ROLE OF AQUEOUS EXTRACT OF RIPE FRUIT'S PULP OF *Cucurbita pepo* Linn. AND RANITIDINE ON ULCER INDEX, MUCOSAL THICKNESS, ENTEROCHROMAFFIN CELL COUNT AND SEROTONIN CONTENT AGAINST CEREBELLAR NODULAR LESION INDUCED GASTRIC AND DUODENAL ULCERATION OF EXPERIMENTAL RAT MODEL

Several neurophysiological defective causations of central nervous system (CNS) such as cerebellar nodular lesion (CNL) and hypothalamic dysfunction along with several neurophysiological defects of enteric nervous system (ENS) hamper normal physiology of gastrointestinal tract (GIT).

Pathogenesis of gastro-duodenal ulcer formation is a complex mechanism due to an imbalance between increased aggressive and decreased defensive factors (Sarkar *et al*., 2006) which are responsible in the loss of gastric mucosal integrity (Ray *et al*., 1990). Many complex and complicated neurogenic mechanisms and their involvement in ulceration have been suggested (Nobrega and Wiener, 1983; Hernandez *et al*., 1985; Ferri *et al*., 1983; Bhargaba *et al*., 1980; Spadaro *et al*., 1987). Cerebellar nodular lesion (CNL) is also involved and responsible for the pathogenesis of gastro-duodenal damage by decreasing the mucus secretion (Guha and Ghosh, 1995; Wolf, 1969). Complex neurotransmitter mechanisms are also involved during such gastric pathology (Grundy, 2006). Serotonin or 5-hydroxytryptamine (5-HT) is a monoamine neurotransmitter which is localized in the enterochromaffin (EC) cells of the gastric and duodenal mucosa (Racke *et al*., 1988). It is reported that serotonin (5-HT) is the specific substance in the enterochromaffin (EC) cells (Gershon, 1999) which are distributed throughout the alimentary canal (Tack and Sarnelli, 2002). Serotonin (5-HT) has also been shown to stimulate mucus production in the gastrointestinal tract (GIT) and it protects gastro-duodenal mucosa against cerebellar nodular lesion (CNL)-induced ulceration (Guha and Ghosh, 1995).
A number of Indian medicinal plants have been extensively used in Indian traditional Ayurvedic system for the protection of gastro-duodenal ulceration. *Cucurbita pepo* Linn. (*C. pepo*), commonly known as pumpkin, is a plant under the family-Cucurbitaceae (Paris et al., 2003; Paris, 2009). The plant is used to prepare infants food formulations (Ezeji and Ojimelukwe, 1993) for the improvements in protein quality (Gibson et al., 2006). Medicinal values of fruit of the plant are high with wide spectrum of biological activities (Caili et al., 2006) and also consumed as a dietary vegetable (Sarkar and Guha, 2008; Koike et al., 2005; Mongkolsilp et al., 2004; Sammon et al., 2003) and medicinal food (Hilgert and Gil, 2007) in various parts of the world. Fruits of *Cucurbita pepo* Linn. are used as vegetable (Sunilson et al., 2009; Linskens and Jorde, 1997) and fruit’s pulp is used in intestinal inflammation or enteritis, dyspepsia (Orlandelli, 1951), intestinal diseases (Francois et al., 2006) and also used to relieve inflammation (Caili et al., 2006). It is also reported that consumption of pumpkin showed a high gastric pH in a rural black African population (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).

Extensive pharmacological investigation suggested that the presence of several major groups of active compounds have been found in fruit's pulp of *C. pepo*. Several antioxidant-type antiulcer compounds 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), sulphhydrils 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 tannin (Ojiako and Igwe, 2008; Silveira et al., 1996), 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; Tadmor et al, 2005; Imaeda et al., 1999; Sedghi et al., 2008) are present in C. pepo fruit have been reported. Furthermore, some antioxidant-type elements such as selenium (Se) (Yoshida et al., 2005; Stibilj et al., 2004), Zinc (Zn) (Fan et al., 2006) and cadmium (Cd) (Qadir et al., 2000) have been found in C. pepo fruit.

