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PREVENTION OF GASTRIC EROSION IN INDOMETHACIN ADMINISTERED RATS BY SELECTED ANTIOXIDANTS, VITAMINS AND MINERALS

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Keywords:

Antioxidants, Histopathology, Indomethacin, Myeloperoxidase, Minerals, Vitamins

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ABSTRACT: This investigation was aimed to study the effect of few selected antioxidants, Vitamins and minerals for prevention of gastric erosion in indomethacin induced gastric mucosal damage in rats. Male 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 disease control received normal saline, Group III was treated with standard drug Omeprazole, Group IV to IX received test substances for 7 days. Ulceration was induced by a single oral administration of indomethacin (30 mg/kg). Various parameters like, the volume, pH of gastric juice, total acidity, ulcer index, percentage protection, mucin content, pepsin activity and antioxidant enzymes 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 pre-treated with test substances. Mucin content was found to be restored significantly in all rats pre-treated with test substances, and pepsin activity was decreased significantly ($p < 0.05$) when compared with disease control treated 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 gastro-protection by test substances. The study reveals antiulcerogenic effects were observed in all test groups antioxidants, Vitamins and minerals probably by its free radical scavenging activity, antisecretory activity, cytoprotection and mucin preventing nature.

INTRODUCTION: Gastric hyperacidity and ulceration of the stomach mucosa due to various factors are serious health problems of global concern.

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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 non-steroidal 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 few selected antioxidants, Vitamins and minerals on indomethacin induced gastric mucosal damage in rats.

MATERIALS AND METHODS:

Experimental Animals: Male wistar rats weighing between 200 to 250g used in the study were purchased from National institute of Biosciences Pune (NIB). The animals were acclimatized for seven days and maintained in standard laboratory conditions. The animals were fed with standard pellet diet (NIB Pune) and water *ad libitum*. Approval of our 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 experiment.

Indomethacin Induced Ulcer Model: [16]

Induction of Ulcer: Gastric ulcer in male wistar rats was induced according to the procedure described by Sayanti *et al.* Rats were administered with a single oral dose of indomethacin 30 mg/kg. They were fasted but had free access to water 24 hr prior to ulcer induction. Various degrees of ulceration have manifested 6 hr after indomethacin administration [17].

Grouping of Animals and Treatment: Male Wistar rats weighing between 200 to 250g were divided into 9 groups of 6 animals each.

- **Group I:** Normal Control, Normal saline treated 0.2 ml / rat.
- **Group II:** Disease Control, Normal saline treated 0.2 ml / rat.
- **Group III:** Standard drug treated Omeprazole 20 mg/kg.
- **Group IV:** Vitamin E 45mg/kg in 2% gum acacia orally.
- **Group V:** L Cystine 54mg/kg in 2% gum acacia orally.
- **Group VI:** Thiamin 1.05mg/kg in 2% gum acacia orally.
- **Group VII:** Niacinamide 1.4 mg/kg in 2% gum acacia orally.
- **Group VIII:** Zinc 1mg/kg in 2% gum acacia orally.

- **Group IX:** Iron 1mg/kg in 2% gum acacia orally.

The drug and chemicals were administered daily for 7 days. On 7th day after 12 hr fasting, all groups (except Group I), of rats were administered indomethacin in order to induce gastric ulcers [18]. After six hours, the rats were sacrificed. The stomachs were immediately excised and rapidly immersed in 10% buffered formalin solution.

Determination of Volume and pH of Gastric Juice: 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 [19].

Determination of Total acidity of Gastric Juice: 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 [20, 21]. 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: [22] Procedure as described by Kulkarni was followed [23]. 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: [20] 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: [24] 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 °C 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): [25] 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): [26] 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.
Δ 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): [27] 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 [18]. 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): [28] 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.

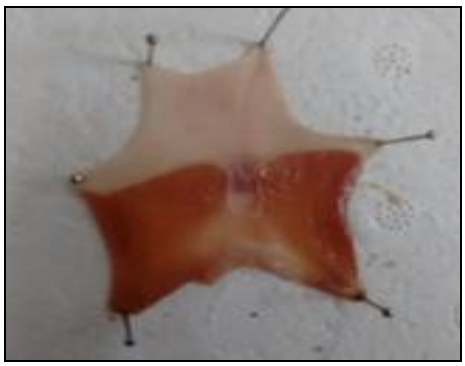
Myeloperoxidase activity (MPO): [29] Myeloper oxidase (MPO) activity in the duodenal mucosa was measured according to the method of Bradley *et al.*[14]. Pre-weighed tissue was homogenized (1:10 wt/vol) in 0.5% hexadecyltrimethyl ammonium bromide in 50 m*M* potassium phosphate buffer (*p*H 6.0) before 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: [29] 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 indomethacin administration to rats caused red coloration, spot ulcers, hemorrhagic streaks and small & large ulcers. However, pretreatment with test substances significantly reduced severity of indomethacin induced ulcer and oozing of blood into lumen of the stomach. (**Fig. 1 A - I**).



