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GASTROPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF *TRACHYSPERMUM AMMI* FRUIT

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ABSTRACT

Trachyspermum ammi fruit have traditionally been used in India as medicinal plant for the treatment of indigestion and dyspepsia and many other gastric disorders. In the present study ethanolic extract of *Trachyspermum ammi* fruit was used for investigation of antiulcer activity by using pylorus ligation, as anti-secretary model and Indomethacin induced ulcer model, ethanol induced ulceration model, cold restraint stress induced ulcer model as cytoprotective model. Animals pretreated with ethanolic extract of *Trachyspermum ammi* fruit at the dose 100mg/kg and 200mg/kg showed significant decrease in ulcer index and percentage ulcer protection in all models. The results suggests that the extract at 100mg/kg and 200mg/kg showed significant protection ($p<0.001$) by reducing ulcerative lesions when compared with control group of animals. These findings indicate that *Trachyspermum ammi* fruit extract shows significant antiulcer activity.

KEY WORDS

Cytoprotective, Anti-secretory, Anti-ulcer and *Trachyspermum ammi* fruit.

INTRODUCTION

Trachyspermum ammi (Umbelliferae), known in India as *Ajowan*, is widely distributed in northern part of the India. In India, the fruit are used as remedy for indigestion and colic and also used in poultices to relieve asthma and arthritis. It is also having aphrodisiac properties. It is used in a steeped

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liquid form against diarrhea and flatulence. It is mostly used for indigestion and dyspepsia[1].

Peptic ulcer is the term designated to localized destruction of the inner wall or mucosa of the stomach (gastric ulcer) or the upper part of the small intestine (duodenal ulcer). Peptic ulcer generally occur when there is an imbalance between aggressive gastric factors (acid, pepsin, *Helicobacter pylori* and refluxed bile salts) and defensive mucosal factors[2] (gastric mucosal barrier, bicarbonate secretion, rapid cell turnover, high blood flow). The treatment of peptic ulcer is directed against reduction of aggressive factors or enhancement of mucosal defense of stomach and duodenum with cytoprotective agents. Endogenous non-protein sulfhydryl (SH) compounds are presumed to participate in gastric mucosal adaptive cytoprotection[3]. Peptic ulcer as an important chemical entity, various efforts have been made to find suitable remedial measures. For several decades the adage 'NO ACID – NO ULCER' and the drugs used to reduce acid secretion has dominated the pharmacological basis of ulcer therapy. More recently, the role of mucosal factor in peptic ulceration has received much attention and the term "Cytoprotection" was first introduced by Andre Robert in 1979. In general it can be said that there is a plethora of mechanisms of gastric cytoprotection, their relative importance and interdependence being far from clarity. This itself is a point that gastric cytoprotection may be a multifactorial phenomenon.

The term "Ayurveda" means "Science of Life", ayur means life and veda means knowledge. Ayurveda deals with physical body, herbal medicine, diet, surgery, psychology, spirituality and religion. Ayurveda has a well-classified materia medica consisting mainly of drugs of plant origin. Various parts of plants, whole plant, root, rhizome, stem, leaf, flowers, fruit, bark, exudates have been employed for therapeutic purpose. Currently, some 1250 plants find usage in ayurvedic medicines[4].

The aim of the study is to evaluate anti-ulcer activity of the ethanolic extract of *Trachyspermum ammi* Fruit.

MATERIALS AND METHODS

Animals

Healthy adult albino rats of Wistar strain weighing 180-250g were used for this study. The animals were obtained from animal house, IRT Perundurai Medical College, Erode, Tamilnadu, India. On arrival, the animals were placed randomly and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at temperature of 24±2 ^{o}C and relative humidity of 30-70%.A12:12 light: day cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pelleted rat chaw (M/S Hindustan Lever Ltd., Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics committee (688/2/C-CPCSEA) and were in accordance with the guidelines of the CPCSEA.

Preparation of the Extract

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Dried fruit of *Trachyspermum ammi* was purchased from local market. The fruit was grinded in mixer grinder to obtain particle size of coarser size. The grinded product is macerated with absolute ethanol for 7 days following the process called simple maceration. After 7 days of maceration, evaporation of solvent was done to obtain semisolid product which was used for further studies.

