

SCREENING FOR ANTI ULCER ACTIVITY OF CONVULVULUS PLURICAULIS USING PYLORIC LIGATION METHOD IN WISTER RATS.

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ABSTRACT:

Peptic ulcer disease is a chronic problem of the gastrointestinal tract characterized by mucosal damage secondary to pepsin and gastric acid secretion. Herbal medicines are generally used in cases when drugs are to be used for chronic periods. The study was conducted to evaluate antiulcerogenic activity of convolvulus pluricaulis in wistar rats. The antiulcer activity was carried out employing - Pyloric ligation method. The rats were randomly divided into 4 groups of 6 animals each. First group was given 1ml of 0.1% of vehicle (Tween 80); the second group was treated with ranitidine 50 mg/kg in 1 ml Tween 80. The remaining groups receive 200 mg/kg and 400 mg/kg of Convolvulus pluricaulis alcoholic extract (CPAE) in 1 ml Tween 80 respectively. All the drugs were administered orally for five days duration daily in two divided doses. Evaluation of antiulcer activity was done by Ulcer score/ulcer index. Volume of gastric juice secreted, gastric free acidity, total acidity, total protein, pepsin mucin and pH were also estimated. CPAE at a dose of 200 mg/kg inhibited ulcer index by 40.87% and at a dose of 400 mg/kg inhibited ulcer index by 26.64 %. Alcoholic extracts of Convolvulus pluricaulis have shown a significant protection against gastric ulcers in pyloric ligation model.

KEYWORDS- Convolvulus pluricaulis, Gastric ulcers, Pyloric Ligation Method

INTRODUCTION

Peptic ulcer disease is a problem of the gastrointestinal tract characterized by mucosal damage secondary to pepsin and gastric acid secretion. It usually occurs in the stomach and proximal duodenum; less commonly, it occurs in the lower esophagus, the distal duodenum, or the jejunum, as in unopposed hypersecretory states such as Zollinger-Ellison syndrome, in hiatal hernias (Cameron ulcers), or in ectopic gastric mucosa (e.g., in Meckel's diverticulum).¹

The etiology of peptic ulcer is unknown in most of the cases, yet it is generally accepted that it results from an imbalance between aggressive factors (acid, pepsin and H. pylori) and the maintenance of mucosal integrity through the endogenous defence mechanisms (gastric mucus and bicarbonate secretion, prostaglandins, innate resistance of the mucosal cells). To regain the balance, different therapeutic agents including plant extracts may be used.²

Peptic ulcer disease is one of the most common gastrointestinal disorders, which causes a high rate of morbidity particularly in the population of nonindustrialized countries.³ Peptic ulcer is a common global problem with increasing incidence and prevalence. Worldwide 14.5 million people have the ulcers with a mortality of 4.08 million. The increasing incidence and prevalence of ulcers have been attributed to several factors encountered during day to day life, such as stress, exposure to bacterial infection, and use of NSAIDs.⁴

Number of drugs including proton pump inhibitors, prostaglandins analogs, histamine receptor antagonists and cytoprotective agents are available for the treatment of peptic ulcer. But most of these drugs produce several adverse reactions including toxicities and even may alter biochemical mechanisms of the body upon chronic usage.⁵ Hence, herbal medicines are generally used in such cases when drugs are to be used for chronic periods. Several natural drugs have been reported to possess antiulcerogenic activity by virtue of their predominant effect on mucosal defensive factors.^{6,7}

Convolvulus pluricaulis is a rasayana drug which is mainly advocated for use in mental stimulation and rejuvenation therapy. The shape of the flower is like a shankh (a marine shell) giving it the name,

Shankhpushpi. Chemically it contains several alkaloids, flavanoids and coumarins as active chemicals that bring about its biological effects. The pharmacological activity previously reported are nootropic, anxiolytic, tranquillising, antidepressant, antistress, neurodegenerative, anti-amnesic, antioxidant, hypolipidemic, immunomodulatory, anti-inflammatory, analgesic, antimicrobial, insecticidal, antifungal, antibacterial, antidiabetic, antiulcer, antitachycardic and cardiovascular activity.⁸

The present study was carried out for the screening the antiulcer activity of alcoholic extract of *Convolvulus pluricaulis* in pylorus ligated method in the Wistar rats.

