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Anti-ulcer Effect of *Chenopodium album* Linn. Against Gastric Ulcers in Rats

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ABSTRACT
The effect of alcoholic extract of *Chenopodium album* Linn. (Chenopodiaceae) was investigated in rats to evaluate the anti-ulcer activity by using three models, i.e., pyloric ligation, ethanol and cold restraint stress induced ulcers. Ranitidine was used as reference standard. The parameters taken to assess anti-ulcer activity were volume of gastric secretion, pH, free acidity, total acidity and ulcer index. The results indicate that the alcoholic extract significantly decreases the volume of gastric acid secretion, free acidity, total acidity and ulcer index with respect to control. Sections of ulcerated area revealed that there was a significant increase in regenerated glandular epithelium width after treatment with the alcohol extract. The collagen content in the ulcerated tissue was significantly increased by alcohol extract and ranitidine, showing the maximum effect. No significant difference on capillary density in scar tissue was observed after treatment with alcohol extract or ranitidine.

Keywords: *Chenopodium album* Linn., anti-ulcer activity, pylorus ligation model, ethanol induced ulcer, cold restraint stress, ulcer index.

INTRODUCTION

Peptic ulcers are craters or open sores in the lining of the upper gastrointestinal tract. They include duodenal ulcers (those that are located in the top of the small intestine or duodenum) and gastric ulcers (those found in the stomach). [1] Gastric ulcer is among the most serious diseases in the world. The etiology of gastro duodenal ulcers is influenced by various aggressive and defensive factors such as acid–pepsin secretion, parietal cell, mucosal barrier, mucus secretion, blood flow, cellular regeneration and endogenous protective agents such as prostaglandins and epidermic growth factors. [2] Some other factors, such as inadequate dietary habits, excessive ingestion of non-steroidal anti-inflammatory agents, stress, hereditary predisposition and infection by *Helicobacter pylori*, may be responsible for the development of peptic ulcer. [3] In spite of the progress in conventional chemistry and pharmacology in producing effective drugs, the plant kingdom might provide a useful source of new antiulcer compounds for development as pharmaceutical entities or, alternatively, as simple dietary adjuncts to existing therapies.

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Drugs with multiple mechanisms of protective action, including antioxidant properties, may be one way forward in minimizing tissue injury in human disease. [4] Drugs of plant origin are gaining popularity and are being investigated for a number of disorders, including peptic ulcer. An indigenous drug possessing fewer side effects is the major thrust area of the present day.

In such context, one such drug is *Chenopodium album* Linn (Family: Chenopodiaceae) which has ethno pharmacological relevance to be used for ulcers. [5] *Chenopodium album* Linn (Chenopodiaceae) found wild up to an altitude of 4700 m and cultivated throughout India particularly Western Rajasthan, Kulu valley and Shimla. It is commonly known as Lamb's quarte, wild spinach and white goosefoot in English. [6-7] In Tradition System of Medicine, it is used as an anthelmintic, antidiarrhoeal, antiphlogistic, antirheumatic, contraceptive, odontalgic, laxative, cardiotonic, antiscorbutic, blood purifier, hepatic disorder, spleen enlargement, biliousness, carminative, aphrodisiac, dyspepsia, flatulence, strangury, seminal weakness, pharyngopathy, splenopathy, hemorrhoids, ophthalamopathy, cardiac disorder and general debility. [8-11] The phytoconstituents isolated so far from the plant are ascorbic acid, β-carotene, catechin, gallocatechin, caffeic acid, p-coumaric acid, ferulic acid, β-sitosterol, campesterol, xanthotoxin, stigmasterol, n-triacontanol, imperatorin, ecdysteroid [12], cinnamic acid amide alkaloid [13], phenol,

saponin, apocartenoids [14], crytomeridiol [15], n-trans-feruloyl-4-O-methyl dopamine and syringaresinol [16] and β-sitosterol, lupeol and 3 hydroxy nonadecyl henicosanoate. [17] The pharmacological activity reported so far from this plant are antipruritic and antinociceptive activity [18], anthelmintic activity [19] and as vaginal contraceptive. [20]

As there is not report on gastric ulcer activity, this prompted us to investigate the anti-ulcer activity of aerial parts of *Chenopodium album* extract.

MATERIALS AND METHODS

Plant material

Plant material used in the study consisted of aerial parts of *Chenopodium album* Linn. collected from the local area of Nadaun, Dist. Hamirpur (H.P.), and authenticated by Dr. Sushil Vashi, Reader, Department of Botany, Govt Degree College of Arts, Commerce and Science, Hamirpur (H.P.). A voucher specimen is preserved in the Department.

