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Antiulcer and Antioxidant Effect of Enteric Coated Sodium Alginate Beads of Substituted Benzimidazole Proton Pump Inhibitor

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Abstract:
In the present study modified released formulation of Rabeprazole sodium was formulated to study the gastric and duodenal ulcer activity in rats. Ulcers were produced by ethanol induced ulcers in rats. The animals were randomly divided into 4 groups with 6 animals in each group. In group 1 animals were of the control group and they received only water and in group 2 animals received ethanol (70%) at the dose of 10ml/kg body weight, the animals of group 3 received Rabeprazole Sodium at the dose of 20mg/kg body weight and the group 4 animals received the aqueous solution of enteric coated alginate bead of Rabeprazole sodium + ethanol at the dose of 20mg/kg body weight. The antiulcer activity was correlated for reduction in ulcer levels. The parameters like ulcer index, histopathological Evaluation, macroscopic evaluation and antioxidant parameters were studied and the results obtained from study were analyzed using one way ANOVA followed by Dunnet's multiple comparison test. From the results it can be concluded that Rabeprazole sodium exhibited significant antiulcer effect and histopathology reports along with biochemical parameters studies supports the results.

Key words*: Rabeprazole sodium, alginate beads, ethanol induced ulcers, antiulcer activity, antioxidant parameters*

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I. Introduction

Stomach produces HCl and pepsin, which collectively start the digestion of food. The ulcers that develop in area of gastrointestinal tract exposed to acidic gastric juices and pepsin are called peptic ulcers. Ulcers mainly occur in either duodenum or in stomach in ratio of 4:1. Ulceration of gastrointestinal tract mucosa is caused by disruption of normal balance of corrosive effect of gastric juice and the shielding effect of mucus on gastric epithelial cells. Gastric secretion is a complex continuous process controlled by multiple central and peripheral factors. Parietal cells present in the stomach secrete H^+ ions. There are two pathways, which activate the process of gastric secretion viz., AMP dependent pathway and calcium dependent pathway. The H^+K^+ ATPase pump (proton pump), which secretes H^+ ions in parietal cells, can be activated by histamine, Ach and gastrin via these two pathways. Ulcers may be associated with Helicobacter pylori infection of stomach. This infection may lead to impaired production of somatostatins by D cells and in time inhibition of gastrin with the resulting higher acid production as well as duodenal bicarbonate production.

For the management of acid related disorders proton pump inhibitors (PPIs) are very useful and preferred drug therapy. They are superior to Histamine-2 receptor antagonist in demonstrating the more acid suppression. PPIs are recommended as first line of drug therapy for the treatment of severe GERD- related symptoms like esophagitis etc.

Rabeprazole is substituted benzimidazole derivatives that reduce gastric secretion by specifically inhibiting the proton pump (H^+/K^+ ATPase) at the secretory surface of the gastric parietal cells. It is a prodrug which requires activation in order to inhibit gastric acid secretion. After oral administration it is absorbed into systemic circulation and ultimately enters actively secreting parietal cells. At highly acidic pH the agents are activated by conversion to a sulfonamide moiety that binds to the luminal surface of H^+/K^+ ATPase, thereby irreversibly inhibiting the gastric proton pump

Rabeprazole is degraded by gastric acid and the drug formulations must therefore withstand the degradation due to acid to deliver active drug to the stomach for absorption. Pharmacokinetics studies indicate that plasma concentrations vary considerably from individual to individual, and there is poor correlation between maximal plasma concentration and degree of gastric acid suppression. Various types of enteric coating have been developed to protect the PPIs, but they all delay absorption. The use of hydrogel systems for

controlling the release of drugs has increasingly well known respond to surrounding conditions such as pH, ionic strength, temperature and frequent changes in the environmental conditions in the GI-tract, which has a variations of pH from the stomach to intestine.

