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# IN-VIVO ANIMAL EVALUATION OF PLANT EXTRACT OF CUCURBITA PEPO FOR DETERMINATION OF GASTROPROTECTIVE ACTIVITY

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**Abstract:**

The causes of gastric ulcer pyloric ligation are believed to be due to stress induced increase in gastric hydrochloric acid secretion and/or stasis of acid and the volume of secretion is also an important factor in the formation of ulcer due to exposure of the unprotected lumen of the stomach to the accumulating acid. The gastric tissue in treated with Cucurbita pepo fruit extract showed regains of cellular structure, less hemorrhage condition, and with less edema which is almost equal to that of the standard group, exhibiting high significant action. Gastro Protective Activity of ethanol extract of Cucurbita pepo fruit were performed on ulcer induce model including aspirin + Pylorus ligation induced, acetic acid induced chronic ulcer, HCl- ethanol induced ulcer. Ethanol extracts of Cucurbita pepo have exhibited significant gastroprotective activity. The ethanol extract of cucurbita pepo at a dose of 100, 200 and 300 mg/kg b.w has showed highly significant gastroprotective activity copared to standard drug ranitidine. Gastroprotective actions of certain phytoconstituents like flavonoids, alkaloids, tannins have been well documented in the literature. The above mentioned phytoconstituents alone or in combination may be responsible for the gastroprotective activity of the selected plants.

**Introduction:** Peptic ulcers are sores or lesions in the gastrointestinal mucosa extending throughout the muscularis mucosae, typically characterized by different stages of necrosis, neutrophil infiltration, blood flow reduction, increased oxidative stress and inflammation [1]. Studies have shown that peptic ulcer disease occurs because of an imbalance between aggressive injurious (e.g., pepsin, HCl) and defensive mucosa-protective factors (e.g., prostaglandins, mucus and bicarbonate barrier and adequate blood flow). All ulcers of the upper gastrointestinal tract were originally thought to be caused by the aggressive action of pepsin and gastric acid on mucosa [2]. A special emphasis was given on plant products safety and security, in order to trigger the interest in deepening skills on this matter and to ensure an effective managing competence for health-related systems [3]. Peptic ulcer describes a condition in which there is a discontinuity in the entire thickness of gastric or duodenal mucosa that persists as a result of acid and pepsin in the gastric juice. Peptic ulcer is caused due to several causes of abnormal acid secretion, abnormal mucosal defence, reflux of bile and pancreatic juice, genetic predisposition, microbial attack, etc. Several rare genetic syndromes are associated with peptic ulcer, e.g. multiple endocrine neoplasias [4]. The bacteria Helicobacter pylori have revolutionized the approach of peptic ulcer diseases and gastritis. Almost all patients with duodenal ulcer and 80% of patients with gastric ulcers are infected with H. pylori. The organism is also associated with chronic active gastritis (stomach inflammation) and may also play a role in non-ulcer dyspepsia (the belching and bloating symptoms of indigestion). H. pylori infection has also been associated with a slightly increased risk of gastric cancer**.** Since ancient times, plants and plant derived-products have been used in folklores around the world for the treatment of several ailments and diseases. Nowadays, herbal medicine is becoming a viable alternative treatment over the commercially available synthetic drugs on PU management/treatment. This is premised on its lower cost, perceived effectiveness, availability as well as little or no adverse effects. A number of these herbal remedies have demonstrated gastroprotective properties and have been used in the treatment of

PU, digestive disorders and other related ailments for several centuries [5, 6]. The experiments performed in animal models of gastric ulcer confirm the gastroprotective and healing properties of many herbs traditionally used in folk medicine [7]. A variety of biochemical products were established, many of which are extractable and used as chemical feed stocks or as a raw materials for various scientific investigations. Many secondary metabolites of plant are commercially important and find use in a number of pharmaceutical compounds. The use of herbal medicines is an evidence or science based approach for the treatment and prevention of disease is known as phytotherapy. Fruits of Cucurbita pepo Linn, are used as vegetable and fruit s pulp of Cucurbita pepo Linn. is used for the treatment of digestive system such as dyspepsia and enteritis or intestinal inflammation intestinal diseases. It has been reported that pumpkin is consumed as a diet to increase the pH of fasting gastric sample and the dietetic management of patients undergoing gastric operations is also carried out by supplementation of pumpkin. So, the aim of our study is to elucidate the gastro-protective role of extract of ripe fruit s pulp of Cucurbita pepo Linn. keeping several objectives for the prevention of different experimental peptic ulcer (drug such as- aspirin induced; alcohol such as- ethanol induced; stress such as- immobilized and cold induced; surgery such as- cerebellar nodular lesion induced; CNL) in rat model.

**Material and Methods:** The proposed work includes fruits of Cucurbita pepo Linn is used as vegetable for the treatment of digestive system such as dyspepsia and enteritis or intestinal inflammation intestinal diseases.

**Procurement and authentication of Cucurbita pepo fruit:** As the crude drugs form the basis for the manufacture of wide range of medicinal preparations needed by people, the development of pharmacognostical research has become indispensable for procuring therapeutically potent medicine prepared from genuine drug material. Fresh and fully grown fruits of Cucurbita pepo were purchased from local market of Sagar India. The plants were authenticated Botanist of Department of Botany, Government College Khimlasa, Sagar. The fruits were washed properly with water. The seeds from fruits of Cucurbita pepo were separated. The fruit were cut into small pieces, dried in sun and ground with the help of an electrical grinder to get powder, stored in airtight containers and used for phytochemical and pharmacological studies.

