CHAPTER- VIII

ANTIOXIDANT AND ANTIULCER ACTIVITY OF AQUEOUS EXTRACT OF RIPE FRUIT’S PULP OF Cucurbita pepo Linn. AND RANITIDINE AGAINST IMMOBILIZED COLD-STRESS INDUCED GASTRIC AND DUODENAL ULCERATION OF EXPERIMENTAL RAT MODEL

A deleterious effect of ulcerogen such as stress (Yoshikawa et al., 1990) produces the reactive oxygen species (ROS) (Bandyopadhyay et al., 2000; Yoshikawa et al., 1990; Cochron et al., 1982; Das et al., 1997; Biswas et al., 2003) which result in damage to bio-molecules such as lipids, proteins, enzymes, amino acids and DNA (Halliwell and Gutteridge, 1990; Imlay and Linn, 1988; Simmonds et al., 1992; Biswas et al., 2003). Free radicals such as superoxide anion (O$_2^-$), singlet oxygen, hydroxyl radicals (•OH) and other reactive oxygen species (ROS) such as hydrogen peroxide (H$_2$O$_2$), peroxynitrite and hypochlorus acid (HClO) etc are produced in the body, primarily as a result of aerobic metabolism. Dietary deficiencies of protein, vitamins (Fang et al., 2002; Granger et al., 1986) minerals such as selenium (Se) and zinc (Zn) give rise to the oxidation of bio-molecules and cell injury (Izgut-Uysal et al., 1993; Jamal and Sprowls, 1987).

"Oxidative stress" is a condition when antioxidant defense mechanisms are hampered due to generation of some reactive oxygen species (ROS) namely superoxide anion (O$_2^-$), hydroxyl radicals (•OH) and hydrogen peroxide (H$_2$O$_2$) in the cell by stress. Reactive oxygen species (ROS) have been regarded as highly toxic agents responsible for a wide variety of tissues damage (Halliwell and Gutteridge, 1986).

Peptic ulcer develops due to a break down of mucosal defense mechanisms (Sonnenberg, 1995) and epithelial lining cells of gastric mucosa (Debnath and Guha, 2007) when the balance between some aggressive and defensive factors is lost (Sarkar et al., 2006). Exogenous aggressive factors such as environment factor like cold stress, immobilize induced mental stress and various tension in our daily life (Yoshikawa et al., 1990).
1990), intake of NSAIDs (Vaanannen et al., 1991; Yoshikawa et al., 1993; Pihan et al., 1987), chronic alcohol consumption (Peskar et al., 1986; Pihan et al., 1987; Szelenyi and Brune, 1988) and *H. pylori* infection (Demir et al., 2003) influence to produce endogenous aggressive factors like HCl, pepsin, bile, leukotrienes, oxygen derived free radicals and reactive oxygen species (ROS) such as O$_2^*$, H$_2$O$_2$ and OH etc and cause to disrupt the mucosal barrier with lining cells by damaging bio-molecules (Halliwell and Gutteridge, 1990) which lead to pathology of gastro-duodenal ulceration due to fall of activity of endogenous antioxidant systems of the gastric and duodenal tissues. Epigastric pain is the common clinical feature of peptic ulcer and in severe cases blood appears in the vomiting substances. Management of this painful disease, its prevention or cure is one of the challenging problems today globally by non-toxic dietary herbal products of vegetables and fruits.

However, the mucosal defense mechanisms that offer cytoprotection are included the mucus-HCO$_3^-$ barrier, surface active phospholipids, mucosal blood flow, cell restitution and regeneration, cellular antioxidant reduced glutathione (GSH), antioxidant enzymes such as superoxide dismutase (SOD), catalase and other enzymes such as, PG synthase, nitric oxide synthase (NOS) and epidermal growth factors (EGF) etc against these exogenous and endogenous aggressive factors.

A large body of the literature supports the notion that dietary antioxidants are useful and which play an important role in preventing many human diseases (Fang et al., 2002) like peptic ulcer disease (PUD). Origin of several anti-oxidative dietary fruits (Chun et al., 2005) and anti-ulcer compounds from some medicinal plants (Lewis and Hanson, 1991) has been reported.

