

Antiulcerogenic Activity of Vitamin E on Gastric Ulcers produced by Indomethacin

Ayesha Yousaf¹, Ifra Saeed², Tehzeeb ul Hassan³, Rana Fahd Intisar⁴, Mohammad Amin Shiekh⁵

^{1,2} Associate Professor, Department of Anatomy, Rawalpindi Medical University, Rawalpindi.

⁴ House Officer, Military Hospital, Rawalpindi.

³ Professor, Department of Anatomy, Rawalpindi Medical University, Rawalpindi.

⁵ Head of Department, Department of Anatomy, King Edward Medical University, Lahore.

Author's Contribution

¹ Conception of study

¹ Experimentation/Study conduction

^{1,2} Analysis/Interpretation/Discussion

² Manuscript Writing

^{3,4} Critical Review

^{4,5} Facilitation and Material analysis

Corresponding Author

Dr. Ayesha Yousaf,

Associate Professor,

Department of Anatomy,

Rawalpindi Medical University,

Rawalpindi

Email: Ayesha_intisar@yahoo.com

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Abstract

Objective: To study the morphological and histological effects of vitamin E on gastric lesions produced by indomethacin.

Materials and Methods: This was an animal interventional study, 48 adult healthy albino mice were selected and were split into four groups A, B, C and D. Number of animals in each group was 12. Group A was categorized as control. Evion 400mg/kg was administered to Group B. Indocid 25mg/kg was given to group C. Indocid and Evion both (25mg/kg & 400 mg/kg respectively) were administered to Group D. In all group, six animals were selected and treated for three days and rest for eight days with calculated doses of drugs. Mice were sacrificed and dissection was done 24 hours after the last dose. The stomach was identified, washed, and observed under dissecting microscope to study the number and shape of ulcers. The dimension of ulcers was measured under a compound microscope.

Results: No ulcers were seen in groups A and B. 35 and 10 ulcers were observed in groups C and D respectively. The mean number of ulcers in groups C and D was statistically significant (p-value=0.000). In comparison to group D, Pindot, linear, Irregular, and punched-out ulcers were more prevalent in group C and were statistically significant (p-value < 0.05). The mean linear dimension of ulcers in group C was much greater than in group D. The mean dimension of ulcers in group C1 and C2 was 262.50µm and 232.5µm respectively. Whereas in group D1 and D2 given both Indocid and vitamin E the dimensions were 56.5µm and 50µm.

Conclusion: Vitamin E has an anti-ulcerogenic effect on stomach mucosa by reducing the number and dimension of ulcers.

Keywords: gastric ulcers, Indomethacin, Vitamin E.

Introduction

Vitamin E is an important lipid-soluble antioxidant exclusively derived from the diet. Vitamin E through oxidation of free radicals saves constituents of the cell membrane and polyunsaturated fatty acids along with lipoproteins. It lies in the lipid bilayer of cell membranes. α -tocopherol is the dominant form of Vitamin E.¹ Initially Vitamin E was known as a dietary factor and was considered important for rats reproductive activity, but with advance research, it was discovered that it has other important functions like searching of reactive nitrogen and oxygen species that prevent oxidative injuries related with many diseases.²

Extensive work has been done over the past few decades regarding the role of various vitamins in different gastrointestinal ailments. Many vitamins exhibit an opposite association with gastric malignancies, but different trials have not proved their defensive role.³ Reboul⁴ elaborates the providence of vitamin E during digestion in the lumen of the human gastrointestinal tract. She focused on the cellular passage of vitamin E across the enterocytes and describe different elements modifying absorption of Vitamin E. 13'-hydroxychromanol and 13'-carboxychromanol are produced during the metabolism of vitamin E, by oxidative modification of the side-chain

Naohito⁵ explored the action of vitamin E on gastric mucosal lesions produced by an infection caused by *Helicobacter pylori*. His results proved that vitamin E has an antiulcerogenic effect on gastric mucosa caused by *H. pylori* infection by preventing aggregation of highly active neutrophils.

Materials and Methods

Study Design: Animal interventional study.

The study was conducted at King Edward Medical University and Post Graduate Medical Institute, Lahore. 48 adult swiss webster albino male mice weighing 21-35g were procured from the Veterinary Research Institute of Lahore. International and institutional guidelines were observed for housing and treatment of mice. The room temperature was controlled at 25 + 2°C and humidity was around 45-65%. Mice were fed on a commercially available diet.

