

ANTIULCER ACTIVITY OF AQUEOUS EXTRACT OF *SPINACIA OLERACIA* IN RATS

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ABSTRACT

The main objective is to study the antiulcer activity of AESO using different models of gastric ulceration in rats. Antiulcer activity of AESO was studied in rats in which gastric ulcers were induced by oral administration of ethanol or aspirin or by pyloric ligation. AESO was administered in the dose of 500 mg/kg and 1000 mg/kg orally 30 min prior to ulcer induction. The antiulcer activity was assessed by determining and comparing the ulcer index in the test drug group with that of the vehicle control group. Gastric total acid output and pepsin activity were estimated in the pylorus ligated rats. Ranitidine and Sucralfate were used as a reference drug. The ulcer index in the AESO treated animals was found to be significantly less in all the models compared to vehicle control animals. This antiulcer property was more prominent in animals in which ulcers were induced by ethanol, Aspirin and pyloric ligation. Ranitidine (30 mg/kg) produced a significant gastric ulcer protection when compared with the control group. The anti-ulcer activity of AESO was however less than that of ranitidine. Our results suggest that AESO possesses significant antiulcer property which could be either due to cytoprotective action of the drug or by strengthening of gastric mucosa and thus enhancing mucosal defense.

Keywords: Cytoprotection, mucosal defense, ulcer protection.

INTRODUCTION

Peptic ulcer is an excoriated area of the gastric or duodenal mucosa caused by action of the gastric juice. It is a chronic and recurrent disease, and is the most predominant of the gastrointestinal diseases¹. It is generally recognized that peptic ulcer is caused by a lack of equilibrium between the gastric aggressive factors and the mucosal defensive factors². Gastric ulcer is among the most serious diseases in the world. The etiology of gastroduodenal 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 factors³. 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 ulcer⁴.

Spinacia oleracia L. (SO), commonly named as spinach (family Amaranthaceae) is an annual plant having medicinal property native to central and southwestern Asia. It is cultivated for sake of its succulent leaves and was introduced in Europe in 15th century and is probably of Persian origin. It is favorite food vegetable among Indian in winter season and is a dietary powerhouse, full of vitamins and minerals such as vitamin C, iron and vitamin

E. it also contain magnesium, anisene, calcium, vitamin K, vitamin A and folic acid. Presence of different carotenoids such as lutein, β -carotene, violaxanthin, 9-(Z)-neoxanthin has also been reported in originally grown spinach. Spinach leaves contain several active components, including various flavonoid, which exhibit antioxidant and anti-inflammatory property. Quercetin is most abundant flavonoid of spinach⁵. The plant is sweet, cooling, carminative, laxative, alexipharmic; useful in diseases of blood and brain, asthma, leprosy, biliousness; causes "kapha" (Ayurveda). In experiments it has been shown to have hypoglycemic properties. It has been used in the treatment of urinary calculi. The leaves are cooling, emollient, wholesome, antipyretic, diuretic, maturant, laxative, digestible, anthelmintic, useful in urinary concretion, inflammation of the lungs and the bowels, sore throat, pain in joints, thirst, lumbago, cold and sneezing, sore eye, ring worm scabies, leucoderma, soading urine, arrest vomiting, biliousness, flatulence. And have been used in the treatment of febrile conditions⁶. The seeds are useful in fevers, leucorrhoea, urinary discharges, lumbago, diseases of the brain and of the heart (Yunani). Seeds are laxative and cooling. They have been used in the treatment of difficulty in breathing, inflammation of the liver and jaundice. The green plant is given for the urinary calculi⁷. It has shown that the antioxidants play an important role in the treatment of ulcer⁶. As synthetic drugs having various adverse effect, so to overcome or counteract this effect herbal medicines are used now a days. Therefore *Spinacia oleracia* is used in present study.

MATERIAL AND METHODS

Plant Collection and authentication of Leaves

The dried leaves of *Spinacia Oleracia* was procured from Mumbai region in the month of September-October (2008-09) and air-dried at room temperature ($28 \pm 2^\circ\text{C}$) for a one week.

