DOSE DEPENDENT ANTIULCER ACTIVITY OF AQUEOUS EXTRACT OF RIPE FRUIT’S PULP OF *Cucurbita pepo* Linn. AND RANITIDINE AGAINST ASPIRIN INDUCED GASTRIC AND DUODENAL ULCER INDEX AND MUCOSAL THICKNESS

Fruits of *Cucurbita pepo* Linn. (*Cucurbitaceae*) (Paris et al., 2003; Paris, 2009) are used as vegetable (Sunilson et al., 2009; Sarkar and Guha, 2008; Koike et al., 2005; Mongkolsilp et al., 2004; Sammon et al., 2003; Linskens and Jorde, 1997). Fruit’s pulp of pumpkin is used in dyspepsia, enteritis or intestinal inflammation (Orlandelli, 1951) intestinal diseases (Francois et al., 2006) and used to relieve inflammation (Caili et al., 2006). Fruits are applied to treat liver disorder (Sezik et al., 2004). It has been reported that pumpkin is consumed as a diet to increase the pH of fasting gastric sample (Sammon et al., 2003) and the dietetic management of patients undergoing gastric operations is also carried out by supplementation of pumpkin (Loraskaia et al., 1986).

Some antiulcer compounds such as triterpenoids (Wang et al., 2008), glycosides such as cucurbitacin B, cucurbitacin D, cucurbitacin E, cucurbitacin F (Feng et al., 2007), cucurbitacin L and cucurbitacin K (Wang et al., 2007), sulfhydryls such as glutathione (Alosi et al., 1988), cysteine (Cys or C) (Fahmy et al., 2008; Pham et al., 1985; Allen, 1979; Kleinig et al., 1975; Ogura et al., 1972; Walker, 1972; Katoaka et al., 1998), S-adenosyl methionine (SAM) (Huang et al., 1991), methionine (Met or M) (Yoshida et al., 2005), alkaloid such as tannin (Ojika and Igwe, 2008; Silveira et al., 1996), phenols such as syringic acid (Dragovic-Uzelac et al., 2005), phenolic phytochemicals (Kwon et al., 2007), polyphenol and phenolic contents (Mongkolsilp et al., 2004), polyamines (Nishimura et al., 2006; Martinez-Tellez et al., 2002), beta-carotene (Veda et al., 2006), pro-vitamin A carotenoids (Azevedo-Meleiro et al., 2007; Priyadarshani and Chandrika, 2007; Seo et al., 2005; Mongkolsilp et al., 2004; Manzi et al., 2002; Gonzalez, 2001; Rodriguez-Amaya, 1999; Kune et al., 1992; Arima and Rodriguez-Amaya, 1990; Arima and Rodriguez-Amaya, 1988), vitamins A (Lans et al., 2007; Ahmed et al., 2003; Ribaya-Mercado et al., 1999), vitamin C (Hancock et al., 2008; Mongkolsilp et al., 2004; Hancock et al., 2003) and vitamin E (Franke et al., 2007).
fruit have been reported. Furthermore, some antioxidant-type elements such as selenium (Se) (Yoshida et al., 2005; Stibilj et al., 2004), cadmium (Cd) (Qadir et al., 2000) and multi-trace elements such as zinc (Zn), copper (Cu) and manganese (Mn) (Fan et al., 2006) are present in C. pepo fruit have been reported although compounds of other chemical nature are also evident.

It has been reported that multi-trace elements such as Zn, Cu and Mn are different parts and different growth periods of pumpkin. Some elements essential to human such as Zn, Cu and Mn in pumpkin are abundant, implying that the nutritive value of pumpkin is high (Fan et al., 2006).

Some proteins such as pepocin, a type-1 ribosome-inactivating protein (RIP) (Yoshinari et al., 1996), lectin (Allen, 1979), macvicyanin (Xie et al., 2005; Xie et al., 2003; Marchesini et al., 1979; Kataoka et al., 1998), CpNIP 1, a Nod 16-like protein, patellin 1, a novel sec14-related protein (Peterman et al., 2006), sieve tube proteins (Kleinig et al., 1975; Walker, 1972), aspartic proteinase inhibitor (Christeller et al., 1998) trypsin inhibitor (Pham et al., 1985) and some enzymes such as ascorbic oxidase (Altmann, 1998; Pitari et al., 1998; Kisu et al., 1997; Esaka et al., 1992; Lin et al., 1991; Esaka et al., 1990; Esaka et al., 1989; Chichiricco et al., 1989; D’Andrea et al., 1989; Casella et al., 1988; Esaka et al., 1988; Avigliano et al., 1983; Marchesini et al., 1979; Marchesini and Kroneck, 1979; Marchesini et al., 1977; Porat et al., 1967), glutathione reductase (Alosi et al., 1988), esterase (Fahmy et al., 2008) and S-adenosyl L-methionine methylthioadenosine-lyase (Huang et al., 1991) have been found in the fruit of pumpkin.