II. Materials And Methods

2.1 Materials

Rabeprazole Sodium was obtained fron API Private Limited, Hyderabad and Albion wistar rats of either sex were used and purchased from Disease Free Small Animals, Lala Lajpat Rai University of Vetenary and Animal Sciences, Hisar (India). Before the experiment the albino wistar rats (100-150 gm.) were exposed to light and dark cycle at room temperature for 12 hours. The rats were fasted for 16 hrs with free access to water before the experiment. The experiment protocol was approved by Institutional Animal Ethical Committee (IAEC) of the department and care of animals was taken according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India

2.2

Methods

The antiulcer activity was carried out on ethanol induced ulcers. The animals were divided into posfive groups each having six animals. All other materials, reagent and chemicals used were of analytical grade

Model: Ethanol Induced Gastric Ulcers

1. Control group(distilled water) Group 1
2. Ethanol group(70% ethanol) Group 2
3. Pure Drug control group Group 3
4. Enteric coated alginate beads formulation treatment group Group 4

Ethanol Induced Ulcers

The animals were randomly divided into 5 groups with 6 animals in each group. During the fasting period rats were given to avoid excessive dehydration a nutritive solution of 8% sucrose in 0.2% NaCl. Control group received only water and group 2 received 70 % ethanol at the dose of 10 ml /kg body weight. Pure Rabeprazole sodium at the dose of 20 mg/kg body weight was given to the group 3 animals. The group 4 received the aqueous solution of alginate beads+ ethanol at the dose of 20 mg / kg body weight. The animals were sacrificed after 6 hrs by cutting the stomach along the greater curvature, washed carefully with 0.9% sodium chloride and the ulcers were scored.

The ulcers were scored as given below

Scoring of severity of ulcers

0= Normal colored stomach

0.5= Red coloration

1= ulcers in spots

Mean ulcer score for each animal was expressed as ulcer index .The ulcer index was determined using the following formula

Ulcer index = 10/x

Where X = $\mathbf{total\ mucosal\ area}/\mathbf{total\ ulcerated\ area}$

The results were presented as mean ± SD.The statistical significance was determined using one way ANOVA followed by Dunnett's multiple comparison test. Stomachs were collected and were subjected to macroscopic evaluation and biological estimation of antiulcer activity.

III. Histopathological Evaluation

For macroscopic evaluation, a portion of stomach from each experimental group was fixed in 10% formalin and then immersed in the paraffin. Sections of stomach of 5mm were made with a standard microtome and were stained with hematoxylin and eosin. The sections of stomach were examined for edema/erosion/necrosis and ulceration. Histopathological analysis was done using homogenized tissue of stomach in 9 ml of 0.1 mol/L potassium phosphate buffer (pH 7.4).

IV. Macroscopic Evaluation

The stomach was opend from greature curvature and washed with 0.9% NaCl to study the lesions using dissecting microscope. The grading was assigned to severity of lesions to calculate the ulcer index.

V. Biochemical Estimation For Antiulcer Activity

5.1 Estimation of Lipid Peroxidation

The estimation of lipid peroxidation was done by method given by Karmakar and Chaterjee. The reaction mixture prepared by mixing the homogenate (0.2 ml) with the 1.5 ml of aqueous solution of thiobarbituric acid, 8.1% dodecylsulfate, 1.5 ml of 20% acetic acid having pH 3.5 and 0.6 ml of distilled water. The incubation of mixture was done at 95 °C by keeping in water bath. After cooling under tap water to the mixture 1.0 ml distilled water and 5 ml. of butanol: pyridine (15:1) was added. The mixture was shaken and centrifuged at 4000 rpm for 10 minutes. After centrifugation the organic was layer obtained and the absorbance of this layer was measured at 532nm. The results were calculated as n moles of malondialdehyde per minute per mg protein[11].

5.2 Estimation of Reduced Glutathione

The tissue homogenate was mixed with the 10 % w/v of trichloroacetic acid in ration of 1:1 and centrifuged at 4^0C for 10 minutes at 5000rpm. The 0.5 ml. of supernatant was obtained and it was mixed with 2.0 ml of 0.3 M disodium hydrogen phosphate buffer (pH 8.4) and 0.4 ml of double distilled water. Then 0.25 ml of 0.001 M freshly prepared DNTB [5, 5- dithiobis (2- nitro benzoic acid) was dissolved in 1% sodium citrate solution and this was added to the above mixture. The reaction mixture was incubated for 10 minutes and absorbance of yellow colored complex was noted using spectrophotometer at 412 nm. The results were calculated as n moles of GSH per mg of protein. [12]

5.3 Estimation of Catalase Activity

From the homogenate 50 µl the supernatant was added to 1.95 ml of 50 mM phosphate buffer having pH 7.0 placed in 3.0 ml cuvette. To this mixture 1.0 ml of 30 Mm hydrogen peroxide was added and change in absorbance was noted at 240 nm. The results were calculated as n moles of H_2O_2 used / min / mg of protein.