Preparation of plant extract

Crude aerial parts of *Chenopodium album* were subjected to pulverizations and passed through sieve no. 40. The powder [300 g] was packed into a soxhlet apparatus and extracted with petroleum ether (60-80ºC) for 18 h. The same marc was successively extracted with alcohol for 18 hours. All the extracts were concentrated by rotary vacuum evaporator and evaporated to dryness and the percentage yield was found to be 2.3 and 15.3 % w/w respectively.

Chemicals

Ranitidine and alcohol were purchased from M/s CDH, Mumbai. Other chemicals and reagents used were of AR grade.

Experimental animal

Wistar albino rats (150-200 g) were maintained in the animal house of Despanday labs, M.P. Nagar, Bhopal (M.P.) for experimental purpose. Then all the animals were acclimatized for seven days under standard husbandry conditions, i.e. room temperature of 25 ± 1°C; relative humidity 45-55% and a 12:12h light/ dark cycle. The animals had free access to standard rat pellet, with water supplied *ad libitum* under strict hygienic conditions. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol. All the protocols and the experiments were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Determination of acute oral toxicity (LD_{50})

The acute oral toxicity (AOT) of alcoholic extract of aerial parts of *Chenopodium album* Linn. were determined by using female albino rats (Wistar strains) weighing between 180-220 g. The animals were fasted 3 hrs prior to the experiment, up and down procedure (OECD Guideline no. 425). Animals were administered with single dose of extracts dissolved in 2% w/v acacia and observed for its mortality during 48 hours study period (short term) toxicity. Based on short-term profile of drug, the dose of the next animals was determined as per as OECD guideline 425. All the animals were also observed for long term toxicity (14 Days). The LD_{50} of the test extract was calculated using AOT 425 software provided by Environmental protection agency, USA.

EVALUATION OF ANTI-ULCER ACTIVITY

Pylorus ligation induced ulcers

Eighteen rats of either sex were randomly divided into three groups and fasted for 48 h with free access to water. Pyloric ligation was performed under light ether anesthesia to each animal. [21] Animal were given 1% CMC solution [Group1], alcohol extract 400mg/kg [Group 2] and 50 mg /kg ranitidine [Group 3] orally immediately after pylorus ligation. Animal were scarified 4 h later. The stomach was carefully removed and gastric contents were collected. The stomachs were cut open along the greater curvature and the ulcer index was calculated.

Ulcer Index determination

The ulcer index was determined using the formula

$$\text{Ulcer index} = 10/X$$

Where X = Total mucosal area / Total ulcerated area.

The volume of the gastric juice was measured and gastric contents were centrifuged at 1000 rpm for 10 min. [22] One ml of the supernatant liquid was pipette out and diluted to 10 ml with distilled water. The solution was titrated against 0.01 N NaOH using Topfer's reagent as indicator, to the end point when the solution turned to orange color. The volume of NaOH needed was taken as corresponding to the free acidity. Titration was further continued till the solution regained pink color. The volume of NaOH required was noted and was taken as corresponding to the total acidity.

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH} \times 100}{0.1} \text{ mEq/litre}$$

Ethanol induced ulcers

Eighteen rats of either sex were randomly divided into three groups. The ulcer was induced by administering ethanol. [23] All the animals were fasted for 36 hours before administration of ethanol. The standard drug (Ranitidine 50 mg/Kg p.o.) and the alcoholic extracts (400 mg/Kg p.o.) were administered one hour before ethanol administration. Ethanol (90%) was administered to all the animals at a dose of 1 ml/200 gm rat and after one hour all the animals were sacrificed, stomachs were isolated and ulcer index was determined as mentioned above.

Cold restraint stress induced ulcers

The ulcer was induced by subjecting the animals to cold restraint stress. [24] The alcoholic extracts (400 mg/kg p.o.) or ranitidine (50 mg/kg p.o.) were administered 30 minutes prior to subjection of stress. The animals were placed in a restraint cage and the cage was placed at a temperature of 2°C for 3 hours. After 3 hours, the animals were sacrificed by over dose of ether anesthesia and the stomach was isolated and cut opened along the greater curvature. The ulcer index was determined.

Histopathological studies

Stomach was sliced and pieces were preserved in 10% formalin for proper fixation. These tissues were processed and embedded in paraffin wax. Section of 5- 6 microns in thickness were cut and stained with staining reagents. All the sections of the tissues were examined under microscope [25] which was documented by taking photograph.

Statistical analysis

Results were expressed as mean ± SEM. Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Dunnet's test for significance analysis using Graph Pad Prism software.

RESULTS

Pylorus Ligation Induced gastric ulcer study

The alcoholic extract of *Chenopodium album* and standard drug Ranitidine showed a significant reduction in ulcer index when compared to control (Table 1; $p<0.01$). The alcoholic extract of the plant was most potent; it produced decrease in the ulcer index when compared to control.

