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Cytoprotection mediated antiulcer effect of aqueous fruit pulp extract of *Cucumis sativus*

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ABSTRACT

Objective: The study was aimed to evaluate the gastroprotective potential of *Cucumis Sativus* fruit pulp aqueous extract (CSE) in gastric ulcerated rats. **Methods:** Cytoprotective potential was evaluated via oral administration of CSE at the doses of 250, 500 &1000 mg/kg three times in a day, for 5 days before the induction of ulcers in indomethacin and pyloric ligation induced ulcer model. Further, its effects were studied on various parameters volume of gastric juice, pH, free and total acidity, protein concentration, acid output in gastric juice, lipid peroxide (LPO), and activities of enzymic antioxidants−super oxide dismutase (SOD) and catalase (CAT) in gastric mucosa. The levels of hexose, hexosamine, sialic acid, fucose in gastric mucosa and gastric juice were also examined. The extent of healing was also determined with post administration of CSE at the same doses & dosage schedule in acetic acid induced model. **Results:** In indomethacin and pyloric ligation model, the pretreatment with CSE and ranitidine significantly reduced the lesion index, in comparison with control treated group ($P< 0.05$). The percentages of protection of ulcers were 25.8, 65.7, 80.6 & 93.8 for the treated groups of CSE and ranitidine whereas in pyloric ligation it was 31.26, 55.18, 93.26 & 95.51 respectively. In pyloric ligation model, CSE resulted in significant increase in pH, enzymic antioxidants i.e. SOD & CAT, with a significant decrease in volume of gastric juice, free and total acidity, protein & carbohydrate concentration and LPO levels. In acetic acid inducer model, treatment with *Cucumis sativus* (CSE) caused significant reduction in lesion index in when compared to control treated group, providing evidence for ulcer healing capacity of it. The presence of the flavonoids and polyphenols may be responsible for the gastroprotective effect of CSE. **Conclusions:** The aqueous fruit pulp extract of *Cucumis sativus* (CSE) has a gastroprotective property.

1. Introduction

Cumunis Sativus

  Cucumis sativus (Cucumber, family Cucurbitaceae) is native to India. It is found in forest lands, riversides, and wasteland. Several studies have shown multiple biological activities of sea cucumber species. These include cytotoxicity and exhibiting antibacterial, analgesic and antioxidant properties [1–3]. They are also rich in flavanoids and poyphenols that exhibit antidiabetic activity [4]. Research is currently ongoing in regard to cucumbers possibly lowering cholesterol. The fruit juice has been used as lotion for skin whitening and to treat skin irritations, skin inflammations, bed sores, burns, sunburn, scalds [5]. Documented scientific studies have demonstrated that many of the medicinal plants and their constituent phytoceuticals not only provide gastroprotection against various ulcerogens, but also accelerate ulcer healing[6].

  Exhaustive literature survey revealed that the potential of Cumunis Sativus fruit in gastrointestinal ailment has not been exploited, so an effort is made to evaluate scientifically the gastroprotective potential of Cumunis Sativus in gastric ulcerated rats.

2. Materials & methods

2.1 Experimental

  Fresh *C.sativus* (Cucumber) was purchased from the market of Jaipur (Rajasthan, India) and botanical authentification

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was carried out at the Department of Botany, University of Rajasthan (voucher specimen no. RUBL20685). Fresh fruit pulp was homogenized and dried under shade and thus dark brownish red powder mass obtained was powdered & weighed. The yield was found to be 38%. The dried *C. sativus* fruit pulp was reconstituted in water (CSE) extract so obtained, was subjected to qualitative phytochemical analysis.

Phytochemical analysis

Phytochemical analysis of the CSE was done following the standard methods of Trease and Evans and Kokate, C.K., Purohit A. P., Gokhale S.B [7−8].