Dietary antioxidants play a crucial role in the prevention of stress induced peptic ulcer disease (PUD). A number of Indian medicinal plants and fruits have been extensively used in the Indian traditional Ayurvedic system of medicine in the treatment of peptic ulcer disease (PUD) for their antioxidant properties. Some of these plants have already been reported to possess strong antioxidant activity such as chamomile (Beil et al., 1995), vegetables such as carrot, tomato (Davey et al., 2000) and fruits such as apple, banana, cucumber, guava, melon, orange, (Davey et al., 2000; Scartezzini and Speroni, 2000). Leaves such as *Camellia sinensis* (tea) leaves (Euahelhardt et al., 2001),
and *Osimum sanctum* (tulsi) leaves (Kath and Gupta, 2006) and *Azadirachta indica* (neem) leaves (Bandyopadhyay et al, 2002). Antioxidants, these include large protein molecules antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione reductase and glutathione peroxidases etc and the small molecular weight substances such as arginine, citrulline, (Fang et al., 2002), taurine (Panasenko et al., 2005; Fang et al., 2002), carnitine (Dokme ci et al., 2005; Izgut-Uysal et al., 2007; Izgut-Uysal et al., 2001), sulfhydryls (Loguercio et al., 1991; Szabo et al., 1981) like glutathione (Kimura et al., 2001; Loguercio et al., 1993), cysteine (Cys or C) (Bourdon et al., 2005; Loguercio et al., 1991), SAM (Caro and Cederbaum, 2004; Evans et al., 1997), strong antioxidant elements like selenium (Se), zinc (Zn) and vitamin A, vitamin C, vitamin E and polyphenols exert synergistic actions in scavenging free radicals. Therefore, several biochemical compounds are known to possess antioxidant activity (Fang et al., 2002).

Recently, interest has been focused towards the role of ROS in gastrointestinal disorders, related to antioxidants and gastrointestinal mucosal damage. *Cucurbita pepo* Linn. (*C. pepo*) is one of those plants and in the preliminary investigations of aqueous extract of ripe fruit’s pulp of *C. pepo* exhibited significant protection of gastric and duodenal ulceration at a dose of 400 mg/kg body weight by decreasing ulcer index (UI) and concomitantly increasing mucosal thickness (MT) and increasing alkaline phosphatase (AP) enzyme activity intact in gastric and duodenal tissues which cumulatively increased mucosal defense activity of gastric and duodenal tissues in all rats against treated with aspirin (Sarkar and Guha, 2008). In order to contribute further to the knowledge of Indian traditional plant’s fruits, in the present study on aqueous extract of ripe fruit’s pulp of *Cucurbita pepo* Linn. has been used for the treatment peptic ulcer and has been screened to determine its antioxidant activity.

Several antioxidant-type anti-ulcer compounds such as triterpenoids (Wang et al., 2008), glycosides (Wang et al., 2007), sulfhydryls like glutathione (Alosi et al., 1988), cysteine (Cys or C) (Fahmy et al., 2008; Pham et al., 1985; Allen, 1979; Kleinig et al., 1975; Walker, 1972; Kataoka et al., 1998), S-adenosyl methionine (SAM) (Huang et al., 1991), methionine (Met or M) (Yoshida et al., 2005), phenolic compounds such as tannin (Ojiako and Igwe, 2008; Silveira et al., 1996), syringic acid (Dragovic-Uzelac et
phenolic phytochemicals (Kwon et al., 2007), 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; Arima and Rodriguez-Amaya, 1990; Kune et al., 1992; Arima and Rodriguez-Amaya, 1988) and 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; Tadmor et al., 2005; Imaeda et al., 1999; Sedghi et al., 2008) are present in C. pepo fruit have been reported.

Thus the present study was undertaken to determine the antioxidant activity of aqueous extract of ripe fruit’s pulp of C. pepo against immobilized cold-stress induced gastric and duodenal ulceration in rat model.

Materials and methods:

Animal grouping and treatment:

Thirty (30) rats were divided into five groups of 6 rats each. Group I animals comprised control group. Group II was C. pepo extract treated, Group III was immobilized cold-stress induced, Group IV was C. pepo pretreated and immobilized cold-stress induced and Group V was ranitidine pretreated and immobilized cold-stress induced.