Pumpkin in various systems of traditional medicine has been used for several ailments (antidiabetic, antihypertensive, antitumor, immunomodulation, antibacterial, anti-hypercholesterolemia, intestinal antiparasitia, anti-inflammation, antalgic) (Calli et al., 2006).

Dietary deficiencies of protein, vitamins, minerals such as selenium (Se) and zinc (Zn) give rise to the oxidation of bio-molecules and cell injury (Fang et al., 2002; Granger et al., 1986; Izgut-Uysal et al., 1993; Jamal and Sprowls, 1987) leading to peptic ulcer disease (PUD).
Peptic ulcer disease (PUD) is one of the common diseases affecting mankind. "It kills few but troubles many" (Lawrence and Bennett, 1987) results from an imbalance between luminal injurious factors and mucosal resistance. The pathogenesis of peptic ulcer disease (PUD) is believed to reflect an imbalance between increased aggressive factors and decreased protective factors (Sarkar et al., 2006). Damage or impairment of the protective features and subsequent exposure to digestive juices can lead to irritation and erosion of the stomach or duodenal lining. Peptic ulcer disease (PUD) is the disruption of gastric and duodenal lining by various factors.

Numbers of drugs are available to treat peptic ulcer disease (PUD) such as ranitidine, cimetidine, famotidine, nizatidine as a H₂-receptor blocker (Hansten, 1994; Bilchik et al., 1989; Havu et al., 1990). Since acid aggravates gastric and duodenal (peptic) ulcer, one of the approaches for treating this disease is to block acid secretion, which also helps healing the ulcer. Gastric ulcers are thus treated by ranitidine which acts as a H₂-receptor antagonist. The drug is highly effective in controlling overnight acid secretion.

Factors that influence the development of peptic ulcer disease (PUD) are the resultant some endogenous aggressive factors such as hydrochloric acid (HCl), pepsin, refluxed bile, gastric mucosal ischemia, mental stress and tension in daily life and environmental (cold) stress etc. Gastrointestinal ulceration can be formed in rats by exogenous agents like- non-steroidal anti-inflammatory drugs (NSAIDs) (aspirin, indomethacin, ibuprofen and naproxen), ethanol etc. Aspirin is an anti-inflammatory drug (NSAID). Aspirin induces gastrointestinal mucosal damage and forms gastric hyperacidity and gastrointestinal ulceration (Sonnenberg, 1995).

Thus the present study was undertaken to determine the dose dependent antiulcer activity of aqueous extract of ripe fruit's pulp of Cucurbita pepo Linn. and ranitidine against a single dose of aspirin (500 mg/kg body weight) induced gastric and duodenal ulceration in rat model.
Materials and methods:

Animals and grouping: Seventy eight (78) Holtzman strain adult albino rats of both sexes (180-200g) were used throughout this study. Rats were divided into three schedules; schedule I, schedule II, schedule III. Schedule I contains 6 rats (group I Asp) only for aspirin treated, schedule II contains 36 rats (groups II CP&Asp- VII CP&Asp) for C. pepo pretested and aspirin treated and schedule III contains 36 rats (groups II Ran&Asp- VII Ran&Asp) for ranitidine pretested and aspirin treated.

Preparation of animal and treatment:

Schedule I: Aspirin induced ulcerated group

6 rats (group I Asp) were previously fasted for 24 hours and a single dose of aspirin (500 mg/kg body weight) was administered orally by orogastric cannula (Cho and Ogle., 1979; Bose et al., 2003). After 4 hours, the rats were sacrificed and their stomach and duodenum were collected and opened along the greater curvature to expose the mucosal epithelial surface. To note the distribution of ulcer(s) the stomach and duodenum were washed with 0.9% saline and ulcers were scored (Szabo et al., 1985).

Schedule II: Aspirin induced ulcerated groups pretreated with aqueous extract of ripe fruit’s pulp of Cucurbita pepo Linn.

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) and mucosal thickness (MT). Rats (36) were divided into 6 groups (group II CP&Asp -VII CP&Asp) of 6 rats each. Rats (groups II CP&Asp -VII CP&Asp) 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 (Cho and Ogle., 1979; Bose et al., 2003). After 4 hrs the experiment was terminated and rats were sacrificed by an over dose of thiopentone sodium (NEON, Laboratories Ltd, India).
Schedule III: Aspirin induced ulcerated groups were pretreated with referenced anti-ulcer drug ranitidine:

Preliminary studies were done for the selection of effective dose (ED) of ranitidine by evaluating the ulcer index (UI) and mucosal thickness (MT). Rats (36) were divided into 6 groups (group II Ran&Asp -VII Ran&Asp) of 6 rats each. Rats (group II Ran&Asp -VII Ran&Asp) were treated with 7 mg/kg, 8 mg/kg, 9 mg/kg, 10 mg/kg, 11 mg/kg, 12 mg/kg body weight of ranitidine 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, a single dose of 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 (Cho and Ogle,, 1979; Bose et al., 2003). After 4 hrs the experiment was terminated and rats were sacrificed by an over dose of thiopentone sodium (NEON, Laboratories Ltd, India).