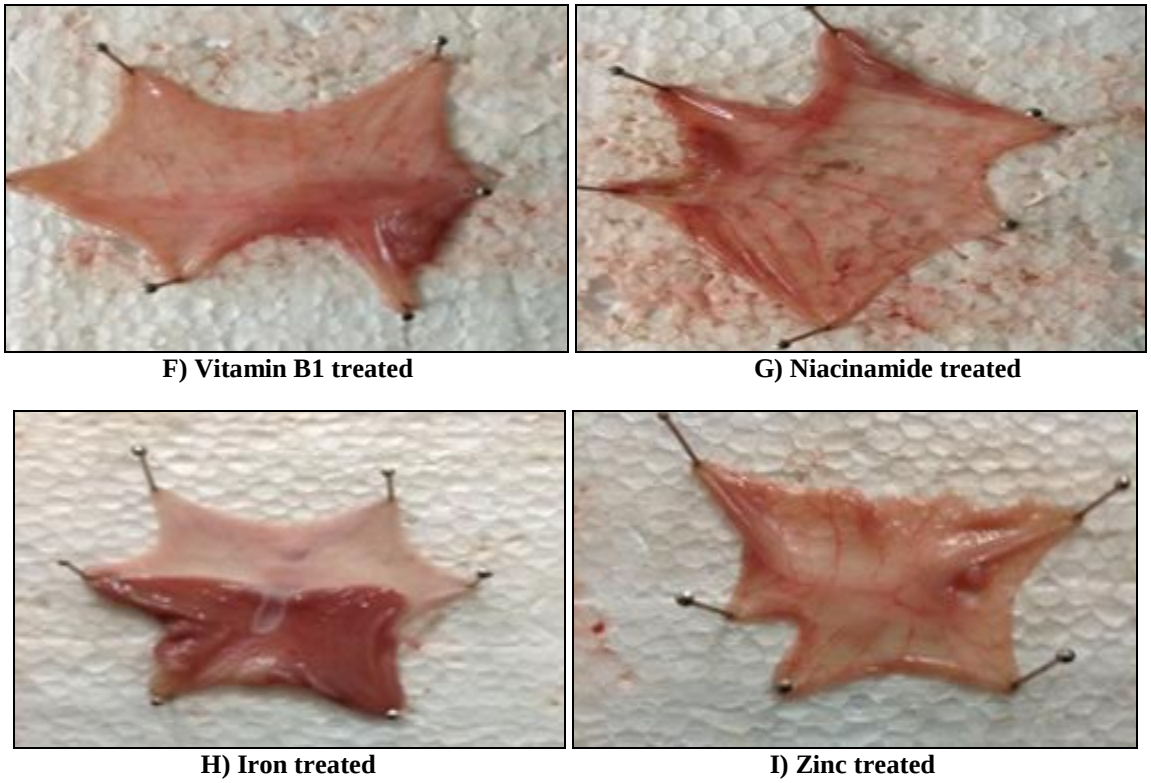
A) Normal control

B) Disease control

C) Standard treated

D) Vitamin E treated

E) Cysteine treated



F) Vitamin B1 treated **G) Niacinamide treated**

H) Iron treated **I) Zinc treated**

FIG. 1: IMAGES OF STOMACH EPITHELIUM OF RATS AFTER INDOMETHACIN INDUCED ULCER

Effect of test substances on Volume and pH of gastric juice: In ulcerated rats, which are pretreated with two antioxidants, vitamins and minerals (Vitamin E 3.23±0.10, Cystine 3.5±0.07) (Vitamin B13.5±0.08, Niacinamide 3.21±0.09) and (Zinc 5.12±0.24, Iron 3.31±0.10) the volume of gastric juice was approximately same when compared with disease control (indomethacin treated 3.23±0.10) & standard drug (omeprazole 3.31±0.10) treated rats (**Fig. 2**).