The dry extract was dissolved in distilled water. Group I and Group III rats were given distilled water orally by orogastric cannula with approximately same volume of C. pepo extract. Group II and group IV rats were pretreated with selected dose of C. pepo extract (400 mg/kg body weight) group V rats were pretreated with selected dose of ranitidine (10 mg/kg body weight) orally by orogastric cannula once daily for 14 consecutive days at a particular time (10:30-11:30 hrs) in every day. From the 8th day of experiment group III, group IV and group V rats were suspended horizontally in restraint case at dark condition and subjected to cold stress by placing them in the refrigerator compartment at 4°C temperature for 3 hours daily and repeated consecutively for 7 days as per procedure (Senay and Levine, 1967). On the 14th day, after feeding the extract,
food was withdrawn from rats of group III, group IV and group V but had free access to water. On the 15th day, the experiment was terminated and rats were sacrificed by an over dose of thiopentone sodium (NEON, Laboratories Ltd, India).

**Parameters studied:**

**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., 1985.

**Superoxide dismutase (SOD):** Stomach and duodenal tissues were homogenized in ice cold phosphate buffer. 0.1 ml of homogenized sample was taken for estimation of SOD activity spectrophotometrically (Marklund and Marklund, 1974).

**Catalase (CAT):** Stomach and duodenal tissues were homogenized in ice cold phosphate buffer. 5 ml of homogenized sample was taken for estimation of CAT activity spectrophotometrically (Aebi, 1974).

**Reduced glutathione (GSH):** reduced glutathione was measured according to the method of Ellman (Ellman, 1959).

**Lipid peroxidation (LPO):** Stomach and duodenal tissues were homogenized in 5 ml ice cold phosphate buffer. Homogenized sample was taken for estimation of LPO activity spectrophotometrically (Rehncrona et al., 1980).

**Mucosal thickness (MT):** 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 (MT) was made on at least two sections from each specimen. The mucosal thickness (MT) 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) (McQueen et al., 1984).
Results:

Ulcer index (Ul): Immobilized cold-stress induced rats showed a significant increase in ulcer index (UI) in both stomach and duodenum tissues as compared to control stomach and duodenum tissues respectively but pretreatment with aqueous extract of ripe fruit’s pulp of *C. pepo* (400 mg/kg body weight) for 14 consecutive days in immobilized cold-stress induced stomach and duodenum tissues showed a significant decrease in the ulcer index (Ul) as compared to immobilized cold-stress induced stomach and duodenum tissues respectively. Therefore, pretreatment with ranitidine (10 mg/kg body weight) for 14 consecutive days in immobilized cold-stress induced stomach and duodenum tissues also showed a significant decrease in the ulcer index (Ul) as compared to immobilized cold-stress induced stomach and duodenum tissues respectively (Table 8.1).

Superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) level and lipid peroxidation (LPO): Treatment with aqueous extract of ripe fruit’s pulp of *C. pepo* (400 mg/kg body weight) significantly increased the SOD, CAT activity, GSH level and decreased LPO of both stomach and duodenum tissues as compared to control stomach and duodenum respectively but the increase was not statistically significant. Immobilized cold-stress induced stomach and duodenal tissues showed significant increase in lipid peroxidation (LPO) and decrease in SOD, CAT activity and GSH level suggesting its damaging effect was mediated by derangement of antioxidant enzymes status as compared to control stomach and duodenum tissues respectively. But pretreatment with aqueous extract of ripe fruit’s pulp of *Cucurbita pepo* Linn. and ranitidine decreased LPO and increased SOD, CAT activity and GSH level thus showed its protective effect was exerted via antioxidant status (Table 8.1).