Phytochemical Evaluation[5]

Trachyspermum ammi fruit was subjected to qualitative analytical test for the detection of various chemical constituents viz. Alkaloids, steroids, carbohydrates, fixed oils, glycosides, tannins, proteins, saponin and flavonoids.

Pharmacological Evaluation

Antisecretory Evaluation

a) Pyloric ligation -induced ulcers[6]:

Four groups of albino Wister male rats (n=6) were selected. In this model, Group 1 served as normal control (vehicle) received 0.5% Carboxy methyl cellulose (CMC), p. o., and group 2 Omeprazole (10mg/kg, p.o), whereas groups 3 and 4 animals received ethanolic extract of *Trachyspermum ammi* Fruit (100 and 200 mg/kg, p.o. respectively) daily for 3 days. Animals were fasted overnight prior to start of the experiment, and water ad libitum. Pyloric ligation was applied by ligating the pyloric end of the stomach of rats on 3rd day under phenobarbital anesthesia a dose of 35 mg/kg b.w. after 30 min of ethanolic extract of *Trachyspermum ammi* Fruit or Omeprazole treatment. Animals were allowed to recover and stabilize in individual cage and were deprived of water during postoperative method. After 4 h of surgery, rats were sacrificed with over dose of ether, stomach was removed and gastric juice was collected for performing gastric secretion study and ulcer scoring was done in stomach as described by the method of Suzuki et al.,[7] The gastric juice that was collected and centrifuged. The volume and pH was recorded and subjected to bio-chemical estimations like free acidity and total acidity[8], total proteins[9], total hexoses[10], hexosamine[11], fucose activity[12] of the gastric juice were calculated.

Cytoprotective Model

a) Indomethacin induced ulcers [13]

Four groups of albino Wister male rats (n=6) were selected. In this model, Group1 served as normal control (vehicle) received 0.5% Carboxy methyl cellulose (CMC), p. o., and group 2 Omeprazole (10mg/kg, p.o), whereas groups 3 and 4 animals received ethanolic extract of *Trachyspermum ammi Fruit* (100 and 200 mg/kg, p.o. respectively) daily for 3 days. Animals were fasted overnight prior to start of the experiment, and water ad libitum On day 3, Indomethacin (30 mg/kg, i.p.,) was given as a single dose to induce gastric ulcers after 30 min of Omeprazole and ethanolic extract of *Trachyspermum ammi*

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treatment. After 5 h, the animals were killed and ulcer index was calculated as described earlier[7]

b) Ethanol-induced ulcer[14]

After 12 h of fasting, albino Wister male rats were divided into four groups of six animals each. Group 1 served as normal control (vehicle) received 0.5% Carboxy methyl cellulose (CMC), and the group 2 was treated with Omeprazole (10 mg/kg). The groups 3 and 4 received 100 and 200 mg/kg of *Trachyspermum ammi Fruit* of ethanolic extract respectively. All are administered orally. One hour after treatment, all rats received ethanol (1ml/200gm/kg., body weight) to induce gastric ulcer. After 4 h the animals were sacrificed by cervical dislocation, the stomachs were removed and opened along the greater curvature. Stomachs were gently rinsed with water to remove gastric contents and the mean ulcer index was calculated as described earlier[7].

c) Cold restraint induced ulcer

Four groups of albino Wister male rats (n=6) were selected. In this model, Group 1 served as normal control (vehicle) received 0.5% Carboxy methyl cellulose (CMC), p. o., and group 2 Omeprazole (10mg/kg, p.o), whereas groups 3 and 4 animals received ethanolic extract of *Trachyspermum ammi Fruit* (100 and 200 mg/kg, p.o. respectively) daily for 3 days. Animal were fasted overnight prior to start of the experiment, and water ad libitum. On day 3, after 30 min of ethanolic extract of *Trachyspermum ammi Fruit* or Omeprazole treatment, rats were immobilized in a stress cage and were placed at 4–6 ^{O}C in an environmental cage[15]. The animals were sacrificed 2 h later and ulcer index was calculated as described earlier[7].

Statistical Analysis

Results were expressed as mean ± SEM. Statistical significance were determined by one way Analysis of Variance (one way ANOVA) followed by Dunnet's 't' test with level of significance set at $P < 0.05$.

RESULTS

Phytochemical evaluation of *Trachyspermum ammi* shows the presence of Carbohydrate, glycoside, proteins, volatile oils and Tannins.