Therefore, compounds of other chemical nature are also evident. One of them is most important bioactive component such as tryptophan amino acid (Trp or W). Some proteins having tryptophan (Trp or W) residue(s) are present in C. pepo fruit such as lectin (Allen, 1979), mavicyanin (Xie et al., 2005; Xie et al., 2003; Marchesini et al., 1979; Kataoka et al., 1998) and pepocin (a ribosome inactivating protein; RIP) having single A-chain (Yoshinari et al., 1996). A-chain from RIP of ricin is similar amino acid sequence compared to A-chain of pepocin isolated from the rice that contains tryptophan (Trp or W) residue at position 211 (Ding et al., 2002). Therefore, some tryptophan (Trp or W) containing enzymes such as 1-aminocyclopropane-1-carboxylate synthase and S-adenosyl L-methionine methylthioadenosine lyase (Huang et al., 1991) have been found in pumpkin fruit.

Dietary source of tryptophan (Trp or W) from the fruit’s pulp of C. pepo may be precursor of serotonin (5-HT) in the enterochromaffin (EC) cells and precursor of melatonin in the pineal gland through serotonin (5-HT). Both serotonin and melatonin protect gastric and duodenal ulceration as a mucus stimulator (Kaufmann et al, 1979; Konturek et al, 1987; Pesker, 1980) and as an antioxidant respectively (Bandyopadhyay et al, 2000). Furthermore, in the preliminary investigation of aqueous extract of ripe fruit’s pulp of C. pepo at a dose of 400 mg/kg body weight exhibited significant protection of gastric and duodenal ulceration 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).

Based on these ethnic reports, the present study was undertaken to observe the role of aqueous extract of ripe fruit’s pulp of C. pepo (400 mg/kg body weight) on
serotonin (5-HT) content, along with enterochromaffin (EC) cells count, ulcer index (UI) and mucosal thickness (MT) against cerebellar nodular lesion (CNL)-induced gastric and duodenal ulcerated tissues on 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 cerebellar nodular lesion (CNL), Group IV was *C. pepo* pretreated and cerebellar nodular lesion (CNL) and Group V was ranitidine pretreated and cerebellar nodular lesion (CNL).

The dry extract was dissolved in distilled water. Group I and Group III rats were given distilled water with approximately same volume of *C. pepo* extract orally by orogastric cannula. 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 8th day of extract treatment group III, group IV and group V rats were anaesthetized with sodium pentobarbital (40 mg/kg body weight; Abbott India Ltd) intraperitoneally (i.p). Cerebellar nodular lesion (CNL) was performed as per stereotaxic co-ordinates (AP=12.8, L=0.4, D=6.8; Pellegrino and Cushman, 1967). Electrolytic lesions were made in the nodular cerebellum by conventional bipolar electrode (insulated by epoxylite with 0.5 mm tip exposed) using 1.5 mA DC (milli Ampere Direct Current) for 20 seconds for consecutive three days.

**Parameters studies:**

**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.

**Estimation of serotonin (5-HT) content:** Stomach and duodenum tissues were weighted and 5-HT level was estimated following the fluorescence spectrophotometrical
method of Amar et al., 1982. Stomach and duodenum were collected in cold condition and homogenized with 5 ml acidified butanol. Then 2 ml homogenate was mixed with 5ml 10% heptane and 2.5 ml 0.003(N) HCl and then shaken for 5 min and centrifuged at 2000 rpm for 10 min. Acid layer (2.25 ml) was eluted and mixed with 100 mg alumina and 0.5 ml of 2(M) sodium acetate. The mixture was shaken for 5 min and centrifuged at 2000 rpm for 10 min. The precipitate was discarded. The supernatant was mixed with 1.5 ml of 10% isobutanol and shaken with 1 ml salt saturated buffer solution at pH=10. Then 1 ml heptane was added and 2.5 ml 0.1(N) HCl was mixed. This was taken for the estimation of serotonin (5-HT). The fluorescence of 5-HT was measured in the Perkin-Elmer MPF 44B fluorescence spectrophotometer with activation and emission wavelength set at 295 nm and 550 nm respectively.