2.2 Animals

Healthy Wistar rats of either sex weighing between 180−250 gm were used in this study. Animals were maintained at 25±2℃ and kept in well ventilated animal house under natural photoperiodic condition in polypropylene cages with paddy husk as bedding with free access to food and water ad libitum. The experimental protocol described in present study was approved by Institutional animal ethical committee (IAEC) of Pinnacle Biomedical Research Institute, Bhopal India (Reg No. 1283/C/09/CPCSEA).

2.3 Acute toxicity studies

Different doses of the CSE were administered orally to seven groups of rats consisting of 6 rats in a group. The animals in group 1 served as control and received 1.0 ml of physiological saline. The animals in groups 2, 3, 4, 5, 6 and 7 received 250, 500, 1000, 1500 and 3,000, 6000 mg/kg body weight respectively through oral administration with a canula attached to a graduated syringe. They were all placed under observation for 24 h after which the number of dead rats was recorded[9].

2.4 Treatment protocol

CSE was administered in the doses of 250 mg/kg, 500 mg/kg & 1000 mg/kg body weight orally for five days for three times in a day before to ulcer induction. Ranitidtne, was taken as the standard drug, in both the ulcer models. The doses of 250 mg/kg, 500 mg/kg, and 1000 mg/kg were administered orally, to different experimental groups thrice daily at 8:00 and 14:00 h, 22:00 h respectively, for 5 days for cytoprotective studies in gastric ulcer index. Normal group of animals received suspension of 1% CMC in distilled water.

1.0 Experimental setup

Indomethacin induded ulcer model− cytoprotective model
The albino rats of either sex weighing between 180 − 200 gm were divided into 6 groups of 6 animals each and fasted for 24 hrs with water ad libitum prior to experiment. The animals of group I were treated with vehicle (1% CMC in distill water) thrice a day, for five days and animals of group III treated with standard i.e. Ranitidine 50mg/kg and the animals of group IV, V and VI were pretreated with CSE at the doses of 250 mg/kg, 500mg/kg & 1000 mg/kg tds for five days respectively before the induction of ulcer. The animals of group II, III, IV, V & VI were treated with indomethacin sodium (20 mg/kg p. o.) once, on day sixth. On sixth day, the animals were sacrificed by cervical dislocation after 12 hrs of last dose. The stomach was taken out and cut open along the greater curvature of stomach. The numbers of ulcers per stomach were noted and severity of the ulcers was observed microscopically and scoring was done [10].

2.0 Pylorus ligation (PL)−induced ulcer model

This study involves the effect of prior administration of CSE on pyloric ligation induced model. Drugs were administered for a period of 5 days as described above. On day 6 after the last dose, the rats were kept for 12 h fasting and care was taken to avoid caprophagy. Pylorus ligation was done according the method as S. Bharti et.al [11]. The animals were deprived of water during the post−operative period. After 4 h, stomachs were dissected out and contents were collected in tubes for estimation of physical & biochemical parameters.

2.1 Physical parameters

Ulcer Index:

The ulcer index was scored for following the method of V. Lakshmi *et al*[12].

2.2 Collection of gastric juice

After post operative period, animals were sacrificed by cervical dislocation and the stomach was dissected out as a whole by passing a ligature at the esophageal end. Gastric content was evacuated into graduated tube by cutting along the greater curvature of the stomach, and was centrifuged at 3000 rpm for 10min.

2.3 Volume of gastric juice

The volume of the centrifuged sample was expressed as ml/100 g body weight.

2.4 pH of gastric juice

pH of gastric juice was measured with the help of pH meter.

2.5 Determination of Total Acidity

Gastric juice (1ml) was pipette into a 100ml conical flask and diluted with 9ml distilled water. Two or three drops of Toepfer's reagent was then added and titrated with 0.01 N sodium hydroxide until all traces of red colour disappeared and the colour of the solution was yellowish−orange. The volume of alkali added was noted. This volume corresponds to free acidity. Two or three drops of phenolphthalein were then added and the titration was continued until a definite red ting appeared; the volume of alkali added was noted. The volume corresponds to total acidity. Acidity was expressed in terms of mEq/L [13].

