Anti-ulcerogenic activity of Ethanolic Extract of *Cucumis sativus* L. against NSAID (Aspirin) induced Gastric Ulcer in wistar albino rats.

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**ABSTRACT**

Peptic disorders like GERD, gastritis, peptic ulcer, duodenal ulcer, etc., are common in today’s lifestyle. This may be due to stressful lifestyle, or improper balance diet. The pathology behind these disorders may be discrepancy between offensive and defensive mechanisms either by excess secretion of acid and pepsin or diminished ability of the gastro-duodenal mucosal barrier to protect against stomach acid–pepsin secretion. Hence this present experiment is designed to evaluate the anti-ulcer activity of *Cucumis sativus* ethanolic extract in wister albino rat model. Pretreatment with ethanolic extract cucumber shows significant ulcer protective effect. Thus, it can concluded that ethanolic extract of cucumber possesses significant antiulcer activity.

**Key words:** *Cucumis sativus* L., Gastric ulcer, NSAID (Aspirin).

1. Introduction

Peptic ulcer is an eroded area of stomach caused principally by the digestive action of gastric juice, upper small intestinal secretions. It is basically an inflamed break in the skin or the mucous membrane lining the alimentary tract [1]. Prolonged use of Non-steroidal anti-inflammatory drugs (NSAIDs) and *Helicobacter pylori* infection are two major factors that can disrupt the mucosal resistance [2, 3]. Cucumbers, *Cucumis sativus* belongs to family Cucurbitaceae, is most widely cultivated vegetable crop all over the world. *Cucumis sativus* is native to the Indian subcontinent [4]. The cucumber is a creeping vine that roots in the ground and grows up trellises or other supporting frames, wrapping around supports with thin, spiraling tendrils. It has monoeccious, bearing staminate and pistillate flower on the same plant. The plant has large leaves that form a canopy over the fruit. The fruit is roughly cylindrical, elongated with tapered ends, and may be as large as 60 centimeters long and 10 centimeters in diameter [5]. The plant is better adapted to low temperatures. *Cucumis sativus* have been evaluated for a wide spectrum of activity including diuretic, antihyperglycemic, antioxidant, amylolytic, anticancer and analgesic activities using various in-vitro and in-vivo models [6-12]. Hence, the objective of the present investigation is to evaluate the anti-ulcer activity of *C. sativus* against NSAID (Aspirin) induced gastric ulcer in wister albino rat model.

2. Material and method

2.1 Preparation of extract of plant material

The fresh cucumbers were collected from rural areas of Berhampur, Odisha in the month of December 2012. The 4kg plant materials sliced into small circular pieces and were shade dried for 6 days. The dried pieces subjected for grinding to coarse powder. The coarse powder was macerated with ethanol for 24hrs followed by Soxhlet extraction for 24-36hrs. The alcoholic layer was concentrated in rotavapor to get a dark brown color semisolid ethanolic extract. The extractive value found to 8.9 %.
2.2 Preliminary Phytochemical Screening

The preliminary phytochemical screening of the ethanolic extract of cucumber was performed for the qualitative analysis of alkaloid, flavonoid, glycoside, steroid, polyphenols, saponin, reducing sugar, and tannin [13, 14].

2.3 Animals

Healthy adult wistar albino rats (120-150gm) were obtained from the National Institute of Biological sciences, Pune, India. The animals were housed in a 6 groups and kept in solid bottom polypropylene cages. They were maintained at (22±1) °C, with relative humidity of 47-55 % and 12:12 h dark/light cycle. The animals were acclimatized for a period of two weeks and were kept under pathogen free conditions. The animals had free access to standard pellet chow which composing of Protein-20.12 %, Total oil-4.38 %, Dietary fiber 3.65 %, Moisture-8.0 % (Pranav Agro industries Ltd., Sangli, India) throughout the experimental protocol, with the exception of overnight fasting before induction of the ulcer. The animals were provided with filtered distilled water.

2.4 Acute Toxicity Testing

Acute oral toxicity in wistar albino rats was performed according to OECD guidelines using AOT 425 software. Graded doses of the ethanolic extract dissolved in distilled water were administered orally and the animals were observed for 2 week following administration. Body weight, food consumption, fluid intake and psycho-motor activities were recorded daily.