**Mucosal thickness (MT):** For determination of mucosal thickness (MT) 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 from muscularis mucosa to the tip of the epithelium 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).

**Enterochromaffin (EC) cells staining and counting:** The stomach and duodenal tissues were stained by silver nitrate method for enterochromaffin cells (EC cells) (Singh, 1964) and counted by the method of Guha and Ghosh, 1995.

**Results:**

**Ulcer index (UI):** Cerebellar nodular lesion (CNL)-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) and ranitidine (10 mg/kg body weight) for 14 consecutive days in cerebellar nodular lesion (CNL)-induced stomach and duodenum tissues showed a significant decrease in ulcer index (UI) as compared to cerebellar nodular lesion (CNL)-induced stomach and duodenum tissues respectively (Table 10.1).
**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. Cerebellar nodular lesion (CNL)-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* and ranitidine (10 mg/kg body weight) for 14 consecutive days in cerebellar nodular lesion (CNL)-induced stomach and duodenum tissues showed a significant increase of mucosal thickness (MT) as compared to cerebellar nodular lesion (CNL)-induced stomach and duodenum tissues respectively (**Table 10.1**).

**Serotonin (5-HT) content:** Treatment with aqueous extract of ripe fruit's pulp of *C. pepo* (400 mg/kg body weight increased the serotonin (5-HT) content of both stomach and duodenum tissues as compared to control stomach and duodenum tissues respectively. Cerebellar nodular lesion (CNL)-induced stomach and duodenum tissues showed a significant decrease of serotonin (5-HT) content as compared to control stomach and duodenum tissues respectively. When *C. pepo* (400 mg/kg body weight) and ranitidine (10 mg/kg body weight) pretreatment rats subjected to cerebellar nodular lesion (CNL)-induced ulceration showed a significant increase in serotonin (5-HT) content as compared to cerebellar nodular lesion (CNL) rats (**Table 10.1**).

**Enterochromaffin (EC) cells count:** Treatment with aqueous extract of ripe fruit’s pulp of *C. pepo* (400 mg/kg body weight) for 14 consecutive days increased the enterochromaffin (EC) cells count per mm² area of both stomach and duodenum tissues as compared to control stomach and duodenum respectively. Cerebellar nodular lesion (CNL)-induced group showed a significant decrease of enterochromaffin (EC) cells count per mm² area as compared to control group. When *C. pepo* (400 mg/kg body weight) and ranitidine (10 mg/kg body weight) pretreatment group subjected to cerebellar nodular lesion (CNL) showed a significant increase of enterochromaffin (EC) cells count per mm² area as compared to only cerebellar nodular lesion (CNL) group (**Table 10.1**).
Table 10.1: 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), enterochromaffin (EC) cell count and serotonin (5-HT) content of stomach and duodenum tissues

