DOI: 10.20472/IAC.2018.041.030

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IN VIVO GASTROPROTECTIVE STUDY OF SELECTED ANTIOXIDANTS, VITAMINS AND MINERALS USING PYLORUS LIGATION INDUCED ULCERETED RATS.

Abstract:

This investigation aimed to study the effect of selected antioxidants, vitamins and minerals on gastroprotection using pylorus ligation induced gastric mucosal damage in rats. The anti ulcerogenic effect of test substances was investigated in Albino Wistar rats, weighing between 200-250 g. were divided into 9 groups of 6 animals each (n=6). The groups were treated respectively as follows Group I normal control and Group II Positive control received normal saline, Group III was treated with standard drug Omeprazole, Group IV to IX received test substances respectively, two antioxidants (Vitamin E and Cystine) two vitamins (Vitamin B1 and Niacinamide) & two minerals (Iron and Zinc) for 5 days. Ulceration was induced by pylorus ligation. Various parameters like, the volume, pH of gastric juice, total acidity, ulcer index, percentage protection, biochemical parameters like mucin content, pepsin activity and antioxidant enzymes like Super oxide dismutase, catalase, reduced glutathione, myeloperoxidase, and malondialdehyde were estimated. Histopathology of stomach epithelium was observed. The ulcer index and total acidity were significantly reduced ($p<0.05$) increase in pH was observed in ulcer induced rats pretreated with test substances. Mucin content was found to be restored significantly in all rats pretreated with test substances, and pepsin activity was decreased significantly ($p<0.05$) when compared with positive control rats. The alteration observed in the level of super oxide dismutase, catalase, reduced glutathione were increased in test substances treated rats and showed significant restoration. The level of myeloperoxidase and malondialdehyde were decreased significantly ($p<0.05$). Histopathology of gastric mucosa confirmed the gastroprotection by test substances. The study reveals antiulcerogenic effects were observed in all test groups antioxidants (Vitamin E and L- Cystine), vitamins (vitamin B1 and Niacinamide) and minerals (Iron and Zinc) probably by its free radical scavenging activity, antisecretory activity, cytoprotection and mucin preventing nature.

Keywords:

Antioxidants, Histopathology, Indomethacin, Myeloperoxidase, Minerals, Vitamins.

JEL Classification: I10

INTRODUCTION

Gastric hyperacidity and ulceration of the stomach mucosa due to various factors are serious health problems of global concern. Gastric ulcer, the most common disorder of GIT has multifunctional causes in its pathophysiology [1]. The pathophysiology of peptic ulcer has been centralized on an imbalance between aggressive and protective factors in the stomach such as acid-pepsin secretion, mucosal barrier, mucus secretion, blood flow, cellular regeneration, prostaglandins and epidermal growth factors. Although hospital admission for uncomplicated peptic ulcers in developed countries had begun to decrease, there was a striking rise in admission for ulcer hemorrhage and perforation among elderly people [2]. This increase has been attributed to the increased use of nonsteroidal anti-inflammatory drugs (NSAIDs), alcoholic beverages, cigarettes and *Helicobacter pylori* infections.

An ulcer is an open sore, or lesion, usually found on the skin or mucous membrane areas of the body. An ulcer in the lining of the stomach mucosa or duodenum, where hydrochloric acid and pepsin are present, is referred to as a peptic ulcer. Usually, the mucosa can withstand the acid-pepsin attack and remain healthy. That is, a “mucosal barrier” to back diffusion of acid is maintained [3]. However, an excess of acid production or an intrinsic defect in the barrier functions of the mucosa can allow the defense mechanism to fail and ulcers to result.

The precise biochemical changes during ulcer generation are not clear yet, although various hypotheses have been proposed from time to time. Vagal over activity [4] mast cell degranulation [5] decreased gastric mucosal blood flow [6] increased gastric motility [7] and decreased prostaglandins level [8] during stress condition are thought to be involved in ulcer generation. Similarly, role of oxygen derived free radicals have been shown to play a role in experimental gastric damage induced by hemorrhagic shock [9]. Ischemia and reperfusion, and ethanol administration [10] *Helicobacter pylori*, a pathogen is known to be the most common and important agent of gastric ulcer in humans. They cause active inflammation with epithelial damage, accompanied by neutrophil migration [11].