Mucosal thickness (MT): Treatment with aqueous extract of ripe fruit’s pulp of *C. pepo* (400 mg/kg body weight) increased the mucosal thickness (MT) of both stomach and duodenum tissues as compared to control stomach and duodenum respectively but the increase was not statistically significant. Immobilized cold-stress induced stomach and duodenum tissues showed a significant decrease of mucosal thickness (MT) as compared to control stomach and duodenum tissues respectively. But pretreatment with aqueous extract of ripe fruit’s pulp of *C. pepo* for 14 consecutive days, immobilized cold-stress induced stomach and duodenum tissues showed a significant increase of mucosal
thickness (MT) as compared to immobilized cold-stress induced stomach and duodenum tissues respectively. Therefore, pretreatment with ranitidine (10 mg/kg body weight) for 14 consecutive days in immobilized cold-stress induced stomach and duodenum tissues also showed a significant increase of mucosal thickness (MT) as compared to immobilized cold-stress induced stomach and duodenum tissues respectively (Table 8.1).
Statistical analysis was done using one way ANOVA followed by multiple comparison t-tests. *, # significantly different from group I (control) and group HI (immobilized-cold stress) respectively at \( p<0.05 \).

Table 8.1: Effect of aqueous extract of ripe fruit’s pulp of *Cucurbita pepo* Linn. (400 mg/kg) and ranitidine (10 mg/kg) on ulcer index (UI), mucosal thickness (MT), superoxide dismutase (SOD), catalase (CAT), lipid peroxidation (LPO) and reduced glutathione (GSH) level of stomach and duodenal tissues against immobilized-cold stress induced ulcer model.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Ulcer index (UI)</th>
<th>Mucosal thickness (MT) (µm)</th>
<th>SOD (units/mg protein)</th>
<th>CAT (µmole of ( \text{H}_2\text{O}_2 )/mg protein)</th>
<th>LPO (nmole of TBARS/gm tissue)</th>
<th>Reduced glutathione; GSH (nmole/gm tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stomach</td>
<td>Duodenum</td>
<td>Stomach</td>
<td>Duodenum</td>
<td>Stomach</td>
<td>Duodenum</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>555.83</td>
<td>739.99</td>
<td>4.45</td>
<td>4.903</td>
</tr>
<tr>
<td></td>
<td>±6.37</td>
<td>±18.55</td>
<td>±6.37</td>
<td>±18.55</td>
<td>±0.08</td>
<td>±0.023</td>
</tr>
<tr>
<td><em>C. pepo</em></td>
<td>0</td>
<td>0</td>
<td>566.666</td>
<td>1093.33</td>
<td>5.722</td>
<td>6.256</td>
</tr>
<tr>
<td></td>
<td>±10.98</td>
<td>±16.44</td>
<td>±10.98</td>
<td>±16.44</td>
<td>±0.247</td>
<td>±0.371</td>
</tr>
<tr>
<td>Immobilized-cold stress</td>
<td>24.00</td>
<td>5.50</td>
<td>326.11</td>
<td>619.44</td>
<td>2.647</td>
<td>3.176</td>
</tr>
<tr>
<td></td>
<td>±0.57*</td>
<td>±0.42*</td>
<td>±8.87*</td>
<td>±15.76*</td>
<td>±0.013*</td>
<td>±0.01*</td>
</tr>
<tr>
<td><em>C. pepo</em> + immobilized-cold stress</td>
<td>2.00</td>
<td>1.00</td>
<td>558.11</td>
<td>758.33</td>
<td>3.375</td>
<td>4.534</td>
</tr>
<tr>
<td></td>
<td>±0.36*</td>
<td>±0.25*</td>
<td>±14.54*</td>
<td>±7.34*</td>
<td>±0.115*</td>
<td>±0.133*</td>
</tr>
<tr>
<td></td>
<td>±0.307*</td>
<td>±0.21*</td>
<td>±6.36*</td>
<td>±6.549*</td>
<td>±0.105*</td>
<td>±0.123*</td>
</tr>
</tbody>
</table>

Values are mean±SE from 6 rats in each group.
Discussion:

Analysis of the results of present experiment showed that immobilized cold-stress produced an increase in ulcer index (UI), lipid peroxidation (LPO) and decrease in antioxidant enzymes activity of SOD, catalase and reduced glutathione (GSH) level and decrease in mucosal thickness (MT) in rat stomach and duodenal tissues. The decrease in antioxidant enzymes activity of SOD, catalase, GSH level (Fang et al., 2002), mucosal thickness (MT) (Sarkar and Guha, 2008; Chang et al., 2005) and increase in lipid peroxidation (LPO) (Fridovich, 1978), ulcer index (UI) (Sarkar and Guha, 2008; Debnath and Guha, 2007; Szabo et al., 1985) of gastro-duodenal tissues may be relevant to gastro-duodenal ulceration.

