ORIGINAL ARTICLE

HISTOPROTECTIVE EFFECTS OF VITAMIN E ON ALENDRONATE INDUCED GASTRIC ULCERS IN ALBINO RATS

AYESHA RAHEEM[1], FARHANA SAJJAD[2], SANIA ASIF[3], ANUM FAROOQ4, SARAH KHAN[5], AREIBA HAIDER [6]

[1]Sr. Demonstrator, Bakhtawar Amin Hospital, Multan.[2]Professor King Edward Medical University, Lahore, [3]Associate Prof, Faisalabad Medical university, Faisalabad.[4] Demonstrator, Fatima Memorial Hospital, Lahore. [5]Associate Prof, King Edward Medical University, Lahore ,[6]Associate Prof, Narowal Medical College, Narowal.

ABSTRACT

Background: Gastric ulcer is a serious precancerous disease. The pathogenesis of gastric ulcer is due to the imbalance of shielding factors and risk factors. Oxidative damage also plays an important part in its pathogenesis. Alendronate is the main bisphosphonate used for Osteoporosis but affects GIT badly. Vitamin E is the main antioxidant which prevents the oxidative damage.

Objectives: To evaluate the gastro protective effects of Vitamin E on gastric mucosal injury produced by Alendronate.

Methods: Twenty-one adult albino rats were divided into three groups. Group I was the control group, group II was given Alendronate 3mg/kg for 28 days and group III was given Vitamin E 400IU/100gm followed by Alendronate 3mg/kg after 1 hour, for 28 days. On 29th day, all rats were sacrificed and specimens of stomach were preserved for tissue processing and sectioning. H&E staining was used to see epithelial integrity, extent of ulcer, inflammatory infiltrates and hemorrhage.

Results: The epithelial integrity was disrupted in Group II (Alendronate) while Group I (Control) and Group III (Vitamin E and Alendronate) had intact epithelium. Most of the ulcers were extended up to epithelium of Group II (Alendronate). Neutrophil infiltrate and microscopic hemorrhages were also seen in Group II (Alendronate) but not in Group III (Vitamin E and Alendronate).

Conclusion: The study showed that Vitamin E had provided gastro protection against Alendronate induced gastric ulcer in rats. There was reduction in gastric mucosal erosion in the Group III which was given Vitamin E along with Alendronate.

Keywords: Vitamin E, gastric ulcer, epithelium, alendronate, hemorrhage,

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Correspondence to: *Ayesha Raheem*
Senior Demonstrator, Bakhtawar Amin Hospital, Multan, Pakistan

Email: *ayesharaheem6@gmail.com*

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INTRODUCTION

Gastric ulcer affects 10% of the world's population and in 1% of them, it leads to gastric cancer. So, it is classified as precancerous disease by WHO.[1] Gastric cancer is the fifth commonest cancer and this malignancy ranked third in being fatal.[2] The Peptic ulcer disease is more common in females worldwide, commonly associated age limit is 65 to 69 years in female and 55 to 59 years in males.[3] Ulcers are caused mostly by H. pylori, NSAIDS, Bisphosphonates mainly Alendronate Sodium, overconsumption of alcohol, stress, tobacco smoking and Selective Serotonin Reuptake Inhibitor (SSRI).[4] Another cause of ulcer is

the oxdative stress because of the toxic free radicals.[5] The imbalance manifests as lower level of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase and raise level of oxidative stress product such as malondialdehyde and 8-hydroxy-2' –deoxyguanosine.[1]

Alendronate Sodium is the main bisphosphonate that is used most commonly for the treatment of many bones related conditions including Osteoporosis (post-menopausal, corticosteroid induces, age related and male osteoporosis), Paget's disease, Periodontal defects and bone metastasis.[6] Bisphoshonates are considered as main therapy for osteoporosis but also cause GIT irritation. They disturb the mucosal barrier mechanism.[7] Nitrogen containing bisphosphonate (Alendronate) targets an enzyme Farnesyl PyroPhosphate synthase. This enzyme is required for protein prenylation which is a process essential for the function of small GTPase proteins. These small GTPase are vital for the activity of osteoclast.[6] Reactive oxygen species (ROS) play an important role in alendronate induced gastrointestinal injury as it increases lipid per oxidation and superoxide production.[8] Since there are many health benefits of it, the alendronate related GIT injuries need more effort and attention to avoid such problems.