Since its recognition various efforts have been made to find suitable remedial measures. For several decades the adage “no acid-no ulcer” and the drugs used to reduce acid secretion have dominated the pharmacological basis of ulcer therapy [12]. More recently, the role of mucosal factor in peptic ulceration has received much attention and the term “cytoprotection” has been coined. Cytoprotection has been defined as an ability of an agent to prevent gastric tissue [13, 14]. It was hypothesized [15] that the stomach may synthesize and release cytoprotective PGs to maintain cellular integrity of gastric epithelium, in spite of constant presence of noxious agents in lumen. It is now well

established that peptic ulcer disease can be prevented by strengthening the defensive mechanisms of gastric and duodenal mucosa rather than attenuating factors of aggression causing ulceration.

Therefore, the present investigation is undertaken to study the effects of selected antioxidants, vitamins and minerals on pylorus ligation induced gastric mucosal damage in rats.

MATERIALS AND METHODS

Experimental Animals

Albino Wistar rats of either sex weighing between 180 to 250 g were purchased as and when needed from National institute of Biosciences Pune (NIB). The animals were acclimatized for seven days and housed under standard conditions of temperature (25 ± 2^0C) and relative humidity (30-70%) with a 12:12 light-dark cycle. The animals were fed with standard pellet diet (NIB Pune.) and water *ad libitum*. Approval of the Institutional Animal Ethics Committee (IAEC) of H.S.B.P.V.T.'s College of Pharmacy, Kashti (CPCSEA: 1697/PO/a/13/CPCSEA) was taken for conducting gastroprotective activity. The animal studies were performed in accordance to guidelines of CPCSEA.

Pylorus ligation induced ulcer model [16]

Albino Wistar rats of either sex weighing between 180 – 250 g were divided into 9 groups of 6 animals each

Group 1 Control treated with saline 0.2 ml/ rat

Group 2 Positive control treated with toxicant.

Group 3 Standard drug treated, omeprazole 20 mg/kg

Group 4 Vitamin E 45 mg/kg, in 2% gum acacia P.O.

Group 5 Cystine 54 mg/kg, in 2% gum acacia P.O.

Group 6 Thiamin 1.05mg/kg, in 2% gum acacia P.O.

Group 7 Niacinamide 1.4 mg/kg, in 2% gum acacia P.O.

Group 8 Zinc 1mg/kg, in 2% gum acacia P.O.

Group 9 Iron 1 mg/kg, in 2% gum acacia P.O.

The drugs and chemicals were administered daily for 5 days. On 5th day, the rats were fasted for 24 h before pyloric ligation. Care was being taken to avoid coprophagy. At the end of 24 h, the rats were anaesthetized with anesthetic ether. Abdomen was opened by a midline incision. The stomach was lifted out and a ligature was placed at the pyloric sphincter without causing any damage to its blood supply. The stomach was replaced carefully and abdomen wall was sutured in two layers. After 6 h, the rats were sacrificed with excess of anesthetic ether, and the stomachs were dissected out. Gastric juice was collected in centrifuge tubes. The gastric mucosa was then washed under running tab water. The stomachs were immediately excised and rapidly immersed in 10 % buffered formalin solution and various parameters like the volume and pH of gastric juice, total acidity, ulcer index, percentage protection, biochemical parameters like mucin content, pepsin activity and antioxidant enzymes like super oxide dismutase, catalase, reduced glutathione, myeloperoxidase, and malondialdehyde were estimated as per the methods described in ethanol induced ulcer model in rat. Histopathology of stomach epithelium was observed.

Determination of Volume and pH of gastric juice [17]

The animals were sacrificed, stomach was dissected out, and the gastric juice collected was centrifuged for 5 min at 2000 rpm. The volume of the supernatant was expressed as ml and pH was measured using pH meter.