MATERIAL AND METHODS

Animals:

60 healthy Wistar albino rats of either sex weighing 150 -250 g were obtained from the central animal house, PES Institute of Medical Sciences and Research Institute and were kept in the laboratory for about 10 days. Throughout the experiment the animals were fed with laboratory chow and water ad libitum. All the animals were placed individually in cages with wide mesh wire bottoms to prevent coprophagy. All animals were fasted overnight prior to and during the day of experiment and the experiments were carried out during light period between 08.00-16.00 hrs. The study was approved by Institutional Animal Ethical Committee. The animals were divided into 4 groups, for each model and each group consists of 6 rats.

Drugs and chemicals:

Absolute alcohol, ranitidine (R-LOC, Zydus Alidac pharmaceuticals), Tween 80 (polyoxyethylene sorbitan monooleate MERCK), ketamine (Aneket, Neon laboratories limited) and alcoholic extract of *Convolvulus pluricaulis* was procured from Department of Pharmacognosy, P.E.S College of Pharmacy, Hanumanth nagara, Bengaluru. The experiment was carried out in laboratory of the Department of Pharmacology PESIMSR, Kuppam.

Laboratory model for testing antiulcer activity:

The antiulcer activity was carried out employing - Pyloric ligation method (Shay Rat model)⁹

PROCEDURE: The experiment was performed using the method of Shay et al, with a few modifications. All the rats in all four groups were pretreated with respective drugs for five days in two daily divided doses, the vehicle Tween 80, CPAE of 200 mg/kg and 400 mg/kg, and ranitidine 50 mg/kg was administered to the respective groups. All the drug dosages were administered orally. The animals after 24 hrs of fasting were anesthetized with ketamine (0.1 ml/kg) intra peritoneal route, abdominal fur was trimmed and under asepsis the abdomen was incised. The incision was extended till the lower end of sternum with a scalpel and then with a curved blunt artery forceps the stomach was looked for and identified, then it was slightly pulled out to the centre which was anatomically present left laterally in the rats abdomen and then pylorus was identified and ligated without compromising its vascularity, the thin layer of omentum attached to the pylorus was pierced by blunt artery forceps and freed making a small gap through which surgical thread is passed below the pylorus and then tied around the pyloric end and then stomach was put back carefully and then abdomen was closed by suturing layer by layer and kept in rat restrainers for 16 hrs, later the animals were sacrificed by over dose of ether; the abdomen was opened and another ligature was placed at the esophageal end. The whole stomachs now were dissected out and the gastric content collected and centrifuged at 3000 rpm (8000×g, 25^o C, 10 min). The gastric juice was collected and its volume, pH and total acidity were measured. The quantity of total proteins, pepsin and mucin were estimated. The glandular portion of the stomach was opened along the greater curvature and the severity of hemorrhagic erosions in the acid secreting mucosa was assessed on a scale of 0-3 (ulcer scoring). Ulcer index was determined for each group. (Photograph)

Evaluation of antiulcer activity:

Ulcer score was calculated as follows:

0 = No ulcer / Normal stomach

1 = Spot ulceration

2 = Haemorrhagic streaks

3 = Perforation ulcer

An ulcer index (UI) was calculated:

$$UI = UN + US + UP \times 10^{-1}$$

UN = average number of ulcers per animal

US = average of severity score

UP = percentage of animals with ulcers

The following parameters are evaluated in the gastric content of pyloric ligated rats: ¹⁰

i) Amount (volume) of gastric acid (ml): The gastric content/juice collected was centrifuged at 3000 rpm (8000×g, 25°C and 10 min) and the volume of gastric juice was noted in millilitres.

ii) Determination of pH: An aliquot of 1ml gastric juice was diluted with 1ml of distilled water and the pH of solution was measured using digital pH meter.

iii) Determination of free and total acidity (mEq/l/100gm): One millilitre of gastric juice was pipetted into a conical flask. Two or three drops of Topfer's reagent were added and this was titrated with 0.01N sodium hydroxide until all traces of red colour disappeared and the colour of the solution became yellowish-orange.

The volume of alkali added was noted. This volume corresponds to free acidity. Two or three drops of phenolphthalein solution were added and titration was continued until a definite red tinge appeared. The total volume corresponds to total acidity.