The pathophysiology of experimental peptic ulcer formation is not clearly known (Dhikav et al., 2003), so an unified concept for development of gastric lesions by various factors has not yet developed, but it is generally agreed multimechanisms (Goodwin et al., 1986; Konturek et al., 1999) and multifactorial process (Guzel et al., 1998). Multifactors like increase in acid secretion, reduction of gastro-duodenal mucosal blood flow, inhibition of prostaglandins (PGs) synthesis, disruption of mucosal barrier, inhibition of mucus and bicarbonate (HCO₃⁻) secretion (Aase, 1989; Allen and Leonard, 1988), increase in reactive oxygen species (ROS) generation (Mizui and Doteuchi, 1983; Mizui et al., 1987b; Yoshikawa et al., 1990; Cochran et al., 1982; Das et al., 1997; Biswas et al., 2003; Pihan et al., 1987; Szelenyi and Brune, 1988), decreased antioxidant enzymes activity of SOD, catalase and GSH level and increase in LPO in the gastro-duodenal mucosal tissues have been suggested (Fang et al., 2002; Phull et al., 1995; Guslandi, 1999). ROS also decrease the level of endogenous antioxidants and cause oxidative damage of gastric mucosa (Das et al., 1997; Phull et al., 1995; Banerjee et al., 2002; Biswas et al., 2003).

The pathogenesis of peptic ulcer disease (PUD) is believed to be reflect an imbalance between increased aggressive factors and decreased protective factors (Sarkar et al., 2006). The decreased antioxidant enzymes activity of SOD, catalase, GSH level, decrease in mucosal thickness (MT) and increase in ulcer index (UI) and lipid peroxidation (LPO) observed in the present study after providing immobilized cold-
stress may be due to failure in gastro-protective and repair mechanisms leading to disrupted mucosal barrier (Biswas et al., 2003; Das et al., 1997; Cochron et al., 1982).

Pretreatment with *C. pepo* at the dose of 400 mg/kg body weight significantly decreased the ulcer index (UI) and LPO and increased mucosal thickness (MT), antioxidant enzymes activity of SOD, catalase and GSH level in all rats after providing immobilized cold stress. The ability of gastric mucosa to resist injury by immobilized cold-stress is attributed to number of factors such as antioxidant enzymes activity of SOD, catalase and GSH level that have been referred to collectively as mucosal defense activity (Biswas et al., 2003; Das et al., 1997; Cochron et al., 1982). The gastric mucosal lesion is induced by immobilized cold-stress is due to depression of this defense mechanisms (Debnath and Guha, 2007).

The gastric and duodenal protective activity such as decrease in ulcer index (UI) and lipid peroxydation (LPO), increase in mucosal thickness (MT) and antioxidant enzymes activity of SOD, CAT and GSH level may be associated with correction or normalization of the altered balance between increased aggressive and harmful activity of oxygen derived free radicals or ROS and decreased defensive activity of gastric and duodenal mucosal tissues. The gastric and duodenal mucosa is thought to play an important role as a defensive factor against mucosal damage preventing toxic effects of oxygen derived free radicals or ROS (Imlay and Linn, 1988).

In the present investigation, *C. pepo* caused a significant enhancement of gastric and duodenal mucosal length as evidenced by increased mucosal thickness (MT) which plays an important role as a defensive factor against mucosal damage, thus confirms the ability of *C. pepo* to prevent the toxic effects of oxygen derived free radicals or ROS (Imlay and Linn, 1988) damaging by mucosal barrier with the help of LPO after applying immobilized cold-stress (Das et al., 1997; Biswas et al., 2003). These findings indicate that *C. pepo* pulp extract strengthens the gastric mucosal defense factors in experimental rats.

