

Ulcer Protective Activity of Ethanolic Extract of *Homalomena aromatica* (Spreng.) Schott. (Araceae) Root

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Abstract

Homalomena aromatica (spreng) Scott (Araceae) is naturally localized in Assam, Arunachal Pradesh, Nagaland and Tripura. The plant is used by local people in various inflammatory conditions and gastric disorders, jaundice, diarrhoea etc. The preset study was undertaken to evaluate the antiulcer properties of the ethanolic extract of the root of *Homalomena aromatica* using HCl-Ethanol, cold restraint stress and indomethacin induced ulcer models in Wistar rat. Various biochemical and antioxidant enzymes in gastric mucosa, liver and serum were analyzed along with histological study. The extract showed ulcero protective activity in all the models at the highest dose i.e. 200 mg/kg. The levels of various biochemical enzymatic and ulcer parameters were normalized after the treatment regime. HPTLC data showed the presence of gallic acid and quercetin amongst other constituents. The extract showed potential ulcero protective property in animal models of ulcer.

Keywords: *Homalomena aromatica*; Antioxidant; Cold stress; HCl-ethanol; Indomethacin

Introduction

Gastric hyperacidity and ulcer are very common causes of human suffering today. Some of these factors are acid secretion, *Helicobacter pylori* infection, mucus secretion, blood flow, cell regeneration and prostaglandins [1]. Extensive studies were undertaken to evaluate antiulcer properties of medicinal plants for the development of herbal alternatives. Studies have also been undertaken to define exact mechanisms responsible for ulceration. The involvement of neural mechanism in the regulation of stress response and complex neurotransmitter interactions were reported to cause gastric ulceration [2]. It has been stated that, an imbalance between aggressive and defensive mechanisms within the gastric mucosa causes ulcer [3]. The main therapeutic aim in the treatment of ulcers is to control acid secretion using antacids, H₂ receptor blockers, anti cholinergics or proton pump blockers [4]. However, most of the drugs currently available show limited efficacy and are often associated with severe side effects like hypersensitivity, arrhythmia, impotence, gynaecomastia and hematopoietic changes. Therefore there is a need to develop more effective and less toxic agents [5].

From times immemorial, plants have been used as a valuable source of natural products and molecules with therapeutic properties [6-8]. Development of herbal medicines has come to importance due to their pharmacological and commercial significance. At present, about 121 active compounds of plant origin have been reported. Among the 252 drugs, considered as basic and essential by the World Health Organization (WHO), 11% are exclusively from plant origin. The north eastern region of India which lies between 87°32'E to 97°52'E latitude and 21°34'N to 29°50' N latitude with its favorable climatic condition, is a rich repository of several medicinal and aromatic plants [9-10]. The region also preserves a rich ethno-medicinal and ethno pharmacological knowledge owing to its diverse ethnic communities.

One of the plants that have been studied in our laboratory for

its antiulcer activity is *Homalomena aromatica* (Spreng.) Schott, which belongs to the family of Araceae and commonly known as "Sugandhmantri" (vernacular name). It is a rhizomatous perennial herb found in Assam, Chittagong hill of Bangladesh and Jampui in Tripura. It has also been reported from the foothills of Arunachal Pradesh, Nagaland, Mizoram and Manipur [11]. The leaves and rhizomes of this plant find application amongst the aboriginal people of north eastern region as remedy of joint-pains and skin infections [12]. Its rhizomes also possess many medicinal properties like analgesic, antidepressant, anti-inflammatory, antiseptic, antispasmodic and sedative [13-16]. Three new sesquiterpene alcohols, 1-β, 4-β, 7-∞- trihydroxyeudesmane, homalomenol A and homalomenol B were isolated from the roots of this plant along with oplopanone, oplodiol and bullatantriol. The chemical profiles of the essential oil suggest the pharmacological activities such as anti-inflammatory, anti-gastric ulcer and anti-microbial activities, relaxing and calming effects [17]. Its rhizome is used as a paste to treat stomach ailments [18]. In view of the above literature review, it was considered worthwhile to conduct a detailed experimental study on the ulcero protective activity of ethanolic extract of *H. aromatica* (EEHA) root which is not reported previously.

Material and Methods

Animals

Male Wistar rats, weighing 180-250 g, kept in controlled environment (temperature 22.2°C; humidity 60.4%; natural light),

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maintained on a standard pellet diet and water ad libitum were used. Such conditions were maintained for one week before the experiments started. The food was withdrawn 18-24 hour before the experiment, though water was added ad libitum. All experiments were performed in the morning according to current guidelines for the care of laboratory animals of IAEC (No.773/03/ac/CPCSEA/FVSc, AAU/IEC/06/22). Effort was made to minimize suffering of the experimental animals throughout the study.

Plant material and extract preparation

Voucher specimens (IC Barua, 4915) were prepared by following the guidelines of Botanical Survey of India, poisoned with mercuric chloride and processed to deposit in the Central National Herbarium (CAL), Howrah, and the Kanjilal Herbarium (Assam), Shillong, Meghalaya.