Acidity was calculated using the following formula:

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH} \times 100}{0.1} \text{ mEq/l/100gm}$$

iv) Estimation of pepsin: Aliquots of 20 µl of the gastric content were incubated with 500 µl of albumin solution (5 mg/ml in 0.06 N hydrochloric acid) at 37 °C for 10 min. the reaction was stopped with 200 µl of 10% trichloroacetic acid and the samples were centrifuged at 1500g for 20 min. the supernatant was alkalised with 2.5 ml of 0.55 M sodium carbonate and 400 µl of 1.0 N Folin's reagent was added to the tubes, which were incubated for 30 min at room temperature. The absorbance of the samples was determined by spectrophotometer at 660 nm. The concentration of pepsin is determined by a standard curve.

v) Estimation of total proteins: The dissolved proteins in gastric juice was estimated in the alcoholic precipitate obtained by adding 90% alcohol with gastric juice in a 9:1 ratio. Then 0.1 ml of the alcoholic precipitate of gastric juice was dissolved in 1ml of 0.1 N NaOH and from this, 0.05 ml was taken into another test tube. To this 4ml of alkaline mixture was added and after 10 min, colour started developing again. A reading was taken against blank prepared with distilled water at 610 nm using a Thermo scientific UV spectrophotometer. The protein content was calculated from the standard curve prepared with bovine albumin and expressed as micrograms/millilitre of gastric juice.

vi) Estimation of mucin: After the collection of gastric juice. The everted stomachs were soaked for 2hr in 0.1% alcian blue 8GX dissolved in 0.16 M sucrose buffered with 0.05 M sodium acetate adjusted to a pH with hydrochloric acid.

Uncomplexed dye was removed by two successive washes at 15 and 45 min in 0.25 M sucrose solution. Dye complex with mucus was diluted by immersion in 10 ml aliquots of 0.5 M magnesium chloride for 2 hr, the resulting blue solutions were shaken briefly with an equal volume of diethyl ether and the optical density of the aqueous phase was measured at 540 nm using Thermo scientific UV spectrophotometer. Later optical density values of standard, CPAE 200, CPAE 400 was compared with control.

The rat pre-treated with ranitidine, CPAE 200, CPAE 400 was compared for ulcer index, gastric secretion volume, free acidity, total acidity, total protein, pepsin, mucin and pH in comparison with control group.

STATISTICAL ANALYSIS

Data was entered into excel spread sheet 2007 and analyzed by using Graph pad version-6. All continuous data will expressed as Mean±SD. Between group analysis was done using one way Analysis of Variance (ANOVA) followed by post hoc Dunnet's test.. A two tailed probability value less than 0.05 was considered statistically significant.

RESULTS

The mean ulcer index with control, standard, CP 200 and CP400 was 11.95(6.65), 5.37(2.28), 7.07(1.94) and 8.77(2.16) mm² respectively. CPAE at a dose of 200 mg/kg inhibited ulcer index by 40.87% and at a dose of 400 mg/kg inhibited ulcer index by 26.64 %. (Table 1)

The mean volume of gastric secretion with control, standard, CP 200 and CP400 was 9.45(3.8), 5.95(2.57), 6.61(1.08) and 7.10(1.22) ml respectively. Volume of gastric secretion was reduced by 29.98% and 24.87% by CPAE at a dose of 200 mg/kg and 400 mg/kg respectively and was statistically significant on comparison with control. (Table 2)

The mean pH of gastric secretion with control, standard, CP 200 and CP400 was 1.66(0.44), 3.95(0.23), 3.06(0.65) and 2.75(1.03) respectively. pH of gastric acid was increased by 184.34% and 165.66% by CPAE at a dose of 200 mg/kg and 400 mg/kg respectively and was statistically significant. (Table 3)

The mean free acid with control, standard, CP 200 and CP400 was 6.13(1.33), 3.95(0.96), 4.01(0.44) and 5.21(0.85) mEq/l/100g respectively. CPAE at a dose of 200 mg/kg reduced free acid by 34.51% and at a dose of 400 mg/kg by 14.94% significantly. (Table 4)