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Stomach Ulcer index (UI)</th>
<th>Mucosal thickness (MT) (μm)</th>
<th>Enterochromaffin cells count/ mm² area</th>
<th>Serotonin (5-HT) (μg/gm tissue)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Stomach</td>
<td>Duodenum</td>
<td>Stomach</td>
<td>Duodenum</td>
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<tr>
<td>Control</td>
<td>0</td>
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<td>555.832 ±6.37</td>
<td>739.999 ±18.559</td>
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<td>2638.33 ±37.52</td>
<td>3225.50 ±13.647</td>
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<tr>
<td></td>
<td>1.527 ±0.008</td>
<td>3.660 ±0.008</td>
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<td><em>C. pepo</em></td>
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<td>566.666 ±10.98</td>
<td>1093.333 ±16.442</td>
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<td>2800.00 ±16.733</td>
<td>3357.50 ±13.086</td>
<td>3225.50 ±13.647</td>
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<td></td>
<td>2.037 ±0.011</td>
<td>4.163 ±0.015</td>
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<tr>
<td>CNL</td>
<td>38.500 ±0.763*</td>
<td>30.833 ±1.166*</td>
<td>327.777 ±7.349*</td>
<td>561.110 ±3.743*</td>
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<td>513.333 ±14.20*</td>
<td>2115.833 ±17.292*</td>
<td>3238.33 ±12.52</td>
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<tr>
<td></td>
<td>0.890 ±0.006*</td>
<td>2.353 ±0.014*</td>
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<tr>
<td><em>C. pepo</em> + CNL</td>
<td>2.166 ±0.600*</td>
<td>1.833 ±0.654*</td>
<td>558.333 ±2.545*</td>
<td>750.694 ±4.861*</td>
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<td>2623.666 ±22.944*</td>
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<td>1.336 ±0.045*</td>
<td>3.415 ±0.135*</td>
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<td>Ranitidine</td>
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<td>561.666 ±2.545*</td>
<td>751.110 ±4.937*</td>
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<td>2623.666 ±22.944*</td>
<td>3247.50 ±14.068*</td>
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<tr>
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<td>1.219 ±0.0274*</td>
<td>3.337 ±0.193*</td>
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</table>

UI= Ulcer index; MT= Mucosal thickness; EC= Enterochromaffin cell; 5-HT= 5-hydroxytryptamine; *C. pepo*= *Cucurbita pepo*; CNL= Cerebellar nodular lesion.

One way ANOVA; *, # significantly different from group I (control) and group III (Cerebellar nodular lesion) respectively at *p*<0.05

**Histological analysis of enterochromaffin (EC) cells:**

Micro-photographic observation of Fig 10.1 and Fig 10.6 showed the distribution of EC cells (×100) and serotonin (5-HT) vesicles (×1000) of control stomach and duodenum tissues respectively. Black colored-precipitations (serotonin vesicles) were argentaffin reaction resulted from the reaction-products of aldehyde and biogenic amines with ammonical silver solution.

Treatment of *C. pepo* extract (400 mg/kg body weight) in both the stomach (Fig 10.2) and duodenum (Fig 10.7) showed increased black colored-precipitations of argentaffin reaction products as compared to control stomach (Fig 10.1) and control duodenum (Fig 10.6) respectively.

The black colored-precipitations of argentaffin reaction products were diminished in the cerebellar nodular lesion (CNL)-induced stomach (Fig 10.3) and duodenum (Fig 10.8) tissues as compared to control stomach (Fig 10.1) and control duodenum (Fig 10.6). A small number of black colored-precipitations of argentaffin reaction products were found in both cerebellar nodular lesion (CNL)-induced stomach (Fig 10.3) and duodenum (Fig 10.8) tissues.
When *C. pepo* pretreatment rats subjected to cerebellar nodular lesion (CNL)-induced ulceration, stomach (Fig 10.4) and duodenum (Fig 10.9) also showed the black colored-precipitations of argentaffin reaction products though less as compared to only *C. pepo* treated stomach (Fig 10.2) and duodenum (Fig 10.7) respectively.

Therefore, ranitidine pretreatment rats subjected to cerebellar nodular lesion (CNL)-induced ulceration, stomach (Fig 10.5) and duodenum (Fig 10.10) also showed the black colored-precipitations of argentaffin reaction products though less as compared to only *C. pepo* treated stomach (Fig 10.2) and duodenum (Fig 10.7) respectively.
Histology of stomach EC cells by silver nitrate stain:

Stomach EC cells at (×100) magnification:

Fig 10.1: Control (×100) Fig 10.2: *C. pepo* treated Fig 10.3: CNL-induced Fig 10.4: *C. pepo*+ CNL Fig 10.5: Ran+ CNL

Stomach serotonin vesicles (granules) in EC cells at (×1000) magnification:

Fig 10.1: Control (×1000) Fig 10.2: *C. pepo* treated Fig 10.3: CNL-induced Fig 10.4: *C. pepo*+ CNL Fig 10.5: Ran+ CNL

Duodenum EC cells at (×100) magnification:

Fig 10.6: Control (×100) Fig 10.7: *C. pepo* treated Fig 10.8: CNL Fig 10.9: *C. pepo*+ CNL Fig 10.10: Ranitidine+ CNL

Duodenum serotonin vesicles (granules) in EC cells at (×1000) magnification:

Fig 10.6: Control (×1000) Fig 10.7: *C. pepo* treated Fig 10.8: CNL-induced Fig 10.9: *C. pepo*+ CNL Fig 10.10: Ran+ CNL
Fig 10.1: Shows the distribution of enterochromaffin (EC) cells of control stomach (×100). Black colored-granules were argentaffin reaction resulted from the reaction-products of aldehyde and biogenic amines with ammonical silver nitrate solution (×1000).

Fig 10.2: Shows the EC cells of *C. pepo* extract treated stomach (×100) that increased granules of argentaffined amines as compared with control stomach (×1000).

Fig 10.3: Shows the EC cells of cerebellar nodular lesioned stomach (×100) that decreased granules of argentaffined amines as compared with control stomach (×1000).

Fig 10.4: Shows the EC cells of *C. pepo* pretreated cerebellar nodular lesioned stomach (×100) that decreased granules of argentaffined amines though less as compared to only *C. pepo* treated stomach (×1000).

Fig 10.5: Shows the EC cells of ranitidine pretreated in cerebellar nodular lesioned stomach (×100) that decreased granules of argentaffined amines though less as compared to only *C. pepo* treated stomach (×1000).

Fig 10.6: Shows the distribution of enterochromaffin (EC) cells of control duodenum (×100). Black colored-granules were argentaffin reaction resulted from the reaction-products of aldehyde and biogenic amines with ammonical silver nitrate solution (×1000).

Fig 10.7: Shows the EC cells of *C. pepo* extract treated duodenum (×100) that increased granules of argentaffined amines as compared with control duodenum (×1000).

Fig 10.8: Shows the EC cells of cerebellar nodular lesioned duodenum (×100) that decreased granules of argentaffined amines as compared with control duodenum (×1000).

Fig 10.9: Shows the EC cells of *C. pepo* pretreated in cerebellar nodular lesioned duodenum (×100) that decreased granules of argentaffined amines though less as compared to only *C. pepo* treated duodenum (×1000).

Fig 10.10: Shows the EC cells of ranitidine pretreated in cerebellar nodular lesioned duodenum (×100) that decreased granules of argentaffined amines though less as compared to only *C. pepo* treated duodenum (×1000).
Serotonin vesicles (granules) in EC cells at high magnifications

Fig. A: Serotonin vesicles (×1200)  Fig. B: Serotonin vesicles (×1500)
Discussion:

Analysis of the results showed that cerebellar nodular lesion (CNL) produced an increase in ulcer index (UI), decrease in mucosal thickness (MT) and decrease in serotonin (5-HT) content along with enterochromaffin (EC) cells count in rat stomach and duodenal tissues. The decrease in serotonin (5-HT) content along with decrease in EC cells count (Guha and Ghosh, 1995; Debnath and Guha, 2007), decrease in mucosal thickness (MT) and increase in ulcer index (UI) (Sarkar and Guha, 2008; Debnath and Guha, 2007; Szabo et al., 1985) may be relevant to gastro-duodenal ulcer pathogenesis. The pathophysiology of experimental peptic ulcer formation is not clearly known (Dhikav et al., 2003) but is believed to be multimechanisms (Goodwin et al., 1986; Konturek et al., 1999) and multifactorial (Guzel et al., 1998) process. The various factors like an increase in acid secretion, increase in peptic activity (Manonmani et al., 1995), reduction of gastro-duodenal mucosal blood flow, inhibition of prostaglandins (PGs) synthesis, disruption of mucosal epithelium, inhibition of mucus secretion, inhibition of bicarbonate secretion (Aase, 1989; Allen and Leonard, 1988), inhibition of 5-HT synthesis and reduction of EC cells count in gastro-duodenal mucosa have been suggested (Guha and Ghosh, 1995; Debnath and Guha, 2007).