5.4 Protein Determination

The protein concentration in stomach was determined using Lowery's method.This method is used widely for quantitative determination of protein concentration. Folin-Ciocalteau reagent contains phosphomolybdic/tungstic acid and it produces blue/purple colour on reaction with phenolic moiety of tyrosine present in protein at 660 nm. In the above mixture copper reagent is added to enhance the colour formation by chelating with the peptide bond and helps in electron transfer to the chromophore formed.

Table 1: Assay procedure for protein determination (Lowery's Method)

Sr. No.	Pipette in marked tubes	Blank Solution	Standard Solution	Test Solution
1	Lowery's Reagent	5 ml.	5 ml.	5 ml.
2	Distilled Water	1 ml.	0.9 ml.	¨
3	Standards	¨	100 µl.	¨
4	Test	¨	¨	100 µl.
Incubation for 10 min.				
5	Folin-Ciocalteau reagent	0.5 ml.	0.5 ml.	0.5 ml.

After adding Folin-Ciocalteau reagent incubate the above mixture for 30 min at 37 °C. The absorbance of standard and test solution was taken at 750 nm against reagent blank.

VI. Statistical Analysis

The results were expressed as mean ± SEM

The all values were expressed as mean ± SEM and data were analyzed using one way ANOVA followed by Dunnet's multiple comparison test. A level of $p<0.05$ was considered as statistically significant.

VII. Results And Discussion

The antiulcer activity of the selected formulations was studied on albino Wistar rats.

7.1 Ulcer Index

The stomachs of the rats were opened from the greature curvature and washed with 0.9% NaCl to study the lesions using dissecting microscope. Further grading was assigned to the lesions to calculate the ulcer index as shown in Table 2.

Table 2: Ulcer Index for Ethanol Induced Ulcers in Rats

Treatment Group	Ulcer Index ± S.D.
Group 1(Normal group)	
Group 2 (ethanol group)	0.84 ± 0.615
Group 3 (Pure drug group)	0.216 ± 0.119
Group 4 (alginate beads group)	0.378 ± 0.107

N=3, (S.D.= Standard Deviation)
The ulcer index for the groups ethanol, Rabeprazole standard, Rabeprazole alginate beads(20 mg/kg), Rabeprazole pellets (20 mg/kg) were found to be 0.84 ± 0.615, 0.216 ± 0.119, 0.378 ± 0.107, 0.455 ± 0.17 respectively.

7.2 Macroscopic Analysis

There was presence of more ulcerative hemorrhage in animals which received 70% ethanol. It was attenuated by the prior administration of RAB STD 20, RAB 20 beads and RAB 20 pellets with a few fields of hyperemia. Furthermore, the animals treated with RAB 20 beads and RAB 20 pellets were able to prevent the damage induced by ethanol with similar aspect to the control group. The administration of ethanol causes the significant increase of ulcer index in relation to control animals. Moreover, animals that received RAB 20 pellets did not show any significant difference of ulcer index as compared to control animals

7.3 Histopathological Evaluation

The results of histological evaluation revealed that ethanol causes the deep alteration od glandular epithelium cells and loss of histological structure. The administration of 70 % ethanol induced the consistent microscopic damage with presence of severe swelling in the tissue structure. It also causes loss of continuity of epithelial cells. Furthermore , the RAB STD group at 20 mg/kg showed a relative protection against ethanol, with swelling similar to animals which received ethanol only.
The lesions of stomach were characterized by many granulation tissues and intense inflammation. Pre treatment with RAB 20 prevented the hemorrhage, edema, necrosis and deep ulcerations induced by ethanol.