Determination of Total acidity of Gastric juice [18, 19]

The supernatant of gastric juice was taken and diluted 10 times and a few drops of phenolphthalein were added to the solution. Titration was done using 0.01 M NaOH solutions until the color of the test solution changed to light pink, indicating pH 7.0. The volume of sodium hydroxide (NaOH) needed for titration was used in the calculation to derive the hydrogen ion concentration. The total acidity is expressed as m equiv /L using the following formula:

$$n \times 0.01 \times 40 \times 1000$$

Where,

n = volume of NaOH quantified

40 is the molecular weight of NaOH

0.01 is normality of NaOH and 1000 is the factor represented in liter.

Determination of Ulcer Score [20]

Procedure as described by Kulkarni was followed[21]. The ulcer index is measured or registered using the following scores involving the number and severity of ulcers:

0.0 = normal colored stomach,

0.5 = red coloration,

1.0 = spot ulcers,

1.5 = hemorrhagic streaks,

2.0 = ulcers with area >3 but ≤5mm,

3.0 = ulcers > 5mm,

The ulcer index and percentage protection given by the following equation:

Ulcer index (UI) = UN + US + UP × 10,

Where UI = ulcer index,

UN = average number of ulcers per animal,

US = average of severity score,

And UP = percentage of animals with ulcer.

$$\text{Percentage Protection} = 100 - \frac{\text{UI of Pretreated}}{\text{UI of Control}} \times 100$$

Determination of Mucin Content [18]

The glandular portion was excised and opened down along the lesser curvature of animals from both models. The reverted stomach was soaked for 2 h in 0.1% alcian blue (0.16M sucrose buffered with 0.05M sodium acetate). The uncomplexed dye was removed by two successive washes of 15 and 45 min in 0.25M sucrose solution. The dye complexes with mucus were diluted by immersion in 10 ml of 0.5M magnesium chloride for 2 h. The resulting blue solution was shaken briefly with equal volume of diethyl ether and the optical density of aqueous phase was measured at 605 nm. The mucin content of the sample was determined from the standard curve obtained with different concentrations of mucin.

Determination of Pepsin Activity [22]

Pepsin activity was determined by the Anson-Mirsky revised method using bovine hemoglobin as a substrate. One gram of hemoglobin was added to 10 ml of 0.3 mol/L HCl solution, then the solution was diluted to 50 ml as the hemoglobin substrate. Gastric juice was diluted 50-fold with 0.04 mol/L HCl solution to produce the sample solution. The hemoglobin substrate and 0.5 ml of the sample solution were stored at 37 ℃. The sample solution was added to 2 ml of the hemoglobin substrate, and the solution was mixed. Then, the solution was incubated at 37 ℃ for digestion. After 10 min, the solution was added to 5 ml of 5% trichloroacetic acid and mixed. The sample solution was added to 5% trichloroacetic acid, and then hemoglobin solution was added as a blank. After 30 min, the solution was centrifuged, and 1 ml of supernatant was added to tube. The supernatant was added to 5 ml of 0.5 mol/L sodium hydroxide and phenol reagent. optical density of the color was determined using a tyrosine standard at 640 nm after 60 min. Acidity and pepsin activity were expressed as mEq/L and tyrosine μg/ml/min, respectively.

Pepsin activity was calculated using following formula.

Pepsin activity= (A－B) × 50 × 7.5/0.5 × 1/10 = (A－B) × 75,

Where, A is the concentration of tyrosine in the sample, and B is the concentration of tyrosine in the blank.

Estimation of Antioxidant enzymes

Serum Superoxide Dismutase (SOD) [23].

Superoxide dismutase was assayed in gastric juice by the method devised by Marklund S, Marklund G modified by Nandi and Chatterjee.

Principle

Pyrogallol auto oxidises rapidly in aqueous or alkaline medium solution and this has been employed for the estimation of superoxide dismutase. SOD inhibits the auto oxidation of pyrogallol. This principle was employed in a rapid and convenient method for the determination of the enzyme concentration.