Grouping of Animals: Four cages were taken and labelled as control group-A, and experimental groups-B, C, and D each having twelve mice.

Dosage and Route of Administration: The dose of indocid (Indomethacin) and Evion (Vitamin E) for each mice was measured by the following formula:

Dose (in ml) = 0.002 x Wt(mice in grams)

The calculated dose of Indocid was administered as 25 mg/kg body weight/day daily by combining powder of 1 indocid capsule in 2ml of distilled water. A calculated dose of Evion was given as 400mg/kg body weight/day by mixing liquid of 1 Evion capsule in 2ml of corn oil.

Control Group: Control group-A was further subdivided randomly into two subgroups A1, A2 having 6 animals each. The animals of the A1 and A2 subgroups were given a commercial diet and water for 3 and 8 days and then sacrificed on the fourth and ninth day respectively.

Experimental Groups: Group B was further subdivided into subgroups B1 and B2 comprising 6 animals each. Group B1 and B2 were given Evion in a dose of 400mg/kg/day in 2ml solution orally for 3 and 8 days respectively and sacrificed 24 hours after the last dose. Group C was divided into C1 and C2. C1 subgroup received Indocid 25 mg/kg orally dissolved in 2 ml of distilled water for 3 days and sacrificed on the fourth day while C2 received Indocid 25mg/kg for 8 days and sacrificed on the ninth day. Animals in group D were subdivided into subgroups, each comprising of six male mice. D1 and D2 were given Indocid 25mg/kg and Evion 400mg/kg simultaneously for 3 days and 8 days respectively. D1 was sacrificed on the fourth day and D2 was sacrificed on a ninth day.

The number and shape of ulcers were observed after washing and dissecting the stomach of sacrificed mice under a dissecting microscope. The stomach of subgroups was placed in tagged glass jars for 48 hours containing 10% formalin. One specimen was randomly selected for histological study. Tissue processing was done and Harris's Haematoxylin and Eosin (H & E) were used for staining purposes. The linear Dimension of ulcers was measured with the help of an oculometer under a light microscope at a magnification of 10X.

Statistical Analysis: The data were analyzed in SPSS version 23. The qualitative data (shape of ulcers) was presented in form of cross-tabulation and multiple bar charts with respect to study groups. The quantitative data (number of ulcers and linear dimension) was given in mean \pm S.D along with its minimum and maximum values. The standard error (S.E) was also given when S.D was too high. The comparison in qualitative data (mentioned above) was made by the

chi-square test and Fisher’s exact test. The comparison in quantitative data was done using Analysis of variance (ANOVA). The p-value was considered as significant at < 0.05.

Results

No ulcer developed in group A1, A2, B1, and B2. (Table 1, Graph. 1). The mean number of ulcers in subgroup C1 and C2 was 3 ± 1.10 and 2.83 ± 0.75 . The total number of ulcers in subgroup C1 and C2 were 18 and 17 respectively. (Table 1, Graph 1)
 The mean number of ulcers in subgroup D1 and D2 was 1 ± 1.55 and 0.67 ± 1.03 respectively. The total number of ulcers in D1, D2 was 6 and 4 respectively. (Table 1, Graph 1).
 There were 5 irregulars, 5 pin dot, 6 linear, and 2 punched-out ulcers in the C1 subgroup making a total

of 18 ulcers. There were 17 ulcers in C2, out of which 6 were irregular, 8 pin dot, 2 linear, and 1 punched-out ulcers in subgroup C2. (Table 2, Figure 1)

There were 6 ulcers in D1, 5 were pin dot and 1 was linear. There were 2 ulcers with irregular margins and 2 pin dot in D2. (Table2, Figure 1)

