ANTIOXIDANT ACTIVITY OF AQUEOUS EXTRACT OF RIPE FRUIT'S PULP OF Cucurbita pepo Linn. AND RANITIDINE AGAINST ASPIRIN INDUCED GASTRIC AND DUODENAL ULCERATION OF EXPERIMENTAL RAT MODEL

Dietary antioxidants play a crucial role in the prevention of peptic ulcer disease (PUD). Peptic ulcer disease (PUD) is caused by many endogenous and exogenous aggressive factors. Exogenous aggressive factors such as excessive intake of non-steroidal anti-inflammatory drugs (NSAIDs; aspirin, indomethacin and ibuprofen etc), mental stress and tension in our daily life and chronic alcohol consumption influence the endogenous aggressive factors like excessive generation of reactive oxygen species (ROS) (Pihan et al., 1987; Miura et al., 2002; Yoshikawa et al., 1993; Vaananen et al., 1991; Salim, 1992), 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. The break down and the falls of activity of endogenous antioxidant systems of the gastric and duodenal tissues is the result of peptic ulcer disease (PUD). Some endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione reductase, glutathione peroxidases and antioxidants such as arginine, citrulline (Fang et al., 2002), taurine (Panasenko et al., 2005; Fang et al., 2002), carnitine (Dokmeci et al., 2005 Izgut-Uysal et al., 2007; Izgut-Uysal et al., 2001), exogenous antioxidants such as polyphenols, vitamin A, vitamin C, vitamin E (Fang et al., 2002), sulphhydrlyls (Loguercio et al., 1991; Szabo et al., 1981), glutathione (Fang et al., 2002; Loguercio et al., 1993; Boyd et al., 1981), cucurbitacin glucosides (Tannin-Spitz et al., 2007) and 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 which are produced by decreased activity of endogenous antioxidant enzymes such as SOD, catalase, GSH level and increase in lipid peroxidation (LPO).
Some ulcerogens such as non-steroidal anti-inflammatory drugs (NSAIDs) have been reported to produce free radical generation leading to the generation of reactive oxygen species (ROS) (Miura et al., 2002) by decreased activity of antioxidants enzymes such as SOD, catalase, GSH level and increase in lipid peroxidation (LPO) resulting in various types of bio-molecules such as lipids, proteins, amino acids and DNAs damage (Halliwell and Gutteridge, 1990; Pihan et al., 1987; Imlay and Linn, 1988; Simmonds et al., 1992; Biswas et al., 2003). ROS have been implicated gastric mucosal damage by aspirin (NSAID: non-steroidal anti-inflammatory drug) (Pihan et al., 1987). Dietary deficiencies of amino acids, proteins, vitamin A, vitamin C, vitamin E and antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione reductase, sulphydryls (Fang et al., 2002) and selenium (Se), zinc (Zn) cadmium (Cd) (Izgut-Uysal et al., 1993; Jamal and Sprowls, 1987) give rise to the oxidation of bio-molecules and cell injury (Granger et al., 1986) which leads to peptic ulcer disease (PUD).

Gastric and duodenal ulceration develops due to a break of the epithelial lining cells of mucosa of these tissues (Debnath and Guha, 2007; Sarkar and Guha, 2008) when the balance between some aggressive and defensive factors is lost (Sarkar et al., 2006). Various endogenous aggressive factors like excessive HCl secretion, pepsin, excessive bile (Schoen and Vender, 1989; Sonnenberg, 1995) secretion, excessive generation of leukotrienes (Hudson et al., 1993) and reactive oxygen species (ROS) such as O$_2^-$, H$_2$O$_2$ and ·OH etc (Peskar et al., 1986; Pihan et al., 1987; Szelenyi and Brune, 1988) are influenced by various exogenous aggressive factors such as excessive intake of non-steroidal anti-inflammatory drugs (NSAIDs; aspirin, indomethacin and ibuprofen etc), mental stress and tension in our daily life and chronic alcohol consumption and these generation of ROS cause the disruption of mucosal barrier (Phull et al., 1995; Guslandi, 1999) which leads to peptic ulcer disease (PUD).

