Evaluation of Antioxidant and Antiulcer Activity of Traditionally Consumed Cucumis melo Seeds

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ABSTRACT

The present study was to investigate the anti-ulcer activity of methanolic extract of Cucumis melo (MECM) seeds. The seeds were extracted with methanol and the extract was evaluated for its free radical scavenging activity by DPPH. It shows maximum antioxidant activity. Thus extract was further evaluated against gastric ulcerations by Pyloric Ligation (PL), Water Immersion Stress (WIS) and NSAID (indomethacin) induced ulcer (NIU) models. In case of PL method, the extract inhibited gastric ulcerations in a dose-related manner (150 and 300 mg kg\(^{-1}\)). Then it was evaluated for gastric volume, free acidity, total acidity, ulcerative index. The ulcerative index was also measured in WIS model and NIU with same doses. The MECM caused 57.6, 67.6 and 61.9% inhibition of ulcers in pyloric ligation, water immersion stress, NSAID induced ulcer models respectively at 300 mg kg\(^{-1}\). The results concluded that MECM possess high antiulcer potential which is due to its higher antioxidant activity.

Key words: Cucumis melo, antiulcer, pyloric ligation, water immersion stress, NSAID induced ulcer

INTRODUCTION

Medicinal plants have an important therapeutic role in the treatment of many human diseases thus they have been used as a major source in many medicines. Nowadays almost 25% of the active components of currently prescribed medicines were identified from plants sources. Gastric ulcer is among the most serious diseases in the world (Pezzuto, 1997).

The etiology of 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 factors (Repetto and Llesuy, 2002). Some other factors such as inadequate dietary habits, excessive ingestion of non-steroidal anti-inflammatory agents, stress, Helicobacter pylori, reactive oxygen species generated by the metabolism of arachidonic acid, platelets, macrophages and smooth muscle cells may contribute to gastric mucosal damage (Peckenaugh and Poleman, 1997). Therefore, treatment with antioxidants and synthetic drugs such as H\(_2\)-receptor blockers, proton pump inhibitors can decrease gastric mucosal damage (Salim, 1994; Waldum et al., 2005). But due to side effects of synthetic drugs (diarrhea, headache, fatigue and muscular pain) the use of herbal drugs
is preferred (Zimmerman, 1984). Various herbal drugs notably Musa sapientum, Tectona grandis, Rhamnus procumbens, Shilajit, Dhatura Fastuosa, Withania somnifera, Zingiber officinale, Ocimum sanctum and Curcuma longa have been used for treatment of ulcers. Some of plants namely Withania somnifera, Bacopa monnieri, Asparagus racemosus, Ocimum sanctum and Terminalia chebula have been classified in Ayurveda as rasayanas claimed for their ulcer protective effects both experimentally and clinically (Goel and Bhattacharya, 1991; Wagner et al., 1994; Kasture et al., 2007). Our present study was carried out to evaluate the antioxidant activity, antiulcer activity of methanolic extract of Cucumis melo in pylorus ligation, water immersion stress and NSAID induced ulcer model in rats.

MATERIALS AND METHODS

Plant material: The seeds were purchased from the local market of Ropar (India) in July, 2009. The healthy looking seeds were selected for authentication and voucher specimen number 0688 has been deposited in the Botanical and Environmental Science Department, Guru Nanak Dev University, Amritsar (India). The seeds were cleaned, washed, dried at room temperature and powered at low temperature.

Drugs and chemicals: Ranitidine and indomethacin were 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.

Experimental animals: The wistar albino rats and swiss albino mice of either sex were obtained from Sanjay biological, Amritsar. Institutional Animal Ethics Committee (IAEC) duly approved the experimental protocol and care of the animals was carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India (Reg No. 874/ac/05/CPCSEA).

Extraction: The seeds of Cucumis melo (500 g) were cleaned, shade dried, coarsely powdered and extracted with various solvents (methanol, ethyl acetate, chloroform etc.) for 72 h at room temperature. The extract was evaporated and concentrated under reduced pressure in rotary evaporator. The concentrated filtrate was suspended in distilled water and partitioned successively with hexane. The aqueous layer was separated and concentrated on the water bath. These crude extracts were further used for various investigations (Uchikoba et al., 1998; Banerjee et al., 2008).

