British Journal of Pharmaceutical Research
5(1): 42-51, 2015, Article no.BJPR.2015.004
ISSN: 2231-2919

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Evaluation of Anti-ulcer Activity of Hydro Alcoholic Extracts of *Abutilon indicum*, *Helianthus annuus* and Combination of Both against Ethanol and Pyloric Ligation Induced Gastric Ulcer in Albino Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author KV designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors KVB and OSK managed the animals and experimental analyses of the study. Authors ND and KBCS managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2015/14386
Editor(s):
(1) Sami Nazzal, College of Pharmacy, University of Louisiana at Monroe, USA
Reviewers:
(1) Anonymous, Brazil.
(2) Arif H. Shah, Akamai University, USA.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=860&id=14&aid=7078

Original Research Article

Received 29th September 2014
Accepted 11th November 2014
Published 2nd December 2014

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ABSTRACT

Background: *Abutilon indicum* and *Helianthus annuus* consists of various compounds which have different therapeutic activities.
Objective: The main aim of the present study was to compare the anti-ulcer activity of hydroalcoholic extracts of *A. indicum*, *H. annuus* and combination of both against Ethanol induced gastric ulcer and pyloric ligation induced gastric ulcer in Albino Wistar Rats.
Methodology: The method used was OECD 423 (Acute Toxic Class Method), which allowed evaluating the toxic dose of the crude drugs. Furthermore, the method adopted, provided the selection of proper dose regime to investigate the activity against both types of stomach ulcers in animal models. The omeprazole was used as standard drug for comparison.
Results: Three different extracts were prepared for evaluation namely: HAEAI, HAEHA and C-HAEAIHA which showed a statistically significant anti-ulcer activity. The protective effect was found to be in the following order: C-HAEAIHA > HAEHA > HAEAI.
Conclusion: Among the tested extracts, the combined mixture of hydroalcoholic extract of *A. indicum* and *H. annuus* (C-HAEAIHA) showed better anti-ulcer activity.

Keywords: Acute Toxic Class Method; Abutilon indicum; Helianthus annuus; stomach anti-ulcer activity; animal model.

1. INTRODUCTION

A gastric ulcer is an inflamed break in the mucus membrane of Gastrointestinal Tract. The main cause of gastrointestinal track ulceration is either by aggression factors activity or decreased mucosal resistance. Gastric ulcers are found in the stomach wall and are less common in occurrence [1]. The agents known to cause ulcers are as follows: bile acids, pepsin, *Helicobacter pylori*, alcohol, various food constituents and some pharmaceutical drug products. These agents may play a key role in the pathogenesis of gastric ulcer. These agents usually cause harm by enhancing gastric acid secretion or inhibition of prostaglandin synthesis or by increasing pepsin [1,2,3].

Various plants used in traditional medicine showed stomach antiulcer activity and some toxicity due to the presence of different phytochemical constituents [4-6]. The literature review on traditional medicine indicated that no gastric antiulcer activity studies were conducted earlier on the popular herbal drugs such as *Abutilon indicum* L. (Malvaceae) and *Helianthus annuus* L. (Asteraceae). Both the herbal drugs are known to possess several biologically active chemical constituents [7-12]. Hence, the study design included the extracts of these plants to be subjected to toxicity test in order to find the appropriate dose for current study. Current gastric anti-ulcer study was conducted by following the standard procedures: protection against ethanol induced and Pyloric Ligation (PL) induced gastric ulcers using rats as experimental animal model and the results are presented in present communication [13-17].

2. MATERIALS AND METHODS

2.1 Collection of Plants Material

Helianthus annuus (HA) was collected in the month of November, from the fields of Narasapuram, Kurnool District, Andhra Pradesh state, India and *Abutilon indicum* (AI) was collected in the month of November, from the fields of North Rajupalem, Nellore District, Andhra Pradesh state, India.

