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Research Article

Pharmacological & Phytochemical Evaluation of Gastric Anti-Ulcer Activity of *Parthenium hysterophorus* in Different Models

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ABSTRACT

The cause of ulceration in patients is mainly due to hyper secretion of gastric juice and also due to hyper secretion of pepsin. In traditional system of medicine a number of herbal preparations have been used for the treatment of peptic ulcers. There are various medicinal plants has been used for the treatment of gastrointestinal disorders. In view of this, in present study we have to evaluate the anti-ulcer activity of *parathenium hysterophorus*. Study was carried out, by using three methods i.e., alcohol, paracetamol and stress induced ulcers in rats pretreated with the doses of 250 mg/kg AQPH and ALPH, 20mg/kg Omeoprazole and 50 mg/kg Ranitidine. To evaluate the antiulcer activity of aqueous and alcoholic extracts of *parathenium hysterophorus* leaves (AQPH and ALPH) at 250 doses using different experimentally induced gastric ulcer models in rats. Gastric ulcers were induced in rats by 80% alcohol, paracetamol and forced immersion stress induced methods. In alcohol induced ulcer model, paracetamol induced ulcer model and stress induced model the ulcer index was determined. Where as in stress induced ulcers stress plays an important role in ulcerogenesis. In alcohol-induced ulcers, AQPH and ALPH were effective in reducing lesion index and increasing the gastric mucus content. It was also effective in decreasing ulcer index in paracetamol-induced ulcers. All the results obtained with *parathenium hysterophorus* were dose dependent. The results suggest that AQPH and ALPH possesses significant and dose dependent antiulcer activity. The antiulcer activity of AQPH and ALPH can be attributed to its cytoprotective and antisecretory action.

Keywords: *parathenium hysterophorus*, antisecretory, cytoprotective, gastric ulcer, alcohol induced ulcers, paracetamol-induced ulcers and stress induced ulcers.

INTRODUCTION

In order to digest food, absorb nutrients and excrete unabsorbed waste products, the GI tract has to perform a no. of coordinated activities and the tract has to provide the whole body with a continual supply of water, electrolytes and nutrients[1]. In order to achieve these objects, several organs have to integrate with each other and are regulated by nervous rand hormonal systems, as well as the central nervous system[2]. Thus for fully understanding physiological functions of GI tract and its various pathological states including peptic ulcer, diarrhea and constipation, etc.,

Control and Co-ordination of GI Tract

In addition to the main function of assimilation of food, the GI tract has to perform endocrine function and also gut has its own integrative neuronal network, the enteric nervous system (ENS) that shares about the same no. of neurons as those of spinal cord. Many of the neurotransmitters or neuromodulators and hormones in GI tract are peptides. The smooth muscles, blood vessels and glands (exocrine, endocrine and pancrine) are main elements under the neuronal and hormonal control.

Gastric Exocrine secretions

Normally human stomach secretes about 2.5 liter of gastric juice in 24 hours. The principle exocrine secretions include pepsinogens, which are released from zymogens, or peptic or chief cells and hydrochloric acid (HCL) in addition to the intrinsic factor from the parietal cells. The gastric mucosa contains many deep as well as surface glands. Chief and parietal cells are located in the body fundus of stomach, while glands in the pyloric and cardiac regions secrete the mucosa. Thus, mucus is secreted throughout the gastric mucosa. In addition to mucus, bicarbonate ions (HCO_3^-) are secreted by mucus cells on the surface of epithelium in between the gastric glands. The gastric secretions released in response to food involve both neuronal and hormonal mechanisms. They can be divided into two phases: 1. Gastric phase, 2. Intestinal phase.

Gastric Motility and Emptying

The fundus and upper portion of body of stomach relax and accommodate the food upon its entry with little increase in pressure, which is known as “receptive relaxation”. In the lower portion of body of stomach, motility begins which mixes and grinds the food. The small portions of semisolid food are then passed through the pylorus and enter into duodenum. This receptive relaxation is triggered by pharynx and esophageal movements and is mediated by vagus nerve. The gastric basic electrical rhythm is controlled by the peristaltic waves, which initiate soon thereafter and sweeps towards the pylorus. This distal stomach contraction or “systole” lasts up to 10 seconds, which occurs 3-4 times per minute. Therefore, the antrum,

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pylorus and duodenum work apparently as a unit. So, the contraction waves of antrum pass down to pylorus and then duodenum in a sequence and propelling the crushed, well-mixed and semisolid food into the intestine.