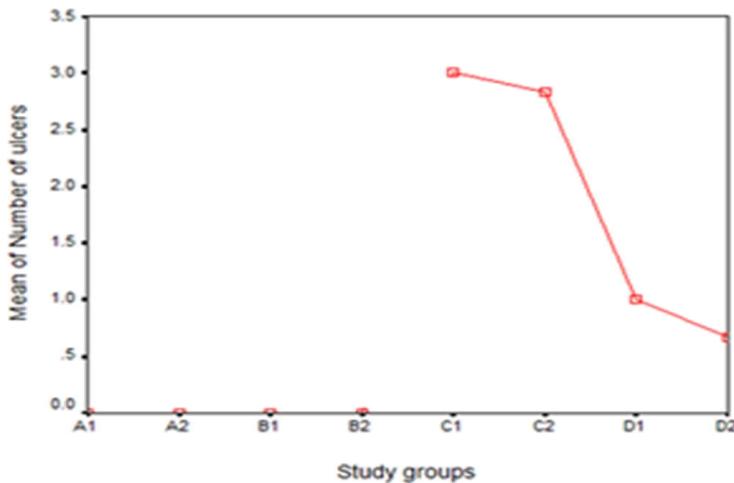
In subgroup C1 the mean linear dimension of ulcers was $262.50\mu\text{m}$ with the largest dimension $1400\mu\text{m}$ and the smallest dimension $30\mu\text{m}$. In C2 mean dimension of ulcers was $232.5\mu\text{m}$ ranging from $1200\mu\text{m}$ to $60\mu\text{m}$. (Table 3, Figure 2, Graph 2)

In subgroup D1 the mean dimension of ulcers was $56.5\mu\text{m}$ with the biggest ulcer of $290\mu\text{m}$ and the smallest ulcer of $30\mu\text{m}$. In subgroup D2 the largest ulcer and smallest ulcers were $280\mu\text{m}$ and $50\mu\text{m}$ respectively having a mean dimension of $50\mu\text{m}$. (Table 3, Figure 3, Graph 2)

Table 1: Mean Number Of Ulcers In Groups

		No of ulcers	Mean	S.D	Maximum	Minimum
Study Groups	A1	--	0	0	0	0
	A2	--	0	0	0	0
	B1	--	0	0	0	0
	B2	--	0	0	0	0
	C1	18	3.00	1.10	5.00	2.00
	C2	17	2.83	.75	4.00	2.00
	D1	6	1.00	1.55	.00	3.00
	D2	4	0.67	1.03	.00	2.00
	Total	45	0.94	1.42	.00	5.00

P-value =0.000, i.e. < 0.05



Graph 1: Comparison of the number of ulcers in study groups, C vs. D P-value < 0.05

Table 2: Shape of Ulcers in groups

Shape of Ulcers	Study Groups					Total No of Ulcers
	A1, A2, B1, B2	C1	C2	D1	D2	
Irregular margins	-	5 (27.7%)	6 (35.29%)	-	2 (50%)	13(28.8%)
Pin dot	-	5 (27.7%)	8 (47.05%)	5 (83.33%)	2 (50%)	20 (44.44%)
Linear	-	6 (33.3%)	2 (11.76%)	1(16.67)	-	9(20%)
Punched out	-	2 (11.11%)	1 (5.88%)	-	-	3(6.667%)
Total No of Ulcers	-	18 (100%)	17 (100%)	6(100%)	4 (100%)	45(100%)

P-value = 0.035, i.e. < 0.05

Table 3: Linear Dimension of Ulcers in Groups (µm)

		No.	Mean	S.D
Study Groups	A1	6	.00	.00
	A2	6	.00	.00
	B1	6	.00	.00
	B2	6	.00	.00
	C1	6	262.50	261.53
	C2	6	232.50	129.53
	D1	6	56.50	87.63
	D2	6	50.00	79.75
Total	48	75.19	145.62	

P-value = 0.000, i.e. < 0.05

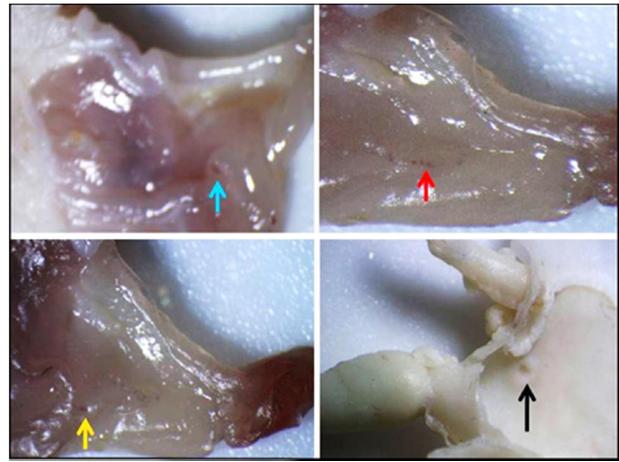
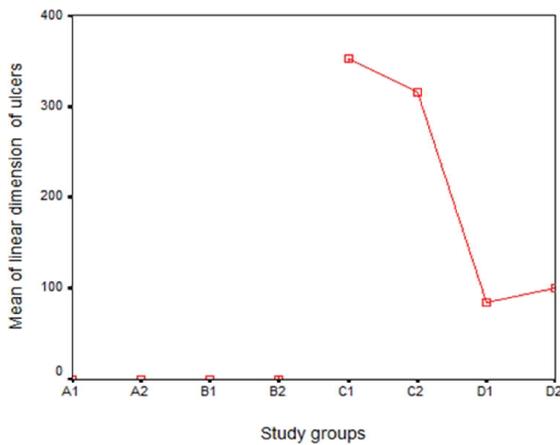


