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Evaluation of Antioxidant and Antiulcer Potential of *Cucumis sativum* L. Seed Extract in Rats

[1]N.S. Gill, [1]M. Garg, [1]R. Bansal, [1]S. Sood, [1]A. Muthuraman,
[2]M. Bali and [3]P.D. Sharma
[1]Rayat Institute of Pharmacy, Near Railmajra, Punjab Technical University,
Ropar-144533, India
[2]Rayat Institute of Engineering and Technology, Near Railmajra,
Punjab Technical University, Ropar-144533, India
[3]University Institute of Pharmaceutical Sciences,
Punjab University, Chandigarh, India

Abstract: The study was aimed to investigate the antioxidant and anti-ulcer effect of methanolic extract of *Cucumis sativum* L. seeds. Extraction was done using different solvents of increasing polarity (chloroform, ethyl acetate and methanol). The antioxidant activity of all the extracts was measured by DPPH method. The methanolic extract of *Cucumis sativum* L. (MECS) seeds showed maximum antioxidant potential. Hence, it was further evaluated for its *in vivo* anti-ulcer activity by Pyloric Ligation (PL) and Water Immersion Stress (WIS) induced ulcer models in rat. In PL model gastric volume, free and total acidity of MECS were measured at 150 and 300 mg kg^{-1} doses. The ulcerative index was measured in both the models at the same doses. The MECS showed maximum reduction of gastric acid volume, free and total acidity such as 41, 48 and 29% at 300 mg kg^{-1} dose, respectively. The ulcerative index inhibition in PL and WIS models was found to be 52.5 and 62.7%, respectively at higher dose. The results suggested that methanolic extract of *Cucumis sativum* L. seeds possessed significant antiulcer potential which could be due to its antioxidant activity.

Key words: *Cucumis sativum*, antiulcer, pylorus ligation, water immersion stress

INTRODUCTION

Worldwide interest in natural products as preventive and therapeutic agents has led to a greater appreciation of the rich heritage of traditional systems of medicine (Chaturvedi *et al*., 2007). The plant products today symbolise safety in contrast to the synthetics products (Allison *et al*., 1968). These compounds can be used directly as lead for the development of new medicines or as pharmacological tools to discover new active compounds, so they can be life-saving or determine the quality of life in long-lasting diseases (Kaplan and Gottlieb, 1990; Elisabetsky and Costa-Campos, 1996; Falcao *et al*., 2008). For over a century, peptic ulcer disease has been one of the leading causes of gastrointestinal surgery, with high morbidity and mortality rates. The prevalence of duodenal ulcers is dominant in Western populations and gastric ulcers are more frequent in Asia

Corresponding Author: Naresh Singh Gill, Rayat Institute of Pharmacy, Near Railmajra,
Punjab Technical University, Ropar-144533, India
Tel: +91-9888611679 Fax: +91-1881-270501

(Yuan *et al*., 2006; Falcao *et al*., 2008). The treatment of peptic ulcers with plant products and the protection of induced gastric ulcer in laboratory animals using medicinal plants was reported by Disi *et al*. (1998) and Muralidharan and Srikanth (2009). Various herbal drugs notably *Musa sapientum*, *Tectona grandis*. *Rhamnus procumbens*, *Rhammis triquerta*, *Withania somnifera*, Shilajit, *Dhatura fastuosa*, *Fluggea macrocarpa*, *Aegle marmelos*, *Zingiber officinale* and *Asparagus recemosus* have been tried for their ulcer protective effects both experimentally (Goel and Sairam, 2002; Chaturvedi *et al*., 2007) and clinically (Arora and Sharma, 1990; Chaturvedi *et al*., 2007). On the basis of literature our present study was to evaluate the antiulcer activity of methanolic extract of *Cucumis sativum* L. against pylorus ligation and water immersion induced ulcer in rats.

MATERIALS AND METHODS

Plant Material

The present study was conducted at Rayat Institute of Pharmacy Department, Near Railmajra, Ropar-144533, India. Seeds were purchased from Local Grain Market Sector 26, Chandigarh (UT). Healthy looking seeds were chosen for examination (without mechanical damages and bacterial infection). The seeds were authenticated and the voucher specimen No. 0355 has been deposited in the Botanical and Environmental Science Department, Guru Nanak Dev University, Amritsar (PB). The seeds were cleaned, washed, dried at room temperature for two days and powdered. The sample was kept in air tightened light-protected containers. Further, extraction process and pharmacological evaluation was carried out during March, 2008 to Jan, 2009 period.