Preparation of crude extract

Leaves of *Spinacia Oleracia* were shade dried and coarsely powdered by using grinder mixer. The powdered material was macerated in sufficient quantity of distilled water with small quantity of chloroform to prevent fungal growth and kept for 7 days. During maceration it was shaken twice daily. On

seventh day it was filtered & the filtrate was concentrated on water bath (45°C) to remove the solvent and to get sticky brown coloured extract i.e. aqueous extract of *Spinacia Oleracia* (AESO). The extractive value of the extract was 16%.

Experimental animals

The study was conducted on Albino rats (Wistar) of 200-250 g and maintained under standard conditions (room temperature $24-27^\circ\text{C}$ and humidity 60-65%) with 12 h light and dark cycle. The food in the form of dry pellets (Amrut Lab., Pune) and water were available ad libitum. The animal experiments were approved by the ethics committee of the institute.

Chemicals and drugs

Ethanol (Yashchem.,pune), Aspirin (Researchlab,Mumbai), Ranitidine (cipra,Mumbai), Sucralfate (Dr.Reddys lab,Mumbai), Carboxy methyl cellulose (CMC), Trichloroacetic Acid (Researchlab,Mumbai), Phenol reagent (Research lab,pune), Bovine albumin serum (Research lab.fine chem.industries,Mumbai), Std. phenol solution (Research lab,pune), Conc.HCL (Research lab.fine chem. industries,Mumbai), NAOH (Research lab.fine chem. industries,Mumbai) were used in the study.

Toxicity Studies

The acute toxicity study was done as per the OECD guidelines (407). The compounds were administered orally in different doses, where 24h toxicity was recorded to identify the toxic dose. No mortality and no signs of toxicity were found at the dose of 5000 mg/kg body weight of AESO. Therefore, it might be considered that AESO have an LD50 value above 5000 mg/kg. Two doses 500 mg/kg and 1000 mg/kg were selected for present study.

EXPERIMENTAL DESIGN

Gastric cytoprotection methods (Ethanol induced ulcers)

Albino rats (Wistar) of 200-250 g are maintained under standard conditions (room temperature $24-27^\circ\text{C}$ and humidity 60-65%) with 12 h light and dark cycle. The food in the form of dry pellets (Amrut Lab., Pune) and water were available ad - libitum. Rats of

either sex, were randomly divided in to 5 groups of 6 animals in each group.

- Group-I :** Normal control .
Group-II : Ulcerated control (1 ml ethanol, p.o.).
Group-III : Sucralfate (400 mg/kg).
Group-IV : AESO (500 mg/kg, p.o.).
Group-V : AESO (1000 mg/kg, p.o.).

Thirty minutes after the test or reference drug or the control vehicle treatment, 1 ml of ethanol was orally administered to each rat. After 1 h the rats were euthenised with excess of anesthetic ether and stomach was cut open along the greater curvature, cleared of residual matter with saline and the inner surface was examined for ulceration. Ulcer index and % ulcer protection were calculated by using the methods described earlier ^{8,9}.

Aspirin-induced gastric mucosal damage

Albino rats (Wistar) of 200-250 g were selected, And fasted for 36 h. Rats of either sex, were randomly divided in to 5 groups of 6 animals in each group.

- Group-I :** Normal control .
Group-II : Ulcerated control (1% CMC, 5 ml/kg, p.o.).
Group-III : Ranitidine(50 mg/kg).
Group-IV : AESO (500 mg/kg, p.o.).
Group-V : AESO (1000 mg/kg, p.o.).

After 30 min., aspirin suspended in 1% CMC in water(20 mg/ml) at a dose of 500 mg/kg was administered orally to all the animals and 4 h later, the animals were sacrificed. The stomach was removed and open along the greater curvature. The number of ulcer spots in the glandular portion of the stomach were counted in both control and drug treated animals and the ulcer index was calculated^{10,11,12}.

Pyloric ligation method

In this method albino rats were fasted in individual cages for 24 h. Care was being taken to avoid coprophagy. Rats of either sex, were randomly divided in to 5 groups of 6 animals in each group.

