Research Article

Evaluation of gastroprotective role of alpha-tocopherol in indomethacin induced peptic ulcer in albino rats

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ABSTRACT

Background: Oxidative free radicals induce gastric injury in animal models of peptic ulcer. Hence antioxidants may provide gastroprotection.

Objectives: The present study was undertaken to evaluate the gastroprotective role of antioxidant alpha-tocopherol in indomethacin induced gastric ulceration in albino rats.

Materials and methods: Animals were divided into 3 groups (n=6), group I (control which received distilled water), group II (alpha-tocopherol 12.5 mg/kg) and group III (omeprazole 3.6 mg/kg). Each group received the corresponding drug orally for 5 days. On fifth day, animals were administered indomethacin 20 mg/kg orally to induce ulceration. After 4 hours, animals were sacrificed and their stomachs were studied for ulceration, adherent mucin content, and ulcer index was calculated. The results of alpha-tocopherol were compared with those of control and omeprazole treated groups, and data was analyzed manually using Student 't' test. A p value of < 0.05 was considered as statistically significant.

Results: Alpha-tocopherol administration reduced ulceration and ulcer index compared to control group (t_{cal} 5.2 > t_{tab} 2.23 at 5% level of significance, $p < 0.05$). Omeprazole treatment also reduced ulcer index and ulceration in comparison to control group (t_{cal} 6.2 > t_{tab} 3.16 at 1% level of significance, $p < 0.01$). Thus results of test drug were similar to omeprazole treated group.

Conclusion: Alpha-tocopherol treatment provides gastroprotection in indomethacin induced gastric ulceration.

Key words: Indomethacin, alpha-tocopherol, antioxidant, gastroprotection

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INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) induce gastric damage ranging from mere irritation and inflammation to severe ulceration and perforation. Peptic ulcer appears to be the major complication with NSAIDs till date.[1] Recently oxidative free radicals have been implicated in NSAID, ethanol, and cold restraint stress induced gastric injury.[2-4] McAlindon ME et al in their study had proposed that NSAIDs caused inflammation and neutrophil infiltration leading to free radical mediated gastric injury.[5] Pihan G et al in their study reported the possible role of oxidative free radicals and lipid peroxidation in mediating NSAID induced gastric injury in albino rats.[6] Jaarin K et al evaluated the effects of various doses of vitamin E and tocopherol for mucosal integrity, gastric acid, malondialdehyde (MDA) concentration and gastric lesion index in post aspirin treated albino rats.[7] In another study, authors had evaluated the anti ulcerogenic potential of antioxidant *Aloe vera* leaf gel in indomethacin and ethanol induced gastric lesions.[8] Alpha-tocopherol is an important constituent of vitamin E present in animal tissues.[9] It is a redox agent with antioxidant and biological activity, which had shown promising results in coronary heart disease, cancer and peptic ulcer

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disease.[10-12] Halliwell S in his review report has suggested alpha-tocopherol protects gastric mucosa by its "chain breaking activity".[13]

Early few reports suggest the possible role of antioxidants in gastroprotection, however till date the gastroprotective role of antioxidant alpha-tocopherol in peptic ulcer was not proved, hence the present study was undertaken to evaluate the possible role of alpha-tocopherol in indomethacin (NSAID) induced gastric injury.

MATERIALS AND METHODS

Animals

The present study was undertaken at Al -Ameen Medical College, Bijapur, Karnataka, India. The study was conducted from December 2010 to December 2011. The experimental protocol was approved by Institutional Animal Ethics Committee, Al-Ameen Medical College, Bijapur. Adult albino Wistar rats of age 8-10 months of either sex, weighing 150-200 gms were selected from central animal house of the college. Each rat was housed singly in a polypropylene cage (UN Shah manufacturers, Mumbai, India) and provided with standard rat feed (Hindustan lever ltd, Mumbai, India) and water ad libitum. The rats were maintained under standard conditions in animal house approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The temperature maintained was $(23 \pm 2)^0$C, humidity 50 ± 5%, light and dark cycle of 10-12 hours.