The mean total acidity with control, standard, CP 200 and CP400 was 10.15(1.72), 6.46(1.13), 7.01(0.88) and 9.08(1.31) mEq/l/100g respectively. Total acidity was reduced by 22.97 % and 13.11% by CPAE at a dose of 200 mg/kg and 400 mg/kg respectively and was statistically significant. (Table 5)

The mean pepsin with control, standard, CP 200 and CP400 was 38.83(9.34), 18.83(3.81), 20.66(3.81) and 22.00(4.19) μ mol/ml respectively. CPAE at a dose of 200 mg/kg reduced pepsin by 46.78% and at a dose of 400 mg/kg by 43.35 % significantly. (Table 6)

The mean protein with control, standard, CP 200 and CP400 was 148.33(50.76), 70.00(21.90), 73.33(17.51) and 75.00(56.48) μ g/ml respectively. Total protein in gastric juice was reduced by 50.56% and 49.44 % by CPAE at a dose of 200 mg/kg and 400 mg/kg respectively and was statistically significant. (Table 7)

The mean mucin with control, standard, CP 200 and CP400 was 0.08(0.01), 0.12(0.02), 0.15(0.03) and 0.15(0.01) (Optical density in 540 nm)respectively. CPAE at a dose of 200 mg/kg increased mucin by 166.67% and at a dose of 400 mg/kg by 178.77% significantly. (Table 8)

The results can indicate the presence of antiulcer activity, but more studies with larger number of animals are essential to confirm our findings.

DISCUSSION

Peptic ulcer and gastritis have been associated with mutipathogenic factors and could be due to disturbances in natural balances between the aggressive factors (e.g. acid, bicarbonate, pepsin) and maintenance of the mucosal integrity through the endogenous defense mechanism (e.g. defensive mechanisms of mucus, mucosal turnover and blood supply (mucosal barrier)).^{11,12}

Generally various non-specific methods are used to restore these imbalances including regular food intake, adequate rest and avoidance of ulcerogenic agents (e.g. tobacco, alcohol and coffee). Their aims are to attenuate and possibly block the gastric acid secretion or to enhance the mucosal defense mechanisms.¹³

The enhancement of the mucosal defense mechanisms can be achieved through increasing mucus production, stabilizing the surface epithelial cells or interfering with the prostaglandin synthesis. In addition, there are also drugs, such as PPI, H₂ -antagonists, anticholinergics and antacids, used in the treatment of ulcer.¹⁴

Due to the reported side effects of available antiulcer drugs, focused have been shifted towards natural products as the new sources of antiulcer agents. Medicinal plants have been known to be amongst the most attractive sources of new drugs, and have been shown to give promising results in treatment of various diseases including gastric and duodenal ulcers.¹⁵

Convolvulus pluricaulis has been widely screened for its various pharmacological activities. It has relatively well documented neuropharmacological actions such as nootropic, antistress, anxiolytic, antidepressant, anticonvulsant, tranquilising and sedative activities which justify its use in central nervous system diseases in the Ayurvedic system of medicine. It has antimicrobial, antipyretic, antiinflammatory, analgesic, diuretic, antidiabetic and insecticidal properties. It is reported to contain several types of alkaloids, flavanoids and coumarins as active chemicals that bring about its biological effects.¹⁶

Flavonoids are phenolic compounds found in many green plants. A wide variety of pharmacodynamic effects such as anti inflammatory, antimicrobial, antiallergic, hepatoprotective, antithrombotic and antineoplastic activities have been attributed to flavonoids. Flavonoids are known to inhibit several enzymes e.g. alkaline phosphatase, cAMP phosphodiesterase, lipases, hydrolases, lysosomal H⁺ - ATPase and Na⁺ / K⁺ - ATPase. Since flavonoids antagonize aggressive factors which play a crucial role in the pathogenesis of gastric lesions and also augment defensive factors to protect the gastric mucosa from injury. Flavonoids decrease histamine secretion from mast cells by inhibition of histidine decarboxylase and stimulate prostaglandin biosynthesis, it was postulated that these mechanisms of action may be responsible for the anti-ulcer activity of flavonoids. All flavonoids blocked acid formation in parietal cells in response to histamine. H⁺ / K⁺ - ATPase, the gastric proton pump are also inhibited by all flavonoids. These findings indicate that the site of action for inhibition of acid formation by parietal cells is the H⁺ / K⁺ - ATPase. Flavone and flavonone were effective to stimulate PGE₂ production in gastric mucosal cells. Exogenous prostaglandins particularly E series protects gastro intestinal mucosa from the damage induced by a wide range of irritants and there is evidence that endogenous prostaglandins are important in maintaining gastro duodenal integrity.¹⁷