2.2 Identification of Plants

The plant specimens were identified and were authenticated by Dr. P. Jayaraman, Director, Institute of Herbal Botany, Plant Anatomy Research Center, (PARC) Tambaram, Chennai, India where plant specimen were kept for record.

Voucher Specimen Number:

PARC/2013/1454 for *Helianthus annuus* L,
PARC/2013/1455 for *Abutilon indicum* (L) Sweet.

2.3 Plant Extraction

The leaves of HA and AI were shade dried and coarsely powdered. The powdered materials about 500 g of HA, 500 g of AI and combined extract of AI+HA 500g (the mixture contained: AI

250 g and HA 250 g) were taken into Stoppard glass jars and soaked with Petroleum Ether for 8 h to remove the wax content from the powder [18,19,22]. Petroleum Ether layer was later separated from the jars. The powder left was extracted with Hydro alcoholic solvent (mixture of 60% Ethanol and 40% water) by cold maceration method [20].

The solvent was filtered and distilled off. Final traces of solvent were removed under vacuum. The hydro alcoholic extracts (HAE) were concentrated and the yield was reported in Table 1.

2.4 Experimental Animals

Albino rats (150–200 g) of either sex were maintained in a 12 hour light/dark cycle at a constant temperature 25ºC with free access to feed and water. All animals were fasted prior to all assays and were allocated to different experimental groups each of 6 rats. Moreover the animals were kept in specially constructed cages to prevent coprophagia during the experiment. All these experiments were carried out according to the guidelines for care and use of experimental animals and these experiments were approved by CPSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals). Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPSEA.

2.5 Toxicity Evaluation

2.5.1 Acute oral toxicity study

The procedure was followed by using OECD 423 (Acute Toxic Class Method). This is a step wise procedure with three animals of same sex per each step. Depending on the mortality or moribund status of the animals, the average two to four steps may give the decision on test substances about their acute toxicity. This official procedure needs relatively small numbers of animals. As defined the following doses were used: 5 mg/kg, 50 mg/kg, 500 mg/kg, and 2000 mg/kg body weight. The toxicity signs were recorded and the test substances were classified and ranked according to the 'Globally Harmonized System'.

2.5.2 Experimental procedure

Female Wistar rats weighing 150-250 g were used in the present study. The starting dose level of Hydro alcoholic extract of *A. indicum* (HAEAI), Hydro alcoholic extract of *H. annuus* (HAEHA) and combined hydro alcoholic extract of *A. indicum* and *H. annuus* (C-HEAIHA) was 2000 mg/kg body weight p.o (per oral) because most of the crude extracts possess LD_{50} value more than 2000 mg/kg (p.o). The results of the present study were depicted in Table 2.

Table 1. Percentage yield and colour of total extracts

Hydro alcoholic extract (HAE)	Percentage yield (%)	Colour	Consistency
HAEAI	12.45	Dark green	Greasy
HAEHA	10.69	Dark brown	Semi solid
C-HAEAIHA	13.84	Brown	Greasy

Table 2. Results of acute toxicity class method OECD guidelines 423

S. no	Groups	Dose/kg b.wt.	Weight of animals		Signs of toxicity	Onset of toxicity	Duration of study
			Before test	After test			
1.	HAEAI	2000 mg	180 g	190 g	No signs	Nil	14 days
2.	HAEAI	2000 mg	185 g	195g	No signs	Nil	14 days
3.	HAEAI	2000 mg	200 g	210 g	No signs	Nil	14 days
4.	HAEHA	2000 mg	200 g	215 g	No signs	Nil	14 days
5.	HAEHA	2000 mg	190 g	205 g	No signs	Nil	14 days
6.	HAEHA	2000 mg	195 g	210 g	No signs	Nil	14 days
7.	C-HAEAIHA	2000 mg	185 g	200 g	No signs	Nil	14 days
8.	C-HAEAIHA	2000 mg	175 g	185 g	No signs	Nil	14 days
9.	C-HAEAIHA	2000 mg	180 g	195 g	No signs	Nil	14 days

Dose volume was administered 0.2 ml per 100 g body weight to overnight fasted rats with water ad libitum. Then the Hydro alcoholic extract of *A. indicum* (HAEAI), Hydro alcoholic extract of *H. annuus* (HAEHA) and combined Hydro alcoholic extract of *A. indicum* and *H. annuus* (C-HEAIHA) were administered, the feed was given after 3-4 hours of drug administration and during this time the signs of toxicity were observed. The body weights of rats were recorded before and after drug administration.