**Selection of animals and preparation of groups:** Healthy albino rats of either sex, weighing between 180-250g were procured from the disease free animal house of Zydus Cadila Research Center, Ahmedabad. They were housed in standard environmental conditions of temperature, humidity, and light and provided with standard rodent food and water ad libitum.

**Acute Toxicity studies of Cucurbita pepo fruit extracts as per OECD guideline:**

Acute toxicity studies of extracts of *Cucurbita pepo* fruit were performed in Swiss Albino rats dose levels of 50, 300 and 2000 mg/kg as per OECD guide lines. No mortality was observed in animals dosed with the extracts of *Cucurbita pepo* fruits at dose levels of 50, 300 and 2000 mg/kg (p.o). The treated animals did not demonstrate any significant changes in behavioral pattern and exhibited normal activity. The animals were examined for long term toxicity (14 days).

**Preparation of extract:** Cucurbita pepo fruit powdered (100 g) was successively extracted with the following solvents of increasing polarity (petroleum ether, ethyl acetate and ethanol) in a soxhlet apparatus. The dried extracts were redissolved in water using carboxymethyl cellulose (CMC) as suspending agent and this suspension was used for gastro-protective activity. Sources of chemicals and drugs Ranitidine - standard drug-gift sample from Zydus Cadilla, Ahmedabad.

**Plant extracts:**

**EOCP**: Ethanol extract of Cucurbita pepo (100, 200, 300 mg/kg)

**Evaluation of Gastroprotective activity:** Gastro Protective Activity of ethanol extract of Cucurbita pepo fruit were performed on ulcer induce model including aspirin + Pylorus ligation induced, acetic acid induced chronic ulcer, HCl- ethanol induced ulcer. Ethanol extracts of Cucurbita pepo fruit were found more potent in antioxidant activity so ethanol extract were selected for further gastroprotective activity. Ethanol extracts of both plant at dose of 100 200 and 300 mg/kg were tested, 100 and 200 and 300 mg/kg b.w in 2% gum acacia for gastroprotective activity using above ulcer model [8]. The wistar albino rats of either sex were divided in following groups of six animals (n=6) each.

Group I served as control and received 2% gum acacia suspension 1ml/kg b.w p.o

Group-II served as standard and received Ranitidine (50mg/kg b.w, p.o) 2% gum acacia suspension

Group-III served as Ethanol extract of Cucurbita pepo (100 mg/ml)

Group-IV served as Ethanol extract of Cucurbita pepo (200 mg/ml)

Group-V served as Ethanol extract of Cucurbita pepo (300 mg/ml)

**Aspirin + Pyloric ligation (PL) model:** The animals were divided into 5 groups, each containing six animals. Group I served as Aspirin (200 mg/kg, p.o.)+PL control. Group II received Ranitidine (50 mg/kg, p.o.) as standard drug+Aspirin+ PL. Groups III and IV and V received plant extract at the dose of 100, 200 and 300 mg/kg, p.o+PL. Groups II V received the assigned drug treatment for the respective 10 days daily. From days 8 to 10, animals of all groups received aspirin orally as an aqueous suspension at the dose of 200 mg/kg, 2 h after the administration of the drugs. Animals in all groups were fasted for 18 h after the assigned treatment, anesthetized and the pyloric was ligated. The rats were sacrificed after 4 h by excess anesthesia (ether). The stomach was removed, opened along greater curvature and the gastric lesions were observed. The gastric ulcers were counted and the ulcer index was determined. The gastric juice was collected, centrifuged and the volume of the supernatant was expressed as mL/ 100 gm.b.wt. Free acidity and total acidity were determined by titrating with 0.01N NaOH using Topfer s reagent and phenolphthalein as indicators. The free and total acids were expressed as mEq/L. The total acid output was determined and expressed as mEq/L [9].

**Acetic acid-induced ulcer model:** The ulcers were induced by the local application of acetic acid to serosal surface of the stomach. Under anesthesia, the midline incision was made, and the stomach was taken out. On the serosal surface of the glandular portion of the stomach, 0.2 ml of 100% acetic acid (anterior gastric wall) was injected. After the treatment, the rats were sacrificed, and stomachs were removed and weighed, fixed in 10% neutral buffered formalin, embedded in the paraffin wax, sectioned at 5 μm, stained with hematoxylin and eosin and then examined by the light microscopy [10-11].

**HCl and ethanol induced model:** Animals were deprived of food for 24 h in a cage with wide-mesh wire bottoms to prevent coprophagia before conducting the experiment. One hour after the last (the 14th) administration of vehicle, an HCl and ethanol mixture (98% ethanol containing 150 mM HCl) was orally administered to mice at 5 mL/kg of body weight according to a previous report**.** Untreated control mice were administered an equal volume of distilled water instead of ethanol mixture solution. ethanol (EtOH)-induced ulcer model is most preferential used animal model because it enables rapid induction and can be widely employed to test the efficacy of potential drugs independent of gastric acid secretion [12].

**Estimation of biochemical parameters**

**Free acidity and total acidity determination**: One ml of gastric juice was pipetted out in to 100ml conical flask, add 2 to 3 drops of Topfer s reagent and titrated with 0.01N NaoH until all traces of red colour disappears and the colour of the solution turns to yellowish orange. The volume of the alkali added was noted, this volume corresponds to free acidity. Then 2 to 3 drops of phenolpthalein solution was added and titration was continued until a define red tinge reappears. Again the total volume of alkali added was noted. The volume corresponds to total acidity.

