Effect of ripe fruit pulp extract of *Cucurbita pepo* Linn. in aspirin induced gastric and duodenal ulcer in rats

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A significant decrease in alkaline phosphatase (AP) activity and mucosal thickness and increase in ulcer index (UI) was observed in aspirin treated stomach and duodenum of albino rats. However, pretreatment with *C. pepo* fruit pulp extract for 14 consecutive days showed increase in AP activity and mucosal thickness along with decrease in UI, suggesting gastro-duodenal protective and anti-ulcerogenic properties of *C. pepo*.

**Keywords:** Alkaline phosphatase, Aspirin, *Cucurbita pepo*, Gastric and Duodenal ulcer, Mucosal thickness, Ulcer index

*Cucurbita pepo* Linn. (Cucurbitaceae), commonly known as pumpkin is available throughout India and consumed as vegetable in various parts of the world. Different parts of the plant have been used as medicine in Ayurveda. The pulp of ripe fruit of *C. pepo* is used to relieve intestinal inflammation or enteritis, dyspepsia and stomach disorders. Its pulp is used as dietary supplements for vitamin-A and is also used to treat liver disorder such as jaundice.

Extensive pharmacological investigation led to isolation of several active compounds from *C. pepo* e.g. phenolic compound such as syringic acid, cucurbitane and hexanocucurbitane glycoside such as cucurbitacin L 2-O-β-D-glucopyranoside and cucurbitacin K 2-O-β-D-glucopyranoside, 2, 6-dihydroxy-22, 23, 24, 25, 26, 27-hexanorcucurbit-5-en-11, 20-dione 2-O-β-D-glucopyranoside and 16-hydroxy-22, 23, 24, 25, 26, 27-hexanorcucurbit-5-en-11, 20-dione 3-O-α-l-rhamnopyranosyl-(1, 2)-β-D-glucopyranoside respectively, β-carotene, provitamin A carotenoids, vitamins A, vitamin E, vitamin C and alkaloid such as tannin. Some of these compounds have been reported to have a role in protection against gastric mucosal damage.

The present study has been undertaken to evaluate the role of the ripe fruit pulp of *Cucurbita pepo* against aspirin induced ulcer model as evidenced by alteration of the AP activity, mucosal thickness and ulcer index in aspirin induced gastric and duodenal ulcer in rats.

**Materials and Methods**

**Animals**—Inbred Holtzman strain adult albino rats (180-200g) of either sex were obtained from Indian Institute of Chemical Biology, Jadavpur, Kolkata, West Bengal and housed individually in a room (28ºC; 60% RH with 12:12 hrs L:D cycle) and both the control and experimental rats were maintained on a daily schedule of standard laboratory diet. Drinking water was supplied *ad libitum*. Food intake (g/day/rat) and body weight of the rats were recorded daily and maintained throughout the experimental period. Experiments were carried out after the approval of the experimental design by the Animal Ethical Committee of the institute.

**Preparation of extract**—The ripe fruit of *C. pepo* was purchased locally and its identity was authenticated by the Botanical Survey of India, Howrah, West Bengal. The fruit kept at room temperature (28ºC). During extraction, the outer surface of the ripe fruit was washed with distilled water, the bark was discarded and seeds were removed. The pulp (1 kg) was cut into pieces, sun dried and ground with the help of an electrical grinder to get a free flowing powder. This powder was subjected to extraction with water (1:3) at room temperature for 48 hrs then filtered through Whatman No.1 filter paper and vacuum dried in a lyophilizer at 40º-50ºC. A viscous and sticky substance was obtained. It was kept in cold (4ºC) and dissolved in double distilled water for future use. The final yield

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was 29.3% (w/w).

**Animal treatment**

Schedule I: Preliminary studies were done for the selection of effective dose (ED) of ripe fruit pulp of *C. pepo* extract by evaluating the ulcer index (UI). Rats (42) were divided into 7 groups of 6 rats each. The control rats (group I) were fed orally with distilled water for 14 days and the experimental rats (groups II-VII) were treated with 200, 300, 350, 400, 450 and 500 mg/kg body weight of aqueous extract of *C. pepo* respectively, orally by orogastric cannula once daily for 14 consecutive days at a particular time (10:30–11:30 hrs every day). On the 14th day, after feeding the extract, the food was withdrawn but rats had free access to water. On the 15th day, aspirin (German–Remidies Ltd) was dissolved in distilled water and given to all groups of rat at a dose of 500 mg/kg body weight orally. After 4 hrs the experiment was terminated and rats were sacrificed by an over dose of thiopentone sodium (NEON, Laboratories Ltd, India).