Throughout the study, official procedure was followed as defined under OECD guidelines 423 (acute toxic class method). The data collected included: changes in mucous membranes, fur, respiratory system, eyes, circulatory system, skin, autonomic and central nervous system and motor activity besides behavioral changes. The signs of tremors, diarrhea, convulsions, salivation, sleep, lethargy and coma were also noted. The onset of toxicity and signs of toxicity noticed are presented in Table 2. Based on the results of present study, 1/5th (400 mg/kg), 1/10th (200 mg/kg) of these oral doses was selected for further study.

2.6 Materials

1. Hydro alcoholic extract of *A. indicum* (HAEAI)
2. Hydro alcoholic extract of *H. annuus* (HAEHA)
3. Combined Hydro alcoholic extract of *A. indicum* and *H. annuus* (C-HAEAIHA), 1%v/v tween 80
4. Standard drug Omeprazole

2.7 Antiulcer Activity

2.7.1 Ethanol induced gastric ulcer

Animals were randomly assigned to 8 groups each of 6 rats.

Group I [control] - Animals treated with 1% v/v aqueous tween 80 (10 ml/kg, p.o)
Group II - Animals treated with Hydro alcoholic extract of *A. indicum* (HEAI) (200 mg/kg, p.o)
Group III - Animals treated with Hydro alcoholic extract of *A. indicum* (HEAI) (400 mg/kg, p.o)
Group IV -Animals treated with Hydro alcoholic extract of *H. annuus* (HEHA) (200 mg/kg, p.o)
Group V - Animals treated with Hydro alcoholic extract of *H. Annuus* (HAEHA) (400 mg/kg, p.o)
Group VI - Animals treated with Combined Hydro alcoholic extract of *A. indicum* and *H. annuus* (C-HEAIHA) (200 mg/kg, p.o)
Group VII - Animals treated with Hydro alcoholic extract of *A. indicum* and *H. annuus* (C-HEAIHA) (200 mg/kg, p.o) respectively for 7 days.
Group VIII [Standard Control Group] - Animals treated with omeprazole (20 mg/kg, p.o) were administered 30 min prior to induction of gastric ulcer.

On the 7th day, Gastric ulcers were induced with ethanol at a dose of 8 ml/kg administered to all groups orally [14,20]. The animals were anaesthetized 6 h with ether and stomachs were incised along the greater curvature and the ulcer index for each rat was taken as the mean ulcer score shown in Table 3.

2.7.2 Pyloric ligation induced gastric ulcer

Animals were randomly divided into 8 groups each of 6 rats.

Group I [Normal control] - Animals treated with 1% v/v aqueous tween 80 (10 ml/kg, p.o)
Group II - Animals treated with Hydro alcoholic extract of *A. indicum* (HEAI) (200 mg/kg, p.o)
Group III - Animals treated with Hydro alcoholic extract of *A. indicum* (HEAI) (400 mg/kg, p.o)
Group IV - Animals treated with Hydro alcoholic extract of *H. annuus* (HEHA) (200mg/kg, p.o)
Group V - Animals treated with Hydro alcoholic extract of *H. annuus* (HEHA) (400 mg/kg. p.o)
Group VI - Animals treated with Combined Hydro alcoholic extract of *A. Indicum* & *H. annuus* (C-HEAIHA) (200 mg/kg, p.o)
Group VII - Animals treated with Hydro alcoholic extract of *A. Indicum* & *H. annuus* (C-HEAIHA) (200 mg/kg, p.o) respectively for 7 days.
Group VIII [Standard Group] - Animals treated with Omeprazole (20 mg/kg, p.o) were administered 1 h prior to induction of gastric ulcer and results were shown in Table 4.

