

Research article

Aqueous extract of whole plant of *hyptis suaveolens* (L.) poit: An anti-ulcer agent

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ABSTRACT

The aim of current experimental study was to determine anti ulcer potential of Watery extricate of *Hyptis suaveolens* entire plant (AEHS) on the Wister strain albino rats. The Watery extricate of *Hyptis suaveolens* entire plant (in two different doses, 250mg/kg and 500 mg/kg) was allowed for anti-ulcer activity against Pylorus ligation induced model in Wistar albino rats. Omeprazole (20mg/kg) was used as standard for the evaluation of activity. Parameters like Proportion of Ulcer protection was reckoned based on Ulcer index and Digestive juice volume, pH and acidity of gastric juice, Mucus production and Pepsin estimation, tensile strength. The AEHS with 500 mg/kg dose was shown significant anti ulcer activity when compared with the control group.

Keywords: *Hyptis suaveolens* Omeprazole, ulcer index, ulcer protection, free acidity, total acidity.

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INTRODUCTION

Now days, Ulcer is one of the widespread gastrointestinal disorder diagnosed in most of the population. It is primarily an inflamed discontinuity in the skin or the mucus membrane which lines the alimentary tract. Abnormal equilibrium raised due to either increased aggression or diminished mucosal resistance results in the Ulceration. It may be because of regular drugs usage, food habits, stress. Now days, Peptic ulcers are foremost common ulcers of gastrointestinal tract. Peptic ulcers formation depends on the presence of acid and peptic activity in gastric fluid plus a breakdown in mucosal defenses. Huge quantity of synthetic drugs are available in the market for the treatments of ulcers, but these drugs cause more side effects after prolong usage, so there is a huge demand for herbal drugs and alsosynthetic drugs are expensive hen compared to herbal.^[1]

Hyptis belongs to the genus of Lamiaceae with 400 species, among the species, one named as *Hyptis suaveolens* Poit, most of the species are ancient to the tropical and subtropical regions.^[2] The plant *Hyptis suaveolens* (*H. suaveolens*) (L.) Poit. is a pharmacognostically major medicinal plant. *Hyptis suaveolens* commonly named as “Wilayati tulsi.” Most of plant parts are being utilized in the conventional medicine to treat variety of diseases. Extracts of *Hyptis suaveolens* plant have been found to be the one of

the important pharmacologically active plant with wide range of activities. Traditionally, *Hyptis suaveolens* has been reported to active as tonics, emmenagogues, diaphoretics, antispasmodics, burns and wounds, stimulant, carminative, antimicrobial, antibacterial, antispasmodic, analgesic, anti-inflammatory, headaches, anti-cutaneous, anti-catarhal, Insecticidal Effect, Acaricidal Effect, antifertility agent.^[3-9]

In this study, Omeprazole was chosen as the reference anti-ulcer drug (a proton pump inhibitor), which is one of the first line acid inhibitor used to treat gastric disorders for about 15 years (Li et al., 2004). The Current study was carried out to evaluate anti-ulcerogenic activity of watery extricate of *Hyptis suaveolens* in rats.

MATERIALS AND METHODS

Collection of Plant material and extraction

The entire plant of *Hyptis suaveolens* was collected from the forests of Maisammaguda, Secunderabad, from the state of Telangana (India) and shade dried and powdered. The plant specimen was attested by the natural historian of Osmania University and voucher specimen Number 276 of the plant has been filed in the department for the future use. The dried plant powder was successively extracted using different solvents based on their polarity using petroleum ether, trichloroMethane, ethyl acetate, ethanol by

Soxhlet extraction process and method of maceration using water for a about 72 hours. The crude extracts were allowed to dry under vacuum, dried in vacuum desiccators and stored in refrigerator. Crude suspension obtained from Watery extricate of the plant powder(dried and milled) was went through filtration process using a muslin cloth and the further concentrated on a water bath by boiling for one hour at 100°C. This was later freeze dried Preliminary phytochemical investigation was performed and revealed that watery extricate was rich in Phyto constituents among the other extricates. From the above information provided, watery extricate of Hyptis suaveolens was selected for screening anti-ulcer potential in experimental animals.

Experimental Animals

Healthy male and female rats will be procured, acclimatized to the college laboratory conditions (1week or 10 days), they are provided with standard food pellets and water. Wistar albino rats weighing 150-250 gm of either gender were maintained in a 12 hr light/dark cycle at a constant temperature 25°C with free access to feed and water. All animals were on fasting prior to all assays and were assigned to four different experimental groups, each group consists of 6 rats. Moreover the animals were housed in respective cages to prevent coprophagia during the experiment. All animal experiments were performed according to NIH guidelines, after getting the approval of the Institutional Animal Ethics Committee with an approval number CPCSEA/IAEC/JLS/11/11/19/13. Food was served in the form of dry pellets and water ad libitum.

EXPERIMENTAL METHODOLOGY

The rats were randomly assigned into 4 different groups (n=6). All the animals were received 200 mg/kg of aspirin once daily for three days.