Aspirin has been found to be the inhibitor of PG synthetase (Vane, 1971) and consequently interferes with the gastroprotective mechanism through PG (Langman et al., 1991). Due to excessive intake of exogenous aggressive factors like non-steroidal anti-inflammatory drugs (NSAIDs; aspirin) influences endogenous aggressive factor such as excessive generation of reactive oxygen species (ROS) and oxygen derived free radicals which damage the gastric mucosal barrier allowing back-diffusion of H$^+$ and decrease
both of the mucus and HCO$_3^-$ secretion and thereby reduces surface hydrophobicity, decrease mucosal blood flow and cellular regeneration (Schoen and Vender, 1989). Back diffusion of acid through the breached mucosa destroys the cells of capillaries and veins causing hemorrhagic ulcer. Excessive intake of NSAIDs also enhances leukotrienes biosynthesis (Hudson et al., 1993), decrease ATP biosynthesis and cell turnover process (Ivey, 1988) which is related to peptic ulcer disease (PUD). Epigastric pain is the common clinical feature of peptic ulcer disease (PUD) and in severe cases blood appears in the vomiting substances. Since in majority, it is aggravated due to HCl, pepsin and bile etc it is also termed as peptic ulcer disease (PUD) (Schoen and Vender, 1989; Sonnenberg, 1995). 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.

Different treatment strategies have been used to prevent the peptic ulcer disease (PUD). The different anti-ulcer drugs such as ranitidine, famotidine, cimetidine, nizatidine (Hansten, 1994; Bilchik et al., 1989; Havu et al., 1990), omeprazole (Howden and Hunt, 1994; Wolfe and Sachs, 2000; Qi et al., 2009), sucralfate (Slomiany et al., 1985; Tytgat, 1984), misopristol (Graham et al., 1988) etc have been widely used for the treatment of peptic ulcer disease (PUD) but these drugs have many side effects such as confusion, restlessness, convulsions, confusion, restlessness, convulsions, nausea, vomiting, headache, diarrhea, uterine bleeding, abortion, constipation, dry mouth, liver disorders, kidney damage etc. These side effects have prompted the scientific world for the search of alternative herbal remedies of peptic ulcer disease (PUD). A number of Indian medicinal plants (Dharmani et al., 2006; Davey et al., 2000), fruits and vegetables (Sarkar and Guha, 2008; Chun et al., 2005) have been extensively used in the Indian traditional Ayurvedic system of medicine for the management of peptic ulcer disease (PUD) (Lewis and Hanson, 1991) due to their antioxidant properties (Scartezzini and Speroni, 2000; Mongkolsilp et al., 2004).

Some medicinal plants have already been reported to possess strong antioxidant activity such as chamomile (Beil et al., 1995), carrot, tomato (Davey et al., 2000) and fruits such as apple, banana, cucumber, guava, melon, orange (Scartezzini and Speroni, 2000) and Vitis trifolia (Kumar and Kumar, 2009). Leaves such as tea leaves (Euaelhardt
et al., 2001), tulsi leaves (Kath and Gupta, 2006), Neem (Azadirachta indica) leaves (Bandyopadhyay et al., 2002) and Chenopodium album leaves (Kumar and Kumar, 2009). C. pepo is one of those plants and in the preliminary investigations of aqueous extract ripe fruit’s pulp 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).

Several dietary antioxidant-type antiulcer compounds have been identified from fruit’s pulp of Cucurbita pepo Linn. (commonly known as pumpkin) such as terpenoids and 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), sulfhydryl like 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; Kataoka et al., 1998), S-adenosyl methionine (SAM) (Huang et al., 1991), methionine (Met or M) (Yoshida et al., 2005), phenolic compounds such as syringic acid (Dragovic-Uzelac et al., 2005), tannin (Ojiako and Igwe, 2008; Silveira et al., 1996), 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), vitamin 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), vitamin E (Franke et al., 2007; Tadmor et al., 2005; Imaeda et al., 1999; Sedghi et al., 2008). 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. From the previous experiments, it has been emphasized that ripe fruit’s pulp of this C. pepo plant may exhibit strong antioxidant with free radical scavenging action for the presence of several
antioxidant compounds which have been mentioned in above from various ethnic scientific reports. Thus the present study was undertaken to determine the antioxidant activity of aqueous extract of ripe fruit’s pulp of *Cucurbita pepo* Linn. (*C. pepo*) against aspirin induced gastric and duodenal ulceration in rat model.