On the 7th day, all group rats were fasted 24 h prior to induction of gastric ulcer. Pyloric ligation was done by ligating the pyloric end of the stomach of rats after 1 h of drug administration [16]. Animals were allowed to recover and stabilized in individual cage and were deprived of water during post-operative period. After 4 h of surgery, rats were sacrificed by cervical dislocation and ulcer index were examined on the dissected stomachs as described below.

2.8 Measurement of Ulcer Index

The stomachs were excised and were examined for hemorrhagic lesions in glandular mucosa. After the animals were sacrificed, their stomachs dissected out, and then cut along the greater curvature and the mucosa was rinsed with cold normal saline to remove blood contaminant, if any. The sum of the length (mm) of all lesions for each stomach was used as the ulcer index (UI), and the percentage of inhibition (%I) was calculated as described by using the following formula [21].

$$\%I = \frac{(USc - USt)}{USc} \times 100$$

Where USc = ulcer surface area in control and USt = ulcer surface area in treated animals.

2.9 Evaluation of Anti-oxidant Potential

2.9.1 Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M). The significance of differences among the group was assessed using one way and multiple way analyses of variance (ANOVA). The test followed by Dunnett's test was used and value $p<0.05$ was considered statistically significant.

3. RESULTS

3.1 Effect of Haeai, Haeha & C-Haeaihaon Ethanol Induced Gastric Ulcer

The HAEAI, HAEHA & C-HAEAIHA had shown a significant anti-ulcer effect against Ethanol induced ulcer in a dose dependent manner. In this Ethanol induced ulcer model, HAEAI, HAEHA and C-HAEAIHA at a dose of 200 mg/kg body weight showed protective effect as follows: 46.78%, 55.97% and 57.79% respectively. However, at a dose of 400 mg/kg body weight, the protective effect was as follows: 62.41%, 65.16% and 75.23% respectively. During present study the standard drug omeprazole showed protection index of 81.67% at a dose of 20 mg/kg body weight. The protective effect of HAEAI, HAEHA and C-HAEAIHA were in the following order: C-HAEAIHA > HAEHA > HAEAI **(**Table 3 and Figs. 1, 2 and 3).

3.2 Effect of Haeai, Haeha & C-haeaihaon Pylorus Ligation (PL) Induced Gastric Ulcer

The HAEAI, HAEHA & C-HAEAIHA had shown significant anti-ulcer effect against pylorus ligation induced ulcer in a dose dependent manner. In PL induced ulcer model, HAEAI, HAEHA & C-HAEAIHA at a dose of 200 mg/kg body weight has shown a protective effect as follows: 51.57%, 60.95%, 61.69% respectively and at a dose of 400 mg/kg body weight has shown a protective effect as follows 67.18%, 67.18%, 74.21% respectively, whereas omeprazole has shown protection index of 78.90% at a dose of 20 mg/kg body weight. The HAEAI, HAEHA & C-HAEAIHA showed the protective effect in following order C-HAEAIHA > HAEHA >HAEAI (Table 4 and Figs. 4, 5 and 6) [16].

Table 3. Effect of HAEAI, HAEHA and C-HAEAIHA on ethanol (8 ml/kg) induced gastric ulcer in rats

Group	Design of Treatment (Oral route)	Ulcer index	Percentage inhibition (% I)
I	Control (1% v/v aqueous tween 80, 10 ml/kg b.wt.)	18.17±0.30	---
II	HAEAI (20 0mg/kg b.w)	9.67±0.21**	46.78
III	HAEAI (400 mg/kg b.w)	8.0±0.36**	55.97
IV	HAEHA (200 mg/kg b.w)	7.67±0.33**	57.79
V	HAEHA (400 mg/kg b.w)	6.83±0.30**	62.41
VI	C-HAEAIHA (200 mg/kg b.w)	6.33±0.33**	65.16
VII	C-HAEAIHA (400 mg/kg b.w)	4.50±0.22**	75.23
VIII	Omeprazole (20 mg/kg b.w)	3.33±0.21**	81.67