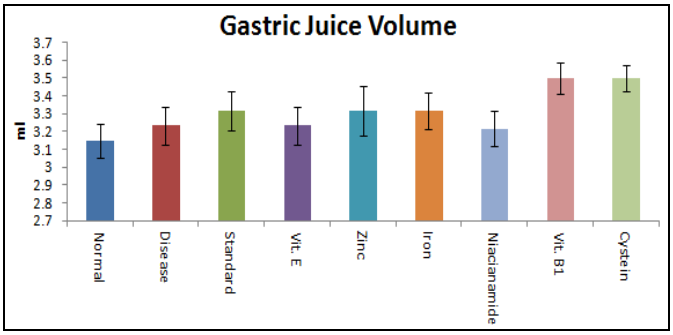


FIG. 2: EFFECT OF TEST SUBSTANCES ON VOLUME OF GASTRIC JUICE OF INDOMETHACIN ULCERATED RATS

(n= 6) Mean ± S.E.M. by one way ANOVA followed by Tuckey's test.

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

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

The pH of gastric juice collected at the time of sacrifice was less 3.16±0.26 in disease control (Indomethacin treated) rats. Standard drug (omeprazole) treated rats shows maximum pH 6.40±0.13 and there was significant ($p<0.05$) increase in pH in pretreated rats when compared with disease control. Cystine 4.86±0.29, Vitamin B1 5.59±0.11 and Iron 5.44±0.13 treated rat's shows maximum pH than Vitamin E 4.83±0.18, Niacinamide 5.49±0.09 and Zinc 5.12±0.24 treated rats respectively (**Fig. 3**).

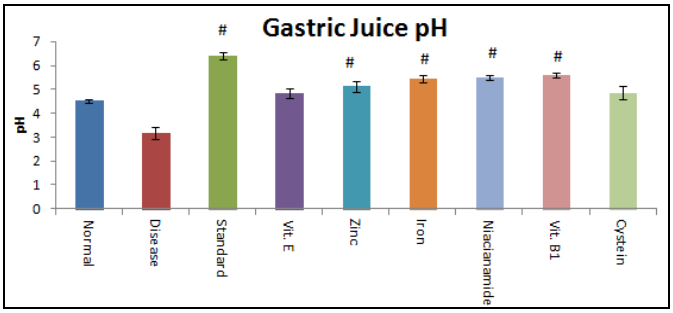


FIG. 3: EFFECT OF TEST SUBSTANCES ON pH OF GASTRIC JUICE OF INDOMETHACIN ULCERATED RATS (n= 6)

Mean ± S.E.M. by one way ANOVA followed by Tuckey's test.

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

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

Effect of test substances on Total acidity of Gastric juice: Total acidity is highest in disease control (Indomethacin treated) rats 128.39.±2.45 and minimum in standard drug (Omeprazole) treated rats 58.64±1.09 In pretreated test groups, total acidity is significantly ($p<0.05$) reduced than disease control rats. Cystine 83.30±5.40, Vitamin B 80.15±3.58 and Iron 87.08±6.19 treated rat's shows less total acidity, than Vitamin E 99.06±3.70, Niacinamide 81.90±5.26 and Zinc 94.28±5.14 treated rats respectively (**Fig. 4**).

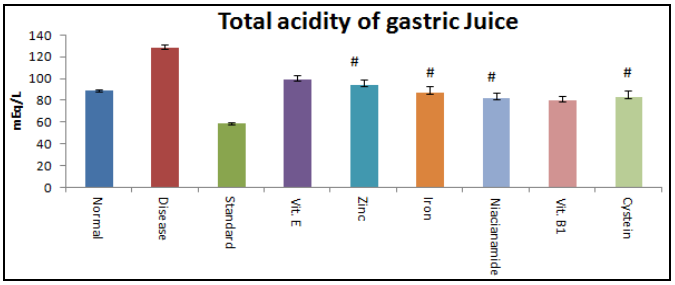


FIG. 4: EFFECT OF TEST SUBSTANCES ON TOTAL ACIDITY OF GASTRIC JUICE OF INDOMETHACIN ULCERATED RATS

(n= 6) Mean ± S.E.M. by one way ANOVA followed by Tuckey's test.

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

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

Effect of test substances on ulcer index and percentage protection: Ulcer index of disease control (Indomethacin treated) rats is 20.5±0.99 which is significantly ($p<0.05$) reduced in standard drug (Omeprazole) treated rats 1.94±1.23. In Iron treated rats it was totally reduced. In vitamins treated groups Vitamin B1 3.66±1.64 Niacinamide 3.83±1.74 shows significant reduction in ulcer index than antioxidant treated groups Vitamin E 4.5±2.02 Cystine 6.00±2.69 (**Fig. 5**).