The antiulcerogenic effect of *Convolvulus pluricaulis* was found to be due to augmentation of mucosal defensive factors such as mucin secretion, lifespan of mucosal cells and glycoprotein rather than on the offensive factors such as acid pepsin.⁷

Pyloric ligation induced ulcers caused due to imbalance between offensive and defensive mucosal factors are ideal model to infer the mechanism by which a drug works as an anti ulcerogenic agent.¹⁸ The activation of the vagus-vagal reflux by stimulation of pressure receptors in the antral gastric mucosa in the hyper secretion model of pylorus ligation is believed to increase gastric acid secretion.¹⁹ The causes of gastric ulcer in 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.²

Studies have shown alterations in the antioxidant status following ulceration, indicating that free radicals seem to be associated with the pylorus ligation-induced ulceration in rats.²⁰

The present study demonstrated the potential of CPAE 200 and CPAE 400 to significantly reduced gastric ulceration as indicated by the reduction in ulcer index in pyloric ligation model by 40.87% and 26.64 % when compared to control.

Based on further findings using the pyloric ligation assay, the extract was suggested to act by reducing the volume of gastric juice secreted, gastric free acidity, total acidity, total protein, pepsin and increasing the pH, mucin.

Thus, the possible mechanism of gastric mucosal protection by CPAE may be partly due to the reinforcement of resistance of the mucosal barrier by a protective coating. CPAE has shown increased pH and decreased total acidity of gastric fluid.

The antiulcer effect was also supported by a decrease in aggressive factors like pepsin and an increase in defensive factors like mucin. The decrease in the protein content of gastric ulcer by CPAE suggests a decrease of leakage of plasma proteins into gastric juice. This also suggests an increase in the glycoprotein content of the gastric mucosa acting as a coating and protective barrier on the gastric mucosa.

The concentration of flavonoids is what makes *Convolvulus pluricaulis* an important medication for the treatment of this disease. This is because flavonoids possess anti oxidant properties, antihistaminic and anticholinergic activity which play a major role in repairing the gastric damage.^{21,22}

It was observed in this study that the alcoholic extracts of *Convolvulus pluricaulis* shows protection against characteristic lesions produced by ethanol administration. This antiulcer effect of CPAE may be due to both reductions in gastric acid secretion and gastric cytoprotection.

This study indicates that *Convolvulus pluricaulis* extract has a potential anti ulcer activity. However further study is required to isolate the active molecule responsible for the activity.

CONCLUSION

CPAE at a dose of 200mg/kg and 400mg/kg was found to inhibit ulcers in pyloric ligation model (40.87% & 26.64% respectively). Alcoholic extracts of *Convolvulus pluricaulis* have shown a significant protection against gastric ulcers in pyloric ligation model. This protective effect might have been due to the presence flavonoids in the extract with antioxidant, anti-secretory and cytoprotective mechanisms.

CONFLICT OF INTREST: NONE

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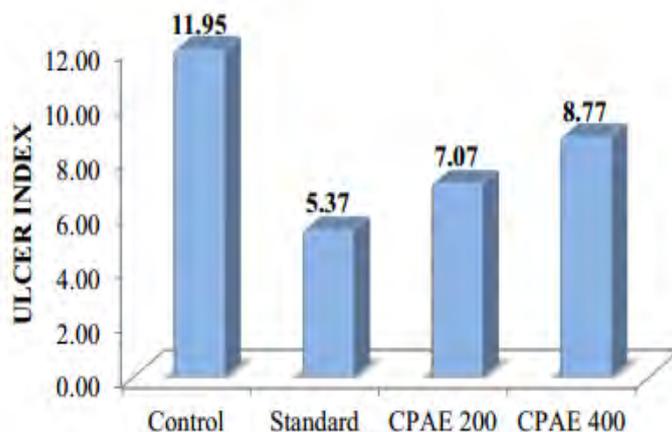
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LEGENDS:

Table 1 : Effect of alcoholic extract of Convolvulus pluricaulis on gastric ulcer induced by pylorus ligation in rats (Ulcer index).