$^{*}P < 0.01$ and $^{**}P < 0.001$ as compared to control (n = 6 in each group). - The extracts were compared with the control omeprazole group. -Data are represented as mean ± S.E.M. Statistical analysis was done by one-way ANOVA followed by Dunnett's multiple comparison test.

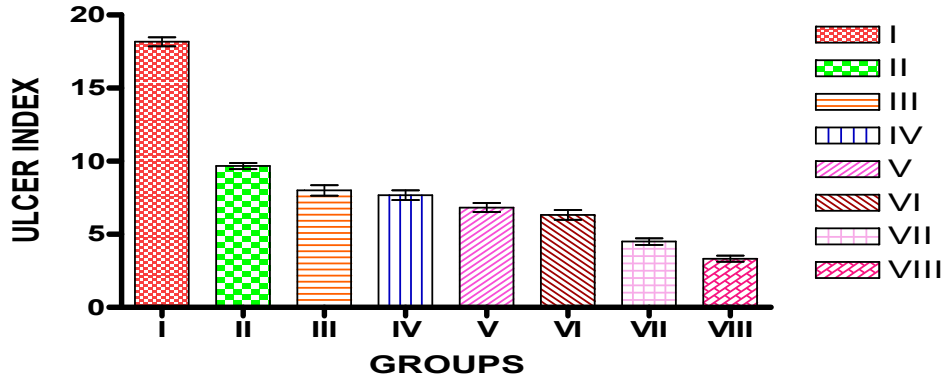

Fig. 1. Effect of HAEAI, HAEHA & C-HAEAIHA on ethanol induced ulcer model

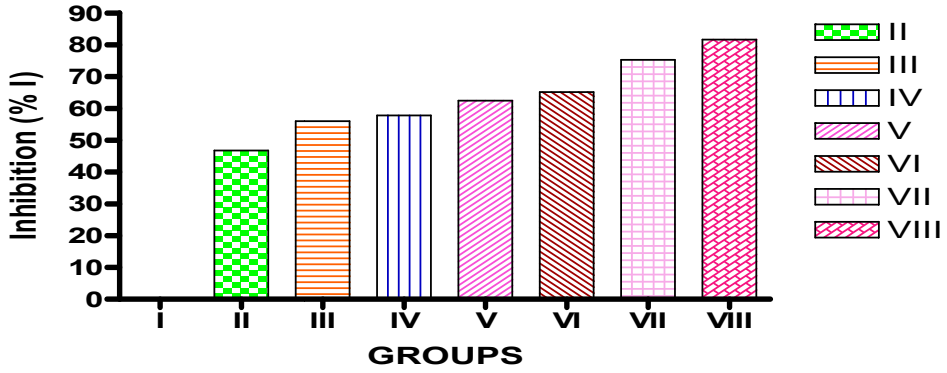

Fig. 2. Percentage Inhibition of HAEAI, HAEHA & C-HAEAIHA on ethanol (8 ml/kg) induced gastric ulcer in rats