Vitamin E is a lipid soluble antioxidant that is present in the cell membrane of all the tissues. Vitamin E acts as a chain breaking antioxidant that removes reactive oxygen radicals especially lipoperoxide to maintain cellular integrity.[9] It is the most effective lipid soluble antioxidant that prevents lipid per oxidation and oxidative damage. It also inhibits the oxidation of polyunsaturated fatty acids and suppresses the platelet aggregation.[10] Vitamin E increases the antioxidant resources of cell.[11] It has positive effects in gene regulation by protein modification and transcription. It also has an impact on cell proliferation and regulation, signaling pathway of cell death, detoxification and even on inflammatory response of the cell.[12] Anti-oxidant and anti-inflammatory properties of Vitamin E are the main reason for its anti-ulcer characteristics. This study aims to see the histo morphological effects of Vitamin E in Alendronate induced gastric ulcer.

METHODS

Twenty-one Wistar Albino rats, weighing between 150 and 200 grams and age between 8 to 12 weeks, were selected. All procedures were adhered strictly to both international and institutional guidelines, with approval granted by the Ethical Committee of King Edward Medical University, Lahore. The rats were kept in the Experimental Research Lab (Animal House) of University of Veterinary & Animal Sciences, Lahore, under controlled environmental conditions, including a temperature of 25 ± 2°C, humidity of 60 ± 5%, and 12-hour light/dark cycle. Food and water were given *ad libitum*. Animals were divided into three groups of seven animals by random number generator. Group I was given 1ml distilled water daily by oral gastric gavage method for 28 days.[13] Group II Animals were given single oral dose of Alendronate sodium 3mg/kg (0.003mg/gm) daily by oral gastric gavage method, for 28 days.[13] Group III Animals were given vitamin E 400IU/100gm (180mg/100gm) body weight once daily for 28 days. After an interval of one hour, 3mg/kg (0.003mg/gm) Alendronate was given daily by oral gastric gavage method for 28 days.[13,14] Alendronate (Tab Drate D 70mg a product of Solace pharma) and Vitamin E (Cap Evion 400mg by the company Martin Dow) were used in the study.

After 24 hours from their last administered dose, the animals underwent dissection. Stomach was then excised from gastro esophageal to gastro duodenal junction. An incision was made along the greater curvature of stomach and the interior was rinsed with cold saline to eliminate the traces of food particles. The stomachs of all animals within each group were individually placed in separate labeled plastic jars filled with 10% formalin fixative fluid and left for 48 hours. Each stomach was transversely cut and tissue strips containing ulcers were carefully separated. The sections were stained with Hematoxylin and Eosin. The prepared slides were studied under the light microscope using the magnification of 10X and 40X objectives. Data was analyzed by SPSS-25. Comparison of three groups was made by applying Chi-square test were applied to compare the association between the groups. p-value ≤0.05 was taken as significant.

Statistical Analysis: Data was analyzed by SPSS-25. Comparison of three groups was made by applying Chi-square test were applied to compare the association between the groups. p-value ≤0.05 was taken as significant.

RESULTS

The Epithelium was intact for all the 7 animals of Group I (control) and Group III (vitamin E plus Alendronate). But the epithelium was disrupted in all the 7 animals of Group II (Alendronate). A Chi-square test revealed a highly significant difference among the groups regarding epithelial integrity ($\chi 2 = 21.00$, $p < 0.001$) which is highly significant. (Table 1) Hemorrhage was not found in any animal of group I. In Group II, hemorrhage was present in six of the seven animals (85.7%), while one animal (14.2%) showed no hemorrhage. In Group III (vitamin E plus Alendronate), 6 (85.7%) animals were not having hemorrhage but 1 (14.2%) animal exhibiting hemorrhage. The results are highly significant with Chi-square test value of 13.28 and P value of <0.001. (Table 1) The neutrophil

infiltrates were absent in all 7 animals of Group I (control) and were present in all 7 animals of Group II (Alendronate). In Group III (vitamin E plus Alendronate), neutrophil infiltrates were absent in five animals (71.4%) and present in two animals (28.5%). The differences in neutrophil infiltration among the groups were highly significant with chi-square test value of 15.16 ($p < 0.001$). (Table 1)

Table1: Comparison of epithelial integrity, hemorrhage and neutrophil infiltrate among groups

Groups	Epithelial integrity				Hemorrhage				Neutrophil Infiltrate			
	Intact		Disrupted		Absent		Present		Absent		Present	
	n	%	n	%	n	%	n	%	n	%	n	%
I	7	100	0	0	7	100	0	0	7	100	0	0
II	0	0	7	100	1	14.2	6	85.7	0	0	7	100
III	7	100	0	0	6	85.7	1	14.2	5	71.4	2	28.5
Total	14		7		14		7		12		9	0
	Chi-square = 21.00 (p =<0.001)*				Chi-square = 13.28 (p =< 0.001) *				Chi-square = 15.16 (p = <0.001) *			

