



Evaluation of Antiulcer Activity of *Lepidium sativum* L. Seeds in Albino Wistar Rats

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ABSTRACT

Lepidium sativum L has been referred in Indian traditional medicine system (Ayurveda) for treatment of various diseases. In the present study ethanolic extract of *Lepidium sativum* L seeds has been studied for Anti ulcer activity in animal models. The ethanolic extract was evaluated against gastric ulcerations by pylorus ligation; NSAIDs induced ulcer models, water immersion stress models. The ethanolic extract of the leaves of *Lepidium sativum* L was given by oral route at a dose of 200 & 400 mg/kg body weight. Ethanolic extract of seeds of *Lepidium sativum* L dose dependently reduced, ulcer index, and an increase in pH of gastric juice in pylorus ligated ulcer model. Extract of *Lepidium sativum* L may be useful in the treatment of ulcer.

Keywords: Antiulcer, *Lepidium sativum*, NSAIDs induced ulcer, Pylorusligation, Water immersion stress.

INTRODUCTION

Herbal drugs constitute a major part of therapeutics in all the traditional systems of medicine. Herbal medicine is a triumph of popular therapeutic diversity. "Peptic ulcer disease" refers to breaks in the mucosa at the stomach and small intestine, principally the proximal duodenum, which are produced by the action of gastric secretion. Peptic ulcer is one of the major gastro intestinal disorders, which occurs due to an imbalance between the offensive (gastric acid secretion) and defensive (gastric mucosal integrity), from an imbalance between acid-pepsin Secretion and mucosal defence factors.

Peptic ulcer disease occurs mainly due to consumption of NSAIDs, infection by *H. pylori*, stress or due to pathological condition such as Zollinger –Ellison Syndrome.^{1,2} Consequently, reduction of gastric acid production as well as re-improvement of gastric mucosal production has been the major approaches for therapy of peptic ulcer disease. As a result drugs, of both herbal and synthetic origin are coming up offering newer and better options for treatment of peptic ulcer.³

Lepidium sativum L (Garden cress) is an annual herb, belonging to Brassicaceae family, it is also known as garden cress, mustard and cress, garden pepper cress, pepperwort pepper grass, or poor man's pepper. It is a fast-growing, edible plant botanically related to watercress and mustard and sharing their peppery, tangy flavour and aroma. Seeds, leaves and roots are economically important, however, the crop is mainly cultivated for seeds.

In some regions garden cress is known as garden pepper cress, pepper grass or pepperwort.^{4,5} It is also known as Asalio or Chandrasur in India and it is an important medicinal crop in India. Garden cress is a perennial plant, and an important green vegetable consumed by human

beings, most typically as a garnish or as a leaf vegetable.⁶ *Lepidium sativum* L have been widely used to treat a number of ailments in traditional system of medicine throughout India. Thought to be an effective medicinal remedy to cure respiratory disorders, like bronchitis and asthma.⁷

The plant is cultivated as culinary vegetable all over Asia. In South Asia, it is used in traditional medicine to treat asthma, bronchitis, and cough and is considered useful as abortifacient, antibacterial, aphrodisiac, diuretic, expectorant, gastrointestinal stimulant, gastro protective, laxative and stomachic.^{8,9}

MATERIAL AND METHODS

Extraction

The seeds of *Lepidium sativum* L were collected and authenticated, seeds are processed and powdered. The powdered material was subjected to batch extraction in Soxhlet apparatus. The solvent used was ethanol. The powdered material of *Lepidium sativum* L seeds were evenly packed in Soxhlet extract or for extraction with solvent. The temperature was maintained on an electric heating mantle with thermostat control. Appearance of colourless solvent in the siphon tube was taken as the termination of extraction. The extracts were then concentrated by distilling the solvent. The concentrated extracts were evaporated on a water bath (40-50°C) to dryness. Each extract was weighed and percentage yield was calculated. The colour and consistency of the extracts were noted.¹⁰

Animals

The healthy Wistar albino rats of either sex weighing between 160-200 g were taken for the study. They were housed under controlled conditions of temperature (23±2°C), humidity (55±5%) and 12 h light and 12 h dark

cycles. The animals were fed with standard pellet diet and water *ad libitum*.