Group	Ulcer index	Standard deviation	P value	% of inhibition
Control	11.95	6.65	0.04	-
Standard	5.37*	2.28		55.09
CP 200	7.07	1.94		40.87
CP 400	8.77	2.16		26.64

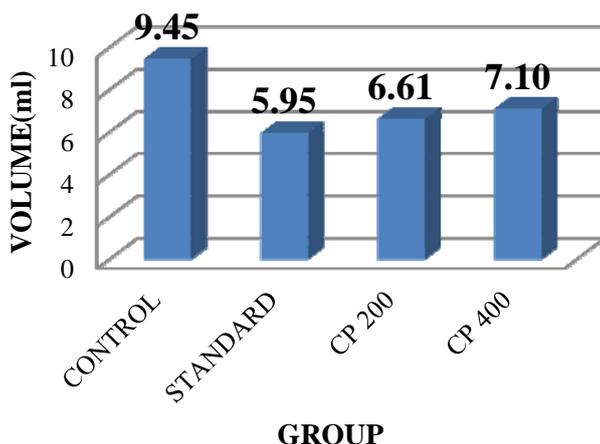
* P<0.05 when compared to control group.



Graph 1 : Effect of alcoholic extract of Convolvulus pluricaulis on gastric ulcer induced by pylorus ligation in rats (Ulcer index).

Table 2 : Estimation of volume (ml) of gastric secretion in pyloric ligation model

Group	N	Mean(ml)	SD	P Value	% of Inhibition
CONTROL	6	9.45	3.80	0.026	
STANDARD	6	5.95	2.57		37.04
CP 200	6	6.61	1.08		29.98
CP 400	6	7.10	1.22		24.87

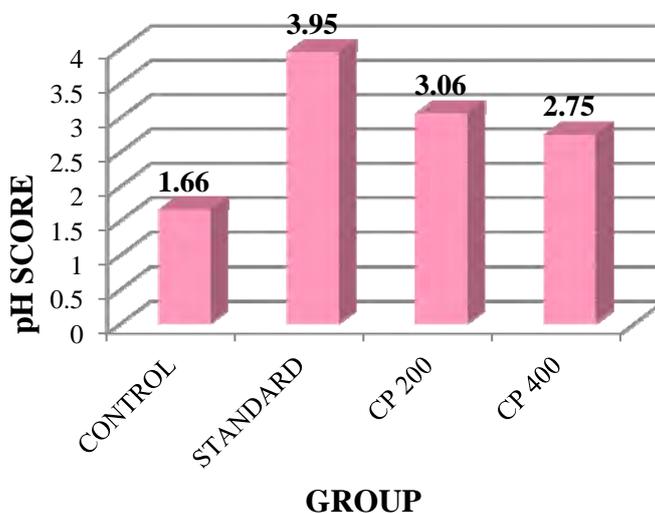


Graph 2 : Estimation of volume (ml) of gastric secretion in pyloric ligation model

Table 3 : Estimation of pH of gastric acid in pyloric ligation model

Group	N	Mean	Std. Deviation	P value	% of inhibition
CONTROL	6	1.66	0.44	0.027	
STANDARD	6	3.95*	0.23		-137.70
CP 200	6	3.06*	0.65		-84.14
CP 400	6	2.75*	1.03		-65.69

* P<0.05 when compared to control group.

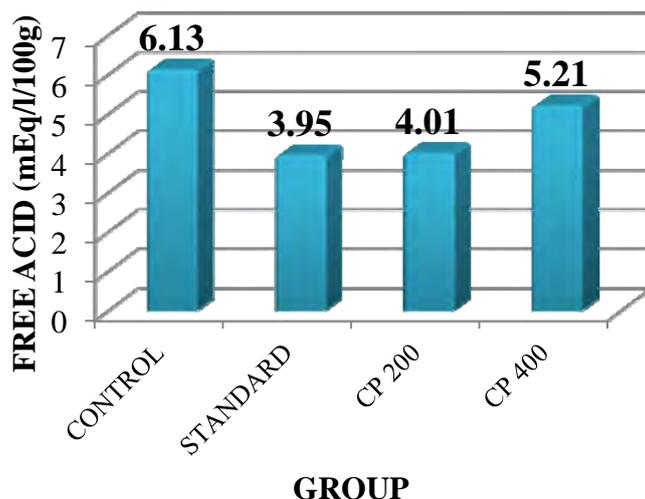


Graph 3: Estimation of pH of gastric acid in pyloric ligation model

Table 4 : Estimation of free acidity (mEq/l/100g) of gastric acid in pyloric ligation model