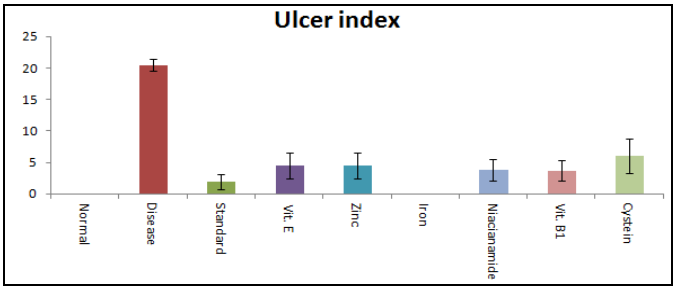


FIG. 5: EFFECT OF TEST SUBSTANCES ON ULCER INDEX OF INDOMETHACIN ULCERATED RATS

(n= 6) Mean ± S.E.M. by one way ANOVA followed by Tuckey's test.

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

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

100 % Protection was observed in Iron treated rats, which were highly significant than all other groups. Standard drug (Omeprazole) treated rats shows 88.48±7.61 % protection. In vitamins treated groups, Vitamin B1 82.11±8.03, Niacinamide 81.30±8.48 shows significant ulcer index than antioxidant treated groups *i.e.* Vitamin E 78.04±9.89, Cystine 70.73±13.14 (**Fig. 6**).

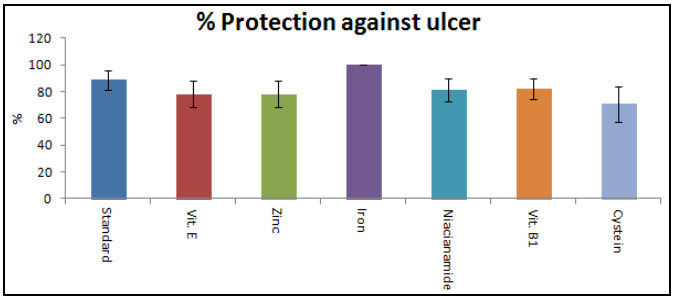


FIG. 6: EFFECT OF TEST SUBSTANCES ON PERCENTAGE PROTECTION AGAINST ULCER OF INDOMETHACIN

ulcerated rats (n= 6) Mean ± S.E.M. by one way ANOVA followed by Tuckey's test.

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

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

Effect of test substances on Mucin Content and Pepsin Activity: It was found that mucin content in all rats pretreated with test groups Vitamin E 483.12±25.36, Cystine 469.57±9.10, Vitamin B1 472.41±10.14, Niacinamide 463.11±10.97, Iron 512.95±9.69 and Zinc 476.76±6.30 were significantly increased ($p<0.05$) when compared with disease control 441.9±8.44 treated rats (**Fig. 7**).

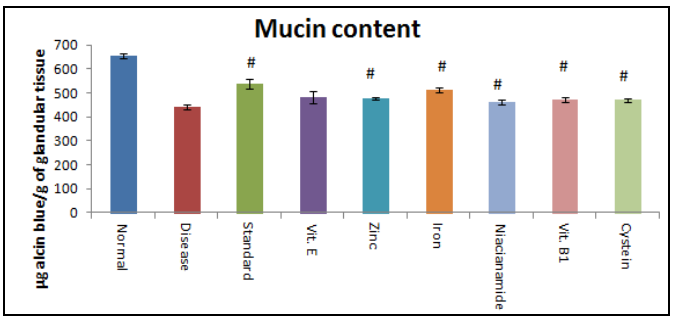


FIG. 7: EFFECT OF TEST SUBSTANCES ON MUCINE CONTENT OF INDOMETHACIN ULCERATED RATS

(n= 6) Mean ± S.E.M. by one way ANOVA followed by Tuckey's test.

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

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

Pepsin activity of all rats pretreated test groups Vitamin E 21.56±2.11, Cystine 20.62±1.91, Vitamin B1 20.29±2.01, Niacinamide 20.46±2.07, Iron 15.52±0.76 and Zinc 18.21±1.88 were decreased significantly ($p<0.05$) when compared with disease control 25.01±0.74 treated rats **(Fig. 8)**.