Plant Profile

Botanical Name: Parathenium Hysterophorus, Kingdom: Plantae, Order: Asterales, Family: Asteraceae, Genus: parthenium, Species: p. Hysterophorus, Common name: Santa Maria feverfew, Whitetop weed.

Chemical constituents

germacrene-D (35.9%), *trans*-β-ocimene (8.5%) and β-myrcene (7.6%). In the essential oil from *A. Polystachya*, 40 constituents were identified and the principal compounds were germacrene-D (29.3%), *trans*-β-ocimene (13.6%) and β-caryophyllene (9.8%).

MATERIALS AND METHODS

Drugs and Chemicals

Cimetidine, Alcohol was purchased in Taj pharmaceuticals Ltd, Omeoprazole and Ranitidine gifted by Aurobindo Pharma Ltd.

Experimental animals

Wistar rats (150-200 g) and were procured from KIMS medical college 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

Preparation of Aqueous Extract

Fresh leaves of *Parathenium hysterophorus* were collected and washed under tap water. The leaves extract used was prepared by taking 20gms of finely cut leaves into 250ml beaker containing 200ml of water. The contents were mixed well and then the mixture was boiled up to 80-100^{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.

Preparation of Alcoholic Extract

Fresh leaves of *Parathenium hysterophorus* were collected and washed under tap water. The leaves extract used was prepared by taking 20gms of finely cut leaves into 250ml beaker containing 200ml of alcohol. The contents were mixed well and then the mixture was boiled up to 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 *Parathenium hysterophorus* (3-5 g/kg). The conversion factor of human dose (per 200 g body weight) is 0.018 for rats (Ghosh 1984). Hence the calculated dose for the rats (considering human dose3 and 5 g/kg) is 200 mg/kg. Acute toxicity was done at dose of 2000mg/kg body weight.

Pharmacological evaluation

Preparation of extracts

The aqueous and alcoholic extracts of *Parathenium hysterophorus* 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.

Screening For Anti-Ulcer Activity

The aqueous and alcoholic extracts of *Parathenium hysterophorus* leaves 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 aqueous extract of *Parathenium hysterophorus (*250mg/kg p.o).

Group V - Test group received alcoholic extract of *Parathenium hysterophorus (*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, sub mucosal, punctiform hemorrhages;

II = presence of sub mucosal, 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 *Parathenium hysterophorus* 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.

Table 1: Effect on alcohol induced gastric ulcers.

Treatment (n=6)	Dose mg/kg	Lesion index	% Inhibition of ulcer	Mucus content
1% CMC	-	27.48 ± 0.38	-	0.48 ± 0.02
Ulcer control	-	38.65±0.54	-	0.52±0.01
Omeprazole	20	21.11± 0.26	23.18	0.61 ± 0.02
AQPH	250	25.12 ± 0.35	8.58	0.55 ± 0.01
ALPH	250	16.2 ± 0.13	41.04	0.90 ± 0.02

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 $p<0.001$).