Group	N	Mean (mEq/l/100g)	Std. Deviation	P Value	% of inhibition
CONTROL	6	6.13	1.33	0.001	
STANDARD	6	3.95*	0.96		35.60
CP 200	6	4.01*	0.44		34.51
CP 400	6	5.21	0.85		14.94

* P<0.05 when compared to control group.

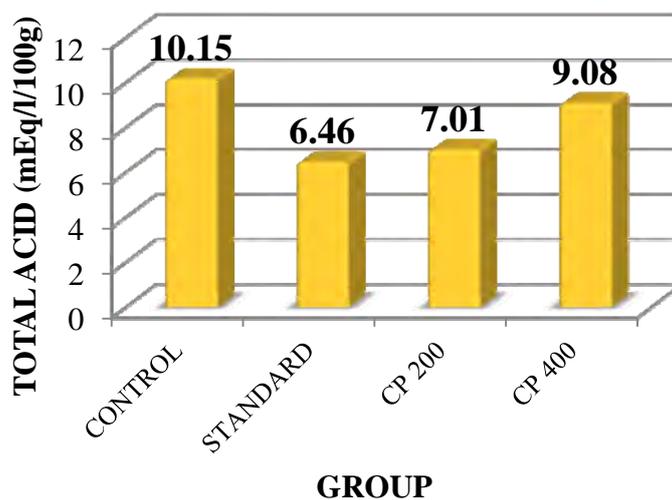


Graph 4 :Estimation of free acidity (mEq/l/100g) of gastric acid in pyloric ligation model

Table 5 : Estimation of total acidity (mEq/l/100g) of gastric acid in pyloric ligation model

Group	N	Mean(mEq/l/100g)	Std. Deviation	P value	% of inhibition
CONTROL	6	10.15	1.72	0.016	
STANDARD	6	6.46*	1.13		36.29
CP 200	6	7.01*	0.88		30.87
CP 400	6	9.08	1.31		10.51

* P<0.05 when compared to control group.

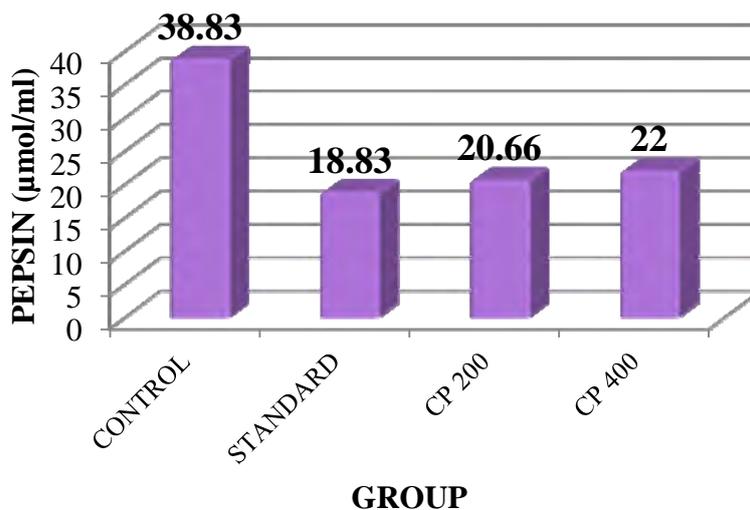


Graph 5 : Estimation of total acidity (mEq/l/100g) of gastric acid in pyloric ligation model

Table 6 : Estimation of pepsin activity ($\mu\text{mol/ml}$) in pyloric ligation model

Group	N	Mean($\mu\text{mol/ml}$)	Std. Deviation	P value	% of inhibition
CONTROL	6	38.83	9.34	0.0009	
STANDARD	6	18.83*	3.81		51.50
CP 200	6	20.66*	3.81		46.78
CP 400	6	22.00*	4.19		43.35

* $P < 0.05$ when compared to control group.