2.6 Estimation of Total Proteins

The dissolved protein in gastric juice was estimated in the alcoholic precipitate obtained by adding 90% alcohol with gastric juice 9:1 ratio respectively. Then 0.1 ml of alcoholic precipitate of gastric juice was dissolved in 1ml of 0.1N NaOH and from this 0.05 ml was taken in another test tube. To this 4 ml of alkaline mixture was added and kept for 10 minutes. Then 0.4 ml of phenol reagent was added and again 10 minutes was allowed for colour development. Absorbance was measured against blank prepared with distilled water at 610 nm using spectrophotometer. The protein content was calculated in terms of μg/ ml of gastric juice [14].

2.7 Estimation of Total Carbohydrates

The dissolved mucosubstances in gastric juice were estimated in the alcoholic precipitate obtained by adding 1 ml of gastric juice to 9 ml of 90% alcohol, the mixture was kept for 10 minutes, and the supernatant was discarded. The precipitate separated was dissolved in 0.5 ml of 0.1N sodium hydroxide. To this 1.8ml of 6N, HCl was added. This mixture was hydrolyzed in the boiling water bath for 2 hrs. The hydrolysate was neutralized by 5N sodium hydroxide using phenolphthalin as indicator and the volume was made up to 4.5 ml with distilled water and used for the estimation of total hexoses, hexosamine and fucose as described below[15].

2.8 Estimation of Total Hexoses

To 0.4 ml of hydrolysate, 3.4 ml of orcinol reagent was added. The mixture was then heated in the boiling water bath for 15 min. this was then cooled under running tap water and intensity of the colour was read in spectrophotometer at 540 nm against the blank by using distilled water instead of hydrolysate. Total hexoses content was determined from the standard curve of D (+)−galactose−mannose and has been expressed in μg/ml of gastric juice[16].

2.9 Estimation of Hexosamine

0.5 ml of the hydrolysate fraction was taken. To this 0.5 ml of acetylacetone reagent was added. The mixture was heated in boiling water bath for 20 minutes and then cooled under running tap water. 1.5 ml of 90% alcohol was then added followed by an addition of 0.5 ml of Ehrlich's reagent. The reaction was allowed for 30 minutes. The colour intensity was measured in spectrophotometers at 530 nm against the blank prepared by using distilled water instead of hydrolysate. Hexosamine content of the sample was determined from the standard curve of D (+)−galactose−mannose and concentration has been expressed in μg/ml of gastric juice [17].

2.10 Estimation of Fucose

In this method, three test tubes were taken. In one tube 0.4ml of distilled water was taken to serve as control and in each of the other two tubes 0.4 ml of hydrolysate were taken. To all three tubes 1.8 ml of sulphuric acid and water in the ratio of 6:1 was added by keeping the tubes in icecold water bath to prevent breakage due to strong exothermic reaction. This mixture was then heated in boiling water bath for exactly 30minutes. The tubes were then taken out and cooled. In the blank and one of the hydrolysate containing tubes (unknown) 0.1ml of cysteine reagent was added, while cysteine reagent was added to the last test tube containing the hydrolysate (unknown), it was then allowed for 90mm to complete the reaction. The reading was taken in Hitachi 15−20 spectrophotometer at 396 and 430 nm setting the zero with the distilled water. The optical density for the fucose in the hydrolysate was calculated from the differences in the reading obtained at 396 and 430 nm and subtracting the values without cysteine. This was read against standard curve prepared with D (+)−fucose. The fucose content was expressed in terms of μg/ml of gastric juice [18].

2.11 Estimation of Sialic acid

To 0.5 ml of the hydrolysate in 0.1N sulphuric acid, 0.2 ml of sodium periodate was added and mixed thoroughly by shaking. A time of 20minutes was allowed to elapse before addition of 1 ml of sodium arsinate solution mixture. The developed brown colour was disappeared by shaking. Then 3 ml of thiobarbituric acid was added and while shaking 4.5ml of cyclohexanone was added, till the entire colour was taken up by the cyclohexanone supernatant. The resulting mixture was centrifuged to get a clear pink layer of cyclohexanone. The supernatant was pipette out and intensity of colour was measured using spectrophotometer at 550 nm. The sialic content of the sample was determined from the standard curve of sialic acid and has been expressed in μg/ml of gastric juice [18].