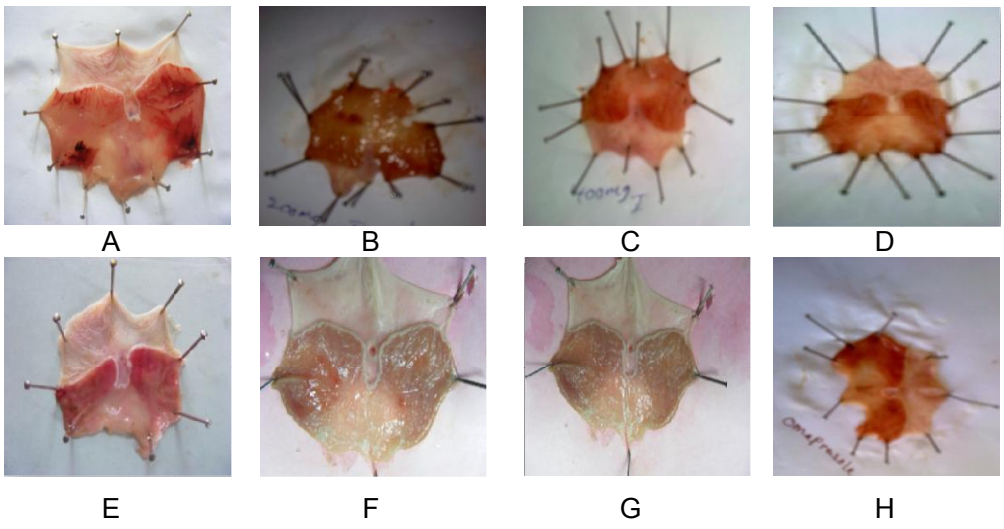

Fig. 3. Effect of HAEAI, HAEHA & C-HAEAIHA on ethanol (8 ml/kg) induced gastric ulcer in rats

(A) *Stomach after ethanol treatment;* ***(B)*** *Stomach treated with HEAI-200 mg/kg plus ethanol;* ***(C)*** *Stomach treated HAEAI-400 mg/kg plus ethanol;* ***(D)*** *Stomach treated with HAEHA-200 mg/kg plus ethanol;* ***(E)*** *Stomach treated with HAEHA-400 mg/kg plus ethanol;* ***(F)*** *Stomach treated with C-HAEAIHA-200 mg/kg plus ethanol;* ***(G)*** *Stomach treated with C-HAEAIHA-400 mg/kg plus ethanol and* ***(H)*** *stomach treated with omeprazole-20 mg/kg plus ethanol.*

Table 4. Effect of HAEAI, HAEHA & C-HAEAIHA on pylorus ligation induced ulcer model

Group	Design of treatment (Administration route: oral)	Ulcer index	% inhibition (% I)
I	Control (1% v/v aqueous tween 80, 10 ml/kg b.wt)	21.33±0.42	-
II	HAEAI (200 mg/kg b.wt.)	10.33±0.33**	51.57
III	HAEAI (400 mg/kg b.wt.)	8.33±0.21**	60.95
IV	HAEHA (200 mg/kg b.w) p.o	8.17±0.30**	61.69
V	HAEHA (400 mg/kg b.w) p.o	7±0.25**	67.18
VI	C-HAEAIHA (200 mg/kg b.w) p.o	7.33±0.33**	65.64
VII	C-HAEAIHA (400 mg/kg b.w) p.o	5.50±0.42**	74.21
VIII	Omeprazole (20 mg/kg b.w) p.o	4.50±0.22**	78.90

**P < 0.01 and **P < 0.001 as compared to control (n = 6 in each group).*
- Data are represented as mean ± S.E.M.
- Statistical analysis was done by one-way ANOVA followed by Dunnett's multiple comparison tests.