**Materials and methods:**

**Animal grouping and treatment:**

Thirty (30) rats were divided into five (5) groups of 6 rats each. Group I animals comprised control group. Group II was *C. pepo* extract treated, Group III was aspirin treated, Group IV was *C. pepo* pretreated and aspirin treated and Group V was ranitidine pretreated and aspirin treated.

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) and 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. 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, aspirin (German-Remidies Ltd) was dissolved in distilled water and group III, group IV and group V rats were given a single dose of aspirin at 500 mg/kg body weight orally by orogastric cannula (Cho and Ogle, 1979) and waited for 4 hrs. After 4 hrs, the experiment was terminated and rats were sacrificed by an over dose of thiopentone sodium (NEON, Laboratories Ltd, India).

**Parameters studied:**

**Superoxide dismutase (SOD):** Superoxide dismutase (SOD) was estimated by the method of Marklund and Marklund, 1974. Stomach and duodenum tissue samples were homogenized with 5 ml of ice-cold 0.1(M) phosphate buffer (pH=7.4). The homogenates was then centrifuged at 3000 rpm for 10 min. Then 0.1 ml of sample was mixed with 0.8 ml of TDB solution (triethyleamine, diethyleamine and buffer mixture). Reaction started by the addition of 4 μl of NADH. Then 25 μl of EDTA-MnCl₂ mixture was added to it. Thereafter spectrophotometric readings were recorded at 340 nm. After
recording of spectrophotometric reading, 0.1 ml of mercaptoethanol was added with those mixture and again spectrophotometric reading were recorded at 340 nm wave length.

**Catalase (CAT):** Catalase activity was estimated by the method of Aebi, 1974. Stomach and duodenum tissue samples were homogenized with 5 ml of ice-cold 0.1(M) phosphate buffer (pH=7.4). The homogenates was then centrifuged at 3000 rpm for 10 min. The precipitate was then stirred with the addition of 15 ml of ice-cold 0.1(M) phosphate buffer and allowed to stand in cold condition with occasional shaking. The shaking procedure was repeated for thrice. 1 ml of sample was added with 9 ml of H$_2$O$_2$. The rate of decomposition of H$_2$O$_2$ was measured spectrophotometrically from the changes in absorbance at 350 nm wave length.

**Reduced glutathione (GSH):** Glutathione was measured according to the method of Ellman (1959). Stomach and duodenum tissue samples were homogenized with 5 ml of ice-cold 0.1 M phosphate buffer (pH=7.4). The equal quantity of homogenate was mixed with 10% trichloroacetic acid (TCA) and centrifuged to separate the proteins. To 0.01 ml of this supernatant, 2 ml of phosphate buffer (pH= 8.4), 0.5 ml of 5' 5'-dithiobis (2-nitrobenzoic acid) and 0.4 ml of double distilled water were added. The mixture was vortexed and the absorbance was read at 412 nm wave length within 15 min.

**Lipid peroxidation (LPO):** Lipid peroxidation (LPO) was measured according to the method of Rehncrona et al., 1980. Stomach and duodenum tissue samples were homogenized with 5 ml of ice-cold 0.1 M phosphate buffer (pH=7.4). The homogenates was then centrifuged at 3000 rpm for 10 min. 0.5 ml of sample was mixed with 1 ml of TDB solution (triethyleamine, diethyleamine and buffer mixture) and then the mixture was incubated at 37°C for 1 hour. To it, 0.5 ml of trichloroacetic acid (TCA) was added, vortexed and the absorbance was read at 350 nm wave length. After recording of spectrophotometric reading, 1 ml sample was mixed with 500 µl mercaptoethanol and again the absorbance was read at 350 nm wave length.
Results:

Superoxide dismutase (SOD), catalase (CAT) activity, lipid peroxidation (LPO) and reduced glutathione (GSH) level:

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. Aspirin 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 6.1).