Antioxidant and antiulcer compounds which have free radical scavenging activity and may be classified as terpenoids (Rao et al., 2004), diterpenoids (Feliciano et al., 1993; Maciel et al., 2000; Schmeda-Hirschmann et al., 2002), triterpenoids (Siqueira et al.,
Amino acids also function as antioxidants (Fang et al., 2002), arginine, citrulline (Fang et al., 2002), taurine (Panasenko et al., 2005; Fang et al., 2002), carnitine (Dokmeci et al., 2005 Izgut-Uysal et al., 2001; Izgut-Uysal et al., 2001), vitamin A (Kasper et al., 1975; Mozsic et al, 1989), vitamin C (Smirnoff et al., 2001; Brzozowski et al., 2001; Davey et al., 2000; Kitano et al., 1997) and vitamin E (Wu et al., 2007; Azliina et al., 2005; Ichikawa et al., 2003; Saad et al., 2002; Nafeeza et al., 1999; Guzel et al., 1998, O’Brien, 1992; Zaror-Behrens et al., 1992; Yoshikawa et al., 1991; Armario et al., 1990; Granger et al., 1986; Brady et al., 1979; Young et al., 1976; Toshikazu et al., 1991; Okuma et al., 1980; Nakamoto et al., 1997) lipid peroxidation, phenols such as tannin (Banerjee et al., 2008; Souza et al., 2006; Ramirez and Roa, 2003; Czinner et al., 2001; Ezaki et al., 1985), polyphenols such as catechin and epigallocatechin, gallate (Eu et al., 2001), polyamines (Cheng et al., 2004; Ma et al., 2000; Brzozowski et al., 1993), selenium (Se) (Combs, 2001; Combs, 1999; O’Brien, 1992; Jamal and Sprowls, 1987; Brady et al., 1979; Young et al., 1976), cadmium (Cd) (Izgut-Uysal et al., 1993; Jamal and Sprowls, 1987) have antioxidant activities.

The chemical constituents of ripe fruit's pulp of *C. pepo* responsible for its antioxidant and antiulcer activity are not known. However, extensive pharmacological investigation suggested that the presence of several major groups of bioactive compounds such as triterpenoids (Wang et al., 2008), glycosides cucurbitacin B, cucurbitacin D, cucurbitacin E, cucurbitacin F (Feng et al., 2007), cucurbitacin L and cucurbitacin K (Wang et al., 2007) were isolated from *C. pepo* fruit. It has been reported that some of these anti-ulcer compounds such as terpenoids (Rao et al., 2004),
diterpenoids (Feliciano et al., 1993; Maciel et al., 2000; Schmeda-Hirschmann et al., 2005), triterpenoids (Siqueira et al., 2007) and flavonone-glycosides (Naserifar, 2007), flavonoid-glycosides (Dharmani and Palit, 2006; Yesildada et al., 2000; Lewis and Hanson, 1991), flavonoid (Alarcon et al., 1994; Reyes-Ruiz et al., 1998; Alvarez et al., 1997; Zayakhivska et al., 2005; Suzgec, 2005) protected ulceration in various animals model.

Another extensive pharmacological investigation suggested that the presence of several major groups of antioxidants like sulfhydryl compounds 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; Walker, 1972; Kataoka et al., 1998), S-adenosyl methionine (SAM) (Huang et al., 1991), methionine (Met or M) (Yoshida et al., 2005) and antioxidant like phenolic compounds such as tannin (Ojiako and Igwe, 2008; Silveira et al., 1996), syringic acid (Dragovic-Uzelac et al., 2005), phenolic phytochemicals (Kwon et al., 2007), phenolic contents (Mongkolsilp et al., 2004), polyamines (Nishimura et al., 2006; Martinez-Tellez et al., 2002) were isolated from C. pepo fruit.

It has been reported that some of them antioxidant compounds such as sulfphydrys (Loguercio et al., 1991; Szabo et al., 1981), glutathione (Kimura et al., 2001; Loguercio et al., 1993), cysteine (Cys or C) (Loguercio et al., 1991) and methionine (Met or M) (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), Amino acids also function as antioxidants (Fang et al., 2002) offer special protection especially against oxidative damage by oxygen derived free radicals and reactive oxygen species (ROS). Sulphydryls also help in prostaglandins (PGs) mediated gastro-protection (Szabo et al., 1981). Phenols such as 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), 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) polyamines (Cheng et al., 2004; Ma et al., 2000;
Brzozowski et al., 1993) protect ulcerated injury preventing oxidative damage by scavenging the oxygen derived free radicals and reactive oxygen species (ROS).