- Group I : Normal control receiving Distilled water
- Group II : Omeprazole (20mg/kg) p.o.
- Group III : Watery extricate of Hyptis suaveolens Linn. (250mg/kg) p.o
- Group IV : Watery extricate of Hyptis suaveolens Linn. (500mg/kg) p.o

On the 3rd day, all group rats were fasted 24 h prior to induction of gastric ulcer. After the pretreatment period (1h), animals were anaesthetized by giving pentobarbitone with a dose of 35 mg/kg (i.p.), stomach was opened by a tiny midline slit below the xiphoid process; the pyloric region of the stomach was slightly lifted out and ligated carefully, without any traction to the pylorus or damage to its blood circulation. The stomach was replaced warily and the abdominal layer was closed in two layers with sutures. After 4 hrs, stomachs were dissected out carefully and open along the greater curvature, examined for ulcers index (Shay et al., 1945). Stomach was washed with water, pinned on a corkboard. Erosions on the glandular portion of stomach were counted and each ranked on 1-3 scale based on size of ulcers (diameter). The ulcer index (UI) is assessed by dividing the overall total diameter of ulcers in one

stomach with a factor 10. Free acidity and total acidity were assessed by titrating the Digestive juice against with 0.01N NaOH using Topfer's reagent as indicator.

The % Ulcer protection was determined using the formula = $(1 - (U_t/U_c)) * 100$

Where U_t = Ulcer index of treated group and U_c = Ulcer index of the control group.

Ulcer index has been calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach.

Measurement of mucus production

Gastric mucus production was measured in rats subjected to pylorus ligation. The mucus covering of each stomach was gently scraped using a glass slide and weighed immediately using a digital precision electronic balance.

Estimation of pepsin activity

The centrifuged gastric juice (0.1 mL) was added to 1 mL bovine albumin (0.5% w/v in 0.01 N HCl pH 2.0) and allow to incubate for about 20 minutes at 37°C. A gastric juice blank tube, in which 1 mL 0.01 N HCl was taken in the place of 1 mL of albumin, was simultaneously run in the same process. Hydrolysis was hindered by adding 10% TCA (2 mL). All these tubes were allowed to heat for 5 minutes in boiling water bath, then allowed to cool. Protein's denaturation was done by the process of heating; the formed precipitate was separated by centrifugation process. 1 mL of the supernatant liquid (upper layer) was added to mixture of 0.4 mL of 2.5 N Sodium Hydroxide and 0.1 ml of the Folin-Ciocalteu reagent, then the final volume was rise up to 10 mL by using distilled water. Absorbance was measured at 700 nm. The peptic activity was assessed in terms of number of μg of liberated tyrosine per ml of digestive juice.^[10]

RESULTS

The significant antiulcer activity (ulcer index, %ulcer inhibition, volume of gastric juice, free acidity, total acidity) of Watery extricate of Hyptis suaveolens (AEHS) at different doses(250mg/kg and 500mg/kg) on aspirin plus pylorus ligation induced gastric ulcers in rats is evaluated using Omeprazole as standard and the results were shown in table1. The results were shown graphically. Ulcer index, % ulcer inhibition, Free acidity and total acidity were calculated.

DISCUSSION

The Ulcer index was measured at both doses 250mg/kg and 500mg/kg, but the dose 500 mg/kg shows closer response to standard drug.(Omeprazole) Percentage of ulcer protection in 500 mg/kg treated group was higher when compared to 250 mg/kg treated group, but not as standard drug treated group. Gastric juice was measured and the values were given in Table 1, figure 1 and 2. Free acidity, total acidity (table 1, figure 3 and 4) were also measured, it reveals, the 500mg/kg dose extract shows better activity compared to

250mg/kg dose extract. Hence, it can be said that both extracts have anti-ulcer activity, but AEHS at 500 mg/kg is more potent, but not like standard (Omeprazole). pepsin and HCl were crucial for pylorus ligated ulcers formation, the ulcer protective effect of the watery extract of *H. Suaveolens* could arguably have been achieved either through the enhanced mucus secretion that would re-enforce gastric mucous defenses, or through the depletion of the proteolytic activity of the pepsin in the digestive juice. Is evident from the results, as

Table 1: Effect of AEHS on various parameters

Treatment	Ulcer index (mean \pm SEM)	% Ulcer protection	pH of gastric juice (ml)	Gastric juice (ml)	Free acidity meq/ltr	Total acidity meq/ltr
Control	14.8 \pm 0.15	3.1 \pm 0.25	8.8	91.61	110.34
Omeprazole(20mg/kg)	2.6 \pm 0.06***	82%	5.85 \pm 0.05	2.6	35.53	62.61
AEHS(250 mg/kg)	3.3 \pm 0.04*	78%	3.31 \pm 0.13	4.2	42.21	65.45
AEHS (500mg/kg)	2.8 \pm 0.05**	81%	4.28 \pm 0.15	4	38.6	63.82

Values are expressed as mean \pm SEM. Test and standard groups were compared with control group. Statistical comparison was done using ANOVA followed by t- test. *P < .05; **P < .01; ***P < .001, when compared with control groups.