*p-value ≤0.05 is considered statistically significant

The gastric ulcers were absent in group I (control) and Group (III vitamin E plus Alendronate). However, ulcers were present in Group II (Alendronate) and all were extending the Epithelium. The Chi-square test value is 21.00 and p value is <0.001 which is highly significant. (Fig 1)

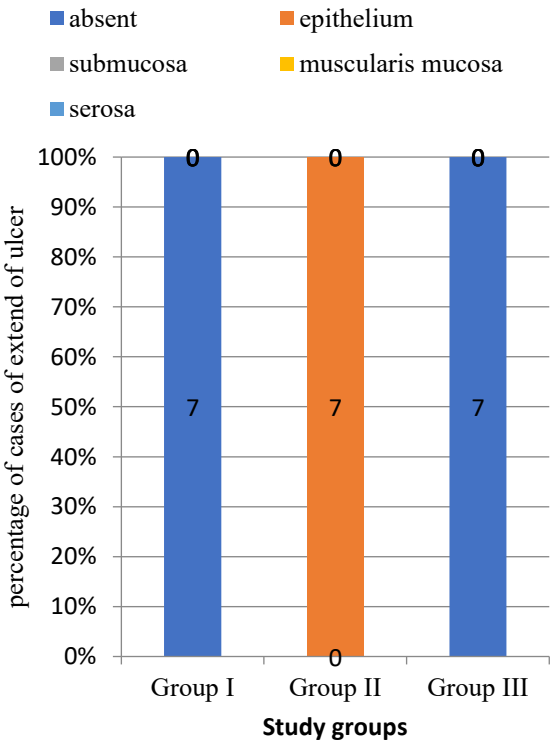

Figure 1: Bar diagram presenting the percentage component of extent of ulcer in gastric mucosa for various groups

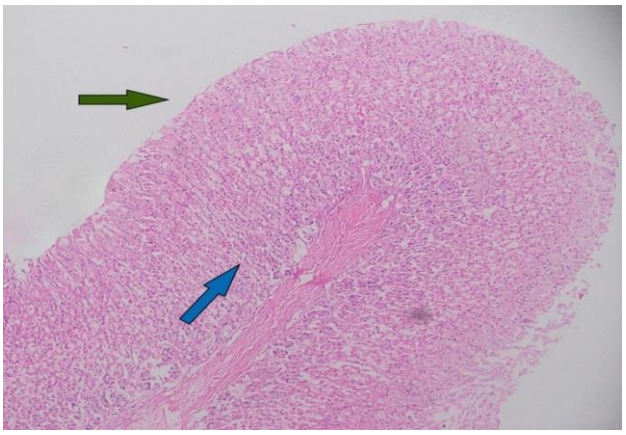

Figure 2a: Photomicrograph showing epithelial integrity of Group I, green arrow shows intact epithelium, blue arrow shows normal gastric pits, no infiltrate or hemorrhage. H&E stain. Magnification 10X

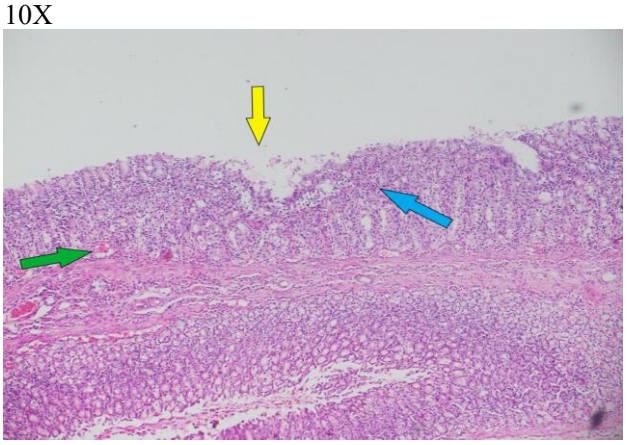

Figure 2b: Photomicrograph showing epithelial integrity of Group II having epithelial disruption (yellow arrow) with neutrophil infiltrate (blue arrow) and hemorrhage (green arrow). H&E stain. Magnification 10X