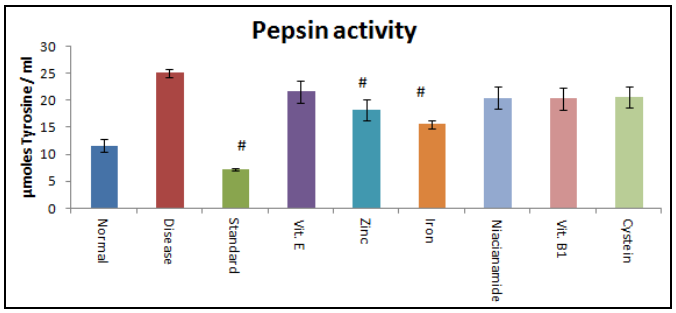


FIG. 8: EFFECT OF TEST SUBSTANCES ON PEPSIN ACTIVITY OF INDOMETHACIN ULCERATED RATS

(n= 6) Mean ± S.E.M. by one way ANOVA followed by Tuckey's test.

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

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

Effect of test substances on antioxidant enzymes:

Effect of test substances on SOD: Rats treated with disease control group 0.77±0.08 shows decrease in the levels of SOD when compared with normal control 1.42±0.06 treated rats, but test substances Vitamin E 1.05±0.01, Cystine 1.15±0.10, Vitamin B1 1.05±0.01, Niacinamide 1.03±0.00, Iron 1.04±0.00 and Zinc 1.06±0.00 treated rats showed significant restoration ($p<0.05$) *i.e.*, increased the level of SOD (**Fig. 9**).

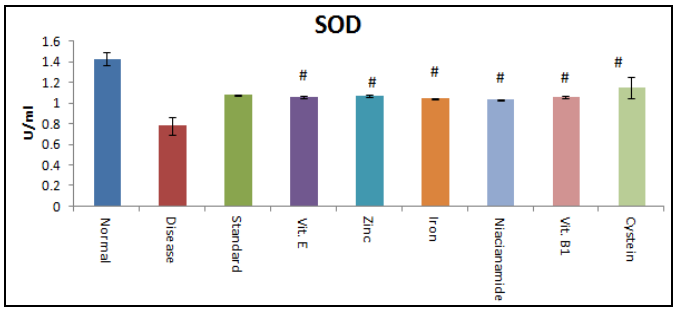


FIG. 9: EFFECT OF TEST SUBSTANCES ON ANTIOXIDANT ENZYME, SUPEROXIDE DISMUTASE (SOD) OF INDOMETHACIN ULCERATED RATS

(n= 6) Mean ± S.E.M. by one way ANOVA followed by Tuckey's test.

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

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

Effect of test substances on CAT: Rats treated with disease control group 1.29±0.10 shows decrease in the levels of CAT when compared with normal control 4.47±0.10 treated rats, but test substances Vitamin E 2.35±0.58, Cystine 2.16±0.27, Vitamin B1 2.36±0.37, Niacinamide 2.31±0.07, Iron 2.55±0.07 and Zinc 2.73±0.40 treated rats showed significant restoration ($p<0.05$) *i.e.*, increased the level of CAT (**Fig. 10).**

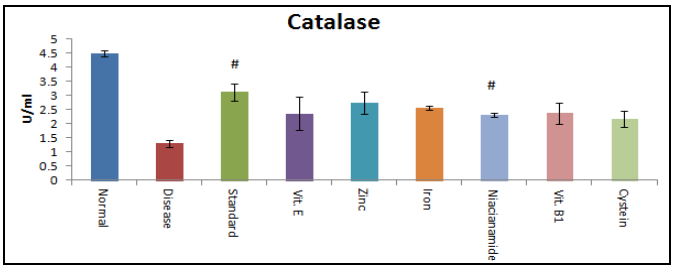


FIG. 10: EFFECT OF TEST SUBSTANCES ON ANTIOXIDANT ENZYME, CATALASE (CAT) OF INDOMETHACIN ULCERATED RATS

(n= 6) Mean ± S.E.M. by one way ANOVA followed by Tuckey's test.

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

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

Effect of test substances on GSH: Rats treated with disease control group 129.06±6.05 shows decrease in the levels of GSH when compared with normal control 259.40±6.27 treated rats, but test substances Vitamin E 225.66±9.66, Cystine 205.64±19.43, Vitamin B1 208.10±12.20, Niacinamide 220.57±12.17, Iron 214.09±16.35 and Zinc 213.56±11.04 treated rats showed significant restoration ($p<0.05$) i.e., increased the level of GSH (**Fig. 11**).