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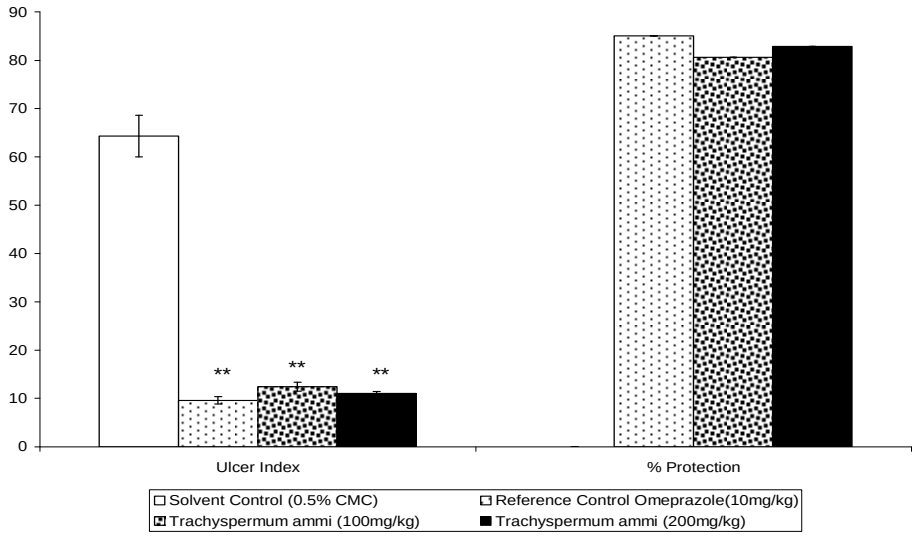
Figure 1

Effect of Trachyspermum ammi extract on Pylorus Ligated (Shay) Rat Model indicating Ulcer index & Percentage ulcer protection.



Values are presented as mean ± SEM (n = 6)
$^{***}P<0.001$, $^{**}P<0.01$ and $^{*}P<0.05$ Vs control

The results of effect of ethanolic extract of *Trachyspermum ammi* fruit in Pylorus ligation model was shown on Figure.1. It indicates that *Trachyspermum ammi* fruit extract at the dose levels of 100 mg/kg and 200 mg/kg produced a significant decrease in the ulcer index, which was also evidenced by significant increase in percentage protection from ulcers at both the dose levels (80.65 & 82.87) respectively. The activity was comparable and equipotent with that of standard drug Omeprazole (85.3%).

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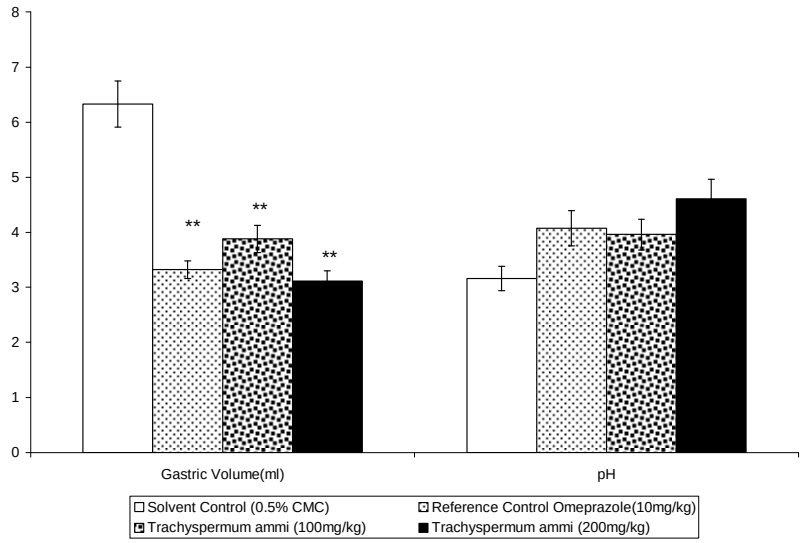
Figure 2

Effect of Trachyspermum ammi on Pylorus Ligated (Shay) Rat Model indicating pH and Gastric volume of gastric juice.



Values are presented as mean ± SEM (n = 6)
$^{***}P<0.001$, $^{**}P<0.01$ and $^{*}P<0.05$ Vs control

Figure. 2, shows the results of gastric volume determination of *Trachyspermum ammi* fruit extract treated groups indicate that there was a significant decrease in the volume of the gastric juice. The activity was comparable and equipotent as that of Omeprazole ($p<0.01$).