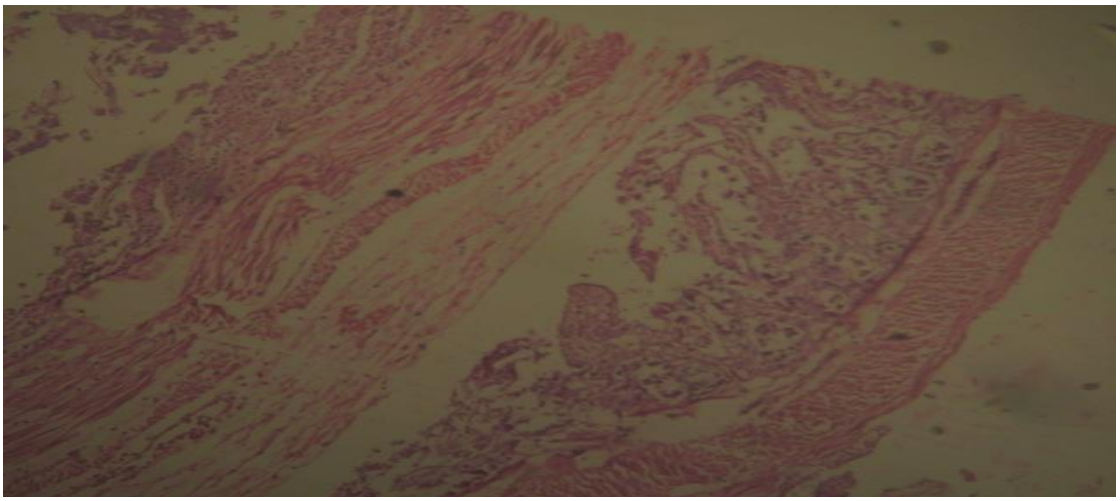

Fig1: Histological Section of Gastric Mucosa of Rat (ethanol group). There is severe disruption of the surface epithelium (H&E stain, 10 X)

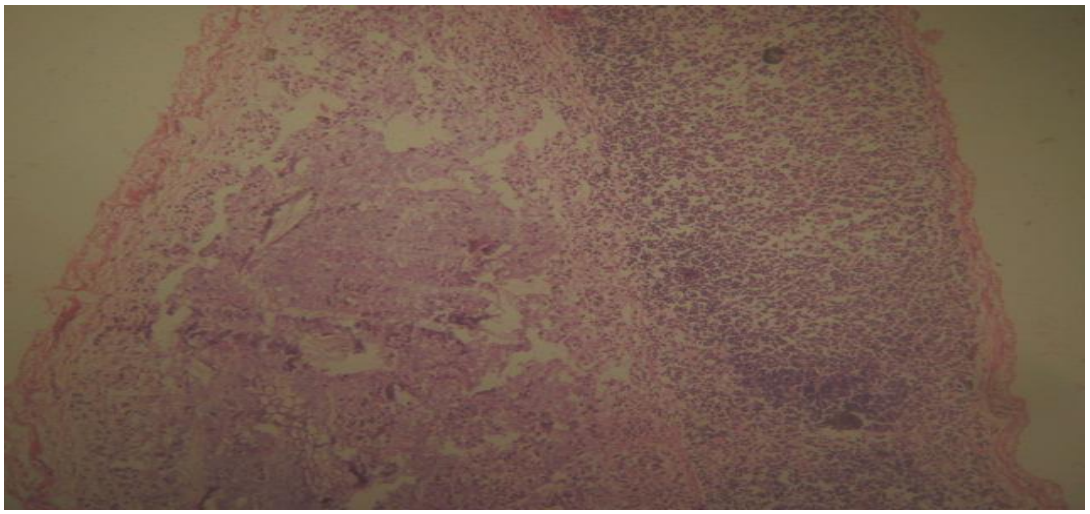

Fig 2: Histological Section of Gastric Mucosa of Rat Pre-treated Rabeprazole pure 20 mg/kg). There is no disruption of surface epithelium (H&E stain, 10 X)