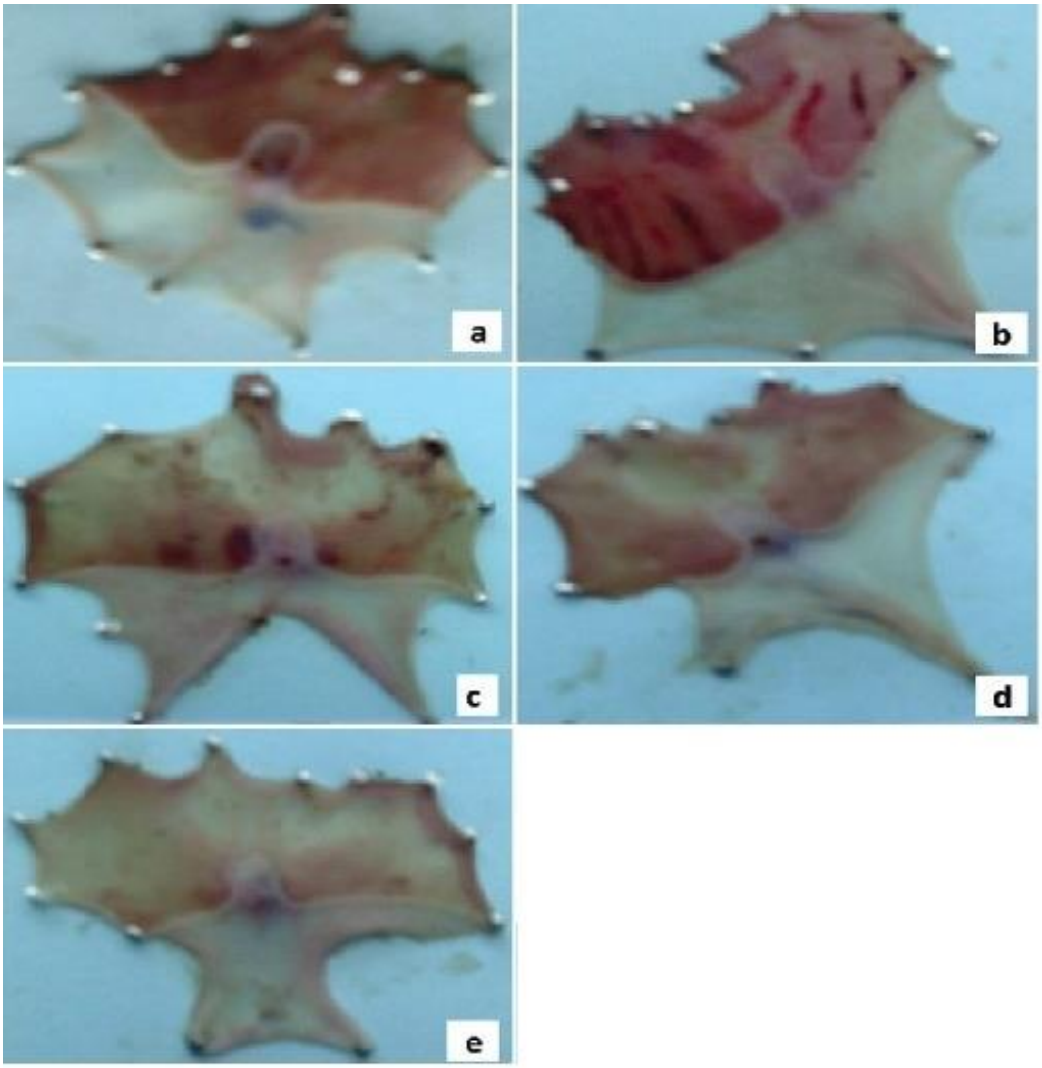

Figure 1: Effect of *Parathenium hysterophorus* on alcohol induced ulcers in the rats in the study (a) Normal Control (b) Ulcer Control (c) *AQPH* (250 mg/kg) treated (d) *ALPH* (250 mg/kg) treated (e) Omeprazole (20 mg/kg treated).

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 aqueous extract of *Parathenium hysterophorus* (250mg/kg p.o) for seven days.
Group V - Test group received alcoholic extract of *Parathenium hysterophorus* (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 hemorrhagic 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

Table 2: Effect of *Parathenium hysterophorus* at various dose levels on paracetamol induced gastric ulcer in rats.

Treatment (n=6)	Dose mg/kg (p.o.)	Ulcer index	% Inhibition of ulcer
1% CMC	-	0.66 ± 0.01	-
Ulcer control	-	0.89±0.02	--
Ranitidine	50	0.23 ± 0.01	65.2
AQPH	250	0.40 ± 0.01	39.4
ALPH	250	0.29 ± 0.01	56.1

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. ($p<0.001$).