Drugs and Chemicals

DPPH (1,1-diphenyl-2-picrylhydrazyl) was obtained from Sigma Chemical Co. Ranitidine was obtained as free sample from Jackson Laboratories, Amritsar. Pentobarbitone (Neon pharmaceuticals), methanol, ethyl acetate, hexane and sodium hydroxide were of analytical grade and purchased from SD fine chemicals, Merk, Qualigen and Loba chemicals.

Preparation of Extract

Powdered seeds were defatted with hexane. Then extraction was carried out with different solvents of increasing polarity such as chloroform, ethyl acetate and methanol by maceration process for 16 h at room temperature. The solvent was evaporated to dryness under reduced pressure and obtained crude extracts were used for further investigation.

Preliminary Phytochemical Screening

The preliminary phytochemical screening of the extracts was carried out to know the different constituents present as per the standard procedures. The extracts were tested for alkaloids, sterols, triterpenes, saponins, flavonoids, tannins, carbohydrates, protein and amino acids (Harborne, 1973).

Experimental Animals

Wistar albino rats (160-180 g) of either sex were purchased from Agricultural University, Ludhiana (PB). Animals were maintained under standard environmental condition like temperature (24.0±1.0°C), relative humidity: 55-65% and 12 h light/dark cycle and had free access to feed and water *ad libitum*. The animals were acclimatized to laboratory condition

for 1 week prior to experimentation. All animal experiments were carried out in accordance with the guidelines of CPCSEA and study was approved by the IAEC (Institutional Animal Ethical Committee) with registration No. 874/ac/05/CPCSEA).

Quantitative Scavenging Activity of DPPH Radical

Cucumis sativum L. seed extracts (0.5 mL) of different concentration (500-700 μg mL^{-1}) were added to 1.5 mL of freshly prepared methanolic solution of DPPH (0.05 mM). The change in absorbance at 517 nm was measured 30 min later by a spectrophotometer (Shimadzu UV-1700 Pharma Spec). A blank was used to remove the influence of the colour of the samples. A methanolic solution of DPPH was used as negative control. Ascorbic acid was used as a reference drug. Percentage inhibition was calculated by using the following equation:

$$I\,(\%) = 100 \times (A_0 - As)/A_0$$

where, A_0 and As are the values for the absorbance of the negative control and the absorbance of the sample respectively. Tests were carried out in triplicate.

Antiulcer Activity

Experimental Design for Pyloric Ligation Induced Gastric Ulcer

Animals were divided into 6 groups, each comprising of 6 rats.

Group I: Administered vehicle (normal saline 0.9% w/v, p.o.) 1 h before pyloric ligation on the day of experiment

Group II: Sham control group subjected to surgical procedure without pyloric ligation

Group III: Subjected to pyloric ligation for the induction of ulcer

Group IV: Administered standard (ranitidine 50 mg kg^{-1}, p.o.) 1 h before pyloric ligation on the day of experiment

Group V: Administered methanolic extract (150 mg kg^{-1}, p.o.) 1 h before pyloric ligation on the day of experiment

Group VI: Administered methanolic extract (300 mg kg^{-1}, p.o.) 1 h before pyloric ligation on the day of experiment

Methanolic seed extract (150 and 300 mg kg^{-1}) was administered for a period of 8 days. On 8th day normal saline, ranitidine and MECL were administered 1 h prior to pyloric ligation. Animals were anaesthetized using pentobarbitone (35 mg kg^{-1}, i.p.) and the abdomen was opened and pylorus was ligated without causing any damage to its blood vessels. The stomach was replaced carefully and the abdominal wall was closed with interrupted sutures. (Shay *et al*., 1945; Muralidharan and Srikanth, 2009). After 4 h of ligation, the animals were sacrificed by cervical dislocation. The abdomen was opened and a ligature was placed around the cardiac sphincter. The stomach was removed (Khayum *et al*., 2009). Gastric volume, free and total acid content of gastric juices were determined. Mean ulcer score for each animal was expressed as ulcerative index and the percentage ulcer protection was also calculated (Bose *et al*., 2003).

Experimental Design for Water Immersion Stress Induced Gastric Ulcer

Animals were divided into 5 groups, each comprising of 6 rats.

Group I: Administered vehicle (normal saline 0.9% w/v, p.o.) 1 h before water immersion stress

Group II: Subjected to water immersion stress for the induction of gastric ulcer

Group III: Administered standard (ranitidine 50 mg kg^{-1}, p.o.) 1 h before water immersion stress

Group IV: Administered methanolic extract (150 mg kg^{-1}, p.o.) 1 h before water immersion stress

Group V: Administered methanolic extract (300 mg kg^{-1}, p.o.) 1 h before water immersion stress

Gastric ulcer was induced by water immersion stress as described by Paul *et al.* (2004). Briefly, they were immersed vertically up to the level of xiphoid in plastic containers containing water maintained at 23°C for 4 h. Animals were fasted for 24 h prior to the experiment. All the drugs were administered by oral route. After 4 h animals were removed, they were then sacrificed and stomach was opened along the greatest curvature, washed with normal saline (0.9% w/v NaCl). Then ulcerative index and percentage ulcer protection were calculated.