2.12 Estimation of free radical generation

The fundic part of the stomach was homogenized (5%) in ice cold 0.9% saline with a glass homogeniser for 30 sec. The homogenate was then centrifuged at 800 g for 10 min followed by centrifugation of the supernatant at 12,000 g for 15 min and the obtained fraction was used for the following estimations .

2.13 Lipid peroxidase (LPO)

LPO product malondialdehyde (MDA) was estimated using 1,1,3,3−tetraethoxypropane as the standard and is expressed as nmol/mg protein. The glandular portion of the gastric mucosa was homogenized with cold 0.15 M Tris−HCl (pH 7.4) to give a 10% (w/v) homogenate. After 10min, 0.2 ml of tissue homogenate, 0.2 ml of 8.1% sodium dodecyl sulphate (SDS), 1.5 ml of 20% acetic acid and 1.5 ml of 8% TBA were added. The volume of the mixture was made up to 4 ml with distilled water and then heated at 95℃ on a water bath for 60 min using glass balls as condenser. After incubation the tubes were cooled to room temperature and final volume was made to 5 ml in each tube. 5.0 ml of butanol: pyridine (15:1) mixture was added and the contents were vortexed thoroughly for 2 min. After centrifugation at 3000 rpm for 10 min, the upper organic layer was taken and its OD read at 532 nm against an appropriate blank without the sample [19].

2.14 Superoxide dismutase (SOD) activity

SOD estimation involve the inhibition of reduction of nitro blue tetrazolium (NBT) to blue coloured Formosan in presence of phenazine metha sulphate (PMS) and NADH was measured at 560 nm using n−butanol as blank. One unit of enzyme activity was defined as the amount of enzyme that inhibits rate of reaction by 50% in one min under the defined assay conditions and the results have been expressed as units (U) of SOD activity/mg protein [19].

2.15 Catalase (CAT) activity

100μ l of supernatant was added to cuvette containing 1.9 ml of 50 mM phosphate buffer (pH 7.0). Reaction was started by the addition of 1.0 ml of freshly prepared 30 mM H2O2. Decrease in absorbance was read at 240 nm for 3min at interval of 30 sec. The activity was calculated using extinction coefficient of H_2O_2 0.041micromole/cm2. Results were expressed as micromole of H_2O_2 utilized/min/ gm tissue. The rate of Decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm [19].

3. Acetic acid−induced ulcer model

The rats were anaesthetized with pentobarbitone (35 mg/ kg, i.p.). The abdomen was opened and the stomach was visualized. A cylindrical glass tube of 6 mm in diameter was tightly placed upon the anterior serosal surface of the glandular portion of stomach 1 cm away from the pyloric end. A total of 50% acetic acid (0.06 ml/ animal) was instilled into the tube and allowed to remain 60 s on the gastric wall. After removal of the acid solution, the abdomen was closed in two layers and animals were caged and fed normally. CSE was given in the doses of 250,500 & 1000 mg/kg orally, thrice daily at 8:00 and 14:00 h, 22:00 h for five days, after the application of acetic acid and continued up to 5 days after induction of the ulcer. The animals were then sacrificed after 18 h of the last dose of drug either on day 6 of experiment to assess the ulcer size and healing. Ulcer index was calculated based upon the product of length and width (mm2/rat) of ulcers. Carbenoloxone sodium (100 mg/kg) was used as the standard drug [20].

4. Statistical Analysis

Results were expressed as the mean ±S .E.M. Statistical significance was determined by one−way analysis of variance followed by Dunnett′s test, with the level of significance set at $P < 0.05$.