**Ulcer scoring**—The stomach and duodenum were collected, opened along the greater curvature to expose the mucosal surface, stretched on a flat paraffin bed and washed with normal saline to remove the food particles to note the distribution of ulcers. The ulcer scoring was performed by the method of Szabo et al.16

<table>
<thead>
<tr>
<th>Severity score</th>
<th>Ulcer type</th>
<th>Length of ulceration (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>no pathology</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>small ulcer</td>
<td>1-2</td>
</tr>
<tr>
<td>2</td>
<td>medium ulcer</td>
<td>3-4</td>
</tr>
<tr>
<td>4</td>
<td>large ulcer</td>
<td>5-6</td>
</tr>
<tr>
<td>8</td>
<td>larger ulcer</td>
<td>&gt;6</td>
</tr>
</tbody>
</table>

The sum of the total severity scores in each group of rats divided by the number of rats was expressed as the mean ulcer index (MUI).

**Dose selection**—The group which showed the lowest ulcer index (UI) was selected as the effective dose (ED) of *C. pepo*. In the present experiment, the dose of 400 mg/kg body weight showed the lowest UI after aspirin treatment and therefore all experiments were carried out with this dose.

Schedule II: Twenty four rats were divided into four groups of 6 rats each. Gr I animals comprised control group. Group II was *C. pepo* extract treated, Group III was aspirin treated and Group IV was *C. pepo* pretreated and aspirin treated.

The dry extract was dissolved in distilled water. Group I rats were given distilled water orally with approximately same volume of *C. pepo* extract. Group II and group IV rats were treated with selected dose of *C. pepo* extract (400 mg/kg body weight) by orogastric cannula once daily for 14 consecutive days at a particular time (10:30-11:30 hrs) in every day. On the 14th day, after feeding the extract, food was withdrawn from rats of group III and group IV but had free access to water. On the 15th day, aspirin (German–Remidies Ltd) was dissolved in distilled water and given at a dose of 500 mg/kg body weight orally and waited for 4 hrs, then procedure of the schedule I was followed for ulcer scoring as per the method of Szabo et al.16

**Staining of alkaline phosphatase (AP) enzyme**—The stomach and duodenum tissues were processed for routine paraffin sections. The paraffin sections were stained by the modified calcium method of Gomori17 for AP activity.

**Mucosal thickness**—For determination of mucosal thickness 5 μm thick transverse sections of stomach and duodenum tissues were taken. The sections were stained with H & E. At least 10 determination of mucosal thickness was made on at least two sections from each specimen. The mucosal thickness of both tissues was measured by a stage micrometer. Sections were examined with objective 10× (visual field diameter, 2.5mm) and eyepiece 5× (with scale bar inserted)18.

**Statistical analysis**—All results were expressed as mean±SE. Comparisons of groups were evaluated by one-way ANOVA. The results were considered statistically significant when *P*<0.05.

**Results**

**Schedule I:**

**Ulcer index**—Aqueous extract of ripe pulp of *C. pepo* (200, 300, 350, 400, 450 and 500 mg/kg body weight) dose dependently decreased the ulcer index (Table 1). At lower doses of extract (200, 300 and 350 mg/kg body weight) the decrease was not statistically significant compared to aspirin treated group but at higher doses (400, 450 and 500 mg/kg body weight) it decreased the UI in the stomach and duodenum tissues. The dose of 400 mg/kg body weight of *C. pepo* extract was most effective and thus this dose
was selected for the following experiments.

Schedule II:

Ulcer index—Aspirin treated rats showed a significant increase in ulcer index as compared to control group but pretreatment with *C. pepo* pulp extract (400 mg/kg body weight) for 14 days in aspirin treated group showed a significant decrease in the UI as compared to aspirin treated group (Table 2).

Alkaline phosphatase (AP) activity in stomach and duodenum—Distribution of AP activity along the mucosal brush border of control stomach and duodenum tissues is shown Fig. 1 and Fig. 5 (arrows)

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Table 1—Effect of different dose of *C. pepo* extract on ulcer index of stomach and duodenum (Schedule I)

[Values are mean±SE from 6 rats in each group]

<table>
<thead>
<tr>
<th>Groups</th>
<th>Stomach (Ulcer index)</th>
<th>Duodenum (Ulcer index)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Distilled water + aspirin, 500 mg/kg)</td>
<td>41.19±0.69</td>
<td>31.55±1.07</td>
</tr>
<tr>
<td>Group II (<em>C. pepo</em>, 200 mg/kg + aspirin (500 mg/kg))</td>
<td>27.76±0.24</td>
<td>20.76±0.25</td>
</tr>
<tr>
<td>Group III (<em>C. pepo</em>, 300 mg/kg + aspirin (500 mg/kg))</td>
<td>14.76±0.23</td>
<td>10.86±0.28</td>
</tr>
<tr>
<td>Group IV (<em>C. pepo</em>, 350 mg/kg + aspirin (500 mg/kg))</td>
<td>7.03±0.25</td>
<td>5.75±0.23</td>
</tr>
<tr>
<td>Group V (<em>C. pepo</em>, 400 mg/kg + aspirin (500 mg/kg))</td>
<td>1.27±0.35*</td>
<td>1.58±0.51*</td>
</tr>
<tr>
<td>Group VI (<em>C. pepo</em>, 450 mg/kg + aspirin (500 mg/kg))</td>
<td>1.25±0.30</td>
<td>1.55±0.40</td>
</tr>
<tr>
<td>Group VII (<em>C. pepo</em>, 500 mg/kg + aspirin (500 mg/kg))</td>
<td>1.20±0.10</td>
<td>1.35±0.35</td>
</tr>
</tbody>
</table>