Roots of *Homalomena aromatica* were collected and air dried for 10 days. The roots were then grinded into small pieces. One kg of small pieces of roots was macerated with occasional agitation with ethanol for 72 hours at room temperature ($25 \pm 3^\circ\text{C}$). The mixture was then filtered using Whatman No. 1 filter paper to obtain the filtrate. The extraction process was repeated for three times using the same filtrate. Then the solvent was evaporated using rotary evaporator (BUCHI, ROTAVAPOR R210, Switzerland) to obtain the extract. The percent recovery of the extract was 0.71%.

Acute toxicity studies

The acute toxicity study of EEHA was performed according to the Organization of Economic Corporation Development (OECD) Guidelines No. 425. EEHA was administered orally at 2000 mg/kg to the group of mice (n=3) and the percentage mortality, if any was recorded for a period of 24 hours. After the first hour of drug administration, the mice were observed for any gross behavioral changes in the parameters like hyperactivity, grooming, convulsions, sedation and loss of righting reflex, respiration, salivation and defecation [19]. The animals were fasted for 24 hours before oral administration of EEHA. The control group received distilled water as vehicle. The animals were kept under observation for the next 14 days. No mortality or gross abnormality was observed with the given dose. Hence, based on the acute toxicity study, three oral doses viz. 50, 100 and 200 mg/kg, were selected for dose response study.

Anti-ulcerogenic activity

HCl/ETH- induced ulcer [20]: EEHA (50, 100 and 200 mg/kg) was administered orally to fasted rats, while omeprazole (4 mg/kg) was given p.o to the standard group (positive control). The control group (negative control) received distilled water. One hour after drug treatment, 1 ml of the necrotizing solution (150 mM HCl in 60% ethanol) was administered to each rat. The rats were euthanatized after an hour; stomachs were opened along the greater curvature and observed for ulcers in the glandular region. The gastric content [21] was measured and total acidity [21] was estimated. The stomach and liver samples were collected for biochemical tests. The surface area of each lesion was measured and scored for ulcer index using the formula [20] [Ulcer index = $10/X$ where X = total mucosal area/total ulcerated area]. Based on their intensity, ulcer scores were given arbitrarily as, 0: Absence of any detectable lesion, 0.5: Small haemorrhagic effusion, 1.0: Haemorrhagic effusion, 1.5: Mucosal ulceration of limited diffusion involving more than 1/3 of the whole surface, 2.0: Mucosal ulceration of limited diffusion involving more than 2/3 of the whole surface, 2.5: Mucosal ulceration of generalized

diffusion, 3.0: Deep ulcerations of limited diffusion, 3.5: Deep ulcerations of generalized diffusion, 4.0: Perforated ulcer.

Percentage protection was calculated as:

$$\frac{\text{Ulcer index of control} - \text{Ulcer index of test}}{\text{Ulcer index of control}} \times 100$$

Cold restraint stress (CRS) induced ulcer [22]: For this study, the animals were fasted for 48 hour. EEHA (50, 100 and 200 mg/kg) was administered orally while omeprazole was given at a dose of 4 mg/kg p.o. to the respective groups; the control group received distilled water, 30 min prior to subjecting to stress. The animals were placed in restraint cage at a temperature 4°C for 3 hours. Animals were then euthanatized and ulcer score, ulcer index and percentage protection were determined as described above. The gastric volume [21], pH, free and total acidity [21] were estimated. The stomach and liver samples were collected for biochemical estimations to study the anti-ulcer effect of the extract in both the organs.

Indomethacin induced ulcer [23]: In this protocol, following overnight fasting, Indomethacin was administered orally at 40 mg/kg per os. After an hour, EEHA (50, 100 and 200 mg/kg) was administered to the test rats, while omeprazole was given at a dose of 4 mg/kg p.o. to the standard group and control group received distilled water. Five hours later, the animals were euthanatized, the stomach and liver were removed for analysis of biochemical parameters. The ulcer score and index were determined based on the extent of gastric lesions. The pH and gastric volume were also determined. Percentage protection was determined as described previously.

Estimation of biochemical parameters

Estimation of enzymatic Catalase (CAT), Superoxide dismutase (SOD), Non-enzymatic reduced glutathione (GSH) anti-oxidant system, Lipid peroxidation (LPO): CAT, SOD, GSH and LPO assays were performed taking both liver and mucosal scrapings to study the effect of the extract on anti-oxidant enzymes in the organs.

Tissue sample preparation for CAT, SOD, GSH and LPO assay: The liver samples were prepared at a concentration of 200 g/L and the mucosal scrapings were prepared at a concentration of 100 g/L in 20 mM Tris buffer (pH 7.4) and centrifuged at 3000 g at 4°C for 30 min. The supernatant was collected to estimate SOD [24], CAT [25], GSH [26], and LPO [27].