- Group-I :** Normal control
Group-II : Ulcerated control (1% CMC, 5 ml/kg, p.o.).
Group-III : Ranitidine(30 mg/kg).
Group-IV : AESO (500 mg/kg, p.o.).
Group-V : AESO (1000 mg/kg, p.o.).
- AESO or reference drug or control vehicle was administered 30 min prior to pyloric ligation. Under light ether anesthesia, the abdomen was opened and the pylorus was ligated. The abdomen was then sutured. At the end of 4 h after ligation, the animals were sacrificed with excess of anesthetic ether, and the stomach was dissected out. Gastric juice was collected and its volume was measured. The glandular portion was then exposed and examined for ulceration. Ulcer index was determined^{12,13,14}.

Determination of various parameters

a) Determination of Ulcer Index (UI)

The ulcerative index was calculated by severity of gastric mucosal lesions and graded as follows;

Erosions	Score
1 mm or less	1
1-2 mm	2
More than 2 mm	3

Then the UI was calculated by using the formula:

UI = 1 (no. of lesions of grade 1) + 2 (no. of lesions of grade 2) + 3 (no. of lesions of grade 3).

Then the overall score was divided by a factor 10, which was designed as ulcer index^{15,16}

% gastro protection was calculated according to;
% gastro protection = (UIC-UIT)/UIC*100

Where, UIC-ulcer index of control.
 UIT-ulcer index of test¹⁷.

b) Collection of gastric juice

Gastric juice was collected from pylorus ligated rats. The gastric juice collected was centrifuged at 1000 rpm for 10 min. And the volume of gastric juice was measured. The gastric juice was used for biochemical estimation.

c) Determination of free acidity and total acidity

1. Gastric juice (1 ml) was taken in to a 100 ml conical flask, to this 2-3 drops of Topfer's reagent was added and titrated with 0.01 N NAOH until all traces of red colour disappears and the colour of the solution turns yellowish orange (end point).
2. The volume of alkali added was noted. This volume corresponds to free acidity.
3. 2-3 drops of phenolphthalein solution was added and titration was continued until a definite red tinge reappears.
4. The volume of alkali added was noted which corresponds to total acidity^{13,18}.

Acidity was calculated by using the formula;
 Acidity (mEq/litre) = Volume of NAOH*
 Normality of NAOH * 100/0.1

d) Estimation of pepsin

1. For estimation pepsin, placed 4 test tubes (1) and (2) containing 5 ml of 1% bovine albumin in 0.01 M HCL, (3) and (4) containing 10 ml of 0.35 M trichloroacetic acid.
2. The gastric juice was mixed with an equal volume of 0.01 M HCL, warmed to 37°C. 1 ml of this mixture was added to each of test tubes of (1) and (4).
3. Incubated for 15 min. At the end mixed content of tube (1) with (3). Allow to stand for about 4 min. (1) + (3) gives test and (2) + (4) gives blank.
4. The mixture was filtered. To 2 ml of the filtrate, 10 ml of NAOH was added. Then 1 ml of phenol reagent was added and mixed by gentle rotation. After 30 min., The absorbance was measured at 680 nm. The difference between test and blank gives the measures of peptic activity.
5. As standard, mixed 2 ml of freshly prepared phenol solution containing 50 mcg/ml with 10 ml NAOH and 1 ml of phenol reagent was added and was measured at 680 nm after 5 to 10 min^{14,19}.

Statistical analysis

The Statistical analysis was performed by using One Way ANOVA followed by Dunnet's comparison test and student t-test

(unpaired). The values are expressed as mean \pm SEM.

RESULT

Effect of AESO in pylorus ligated rats

Pylorus ligation in ulcerated control group had produced ulcer in all animals and the mean ulcer index was 4.7 ± 0.29 indicating the ulcerogenic effect. Another ulcerogenic effect as compared to normal control was measured as follows; mean gastric content volume as 5.28 ± 0.12 , pH as 1.97 ± 0.67 , free acidity as 37.33 ± 1.80 , total acidity as 74.13 ± 1.70 , pepsin content as 5.56 ± 0.69 , indicating the ulcer production in animals. Pylorus ligation also produced ulcers in all the AESO pretreated animals. However, the ulcer index showed significant dose dependent reduction in the animal pretreated with AESO 500 mg/kg (UI; 4.21 ± 0.11) and 1000 mg/kg (UI; 2.35 ± 0.15). It indicated 29.70% gastroprotection at 500 mg/kg and 62% gastro protection at 1000 mg/kg as compared with ulcerated control. The results indicate that the higher dose of AESO i.e. 1000 mg/kg was effective in protecting ulcers in pylorus ligated rats. Pylorus ligation had produce ulcers in all animals pretreated with Ranitidine 30 mg/kg. However, ulcer index (1.88 ± 0.11) showed significant reduction as compared with ulcerated control and showed 60 % gastro protection.