Parameters studied:

Ulcers scoring:

The stomach(s) and duodenum(s) were collected and stomach(s) were opened along the greater curvature to expose the mucosal surface and stretched on a flat paraffin bed. The stomach(s) and duodenum(s) were washed with 0.9% normal saline to remove the food particles and to note the distribution of ulcer(s) and the ulcer scoring was performed (Szabo et al., 1985).

Measurement of mucosal thickness (MT) of gastric and duodenal tissues:

For determination of mucosal thickness (MT) the transverse sections (5 µm thick) of stomach and duodenum tissues were taken. The sections were stained with haematoxylin and eosin (H & E). At least 10 determination of mucosal thickness (MT) was made on at least two sections from each specimen. The mucosal thickness (MT) of both tissues was measured from the mucularis mucosa to the tip of the epithelium by a stage micrometer. Sections were examined with objective 10X (visual field diameter, 2.5 mm) and eyepiece 5X (with scale bar inserted) (McQueen et al., 1984).
Results:

Ulcer index (UI): Results showed a significant increase in the extent of ulcerations in gastric and duodenal tissues evidenced by increased ulcer index (UI) in aspirin treated rats. But pretreatment with aqueous extract of fruit’s pulp of *C. pepo* showed significant protection at a dose of 400 mg/kg body weight (*Table 4.1*) and pretreatment with ranitidine showed significant protection at a dose of 10 mg/kg body weight (*Table 4.2*) against a single dose of aspirin at 500 mg/kg body induced ulceration.

Mucosal thickness (MT): Results showed a significant decreased mucosal thickness (MT) in gastric and duodenal tissues in aspirin treated rats. But pretreatment with aqueous extract of fruit’s pulp of *C. pepo* showed significant protection at a dose of 400 mg/kg body weight (*Table 4.1*) and pretreatment with ranitidine showed significant protection at a dose of 10 mg/kg body weight (*Table 4.2*) against a single dose of aspirin at 500 mg/kg body induced ulceration.

*Table 4.1: Effect of different dose of aqueous extract of ripe fruit’s pulp of *Cucurbita pepo* Linn. on ulcer index (UI) and mucosal thickness (MT) against aspirin treated stomach and duodenum*  

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ulcer index (UI)</th>
<th>Mucosal thickness (MT) (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stomach</td>
<td>Duodenum</td>
</tr>
<tr>
<td>Group I-Asp (Distilled water+ aspirin, 500 mg/kg)</td>
<td>41.19±0.69</td>
<td>31.55±1.07</td>
</tr>
<tr>
<td>Group II-CP &amp; Asp (C. pepo, 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-CP &amp; Asp (C. pepo, 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-CP &amp; Asp (C. pepo, 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-CP &amp; Asp (C. pepo, 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-CP &amp; Asp (C. pepo, 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-CP &amp; Asp (C. pepo, 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 and when compared to *(Distilled water+ aspirin) in which minimum mucosal thickness (MT) was produced.

Table 4.2: Effect of different dose of ranitidine on ulcer index (UI) and mucosal thickness (MT) against aspirin treated stomach and duodenum
[Values are mean±SE from 6 rats in each group]

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ulcer index (UI)</th>
<th>Mucosal thickness (MT) (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stomach</td>
<td>Duodenum</td>
</tr>
<tr>
<td>Group I-Asp (Distilled water+ aspirin, 500 mg/kg)</td>
<td>41.19±0.69</td>
<td>31.55±1.07</td>
</tr>
<tr>
<td>Group II-Ran &amp; Asp (Ranitidine, 7 mg/kg+ aspirin, 500 mg/kg)</td>
<td>27.68±0.30</td>
<td>20.15±0.37</td>
</tr>
<tr>
<td>Group III-Ran &amp; Asp (Ranitidine, 8 mg/kg+ aspirin, 500 mg/kg)</td>
<td>13.5±0.53</td>
<td>10.73±0.25</td>
</tr>
<tr>
<td>Group IV-Ran &amp; Asp (Ranitidine, 9 mg/kg+ aspirin, 500 mg/kg)</td>
<td>6.76±0.24</td>
<td>5.81±0.23</td>
</tr>
<tr>
<td>Group V-Ran &amp; Asp (Ranitidine, 10 mg/kg+ aspirin, 500 mg/kg)</td>
<td>1.55±0.38*</td>
<td>1.38±0.33*</td>
</tr>
<tr>
<td>Group VI-Ran &amp; Asp (Ranitidine, 11 mg/kg+ aspirin, 500 mg/kg)</td>
<td>1.51±0.38</td>
<td>1.28±0.07</td>
</tr>
<tr>
<td>Group VII-Ran &amp; Asp (Ranitidine, 12 mg/kg+ aspirin, 500 mg/kg)</td>
<td>1.25±0.35</td>
<td>1.05±0.22</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 (ulcer index; UI) was produced and when compared to *(distilled water+ aspirin) in which minimum mucosal thickness (MT) was remained.