Estimation of serum glutamic oxaloacetic (SGOT), serum glutamic pyruvic transaminase (SGPT) and alkaline phosphatase (ALP): Serum was analyzed for biochemical parameters like serum SGOT, SGPT, ALP [28-30] to study the extent of damage on liver enzymes by ulceration and thereafter protection or reversal of these damages by the extract itself.

Histopathology

For histological studies, tissues were collected and fixed in 10% neutral formalin solution and dehydrated with a series of ethanol-xylene solutions. The materials were processed by conventional paraffin embedding method. Microtome sections were prepared at 6 μm thicknesses, mounted on glass slides, stained with hematoxylin and eosin followed by observation for histopathological changes under light microscope [31].

Phytochemical screening

Preliminary qualitative phytochemical screening [32] of EEHA was performed for alkaloids, triterpenes, flavonoids, glycosides, phenolics, diterpenes and tannins.

High performance thin layer chromatography (HPTLC) analysis

A densitometric HPTLC analysis of EEHA was performed for the characteristic fingerprinting profile of phytochemicals using CAMAG HPTLC System (Switzerland), after preliminary phytochemical study and also to correlate the findings. Standard quercetin and gallic acid (Sigma) were prepared in methanol at 1 mg/ml and 40 mg/ml concentration, respectively. The samples were centrifuged at 3000 rpm for 5 min and supernatants were used for HPTLC analysis. The samples (10 µl) were loaded as 8 mm band length in the 10 x 10 Silica gel 60F254 TLC plate using Hamilton syringe and CAMAG LINOMAT 5 applicator. The plates loaded with samples were kept in TLC twin trough developing chamber (after saturation with solvent vapor) filled with solvent system Toluene: Ethyl acetate: Formic acid (4.5:3:0.2) and chloroform: ethyl acetate: formic acid (7.5:6:0.3) as mobile phase for quercetin and gallic acid respectively. Finally, the plate was dried in air and scanning was done at 254 nm.

High performance liquid chromatography-Diode array detection (HPLC-DAD) analysis

The extracts were also analyzed using an HPLC system, equipped with Binary Gradient Pump, column heater and degasser online, photodiode array detector (Dionex, UHPLC 3000) and Chromeleon Software (version: 6.80 SR12 Build 3578 (207169)). Separation was achieved using a reversed phase column, C18 (4.6x250 mm, 4 µm), PROD, ACCLAIM at temperature of 25 °C. DAD detection was employed at the wavelength range between 210 and 500 nm. Samples were dissolved in the corresponding solvent of the extract at the concentration of 10 mg/mL. The volume of sample injected was 20 µL using an L-7200 auto-sampler. The mobile phase was a mixture of Methanol: Acetonitrile: Water (60:20:20 v/v) and 0.1% O-phosphoric acid: Acetonitrile (400:600 v/v) and the flow rate was 1 mL/min. The elution system was in isocratic mode.

Statistical analysis

Gastro protective and antioxidant activity data were presented as the mean ± SEM of n = 6 rats per group. Statistically significant differences between the treatments and control in ulcer score and ulcer index were tested by Kruskal Wallis test (non-parametric ANOVA) followed by Dunn. Rest of the parameters studied was tested by ANOVA followed by Dunnett's test. P<0.05 was considered statistically significant. Graph-Pad InStat3 software was used for statistics and plotting.