Procedure

a) For Control

To 2.9 ml of Tris buffer, 0.1 ml of pyrogallol solution was added and mixed thoroughly .Then the reading was taken at 420 nm, exactly after 1.5 min and 3.5 min. The absorbance per two minutes was recorded and the concentration of pyrogallol was adjusted (by diluting the pyrogallol solution) so that the rate of change of absorbance per minute was approximately 0.020 – 0.023 nm.

b) For Sample

To 2.8 ml of Tris buffer, 0.1 ml of gastric juice sample was added, mixed and started the reaction by adding 0.1 ml of adjusted pyrogallol solution (as per control). It was read at 420 nm exactly after 1.5 min and 3.5 min and absorbance per 2 min was recorded.

Calculations

Absorbance reading of control - A

Absorbance reading of sample - B

Units of SOD/3 ml of assay mixture = [(A-B) / (A×50)] ×100

Unit×10 = Units /ml of sample solution.

Catalase (CAT) [24]

Catalase activity was measured by the method of Aebi. 0.1 ml of gastric juice was added to cuvette containing 1.9 ml of 50 mM phosphate buffer (pH 7.0). Reaction was started by the addition of 1.0 ml of freshly prepared 30 mM H_2O_2. The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm. Activity of catalase was expressed as units/mg protein. A unit is defined as the velocity constant per second.

Reagents	Sample	Blank
Phosphate buffer solution	1.9 ml	2..9 ml
Supernatant	0.1 ml	0.1 ml
H_2O_2	1 ml	---------

The reaction occurs immediately after the addition of H_2O_2.

Solutions are mixed well and the first absorbance (A1) is read after 15 seconds (t1) and the second absorbance (A2) after 30 seconds (t2).The absorbance is read at wave length 240 nm.

Calculation

$$K = Vt/Vs*2.3/ \Delta t* \log (A1/A2)*60$$

Where,

K= Rate constant of the reaction.

$\Delta t= (t2 – t1) =15$ seconds.

A1= absorbance after 15 seconds.

A2= absorbance after 30 seconds.

Vt = total volume (3 ml).

Vs = volume of the sample (0.1ml).

Lipid Peroxidation (LPO)

The level of thiobarbituric acid reactive substance (TBARS) and malondialdehyde (MDA) production was measured in stomach homogenate by the modified method as described by Draper and Hadley [25]. The gastric juice (50 μL) was deproteinized by adding 1mL of 14% trichloroacetic acid and 1mL of 0.6% thiobarbituric acid. The mixture was heated in a water bath for 30 min to complete the reaction and then cooled on ice for 5 min. After centrifugation at 2000 g for 10 min, the absorbance of the colored product (TBARS) was measured at 535 nm with a UV spectrophotometer. The concentration of TBARS was calculated using the molar extinction coefficient of malondialdehyde (1.56 × 105 mol/L/cm) using the formula,

A = ΣCL, where A = absorbance, Σ = molar coefficient,

C = concentration, and L = path length.

Reduced Glutathione (GSH) [26]

Stomach homogenate was mixed with equal volume of ice cold 5 % TCA and the precipitated proteins were removed by centrifugation. The supernatant was added to equal volumes of 0.5 M Tris-HCl, pH 9.0 containing 20 mM DTNB to yield yellow chromophore of thionitrobenzoic acid, which was measured at 412 nm. GSH was used as a reference standard. The activity of GPx was expressed as nM of GSH oxidized/min/ml.

Myeloperoxidase activity (MPO) [27]

Myeloperoxidase (MPO) activity in the duodenal mucosa was measured according to the method of Bradley *et al.*14. Pre-weighed tissue was homogenized (1:10 wt/vol) in 0.5% hexadecyltrimethyl ammonium bromide in 50 m*M* potassium phosphate buffer (*p*H 6.0) before sonication in an ice bath for 20 sec. Three freeze/thaw cycles were performed followed by sonication (20 sec in ice bath). The samples were centrifuged at 17000 *g* (5 min, 4°C) and myeloperoxidase in the supernatant was assayed by mixing 0.1 ml of supernatant and 2.9 ml of 50 m*M* potassium phosphate buffer (*p*H 6.0) containing 0.167 g/L o-dianisidine dihydrochloride and 0.0005% hydrogen peroxide. The change in absorbance at 460 nm was measured for 4 min using an UV spectrophotometer.