Table 6.1: Effect of aqueous extract of ripe fruit’s pulp of *Cucurbita pepo* Linn. (400 mg/kg) and ranitidine (10 mg/kg) on superoxide dismutase (SOD), catalase (CAT), lipid peroxidation (LPO) and reduced glutathione (GSH) level of stomach and duodenum tissues in aspirin induced ulcer model.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SOD(units/mg protein)</th>
<th>CAT(umole H2O2/mg protein)</th>
<th>LPO(umole 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>
</tr>
<tr>
<td>Control</td>
<td>4.45±0.08</td>
<td>4.903±0.023</td>
<td>3.503±0.048</td>
<td>4.446±0.0127</td>
</tr>
<tr>
<td><em>C. pepo</em></td>
<td>5.722±0.247</td>
<td>6.256±0.371</td>
<td>5.443±0.87</td>
<td>5.746±0.054</td>
</tr>
<tr>
<td>Aspirin</td>
<td>2.595±0.027*</td>
<td>3.168±0.025*</td>
<td>2.496±0.128*</td>
<td>3.193±0.048*</td>
</tr>
<tr>
<td><em>C. pepo</em>+ Aspirin</td>
<td>4.807±0.45*</td>
<td>5.471±0.024*</td>
<td>3.945±0.163*</td>
<td>4.683±0.045*</td>
</tr>
<tr>
<td>Ranitidine+ Aspirin</td>
<td>6.23±0.025*</td>
<td>6.821±0.93*</td>
<td>3.300±0.334*</td>
<td>4.235±0.285*</td>
</tr>
</tbody>
</table>

Statistical analysis was done using one way ANOVA followed by multiple comparison t-tests when *, # significantly different from group I (control) and group III (aspirin) respectively at *p*<0.05.

Discussion:

The present study evaluates the antioxidant activity of aqueous extract of ripe fruit’s pulp of *C. pepo* on the aspirin induced gastric and duodenal ulceration in rat
It is evident from the results of the present investigation that aspirin showed decrease in antioxidant enzymes activity of SOD, catalase, GSH level and increase in lipid peroxidation (LPO) of gastro-duodenal tissues. The decreased antioxidant enzymes activity of SOD, catalase, GSH level and the increased lipid peroxidation (LPO) (Fang et al., 2002) and free radicals generation (Pihan et al., 1987; Miura et al., 2002; Yoshikawa et al., 1993; Vaananenn et al., 1991; Salim, 1992) in 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). Factors like increase in acid secretion, reduction of gastric mucosal blood flow, inhibition of prostaglandins (PGs) synthesis, disruption of mucosal barrier, inhibition of mucus and bicarbonate secretion (Aase, 1989; Allen and Leonard, 1988), ischemia (Itoh and Guth, 1985; Perry et al., 1986; Yoshikawa et al., 1989; Guzel et al., 1998), inhibition of angiogenesis (Jones et al., 1999), decreased activity of SOD, catalase, GSH level and increase in lipid peroxidation (LPO) (Fang et al., 2002) and generation of ROS (Salim, 1992) in the gastric and duodenal mucosal tissues have been suggested.

The pathogenesis of ulcer disease is believed to reflect an imbalance between increased aggressive factors and decreased protective factors (Sarkar et al., 2006). The decreased activity of SOD, catalase activity, GSH level and increase in lipid peroxidation (LPO) observed in the present study after aspirin treatment may be due to failure in gastro-protective and repair mechanisms leading to disrupted mucosal barrier by the generation of reactive oxygen species (ROS) or oxygen derived free radicals (Pihan et al., 1987; Miura et al., 2002; Yoshikawa et al., 1993; Vaananenn et al., 1991; Salim, 1992).

Pretreatment with C. pepo at the doses of 400 mg/kg body weight was the most effective dose (ED) and significantly decreased the ulcer index (UI) and increased the mucosal thickness (MT) and increased the activity of alkaline phosphatase (AP) enzyme activity intact in gastric and duodenal tissues which cumulatively increased mucosal defense activity of gastric and duodenal tissues of all rats against treated with aspirin.
In the present study \textit{C. pepo} increased the activity of SOD, catalase, GSH level and increased lipid peroxidation (LPO).

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 activity (Wallace, 2001a). The gastric mucosal lesions induced by necrotizing agents such as aspirin and strong alkalis are due to depression of this defense mechanisms (Kinoshita \textit{et al}, 1995).