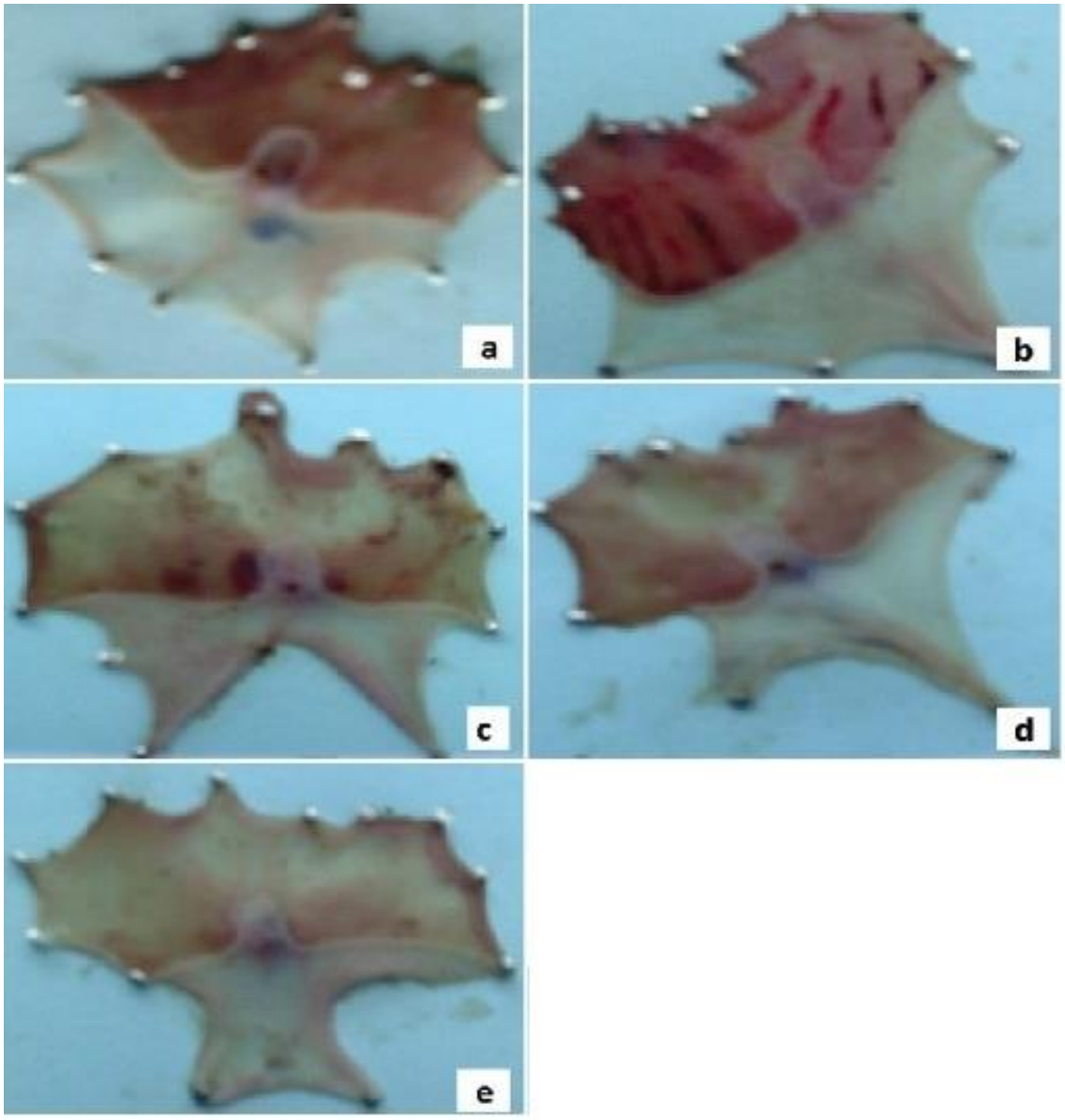

Figure 2: Effect of *Parathenium hysterophorus* on paracetamol induced ulcers in the rats in the study (a) Normal Control (b) Ulcer Control (c) *AQPH*(250 mg/kg) treated (d) *ALPH*(250 mg/kg) treated (e) Ranitidine (50 mg/kg treated).

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

Table 3: Effect of *Parathenium hysterophorus* at various dose levels on Stress induced gastric ulcer in rats.

Group	Ulcer index	Percentage inhibition
Normal Control	00.00±0.00	-----
Ulcer control	26.66±5.82	------
Standard	1.95±0.29	92.68
AQPH	7.70±2.01	71.11
ALPH	3.28±1.93	87.69

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. ($p<0.001$).