Ulcerative photograph in different groups

Ulcer less control stomach tissue has been shown Fig 4.1.1.

After *C. pepo* extract (400 mg/kg body weight) treatment the stomach (Fig 4.1.2) showed ulcer less throughout the epithelial layer.

Aspirin treated stomach (Fig 4.1.3) tissue showed large number of ulcerations throughout the epithelial layer as compared to control stomach (Fig 4.1.1).
*C. pepo* pretreatment in aspirin treated stomach (Fig 4.1.4) also showed few ulcerations throughout the epithelial layer though less as compared to only *C. pepo* treated stomach (Fig 4.1.2).

Ranitidine pretreatment in aspirin treated stomach (Fig 4.1.5) also showed few ulcerations throughout the epithelial layer though less as compared to only *C. pepo* treated stomach (Fig 4.1.2).

**Histology of stomach and duodenum tissues by H & E stain:**

Mucosal thickness (MT) with erosion less control stomach and control duodenum tissues have been shown Fig 4.2.1 and Fig 4.2.6 respectively.

After *C. pepo* extract (400 mg/kg body weight) treatment both the stomach (Fig 4.2.2) and duodenum (Fig 4.2.7) showed erosion less throughout the epithelial layer with increased mucosal thickness (MT) as compared to control stomach (Fig 4.2.1) and duodenum (Fig 4.2.6) respectively.

The erosions throughout the epithelial layer with decreased mucosal thickness (MT) have been shown in the aspirin treated stomach (Fig 4.2.3) and duodenum (Fig 4.2.8) tissues as compared to control stomach (Fig 4.2.1) and duodenum (Fig 4.2.6) tissues.

*C. pepo* pretreatment in aspirin treated stomach (Fig 4.2.4) and duodenum (Fig 4.2.9) also showed few erosions throughout the epithelial layer with reduced mucosal thickness (MT) though less as compared to only *C. pepo* treated stomach (Fig 4.2.2) and duodenum (Fig 4.2.7) respectively.

Ranitidine pretreatment in aspirin treated stomach (Fig 4.2.5) and duodenum (Fig 4.2.10) also showed few erosions throughout the epithelial layer with reduced mucosal thickness (MT) though less as compared to only *C. pepo* treated stomach (Fig 4.2.2) and duodenum (Fig 4.2.7) respectively.
Ulceration photographs in different groups

Fig 4.1.1: Control  Fig 4.1.2: C. *pepo* (ED)  Fig 4.1.3: Aspirin  Fig 4.1.4: C. *pepo* (ED)+Aspirin  Fig 4.1.5: Ran+Aspirin

Stomach: H&E stain at (×100) magnifications

Fig 4.2.1: Control  Fig 4.2.2: CP(ED)  Fig 4.2.3: Aspirin  Fig 4.2.4: CP(ED)+Asp  Fig 4.2.5: Ran(ED)+Asp

Duodenum: H&E stain at (×100) magnifications

Fig 4.2.6: Control  Fig 4.2.7: CP(ED)  Fig 4.2.8: Aspirin  Fig 4.2.9: CP(ED)+Asp  Fig 4.2.10: Ran(ED)+Asp
Legends for ulcer photograph:

Fig 4.1.1: Shows ulcer less control stomach tissue.

Fig 4.1.2: Shows *C. pepo* extract treated ulcer less stomach tissue.

Fig 4.1.3: Shows aspirin treated stomach tissue with large number of ulcerations as compared with control stomach.

Fig 4.1.4: Shows *C. pepo*+aspirin treated stomach tissue with little ulceration throughout the epithelial layer though less as compared to only *C. pepo* treated stomach (Fig 4.1.2).

Fig 4.1.5: Shows ranitidine+aspirin treated stomach tissue with little ulceration throughout the epithelial layer though less as compared to only *C. pepo* treated stomach (Fig 4.1.2).

Legends for H&E stain:

Fig 4.2.1: Shows the mucosal thickness (MT) with erosion less luminal epithelial lining of glandular part of control stomach tissue (×100).

Fig 4.2.2: Luminal epithelial lining of glandular part of *C. pepo* extract treated stomach tissue showed an increased mucosal thickness (MT) with erosion less luminal epithelial lining as compared with control stomach (×100).

Fig 4.2.3: Luminal epithelial lining of glandular part of aspirin treated stomach tissue that decreased mucosal thickness (MT) with mucosal erosions as compared with control stomach (×100).

Fig 4.2.4: Luminal epithelial lining of glandular part of *C. pepo*+aspirin treated stomach tissue showed a decreased mucosal thickness (MT) with erosion less though less as compared to only *C. pepo* treated stomach (×100).

Fig 4.2.5: Luminal epithelial lining of glandular part of ranitidine+aspirin treated stomach tissue showed a decreased mucosal thickness (MT) with erosion less though less as compared to only *C. pepo* treated stomach (×100).