Acidity was calculated by following formula

$$\text{Acidity} = \frac{\text{Volume of NaOH X Normality of NaOH X 100}}{0.1} \quad \text{meq / L / 100 g}$$

**Ulcer index:** The number of ulcers per stomach was noted and severity of the ulcers and scoring of ulcers was done microscopically [13].

Mean ulcer score for each animal will be expressed as ulcer index. The percentage of ulcer protection was determined as follows:

$$\text{Percent protection (\%)} = \frac{\text{Control mean ulcer index - Test mean ulcer index} \times 100}{\text{Control mean ulcer index}}$$

After 45 min of extracts and ranitidine treatment, pyloric ligation was done by ligating the pyloric end of stomach of rats of respective groups under light ether anaesthesia at a dose of 35 mg/kg of body weight. Ligation was done without causing any damage to the blood supply of the stomach. Animals were allowed to recover and stabilize in individual cages and were deprived of water during postoperative period. After 4 h of surgery, rats were sacrificed with excess of anaesthetic ether. Abdominal end was opened, esophageal end of the stomach was opened and entire stomach from the body of the animal was removed. Gastric juice present in each stomach of the respective group was measured by collecting into a graduated centrifugation tube and was centrifuged at 1000 rpm for 10 min and gastric volume was noted. Percentage protection was calculated **[14].**

**Mucin activity:** Mucin activity was estimated in the mucosubstances precipitated twice by treating the gastric secretion with 90%ethanol in a 9:1 ratio. The precipitate thus obtained was divided into two parts, one part was dissolved in 1mL of 0.1N NaOH, and the other part was dissolved in 1mL of 0.1N H2SO4. The former was used for the estimation of protein, total hexoses, hexosamine and fucose, while the latter was used for the estimation of sialic acid. The results were expressed as mg/mL. Finally, the total carbohydrate to protein (TC:P) ratio, i.e., mucin activity was determined.

**Evaluation of pepsin:** Pepsin levels in the gastric effluent were determined as Anson method. Briefly, 2 ml of 2.5% bovine hemoglobin plus 0.5 ml of 0.3 N HCl and 0.5 ml of gastric effluent were maintained in separate tubes at 37°C for 10 min and then mixed. Mixtures were incubated for 10 min at 37°C, and the reaction was stopped by the addition of 5 ml 0.3 N trichloroacetic acid. After agitation and filtration, optical density was measured at 280 nm by using a spectrophotometer (Unikon 930, Kontron Instrument, Italy). The results were compared to a standard curve, which was generated in an identical manner using known amounts of porcine pepsin (1 µg = 3 peptic units), and were expressed as micrograms of pepsin.

**Assessment of antioxidant parameters**: Stomach tissue was assessed for antioxidant assay.

**Superoxide dismutase assay**: 0.6 mM EDTA solution, 0.1 M carbonate bicarbonate (pH 10.2) buffer,

1.8 mM epinephrine. The SOD activity was measured as a degree of inhibition of auto-oxidation of epinephrine at an alkaline pH by the method. 0.1 ml of tissue homogenate was added to the tubes containing 0.75 ml ethanol and 0.15 ml chloroform (chilled in ice) and centrifuged. To 0.5 ml of supernatant, 0.5 ml of 0.6 mM EDTA solution and 1 ml of 0.1 M carbonate bicarbonate (pH 10.2) buffer were added. The reaction was initiated by the addition of 0.5 ml of 1.8 mM epinephrine (freshly prepared) and the increase in absorbance at 480 nm was measured by using Shimadzu UV visible spectrophotometer.

**Catalase:** Phosphate buffer (0.05 M) pH 7.0 Hydrogen peroxide (5.0 mM), Beers and Seizer method was used to determine the activity of the enzyme CAT. Three milliliters of reaction mixture containing 1.9 ml of phosphate buffer (0.05 M) pH 7.0, 1.0 ml of hydrogen peroxide (5.0 mM) and 0.1 ml of diluted enzyme (skin homogenate) was used in this assay. The activity was measured by reading absorbance at 240 nm at 30 second interval for 3 min using UV spectrophotometer.

**Reduced glutathione assay (GSH):** 25% TCA solution, 0.6 mM DTNB solution, 0.2 mM sodium phosphate buffer (pH 8.0). Reduced glutathione (GSH) level was determined by method of Moron (Moron *et al*., 1979). Skin homogenates were immediately precipitated with 0.1 ml of 25% TCA and the precipitate was removed after centrifugation. Free-SH groups were assayed in a total 3 ml volume by the addition of 2 ml of 0.6 mM DTNB and 0.9 ml 0.2 mM sodium phosphate buffer (pH 8.0) to 0.1 ml of the supernatant and the absorbance was read at 412 nm using a UV spectrophotometer.

**Histological studies:** The stomachs were immersed in 10% formalin solution for histopathological examination. These tissues were processed and embedded in paraffin wax. The central part of damaged or ulcerated tissue was cut on half along the long diameter. If the stomach was protected from the damage then the section was taken from basal part using a rotary microtome sections of thickness of about 5µm were cut and stained with Haemotoxylin and Eosin. These were examined under microscope for histopathological changes such as congestion, haemorrhage, necrosis, inflammation, infiltration, erosion and ulcer and photographs were taken.