The alcoholic extract of *Chenopodium album* and ranitidine showed a significant reduction in volume, pH, free acidity and total acidity when compared to control.

Sections of ulcerated area revealed that there was a significant increase in regenerated glandular epithelium width after treatment with the alcohol extract (Table 2; Fig. 1; $p<0.05$).

The collagen content in the ulcerated tissue was significantly increased by alcohol extract and ranitidine, showing the maximum effect (Fig. 2).

No significant difference on capillary density in scar tissue was observed after treatment with alcohol extract or ranitidine (Table 2; Fig. 3).

Ethanol induced gastric ulcer

The alcoholic extract of *Chenopodium album* and ranitidine showed a significant reduction in ulcer index when compared to control [Table 3; $p<0.05$].

Table 1: Effect of *Chenopodium album* extract on free acidity, total acidity and ulcer index

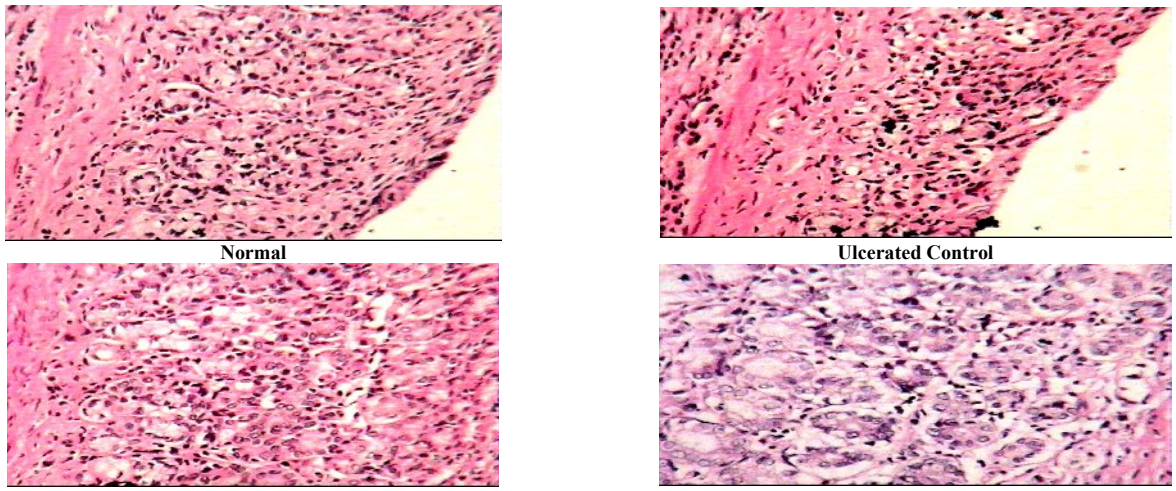
Treatment	Volume	pH	Free acidity mEq/litre	Total acidity mEq/litre	Ulcer index UI/L
Control (CMC 1%)	2.78± 0.30	2.26± 0.61	24.31±3.44	43.11±6.19	5. 22± 0.70
Alcohol (400 mg/Kg) extract	1.82± 0.27*	3.25± 0.30*	15.36±2.06*	29.01± 2.06*	3.46± 0.07**
Ranitidine (50 mg/Kg)	1.42± 0.23*	3.80± 0.23*	7.92±1.37*	17.20± 1.36*	2.57± 0.52**

All values are mean ± SEM; n = 6. *$p<0.05$, **$p<0.01$ when compared to control group

Table 2: Effect of *Chenopodium album* on regenerated glandular epithelium width, capillary density and volume of collagen content