Fig 4.2.6: Shows the mucosal thickness (MT) with erosion less normal duodenum tissue (×100).

Fig 4.2.7: Mucosal epithelial lining of *C. pepo* extract treated duodenum tissue showed an increased mucosal thickness (MT) with erosion less as compared with control duodenum (×100).
Fig 4.2.8: Mucosal epithelial lining of aspirin treated duodenum that decreased mucosal thickness (MT) with erosions as compared with control duodenum (×100).

Fig 4.2.9: Mucosal epithelial lining of C. pepo+aspirin treated duodenum showed a decreased mucosal thickness (MT) with erosion less though less as compared to only C. pepo treated duodenum (×100).

Fig 4.2.10: Mucosal epithelial lining of ranitidine+aspirin treated duodenum showed a decreased mucosal thickness (MT) with erosion less though less as compared to only C. pepo treated duodenum (×100).

Dose selection by correct choice:

From the results, C. pepo at a dose of 400 mg/kg body weight and ranitidine at a dose of 10 mg/kg body weight exhibited significant protection against a single dose of aspirin (500 mg/kg body weight) induced ulceration. So, the dose of C. pepo (400 mg/kg body weight) is equivalent to the dose of ranitidine (10 mg/kg body weight) and hence both these doses such as C. pepo (400 mg/kg body weight) and ranitidine (10 mg/kg body weight) were used for further experiments.

Discussion:

The present study evaluates the dose dependent antiulcer activity of aqueous extract of ripe fruit's pulp of Cucurbita pepo Linn. and ranitidine against the aspirin induced gastric and duodenal ulceration in rat model. Aspirin increased the ulcer index (UI) and decreased mucosal thickness (MT) (Sarkar and Guha, 2008). The increase in ulcer index (UI) and decrease in mucosal thickness (MT) of gastric and duodenal tissues may be related to gastric haemorrhage (Chang et al., 2005), mucosal injury of gut lumen (Gurleyik et al., 2006) and peptic ulcer disease (PUD) (Sarkar and Guha, 2008). The pathophysiology of experimental peptic ulcer formation is not clearly known (Dhikav et al., 2003), so an unified concept for development of gastric and duodenal lesions by various factors has not yet developed, but generally agreed it is multimechanisms (Goodwin et al., 1986; Konturek et al., 1999) and multifactorial process (Guzel et al., 1998). The multifactors like disruption of integrity in mucosal barrier, an increase in acid secretion, reduction of gastric mucosal blood flow, inhibition of prostaglandins (PGs) synthesis, inhibition of mucus synthesis, bicarbonate secretion (Aase, 1989; Allen and Leonard, 1988) and ischemia (Guzel et al, 1998) have been suggested.
It is evident from the results of the present investigation that pretreatment with aqueous extract of ripe fruit's pulp of *Cucurbita pepo* Linn. and ranitidine significantly decreased the ulcer index (UI) and increased mucosal thickness (MT) in a dose dependent manner. The finding can be explained with the possible involvement of the gastric and duodenal protection by defensive mechanisms of gastro-duodenal tissues possibly by increasing mucus production, reduction of ischemia, inhibition of free radical production, scavenging of free radicals and inhibition of oxidative mucosal damage which possibly led to increase MT and decrease UI in the present investigation.

The pathogenesis of ulcer disease is believed to reflect an imbalance between increased aggressive factors and decreased protective factors (Sarkar *et al*., 2006). The increase in ulcer index (UI) and the decrease in mucosal thickness (MT) observed in the present study after aspirin treatment may be due to failure of defense mechanisms and restitution of cells by repair mechanisms in gastro-duodenal protection leading to disrupted mucosal barrier (Dhikav *et al*., 2003; Allen and Leonard, 1988).

Pretreatment with aqueous extract of ripe fruit's pulp of *C. pepo* showed that 400 mg/kg body weight was the most effective dose (ED) and significantly decreased the ulcer index (UI) and increased the mucosal thickness (MT) in all rats treated with aspirin (Sarkar and Guha, 2008). On the other hand, pretreatment with ranitidine showed that 10 mg/kg body weight was the most effective dose (ED) and significantly decreased the ulcer index (UI) and increased the mucosal thickness (MT) in all rats treated with aspirin.

So, pretreatment of *C. pepo* (400 mg/kg body weight) among the doses of 200, 300, 350, 400, 450 and 500 mg/kg body weight and pretreatment of ranitidine (10 mg/kg body weight) among the doses of 7, 8, 9, 10, 11 and 12 mg/kg body weight showed that *C. pepo* at a dose 400 mg/kg body weight and ranitidine at a dose 10 mg/kg body weight were the most effective dose (ED) and both these doses significantly decreased the ulcer index (UI) and increased the mucosal thickness (MT) in all rats against treated with a single dose of aspirin at 500 mg/kg body weight.