Phytochemical screening: The crude extracts were analyzed for alkaloids, tannins, saponins, flavonoids, steroids, terpenoids and phenolic acids using standard procedures of analysis (Harborne, 1973).

Free radical scavenging activity

Quantitative evaluation of the DPPH free radical scavenging activity: DPPH scavenging activity of cantaloupe extract was determined according to the method described with slight modifications. In brief, 1 mL of methanolic extract of Cucumis melo seeds (MECM) at various concentrations were respectively added to 1 mL 0.05 mM DPPH in methanol and was made up to 5 mL with methanol. Then, the mixtures were vortexed vigorously and allowed to stand in the dark
for 60 min. Finally, the absorbance of these mixtures was measured by using a spectrophotometer (Pharmaspec UV-1700, Shimadzu, Kyoto, Japan) at 517 nm using methanol as blank. Ascorbic acid was used as standard. Control was prepared by diluting 1 mL of 0.05 mM DPPH with 4 mL of methanol. The capability to scavenge the DPPH radical was calculated using the following equation:

\[
\text{DPPH scavenged (\%) = } \frac{(A_i - A_f)}{A_i} \times 100
\]

Where:
\(A_i\) = Absorbance of the control reaction
\(A_f\) = Absorbance in presence of the sample of the extracts (Singh et al., 2002)

**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

Seed extract (150 and 300 mg kg\(^{-1}\)) was administered for a period of 8 days. On 8th day normal saline, ranitidine and MECM were administered 1 h prior to pyloric ligation. Animals were anaesthetized using pentobarbitone (35 mg kg\(^{-1}\), i.p.) and the abdomen was cut open through a midline incision. The pylorus was secured and ligated with silk sutures, after which the wound was closed and the animals were allowed to recover from anaesthesia. After ligation of the pylorus, drinking water was withheld and the gastric juice was collected for a period of 4 h. The rats were killed by cervical decapitation and the stomach was removed after clamping the oesophagus. The gastric contents were collected through the oesophagus. The gastric juice was centrifuged and the volume was noted. The stomach was then inflated with formal saline and then incised through the greater curvature and examined for the number of lesions under the dissecting microscope (Mahendran et al., 2003).

**Experimental design for water immersion stress 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

Stress ulcers were induced by forced swimming in the glass cylinder (height 45 cm, diameter 25 cm) containing water to the height of 35 cm maintained at 25°C for 3 h. Animals were fasted for 24 h prior to the experiment. After the drug treatment animals were allowed to swim in water for 4 h. The stomach of each animal was removed 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 (Alphine and Word, 1969).

Experimental design for indomethacin induced ulcer model: Animals were divided into 5 groups, each comprising of 6 rats:

Group I: Administered vehicle (normal saline 0.9% w/v, p.o.) 30 min before indomethacin induced ulcers

Group II: Disease control group administered indomethacin (25 mg kg⁻¹ p.o.) for the induction of gastric ulcers

Group III: Administered standard (ranitidine 50 mg kg⁻¹, p.o.) 30 min before indomethacin induced ulcers

Group IV: Administered methanolic extract (150 mg kg⁻¹, p.o.) 30 min before indomethacin induced ulcers

Group V: Administered methanolic extract (300 mg kg⁻¹, p.o.) 30 min before indomethacin induced ulcers

Normal saline, ranitidine, extract were given orally and 30 min later indomethacin was administrated to all the groups. Six hours later, the animals were killed by decapitation. The stomachs were removed, opened along the great curvature and washed with tap water to remove gastric contents and examined under a dissecting microscope with square-grid eyepiece to assess the formation of ulcers. For each stomach, ulcerated and total areas were measured as mm² and the ulcer indexes for each stomach were calculated (Dengiz and Gursan, 2005).

Estimation of gastric volume, total and free acidity, ulcer index 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.

Determination of total acidity and free 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. 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:

\[
\text{Acidity} = \frac{\text{Volume of sodium hydroxide} \times \text{Normality} \times 100 \text{ mEq/L/100 g}}{0.1}
\]
Ulcer index: The number of ulcers was counted and scoring was undertaken according to the reported method (Desai et al., 1995).