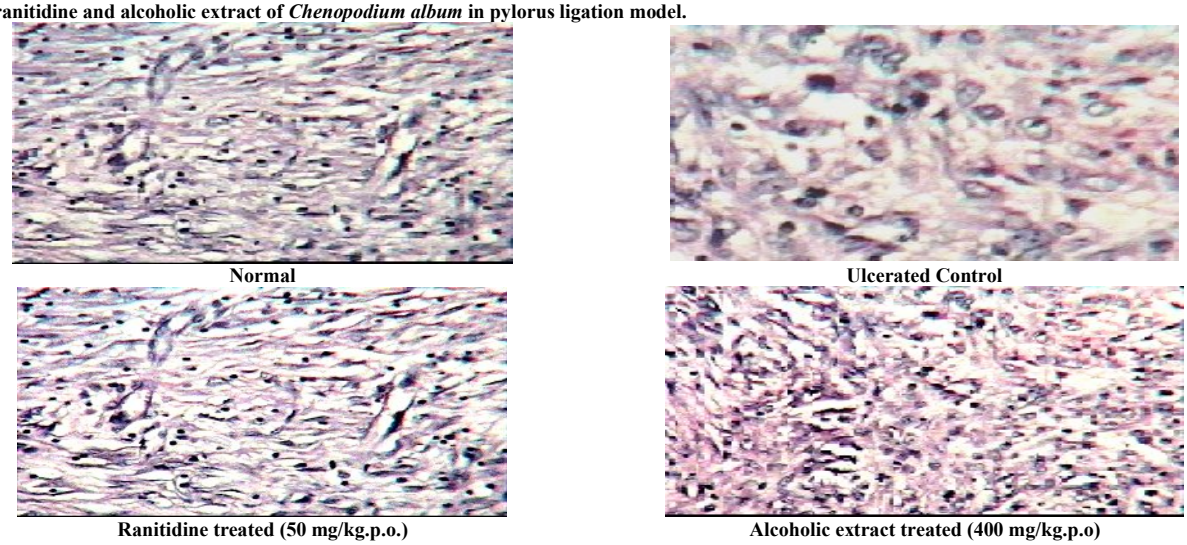
Treatment	Regenerated glandular epithelium width (µm)	Capillary density (No) in 19600 µm^2	Vol. of collagen content
Control	503±60.39	5.4±0.51	0.182±0.018
Ranitidine	638 ±35.33*	5.2±0.38	0.295±0.019**
Alcohol extract	705±20.94	4.6±1.89	0.287±0.009**

All values are mean ± SEM; n = 6. *$p<0.05$, **$p<0.01$ when compared to control group



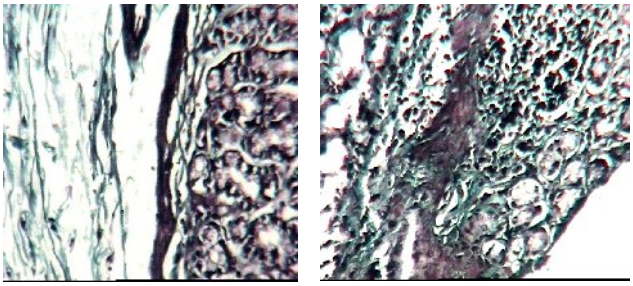
Normal | Ulcerated Control | Ranitidine treated (50 mg/kg.p.o.) | Alcoholic extract treated (400 mg/kg.p.o)

Fig. 1: Sections stained with hematoxylin and eosin (H&E; 100 X) displaying the regenerated glandular epithelium width in stomachs of rats treated with ranitidine and alcoholic extract of *Chenopodium album* in pylorus ligation model.



Normal | Ulcerated Control | Ranitidine treated (50 mg/kg.p.o.) | Alcoholic extract treated (400 mg/kg.p.o)

Fig. 2: Sections stained with periodic acid schiff's stain [100 X] displaying capillary density in stomachs of rats treated with ranitidine and alcoholic extract of *Chenopodium album* in pylorus ligation induced gastric ulcer model.



Normal | Ulcerated Control

Ranitidine treated (50 mg/kg.p.o.) | Alcoholic extract treated (400 mg/kg.p.o)

Fig. 3: Sections stained with masson's stain [100 X] displaying collagen in stomachs of rats treated with ranitidine and alcoholic extract of *Chenopodium album* in pylorus ligation induced gastric ulcer model.

Cold restraint stress induced gastric ulcer

The alcoholic extract of *Chenopodium album* and ranitidine showed a significant reduction in ulcer index when compared to control [Table 4; $p<0.05$].

Table 3: Effect of *Chenopodium album* extract on ulcer index in ethanol induced gastric ulcers

Treatment	Ulcer Index
Control	0.468±0.055
Ranitidine	0.117±0.044*
Alcohol extract	0.137±0.051*

All values are mean ± SEM, n = 5-6. *$p<0.05$, **$p<0.01$, ***$p<0.001$ when compared to control group

Table 4: Effect of *Chenopodium album* extract on ulcer index in cold restraint stress induced gastric ulcers

Treatment	Ulcer Index
Control	0.115±0.037
Ranitidine	0.085±0.015*
Alcohol extract	0.094±0.016*

All values are mean ± SEM; n = 6. *$p<0.05$ when compared to control group

DISCUSSION

It is very clear from the Tables and Figures that the alcoholic extract possesses anti-ulcer activity against pyloric ligation induced ulcer, ethanol and cold restraint stress induced ulcer. The alcoholic extract significantly decreases the volume of gastric acid secretion, free acidity, total acidity and ulcer index with respect to control suggesting that the plant provides significant anti-ulcer activity against gastric ulcers in rats. Sections of ulcerated area revealed that there was a significant increase in regenerated glandular epithelium width, collagen content and no significant difference on capillary density in scar tissue was observed after treatment with the alcohol extract. This study confirms its use as gastro protective as per the ethno pharmacological claims.

Conflict of interest

No Conflict of interest declared.

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