Estimation of Gastric Volume and Free and Total Activity Changes in PL Model

Gastric Volume

Four hours after ligation, stomachs were dissected out and contents were collected into measuring cylinder to measure the volume of gastric content.

Free and Total Acidity

The gastric contents were centrifuged and subjected to titration for estimation of free and total acidity. One milliliter 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 endpoint when the solution turned to orange colour. The volume of NaOH needed was taken as corresponding to the free acidity (Rajkapoor *et al.*, 2002). Titration was further continued by adding 1% solution of phenolphthalein till the solution gained pink colour. The volume of NaOH required was noted and was taken as corresponding to the total acidity. The sum of the two titrations was total acidity (Rajkapoor *et al.*, 2002). Acidity was expressed as:

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

Estimation of Gastric Ulcerative Index Changes in PL and WIS Model

Ulcerative index was measured by method of Takagi *et al.* (1969). Briefly, the stomach was opened along the greater curvature. The stomach was washed with running tap water. Then it was placed on a flat wooden plate to count the ulcerative area.

The ulcer index was determined by using the formula:

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

where, X = Total mucosal area/Total ulcerated area

Percentage ulcer protection was calculated using the formula:

$$\text{Ulcer protection (\%)} = 100 - \text{Ut/Uc} \times 100$$

Where:
Ut = Ulcer index of treated group
Uc = Ulcer index of control group

Statistical Analysis

All the biochemical results were expressed as mean±standard error of means (SEM). Data were analysed by Tukey's multiple range tests using Sigma Stat Version-3.5 software. A probability value of $p<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Preliminary phytochemicals screening of different extracts of *Cucumis sativum* L. seeds showed the presence of sterols, triterpenes, carbohydrates, tannins, protein and amino acid but methanolic extract showed the presence of proteins, amino acids, triterpenoids, carbohydrates, phytosterols and tannins (Table 1). Further, free radical scavenging activity of all the extracts were evaluated by DPPH method. All the extract showed concentration dependent antioxidant activity but maximum activity was reported in the methanolic extract of *Cucumis sativum* L. seeds (MECS). DPPH scavenging effect of MECS extract was 76.2% at a concentration of 700 μg mL^{-1} which was comparable to the scavenging effect of ascorbic acid (Fig. 1). Hence, MECS was further used to evaluate its antiuncerogenic potential in pyloric ligated and water immersion stress induced peptic ulcer models.

Table 1: Phytochemical screening of *Cucumis sativum* L. seed extracts

Chemical test	Chloroform extract	Ethyl acetate extract	Methanolic extract
Alkaloids	-	-	-
Flavonoids	-	-	-
Protein and amino acid	-	+	++
Triterpenoids	+	+	++
Saponin	-	-	-
Carbohydrates	+	+	++
Phytosterols	-	+	++
Tannins	-	-	++

+: Presence of compounds, - : Absence of compounds

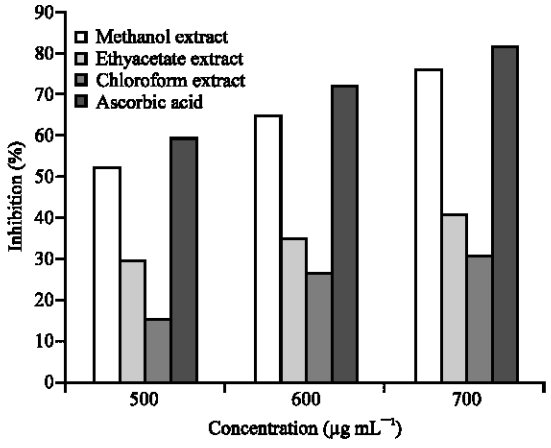

Fig. 1: Percentage DPPH radical scavenging activity

Table 2: Effect of MECS on gastric secretion, free acidity and total acidity in pylorus ligation induced gastric ulcer in rats

Treatment (mg kg^{-1})	Gastric volume (mL/100 g)	Free acidity (mEq/100 g)	Total acidity (mEq/100 g)
Normal	1.18±0.6	23.16±0.94	57.66±2.06
Sham	1.19±0.5	24.06±0.63	55.21±1.04
Disease	2.82±0.2^{a}	61.83±1.99^{a}	101.67±1.38^{a}
Ranitidine (50)	1.14±0.3^{b}	24.83±1.44^{b}	59.12±1.81^{b}
MECS (150)	2.15±0.1^{ac}	43.00±1.41^{ac}	77.33±1.66^{ac}
MECS (300)	1.65±0.5^{b}	31.83±1.42^{b}	72.00±1.65^{b}