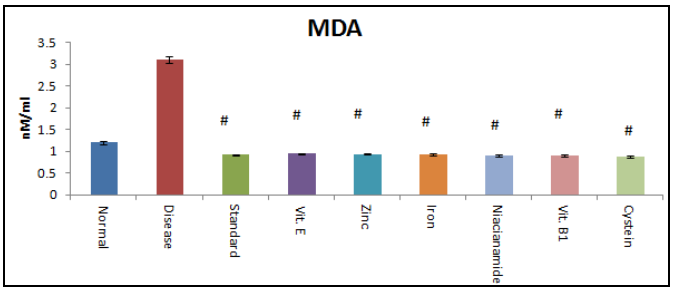


FIG. 11: EFFECT OF TEST SUBSTANCES ON ANTIOXIDANT ENZYME, MELONALDEHIDE (MDA) OF INDOMETHACIN ULCERATED RATS

(n= 6) Mean ± S.E.M. by one way ANOVA followed by Tuckey's test.

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

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

Effect of test substances on MPO: Rats treated with disease control group 7.8±0.08 shows increase in the levels of MPO when compared with normal control 5.82±0.10 treated rats, but test substances Vitamin E 5.92±0.39, Cystine 6.60±0.12, Vitamin B1 6.25±0.10, Niacinamide 6.31±0.13, Iron 5.66±0.28 and Zinc 6.32±0.29 treated rats significantly decreased (p<0.05) the level of MPO (**Fig. 12**).

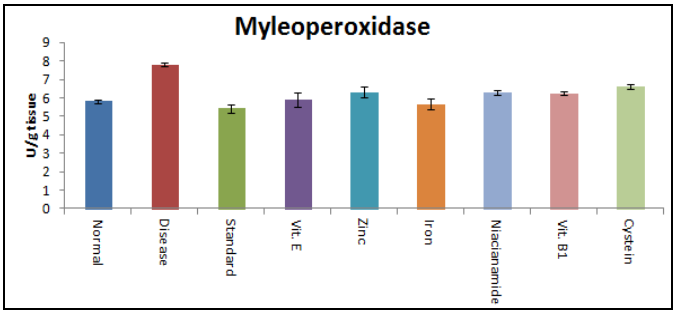


FIG. 12: EFFECT OF TEST SUBSTANCES ON ANTIOXIDANT ENZYME, MYLEOPEROXIDASE (MPO) OF INDOMETHACIN ULCERATED RATS

(n= 6) Mean ± S.E.M. by one way ANOVA followed by Tuckey's test.

* indicates significant difference in data as compare to Normal control group (p<0.05)

# indicates significant difference in data as compare to Disease control group (p<0.05)

Effect of test substances on MDA: Rats treated with disease control group 3.10±0.07 shows increase in the levels of MDA when compared with normal control 1.21±0.03 treated rats, but test substances Vitamin E 0.94±0.01, Cystine 0.87±0.02, Vitamin B1 0.90±0.02, Niacinamide 0.90±0.02, Iron 0.92±0.02 and Zinc 0.93±0.01 treated rats significantly decreased (p<0.05) the level of MDA (**Fig. 13).**

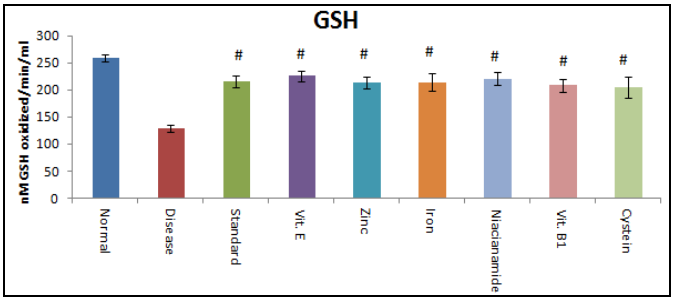


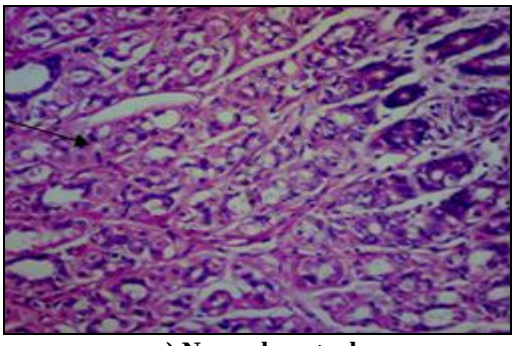
FIG. 13: EFFECT OF TEST SUBSTANCES ON ANTIOXIDANT ENZYME, REDUCED GLUTATHIONE (GSH) OF INDOMETHACIN ULCERATED RATS

(n= 6) Mean ± S.E.M. by one way ANOVA followed by Tuckey's test.