The results of gastric pH determination of *Trachyspermum ammi* fruit extract (Figure.2) treated groups indicate that there was a significant increase in the pH of the gastric juice. The activity was comparable and equipotent as that of Omeprazole ($p<0.01$).

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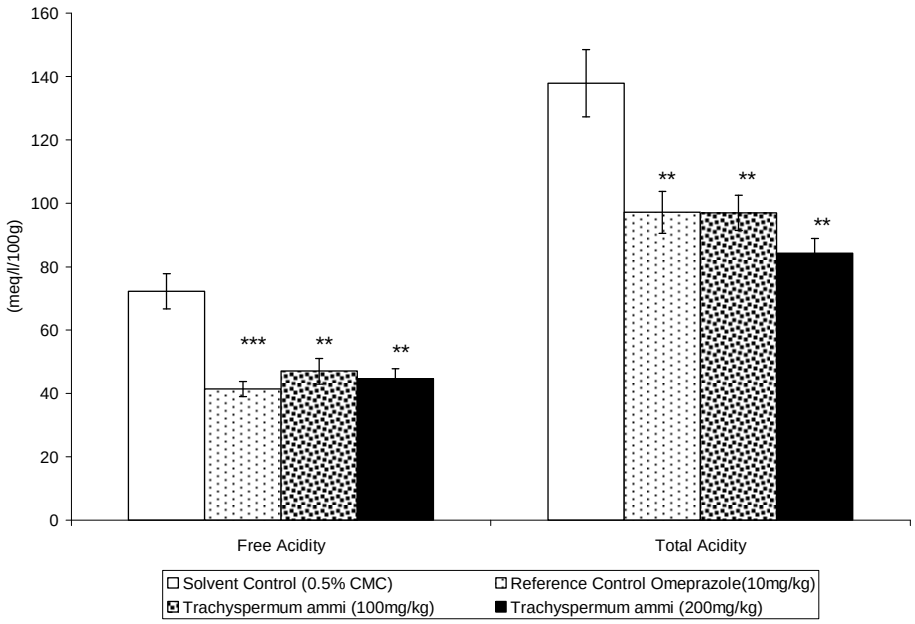
Figure 3

Effect of Trachyspermum ammi on Pylorus Ligated (Shay) Rat Model indicating Free acidity and Total acidity of gastric juice.



Values are presented as mean ± SEM (n = 6)
$^{***}P<0.001$, $^{**}P<0.01$ and $^{*}P<0.05$ Vs control

The results of free acidity and total acidity estimation of gastric juice of *Trachyspermum ammi* (Figure.3) treated groups indicate that there was a significant decrease in the free acidity and total acidity of the gastric juice when compared to control animals.

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Table 1

Effect of Trachyspermum ammi extract on Pylorus Ligated (Shay) Rat Model indicating Total Hexose, Hexosamine, Fucose & Total protein.

Drug Treatment	Total Hexose (µg/ml)	Hexosamine (µg/ml)	Fucose (µg/ml)	Total Protein (µg/ml)
Solvent Control (0.5% CMC)	252.32±18.55	287.17±11.89	74.173.22	894.22±46.44
Omeprazole (10mg/kg)	367.25±15.21**	461.22±21.64**	174.51±11.21**	513.53±31.12**
Trachyspermum ammi (100mg/kg)	341.12±12.26**	426.16±18.88**	117.12±8.42*	552.14±36.33**
Trachyspermum ammi (200mg/kg)	316.64±10.44**	436.52±24.22**	163.80±15.54**	541.16±28.42**

Values are presented as mean ± SEM (n = 6)
***P<0.001, **P<0.01 and *P<0.05 Vs control

The results of total hexoses estimation (Table.1) of gastric juice of *Trachyspermum ammi* treated groups indicate that there is a significant increase in the hexose content of the gastric juice. The activity at both the dose levels was comparable and equipotent as that of Omeprazole treated group (p<0.01). The concentration of Total hexoses of *Trachyspermum ammi* at 100mg/kg and 200mg/kg treated group was found to be 341.12± 12.26µg /ml and 316.64± 10.44µg/ml respectively, and the Omeprazole treated group was 367.25± 15.21µg /ml.