The pathogenesis of peptic ulcer disease is believed to reflect an imbalance between increased aggressive factors and decreased defensive factors (Sarkar et al., 2006). The aggressive factors are acid (Broocks, 1985) and pepsin (Manonmani et al., 1995) and protective factors are mucus, prostaglandins (PGs), serotonin (5-HT) along with enterochromaffin (EC) cells (Guha and Ghosh, 1995; Debnath and Guha, 2007) in gastro-duodenal mucosa has been suggested. The increased ulcer index (UI) and decreased 5-HT along with decreased enterochromaffin (EC) cells count in against cerebellar nodular lesion (CNL)-induced ulceration reduced the gastro-duodenal protection and rapid restitution or repair mechanisms leading to breakdown of mucosal barrier (Guha and Ghosh, 1995; Debnath and Guha, 2007).

Serotonin (5-HT) is a monoamine neurotransmitter in the central nervous system (CNS) and in the enteric nervous system (ENS) especially in the gastrointestinal tract (GIT) (Erspamer, 1966; Vanden et al., 1999) and it is generally considered to be involved in the modulation of motor and sensory functions of gastrointestinal tract (GIT)
Apart from motor and sensory functions, the role of 5-HT in gastric acid secretion has been evaluated. Serotonin (5-HT) inhibits gastric acidity by increasing the gastric mucus secretion with help of prostaglandins (PGs) (Guha and Ghosh, 1995). It has been reported that serotonin (5-HT) stimulates prostaglandins (PGs) synthesis (mainly PGE\(_2\), PGH\(_2\)) and these PGs help in the secretion of mucous along with bicarbonate (Morrow and Roberts, 2001) in rats (Guha and Ghosh, 1995) and prostaglandin (PG) E\(_2\) synthesis in human (Munck \textit{et al.}, 1988) and animals (Beubler \textit{et al.}, 1986).

The cerebellar nodular lesion (CNL) decreases serotonin (5-HT) content along with enterochromaffin (EC) cells count (Guha and Ghosh, 1995; Debnath and Guha, 2007) as well as gastric \textit{hyper acidity} which produces ulceration (Maiti and Guha, 1978; Wolfe, 1969) and mucosal damage of stomach (Debnath and Guha, 2007) and gastro-duodenal tissues (Guha and Ghosh, 1995). The increase in ulcer index (UI), decrease in mucosal thickness (MT) and decrease in serotonin (5-HT) content along with enterochromaffin (EC) cells count observed in the present study against cerebellar nodular lesion (CNL) may be due to failure in gastro-duodenal protection and repair mechanisms leading to disrupted mucosal epithelium (Guha and Ghosh, 1995; Debnath and Guha, 2007).

Serotonin is a monoamine neurotransmitter throughout the gastro-duodenal mucosa (Furness and Costa, 1982) and release of serotonin (5-HT) from the enterochromaffin (EC) cells (Schworer \textit{et al.}, 1987; Racke \textit{et al.}, 1988) acts as chemical and mechanical transducer for the initiation of local reflexes (Tack and Sarnelli, 2002). In all adult mammals there is a significant correlation between the enterochromaffin (EC) cell count and serotonin (5-HT) content in gastro-duodenal tissues (Pentilla, 1966; Nilsson \textit{et al.}, 1983). In the EC cells, serotonin (5-HT) is synthesized from tryptophan (Trp or W) amino acid. Serotonergic terminals located in the gastro-duodenal mucosa are responsible for liberation of serotonin (5-HT) from enterochromaffin (EC) cells. As enterochromaffin (EC) cells produce serotonin (5-HT) (Pentilla, 1966; Nilsson \textit{et al.}, 1983) and lie in close vicinity (proximity) to mucus producing cells (Clara, 1933), it is tempting to assume that local serotonin (5-HT) production might normally influence...
mucus production by paracrine action (Kaufmann et al., 1979; Konturek et al., 1987; Peskar, 1980).