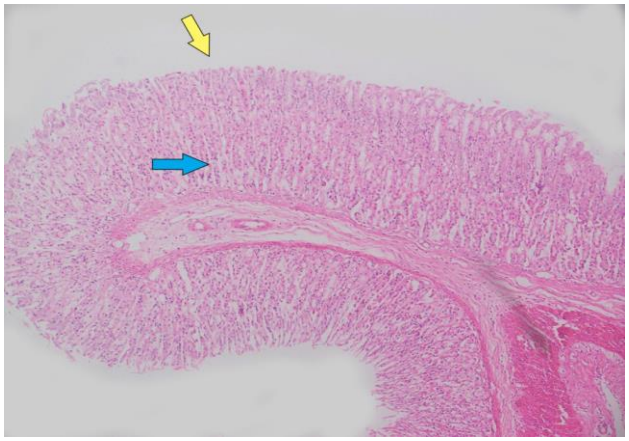

Figure 2c: Photomicrograph showing epithelial integrity of Group III, yellow arrow shows intact epithelium with no infiltrate or hemorrhage and blue arrow shows normal gastric pits. H&E stain. Magnification 10X

DISCUSSION

The adverse effects of Alendronate are known and proven and cause gastric mucosal injury both in animals and humans.[6] The antioxidant effects of Vitamin E have protective effect on gastric mucosa. It maintains the integrity of gastric mucosa.[14] This study was conducted to know the gastro protective effects of Vitamin E against the gastric ulcers or gastric mucosa injury caused by Alendronate.

Histological examination of experimental animals showed that the epithelium of Group I (control) and Group III (Vitamin E plus Alendronate) remained intact. But the epithelium of Group II (Alendronate) was disrupted in all animals of this group with p-value <0.001, (Table 1) Isabela R.S.G. Nolêtoa et al (2019) also shows the same result of epithelium disruption because of alendronate.[15] Renan O. Silva et al (2016) also proved that alendronate causes the breakdown of intact epithelium.[16] Vitamin E protects the epithelium. The protection of the gastric mucosa by Vitamin E is through cytoprotection by formation of prostaglandins, increased blood flow of gastric mucosa, stimulation of cellular growth repairs and scavenging free radical.[14] It is the most effective lipid soluble anti-oxidant that prevent lipid peroxidation and oxidative damage.[17]

Microscopic hemorrhage was not found in any animal of Group I (Control). However, 6 (85.7%) animals of Group II (Alendronate) and 1 (14.2%) animal of Group III (vitamin E plus Alendronate) was having hemorrhage. (p-value <0.001). (Table 1) Another study in 2016 also showed hemorrhagic damage in gastric mucosa due to Alendronate.[18] Ibrahim Abdel Aziz Ibrahim et al (2010) showed that Vitamin E protects the mucosa from gastric ulcer formation and also reduces the hemorrhagic formation in gastric mucosa.[19]

In present study, the overall difference among groups for neutrophil infiltration was found highly significant with p-value <0.001. The neutrophil infiltrates were absent in all 7 animals of Group I (Control) 100% and were present in all 7 animals of Group II (Alendronate) 100% and in 2 (28.5%) animals of Group III (vitamin E plus Alendronate). Nata´lia R.D. Costa et al (2016) also shows the presence of neutrophil infiltrate in the disrupted epithelium of gastric mucosa of rats.[18] Vitamin E shows anticancer properties by anti-apoptotic, anti-angiogenic and antiproliferative activities.[20] It has anti-inflammatory effect by protecting plasma cells, macrophages and lymphocytes.[9]

The extent of the ulcers was noticed in each group. The gastric ulcers were only present in Group II (Alendronate) and all were extending upto Epithelium. (p-value <0.001). L.A.D. Nicolau et al (2013) also showed the presence of gastric ulcer and it also extended upto epithelium.[21] Alendronate disturbs the mucosal barrier mechanism.[7] Reactive oxygen species play an important role in alendronate induced gastrointestinal injury as it increases lipid per oxidation and superoxide production.[22] The antioxidant characteristics of vitamin E are because of its reaction to phospholipid bilayer of cell membrane. It breaks the chain reaction as well as it directly scavenges the free radicals and converts them into less toxic products.[23]

CONCLUSION

This study revealed that administration of Vitamin E showed significant gastro protective effects against the Alendronate induced gastric mucosal damage. Vitamin E increased the amount of mucous output and also showed the decrease in neutrophil infiltrates. This study also proved that ulcers induced by Alendronate were protected by Vitamin E.

CONFLICT OF INTEREST

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICAL APPROVAL

Ethical approval was granted by Institutional Review Board, King Edward Medical University, Lahore. vide reference No.474/RC/KEMU dated: 13/11/2023

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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