The results of hexosamine estimation (Table.1) of gastric juice of *Trachyspermum ammi* treated groups indicate that there is a significant increase in the hexosamine content of the gastric juice. The activity at both the dose levels was comparable and equipotent as that of Omeprazole treated group (p<0.01). The concentration of hexosamine of *Trachyspermum ammi* at 100mg/kg and 200mg/kg treated group was found to be 426.16± 18.88µg /ml and 436.52 ± 24.22 µg/ml respectively, and the Omeprazole treated group was 461.22± 21.64µg /ml.

The results of fucose estimation (Table.1) of gastric juice of *Trachyspermum ammi* treated groups indicate that there is a significant increase in the fucose content of the gastric juice But 200mg/kg showed more significant increase of fucose content (p<0.01) than 100mg/kg (p<0.05)

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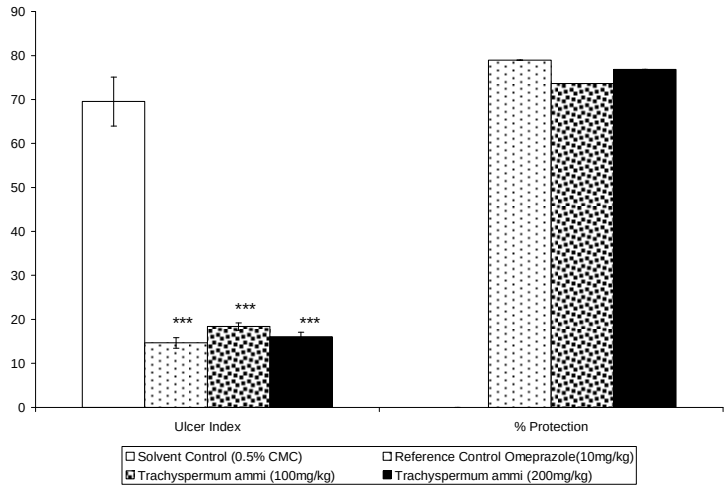
and 200mg/kg was of same potency as that of Omeprazole treated group ($p<0.01$). The concentration of Fucose of *Trachyspermum ammi* at 100mg/kg and 200mg/kg treated group was found to be 117.12± 8.42µg /ml and 163.80± 15.54 µg /ml and the Omeprazole treated group was 174.51± 11.21µg /ml.

The results of total protein (Table.1) estimation of gastric juice of *Trachyspermum ammi* treated groups indicate that there is a significant decrease in the total protein content of the gastric juice. The activity at both the dose levels was comparable and equipotent as that of Omeprazole treated group. The concentration of Total protein of *Trachyspermum ammi* at 100mg/kg and 200mg/kg treated group was found to be 552.14± 36.33µg/ml and 541.16± 28.42µg/ml respectively, and the Omeprazole treated group was 513.53± 31.12µg/ml.

Figure 4

Effect of Trachyspermum ammi extract on Indomethacin –Induced Ulcer Model indicating Ulcer index and Percentage ulcer protection.



Values are presented as mean ± SEM (n = 6)
$^{***}P<0.001$, $^{**}P<0.01$ and $^{*}P<0.05$ Vs control

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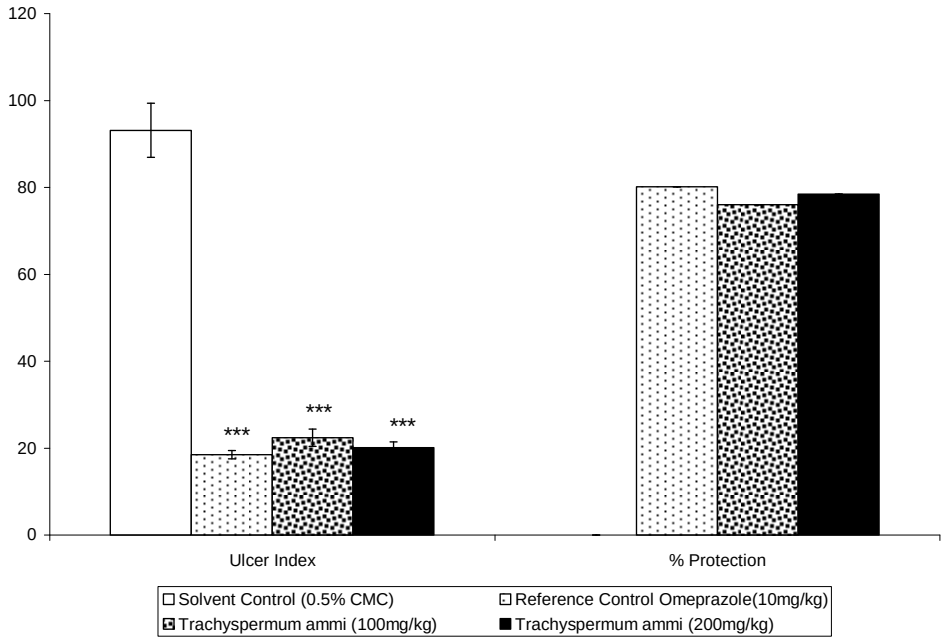
The results indicating the effect of ethanolic extract of *Trachyspermum ammi* fruit (Figure.4) on indomethacin induced ulcer in rats suggests that ethanolic extract at the dose levels of 100 mg/kg and 200 mg/kg produced a significant decrease in the ulcer index ($p<0.01$), which is also evidenced by significant increase in percentage protection from ulcers at the dose of 100mg/kg and 200 mg/kg (73.59 & 76.82) respectively. The activity at both the dose levels was comparable and equipotent as that of Omeprazole treated group ($p<0.01$).