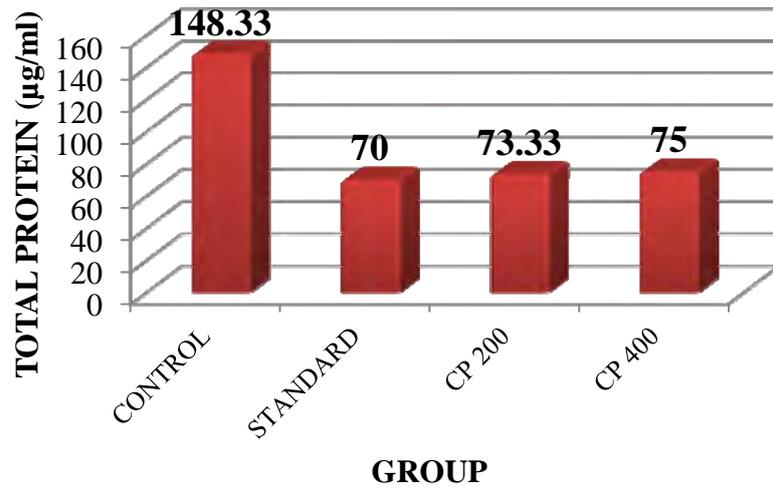


Graph 6 : Estimation of pepsin activity ($\mu\text{mol/ml}$) in pyloric ligation model

Table 7 : Estimation of total protein ($\mu\text{g/ml}$) in gastric juice in pyloric ligation model.

Group	N	Mean($\mu\text{g/ml}$)	Std. Deviation	P value	% of inhibition
CONTROL	6	148.33	50.76	0.007	
STANDARD	6	70.00*	21.90		52.81
CP 200	6	73.33*	17.51		50.56
CP 400	6	75.00*	56.48		49.44

* $P < 0.05$ when compared to control group.

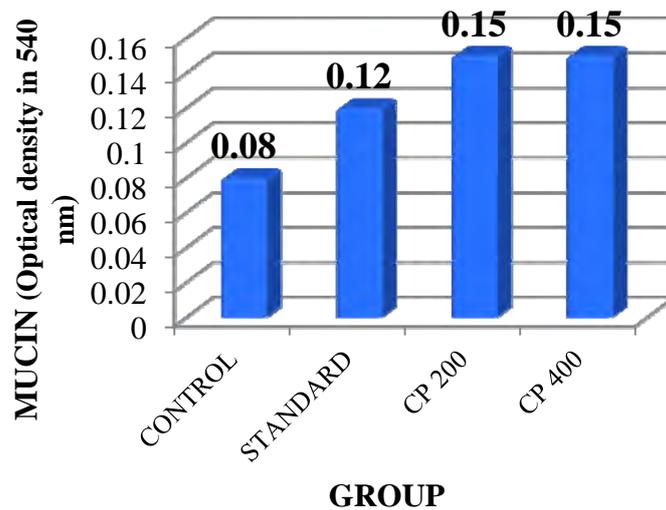


Graph 7 : Estimation of total protein (µg/ml) in gastric juice in pyloric ligation model.

Table 8 : Estimation of mucin (optical density in 540 nm) in pyloric ligation model

Group	N	Mean(Optical density in 540 nm)	Std. Deviation	P value	% increase in mucin
CONTROL	6	0.08	0.01	0.0001	-
STANDARD	6	0.12*	0.02		140.36
CP 200	6	0.15*	0.03		173.01
CP 400	6	0.15*	0.01		178.77

* P<0.05 when compared to control group.



Graph 8 : Estimation of mucin (optical density in 540 nm) in pyloric ligation model

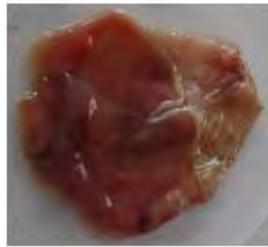
Photograph: Rats stomach specimens showing ulcers in pylorus ligation induced ulcer model.



Control



Standard



CPAE 200



CPAE 400