Histopathological evaluation [27]

The stomachs were immersed in 10 % formalin solution for histopathological examination. These were examined for histopathological changes such as congestion, hemorrhage, necrosis, inflammation, infiltration, erosion and ulcers.

Statistical analysis

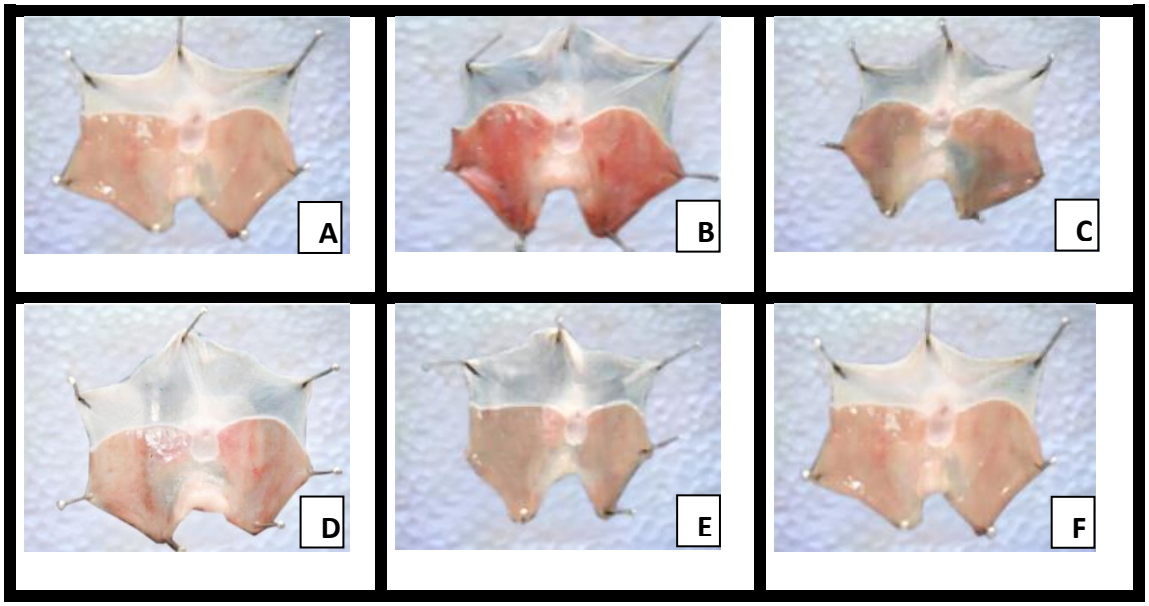
The values expressed as mean ± SEM from six animals. The results were subjected to statistical analysis by using one way ANOVA followed by Tuckey's test to verify the significant difference if any among the groups.

RESULTS

Effect of test substances on stomach epithelium of rats

It was observed that rats with positive control group have blood in the lumen, hemorrhagic streaks, small and large ulcers. However, pretreatment with standard & test substances reduced severity of pylorus ligation induced ulcers and oozing of blood into lumen. Fig: 1(A-I)

Fig: 1 Stomach epithelium of Albino Wistar rats after pylorus ligated gastric ulceration.



A: Control, **B:** Positive control, **C:** Standard (Omeprazole)

D: Vitamin E treated, **E:** Cystine treated, **F:** Thiamin treated,

G: Niacinamide treated, **H:** Zinc treated, **I:** Iron treated

Table 1: Effect of test drugs and chemicals on volume and pH of gastric juice, ulcer index, total acidity and percentage protection in pylorus ligation ulcer model.