Result

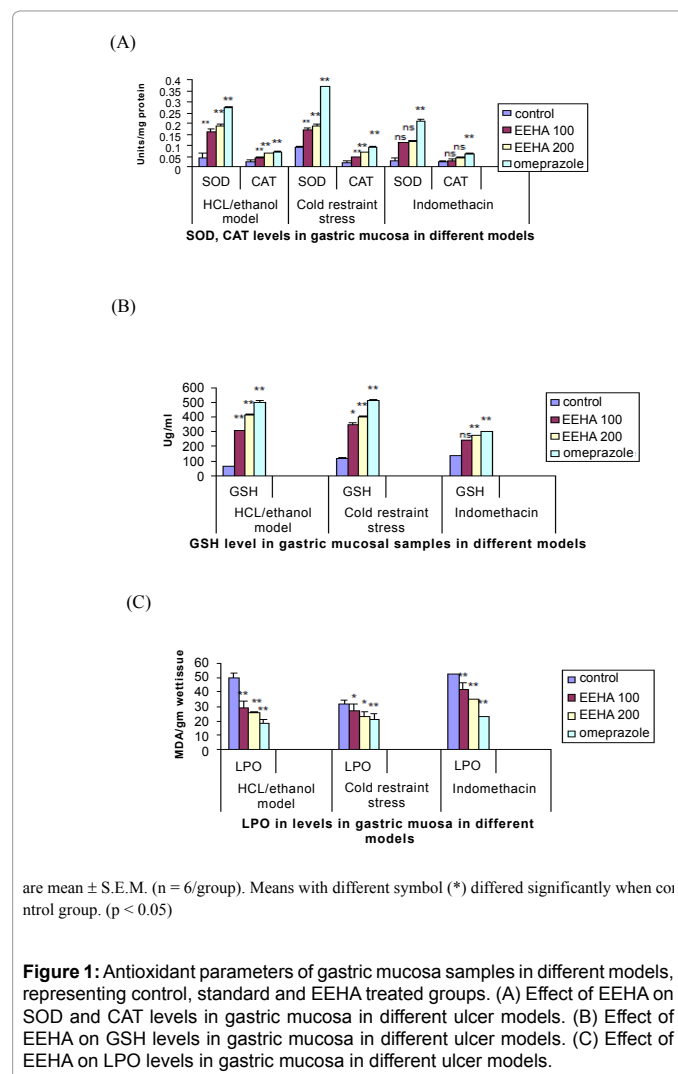
Acute toxicity study

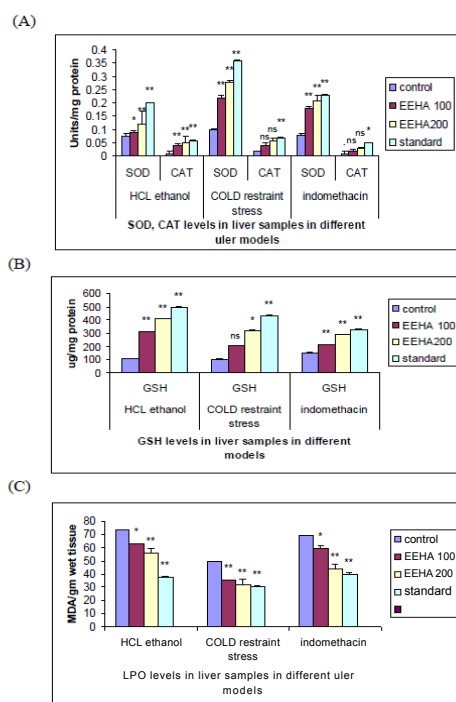
Mice did not show any gross abnormality up to a dose of 2 g/kg of EEHA, based on which 50, 100 and 200 mg/kg doses were selected for different models of anti-ulcer activity. Since there was no effect at 50 mg/kg oral dose of EEHA in all ulcer model studied, hence the result was not shown.

HCL/ETH- induced ulcer: HCL/ETH induced ulcer model is a

well-accepted model for the study of gastric ulcer. In this model, EEHA 200 mg/kg dose showed significant reduction in gastric content (p<0.05), acidity (p<0.05), ulcer score as well as ulcer index (p<0.05) as compared to the control group (Table 1). However the standard drug, omeprazole showed maximum protection in this model of ulcer. Enzymatic antioxidant parameters such as SOD, CAT, and non-enzymatic GSH in the gastric mucosal and liver samples were significantly (p<0.05) increased in EEHA and omeprazole treated group as compared to that of the control group (Figure 1A and 1B). Subsequently, there was a decline in LPO and serum enzymatic parameters such as SGOT, SGPT and ALP in a dose dependent manner in both EEHA treated groups and standard group as shown in Table 2. However, the standard drug, omeprazole treated animals were superior to EEHA treated animals in respect of ulcer protection.

Cold restraint stress (CRS): A reduction in the ulcer index, ulcer score and gastric content and increase in pH (p<0.05) were observed in EEHA and omeprazole treated groups as compared to the control group (Table 1). Antioxidant enzymes like, SOD, GSH and CAT levels were increased (Figures 1A and 2A) in comparison to the control group in CRS induced ulceration (p<0.05) in gastric mucosal and liver samples, while LPO level decreased (p<0.05) as shown in





Values are mean $\pm$ S.E.M. (n = 6/group). Means with different symbol(*) differed significantly when compared to

Figure 2: Antioxidant parameters of liver samples in different models, representing control, standard and EEHA treated groups. (A) Effect of EEHA on SOD and CAT levels. (B) Effect of EEHA on GSH levels. (C) Effect of EEHA on LPO levels.

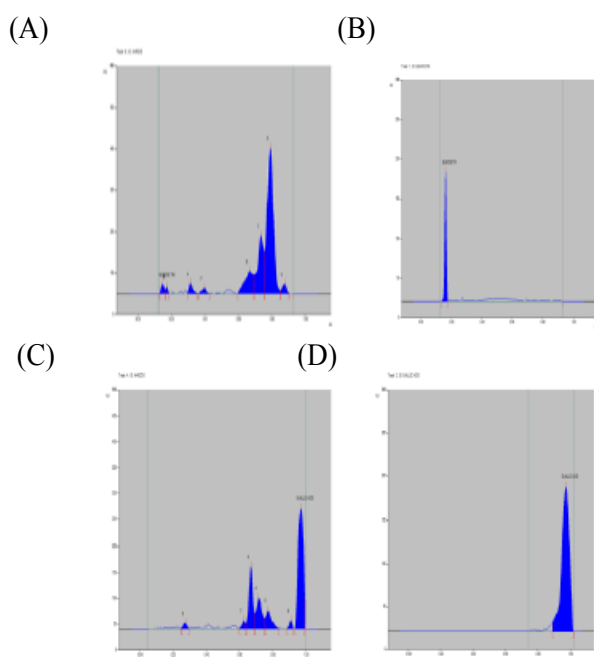


Figure 3: HPTLC fingerprint for EEHA, showing the presence of quercetin (A) and gallic acid (C). Standard quercetin (B) and gallic acid (D) showed the single peak each. The chromatographic analysis has been performed at 254 nm.