Figure 5

Effect of Trachyspermum ammi extract on Ethanol – Induced Ulcer Model Indicating Ulcer index & Percentage ulcer protection.



Values are presented as mean ± SEM (n = 6)
$^{***}P<0.001$, $^{**}P<0.01$ and $^{*}P<0.05$ Vs control

The effect of *Trachyspermum ammi* fruit extract on ethanol induced ulceration model was shown on Figure 5. The results show that the tested extracts have an important protective activity for

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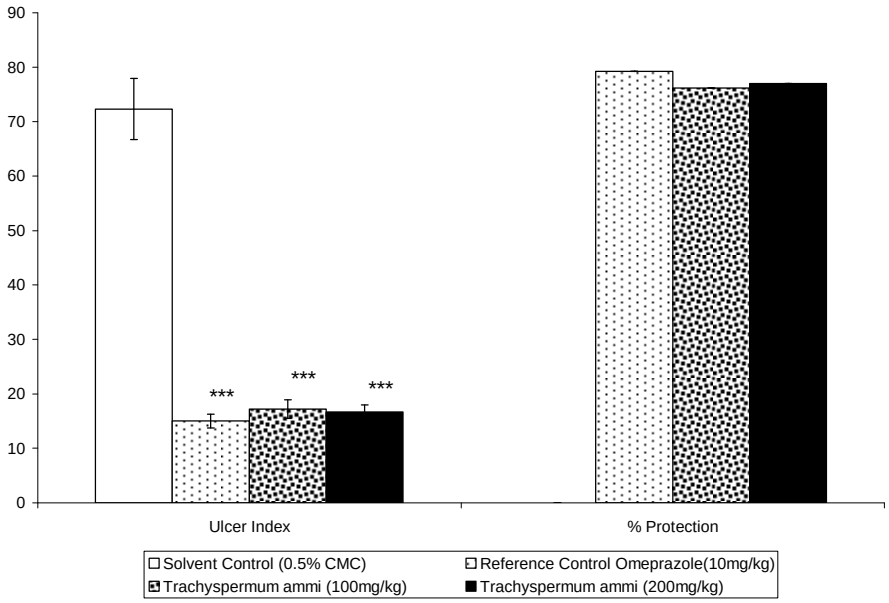
gastric mucosa, since at doses, 100 and 200 mg/kg of fruit extracts, they were effective in reducing ulcer lesion in the ethanol respectively.The results of ethanol induced ulceration model suggests that *Trachyspermum ammi* fruit extract at the dose levels of 100 mg/kg and 200 mg/kg produced a significant decrease in the ulcer index ($p<0.01$), which is also evidenced by significant increase in percentage protection from ulcers at the dose of 100mg/kg and 200 mg/kg (75.98 &78.39) respectively. The activity at both the dose levels was comparable and equipotent as that of Omeprazole treated group ($p<0.01$).

Figure 6

Effect of Trachyspermum ammi extract on cold restraint – Induced Ulcer Model indicating Ulcer index & percentage ulcer protection.