The ulcer index was determined by using the formula:

\[ \text{Ulcer index} = \frac{10}{X} \]

Where: \( X = \frac{\text{Total mucosal area}}{\text{Total ulcerated area}} \)

Percentage ulcer protection was calculated using the formula (Takagi et al., 1969),

\[ \text{Ulcer protection} (\%) = \left( \frac{U_c - U}{U_c} \right) \times 100 \]

Where:
\( U_c \) = Ulcer index of treated group
\( U \) = Ulcer index of disease 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

The results in Table 1 reveals that methanolic extract contain maximum amount of tannins, alkaloids, steroids, triterpenoids, carbohydrates, proteins and amino acids using standard procedures, all the extract showed concentration dependent antioxidant activity but maximum activity was reported in the methanolic extract of \textit{Cucumis melo} seeds. Hence, \textit{Cucumis melo} was further used to evaluate its anti ulcerogenic potential in pyloric ligated, water immersion stress and NSAID induced ulcer models.

DPPH reacts with antioxidants and gets converted into 1,1-diphenyl-2-picrylhydrazine by accepting a hydrogen atom and hence shows decrease in absorbance. The highest antioxidant activity of MECM was found to be 74.9% at concentration of 300 \( \mu \text{g mL}^{-1} \) as shown in Table 2.

It is evident from the results of the present investigation that \textit{Cucumis melo} possesses antulcer activity in pyloric ligation, Water immersion method and NSAID induced ulcer.

In the PL model the pylorus was ligated. This will leads to excessive production of gastric acid secretion. Stomach was removed for observation of ulcerative index, gastric volume, free acidity, total acidity. 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 3, 4). The MECM caused 41.0 and 57.6% inhibition of ulcers at the dose of 150 and 300 mg kg\(^{-1}\), respectively in PL model.

<table>
<thead>
<tr>
<th>Table 1: Phytochemical screening of MECM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical constituents</td>
</tr>
<tr>
<td>Flavonoids</td>
</tr>
<tr>
<td>Amino acid</td>
</tr>
<tr>
<td>Triterpenoids</td>
</tr>
<tr>
<td>Saponin</td>
</tr>
<tr>
<td>Carbohydrates</td>
</tr>
<tr>
<td>Tannins</td>
</tr>
<tr>
<td>Alkaloids</td>
</tr>
</tbody>
</table>

+: Presence of chemical constituent, -: Absence of chemical constituent, ++: Maximum presence of chemical constituents.
Table 2: Percentage scavenging of DPPH radical

<table>
<thead>
<tr>
<th>Conc. of extract (µg mL⁻¹)</th>
<th>Percentage scavenging of DPPH radical</th>
<th>Methanol extract</th>
<th>Ascorbic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td></td>
<td>52.8±0.28</td>
<td>64.7±0.87</td>
</tr>
<tr>
<td>200</td>
<td></td>
<td>63.3±0.54</td>
<td>73.6±0.62</td>
</tr>
<tr>
<td>300</td>
<td></td>
<td>74.9±0.76</td>
<td>82.5±0.58</td>
</tr>
</tbody>
</table>

Values are the average of triplicate experiments and represented as Mean±SEM

Table 3: Effect of MECM on gastric secretion, free acidity and total acidity in pylorus ligation induced gastric ulcer in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (mg kg⁻¹)</th>
<th>Gastric volume (mL/100 g)</th>
<th>Free acidity (mEq/L/100 g)</th>
<th>Total acidity (mEq/L/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal</td>
<td>1.15±0.19</td>
<td>23.9±0.27</td>
<td>58.8±1.03</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Sham</td>
<td>1.26±0.51</td>
<td>23.8±0.31</td>
<td>55.9±0.69</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Disease</td>
<td>3.11±0.19a</td>
<td>63.3±0.50a</td>
<td>162.1±1.11b</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Ranitidine</td>
<td>50</td>
<td>25.8±0.37</td>
<td>61.5±0.22</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>MECM</td>
<td>150</td>
<td>2.44±0.21</td>
<td>46.07±0.55</td>
<td>73.9±0.21</td>
</tr>
<tr>
<td>VI</td>
<td>MECM</td>
<td>300</td>
<td>1.82±0.03</td>
<td>32.5±0.30</td>
<td></td>
</tr>
</tbody>
</table>

Values are Means±SEM, n = 6 animals in each group; *p<0.05 as compared with sham control group. b*p<0.05 compared with disease control groups; *p<0.05 compared with ranitidine treated groups