Figures 1C and 2C in EEHA and omeprazole treated groups, even though effect was better in standard drug treated group. The level of SGOT, SGPT and ALP also decreased significantly ($p < 0.05$) as compared to that of the control group in a dose dependent manner (Table 2).

Indomethacin –induced ulcer

In indomethacin induced gastric ulcer model, the ulcer score, ulcer index, gastric content were dose dependently reduced with elevation in gastric pH indicated ulcero protective property of EEHA. Likewise the biochemical parameters were improved in accordance with the findings of the previously stated models for the extract and standard drug treated groups (Table 1 and Table 2). Significant elevation in the levels of GSH, SOD and CAT in the mucosal and liver samples ($p < 0.05$) and decline in LPO level in both treated and standard groups indicated their ulceroprotective property (Figure 1A and 2A).

In this ulcer model also, standard drug, omeprazole was better than the extract treated group in all the ulcer models.

Histopathological study

In HCL/ETH induced ulcer model, the control group showed severe necrosis and sloughing of epithelial cells of the gastric mucosa making the villi shorter (Figure 5A). In 200 mg/kg dose of EEHA, the mucosal epithelial cell at the lower part of the villi showed mild degree of degeneration and necrosis (Figure 5C). In the standard group, the gastric mucosa was almost normal (Figure 5B).

In CRS model, focal necrosis of epithelial cells of gastric villi without any inflammatory changes was observed in the control group (Figure 5D). In the treated group of 200 mg/kg dose of EEHA, the gastric mucosa showed not much alteration except some focal areas of necrosis of the villous epithelium (Figure 5F). In the standard group, the gastric mucosa was completely intact, though the capillaries in the tunica muscularis and sub-mucosa were slightly congested (Figure 5E).

In indomethacin induced ulcer model, the control group showed massive necrosis and sloughing of the mucosal epithelial cell. The epithelial cells of gastric glands also showed degeneration and necrosis (Figure 5G). In the treated group with 200 mg/kg dose of EEHA, the effect was of much lesser degree with focal necrosis and sloughing of superficial epithelial cells (Figure 5I). In the standard group no visible histopathological alteration was seen in the gastric mucosa (Figure 5H).

Phytochemical screening

Presence of major class of secondary metabolites such as alkaloids, steroids triterpenes, diterpenes, flavonoids, glycosides, phenolic compounds were evident in phytochemical study. In some reports, Homalomena was found to contain high quantity of total phenolic and flavonoid content 33.

HPTLC study

HPTLC study was done taking quercetin and gallic acid as standard to determine the flavonoid and phenolic content of EEHA. As depicted in Figure 3B and 3D, the HPTLC chromatogram of the standards quercetin and gallic acid showed Rf values of 0.16 and 0.97 respectively. As illustrated in figure 3A, out of the eight detected peaks, peak 1 was assigned to quercetin which showed an Rf-value and area of 0.16 and 327.9 respectively. Similarly, in figure 3C, out of the seven detected peaks, peak 7 was assigned to gallic acid which