Values are presented as mean ± SEM (n = 6)
$^{***}P<0.001$, $^{**}P<0.01$ and $^{*}P<0.05$ Vs control

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The results of effect of Treatment of *Trachyspermum ammi* fruit ethanolic extract (Figure.6) indicates that ethanolic extract at the dose levels of 100 mg/kg and 200 mg/kg produced a significant decrease in the ulcer index ($p<0.01$), which is also evidenced by significant increase in percentage protection from ulcers at the dose of 100mg/kg (76.17) and 200 mg/kg (76.96) respectively. The activity at both the dose levels was comparable and equipotent as that of Omeprazole treated group ($p<0.01$).

DISCUSSION

The present study demonstrates that ethanolic extract of *Trachyspermum ammi* fruit exhibits anti ulcer activity, probably as a result of anti-secretary and cytoprotective action.

Gastric ulcers have multiple etiopathogenesis. Ulcers caused by pyloric ligation are due to increased presence of acid and pepsin in the stomach and damage by indomethacin are due to decrease in PG syntheses which are essential for the integrity of mucosa[16]. EtOH-induced gastric lesions are thought to arise as a result of direct damage of gastric mucosal cells[17].

Stress ulcers are due to both physiological and psychological factors, which is crucial for gastrointestinal defense and increased accumulation of acid and pepsin leading to autodigestion of the gastric mucosa[18].

Gastric acid is an important factor for the genesis of ulceration in pylorus-ligated rats[6]. The activation of the vagus-vagal reflux by stimulation of pressure receptors in the antral gastric mucosa in the hypersecretion model of pylorus ligature is believed to increase gastric acid secretion[19]. The current data clearly demonstrated that, *Trachyspermum ammi* dose-dependently decreased the gastric acid, which clearly exhibit the anti secretary activity of *Trachyspermum ammi* fruit.

Further investigations on offensive and defensive factors were carried out in the gastric juice of pyloric ligated rats. Mucus serves as first line of defense against ulcerogens. Mucus is secreted by the mucus neck cells and covers the gastric mucosa thereby preventing physical damage and back diffusion of hydrogen ions[20]. *Trachyspermum ammi* significantly increased mucus secretion as observed from the increase in mucopolysaccharides like hexose, hexosamine and fucose. Further, strengthening of the gastric mucosa is evident from the decrease in the leakage of protein into the gastric juice[21]. This increase was due to increase in mucopolysaccharides, the major constituent of mucus and also which are responsible for viscous nature and gel-forming properties of the mucus. The gel is reported to be resistant to a number of ulcerogens including acid, ethanol and NSAIDs, i.e. indomethacin[22]. Hence increase in synthesis of mucus may be one of the important contributing factors for ulcer protective role of *Trachyspermum ammi* fruit.

Trachyspermum ammi fruit extract is also highly effective in blocking gastric lesions in the Indomethacin induced ulcer model. Indomethacin is known to inhibit cyclooxygenase activity of prostaglandin synthetase and causes gastric damage

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by decreasing the level of prostaglandin, the master molecule for gastroprotection[23].

The anti-ulcer activity of ethanolic extract of *Trachyspermum ammi fruit* was detected in absolute ethanol- lesions in rats. These models evaluate the drug's capacity to protect the gastric mucosa, differentiating only the severity of gastric lesions. Ethanol-induced gastric damage may be due to stasis in gastric blood flow, which contributes to the development of the hemorrhagic and necrotic aspects of tissue injury. In addition, ethanol also induces solubilization of the mucus constituents, decreases the difference of potential in mucosa thus increasing the flow of Na+ and K+ to the lumen and pepsin secretion, and also increases H+ ions and histamine[24]. The results show that the tested extracts have an important protective activity for gastric mucosa, since at doses, 100 and 200 mg/kg of fruit extracts, they were effective in reducing ulcer lesion in the ethanol respectively.

The preliminary phytochemical screening of Trachyspermum ammi fruit revealed the presence of carbohydrate, glycoside, proteins, volatile oils and Tannins. Previous studies proved that tannins and volatile oils possess significant anti-ulcer activity in experimental animal models[25]. The presence of tannin in *Trachyspermum ammi* may also be responsible for its anti ulcer activity.

CONCLUSION

In conclusion, the oral administration of the ethanolic extract of *Trachyspermum ammi* fruit exhibits anti ulcer activity in experimental ulcer models. The probable mechanism for its activity may be due to anti-secretary and cytoprotective property.

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