Results

The CSE revealed the presence of flavonoids, polyphenols, alkaloids, glycosides, steroids and tannins.

The acute toxicity study in mice after oral administration of various doses of CSE (100, 200, 500, 1000 and 2000 mg/kg) indicated no behavioral changes or mortality up to 72 h after treatment.

C. sativus 250−1000 mg/kg, given orally, thrice daily for 5 days showed dose−dependent cytoprotective effect against gastric ulcers induced by indomethacin sodium & pyloric ligation (Table 1 & 2). In indomethacin induced ulcer model it was observed that the treatment with C. sativus extract (250, 500 and 1000 mg/kg) and ranitidine (30 mg/kg) significantly reduced the lesion index, and provide the cytoprotection in comparison with indomethacin treated group ($P< 0.05$). The percentages of protection of ulcers were 25.8, 65.7, 80.6, and 93.8 (Table 1) for the treated groups with 250, 500 and 1000 mg/kg of C. sativus extract and positive control (ranitidine) whereas in pyloric ligation induced ulcer model it was found to be 31.26, 55.18, 93.02 & 95.8 respectively. C. sativus 250 mg/kg, tds, administered orally showed a weak protection against ulcer in both models (Table 2).

In pyloric ligated rats, the extract showed a reduction in the gastric volume at 250, 500 and 1000 mg/kg dosage levels. Other aggressive factors like free acidity, total acidity levels and protein content in comparison to positive control group decreased in all the three dose levels. Ulcer score and carbohydrate/protein ratio also supported the result. But only the highest dose 1000 mg/kg caused significant reduction in

above parameter which was comparable to standard drug ranitidine (Table 3).

Table 1

Effect of dried fruit pulp of *Cucumis sativus* (CSE) on indomethacin–induced gastric ulcers in rats

Group	Treatment (mg/kg,tds for 5 days)	Ulcer indexa	Ulcer protection (%)
I	Vehicle	00.00	00.00
II	Indomethacin 30	3.56 ± 0.10	00.0
III	Ranitidine 50	0.22 ± 0.19*	93.8
IV	CSE 250	2.64 ± 0.08*	25.8
V	CSE 500	1.22 ± 0.04*	65.7
VI	CSE 1000	0.49 ± 0.05*	80.6

a(mean ±S.E.M., n = 6 in each group)* Significant variation as compared to Indomethacin treatment (P<0.05)

Table 2

Effect of dried fruit pulp *Cucumis sativus* (CSE) on pyloric ligation–induced gastric ulcers in rats

Group	Treatment (mg/kg, tds for 5 days)	Ulcer index	Ulcer protection (%)
I	Vehicle	6.02± 0.48	00.00
II	Ranitidine (50)	0.97 ± 0.38*	95.8
III	CSE (250)	4.14 ± 0.23	31.26
IV	CSE (500)	2.67 ± 0.12*	55.18
V	CSE (1000)	0.42 ± 0.15*	93.02

Data are mean ± S.E.M. n = 6 in each group * Significant variation as compared to pyloric ligated group (P<0.05)

Pyloric ligation results in increased lipid peroxidation in the gastric mucosa with decrease in LPO & SOD level with simultaneous increase in CAT level. The CSE in both doses of 500 and 1000 mg/kg significantly protected the animals against pyloric ligation free radical damage as seen from the decrease in LPO and reversal of changes induced by stress on SOD and CAT (Table 4).

Further, CSE in both doses of 500 and 1000 mg/kg significant increase in individual carbohydrates like total hexoses, fucose and sialic acid leading to increase in total carbohydrates in the gastric juice as well (Table 5).