Drugs and chemicals

Pure drug samples of omeprazole and alpha-tocopherol were gifted by Ranbaxy Co Ltd, India. Other chemicals used in this study were indented from Al-Ameen Medical College store.

Study design

Eighteen albino rats were divided into three groups of six each (n = 6). The control group (Group I) received distilled water 1ml daily for 5 days. Group II received alpha-tocopherol 12.5 mg/kg in peanut oil, once daily orally for 5 days.[14] Group III was omeprazole treated group. A solution was prepared by dissolving omeprazole in propylene glycol, in concentration of 5 mg omeprazole in 5 ml of propylene glycol, administered daily in the dose of 3.6 mg/kg, p.o for 5 days.[14] On fifth day, all groups were administered indomethacin in the dose of 20 mg/ kg in distilled water orally to induce ulceration by the method of Goburdhan R et al.[15] At the end of 4 hours animals were sacrificed by cervical dislocation. Stomachs were isolated and washed in normal saline. Each stomach was studied for ulceration and mucosal damage using magnifying lens.

Estimation of mucosal damage

Estimation of Ulcer Index

Ulcer index is the mean of total number of ulcers in particular group. Ulcer Index was calculated by method of Adami et al.[16] 0 = No lesions, 1= haemorrhagic suffusions, 2 = 1 - 5 small ulcers of 3 mm size, 3 – 1 ulcer of more than 3 mm size, 4 = many ulcers > 3 mm size, 5 = Perforated ulcers. Mean ulcer index was calculated for each group.

Estimation of percentage injury (% injury)

This refers to ulcer index converted to percentage for that particular group.

Estimation of adherent mucin

Adherent mucin was estimated by method of Corne et al.[17] Alcian blue, which stains acidic mucin was used for quantitative estimation of adherent mucin. Procedure in brief: as according to Corne et al.[17]

Step1. Isolated stomachs soaked separately in solution of alcian blue (10 mg /10 ml), sodium acetate (200 mg/ 50 ml) and sucrose (2.73 mg / 50 ml) for Two hours.

Step 2. Dye removed by washing with sucrose (4.2 mg / 50 ml)

Step 3. Mucus complexed with dye is diluted in 10 ml of magnesium chloride (5.08 mg/50 ml) for two hours.

Step 4. The resulting blue solution is used for calculating optical density of adherent mucin using spectrophotometer at 605nm. Optical density is measured as absorbance.

The mean absorbance was calculated. Six additional

animals were taken for estimation of normal adherent mucin. Results were analyzed by converting mean absorbance of each group to percentage of mucin content in comparison to normal group.[18]

Statistical analysis

Ulcer index was expressed as mean and SEM. Results were analyzed manually using Student 't' - test.[19] A p value of < 0.05 was considered as statistically significant.

RESULTS

The control group treated with indomethacin presented with features of ulceration. On gross examination, serosal surface of stomachs showed marked induration, dilated blood vessels, ecchymosis and hemorrhagic sites. Mucosal surface presented with features of severe degree of hyperemia, congestion and large number of pin point ulcers of varying sizes with central clots. There was a visible perforation in one of the stomachs (Figure 1A). Histopathology confirmed ulceration (Figure 1B). The ulcer index was very high in comparison to other two groups (UI - 28.0 ± 4.4, Table 1). The adherent mucin content was reduced in control (indomethacin) group (87.5%) in comparison to normal rats (100%) (Table 1).

Animal pretreated with alpha-tocopherol showed few signs of mucosal injury and percentage of damage was less compared to control group treated with indomethacin. Serosal surface revealed very few dilated blood vessels and petechial hemorrhages (Figure 2). Mucosal surface revealed few ulcers of varying sizes, correspondingly ulcer index was also reduced compared to control group (t_{cal} 5.2 > t_{tab} 2.23 at 5% level of significance, $p < 0.05$). Increase in mucin content was observed in this group in comparison to control rats (Table 1). These features were suggestive of ulcero/gastro protective activity of alpha-tocopherol.