**Result and Discussion:**

Gastro Protective Activity of extract of Cucurbita pepo fruit were performed on various ulcer induce model including aspirin + Pylorus ligation induced, acetic acid induced chronic ulcer, HCl- ethanol induced ulcer. Ethanol extracts of Cucurbita pepo fruit and were found more potent in antioxidant activity so ethanol extract were selected for further gastroprotective activity. Ethanol extracts of plant at dose of 100 200 and 300 mg/kg were tested for gastroprotective activity using various ulcer model.

**Effect of ethanolic extract of Cucurbita pepo fruit (EOCP) on Aspirin + Pylorus ligation induced ulcer:** Aspirin+pylorus ligation-induced gastric ulcer model is a useful model to induce severe ulceration in experimental animals. Antiulcer study has been performed using 100, 200 and 300 mg/kg of ethanol extract of Cucurbita pepo fruit against aspirin + Pylorus ligation gastric ulcer models. The ethanol extract were administered to various groups, orally, twice a day as described earlier. The result indicated a dose-dependent antiulcerogenic activity of extract EOCP. The best effect observed was at dose of 300 mg/kg onwards with EOCP. So for further studies on other biochemical parameters of gastric secretion or mucosal studies, a dose of 300 mg/kg was selected.

**Effect of ethanol extract of Cucurbita pepo fruit (EOCP) on volume, acid and pepsin secretion, mucin secretion, mucosal glycoprotein:** The effect of ethanol extract of *Cucurbita pepo* fruit (300 mg/kg) when administered orally, twice daily for 5 days was studied for their effect on volume, acid and pepsin secretion in aspirin + 4hrs pylorus ligation rats. The EOCP showed a trend to decrease in volume,

acid-pepsin concentration and output. The result EOCP were caused significant decrease on volume, acid and pepsin concentration and acid output comparable to standard. Mucoprotein was estimated in the 90% alcoholic precipitate of the gastric juice in aspirin + 4hrs pylorus ligation rats treated with ethanol extract of *Cucurbita pepo* fruit. Treated group with EOCP showed enhance the concentration of total carbohydrates and individual carbohydrates like total hexoses, hexosaamine, fucose and sialic acid with a tendency to decrease protein content leading to a significant incease in TC: P ratio, indicating an increasing in mucin secretion which was comparable with the effect of ranitidine. Gastric mucosal glycoproteins were studied in the 90% alcoholic precipitate of the homogenates of gastric mucosal scraping of the rats. Treated group with EOCP showed enhance the concentration of individual carbohydrates or total carbohydrates with a little change in protein level leading to an increase in total carbohydrate: protein ratio and thus, mucosal glycoprotein in the treated groups. Group I (Aspirin+PL) rats, there was significant increase in protein concentration, but decrease in individual as well as total carbohydrate levels. The drug treatment significantly decreased the protein level and increased the total carbohydrate (TC) level. The index of mucin activity TC:P was found to be decreased in the Aspirin+PL rats. The EOCP and EOBH at the tested dose levels significantly increased the TC:P ratio when compared with control group.

**Effect of ethanolic extract of Cucurbita pepo fruit (EOCP) on 6thday acetic acid- induced chronic ulcer:** The study of healing property of ethanolic extract of Cucurbita pepo fruit (EOCP) on acetic acid induced ulceration has been done using dose of 100, 200 and 300 mg/kg and the healing effect was indicated a dose-dependent antiulcerogenic activity of ethanolic extract. The ulcer area was reduced after 5 day treatment respectively at a dose of 200 and 300 mg/kg while 100 mg/kg did not show significant reduction of ulcer.

**Effect of ethanolic extract of Cucurbita pepo fruit (EOCP) on 6th day acetic acid-induced chronic ulcer on lipid peroxidation (LPO), Catalase (CAT) and Superoxide dismutase (SOD) activities:** The results of the present study on free radical-mediated Lipid peroxidation and alteration in circulating enzymatic antioxidants, CAT and SOD, indicate the involvement of these enzymes in ulcer.

**Histopathology of gastric tissue on 6th day acetic acid- induced chronic ulcers:** Histopathology of the gastric tissue of the control showed focal ulceration and necrosis within the gastric mucosa. The mucosal layer was infiltrated by mixed inflammatory cells. The sub mucosal layer showed scattered inflammatory infiltration along with some congested vascular spaces and areas of hemorrhage. The muscular and serosal layers however appeared within normal limits. Animals treated with EOCB (200mg/kg) showed gastric mucosa with intact lining epithelium. The submucosal slayer showed some congested vascular spaces. The muscular and serosal layers appeared within normal limits. Animals treated with EOCB (200mg/kg) shows gastric mucosa with intact lining epithelium. The mucosal layer and submucosal layer were infiltrated by scattered mononuclear inflammatory cells predominantly compare with ranitidine as standard. The muscularly layer appeared with in normal range.

**Effect of ethanolic extract of Cucurbita pepo fruit (EOCP) on HCl- ethanol induced ulcer:** The result obtained when animals were subjected with HCl ethanol induced ulcer and pretreated with Effect of ethanolic extract of Cucurbita pepo fruit (EOCP) at doses of 100, 200 and 300 mg/kg. The 200 mg/kg and 300 mg/kg were showed inhibitory effect in ulcer index while 100 mg/kg did not show any significant effect in ulcer index.

**Histopathology of Cucurbita pepo fruit (EOCP) ) on HCl - ethanol induced ulcer:** The rats of control group treated with HCl ethanol showed histopathological changes in the gastric mucosa by loss of

glandular architecture, oedema and erosions of the epithelial layer, this infiltration by inflammatory cells. The animals treated with the EOCP (200mg/kg) and EOCP (300mg/kg) showed significant healing of gastric tissue.