From the results, *C. pepo* at a dose of 400 mg/kg body weight and ranitidine at a dose of 10 mg/kg body weight exhibited significant protection against a single dose of aspirin at 500 mg/kg body weight induced gastric and duodenal ulceration. So, the 400
mg/kg body weight dose of *C. pepo* showed an equivalent protective effect to the 10 mg/kg body weight dose of ranitidine. 10 mg/kg body weight ranitidine exhibited significant protection against ethanol induced ulceration in rats (Kath and Gupta, 2006).

The ability of gastric and duodenal mucosa to resist injury by ingested irritants (aspirin) is characterised to number of factors that have been referred to collectively as mucosal defense activity (Wallace, 2001a). The gastric and duodenal mucosal lesions induced by necrotizing agent(s) such as aspirin, ethanol and strong alkalis are due to inhibition of this defense mechanisms (Kinoshita *et al.*, 1995).

Peptic ulcer develops due to disruption of epithelial cell linings of gastric mucosa (Debnath and Guha, 2007) and a break of mucosal defense mechanisms (Sonnenberg, 1995) when the balance between some aggressive and defensive factors is lost (Sarkar *et al.*, 2006). **Exogenous aggressive** factors such as intake of non steroidal anti-inflammatory drugs (NSAIDs; aspirin, indomethacin and ibuprofen etc) influence the **endogenous aggressive** factors like excessive secretion of hydrochloric acid (HCl) (Schoen and Vender, 1989), pepsin, bile (Sonnenberg, 1995) which lead to peptic ulcer disease (PUD) damaging protective mucosal barrier and mucosal defense mechanisms.

Non- steroidal anti-inflammatory drugs (NSAIDs) damage the gastric mucosal barrier which allows back-diffusion of \( H^+ \), decreases the mucus, \( HCO_3^- \) secretion and pH of gastric juice and thereby reduces surface hydrophobicity, decreases mucosal blood flow and cellular regeneration (Schoen and Vender, 1989). Back diffusion of acid through the breached mucosa it destroys the cell of capillaries and veins causing hemorrhagic ulcer (Chang *et al.*, 2005).

Fruits of *Cucurbita pepo* Linn. are used as vegetable (Sunilson *et al.*, 2009; Linskens and Jorde, 1997). It has been reported that pumpkin is consumed as a diet to increase the pH of fasting gastric sample (Sammon *et al.*, 2003) and the dietetic management of patients undergoing gastric operations is also carried out by supplementation of pumpkin (Loranskaia *et al.*, 1986). Fruit’s pulp of pumpkin prevents dyspepsia and enteritis or intestinal inflammation (Orlandelli, 1951), intestinal diseases (Francois *et al.*, 2006) and used to relieve inflammation (Caili *et al.*, 2006).
Thus, it may be suggested that pretreatment of ripe fruit’s pulp of *Cucurbita pepo* Linn. and ranitidine may prevent the gastric mucosal damage by aspirin with the help of ulcer healing activity possibly by decreasing acid secretion and decreasing inflammatory erosion or ulceration as well as by increasing pH of gastric juice which led to decrease ulcer index (UI) and led to increase mucosal thickness (MT) in the present investigation.

Intragastric administration of epidermal growth factor (EGF) promotes gastric ulcer healing, partly through restitution of angiogenesis (Hase *et al.*, 1989). Non-steroidal anti-inflammatory drugs (NSAIDs; aspirin, indomethacin) also induces gastric ulceration by interfering epidermal growth factor (EGF) binding in its receptors in cultured gastric cells, thereby decreasing their proliferative response to EGF (as well as proliferation is under control of EGF) (Fujiwara *et al.*, 1995).

It is reported that polyamines has been associated with cell proliferation during ulcer healing (Wang *et al.*, 1990; Brzozowski *et al.*, 1993) and angiogenesis in placenta (Reynolds and Redmer, 2001). A number of studies have demonstrated that polyamines are involved in epidermal growth factors (EGF) mediated gastro-protection, ulcer healing and inhibition of acid secretion (Konturek, 1991; Wojciechowski *et al.*, 1995; Ray *et al.*, 1982; Aihara *et al.*, 1983). Therefore, polyamines are essential for the normal postnatal development, maintenance, and function of gastrointestinal epithelia (Cheng *et al.*, 2004) and cell growth and differentiation (Aziz *et al.*, 1996). It has been also reported that polyamine-rich food materials of pumpkin as a dietary source of polyamines (Nishimura *et al.*, 2006; Martinez-Tellez *et al.*, 2002).