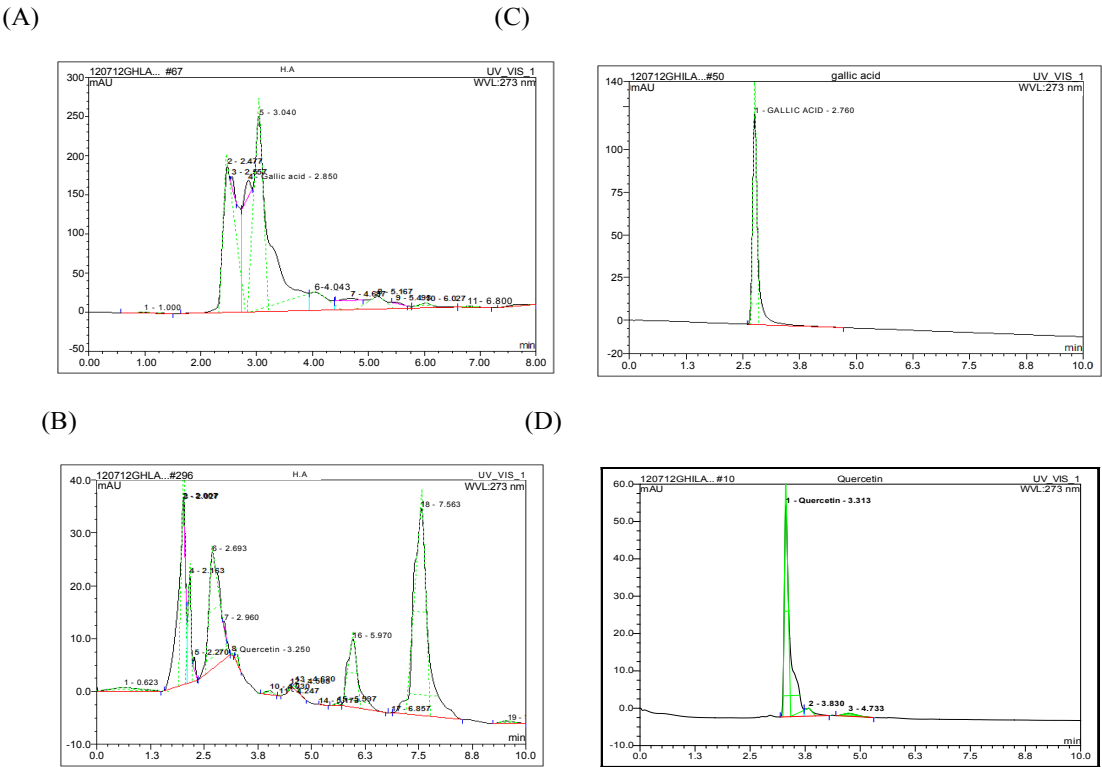


Figure 4: Chromatograms of the extracts of EEHA and standards by HPLC-DAD method at 254 nm. (A) Extract showing phenolic acid; (C) Standard gallic acid; (B) Extract showing flavonoid; (D) Standard quercetin.

Models	Models	Dose mg/kg (p.o.)	Ulcer score	Ulcer index (mm)	PP	pH(unit)	Gastric content(m)
HCL ethanol	Control		3.583	1.68 ± 0.024		2.668 ± 0.22	7.083 ± 0.37
	EEHA	100	1.26 ± 0.11	0.36 ± 0.03	78	3.73 ± 0.22	3.07 ± 18
	EEHA	200	0.80 ± 0.10	0.22 ± 0.03*	86	3.90 ± 0.20	2.95 ± 0.16
	Omeprazole	4	0.31 ± 0.03**	0.16 ± 0.01***	90	4.04 ± 0.21*	1.57 ± 0.29*
CRS	Control		0.69 ± 0.01	0.33 ± 0.06		1.80 ± 0.37	7.22 ± 0.26
	EEHA	100	0.49 ± 0.01	0.23 ± 0.01	30	1.81 ± 0.34	2.06 ± 0.19
	EEHA	200	0.32 ± 0.03*	0.20 ± 0.03*	39	3.51 ± 0.21*	0.97 ± 0.05*
	Omeprazole	4	0.13 ± 0.01**	0.15 ± 0.01*	63	3.78 ± 0.17*	0.88 ± 0.03*
Indomethacin	Control		2.95 ± 0.17	1.41 ± 0.01		2.44 ± 0.26	6.84 ± 0.11
	EEHA	100	1.24 ± 0.05	0.79 ± 0.01	43	4.15 ± 0.19*	2.65 ± 0.14*
	EEHA	200	1.07 ± 0.07*	0.68 ± 0.09*	51	3.883 ± 0.21*	2.41 ± 0.30*
	Omeprazole	4	0.49 ± 0.05**	0.34 ± 0.025***	75	3.967 ± 0.14*	1.13 ± 0.07*

Results presented as mean ±SEM for six rats. Statistical comparisons was performed using Kurskal Wallis (non-parametric ANOVA) followed by Dunn. EEHA treated groups and standard groups were compared with control group. [* P<0.05, **<0.01 and ***<0.001]

Table 1: Ulceroprotective activity of EEHA in 3 three different ulcer models in wister rat.

showed an Rf value and area of 0.97 and 5045.8 respectively.

HPLC-DAD study

To confirm the HPTLC profile, HPLC-DAD was also performed. All the conditions of reversed-phase HPLC-DAD were appropriate to the characterization of the EEHA. Chromatograms at 254 nm of EEHA shown in Figure 4, which contained flavonoids and phenolic acid derivatives in different proportions. In this study also, chromatographic analysis showed presence of gallic acid (Figure 4A) and quercetin (Figure 4B) in EEHA. Gallic acid showed the RT of 2.85 min while quercetin showed it’s RT of 3.31 min (Figure 4C and 4D). Thus, above results confirmed the presence of

quercetin and gallic acid as phytoconstituents of EEHA for exerting ulceroprotective property.