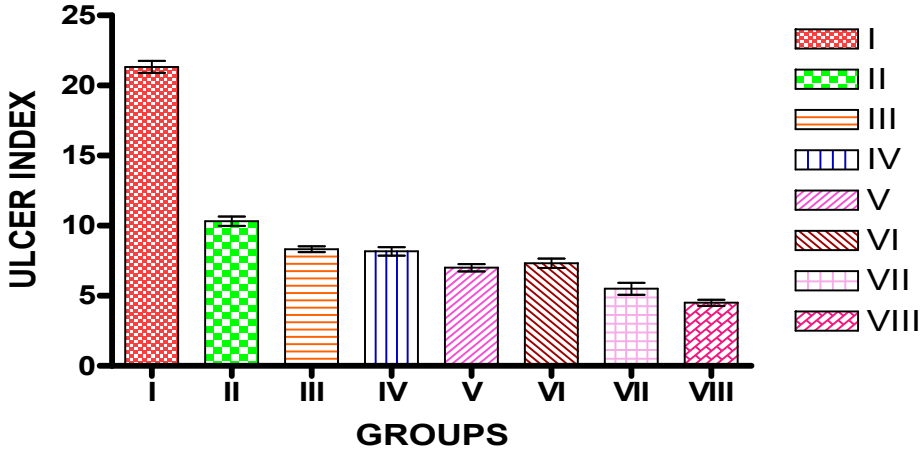

Fig. 4. Effect of HAEAI, HAEHA & C-HAEAIHA on pylorus ligation induced ulcer model

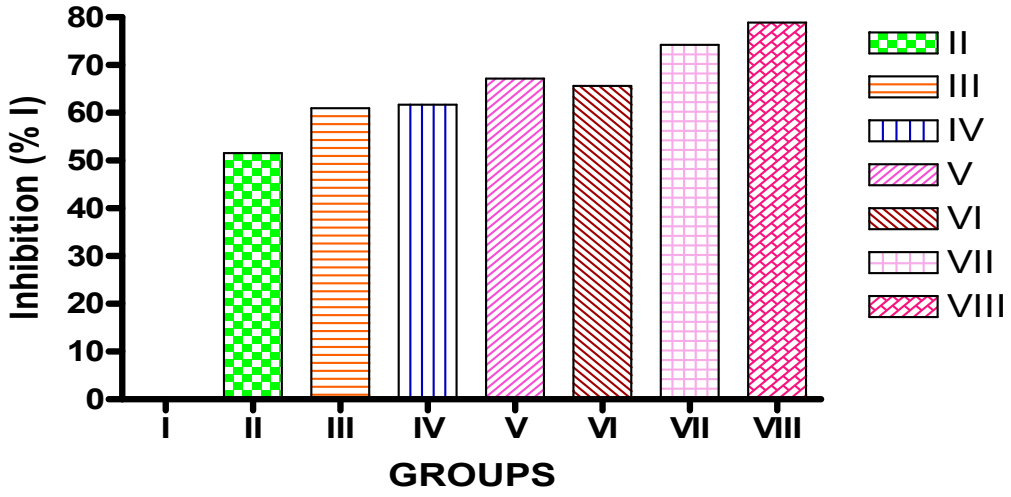

Fig. 5. Percentage Inhibition of HAEAI, HAEHA & C-HAEAIHA on pylorus ligation induced ulcer model

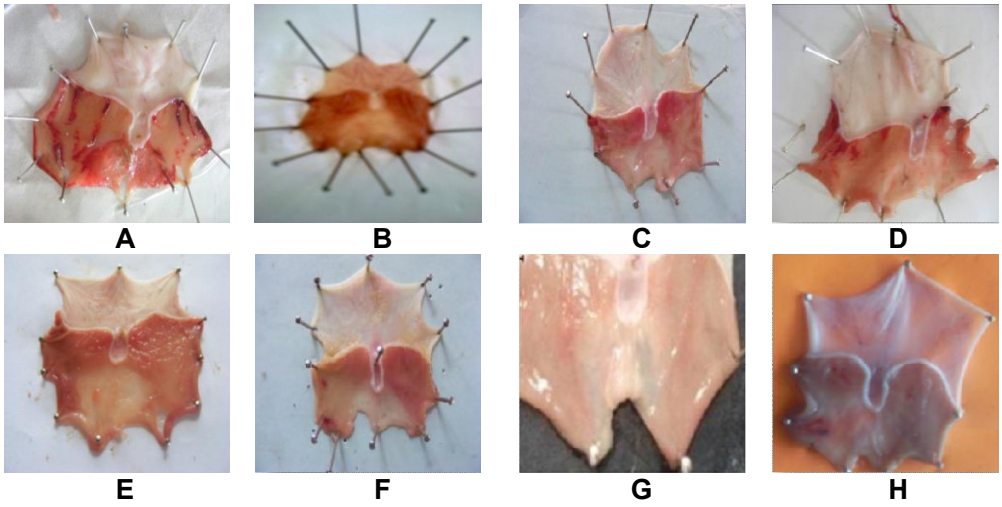

Fig. 6. Effect of HAEAI, HAEHA & C-HAEAIHA on pylorus ligation induced ulcer in rats