Treatment with aqueous extract of ripe fruit's pulp of C. pepo showed that 400 mg/kg body weight was the most effective dose and significantly decreased the ulcer index (UI) and concomitantly increased the mucosal thickness (MT), 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). The ability of gastric mucosa to resist injury by cerebellar nodular lesion (CNL) is attributed to number of factors serotonin (5-HT) that have been referred to collectively as mucosal defense (Guha and Ghosh, 1995). The gastric mucosal erosion induced by cerebellar nodular lesion (CNL) is due to depression of this defense mechanisms (Debnath and Guha, 2007).

Ripe fruit's pulp of Cucurbita pepo Linn. is a major source of tryptophan (Trp or W) containing proteins such as mavicyanin, lectin, pepocin, aspartic proteinase inhibitor, etc. Mavicyanin is a blue copper containing protein with tryptophan (Trp or W) residue(s) isolated from C. pepo medullosa (Xie et al., 2005; Xie et al., 2003; Marchesini et al., 1979; Kataoka et al., 1998). A lectin from the exudate of the fruit of the vegetable marrow (Cucurbita pepo) that has a specificity for beta-1, 4- linked N-acetylglucosamine oligosaccharides (Allen, 1979) having tryptophan (Trp or W) residue(s). Pepocin is a type-1 ribosome inactivating protein (RIP) isolated from the sarcocarp of Cucurbita pepo having single A-chain (Yoshinari et al., 1996). A-chain of ricin, the ribosome inactivating protein (RIP) isolated from the rice, is similar amino acid sequence to A-chain of pepocin that contains tryptophan (Trp or W) residue at position 211 (Ding et al., 2002).

Dietary supplementations of tryptophan (Trp or W) containing proteins are the major source of serotonin (5-HT) precursor especially in the enterochromaffin (EC) cells of the gastrointestinal tract (GIT) (Wheeler and Challacombe, 1984). Therefore, dietary supplementations of tryptophan (Trp or W) containing proteins are the major source of melatonin precursor in the pineal gland is synthesized through serotonin (5-HT). Both serotonin and melatonin protect gastric and duodenal ulceration as a mucus stimulator (Kaufmann et al., 1979; Konturek et al., 1987; Pesker, 1980) and as an antioxidant
respectively (Bandyopadhyay et al, 2000). So, consumption of tryptophan (Trp or W) containing proteins such as mavicyanin, lectin, pepocin, aspartic proteinase inhibitor etc, present in medullosa and sarcocarp of Cucurbita pepo fruit may be a dietary source of tryptophan (Trp or W) and precursor of serotonin (5-HT) synthesis in the enterochromaffin (EC) cells has been shown in the present investigation.

Therefore, it can be explained that serotonin (5-hydroxytryptamine; 5-HT) and 5-methoxytryptamine (5-MT) both 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 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 amino acid 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-hydroxy tryptamine), 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 *OH (Bandyopadhyay et al, 2000). It was suggested that fruit pulp of C. pepo could synthesize endogenous melatonin.
Thus, it may be suggested that pretreatment of *C. pepo* may prevent the gastric and duodenal mucosal damage by immobilized cold-stress increasing mucosal thickness (MT) may synthesize endogenous melatonin, reduce the oxygen derived free radicals production (Bandyopadhyay *et al.*, 2000) which may be due to the presence of tryptophan in the 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).

So, it may be suggested that pretreatment of *C. pepo* may be a source of tryptophan (Trp or W) as well as serotonin (5-HT) of the enterochromaffin (EC) cells which is present in *C. pepo* (Yoshinari *et al.*, 1996; Marchesini *et al.*, 1979; Allen, 1979) and may be responsible for increased serotonin (5-HT) levels and enterochromaffin (EC) cells counts of the gastric and duodenal tissues.

Thus from the present investigation it can be concluded that aqueous extract of ripe fruit's pulp of *C. pepo* protects gastric and duodenal ulceration by modulating serotonin (5-HT) and enterochromaffin (EC) cell of gastroduodenal tissues.