Gr. No.	Treatment	Dose mg/kg	Volume (ml)	pH	Total acidity (mEq/L)	Ulcer Index	% Protection
1	Control	0.2 ml/ rat	0.5±0.15	3.25±0.10	68.10±1.20	0	NA
2	Positive Control	0.2 ml/ rat	6.45 ± 0.15	1.10 ± 0.20	144.22±2.88	7.245 ± 0.22	NA
3	Omeprazole Treated	20 mg/kg	3.65 ± 0.10	7.30 ± 0.12	38.77±0.83	1.007 ± 0.12	86.10
4	Vit. E Treated*	45 mg/kg	3.10 ± 0.20	5.00 ± 0.14	56.18±3.44	1.110± 0.26	84.67

5	**Cystine Treated***	**1.05 mg/kg**	**3.28 ± 0.26**	**4.30 ± 0.30**	**76.45±4.45**	**1.250 ± 0.17**	**82.74**
6	**Thiamin Treated***	**54 mg/kg**	**3.14 ± 0.10**	**5.80 ± 0.64**	**77.75±5.65**	**4.100 ± 0.18**	**43.40**
7	**Niacinamide Treated**	**1.4 mg/kg**	**4.42 ± 0.18**	**4.36 ± 0.34**	**75.92±3.32**	**4.333 ± 0.18**	**40.19**
8	**Zinc Treated***	**1 mg/kg**	**3.36 ± 0.14**	**4.68 ± 0.52**	**84.62±3.88**	**3.242 ± 0.18**	**55.25**
9	**Iron Treated***	**1 mg/kg**	**3.58 ± 0.12**	**4.62± 0.48**	**87.85±1.65**	**3.586 ± 0.14**	**50.50**

Data was expressed as mean ± S.E.M. (n= 6) by one way ANOVA followed by Tukey's test.

* indicates significant difference in data as compared to control group ($p<0.05$)

# indicates significant difference in data as compared to Positive control group ($p<0.05$)

Table 2: Effect of test substance on biochemical parameters in pylorus ligation induced ulcer in rats.

Group No	Treatment	Mucin (µg alcin blue/g of glandular tissue	Pepsin (µmoles Tyrosine / ml)
1	**Control**	**690.60±10.20**	**12.08±1.02**
2	**Positive control**	**426.20±14.60***	**25.35±0.95***
3	**Omeprazole treated**	**652.26±08.44#**	**6.42±0.10#**
4	**Vit. E treated**	**490.42±09.58#**	**17.66±0.88**

5	**Cystine treated**	**465.62±06.18#**	**19.55±2.11**
6	**Thiamin treated**	**455.25±15.85#**	**18.86±2.61**
7	**Niacinamide treated**	**476.58±12.32#**	**21.32±1.68**
8	**Zinc treated**	**490.28±22.72#**	**18.30±0.22#**
9	**Iron treated**	**510.40±12.80#**	**16.34±1.25#**

Data was expressed as mean ± S.E.M. (n= 6) by one way ANOVA followed by Tukey's test.

* indicates significant difference in data as compared to control group ($p<0.05$)

# indicates significant difference in data as compared to Positive control group ($p<0.05$)

Table 3: Effect of test substance on antioxidant enzyme levels in pylorus ligation induced ulcer in rats.

Group No	Treatment	SOD (U/ml)	Catalase (U/ml)	MDA (nM/ml)	Myeloperoxidase (U/g tissue)	GSH (nM GSH oxidized/min/ml)
1	**Control**	**1.30±0.10**	**4.08±0.42**	**1.11±0.04**	**4.56±0.04**	**220.75±4.30**
2	**Positive control**	**0.52±0.05***	**2.25±0.05***	**2.10±0.08***	**8.28±0.04***	**118.61±4.68***
3	**Omeprazole treated**	**1.02±0.04#**	**4.00±0.02#**	**0.95±0.04#**	**5.12±0.08#**	**211.48±8.32#**
4	**Vit. E treated**	**1.09±0.07#**	**3.72±0.38**	**0.98±0.02#**	**5.62±0.18**	**214.62±9.90#**