Pylorus ligation induced ulcer model

Albino rats were fasted in individual cages with measures taken to avoid coprophagy for 24hrs prior to the experiment with free access to water. First animals are divided in to four groups, each contain 6 animals, group I having pyloric ligation and received distilled water orally. Groups II received Ranitidine (20mg/kg) as reference drug for ulcer protective study. Group III and IV received ethanolic extract of *Lepidium sativum* Lin a dose of 200 and 400mg/kg, after 45 min of ethanolic extract of *Lepidium sativum* and Ranitidine treatment.

Pyloric ligation was be done by ligation the pyloric end of stomach of rats of respective groups under ether anaesthesia by stomach is exposed and a thread is passed around the pyloric sphincter and applied a tight knot. Ligation was done without causing any damage to the blood supply of the stomach. Close the abdomen wall by putting the sutures. Clean the skin from any bloodspot and bleeding. Animal were allowed to recover and stabilize in individual case and were deprived of water during post operative surgery. After 4 hours of surgery, rats were sacrificed and ulcer score was done. Gastric juice was collected and gastric fluid was collected for measurement of total gastric volume and estimation of free and total acidity and percentage protection by the formulas given below.¹¹

Scoring of ulcer¹²

0 = Normal coloured stomach

0.5 = Red colouration

1 = Spot ulcer

1.5 = Haemorrhagic streaks

2 = Ulcers ≥ 3 but ≤ 5

3 = Ulcers >5

Calculation of ulcer Index¹³

$U1 = UN + US + UP \times 10^{-1}$

U1 = Ulcer Index

UN = Average of number of ulcer per animal

US = Average of severity score

UP = Percentage of animal with ulcer

Determination of Percentage Protection¹³

% Protection =

$\frac{\text{Control Mean Ulcer Index} - \text{Test Mean Ulcer Index}}{\text{Control Mean Ulcer Index}} \times 100$

Control Mean Ulcer Index

Determination of acidity¹⁴

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

NSAIDs Induced Ulcer¹²

Healthy female Wistar albino rats of weighting between 160-200gm were taken for the studies. The animal were divided in to four groups (each contain 6 animal) us follow.

Group – I (Control): Distilled water

Group – II (Standard): Ranitidine

Group –III: Extract (200mg/kg)

Group –IV: Extract (400mg/kg)

The animal in all the groups were kept for 24 hr fasting after that animal of all groups received diclofenac sodium (NSAIDs, 20mg/kg). The oral feeding of water and diclofenac sodium was continued for 3 days, the animal of II, III and IV were administered with Ranitidine (20mg/kg), seed extract (200mg/kg), seed extract (400mg/kg) respectively after 3hours of diclofenac sodium administration. On 4th day the animals were sacrificed, stomach were removed and cut along the greater curvature to measure the ulcer index

Cold Restraint Model (Stress Induced Ulceration)

Albino Wistar rats of either sex were divided into five groups with six animals in each group as follows:

Group I: Control (untreated) group.

Group II: Standard treatment group (Ranitidine 20 mg/kg)

Group III: Test treatment group (200 mg/kg)

Group IV: Test treatment group (400 mg/kg)

Table 1: Effect of Ethanolic seed extract of *Lepidium sativum* L on NSAIDs – induced ulcer in rats

Group	Dose (mg/kg)	Ulcer index	% Protection
Control	20	4.65±0.41	---
Ranitidine	13.5	1.02±0.26	78.06
Ethanolic extract	200	1.92±0.45	58.71
Ethanolic extract	400	1.15±0.30	75.27

Values are mean \pm S.E.M. (n = 6), Significant as compared to control $P^* < 0.001$

Albino rats between (160-200 gm.) and each group containing 6 animals were divided into 4 groups. After 30 min of oral administration of the vehicle/standard/extracts rats are placed in cold water vertically for 4hrs in individual restraint cages maintained at 22°C then they were taken out, 10 min later, sacrificed with ether and stomachs are removed. Formal –saline (2%v/v) is then injected into the totally ligated stomachs for overnight storage. The next day, the stomachs opened along the greater curvature, were washed in warm water, Mean ulcer score for each animal is expressed as ulcer index. Gastric juice collected into centrifuge tubes and centrifuged at 1000 rpm for 10min and the volume was noted. The pH of the gastric juice was recorded by pH



meter and the gastric content is subjected for analysis of free and total acidity.