Thus, it may be suggested that pretreatment of ripe fruit’s pulp of *C. pepo* may prevent the damage of gastric and duodenal mucosal blood vessels by aspirin by increasing angiogenesis and epidermal growth factors (EGF) mediated gastric and duodenal protection possibly by decreasing acid secretion and decreasing ulceration with haemorrhage leading to ulcer healing activity as evidenced by decreased ulcer index (UI) and increased mucosal thickness (MT) in the present experiment with the help of polyamines which may be due to the presence of polyamines (Nishimura *et al.*, 2006; Martinez-Tellez *et al.*, 2002) in the fruit’s pulp of pumpkin.
Non-steroidal anti-inflammatory drugs (NSAIDs; aspirin, indometacin) also augment gastric lesions by inhibiting angiogenesis (Jones et al., 1999), by developing ischemia (Pihan et al., 1987; Vaananenn et al., 1991; Salim, 1992; Yoshikawa et al., 1993; Miura et al., 2002), by decreasing blood flow (Schoen and Vender, 1989) and by generating reactive oxygen species (ROS) (Itoh and Guth, 1985; Perry et al., 1986; Yoshikawa et al., 1989; Guzel et al., 1998) and lesions by inhibiting angiogenesis in placenta (Reynolds and Redmer, 2001).

Vitamin C (Brzozowski et al., 2001; Bielanski et al., 2001; Pohle et al., 2001; McAlindon et al., 1996) and vitamin E (Saad et al., 2002; Zaror-Behrens et al., 1992) play an important role against NSAIDs in the reduction of pathogenesis of ulcer formation which vitamins led to decrease ulcer index (UI) and led to increase mucosal thickness (MT) by probably reducing the ischemia generated by ROS (Perry et al., 1986; Ichikawa et al., 2003; Kitano et al., 1997; Nakamoto et al., 1997; Toshikazu et al., 1991; Yoshikawa et al., 1991; Yoshikawa et al., 1989). It has been found that the deficiency of dietary vitamin E reduces the synthesis of arterial prostaglandins (PGs) significantly (Okuma et al., 1980) which may trigger ulcer formation in the present experiment.

Thus, it may be suggested that pretreatment of *C. pepo* may prevent the gastric and duodenal mucosal damage by aspirin increasing PGE2 level or by reducing the ischemia (Guzel et al, 1998) which may be due to the presence of vitamin C (Hancock et al., 2008; Mongkolsilp et al., 2004; Hancock et al, 2003) and vitamin E (Franke et al., 2007; Tadmor et al, 2005; Imaeda et al., 1999; Sedghi et al, 2008) in the fruit of *C. pepo* have been found.

It is well known that *exogenous aggressive* factors influence the *endogenous aggressive* factors. Excessive intake of non steroidal anti-inflammatory drugs (NSAIDs) influence the reactive oxygen species (ROS) such as O$_2^*$, H$_2$O$_2$ and *OH etc (Vaananenn et al., 1991; Yoshikawa et al., 1993; Salim, 1992; Pihan et al, 1987) and these ROS cause the disruption of mucosal barrier (Phull et al, 1995; Yoshikawa et al., 1993).

Various antioxidant such as phenolic compounds like *syringic acid* (Dragovic-Uzelac et al., 2005), phenolic phytochemicals (Kwon et al., 2007), phenolic phytochemicals (Kwon et al., 2007), polyphenol and phenolic contents (Mongkolsilp et
alkaloid such as tannin (Ojiako and Igwe, 2008; Silveira et al., 1996), vitamin C (Hancock et al., 2008; Mongkolsilp et al., 2004; Hancock et al., 2003), vitamin E (Franke et al., 2007; Tadmor et al., 2005; Imaeda et al., 1999; Sedghi et al., 2008), triterpenes (Wang et al., 2008) and glycosides such as cucurbitacin B, cucurbitacin D, cucurbitacin E, cucurbitacin F (Feng et al., 2007), cucurbitacin L and cucurbitacin K (Wang et al., 2007), sulphydryl like glutathione (Alosi et al., 1988), cysteine (Cys or C) (Fahmy et al., 2008; Pham et al., 1985; Allen, 1979; Kleining et al., 1975; Ogura et al., 1972; Walker, 1972; Kataoka et al., 1998), S-adenosyl methionine (SAM) (Huang et al., 1991), methionine (Met or M) (Yoshida et al., 2005) are present in fruit’s pulp of *C. pepo*. Some of these components including syringic acid (Aberoumand and Deokule, 2008; Li et al., 2007; Zhoa et al., 2006; Fecht-Christoffers et al., 2006; Que et al., 2006; Chen et al., 2005; Wang et al., 2004; Yrjonen et al., 2003; Baublis et al., 2000; Pecur et al., 2000; Hirota et al., 2000; Masaki et al., 1995), tannin (Banerjee et al., 2008; Souza et al., 2006; Ramirez and Roa, 2003; Repetto and Llesuy, 2002; Czinner et al., 2001; Ezaki et al., 1985), vitamin C (Fang et al., 2002; Alzoghaibi, 2007), vitamin E (Guzel et al., 1998), triterpenes and glycosides (Naseri and Mard, 2007), sulphydryls (Loguercio et al., 1991; Szabo et al., 1981), glutathione (Fang et al., 2002; Kimura et al., 2001; Loguercio et al., 1993; Boyd et al., 1981), cysteine (Cys or C) (Bourdon et al., 2005; Loguercio et al., 1991), SAM (Caro and Cederbaum, 2004; Evans et al., 1997) and methionine (Galey et al., 2007; Bourdon et al., 2005; Panasenko et al., 2005; Erdmann et al., 2005; Slyshenkov et al., 2002; Selvam and Ravichandran, 1991; Levine et al., 2000; Levine et al., 1996; Stadtman et al., 2002; Kroger et al., 1997; Patra et al., 2001; Devasagayam et al., 1991) have been reported to have a role in protection against gastric mucosal damage as a free radicals or reactive oxygen species (ROS) scavengers those are present in *C. pepo* and they may be responsible for reduced ulcer index (UI) and increased mucosal thickness (MT) in the present experiment.