Effect of AESO in Aspirin induced gastric ulcer

Aspirin induced ulcerated control group had produced ulcer in all animals and the mean ulcer index was 2.25 ± 0.21 indicating the ulcerogenic effect. Pylorus ligation also produced ulcers in all the AESO pretreated animals. However, the ulcer index showed significant dose dependent reduction in the animal pretreated with AESO 500 mg/kg (UI; 2.99 ± 0.21) and 1000 mg/kg (UI; 2.16 ± 0.11). It indicated 58.33% gastro protection at 500 mg/kg and 65.27% gastro protection at 1000 mg/kg as compared with ulcerated control. The results indicate that the higher dose of AESO i.e. 1000 mg/kg was effective in protecting ulcers in pylorus ligated rats. Pylorus ligation had produce ulcers in all animals pretreated with Ranitidine 30 mg/kg. However; ulcer index (1.2 ± 0.29) showed significant reduction as compared with

ulcerated control and showed 74.57 % gastro protection.

Effect of AESO in ethanol induced gastric ulcer

Ethanol induced ulcerated control group had produced ulcer in all animals and the mean ulcer index was 4.72 ± 0.07 indicating the ulcerogenic effect. Pylorus ligation also produced ulcers in all the AESO pretreated animals. However, the ulcer index showed significant dose dependent reduction in the animal pretreated with AESO 500 mg/kg (UI; 2.99 ± 0.21) and 1000 mg/kg (UI; 2.16 ± 0.11). It indicated 58.33% gastro protection at 500 mg/kg and 65.27% gastro protection at 1000 mg/kg as compared with ulcerated control. The results indicate that the higher dose of AESO i.e. 1000 mg/kg was effective in protecting ulcers in pylorus ligated rats. Pylorus ligation had produce ulcers in all animals pretreated with Sucralfate 400 mg/kg. However, ulcer index (1.2 ± 0.29) showed significant reduction as compared with ulcerated control and showed 74.57 % gastro protection.

DISCUSSION

The preliminary phytochemical evaluation performed in the present study demonstrated that the AESO contains, Flavonoids *Spinacia oleracea* is very rich in the flavonoids. Various flavonoids have been reported are quercetin, myricetin, kampeferol²⁰. Phenolic compounds The polyphenols isolated from the plant are *para*-coumaric acid, ferulic acid, *ortho*-coumaric acid²¹. Carotinoids: Spinach shows presence of different carotinoids like lutein, β -carotene, violaxanthin and 9'-(Z)-neoxanthin⁵. The finding of the present study demonstrated that AESO possess antiulcer activity against the ulceration caused by pylorus ligation, aspirin and ethanol. In pylorus ligated rats, gastric acid is associated with sever ulceration of the rat gastric mucosa²². The activation of vagus – vagal -reflux by stimulation of pressure receptors in the antral gastric mucosa is believed to increase gastric acid secretion²³.

The digestive site of accumulated gastric juice and interference of gastric blood circulation responsible for ulceration²⁴. It is evident from the present result that AESO has potent ulcer protective activity at a dose 500 mg/kg and 1000 mg/kg, but at the dose 1000 mg/kg was more potent. The ulcer index of castor oil treated rat was comparable to those of ulcerated control rats. There was significant decrease in ulcer index in AESO treated rats and ranitidine treated rats. Several non-steroidal anti-inflammatory drugs like aspirin are known to induce gastric damage by suppression of prostaglandins²⁵. In the stomach, prostaglandins play a vital protective role, stimulating the secretion of bicarbonate and mucus maintaining mucosal blood flow and regulating mucosal cell turn over and repair^{26,27}. Oxy radicals may play important role in the aspirin induced erosive gastritis²⁴. After an initial hydrophobic intermolecular interaction, the free carboxyl group present in all NSAIDs forms a strong electrostatic bond with the positively charged head group of zwitterionic phospholipids of mucus layer and, in doing so, increase the phospholipids solubility, neutralize its surface activity. Thus, NSAIDs topically act on tissue to disrupt the hydrophobic protective lining of the mucus gel layer²⁸. Ethanol produce necrotic lesions in the gastric mucosa by its direct toxic effect reducing the secretion of bicarbonate and production of mucous²⁹. Increase vascular permeability and decreases non-protein sulfhydryl groups (NP-SH) of gastric mucosa. Also, increase xanthine oxidase activity and malonyldialdehyde level³⁰. The ethanol also depresses tissue level of DNA RNA and proteins, leading to flow stasis and injured area³¹. In the present study, AESO has potent ulcer protective activity at a dose 500 mg/kg and 1000 mg/kg, but at the dose 1000 mg/kg was more potent. The ulcer index of *Spinacia Oleracia* treated rat was comparable to those of ulcerated control rats. There was significant decrease in ulcer index in test treated rats and ranitidine treated rats.