Both clinical observations on humans and experimental studies on animals suggest a protective action of vitamin A against gastric ulcer induced either by stress (Kasper et al., 1975). **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; Arima and Rodriguez-Amaya, 1990; Kune et al., 1992; Arima and Rodriguez-Amaya, 1988), vitamins A (Lans et al., 2007; Ahmed et al., 2003; Ribaya-Mercado et al., 1999) and **β-carotene** (Veda et al., 2006) may be responsible against ulceration (UI) for the presence in fruit of *C. pepo* in the present experiment.

Vitamin C (Smirnoff et al., 2001; Davey et al., 2000) and vitamin E (Wu et al., 2007; Oz et al., 2004; Azlina et al., 2005; Guzel et al., 1998; Armario et al., 1990; Granger et al., 1986; Brady et al., 1979; Young et al., 1976) play an important role against lipid peroxidation in stress-induced ulceration. 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’s pulp of *C. pepo* may be responsible for reduced ulcer index (UI), lipid peroxidation (LPO) and increase in mucosal thickness (MT) antioxidant enzymes activity of SOD, catalase and GSH level possibly by scavenging generated oxygen derived free radicals or reactive oxygen species (ROS) in the present experiment.

Therefore, some antioxidant types elements such as selenium (Combs, 2001; Jamal and Sprowls, 1987; Brady et al., 1979), cadmium (Izgut-Uysal et al., 1993; Jamal and Sprowls, 1987), Zn (Fang et al., 2002), have been identified as oxygen derived free radicals or reactive oxygen species (ROS) scavengers. Some of them such as selenium (Se) (Yoshida et al., 2005; Stibilj et al., 2004), cadmium (Qadir et al., 2000), Zn, Cu (Fan et al., 2006) have been found in fruit’s pulp of *C. pepo* which may be responsible for reduced ulcer index (UI), LPO and increase in mucosal thickness (MT), antioxidant enzymes activity of SOD, catalase, GSH level possibly by scavenging generated oxygen derived free radicals or reactive oxygen species (ROS) in the present experiment.
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 stress induced 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 by immobilized cold-stress 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).

Melatonin (N-acetyl-5-hydroxytryptamine), the pineal gland hormone, has been shown to exert antiulcer activity in several experimental animal models (Bandyopadhyay et al, 2000). In stress ulcer model, it is more effective than ranitidine but less effective than omeprazole. It blocks stress ulcer by preventing oxidative damage through scavenging of \( \cdot \)OH (Bandyopadhyay et al, 2000). It is suggested that consumption of fruit’s pulp of C. pepo may synthesize endogenous antioxidant melatonin through serotonin (5-HT) from tryptophan (Trp or W) amino acid which is present 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).

Thus, it may be suggested that pretreatment of C. pepo may prevent the gastric and duodenal mucosal damage by immobilized cold-stress increasing SOD, catalase, mucosal thickness (MT) and reducing reactive oxygen species (ROS), oxygen derived free
radicals production and LPO synthesizing endogenous antioxidant melatonin (Bandyopadhyay et al., 2000) 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 ulcerations induced by immobilized cold-stress as seen by the decreased in ulcer index (UI) is likely by maintaining the structural integrity of gastric epithelial cell membrane and balance of aggressive factors and inherent protective mechanisms.

It is concluded that aqueous extract of ripe fruit’s pulp of *Cucurbita pepo* Linn. by its antiulcer and antioxidant property due to presence of huge numbers of antiulcer and antioxidant components such as vitamin A, C and E, triterpenes, glycosides such as cucurbitacin B, cucurbitacin D, cucurbitacin E, cucurbitacin F, cucurbitacin L and cucurbitacin K, polyamines, syringic acid, sulfhydryls, tryptophan containing proteins mavicyanin and lectin and tannin, selenium (Se), Zinc (Zn), cadmium (Cd) and their ability to protect gastric and duodenal SOD, catalase, glutathione (GSH) against immobilized cold stress induced ROS, free radicals and LPO generating peptic ulcer disease (PUD) in rats thus categorising it as an antiulcerogenic herbal drug. However, further studies on different acute and chronic models of ulceration are necessary to rationalise its therapeutic use as an anti-ulcer herbal drug.