Animals pretreated with omeprazole showed near normal pattern. Serosal surface looked amber colored with few signs of dilated blood vessels and hemorrhagic suffusions (Figure 3). Mucosal surface retained the normal rugae pattern with minimal signs of mucosal injury. The ulcer index was markedly reduced in comparison to control group (Table 1). Concentration of adherent mucin was 106% which was slightly more than normal rats (Table 1). Thus, omeprazole showed gastroprotective activity in the treated group.

The study suggests alpha-tocopherol provides gastroprotection in indomethacin induced gastric ulcer. Thus results of test drug were similar to omeprazole treated group at significance level of < 0.05.

Table 1: Mean Ulcer Index, % injury, mean absorbance and mucin (%) in various treated groups.

Group, treatment	Mean ulcer index	% injury	Mean absorbance	Mucin %
I, Control (indomethacin)	28.0 ± 4.4	100	0.14	87.5
II, Alpha -tocopherol + indomethacin	3.8 ± 1.3*	14	0.15	90.4
III, Omeprazole + indomethacin	1.3 ± 0.6 †	4	0.17	106
IV, Normal rats (distilled water)	-	-	0.16	100

Mean ulcer index values are expressed as Mean ± SEM.
Mucin %: Calculated mean of the absorbance of each group was converted to percentage mucin in comparision to normal rats.
* vs. control (tcal 5.2 > ttab 2.23 at 5% level of significance, $p < 0.05$)
† vs. control (tcal 6.2> ttab 3.16 at 1% level of significance, $p < 0.01$)

Figure 1A: Gross features of stomach in indomethacin treated (control) group.

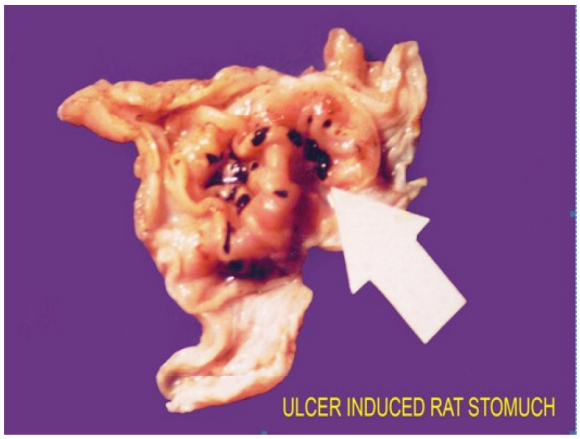


Figure 1B: Histopathology (10X) features of stomach in indomethacin (control) treated group.

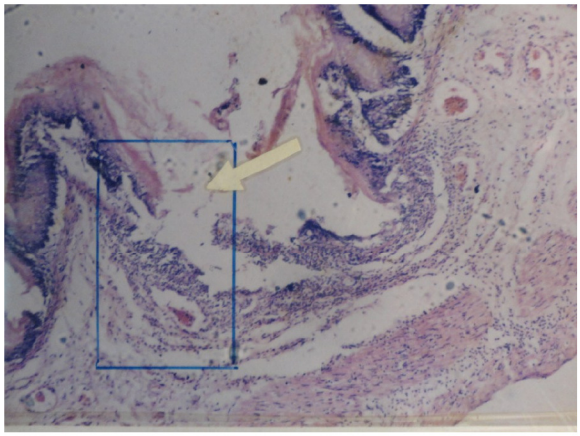


Figure 2: Gross photograph of stomach in alpha-tocopherol treated group.