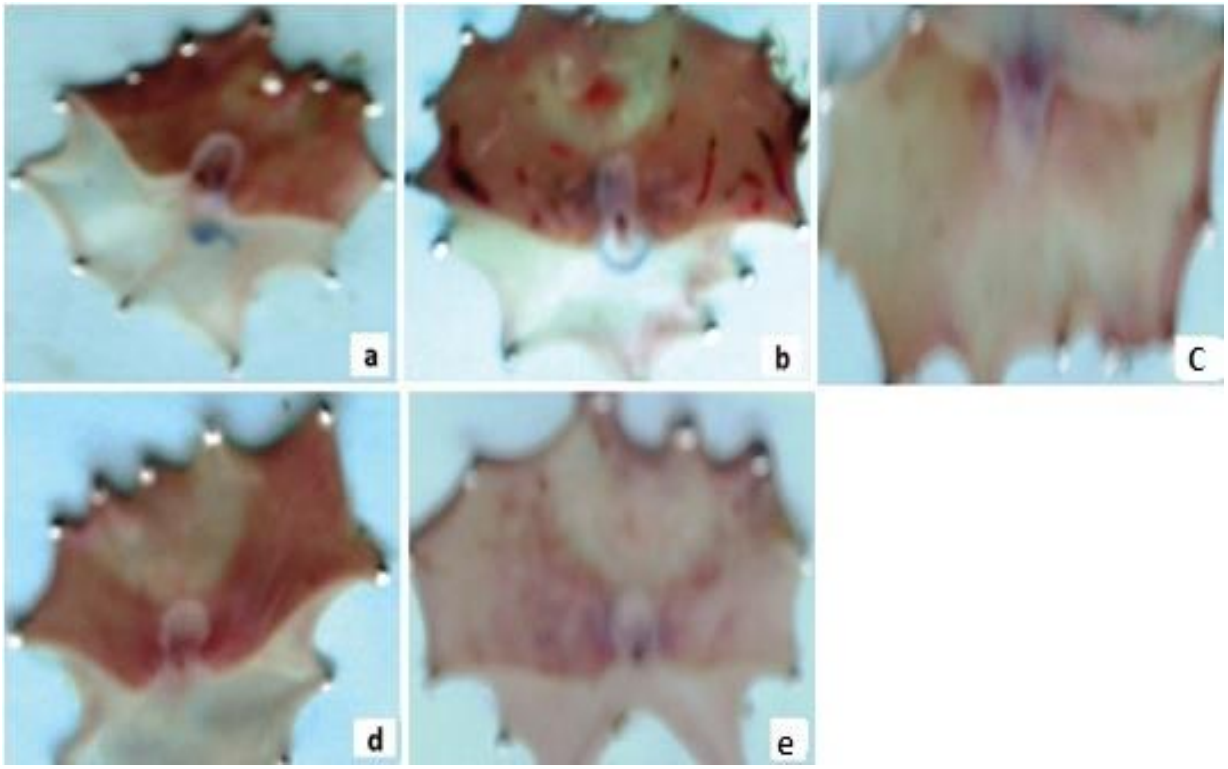

Figure 3: Effect of *Parathenium hysterophorus* on stress induced ulcers in the rats in the study (a) Normal Control (b) Ulcer Control (c) *AQPH*(250 mg/kg) treated (d) *ALPH* (250 mg/kg) treated (e) Omeprazole (20 mg/kg treated).

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 *Parathenium hysterophorus* based on the previous protocol to select the extractives with anti ulcer activity for further evaluation on other anti ulcer models.

Paracetamol 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) + Parcetamol (200 mg/kg)

Group III: Ranitidine (50 mg/kg)

Group IV: AQPH (250 mg/kg)

Group V**:** ALPH (250 mg/kg)

In Paracetamol 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 anesthesia 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 anesthesia. 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 coloration, 1 = Spot ulcer, 1.5 = Hemorrhagic 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.

Acetic Acid Induced Ulcer Model in Rats

The selected plant extractives of both plants were subjected to Acetic acid induced Chronic Ulcer Model in rats. Adult Wistar albino rats weighing 160-220 g of both sexes were selected. Five groups of ten animals each were formed with these rats. To avoid coprophagy they were placed in cages with grating floor. The rats were fasted for 24 h, but allowed free access of water.