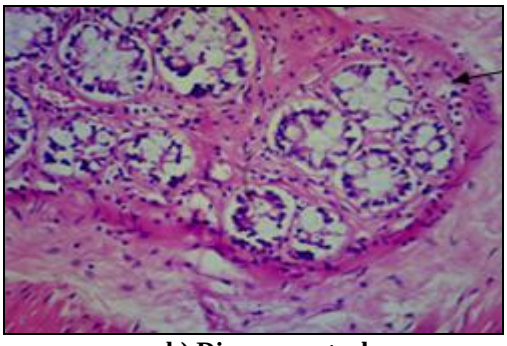
* indicates significant difference in data as compare to Normal control group (p<0.05)

# indicates significant difference in data as compare to Disease control group (p<0.05)

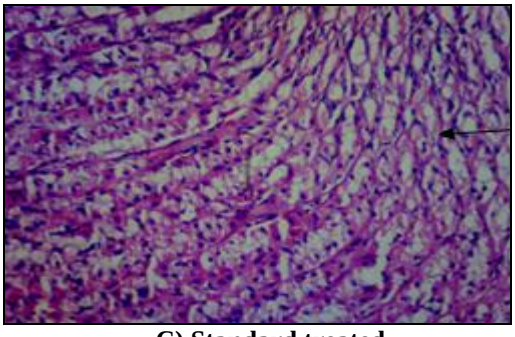
Histopathology: The observations by the histological examination of stomach excised from the experimental rats were presented in (**Fig. 14 a-i)**. Arrow indicates the size of cell; Disease control and all test drugs treated group shows cell hyperplasia (increased cell mass) and congestion (unstained white space within cell), cell hyperplasia and congestion represents damage to stomach wall