Statistical analysis was done using one way ANOVA followed by multiple comparison *t*-tests. 

*P*<0.05, when compared to *(Distilled water + aspirin)* in which maximum ulceration was produced. 

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Table 2—Effect of *C. pepo* extract on ulcer index and mucosal thickness of stomach and duodenum tissues (Schedule II)

[Values are mean ± SE from 6 rats in each group]

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ulcer index</th>
<th>Mucosal thickness (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stomach</td>
<td>Duodenum</td>
</tr>
<tr>
<td>Group I (Control, distilled water)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group II (<em>C. pepo</em>, 400 mg/kg)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group III (aspirin, 500 mg/kg)</td>
<td>41.16±0.65*</td>
<td>31.00±1.06*</td>
</tr>
<tr>
<td>Group IV (<em>C. pepo</em>, 400 mg/kg + aspirin (500 mg/kg))</td>
<td>1.5±0.50*</td>
<td>1.06*</td>
</tr>
</tbody>
</table>

One way ANOVA; *, # significantly different from group I (control) and group III (aspirin) respectively at *P*<0.05

Figs 1 and 2—1, Alkaline phosphatase activity (arrow) of mucosal brush border of glandular part of control stomach tissues (×100). 2, Epithelial lining of glandular part of *C. pepo* extract treated stomach tissues [arrow shows an increased AP activity at luminal brush border as compared with control group] (×100).
Figs 3-8—3, Epithelial lining of glandular part of aspirin treated stomach tissues that decreased AP activity at luminal brush border as compared with control group (arrow) (×100). 4, Epithelial lining of glandular part of C. pepo+ aspirin treated stomach tissues [arrow shows the decreased AP activity at luminal brush border as compared with control group (×100). 5, AP activity of mucosal brush border of normal duodenum tissues (arrow) (×100). 6, Epithelial lining of C. pepo extract treated duodenum tissues [arrow shows an increased AP activity at luminal brush border as compared with control group] (×100). 7, Epithelial lining of aspirin treated duodenum that decreased AP activity at luminal brush border as compared with control group (arrow) (×100). 8, Epithelial lining of C. pepo+ aspirin treated stomach tissues [arrow shows the decreased AP activity at luminal brush border as compared with control group] (×100).

respectively. Black color reaction products were precipitation of cobalt sulfide (CoS).

After C. pepo extract (400 mg/kg body weight) treatment both the stomach (Fig. 2) and duodenum
(Fig. 6) showed increased AP staining reaction products throughout the epithelial layer as compared to control stomach (Fig. 1) and duodenum (Fig. 5) respectively.

The AP activity diminished along the mucosal brush border in the aspirin treated stomach (Fig. 3) and duodenum (Fig. 7) tissues as compared to control stomach (Fig. 1) and duodenum (Fig. 5). A small amount AP reaction product was found in both aspirin treated stomach (Fig. 3) and duodenum (Fig. 7) tissues.

After C. pepo treatment, aspirin treated stomach (Fig. 4) and duodenum (Fig. 8) also showed the endogenous AP staining reaction products though less as compared to only C. pepo treated stomach (Fig. 2) and duodenum (Fig. 6) respectively.

Mucosal thickness—Treatment with C. pepo extract (400 mg/kg body weight) increased the mucosal thickness of both stomach and duodenum tissues as compared to control but the increase was not statistically significant. Aspirin treated group showed a significant decrease of mucosal thickness as compared to control group. But after pretreatment with C. pepo for 14 days, aspirin showed a significant increase of mucosal thickness as compared to aspirin treated group (Table 2).

Discussion

Analysis of the results showed that aspirin produced extensive increase in ulcer index (UI), decrease in mucosal thickness (MT) and decrease in alkaline phosphatase (AP) activity in rat stomach and duodenal tissues. The decrease of AP activity seems to be a general property of all chemicals which are known to provoke severe ulcer. The activity of AP may be relevant to duodenal ulcer pathogenesis or healing. The pathophysiology of experimental peptic ulcer formation is not well known but is believed to be multifactorial. Factors like increase in acid secretion, reduction of gastric mucosal blood flow, inhibition of prostaglandin synthesis, disruption of mucosal barrier, inhibition of mucus and bicarbonate secretion in the gastro-duodenal mucosa have been suggested.