**Conclusion:** Increased mucus secretion by the gastric mucosal cells can prevent gastric ulceration by several mechanisms, including lessening of stomach wall friction during peristalsis and gastric contractions, improving the buffering of acid in gastric juice and by acting as an effective barrier to back diffusion of H+ ions. Plant extract EOCP at dose 200mg/kg and 300 mg/kg were found to augment the mucin secretion as evidenced from the increase in concentration of the individual as well as total carbohydrate levels. The drugs also increased the TC:P ratio, which reflects the functional integrity of the mucosal barrier and has been accepted as a reliable index of mucin secretion. The increase in protein content of the gastric juice resulting in a decrease in the TC:P. ratio in the Aspirin+PL animals indicates damage of the gastric mucosa, as a result of which the plasma proteins may leak into the gastric juice. From the results, it is clear that the drugs exhibited significant antisecretory activity by reducing the secretory parameters when compared with the control group. As the EOCP treatment significantly reduced the protein concentration and increased the total carbohydrate content, it may be suggested that the extract EOCP may act by strengthening the mucosal barrier of the gastric mucosa. The EOCP, 300 mg/kg p.o. showed activity that is comparable to the standard drug, Ranitidine. The presence of polysaccharides in EOCB may be responsible for the mucoprotective action in the specified ulcer model. The antiulcerogenic potential of EOCP was further evidenced by the histopathological studies.In conclusion, EOCP 300 mg/kg p.o. exhibited antiulcerogenic activity, which may be attributed to the presence of mucilaginous polysaccharides.

Ethanol extracts of selected plants have exhibited significant gastroprotective activity. The ethanol extract of cucurbita pepo at a dose of 200 and 300 mg/kg b.w has showed significant gastroprotective activity. Gastroprotective action of certain phytoconstituents like flavonoids, alkaloids, Tannins Have been well documented in the literature. The above mentioned phytoconstituents alone or in combination may be responsible for the gastroprotective activity of the selected plants.

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**Table 1: Effect of ethanolic extract of Cucurbita pepo fruit (EOCP) on aspirin + Pylorus ligation induced ulcer**

| Group | Treatment Dose (mg/kg) | Ulcer index ($mm^2$/rat) | % protection |
|---|---|---|---|
| I | Control | 15.1 ± 2.4 | - |
| II | standard | 2.7 ± 1.5 | 83.78 |
| III | EOCP (100 mg/kg) | 6.9 ± 2.2 | 53.29 |
| IV | EOCP (200 mg/kg) | 4.4 ± 2.2* | 73.18 |
| V | EOCP (300 mg/kg) | 3.1 ± 1.7** | 80.21 |

Values are mean ± SEM for 6 rats

* P < 0.05, **P < 0.01, compared to control group

**Table 2: Effect ethanolic extract of Cucurbita pepo fruit (EOCP) on aspirin + pylorus ligation induced ulcer on volume, acid and pepsin**

| Treatment | Volume (µml/100g) | Acid | | Peptic | |
|---|---|---|---|---|---|
| | | Concentration (µEq/ml) | Output (µEq/4 h) | Concentration (µmol/ml) | Output (µmol/4 h) |
| Control | 2.51 ± 0.12 | 97.8 ± 10.2 | 282.1 ± 12.7 | 289.1 ± 21.7 | 715.4 ± 68.2 |
| Standard | 1.99 ± 0.09 | 74.2 ± 5.6 | 179.6 ± 19.4 | 200.2 ± 19.3 | 440.2 ± 58.4 |
| EOCP (300 mg/kg) | 2.04 ± 0.08 | 78.5 ± 9.7 | 156.0 ± 12.9 | 229.1 ± 9.4 | 475.5 ± 23.7 |

**Values are mean ± SEM of 6 rats in each group**

**Table 3: Effect of ethanolic extract of Cucurbita pepo fruit (EOCP) on gastric secretion in aspirin + pylorus ligation induced ulcer**

| Treatment | Mucoprotein (µg/ml) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Total hexose (A) | Hexosamine (B) | Fucose (C) | Sialic acid (D) | T (A+B+C+D) | Protein (P) | TC : P |
| Control | 248.2 ± 19.2 | 161.6 ± 11.2 | 64.2 ± 2.4 | 25.3 ± 2.5 | 499.3 ± 35.3 | 530.4 ± 39.6 | 1.02 ± 0.12 |
| Standard | 299.2 ± 14.2 | 171.9 ± 10.4 | 71.6 ± 3.8 | 35.2 ± 1.9 | 577.9 ± 30.3 | 385.2 ± 28.5 | 1.55 ± 0.09 |
| EOCP (300 mg/kg) | 329.3 ± 14.2 | 179.7 ± 12.1 | 73.5 ± 3.9 | 33.4 ± 1.7 | 615.9 ± 31.9 | 424.1 ± 31.6 | 1.46 ± 0.10 |