(A) Stomach after Pylorus Ligation; (B) Stomach treated with HAEAI-200 mg/kg plus Pylorus Ligation; (C) Stomach treated HAEAI-400 mg/kg plus Pylorus Ligation; (D) Stomach treated with HAEHA-200 mg/kg plus Pylorus Ligation; (E) Stomach treated with HAEHA-400 mg/kg plus Pylorus Ligation; (F) Stomach treated with C-HAEAIHA-200 mg/kg plus Pylorus Ligation; (G) Stomach treated with C-HAEAIHA-400 mg/kg plus Pylorus Ligation and (H) stomach treated with omeprazole-20 mg/kg plus Pylorus Ligation.

4. DISCUSSION

4.1 Pharmacological Studies: Anti-ulcer Activity

The results of this study shows that the hydroalcoholic extracts of AI, HA and AIHA showed protective effects against ethanol induced gastric mucosal damage as well as in pylorus ligation model. The in an order of activity was: C-HAEAIHA> HAEHA > HAEAI.

4.2 Ethanol Induced Gastric Ulcer

The anti-ulcer effect of hydro alcoholic extracts of AI, HA and AIHA were tested against ethanol induced gastric ulcer. Reactive oxygen species are involved in the pathogenesis of ethanol-induced gastric mucosal injury *in-vivo* [13]. Hydro alcoholic extracts of AI, HA and AIHA prevented the mucosal lesions induced by ethanol. Results in the present study also indicate similar alterations in the anti-oxidant status after ethanol induced ulcers. The gastric mucosal protection against ethanol can be mediated through a number of mechanisms that include enhancement of the gastric mucosal defense through increase in mucus and/or bicarbonate production, reducing the volume of gastric acid secretion or by simply neutralizing the gastric acidity [14].

HAEAI, HAEHA and C-HAEAIHA may either reduce the gastric acid secretion or enhance the barrier defense of the mucosal wall. HAEAI, HAEHA and C-HAEAIHA shows dose dependent inhibition in ethanol induced gastric lesions [16].

4.3 Pylorus Ligation Induced Gastric Ulcer

In order to probe the effectiveness of hydro alcoholic extracts of AI, HA and AIHA in preventing gastric ulcer, they were tested against pylorus ligation-induced ulcer. Pylorus ligation induced ulcers are due to the auto digestion of the gastric mucosal barrier and also probably due to the excess production and accumulation of HCl in the stomach. The main factor involved in facilitation of ulceration in PL rats is gastric acid [15]. The C-HAEAIHA at a dose of 400 mg/kg showed significant activity as compared to Omeprazole which is an established gastric anti-ulcer drug with strong proton pump inhibitor potential. It is worth mentioning that the gastro protective effect of omeprazole is mediated through blockage of acid secretion by inactivation of H^+/K^+-ATPase [17].

Hence, this study reveals that the hydro alcoholic extracts of AI, HA and AIHA are potent inhibitors.

5. CONCLUSION

All the three extracts possessed good antioxidant properties. The mixture of hydro alcoholic extracts of *Abutilon indicum* and *Helianthus annuus* (C-HAEAIHA) showed better inhibition of gastric mucosal lesions caused by ethanol and pylorus ligation method in rats. The study well indicated the combined extract to be a potent anti-ulcer drug and detailed studies are warranted on the chemical constituents responsible for the observed activity.

CONSENT

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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