Strong antioxidant elements such as selenium (Se) (Fang et al., 2002; Combs, 2001; Combs, 1999; O’Brien, 1992; Jamal and Sprowls, 1987; Brady et al., 1979; Young et al., 1976), zinc (Zn), (Fang et al., 2002) and cadmium (Cd) (Izgut-Uysal et al., 1993; Jamal and Sprowls, 1987) etc exert synergistic actions in scavenging free radicals. Therefore, several biochemical compounds are known to possess antioxidant activity. Furthermore, some antioxidant-type element such as selenium (Se) (Yoshida et al., 2005; Stibilj et al, 2004), cadmium (Cd) (Qadir et al., 2000) and multi-trace elements such as
Zn, Cu and Mn (Fan et al., 2006) are present in C. pepo fruit have been reported which have showed a role in protection against gastric and duodenal mucosal damage as a free radicals or reactive oxygen species (ROS) scavengers and they may be responsible for reduced ulcer index (UI) and increased mucosal thickness (MT) in the present experiment.

Both clinical observations on humans and experimental studies on animals suggests 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) (Sarkar and Guha., 2008; Mozsik et al., 1989; Kasper et al., 1975). Aqueous extract of ripe fruit’s pulp of C. pepo exhibits a potential protective activity possibly by increasing the mucosal defensive mechanisms by the presence of beta-carotene (Veda et al., 2006), pro-vitamin A carotenoids (Azevedo-Meleiro et al., 2007; Priyadarshani and Chandrika, 2007; Seo et al., 2005; Mongkolsilp et al., 2004; Manzi et al., 2002; Gonzalez, 2001; Rodriguez-Amaya, 1999; Kune et al., 1992; Arima and Rodriguez-Amaya, 1990; Arima and Rodriguez-Amaya, 1988) and vitamins A (Lans et al., 2007; Ahmed et al., 2003; Ribaya-Mercado et al., 1999).

Therefore, it can be explained that serotonin (5-hydroxytryptamine; 5-HT) and 5-methoxytryptamine (5-MT) are metabolized to the corresponding same amino acid tryptophan (Trp or W) by monoamine oxidase (MAO). N-acetylation of serotonin (5-hydroxytryptamine; 5-HT), followed by O-methylation in the pineal body and forms melatonin, a potent antioxidant and acts against ulceration (Bandyopadhyay et al., 2000). Serotonin has been shown to stimulate mucus production in the gastrointestinal tract (GIT) of dogs (Racke et al., 1988). As enterochromaffin (EC) cells produce serotonin (5-hydroxytryptamine; 5-HT) and lie in close vicinity to mucus producing cells, it is tempting to assume that local serotonin (5-HT) production normally influences mucus production by paracrine action (Kaufmann et al., 1979; Konturek et al., 1987; Pesker, 1980). Melatonin is released from gastrointestinal enterochromaffin cells (Kvetnoy et al., 2002; Raikhlin and Kvetnoy, 1976) and stimulates duodenal bicarbonate secretion via action at enterocyte MT2-receptors (Sjoblom et al., 2001).

Thus, it may be suggested that pretreatment of C. pepo may prevent the gastric and duodenal mucosal damage increasing adherent mucus in gastro-duodenal tissues as
evidenced by increased mucosal thickness (MT) in the present investigation which may be due to the presence of tryptophan in fruit’s pulp of *C. pepo* (Allen, 1979; Huang *et al*., 1991; Yoshinari *et al*., 1996; Xie *et al*., 2005; Xie *et al*., 2003; Marchesini *et al*., 1979; Kataoka *et al*., 1998).

The ability of *C. pepo* to protect the mucosa against lesions induced by aspirin (NSAIDs) as seen by the decrease in ulcer index (UI) and increase in mucosal thickness (MT) are likely by maintaining the structural integrity of gastric epithelium and balance of aggressive factors and inherent protective mechanism.

In conclusion, aqueous extract of ripe fruit’s pulp *C. pepo* exhibits a potential protective activity possibly by increasing the mucosal defensive mechanisms by the presence of vitamin A, C and E or through the triterpenes, glycosides such as cucurbitacin B, cucurbitacin D, cucurbitacin E, cucurbitacin F, cucurbitacin L and cucurbitacin K, polyamines, syringic acid, sulphhydrils, tryptophan containing proteins maviyacianin and lectin and tannin, selenium (Se), Zinc (Zn), cadmium (Cd) etc. which exhibit antioxidant activity.