**Table 4: Effect of ethanolic extract of Cucurbita pepo fruit (EOCP) on gastric mucosal glycoprotein in aspirin + pylorus ligation induced ulcer**

| Treatment | Glycoprotein (µg/100 mg wet tissue) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Total hexose (A) | Hexosamine (B) | Fucose (C) | Sialic acid (D) | TC (A+B+C+D) | Protein (P) | TC : P |
| Control | 2456 ± 156 | 1532 ± 99 | 298 ± 17 | 105 ± 9 | 4626 ± 319 | 6329 ± 245 | 0.75 ± 0.09 |
| Standard | 3629 ± 164 | 2189 ±198** | 369 ± 12 | 169 ± 8 | 6550 ± 399** | 6298 ± 316 | 1.09 ± 0.12 |
| EOCP (300 mg/kg) | 3321 ± 201 | 1889 ± 121* | 364 ± 16 | 237 ± 21 | 6010 ± 343* | 5717 ± 187 | 1.06 ± 0.08 |

Values are mean ± SEM of 6 rats in each group

*P <0.05, **P <0.01 compared to respective control group

**Table 5: Effect of ethanolic extract of *Cucurbita pepo fruit (*EOCP) on 6th day acetic acid- induced chronic ulcer**

| Group | Treatment | Ulcer area ($mm^2$/rat) | Healing Percentage |
|---|---|---|---|
| I | Control | 19.8 ± 2.9 | - |
| II | standard | 3.1 ± 0.9** | 84.21 |
| III | EOCP (100 mg/kg) | 14.1 ± 1.9 | 37.08 |
| IV | EOCP (200 mg/kg) | 9.1 ± 1.4* | 58.33 |
| V | EOCP (300 mg/kg) | 4.3 ± 1.2** | 77.21 |

Values are mean ± SEM for 6 rats * P < 0.05, ** P < 0.01 compared to respective control group

**Table 6: Effect of ethanolic extract of Cucurbita pepo fruit (EOCP) on lipid peroxidation (LPO), superoxide dismutase (SOD), and catalase (CAT) in acetic acid- induced chronic ulcer**

| Group | Treatment | Lipid peroxidation (LPO) | Superoxide dismutase (SOD) | Catalase (CAT) |
|---|---|---|---|---|
| I | Control | 0.70 ± 0.03 | 220.3 ± 9.3 | 19.3 ± 1.7 |
| II | standard | 0.51 ± 0.03** | 96.5 ± 7.3** | 35.3 ±1.8** |
| III | EOCP (100 mg/kg) | 0.65 ± 0.06 | 183.2 ± 2.2 | 23.8 ± 1.1 |
| IV | EOCP (200 mg/kg) | 0.58 ± 0.01** | 142.0 ± 2.4** | 30.2 ± 1.9** |
| V | EOCP (300 mg/kg) | 0.52 ± 0.02** | 109.2 ± 2.9** | 37.0 ± 2.1** |

Values are mean ± SEM * P < 0.05, ** P < 0.01 compared to respective control group

**Table 7: Effect of ethanolic extract of Cucurbita pepo fruit (EOCP) on HCl-ethanol induced ulcer**

| Group | Treatment | Ulcer index ($mm^2$/rat) | Percent protection |
|---|---|---|---|
| I | Control | 17.2 ± 2.2 | - |
| II | standard | 3.6 ± 1.1** | 80.31 |
| III | EOCP (100 mg/kg) | 12.5 ± 1.9 | 30.21 |
| IV | EOCP (200 mg/kg) | 7.9 ± 1.4* | 58.34 |

| | | | |
|---|---|---|---|
| V | EOCP (300 mg/kg) | 4.0 ± 1.9** | 76.21 |

Values are mean ± SEM * P < 0.05, ** P < 0.01 compared to respective control group

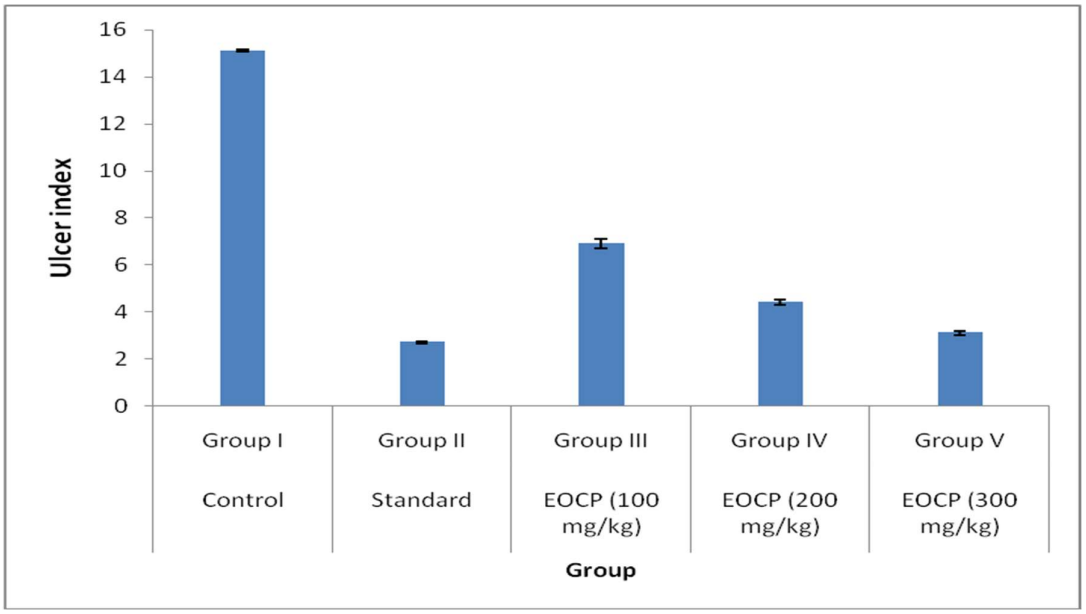


**Figure 1: Effect of ethanolic extract of Cucurbita pepo fruit (EOCP) on ulcer index in aspirin + Pylorus ligation induced ulcer**