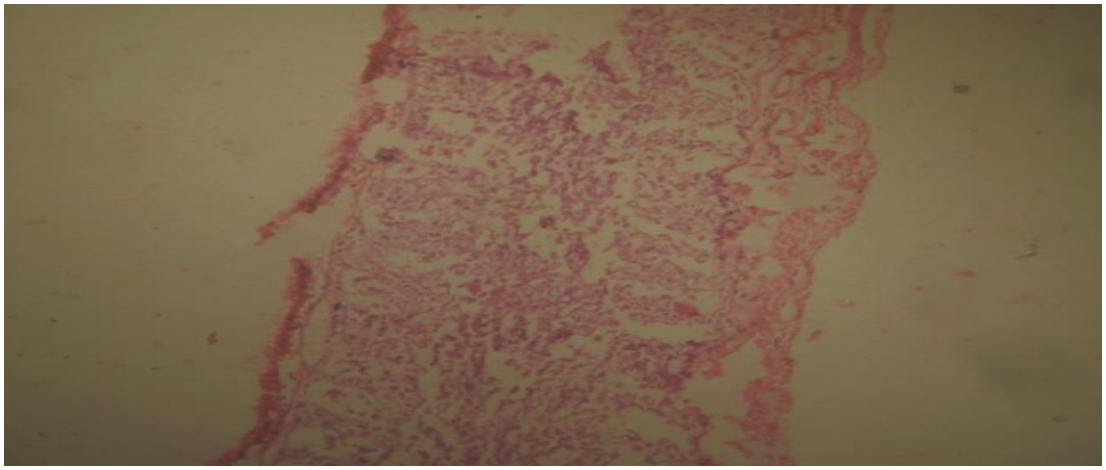

Fig 3: Histological Section of Gastric Mucosa of Rat Pre Treated with Rabeprazole sodium alginate beads 20 mg/kg). There is mild disruption of the surface epithelium with mild edema (H and E stain 10X)

7.4. BIOCHEMICAL ESTIMATION OF ANTIULCER ACTIVITY

7.4.1 Reduced Glutathione (GSH) Estimation

The ethanol group showed significant changes on oxidative markers with a decrease in GSH level (GSH = 3.152 ± 0.6760 n mol/mg protein). However, the animals receiving enteric coated sodium alginate beads of Rabeprazole sodium (RAB 20 BD) (20 mg/kg) completely attenuated the damage induced by ethanol (GSH = 5.885 ± 0.3984, n mol/mg protein, as compared to ethanol group.

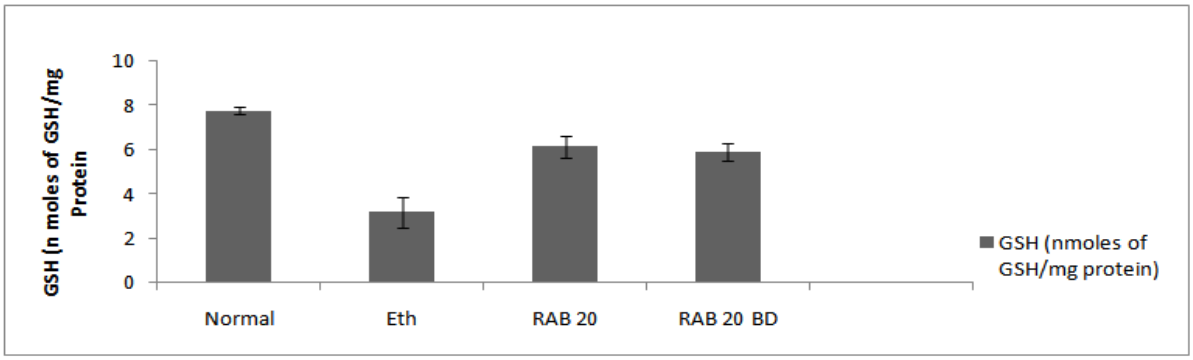

Fig 4: Effect of Formulations on Antioxidant Parameter Reduced glutathione (GSH) in Ethanol Induced Ulcers in Rats. Values are as mean ± SEM; *N* = 6

7.4.2 Lipid Peroxidation (LPO) Estimation

Ethanol administration resulted in marked lipid peroxidation estimated by increased level of TBARs (MDA= 4.524 ± 0.369). However, treatment with enteric coated sodium alginate beads of Rabeprazole sodium (RAB 20 BD) (20 mg/kg) markedly attenuated the TBARS level (MDA = 1.720 ± 0.438) .