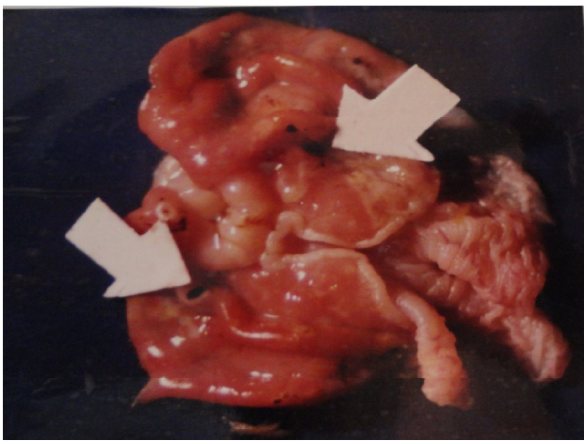
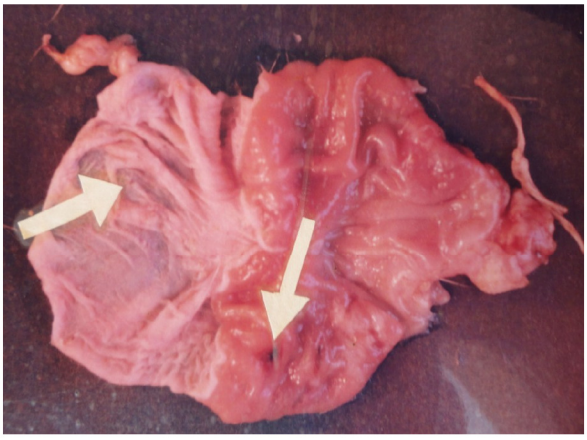


Figure 3: Gross photograph of omeprazole treated group with minimal ulcers



DISCUSSION

The results in present study revealed indomethacin induces marked increase in ulcer index, with a reduction in adherent mucin content associated with mucosal damage, in comparison to omeprazole and alpha-tocopherol treated groups. In contrast, pretreatment with alpha-tocopherol caused a marked reduction in mucosal damage and ulcer index with increased adherent mucin content, suggesting the gastroprotective role of test drug. Similar observations were noted on pretreatment with omeprazole group as evident from results.

The probable mechanisms of mucosal damage due to indomethacin (NSAID) could be explained on following basis:

a. Inhibition of prostaglandin synthesis leading to vasoconstriction and low mucin content.[1]

b. Damage to mucosal barrier leading to back diffusion of H^+ ions causing tissue acidosis and lowering of pH, which in turn brings about uncoupling of oxidative phosphorylation in mitochondria releasing oxidative free radicals.[18]

c. NSAIDs induce inflammation and neutrophil infiltration. With states of low pH and hypoxia, neutrophils get activated to release large number of reactive oxygen species.[20,21]

The gastroprotective role of alpha-tocopherol could be explained on the basis of its lipid soluble property. It diffuses freely into biological membranes, thereby replenishing glutathione and superoxide dismutase (SOD) levels. The peroxyl and alkoxyl radicals generated

during oxidative stress are scavenged by alpha-tocopherol thus preventing peroxidation mediated injury (chain breaking activity).[13] Karmeli et al also in their study had suggested a similar mechanism. Stress and hypoxia cause depletion of alpha tocopherol and gastric peroxidase enzyme while supplementation with antioxidants prevents ulceration.[22] Similar studies conducted suggest the possible role of antioxidants in peptic ulcer. Gill NS et al in their study had proposed pretreatment with methanolic extracts of *Cucumis melo* seeds, an antioxidant, reduced gastric injury in various animal models of gastric ulcer.[23] Salim AS in double blind clinical trial has reported the gastroprotective effect of an antioxidant dimethyl sulfoxide (DMSO) and allopurinol in patients with refractory peptic ulcer disease.[24]

Estimation of endogenous antioxidant enzymes would be an added feature to the study. However, alpha-tocopherol might prove to be useful therapeutic drug in near future, in treatment of refractory peptic ulcers in individuals on long term NSAIDs. Further experimental and clinical studies are needed for confirmation.

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