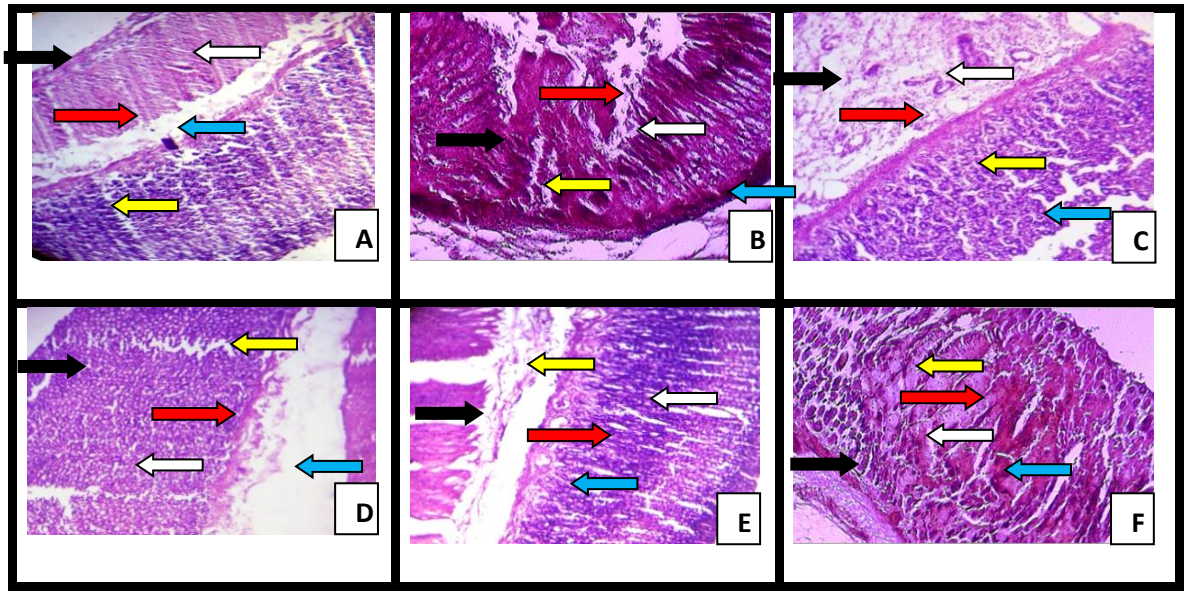
5	Cystine treated	1.10±0.02#	3.15±0.25	0.86±0.06#	6.48±0.194	236.69±8.27#
6	Vit. B1 treated	1.20±0.02#	3.45±0.28	0.89±0.03#	5.95±0.20	220.36±16.26#
7	Niacinamide treated	1.08±0.02#	3.25±0.30	0.80±0.05#	6.90±0.30	222.33±5.55#
8	Zinc treated	1.16±0.01#	3.65±0.56	0.95±0.03#	6.18±0.22	263.39±11.14#
9	Iron treated	1.12±0.02	3.80±0.20	0.96±0.01	5.85±0.15	237.13±10.30

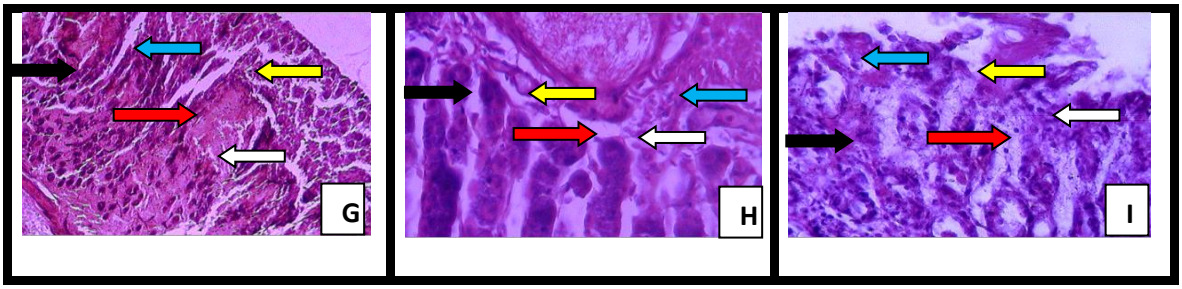
Data was expressed as mean ± S.E.M. (n= 6) by one way ANOVA followed by Tukey's test.