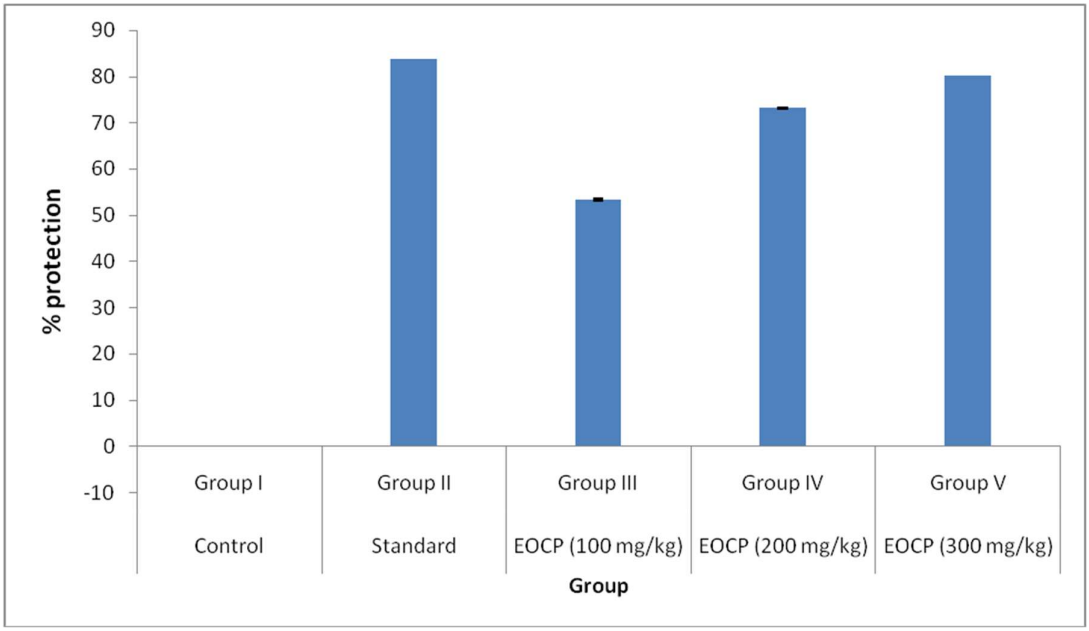


**Figure 2: Effect of ethanolic extract of Cucurbita pepo fruit (EOCP) on percent protection in aspirin + Pylorus ligation induced ulcer**

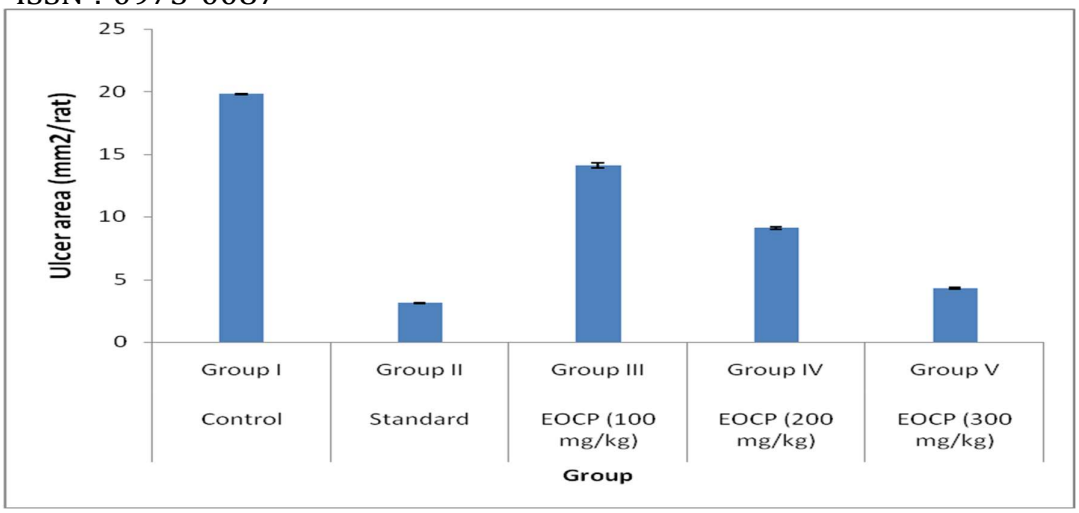


**Figure 3: Effect of ethanolic extract of *Cucurbita pepo fruit (*EOCP) on ulcer index 6th day acetic acid- induced chronic ulcer**