2.5 Dosages of Ethanolic Extract and Standard Drugs Used

The ethanolic extract of cucumber in the three different dosages (100 mg/kg, 200 mg/kg and 400 mg/kg) were administered to animals orally for 6 day. On 7th day, the ulcer was induced by Aspirin. The drug treatment was continued even after administration of Aspirin. Standard drug used for comparison was Ascorbic acid. Ascorbic acid was not given as pretreatment. It was given on the day of Aspirin administration. Ascorbic acid was given in a dose of 50mg/kg/day orally in rats as suspension containing 0.5 % of sodium carboxy methyl cellulose [18].

2.6 Induction of Gastric ulcer

The study comprised 6 groups each containing 6 animals each as follows:

- **Group I**- (served as Control): Received 2 ml of distilled water for 9 days.
- **Group II**- (served as Standard): Received 2 ml of (20mg/ml) Aspirin (once, orally) and 2 ml of distilled water for 9 day.
- **Group III**- Drug treated animals: Received 6 day pretreatment with ethanolic extract of cucumber (100 mg/kg) and 2 ml of (20mg/ml) Aspirin solution, orally on 7th day and this treatment continued till 9th day.
- **Group IV**- Drug treated animals: Received 6 day pretreatment with ethanolic extract of cucumber (200 mg/kg) and 2 ml of (20mg/ml) Aspirin solution, orally on 7th day and this treatment continued till 9th day.
- **Group V**- Drug treated animals: Received 6 day pretreatment with ethanolic extract of cucumber (400 mg/kg) and 2 ml of (20mg/ml) Aspirin solution, orally on 7th day and this treatment continued till 9th day.
- **Group VI**- Ascorbic acid treated animals: Received ascorbic acid (50mg/kg, for 6 day) and 2 ml of (20mg/ml) Aspirin solution once, orally. Ascorbic acid and Aspirin treatment was started on the same day.

On the 9th day the animals were sacrificed, stomachs were isolated and then dissected out through opening along the greater curvature pyloric section, inspected internally for ulcer index [16].

2.7 Determination of ulcer index and % protection

The evaluation of ulcer index was calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach (Table no. 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ulcer Index(UI)</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1(control)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group 2Aspirin(dose)</td>
<td>13.16</td>
<td>0</td>
</tr>
<tr>
<td>Group 3(cucumber extract 100 mg/kg)</td>
<td>6.46</td>
<td>50.91</td>
</tr>
<tr>
<td>Group 4(cucumber extract 200 mg/kg)</td>
<td>5.28</td>
<td>59.87</td>
</tr>
<tr>
<td>Group 5(cucumber extract 400mgkg)</td>
<td>3.51</td>
<td>73.32</td>
</tr>
<tr>
<td>Group 6(Ascorbic acid 50 mg/kg)</td>
<td>2.5</td>
<td>81.00</td>
</tr>
</tbody>
</table>

Severi ty of gastric ulcer score

0 = no ulcers
1 = changes limited to superficial layer of the mucosa with no congestion.
2 = half the mucosal thickness shows necrotic changes.
3 = more than 2/3rd of the mucosal thickness shows necrotic changes.
4 = complete destruction of the mucosa with hemorrhage.

The Ulcer Index (UI) is calculated as given below [17, 18]:

\[ UI = Un + Us + (Up * 10^{-1}) \]

Un = average number of ulcers per animal
Us = average number of severity of scores
Up = percentage of animals with ulcers.

% Protection= (C-T/C) X 100

Where C= ulcer index in control group, T= ulcer index in treated group (Table no. 2).
2.8 Statistical Analysis
The interpretation of the results was done after subjecting the data obtained from various studies to statistical analysis which included one way ANOVA followed by test like Dunnett and Tukey. P<0.05 is considered as statistically significant (Table no 3) \(^{19}\).