* indicates significant difference in data as compared to control group ($p<0.05$)

# indicates significant difference in data as compared to Positive control group ($p<0.05$)

Fig: 2 Histopathological slides of Albino Wistar rats after pylorus ligated gastric ulceration.





A: Control, **B:** Positive control, **C:** Standard (Omeprazole)

D: Vitamin E treated, **E:** Cystine treated, **F:** Thiamin treated,

G: Niacinamide treated, **H:** Zinc treated, **I:** Iron treated

The observations by the histological examination of stomach excised from the experimental rats were presented in Fig: 2(A to I) Photograph showing cellular infiltration (blue arrow), hemorrhages and congestion (red arrow), edema (white arrow), necrosis (black arrow) ulceration (yellow arrow) H&E stain 100X.

DISCUSSION

According to pylorus ligation, ulcers may be due to autodigestion of gastric juice, decreased mucosal blood flow and breakdown of mucosal barrier. The Shay model is simple, reproducible and highly predictable model for the screening and evaluation of antiulcer drugs [28]. It utilizes neither the exogenous ulcerogens nor the induced exogenous interfering factors. In case of pyloric ligation, ulcer formation is mainly due to the stasis at the gastric juice and stress [29] or there is an excess of acid for a given degree of mucosal defense. The Anti-ulcer property of all test substances in pylorus ligation model is evident from its significant reduction in total acidity and ulcer index. Because all test substances treated animals significantly inhibited the formation of ulcers in the pylorus ligated rats and also decreased both the concentration and increased the pH, it is suggested that all test substances can suppress gastric damage induced by aggressive factors.

Test substances in the present study were also found to prevent pylorus ligation induced gastric wall mucus depletion. The gastric mucus coating is thought to be important in both preventing damage and in facilitating the repair of gastric epithelium [30]. Proteolytic activity of pepsin as the primary aggressor in gastric mucosal ulceration has been reported [26]. In present study, the increased pepsin activity with decrease in mucin secretion in the positive control treated rats indicated altered hydrophobicity and reduced protective ability of the mucosal membrane against hemorrhagic erosions, thus resulting

in tissue damage. Besides antioxidant action that protects the mucus layer and arrest ulcer progression, drugs that increase the synthesis and secretion of gastric mucus would facilitate gastric ulcer healing. Accordingly, it was found that mucin content in all rats pretreated with test groups were increased and pepsin activity were decreased significantly ($p<0.05$) when compared with positive control treated rats. This suggests that gastroprotective effect and role of enhancing mucosal resistance to acid and inhibition of pepsin activity may be sufficient to heal the ulcers.

Cells or tissues are in a stable state if the rates of free radical formation and scavenging capacity are essentially constant and in equilibrium. However, an imbalance between them results in oxidative stress which further deregulates cellular functions leading to different pathological conditions [31]. In present study rats treated with positive control group shows decrease in the levels of SOD, CAT and GSH and increase the levels of MPO and MDA when compared with normal control treated rats. However test substances treated rats showed significant restoration i.e., increased the level of SOD, CAT and GSH and significantly reduce the lipid peroxidation and decreased the levels of MPO and MDA when compared with positive control group suggesting that, all test substances treated rats decreased the risk of gastric injury and ulceration in the gastric mucosa.

Our investigation was taken up to study the effect of few selected antioxidants, vitamins and minerals on gastroprotection by using indomethacin induced ulcer model in rats which concludes that, all test groups antioxidants (Vitamin E and L- Cystine), vitamins (Thiamin and Niacinamide) & minerals (Iron and Zinc) can be used as adjuncts in antiulcer drug formulations which act as cytoprotective agents. The antiulcer activity of all test groups may be attributed to its antisecretory and antioxidant activities. The effect of antioxidants, vitamins and minerals are effective in treating peptic ulcer disease when compared to standard omeprazole. The ulcer preventing action of the test substances might probably by restoring the gastric mucin content, depleting pepsin content, and by reducing lipid peroxidation and oxidative damage.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the encouragement of Management, Principal and Staff of HSBPVT’s GOI College of Pharmacy, Kashti, Pune University, Maharashtra, India.

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