Therefore, reactive oxygen species (ROS) are generated by neutrophil accumulation and activation in inflammatory condition of the gastric mucosa caused by various aggressive factors such as NSAIDs and it leads to oxidative damage of biomolecules such as lipids, proteins, amino acids and DNAs damage (Halliwell and Gutteridge, 1990; Imlay and Linn, 1988; Simmonds \textit{et al}, 1992; Biswas \textit{et al}, 2003) and mucosal lesion (Phull \textit{et al}, 1995; Guslandi, 1999). Reactive oxygen species (ROS) also decrease the level of endogenous antioxidants and make a damage of gastric mucosa due to oxidative damage (Phull \textit{et al}, 1995; Das \textit{et al}, 1997; Banerjee \textit{et al}, 2002). Transition metal of iron ions (Fe$^{3+}$/Fe$^{2+}$) in presence of H$_2$O$_2$ which generate OH' (Halliwell and Gutteridge, 1990) can cause lipid peroxidation (LPO) and cellular injury in cultured gastric mucosal cells (Hiraishi \textit{et al}, 1991a; Yajima \textit{et al}, 1995).


Aspirin (NSAIDs) also promote ROS generation and oxidative damage by increasing leukocyte infiltration (Wallace \textit{et al}, 1990; Heeba \textit{et al}, 2009). Gastric damage is also induced through generation of ROS by alteration of antioxidant systems of the gastric mucosa (Levi \textit{et al}, 1990; Vaananenn \textit{et al}, 1991). The decreased antioxidant enzymes activity of SOD, catalase and GSH level imbalanced the mucosal
second lines of defense the gastric and duodenal tissues which observed in the present study after aspirin (NSAIDs) treatment due to failure in gastro-duodenal protection generating free radicals (Vaanannel et al, 1991).

The production of reactive oxygen species (ROS) or oxygen-derived free radicals by NSAIDs like indomethacin causes the pathophysiology of peptic ulcer formation by influencing various protective factors such as reduction of gastric mucosal blood flow by inhibiting angiogenesis (Jones et al, 1999). Angiogenesis, the formation of new capillary blood vessels, is essential not only for the growth and metastasis of solid tumors tissues, but also for wound and ulcer healing, because without restoration of blood flow, $O_2$ and nutrients can not be delivered to the healing site. NSAIDs inhibit angiogenesis through direct effect on endothelial cells (Jones et al, 1999). The possible mechanism of NSAIDs induced gastro-duodenal damage and gastric mucosal cell death has been reviewed (Szabo and Tarnawski, 2000; Hawkey, 2000).

Underlying the surface epithelial layer there is a dense network of capillaries for vascular blood flow. In addition for supplying nutrients and oxygen to the epithelium, the microcirculation also removes, dilutes and neutralizes toxic substances that have diffused into the mucosa from the lumen. The microcirculation also plays a critical role in creating a microenvironment over the site of injury conductive for repair (Wallace and Grænæs, 1996), when the epithelium damaged. The mucosal vascular architecture is ideally suited for delivery of bicarbonate ($HCO_3^-$) to the epithelium. Fenestrated arterioles pass in close proximity to the basolateral membranes of parietal cells, where 1 mole of bicarbonate ($HCO_3^-$) is secreted in to the blood for every mole of hydrochloric acid (HCl) that is secreted into the gland (Cannon et al, 1984).

So, vascular injury to subepithelial capillaries by gastric ulcer induced by well-known gastric-offensive agents like non-steroidal anti-inflammatory drugs (NSAIDs) causing ischemia (Itoh and Guth, 1985; Perry et al, 1986; Yoshikawa et al, 1989; Guzel et al., 1998) by decreasing vascular blood flow (Jones et al, 1999) or by generating reactive oxygen species (ROS) (Yoshikawa et al., 1993; Miura et al., 2002) which increased vascular permeability and circulatory stasis is an early pathogenic factor in experimental gastric lesions. These changes lead to functional impairment of gastric
Pretreatment with aqueous extract of ripe fruit's pulp of *C. pepo* showed an significant increase in antioxidant activity of SOD, catalase and GSH level and reduction of lipid peroxidation (LPO) of gastro-duodenal tissues. The finding can be explained with the possible involvement of the gastro-duodenal protection by defensive mechanisms of gastro-duodenal tissues with the increased antioxidant activity of SOD, catalase and GSH level and reduction of lipid peroxidation (LPO) by scavenging of free radicals or by inhibiting of free radical production. The increased activity of SOD, catalase and GSH level and the reduction of LPO may be relevant to gastro-duodenal antiulcer activity by scavenging of reactive oxygen species (ROS), free radicals or by inhibiting of oxygen-derived free radicals production.