<table>
<thead>
<tr>
<th>Anova Table</th>
<th>Sum of squares</th>
<th>Degree of freedom</th>
<th>MS</th>
<th>F Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment(between columns)</td>
<td>52.83</td>
<td>5</td>
<td>10.57</td>
<td>42.27</td>
</tr>
<tr>
<td>Residual(with in columns)</td>
<td>4,500</td>
<td>18</td>
<td>0.25</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>57.33</td>
<td>23</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

3. Results
3.1 Acute toxicity testing
Acute toxicity studies of the ethanolic extract shows no signs and symptoms such as restlessness, respiratory distress, diarrhea, convulsions and coma and it was found safe up to 5000 mg/kg.

3.2 Preliminary Phytochemical Screening
The ethanolic extract of cucumber was screened through various chemical tests as per the reported methods and was found to contain alkaloids, flavonoid, glycoside, steroids, polyphenols, saponin, and tannins.

3.3 Effect of Ethanolic Extract on Ulcer Index
At the end of the study, the stomach was isolated and washed with saline, it was then observed for ulceration and ulcers were scored. Ulcer index and percentage protection against ulcers was calculated. The mean ulcer index of Aspirin (Group-2) was (3.6±0.289) showed high ulcerogenic effect. Pretreatment with ethanolic extract of dose (400 mg/kg) gives mean value of 1.1±0.289 and with the Ascorbic acid gives mean value 0.33±0.25 (Table no 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Un</th>
<th>Us</th>
<th>Up</th>
<th>UI</th>
<th>Mean± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (Control)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0±0.00</td>
</tr>
<tr>
<td>Group 2 (Aspirin dose )</td>
<td>28</td>
<td>3.6</td>
<td>100</td>
<td>13.16</td>
<td>3.6±0.289</td>
</tr>
<tr>
<td>Group 3(Cucumber extract 100mg/kg)</td>
<td>12</td>
<td>2.6</td>
<td>50</td>
<td>6.46</td>
<td>2.6±0.289</td>
</tr>
<tr>
<td>Group 4 (Cucumber extract 200mg/kg)</td>
<td>6</td>
<td>1.8</td>
<td>45</td>
<td>5.28</td>
<td>1.8±0.289</td>
</tr>
<tr>
<td>Group 5 (Cucumber extract 400mg/kg)</td>
<td>4</td>
<td>1.1</td>
<td>30</td>
<td>3.51</td>
<td>1.1±0.289</td>
</tr>
<tr>
<td>Group 6 (Ascorbic acid 50mg/kg)</td>
<td>0</td>
<td>0.33</td>
<td>25</td>
<td>2.5</td>
<td>0.33±0.25</td>
</tr>
</tbody>
</table>

Fig 1: Morphological representation of pyloric portion treated with distilled water, Aspirin, extract of Cucumber, and Ascorbic acid.
A. Group 1 (Control)
B. Group 2 (Aspirin dose)
C. Group 3 (100 mg/kg Ext + Asp)
D. Group 4 (200 mg/kg Ext + Asp)
E. Group 5 (400 mg/kg Ext + Asp)
F. Group 6 (Ascorbic acid + Asp)

Fig 2: Effect of Ethanolic extract of Cucumber on the Aspirin induced gastric ulcer

4. Conclusion
The current study showed that ulcer index in the group 5 received 400 mg/kg body weight of the ethanolic extracts of cucumber showed a significant less ulcer index when compared to control. Aspirin causes mucosal damage by interfering with prostaglandin synthesis \[28-31\], increasing acid secretion and back diffusion of H\(^+\) ion \[32-35\]. Overall, ethanolic extract of 400mg/kg body weight has shown a substantial and significant protection against gastric ulcers in all the models. The ulceroprotective activity of cucumber may be backed by presence of alkaloids, steroids, flavonoid, polyphenols \[26-28\] have been proven reduction of gastric acid volume, free acidity, total acidity, antioxidant, anti-inflammatory and immunomodulatory activity \[29,30\]. These phytoconstituents of \textit{C. sativus} may have synergistically contributed to the attenuation of peptic ulcer. However, further studies need to be carried out to isolate the desire bioactive phytoconstituents, and underlining the full proof of mechanism of action of \textit{Cucumis sativus} against Gastric ulcer.

5. Conflict of interest statement
We declare that we have no conflict of interest.

6. Reference: