cell and cell membranes. Hemidesmus Indicus has significantly protected the gastric mucosa against alcohol challenge as shown by reduced values of lesion index as compared to solvent control group suggesting its potent cytoprotective effect. This is further substantiated by increase in gastric mucus content produced by Hemidesmus Indicus extract.

NSAID's like *Aspirin*, cause gastric mucosal damage by decreasing prostaglandin levels through inhibition of PG synthesis. *Hemidesmus Indicus* extract was significantly effective in protecting gastric mucosa against *Aspirin* induced ulcers at all the dose level studied. Hence *Hemidesmus Indicus* extract affords effective protection to gastric mucosa against various insults by increasing gastric mucus content and decreasing the acid volume, free and total acidity in rats.

Stress plays an important role in ulcerogenesis. The pathophysiology of stress- induced gastric ulcers is complex. Stress-induced ulcers are probably mediated by histamine release with enhancement in acid secretion and a reduction in mucus production. The aqueous and alcoholic extracts of *Hemidesmus Indicus* were effective in reducing the ulcers induced by stress. The effects in all the 3 models studied were dose dependent. In conclusion, to the best of our knowledge for the first time, we have demonstrated that Hence *Hemidesmus Indicus* extract has gastroprotective activity against experimentally induced ulcers in rats. The mechanism of gastroprotective action can be attributed to its antisecretory and cytoprotective property. However further experiments are required to establish and elaborate the molecular mechanisms of its Anti-ulcer activity.

CONCLUSION

The anti-ulcer activity of the plant Hemidesmus Indicus was evaluated by employing Aspirin, alcohol and stress induced ulcer models. These models represent some of the most common causes of gastric ulcer in humans. Many factors and mechanisms are implicated in the ulcerogenesis and gastric mucosal damage induced by different models employed in the present study involving, depletion of gastric wall, mucin mucosal damage induced by nonsteroidal anti-inflammatory drugs and free radical production. 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 Hemidesmus Indicus was significantly effective in protecting gastric mucosa against Aspirin induced ulcers at all the dose level studied.

Alcohol induced gastric injury is associated with significant production of oxygen free radicals leading to increased lipid peroxidation, which causes damage to cell and cell membrane. The extracts of the *Hemidesmus Indicus* has significantly protected the gastric mucosa against alcohol challenge as shown by reduced values of lesion index as compared to control group suggesting its potent cytoprotective effect.

It has been proposed that in pyloric ligation, the digestive effect of accumulated gastric juice and interference of gastric blood circulation are responsible for induction of ulceration.

The antiulcer activity of *Hemidesmus Indicus* extracts in stress induced

model is evident from its significant reduction in gastric volume, ulcer index and increase in pH of gastric juice. Because of animals treated with *Hemidesmus Indicus* extracts significantly inhibited the formation of ulcer in the stomach and also decreased both acid concentration, gastric volume and increased the pH values.

On the basis of the present results and available reports, it can be concluded that the anti- ulcer activity elucidated by Hemidesmus Indicus 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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