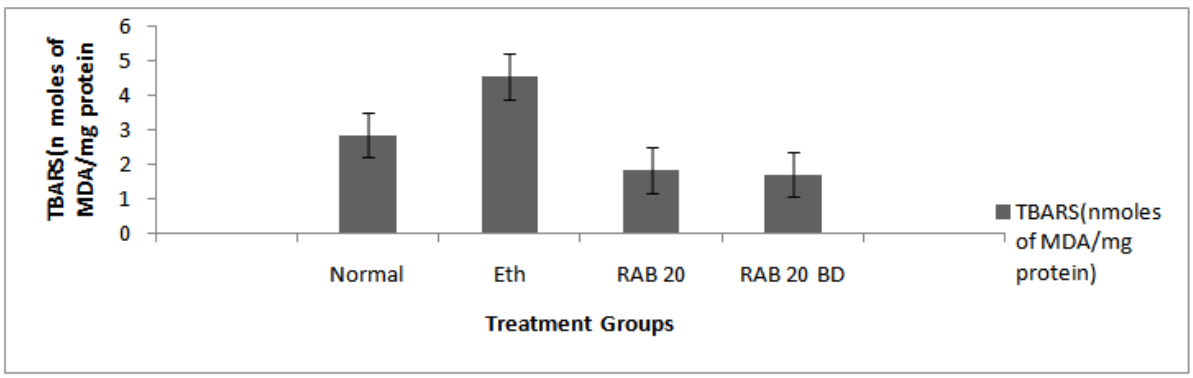

Fig 5 : Effect of Formulations on Antioxidant Parameter Lipid Peroxidation (LPO) in Ethanol Induced Ulcers in Rats. F value= 8.635.Values are mean ± SEM; *N* = 6

7.4.2 Catalase Activity Estimation

Ethanol induced the depletion of antioxidant enzyme Catalase thus in ethanol group of animals the catalase activity decreased (CAT = 1.571 ± 0.2634) and this decrease in Catalase activity was increased on administration of Rabeprazole formulations i.e. enteric coated sodium alginate beads of Rabeprazole sodium (RAB 20 BD) (20 mg/kg) in group 4 and group 5 (CAT = 4.66 ± 0.3984) .

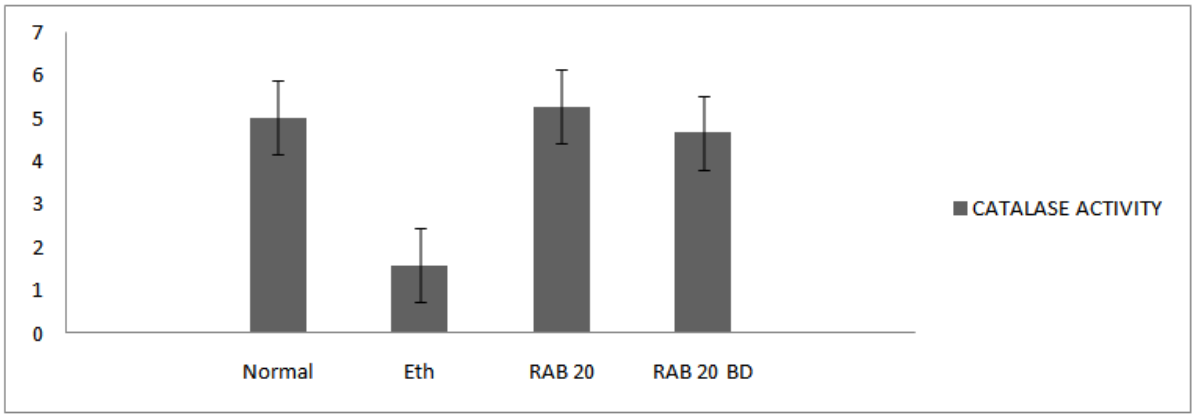

Fig 6: Effect of Formulations on Antioxidant Parameter (Catalase Activity estimation) in Ethanol Induced Ulcers in Rats. F value=7.048. Values are mean ± SEM; *N* = 6

Table 3: List of antioxidant parameters

Antioxidant Parameters	**Groups**			
	Group 1 (Normal group)	Group 2 (Ethanol group**)**	Group 3 (Pure Drug Group)	Group 4 (alginate beads group)
Reduced glutathione (GSH)	7.781 ± 0.1441	3.152 [a] ± 0.6760	6.143 ± 0.4836*	5.885 ± 0.2984**
Lipid peroxidation (LPO)	2.853 ± 0.285	4.524 [a] ± 0.369	1.827 ± 0.610*	1.720 ± 0.430**
Catalase activity	5.0 ± 0.8690	1.571 [a] ± 0.2634	5.261 ± 0.7156*	4.66 ± 0.3984**

Values are presented as mean ± SEM (Standard Error of Mean); N=6 in each group. One way ANOVA followed by Dunnet's test [a]p<0.01 normal group;*P<0.01, **P<0.01 *<0.05 V/S Ethanol group**

VIII. Conclusion

From the study it is concluded that the enteric coated formulation of Rabeprazole have significant antiulcer activity in experimental animal model. It shows the protective and gastric anti- secretary activity when compared with reference drug.

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