In the present experiment, aspirin treated rats pretreated with *C. pepo* extract at a dose of 400 mg/kg body weight demonstrates that the gastro-duodenal protective activity such as an increase in antioxidant enzymes activity of SOD, catalase and GSH level and reduction of LPO observed may be associated with correction or normalization of the altered balance between antioxidants and oxygen derived free radicals or reactive oxygen species (ROS).

Dietary antioxidants play a crucial role in the treatment of peptic ulcer disease (PUD). Vitamin C is a potent antioxidant and it has scavenging activity of free radicals which are generated by NSAIDs (aspirin, indomethacin) (Bielanski *et al.*, 2001; Brzozowski *et al.*, 2001; Pohle *et al.*, 2001; McAlindon *et al.*, 1996; Salim, 1992). Vitamin E (Saad *et al.*, 2002; Zaror-Behrens *et al.*, 1992 play an important role in the reduction of pathogenesis of ulcer formation by probably reducing the ischemia (Ichikawa *et al.*, 2003; Kitano *et al.*, 1997; Nakamoto *et al.*, 1997; Toshikazu *et al.*, 1991; Yoshikawa *et al.*, 1991). It has been found that the deficiency of dietary vitamin E reduces the synthesis of arterial prostaglandins (PGs) significantly with lipid peroxidation (LPO) (Okuma *et al.*, 1980) which may trigger ulcer formation. It has been suggested from the present experiment that *C. pepo* increased the antioxidant enzymes activity of SOD, catalase and GSH level and concomitantly reduction of LPO in the gastric and duodenal tissues may be come after scavenging the free radicals by
exogenous administrations of vitamin C which is present in aqueous extract of ripe fruit’s pulp of *C. pepo* (Hancock *et al.*, 2008; Mongkolsilp *et al.*, 2004; Hancock *et al.*, 2003) and vitamin E also is present in pulp extract of *C. pepo* (Franke *et al.*, 2007; Tadmor *et al.*, 2005; Imaeda *et al.*, 1999; Sedghi *et al.*, 2008) which may be responsible for the increase of antioxidant enzymes activity of SOD, catalase, GSH level and reduction of LPO.

The production of reactive oxygen species (ROS) or oxygen-derived free radicals by NSAIDs cause the pathophysiology of peptic ulcer formation by inhibition of prostaglandins (PGs) synthesis (Langman *et al.*, 1991), inhibition of mucus and bicarbonate (HCO$_3$) secretion (Schoen and Vender, 1989), disruption of mucosal barrier (Szabo and Tarnawski, 2000; Hawkey, 2000).

PGs are the first endogenous compounds implicated in gastric cytoprotection (Robert, 1979). PGs increase mucosal blood flow (Glickman *et al.*, 1982; Kauffman *et al.*, 1979). This has been suggested to be responsible for their gastroprotective effect. However, various other mechanisms have been postulated like dilution of noxious agent by PG stimulated mucus secretion (Johansson and Kollberg, 1979; Domschke *et al.*, 1978), stimulated of basal bicarbonate secretion (Isenberg *et al.*, 1986) increase in the concentration of surface-active phospholipids (Lisachtenberger *et al.*, 1995; Lischtenberger, 1995; Lindell *et al.*, 1997), stimulation of cAMP (Levine *et al.*, 1982; Major and Scholes, 1978), stabilization of lysosome (Fergison *et al.*, 1973), decrease of gastric motility and dissolution of gastric mucosal folds (Mersereau and Hinchey, 1982; Miller, 1983) and maintenance of mucosal sulfhydryl group (Szabo *et al.*, 1981). PGs probably also have a repair function by stimulating rapid resolution of disrupted surface epithelium, injury and lesions (Robert *et al.*, 1979; Brzozowski *et al.*, 1993). It has been shown that prior exposure of gastric mucosa to mild irritants protects it from damage by more noxious agents. This “adaptive cytoprotection” is mediated by PGs (Robert *et al.*, 1983).

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.
Thus, it may be suggested that pre-treatment of *C. pepo* may prevent the gastric mucosal damage by aspirin increasing PGE$_2$ level or by reducing the ischemia (Guzel et al., 1998) and ROS activity as evidenced by increased SOD, catalase activity, GSH level and decreased LPO in the present investigation which may be due to the presence of vitamin E in the pulp of *C. pepo* (Franke et al., 2007; Tadmor et al., 2005; Imaeda et al., 1999; Sedghi et al., 2008).