Aqueous extract of C. Sativus 250–1000 mg/kg, given orally, thrice daily for 5 days showed dose–dependent protective effect via healing of acetic acid–induced gastric ulcers. A total of 50% acetic acid when applied to the serosal surface of rat gastric mucosa in the fundal region near to the pyloric end produced chronic gastric ulcers. CSE 1000 mg/kg significantly healed ulcers induced by 50% acetic acid after 5 days of treatment. Although the extract has showed significant activity in all the three doses used but maximum healing activity has been shown by the aqueous extract at a dose of 1000mg/kg (70.4% ulcer protection after 5 days) as compared to standard carbenoloxone sodium (98.42 % ulcer protection it 5 days). The results are shown in Table 6.

Table 3

Effect of pretreatment of dried fruit pulp of *Cucumis sativus* (CSE) on pH, gastric volume, free acidity and total acidity in pyloric ligated rat

Treatment (mg/kg, tds for 5 days)	Gastric volume	pH	Free acidity Eq/L/100g	Total acidity (m q/L/100g)
Vehicle	5.80± 0.33	1.61 ± 0.13	96.79 ± 1.11	119.78 ± 2.25
Ranitidine (50)	1.61 ± 0.43*	4.38 ± 0.20*	30.95 ± 1.54*	58.03 ± 1.07*
CSE (250)	3.86 ± 0.13*	2.25 ± 0.21*	51.44 ± 0.66*	73.25 ± 1.68*
CSE (500)	3.41 ± 0.32*	2.80 ± 0.20*	47.74 ± 0.73*	67.53 ± 0.70*
CSE (1000)	2.62 ± 0.36*	3.33 ± 0.18*	39.99 ± 1.11*	61.10 ± 0.98*

Data are mean ± S.E.M, n = 6 in each group * Significant variation as compared to pyloric ligated group (P<0.05)

Table 4

Effect of pretreatment of dried fruit pulp of *Cucumis sativus* (CSE) on LPO, SOD and CAT level in pyloric ligated rat

Treatment	LPO(MDA, nmol/g wet tissue)	SOD (units/g wet tissue)	CAT (units/g wet tissue)
Vehicle	44.0 ± 0.79	9.63 ± 0.02	6.38 ± 0.24
Ranitidine (50)	113.92 ± 0.87*	1.37 ± 0.01*	42.47 ± 0.79*
CSE (250)	72.12 ± 0.66*	7.56 ± 0.01*	8.82 ± 0.92*
CSE (500)	64.05 ± 0.66*	5.49 ± 0.01*	22.26 ± 0.69*
CSE (1000)	52.56 ± 0.94*	2.39 ± 0.01*	34.04 ± 0.63*

Data are mean ± S.E.M., n = 6 in each group * Significant variation as compared to pyloric ligation induced ulcer group (P<0.05)

Table 5

Effect of CSE (mg/kg, tds for 5 days) on gastric juice mucoprotein and mucosal glycoprotein in 4 h pyloric ligated rat

Group	Proteins (μg/ml)	Hexosamine (μg/ml)	Hexoses (μg/ml)	Fucose (μg/ml)	Sialic acid (μg/ml)
CONTROL	337.5 ± 12.13	117.5 ± 1.26	87.67 ± 5.87	37.98 ± 2.34	11.4 ± 2.86
STD	165.14 ± 6.24*	197.19 ± 1.27*	362.12 ± 6.34*	174.51 ± 3.56*	54.6 ± 2.23*
CSE 250	321.46 ± 4.13*	137.46 ± 1.28*	98.76 ± 4.32*	46.67 ± 3.12*	14.2 ± 2.78
CSE 500	308.23 ± 3.63*	147.23 ± 1.33*	112.67 ± 4.98*	57.89 ± 3.89*	18.7 ± 2.54*
CSE 1000	246.65 ± 5.42*	166.65 ± 1.21*	131.56 ± 5.12*	65.32 ± 2.97*	26.7 ± 2.89*

Data are mean ± S.E.M., n = 6 in each group * Significant variation as compared to pyloric ligation induced ulcer group (P<0.05)

Table 6

Effect of dried fruit pulp of *Cucumis sativus* (CSE) on acetic acid−induced gastric ulcers in rats.