Table 1: Effect of AESO on volume and pH of gastric content in pylorus ligated rats

S. No.	Treatment (mg/kg)	Volume of gastric juice (ml)	pH of gastric juice
1	Normal control	1.16±0.05	3.87±0.05

2	Ulcerated control	5.28±0.12	1.97±0.67
3	Ranitidine (30)	3.92±0.71	2.48±0.05
4	AESO(500 mg/kg)	4.04±0.07*	3.94±0.08*
5	AESO(1000 mg/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 2: Effect of AESO on free acidity and total acidity in pylorus ligated rats

S. No.	Treatment (mg/kg)	Free acidity (meq/L/100gm)	Total acidity (meq/L/100gm)
1	Normal control	9.74 ± 0.60	27.41 ± 0.68
2	Ulcerated control	37.33 ± 1.80	74.13 ± 1.70
3	Ranitidine (30 mg/kg)	14.40 ± 0.36	27.46 ± 0.76
4	AESO(500 mg/kg)	24.66 ± 0.66	68.06 ± 0.72
5	AESO(1000 mg/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 3: Effect of AESO on pepsin content in pylorus ligated rats

S. No.	Treatment (mg/kg)	Pepsin content (mcg/ml)
1	Normal control	0.89 ± 0.01
2	Ulcerated control	5.56 ± 0.69
3	Ranitidine (30 mg/kg)	2.39 ± 0.63
4	AESO (500 mg/kg)	4.97. ± 0.09
5	AESO (1000 mg/kg)	3.33 ± 0.24**

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 AESO on ulcer index and % gastro protection in pyrolus ligated rats

S. No.	Treatment (mg/kg)	Ulcer index (Mean± SEM)	% Gastro protection
1	Normal control	-	100
2	Ulcerated control	4.7 ± 0.29	-
3	Ranitidine (30 mg/kg)	1.88 ± 0.11	60
4	AESO (500 mg/kg)	4.21 ± 0.11	29.70
5	AESO (1000 mg/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

Table 5: Effect of AESO on ulcer index and % gastro protection in aspirin induced gastric lesion in rats

S. No.	Treatment (mg/kg)	Ulcer index (Mean± SEM)	% Gastro protection
1	Normal control	-	100
2	Ulcerated control	2.25 ± 0.21	-
3	Ranitidine (30)	0.45 ± 0.11	80
4	AESO (500 mg/kg)	2.25 ± 0.07	38.39
5	AESO (1000 mg/kg)	0.95 ± 0.15**	74.77**

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 6: Effect of AESO on ulcer index and % gastro protection in ethanol induced gastric ulcer in rats

S. No.	Treatment (mg/kg)	Ulcer index (Mean± SEM)	% Gastro protection
1	Normal control	-	100
2	Ulcerated control	4.72 ± 0.07	-
3	Sucralfate (400 mg/kg)	1.2 ± 0.29	74.57
4	AESO (500 mg/kg)	2.99 ± 0.21*	58.33*
5	AESO (1000 mg/kg)	2.16 ± 0.11**	65.27**

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.

CONCLUSION

In conclusion, it appears that *Spinacia oleracea* possess anti-ulcerogenic principles like flavanoids, phenolic compound and caratinoid. These phytoconstituents provides protection against gastric mucosal damage induced by pylorus ligation, aspirin and ethanol, through inhibition of gastric acid, pepsin, histamine and free radical and stimulation of mucus secretion.

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