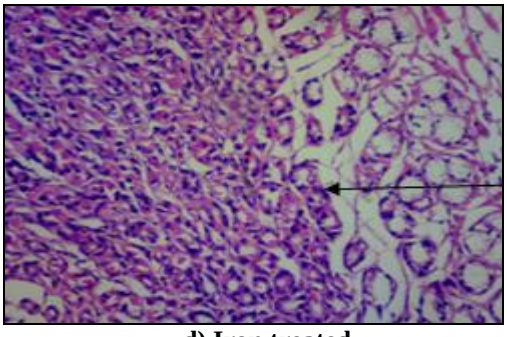
a) Normal control



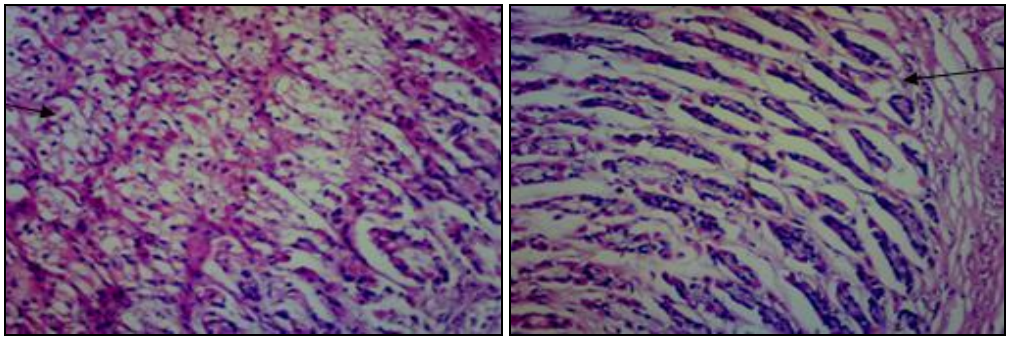
b) Disease control



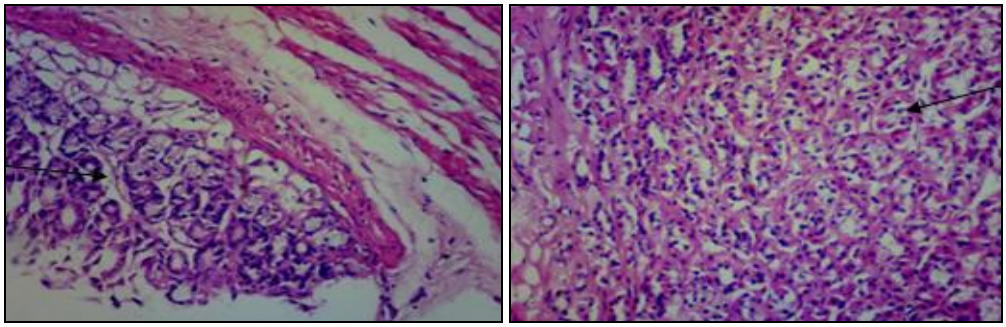
C) Standard treated



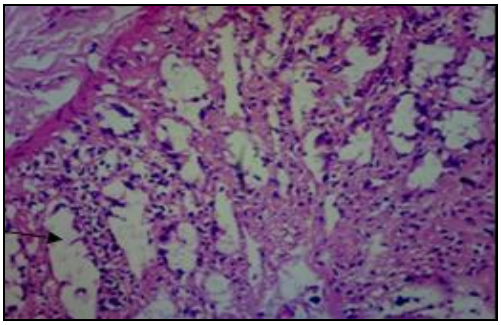
d) Iron treated



e) Niacinamide treated f) Cysteine treated



g) Vitamin E treated h) Vitamin B1 treated



i) Zinc treated

FIG. 14: SLIDE a) TO i) SHOWS HISTOPATHOLOGY OF STOMACHS OF RATS AFTER INDOMETHACIN INDUCED ULCER. ARROWS INDICATES THE SIZE OF CELL

DISCUSSION: The results of the present study demonstrate that all test substances have the capacity to significantly inhibit the basal gastric secretion and ulcerogenicity, induced by Indomethacin in rats. Alteration in gastric secretion[6, 30] has been considered as pathogenic mechanisms responsible for gastric mucosal lesions. Blockade of acid secretion resulted in high healing rates of gastric and duodenal ulcers. The reduced severity of ulcers in this model could be due to its effect in reducing volume and acidity of gastric secretion [31]. Suppressants of gastric acid secretion are known to increase the healing of both human and experimental gastric ulcers. Indomethacin is an established ulcerogen especially in an empty stomach [32] and elevates gastric acid secretion, possibly contributing to its ability to interfere with gastric ulcer healing [33]. The incidence of indomethacin-induced ulceration is mostly on the glandular (mucosal) part of stomach [34]. Although the underlying etiologic mechanisms of indomethacin-induced gastric mucosal lesions are still unclear, indomethacin is known to inhibit endogenous prostaglandin formation [35] and increase gastric motor activity.

The view is supported by the fact that prostaglandins normally serve a protective function in stomach by maintaining gastric microcirculation [35, 36] and causes gastric secretion of biocarbonate [37] and mucus [38]. The test substances inhibited the indomethacin induced ulceration in rats. It has been proposed that mucosal protection induced by test substances may be mediated through the generation of endogenous prostaglandins [39, 40]. It is possible that the observed antiulcer activity of the test

substances against indomethacin injury might be related to antisecretory effect [41], or its ability to mobilize prostaglandins in gastric mucosa or through an unknown mechanism. Test substances in the present study were also found to prevent indomethacin 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 [42]. Proteolytic activity of pepsin as the primary aggressor in gastric mucosal ulceration has been reported [28].

In present study, the increased pepsin activity with decrease in mucin secretion in the disease 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 [43].

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 [16].

In the present study, the increased concentration of MPO & MDA as well as reduced activity of SOD, CAT & GSH in the stomach of indomethacin-ulcerated rats is a manifestation of facilitated lipid peroxidation, and over production of free radicals resulting in mucosal damage. Free radicals thwart antioxidant enzyme activities and initiate lipid peroxidation, which is an important event in the toxicity mechanism of indomethacin. Indomethacin has previously been reported to decrease antioxidant enzymes (SOD, CAT and GSH) activity in rat stomach thereby inducing gastric ulceration. This is associated with overpowering of the cellular antioxidant defense systems by free radical scavenging influence that subsequently results in stomach oxidative injury. However, the significantly reduced concentrations of MPO & MDA coupled with marked increase in the activity of SOD, CAT & GSH in rats pretreated with test substances is an obvious indication of antiperoxidative potential, and thus, antioxidative potential.

The results of histopathological investigation on the gastric mucosa of rats revealed that the pretreatment with test substances absolutely inhibited the indomethacin induced congestion, hemorrhage, edema, necrosis, inflammatory and dysplastic changes, erosions and ulceration.

CONCLUSION: 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.

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CONFLICT OF INTEREST: Authors have no any conflict of interest.

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