Values are Mean±SEM, n = 6 animals in each group; $^{a}p<0.05$ as compared with sham control group. $^{b}p<0.05$ compared with disease control groups, $^{c}p<0.05$ compared with ranitidine treated group

Table 3: Effect of MECS on ulcerative index and percentage inhibition in PL and WIS induced gastric ulcer in rats

	Ulcerative index		Percentage inhibition	
Groups (mg kg^{-1})	PL	WIS	PL	WIS
Normal	0.00±0.00	0.00±0.00	0.0	0.0
Sham	0.00±0.00	0.00±0.00	--	--
Disease	4.82±0.01^{a}	5.81±0.01^{a}	0.0	0.0
Ranitidine (50)	1.91±0.01^{b}	1.26±0.01^{b}	60.0	78.2
MECS (150)	3.84±0.01^{ac}	4.53±0.01^{ac}	42.4	56.5
MECS (300)	2.32±0.01^{b}	1.96±0.01^{b}	52.5	62.7

Values are Mean±SEM, n = 6 animals in each group; $^{a}p<0.05$ compared with sham control group, $^{b}p<0.05$ compared with PL and WIS groups respective coloums, $^{c}p<0.05$ compared with ranitidine treated group

Various mechanisms are thought to be involved in the ulcer production in different experimental models (Parmar and Desai, 1993; Bose *et al*., 2003). Hence, it is not possible to propose a single mechanism for antiulcer effect of a particular drug. Digestive effect of the accumulated gastric juice is believed to be responsible for producing ulcers in the pyloric ligated rats. In addition to gastric acid secretion, reflex or neurogenic effect has also been suggested to play an important role in the formation of gastric ulcer in this model (Goswani *et al*., 1997; Bose *et al*., 2003). Ulcer index parameter was used for the evaluation of anti-ulcer activity since ulcer formation is directly related to factors such as gastric volume, free and total acidity (Khayum *et al*., 2009). In pyloric ligated rats, there was an increase in the gastric volume, free and total acidity and ulcerative index as compared to the sham control group. MECS showed reduction in gastric secretion, free and total acidity and ulcerative index, but only highest dose i.e., 300 mg kg^{-1} showed significant reduction in the above parameters which was comparable to the standard drug ranitidine (Table 2, 3).

Water immersion stress induced ulcer model provides both emotional stress as well as physiological stress (Malairajan *et al*., 2008). Ulcers are formed as a result of disturbance of gastric secretion, alteration in microcirculation of gastric mucosa and abnormal gastric motility (Kitagawa *et al*., 1979; Bose *et al*., 2003). It affect gastrointestinal defence and causes increase accumulation of acid due to influx of H^+ into the lumen of the stomach by parietal cell plasma membrane bound H^+ K^+-ATPase leading to auto digestion of the gastric mucosa and generation of free radicals which further increase the ulcers in the body (Das *et al*., 2008; Goel and Bhattacharya, 1991; Ivy, 1988). There was an increased ulcer formation in WIS model which was indicated by high ulcerative index as compared to the sham control group. Whereas, MECS showed reduction in ulcerative index, but only highest dose i.e., 300 mg kg^{-1} showed significant reduction in the above parameter which was comparable to the standard drug ranitidine (Table 3).

It is well known that free radicals are involved in the progression of ulcers and in PL and WIS models increase in the oxidative and decrease in the antioxidative biomarkers have been reported by Halliwell (1981) and Singh *et al*. (2008). Moreover, triterpenoids isolated from various species of cucurbita family has been reported to possess anti-inflammatory and anti-

cancer activities (Mohammad, 2009). Wound healing property of *Cucumis sativus* L. fruits has also been studied (William *et al*., 1991). Isolation of gibberellins was carried out from seeds of *Cucumis sativus* L. (Delbert *et al*., 1972). Literature also revealed the antioxidant property of various cucurbitacins isolated from cucurbita seeds. Hence, as our results indicated maximum *in vitro* free radical scavenging activity along with ameliorative effect on various ulcerative parameters of MECS extract so, this antioxidant potential may be responsible for its anti-ulcerogenic activity.

CONCLUSION

In the present study it may be concluded that the methanolic extract of the *Cucumis sativum* L. seeds possessed anti-ulcer effect due to its anti-oxidative potential and can be used as a future natural anti-ulcerogenic agent.

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