Table 4: Effect of MECM on ulcerative index and percentage inhibition in PL, WIS and NIU gastric ulcer in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (mg kg⁻¹)</th>
<th>Ulcerative index</th>
<th>Percentage inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>PL</td>
<td>WIS</td>
</tr>
<tr>
<td>I</td>
<td>Normal</td>
<td>00±0.00</td>
<td>00±0.00</td>
<td>00±0.00</td>
</tr>
<tr>
<td>II</td>
<td>Sham</td>
<td>00±0.00</td>
<td>00±0.00</td>
<td>00±0.00</td>
</tr>
<tr>
<td>III</td>
<td>Disease</td>
<td>5.46±0.01a</td>
<td>5.85±0.01a</td>
<td>6.13±0.01a</td>
</tr>
<tr>
<td>IV</td>
<td>Ranitidine</td>
<td>50</td>
<td>1.82±0.01b</td>
<td>1.96±0.01b</td>
</tr>
<tr>
<td>V</td>
<td>MECM</td>
<td>150</td>
<td>3.22±0.01d</td>
<td>2.79±0.01d</td>
</tr>
<tr>
<td>VI</td>
<td>MECM</td>
<td>300</td>
<td>2.31±0.01b</td>
<td>1.89±0.01b</td>
</tr>
</tbody>
</table>

Values are Means±SEM, n = 6 animals in each group; *p<0.05 compared with sham control group. b*p<0.05 compared with PL and WIS groups respective columns. *p<0.05 compared with ranitidine treated group

In WIS induced ulcer model the MECM 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 4). The MECM caused 52.3 and 67.6% inhibition of ulcers at the dose of 200 and 300 mg kg⁻¹, respectively in WIS model.

In NSAID induced ulcer model the MECM 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 4). The MECM caused 51.3 and 61.9% inhibition of ulcers at the dose of 200 and 300 mg kg⁻¹, respectively in indomethacin induced ulcer model (Table 4).

DISCUSSION

The present study reports the antiulcerogenic activity of methanol extracts of Cucumis melo seeds. The mechanism of its gastroprotective activity may be attributed to reduction in vascular permeability, free radical generation and lipid peroxidation along with strengthening of mucosal barrier. Presence of phytoconstituents in this plant like triterpenoids and sterols might be responsible for these actions.
In the present study, the methanolic extract of *Cucumis melo* seeds was evaluated for its free radical scavenging activity followed by in vivo antiulcer activity in pyloric ligation, water immersion stress and indomethacin induced ulcer models. 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. The ulcer formation in each of these models occurs by different mechanisms. Hence, it is not possible to propose a single mechanism for antiulcer effect of a particular drug. Pylorus ligation induces gastric ulcers due to accumulation of gastric secretion in the stomach (Shay et al., 1945). In WIS model stress causes both sympathetic and parasympathetic stimulation of stomach leading to local hypoxia. The ischemic condition caused an increase in the levels H$_2$O$_2$, which in conjugation with O$_2$ generates OH$^-$ ions which oxidized various cellular constituents such as proteins, membrane lipids. Lipid peroxidation causes loss of membrane fluidity and leads to production of ulcers (Tandon et al., 2004). Disturbances of gastric mucosal microcirculation (Guth, 1972), alteration of gastric secretion and abnormal gastric motility have been considered to be the pathogenic mechanisms responsible for stress-induced gastric mucosal lesions and gastric mucus depletion (Koo et al., 1986). Antiulcer activity of methanolic extract of *Momordica charantia* L. has been carried out (Alam et al., 2009). Evaluation of antioxidant and antiulcer potential of *Cucumis sativum* L. seed extract *Cucumis sativum* has been carried out earlier (Gill et al., 2009). Gastroprotective activity of *Trichosanthes cucumerina* has been studied in rats (Arawuwawala et al., 2010). Anti-ulcerogenic and analgesic activities of the leaves of *Wilandra ebracteata, Wilandra ebracteata* has been studied in mice model (Gonzalez and Di-Stasi, 2002). Some radical scavengers have shown to possess a protective effect against the mucosal injuries induced by active oxygen species (Oka et al., 1991).

**CONCLUSION**

In the present study it may be concluded that the methanolic extract of the *Cucumis melo* seeds have antiulcer effect due to its anti-oxidative potential.

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