RESULTS AND DISCUSSION

Ethanollic extract of seeds of *Lepidium sativum* L was evaluated for its anti-ulcer activity against NSAIDs and pylorus ligation induced gastric ulcer model. The results of study are tabulated in Table-I and Table –II&III.

Table 2: Effect of Ethanolic seed extract of *Lepidium sativum* in L Pylorus ligation – induced ulcer in rats

Group	Dose (mg/kg)	Ulcer index	% Protection	Free acidity (mEq/L)	Total acidity (mEq/L)	Volume of gastric juice (mL)	pH of Gastric Juice
Control	---	0.17±0.005	---	40.5±2.88	50.4±4.43	4.52±0.61	2.0±0.08
Ranitidine	13.5	0.05±0.02**	70.59	5.01±1.07**	13.1±1.06*	2.04±0.13**	3.68±0.20**
Ethanollic extract	200	0.09±0.01*	47.06	22.0±1.680	37.0±2.77*	3.15±0.07*	2.85±0.10**
Ethanollic extract	400	0.07±0.005*	58.82	18.022±1.68	20.0±2.15*	2.98±0.1*	2.20±0.08**

Values are mean ± S.E.M. (n = 6), P* < 0.01 P** < 0.05 when compared to control group

Table 3: Effect of Ethanolic Extract of *Lepidium sativum* L seeds on volume and pH of gastric content in stress induced rats

Treatment(mg/kg)	Volume of gastric juice (ml)	pH of gastric juice
Ulcerated control	5.28±0.12	1.97±0.67
Rantidine (20mg/kg)	3.92±0.71	2.48±0.05
Ethanollic Extract (200mg/kg)	4.04±0.07*	3.94±0.08*
Ethanollic Extract (400mg/kg)	4.32±0.06**	3.24±0.10*

Values are expressed as Mean ± SEM. *p<0.05, **p<0.01, as compared with ulcerated control using one way ANNOVA followed by Dunnet test.

Table 4: Effect of ethanolic Extract of *Lepidium sativum* L seeds on free acidity and total acidity in Stress induced rats

Treatment (mg/kg)	Free acidity (meq/L/100gm)	Total Acidity (meq/L/100gm)
Ulcerated control	37.33±1.80	74.13±1.70
Rantidine (20mg/kg)	14.40±0.36	27.46±0.76
Ethanollic Extract (200mg/kg)	24.66±0.66	68.06±0.72
Ethanollic Extract (400mg/kg)	22.60±0.93**	39.83±1.31**

Values are expressed as Mean ± SEM. *p<0.05, **p<0.01, as compared with ulcerated control using one way ANNOVA followed by Dunnet test.

Table 5: Effect of Ethanolic Extract of *Lepidium sativum* L seeds on ulcer index and % gastro protection in stress induced rats

Treatment (mg/kg)	Ulcer index (Mean ± SEM)	% Gastro protection
Ulcerated control	4.7±0.29
Ranitidine (20mg/kg)	1.88±0.11	60
Ethanollic Extract (200mg/kg)	4.21±0.11	29.70
Ethanollic Extract (400mg/kg)	2.35±0.15**	62**

Values are expressed as Mean ± SEM. *p<0.05, **p<0.01, as compared with ulcerated control using one way ANNOVA followed by Dunnet test.