However, various antioxidant phenolic compounds such as 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), alkaloid 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), vitamin C (Brzozowski et al., 2001; Bielanski et al., 2001; Pohle et al., 2001; McAlindon et al., 1996), vitamin E (Saad et al., 2002; Zaror-Behrens et al., 1992; Okuma et al., 1980), triterpenes glycosides (Naseri et al., 2007) and strong anti-oxidant 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) have been identified as oxygen derived free radicals or reactive oxygen species (ROS) scavengers.

Some of these 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), sulfhydryl like 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; Kataoka et al., 1998), S-adenosyl methionine (SAM) (Huang et al., 1991), methionine (Met or M) (Yoshida et al., 2005), phenolic compounds such as *syringic acid* (Dragovic-Uzelac et al., 2005), tannin (Ojiako and Igwe, 2008; Silveira et al., 1996), phenolic phytochemicals (Kwon et al., 2007), polyphenol and phenolic contents (Mongkolsilp et al., 2004), *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) and strong anti-oxidant elements such as selenium (Se) (Yoshida et al., 2005; Stibilj et al., 1998).
2004) and cadmium (Cd) (Qadir et al., 2000) are present in fruit's pulp of *C. pepo* which may be responsible for the increased antioxidant enzymes activity of SOD, catalase, GSH level and reduction of LPO.

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) (Kasper et al., 1975; Mozsik et al., 1989; Sarkar and Guha, 2008).

The ability of *C. pepo* to protect the mucosa against lesions induced by aspirin (NSAID) as seen by the decreased lipid peroxidation (LPO) is likely by maintaining the structural integrity of gastric epithelial cell membrane and balance of aggressive factors and inherent protective mechanism. Furthermore, 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 (Sarkar and Guha, 2008).

Aspirin induced gastric ulceration is also protected by *C. pepo* fruit's pulp extract containing antioxidant compounds such as phenols such as syringic acid (Dragovic-Uzelac et al., 2005), tannin (Ojiako and Igwe, 2008; Silveira et al., 1996), phenolic phytochemicals (Kwon et al., 2007), polyphenol and phenolic contents (Mongkolsilp et al., 2004), 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) which can effectively scavenge *OH (Bors et al., 1990) and block oxidative damage.

The exact mechanism by which *C. pepo* acts is not definitely known. It may be that 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 fruit’s pulp of *C. pepo* may reduce the reactive oxygen species (ROS) or oxygen derived free radicals formation hereby increasing the mucosal defense mechanisms.

Indomethacin, a non-steroidal anti-inflammatory drug, also induces gastric ulceration by interfering epidermal growth factor (EGF) binding it 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) has been suggested. The pathogenesis of ulcer disease is believed to reflect an imbalance between increased aggressive factors and decreased protective factors (Sarkar et al., 2006).

Intragastric administration of EGF promotes gastric ulcer healing, partly through restitution of angiogenesis (Hase et al., 1989).

It is reported that polyamines has been associated with cell proliferation during ulcer healing (Wang et al., 1990; Brzozowski et al., 1993). 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).

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 aqueous extract of ripe fruit’s pulp of C. pepo may prevent the gastric mucosal damage by aspirin possibly by increasing of epidermal growth factors (EGF) mediated gastro-protection ulcer healing as evidenced by increased antioxidant enzymes such as SOD, catalase, GSH level and decreased lipid peroxidation (LPO) in the present experiment and possibly by decreasing of production of oxygen derived free radicals and reactive oxygen species (ROS) which may be due to the presence of polyamines (Nishimura et al., 2006; Martinez-Tellez et al., 2002) in the ripe fruit’s pulp of C. pepo.

In conclusion, aqueous extract of ripe fruit’s pulp of C. pepo exhibits a potential protective activity against aspirin induced peptic ulcer disease (PUD) possibly by increasing the mucosal defensive mechanisms due to presence of vitamin A, C and E or through the syringic acid, triterpenes, glycosides such as cucurbitacin B, cucurbitacin D, cucurbitacin E, cucurbitacin F, cucurbitacin L and cucurbitacin K, tannin, selenium (Se), cadmium (Cd), Zinc (Zn), sulfhydryls and polyamines which exhibit antioxidant activity.