Discussion

Ethanol provoked gastric ulceration by a number of mechanisms that include decrease in amount of gastric mucus and break down of the mucosal barrier, back diffusion of acid, increased gastric mucosal permeability, leading to increase in leakage of H+ from the lumen of gastrointestinal tract (GI), and decreased transmucosal electrical potential difference [34]. It also renders changes in mucosal blood flow, destruction of microvascular and nonvascular cells, mast cell degranulation, neutrophil mediated mucosal injury and depletion

of certain oxygen free radical scavengers [32]. Due to damage of the gastric mucosa, there was a release of marker enzyme, alkaline phosphatase (ALP) [35] in the blood; in our study too, there was a marked increase in the serum ALP activity in the control group and dose dependent decrease of ALP activity in EEHA and standard treated groups was recorded (Table 2). The standard drug used for this experiment was omeprazole, a proton pump inhibitor [36] which prevents the leakage of H⁺ into the GI tract.

Since ethanol causes damage in gastric mucosa as well as in liver tissues, our study indicates that these damages were reverted to normal after treatment. Ethanolic extract of *Oxalis corniculata* leaves significantly increased SOD, CAT levels and percentage of

protection and reduced the ulcer index in ethanol induced ulcer model at 400 mg/kg dose [37]. *Azadirachta indica* bark extracts showed antiulcer activity in ethanol induced gastric ulcer model in albino mice due to presence of flavonoids and phenolics compounds [38]. Rats pretreated with *J. sambac* extract had reduced sub-mucosal edema and leukocyte infiltration along with reversal of liver and kidney functions [39]. Hence, the results of EEHA showed a positive correlation between mucosal ulcer parameters, enzymatic levels, mucosal tissue structure and its phytochemical content in HCL/ETH induced ulcer model.

The cold restrain stress, a purest form of psychological frustration and severe muscular struggling, causes the mucosal

Models	Treatment	Dose mg/kg (p.o.)	SGPT IU/l	SGOT IU/l	Alkaline Phosphatase IU/l
HCL ethanol	Control		3768.8 ± 293.23	3665.5 ± 214.70	88.00 ± 7.42
	EEHA	100	2139.2 ± 191.72*	2789.2 ± 123.1*	70.66 ± 4.32*
	EEHA	200	1726.9 ± 228.61*	2101.5 ± 203.75*	60.667 ± 4.32
	Omeprazole	4	2174.2 ± 232.09*	1754.5 ± 175.00*	36.333 ± 3.02*
	Control		2375.0 ± 277.04	2094.7 ± 262.10	99.3 ± 10.77
CRS	EEHA	100	1344.0 ± 101.59*	1254.2 ± 126.22*	57.5 ± 3.21*
	EEHA	200	1211.2 ± 161.01*	1008.2 ± 68.88*	54.83 ± 6.23*
	Omeprazole	4	1044.8 ± 86.39*	979.00 ± 77.39*	42.167 ± 4.04*
Indomethacin	Control		1515.2 ± 131.30	1080.8 ± 106.54	52.66 ± 5.75
	EEHA	100	501.83 ± 6.51*	426.50 ± 14.33*	38.6 ± 2.17
	EEHA	200	379.67 ± 49.15*	353.83 ± 41.31*	32.1 ± 3.27*
	Omeprazole	4	535.67 ± 82.47*	471.83 ± 15.6*	24.3 ± 2.48*

Results presented as mean ± SEM for six rats. Statistical comparisons was performed using ANOVA analysis followed by Dunnett's test. *p<0.05 compared with control group.

Table 2: SGOT, SGPT and ALP activities in three different ulcer models for control, standard and EEHA treated groups in Wister rats.

(A)

Peak	Rf Value (min)	Peak area (AU)	Area %	Assigned Substances
1	0.16	327.9	1.90	Quercetin
2	0.17	171.8	1.00	P6
3	0.32	405.9	2.35	P4
4	0.40	278.2	1.61	P7
5	0.67	2016.4	11.69	P3
6	0.74	3640.1	21.11	P2
7	0.79	9934.8	57.61	P1
8	0.88	470.1	2.73	P5

(B)

Peak	Rf Value (min)	Peak area (AU)	Area %	Assigned Substances
1	0.27	195.4	1.94	9
2	0.63	231.5	2.29	8
3	0.67	2130.0	21.11	7
4	0.72	1373.6	13.61	2
5	0.77	904.1	8.96	4
6	0.91	209.4	2.08	5
7	0.97	5045.8	50.01	Gallic acid

(C)

Peak	Rf Value (min)	Peak area (AU)	Area %	Assigned Substances
P1	0.16	1313.8	100	Quercetin

(D)

Peak	Rf Value (min)	Peak area (AU)	Area %	Assigned Substances
P1	0.97	2170.4	100	Gallic acid

Table 3: Chromatographic profile of ethanol extract of EEHA at mobile phase. Toluene:Ethyl acetate:Formic acid (4.5:3:0.2) (A) and at mobile phase chloroform:ethyl acetate:formic acid (7.5:6:0.3) (B), Standard Quercetin (C) and Galic acid (D)