The pathogenesis of ulcer disease is believed to reflect an imbalance between increased aggressive factors and decreased protective factors. The decreased AP activity imbalances are one of the first lines of defense protecting the gastro-duodenal mucosa and the mucus bicarbonate barrier overlaying the epithelium. The increase in UI, decrease in MT and decrease in AP activity observed in the present study after aspirin may be due to failure in gastrointestinal and repair mechanisms leading to disrupted mucosal barrier.

Pretreatment with C. pepo (200, 300, 350, 400, 450 and 500 mg/kg body weight) showed that 400 mg/kg body weight dose was the most effective dose and significantly decreased UI, increased MT and AP activity in all rats treated with aspirin. The ability of gastric mucosa to resist injury by ingested irritants (aspirin) is attributed to number of factors that have been referred to collectively as mucosal defense. The gastric mucosal lesions induced by necrotizing agents such as aspirin, ethanol and strong alkalis are due to depression of this defense mechanism.

The gastric protective activity such as decrease in UI, increase in MT and increase in AP activity of C. pepo may be associated with correction or normalization of the altered balance between aggressive activity and defensive gastric mucosal activity. Gastric adherent mucus is thought to play an important role as a defensive factor against mucosal damage.

In the present investigation, C. pepo caused a significant enhancement of gastric adherent mucus which plays an important role as a defensive factor against mucosal damage, thus confirming the ability of C. pepo to prevent the effects of damaging agents. These findings indicate that C. pepo pulp extract strengthens the gastric mucosal defense factors in experimental rats.

The chemical constituents of ripe fruit pulp of C. pepo responsible for its anti-ulcer activity are not known. However, pharmacological investigations have suggested the presence of several major groups of active compounds in C. pepo pulp such as syringic acid, cucurbitane-type triterpenes and hexanocucurbitane glycosides such as cucurbitacin L and cucurbitacin K, vitamins A, carotenoids, pro-vitamin A carotenoids, vitamins A, vitamin E and vitamin C have been reported to have a role in protection against gastric mucosal damage.

Vitamin E and C play an important role in the reduction of pathogenesis of ulcer formation by probably reducing the ischemia. The deficiency of dietary vitamin E reduces the synthesis of arterial
prostaglandin significantly\textsuperscript{35} which may trigger ulcer formation.

Thus, it may be suggested that pre-treatment of \textit{C. pepo} may prevent the gastric mucosal damage by aspirin increasing PGE\textsubscript{2} level or by reducing the ischemia\textsuperscript{21} which may be due to the presence of vitamin E and C in the pulp of \textit{C. pepo}.

However, various antioxidant phenolic compounds such as tannin\textsuperscript{36}, vitamin C and E\textsuperscript{33, 34}, triterpenes and glycosides\textsuperscript{79} have been identified as free radicals or active oxygen scavengers present in \textit{C. pepo} which may be responsible for reduced UI and increase in MT.

Both clinical observations on humans and experimental studies on animals suggest a protective action of vitamin A against gastric ulcer induced either by stress or by well-known gastric-offensive agents like non-steroidal anti-inflammatory drugs (NSAIDs)\textsuperscript{37, 38}. The ability of \textit{C. pepo} to protect the mucosa against lesions induced by aspirin (NSAIDs) as seen by the decreased in UI is likely by maintaining the structural integrity of gastric epithelium and balance of aggressive factors and inherent protective mechanism. Further, the mucus gel and its bicarbonate gradient together with the alkaline environment maintained by AP activity seem to be an important first line defense against harmful stimuli.

The exact mechanism by which \textit{C. pepo} acts is not definitely known. It may be that vitamins C and E in \textit{C. pepo} may reduce the local ischemia and may modulate the prostaglandin synthesis hereby increasing the mucosal defense mechanism. The AP present in the gastric mucosa\textsuperscript{39} and duodenal mucosal brush border\textsuperscript{40} may hydrolyze the phosphate ions (PO\textsubscript{4}\textsuperscript{3-}) from the ATP molecules. The phosphate ions (PO\textsubscript{4}\textsuperscript{3-}) thus liberated may activate the P\textsubscript{2} receptors to secrete more bicarbonate ions (HCO\textsubscript{3}\textsuperscript{-}) thus alkalinizing the microclimate surrounding the AP and increasing its activity\textsuperscript{41}. The increased alkaline microclimate helps in the rapid regeneration of the denuded mucosal barrier and decreases the UI and increases the MT and AP activity.

In conclusion, \textit{C. pepo} pulp extract exhibits a potential protective activity possibly by increasing the mucosal defensive mechanism by the presence of vitamin A, C and E or through the triterpenes, glycosides and tannin which exhibit antioxidant activity.

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\textbf{References}


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