Group	Treatment	Ulcer Index	% Protect
I	Control	5.00 ± 0.44	00
II	Carbenoloxone sodium (100 mg/kg)	0.10 ±0.09*	98.42
III	CSE 250	4.13 ± 0.07*	17.40
IV	CSE 500	3.14 ± 0.43*	37.2
V	CSE 1000	1.48 ± 0.05*	70.40

Data are mean ± S.E.M., n = 6 in each group * Significant variation as compared to control ulcer group (P<0.05)

Discussion

The present study investigated antiulcer activity of aqueous fruit pulp extract of *Cucumis Sativus* in different experimental gastric ulcer models.

Cucumis Sativus is a safe drug as observed from the results of acute toxicity test. Aqueous fruit pulp extract of *Cucumis Sativus* showed significant antiulcer effect against indomethacin sodium induced and pyloric ligation induced gastric ulcers in rats.

Although in most cases the etiology of ulcer is unknown, it is generally accepted that it is the result of an imbalance between aggressive factors and maintenance of the mucosal integrity through the endogenous defense mechanism [21]. The candidate for an effective drug against gastric ulcer should basically act either by reducing the aggressive factors on gastro duodenal mucosa or by increasing mucosal resistance against them. Keeping this view, we have attempted to study the CSE for its antiulcer activity by using different experimental models of gastric ulcer.

Oral administration of the indomethacin sodium clearly produced a mucosal damage characterized by multiple hemorrhage red bands of different sizes along the long axis of the glandular stomach as described in other studies [22]. Treatment with CSE at three times in a day for five days (250, 500 & 1000 mg/kg) to different groups showed significant decrease in the intensity of gastric mucosal damages induced by the necrotizing agent indomethacin sodium compared with vehicle treated group. Cytoprotective action by drugs has been considered to be due to the generation of prostaglandins or blockade of back diffusion of H+ ions [23] may be the major mechanism which is responsible for anti−ulcer activity.

CSE in PL−induced ulcers have showed marked protective effect via reduction in acid secretion, free & total acidity with simultaneous increase in acidic pH. CSE also significantly increased the total carbohydrate content such as hexosamine, sialic acid etc. of the gastric juice, found to be defensive factor in ulcers. Ulcers are caused due to imbalances between offensive and defensive mucosal factors [21] and hence the effects of CSE can be explained based on these factors.

In pyloric ligation model, the role of free radicals in gastric ulcerations is well documented[17]. CSE remarkably reduced lipid peroxidation in rat gastric mucosa. CSE has been reported to possess significant anti−oxidant in vitro [3]. SOD scavenges the super oxide radical one of the reactive oxygen species (ROS) responsible for lipid peroxidation. This reaction leads to increase in generation of peroxyl radical $H_2O_2^-$, which is also capable of producing more oxidative damage [24]. The anti−oxidant activity in gastric mucosal was observed from decrease in LPO may be due to increase in SOD and CAT levels. Pyloric ligation−induced ulceration involves damage by ROS apart from acid and pepsin related factors [25]. CSE were significantly increased SOD & CAT level in all the three groups with simultaneous decrease in LPO level, suggesting decrease in oxidative damage. This may be due to restoration of balance between free radical scavenging enzymes SOD and CAT in the gastric mucosa, effectively counteracting the free radicals generated by cascade of reactions. Thus, the anti−ulcerogenic activity of CSE may also be due its anti−oxidant effects. Acetic acid−induced ulcers better resemble clinical ulcers in location, chronicity and severity, and serve as the most reliable model to study healing process [26]. C.Sativus significantly healed the penetrating ulcers induced by acetic acid after 5 days of treatment. Hence, the cytoprotective and ulcer healing effect of CSE may be associated with its phytoconstituents that has predominant effects on both mucosal offensive and defensive factors.

In conclusion, the aqueous fruit pulp extract of C. sativus has a gastroprotective property against experimentally induced ulcers in rats and hence can be used to treat ulcers.

Conflict of interest statement

We declare that no conflict of interest.

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