Group I - Control (Non-ulcerated) (10 ml/kg, 0.1% CMC *p.o.*)

Group II - Solvent control (Ulcerated) (10 ml/kg, 0.1% CMC *p.o.*)

Group III - Animals received Omeoprazole (20 mg/kg *p.o.*)

Group IV - Animals received AQPH (250 mg/kg *p.o.*)

Group V - Animals received ALPH (250 mg/kg *p.o.*)

Albino rats weighing 160-220 g were fasted for 24 h and abdomen was opened under light ether anesthesia. A cylindrical plastic mould was placed near the region of the lesser curvature of the stomach and 50 µl of 50% glacial acetic acid was administered upon the wall of the stomach corpus. The stomach wall was wiped with cotton wool soaked in saline. Povidone iodine was applied to the abdominal stitches for the next few days to avoid infection. Thereafter the animals were fed with normal diet and access to water. Group I served as nonulcerated control and received only the vehicle while Group II served as the ulcerated control. Rats of Group III served as standard and was administered Omeprazole at 20 mg/kg while groups IV and V were treated with 250 mg/kg of AQPH and ALPH respectively as a suspension in 0.1% CMC. The treatment with the standard drug and the extractives were carried out for 21 days. During the remaining length of the experimental period of 21 days the amount of food and water consumed by the animals were noted. On the final day of the experiment, blood was withdrawn and the blood cell count of all group of animals were estimated by standard methods. The animals were sacrificed by excess anesthesia and Ulcer area of all groups of animals was calculated using the standard formula. Ulcer area was calculated as the product of length and width of ulcer (mm^2). The stomach samples of the treated and the control group of animals were stored in formalin for histopathological studies. The rate of healing of ulcers was calculated by comparing the ulcer index of extractives and Omeprazole treated rats with those of the ulcerated controls.

RESULTS

Effect on alcohol induced gastric ulcers

Oral administration of 80% alcohol produced haemorrhagic gastric lesions in glandular portion of stomach. Pretreatment with AQPH and ALPH at the dose of 250 mg/kg and omeprazole (20 mg/ kg) significantly ($p<0.001$) protected the gastric mucosa as shown by reduced values of lesion index (16.2 ± 0.13 and 21.11 ± 0.26 respectively) against alcohol challenge as compared to solvent control (27.48 ± 0.38).

CONCLUSION

The anti-ulcer activity of the plant *parathenium hysterophorus* was evaluated by employing paracetamol, 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 non-steroidal anti-inflammatory drugs and free radical production. It is suggested that *parathenium hysterophorus* 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 increases 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. On the basis of the present results and available reports, it can be concluded that the anti-ulcer activity elucidated by *parathenium hysterophorus* could be mainly due to the modulation of defensive factors through an improvement of gastric cytoprotection and partly due to acid inhibition.

REFERENCE

1. Nathan C (2002). Points of control in inflammation. Nature 420: 846-852.
2. Vodovotz Y, Csete M, Bartels J, Chang S, An G (2008). Translational systems biology of inflammation. PLoS Computational Biology 4: 1-6.
3. Frank MM, Fries LF (1991). The role of complement in inflammation and phagocytosis. Immunology Today 12: 322-326.

4. Franklin PX, Pillai AD, Rathod PD, Yerande S, Nivsarkar M, Padh H, Vasu KK, SudarsanamV (2008). 2-Amino-5-thiazolyl motif: a novel scaffold for designing anti-inflammatory agents of diverse structures. European Journal of Medicinal Chemistry 43: 129-134.
5. Lucas SM, Rothwell NJ, Gibson RM (2006). The role of inflammation in CNS injuryand disease. British Journal of Pharmacology 147: S232-S240.
6. Kassuya CA, Cremoneze A, Barros LFL, Simas AS, Lapa FR, Mello-Silva R, Stefanello ME, Zampronio AR (2009). Antipyretic and anti-inflammatory properties of the ethanolic extract, dichloromethane fraction and costunolide from *Magnolia ovata* (Magnoliaceae). Journal of Ethnopharmacology 124: 369-376.
7. Kinne RW, Brauer R, Stuhlmuller B, Palombo-Kinne E, Burmester GR (2000). Macrophages in rheumatoid arthritis. Arthritis Research 2: 189-202.
8. White M (1999). Mediators of inflammation and inflammatory process. The Journal of Allergy and Clinical Immunology 103: S378-S381.