DISCUSSION

Peptic ulcer and gastritis have been associated with multipathogenic factors and could be due to disturbances in natural balances between the aggressive factors (e.g. of acid, bicarbonate, pepsin) and maintenance of the mucosal integrity through the endogenous defence mechanism. *Lepidium* L has been reported to exert several pharmacological properties such as anthelmintic, antibacterial, anti-diabetic and antioxidant, wound healing, anti-inflammatory, analgesic and ant lipid peroxidation, anticonvulsant, and anti-amnesic activities. Despite claim of its potential in the treatment of gastric ulcer, this plant has so far not been screened for anti-ulcer activity. Thus, we take this opportunity to report the preliminary findings on anti-ulcer potential of *Lepidium sativum* L seeds ethanolic extract for the first time here. The present study demonstrated the potential of *Lepidium sativum* L to significantly reduced gastric ulceration as indicated by the reduction in ulcer index in the Pylorus induced and Cold stress induced assays. Based on further findings using the pylorus ligation model, the extract was suggested to act by reducing the volume of gastric juice secreted, gastric free and total acidities. These results suggested that *Lepidium sativum* L possesses anti-secretory potency as well as acid neutralizing effect. It is also possible to suggest that the observed antiulcer activity associated with *Lepidium sativum* L. In the present study the control animals showed ulceration, redness, and hemorrhagic streaks, after pylorus ligation. There was also an increase in gastric volume, free acidity, total acidity and p^H. Ethanolic extract at the 200 mg dose and Ranitidine produced significant (P<0.01) reduction in ulcer score when compared to the control. The percentage protection against ulcers by Ranitidine, *Lepidium sativum* L ethanolic extract at 200mg dose and Ranitidine significantly (P<0.01) reduced the gastric volume when compared to control. Extracts produced better (P<0.01) reduction in gastric volume, when compared to ethanolic extracts and Ranitidine. Ethanolic extract of *Lepidium sativum* L at the doses significantly (P<0.01) reduced free acidity when compared to control. Significant (P<0.01) reduction in



ulcer score was production by Ranitidine, ethanolic extract at the does 200mg body weight, when compared to the control.

NSAIDS is known to inactive irreversibly the prostaglandin synthetase system, which mediates synthesis of prostaglandin in the mucosa. An increase in acid secretion and back diffusion at h⁺ ions is also noticed. It is reasonable to assume that the observed gastric mucosal lesions induced by aspirin are due to a deficiency of mucosal prostaglandin.¹⁹ NSAIDS induced ulcer is mediated through tissue damaging free radicals, which are produced from the conversion of hydroperoxyl to hydroxyl fatty acids, which leads to cell destruction. The hydroperoxyl fatty acids are generated from the degeneration of mast cells and generalized lipid per oxidation accompanying cell damage.¹⁷ Pylorus ligation induced ulcers are due to auto digestion at the gastric mucosa and breakdown of the gastric mucosal barrier. in case of pyloric ligation, ulcer formation is mainly due to the stasis at the gastric juice and stress.^{18,19} In NSAIDs induce ulcer model the plant extract at dose of 200 and 400 mg/kg showed significant gastro protective activity 58.71% and 75.27% compared with standard drug Ranitidine showed 78.06% (Table-I). In ligation induced gastric ulcer. Both doses of *Lepidium sativum* extract showed significant reduction in ulcer index, free acidity, total acidity and gastric volume but raised PH of gastric Juice as compared to the control groups. It was showing protection index 47.06% and 58.82% at a dose of 200 and 400 mg/kg respectively compared with standard drugs Ranitidine showed 70.59% (Table-II).

Stress induced ulcerated control group had produced ulcer in all animals and the mean ulcer index was 4.73 ± 0.29 indicating the ulcerogenic effect. However, the ulcer index showed significant dose dependent reduction in the animal pre-treated with ethanolic extract 200 mg/kg (UI; 4.21 ± 0.11) and 400 mg/kg (UI; 2.35 ± 0.15). It indicated 29.70% gastro protection at 200 mg/kg and 62% gastro protection at 400 mg/kg as compared with ulcerated control. The results indicate that the higher dose of ethanolic extract i.e. 400 mg/kg was effective in protecting ulcers in cold stress induced model. Cold stress had produced ulcers in all animals pre-treated with Ranitidine 20 mg/kg. However; ulcer index (1.88 ± 0.11) showed significant reduction as compared with ulcerated control and showed 60 % gastro protection.

CONCLUSION

Ethanolic extract of *Lepidium sativum*L seeds reduced ulcer incidence, when compared to the control as evident by decrease in ulcer score in all the three models. Anti-secretory activity of the extracts was noticed in pylorus ligation induced ulcer model. There was decrease in gastric volume and reduction in free and total acidity in the treated with ethanolic extract in pylorus ligation and

stress induced models, so it is evident that seeds of *Lepidium sativum* L posses the Anti-Ulcer activity.

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