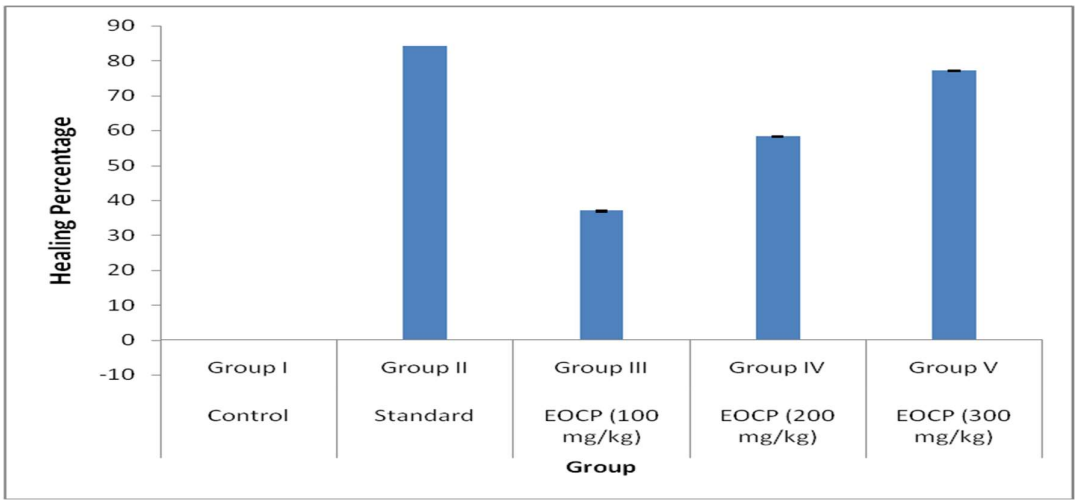


**Figure 4: Effect of ethanolic extract of *Cucurbita pepo fruit (*EOCP) on healing percentage 6th day acetic acid- induced chronic ulcer**

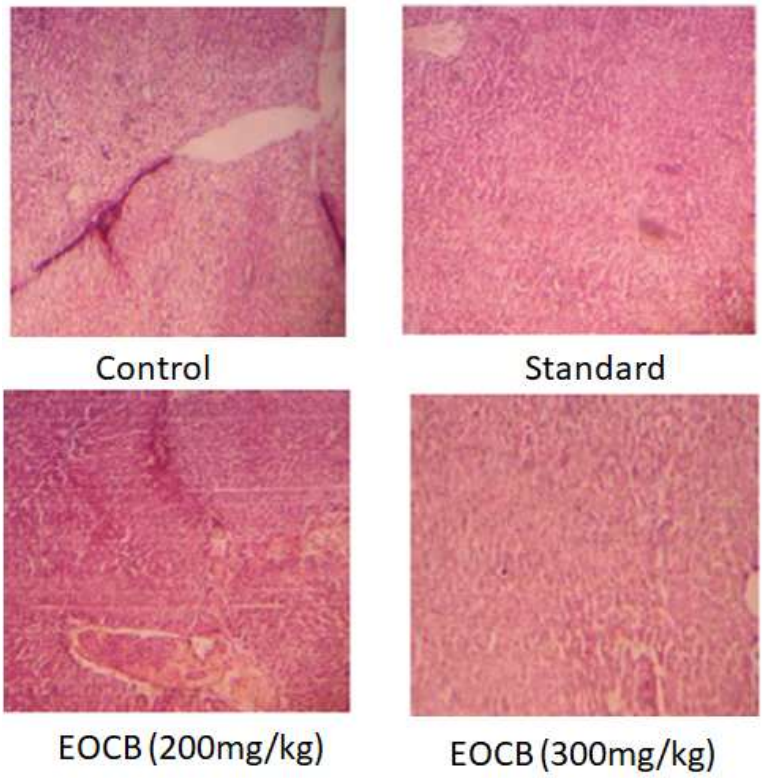


**Figure 5: Effect of ethanolic extract of Cucurbita pepo fruit (EOCP) on 6th day acetic acid-induced chronic ulcers on Histopathology of gastric tissue**

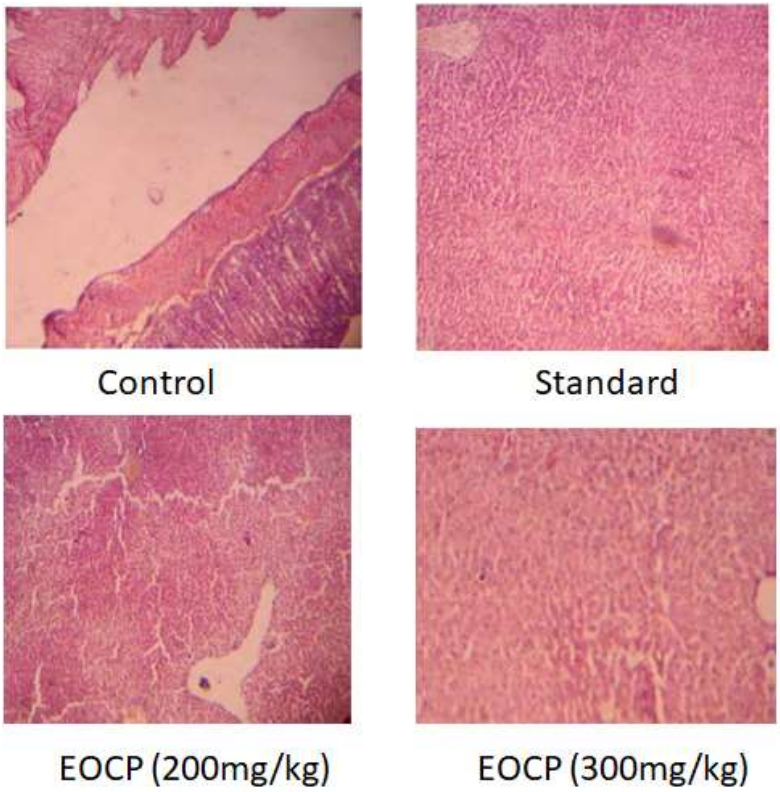


**Figure 6: Effect of ethanolic extract of Cucurbita pepo fruit (EOCP) on HCl ethanol induced ulcer on Histopathology of gastric tissue**