Figure 1: Effect of AEHS (250mg/kg and 500mg/kg) on ulcer index

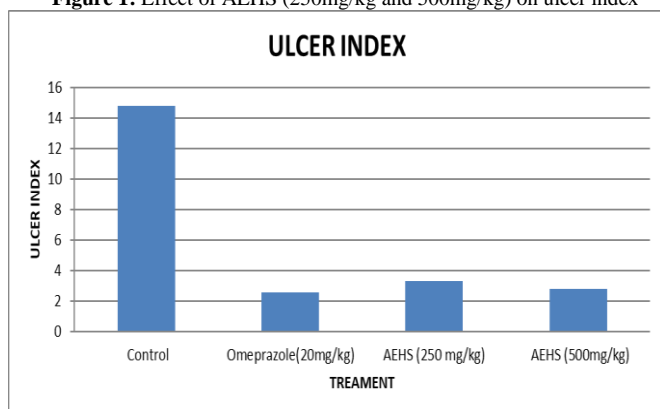


Figure 2: Effect of AEHS (250mg/kg and 500mg/kg) on % ulcer inhibition.

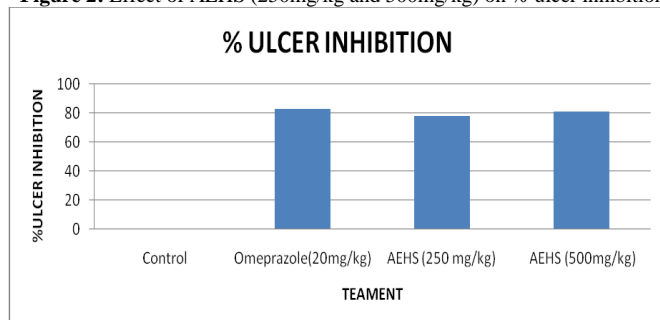
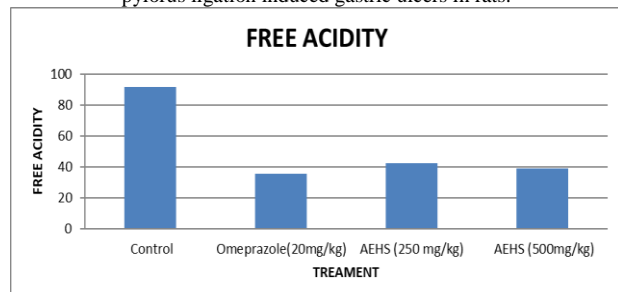


Figure 3: Effect of AEHS (250mg/kg and 500mg/kg) on free acidity in pylorus ligation induced gastric ulcers in rats.



increase in the gastric mucus production and depletion in the pepsin activity (Table 2) seen when increasing doses of the extract were administered to the rats. Thus, by increased mucus production, reduced pepsin content, the extract may possess cytoprotection activity through a mechanism involving the physicochemical re-enforcement of the gastric mucous layer or by effects similar to endogenous Prostaglandins.

Figure 4: Effect of AEHS (250mg/kg and 500mg/kg) on total acidity.

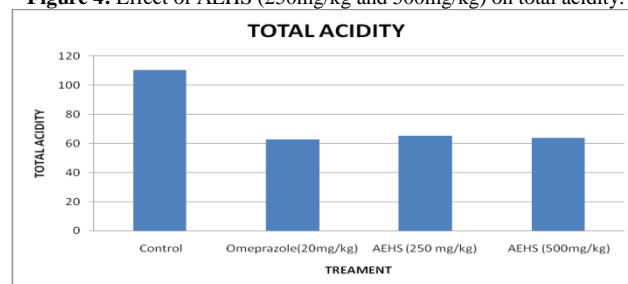


Table 2: Effect of AEHS (250mg/kg and 500mg/kg) on mucus secretion and pepsin content

Treatment	mucus production (μ g)	pepsin content (μ moles of tyrosine/ ml)
Control	34.8 \pm 0.56	21.63 \pm 1.21
Omeprazole (20mg/kg)	64.45 \pm 0.66***	4.57 \pm 1.13***
AEHS (250 mg/kg)	47.3 \pm 0.44*	9.45 \pm 1.22*
AEHS (500mg/kg)	52.81 \pm 0.25**	8.12 \pm 1.21**

Values are expressed as mean \pm SEM. Test and standard groups were compared with control group. Statistical comparison was performed using ANOVA followed by t- test. *P < .05; **P < .01; ***P < .001, when compared with control groups.

CONCLUSION

The results of our study indicate that the Watery extract of *Hyptis suaveolens* entire plant produces significant cytoprotective effects against pylorus ligation-induced ulcers. Thus, the Watery extract of *Hyptis suaveolens* entire plant can be used as a new source for antiulcer drugs.

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