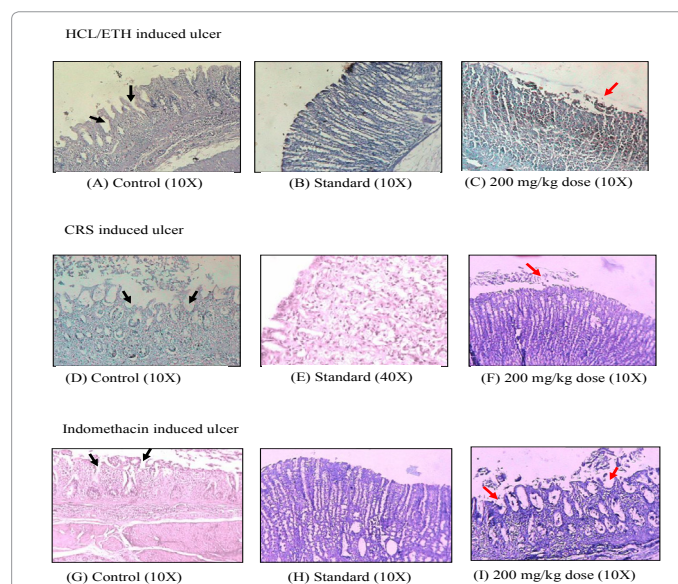


Figure 5: Photomicrographs showing histological changes in the mucosal tissue of stomach of control, standard and EEHA treated groups. (A) Showed the severe necrosis and sloughing (black arrow). (B) Mucosal tissue became almost normal. (C) Lower part of the villi showed mild degree of degeneration and necrosis (red arrow). (D) Focal necrosis of epithelial cells (black arrow). (E) Intact gastric mucosa. (F) Mild necrosis of the villous epithelium (red arrow). (G) Showed massive necrosis and sloughing of the mucosal epithelial cell (black arrow). (H) No visible histopathological alteration (I) lesser degree with focal necrosis and sloughing of superficial epithelial cells (red arrow).

damage in the stomach which was evident by the haemorrhagic red bands on the glandular stomach [40]. The vagus nerve stimulates the stomach acid secretion via interaction of acetylcholine with muscarinic receptor. Acetylcholine causes the secretion of the stomach acids by acting on histamine and parietal cells activity. The stress induced ulcer is also implicated by reactive oxygen species (ROS) apart from the acid and pepsin related factors [41].

In EEHA and omeprazole treated groups, protection from damage of gastric mucosa and liver tissue by scavenging ROS and neutralizing marker enzymes like SGOT, SGPT and ALP were evident by improving tissue structure. Ipomoea batatas (sweet potato) tuber extract exhibited a significant increase in the levels of GSH, SOD and CAT in accordance with mean ulcer score, ulcer index and percentage of protection in cold restrain model of ulcer in Wistar rats [40]. Ethanol extract of leaves of Moringa oleifera exhibited decrease in LPO level and increase in SOD and CAT level with discontinuity in the lining of mucus epithelium and/or no ulcer formation in cold stress restrain induced ulcer model in rat [42]. Thus, the results of EEHA implied that it has ability to restore the antioxidant enzyme activities in this model. Histopathological studies have been shown no visible alteration in gastric mucosa in standard group.

The formation of ulcer induced by indomethacin is caused due to the inhibition of cyclooxygenase action that prevents prostaglandin biosynthesis which in turn inhibits the secretion of mucus, a preventive measure of gastrointestinal tract [43]. The involvement of neutrophil and its activation is also a crucial factor in the indomethacin induced gastric damage [42]. EEHA conferred protection from indomethacin induced gastric ulcer in our study indicating its ulceroprotective property probably due to inhibition of cyclooxygenase, which is however yet to be studied. The methanol extract of Oxalis corniculata revealed the presence of alkaloids, saponin, phenolics, tannins and flavonoids in its preliminary phytochemical screening and conferred gastroprotective activity by reducing the ulcer score and ulcer index in indomethacin induced ulcer model [44]. Assyrian plum (*Cordia myxa* L.) fruit extract (CME) has increased the mucosal CAT level with no histopathological changes of gastric mucosa in indomethacin induced ulcer model in rat and gave positive result in screening of alkaloids, phenolics, flavonoids and saponins [45]. Thus, EEHA has a positive correlation between its phyto-constituents and its ulceroprotective activity in indomethacin induced ulcer model in our study. Histopathological studies in gastric mucosa further confirmed the findings (Table 3).

Conclusion

In conclusion, from the results of our present study, it was evident that the EEHA contains quercetin and gallic acid amongst its other phytoconstituents. EEHA has shown potent ulcer protective activity in each of the ulcer models studied. Although an exact mechanism of ulcer protective activity of EEHA is not derived yet, its ability to combat tissue damage is very significant as compared to vehicle treated control group, probably because of its diverse phytoconstituents like phenolics, flavonoids which have been reported for antioxidant, anti-inflammatory and anti nociceptive property, may contribute to its ulceroprotective activity. The synthetic antiulcer drugs like omeprazole, ranitidine, and cimetidine although are very potent and effective but have adverse side effects, in contrast EEHA, being a plant product, can be used as natural remedial of ulcer without any side effects.

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