Figure 1: A photograph showing Gross morphology of different shape of ulcers under dissecting microscope in groups C and D

- Blue arrow: Irregular ulcer
- Red arrow: Pindot ulcer
- Yellow arrow: Linear ulcer
- Black arrow: Punched out ulcer



Graph 2: Comparison of linear dimension in study groups, C vs. D groups P-value < 0.05

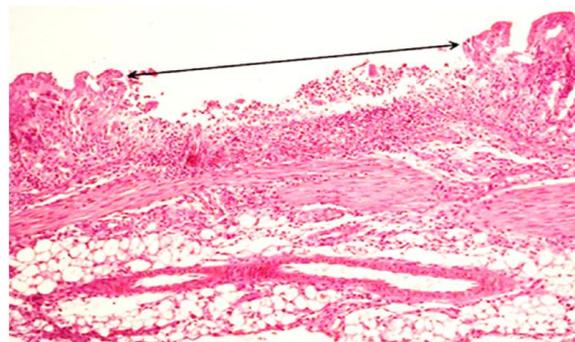


Figure 2: Photomicrograph of stomach showing linear dimensions of ulcer in Group C (H&N Stain Magnification 10x10=100X Black Arrow= Dimensions of Ulcers)

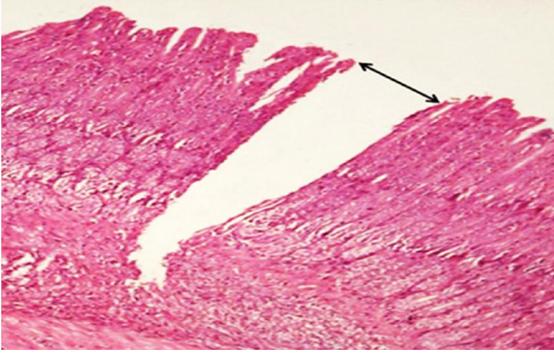


Figure 3: Photomicrograph of stomach showing linear dimensions of ulcer in Group D (H&N Stain Magnification 10x10=100X Black Arrow= Dimensions of Ulcers

Discussion

The detrimental effects of Indomethacin on the musculoskeletal system cannot be ignored but its exposure has been known to cause gastric ulcers in humans and animals.⁶ It also blocks the increased effects of cocaine. When given intraperitoneally, indomethacin before an episode of stress blocks increases in the effects of cocaine in mice.⁷

Vitamin E is a valuable antioxidant present in the human body. Intake of the antioxidant has a fruitful effect on the prevention as well as management of chronic diseases.⁸ The antioxidant effect of Vitamin E also nourishes the gastric mucosa in animals⁽⁹⁾. High-dose vitamin E is not recommended due to its prooxidant actions.¹⁰ In this study the antiulcerogenic role of vitamin E was assessed against experimentally produced gastric ulcers in mice.

In our study, there were 18 ulcers in C1 and 17 ulcers in C2. The mean number of ulcers in C1 and C2 was 3.00 ± 1.10 and 2.83 ± 0.75 respectively. Taiwo observed similar findings in his study. Indomethacin has proved to uncouple oxidative phosphorylation and decreases the production of mucopolysaccharides which are secreted in the stomach and protect the substances from the acidic environment of the stomach. A decreased amount of these substances will not prevent mucosae from acids, thus causing necrosis, lesions, and ulcerations.¹¹ Only 6 ulcers in D1 and 4 in D2 were found saving 4 mice in each D1 and D2 subgroups. The mean number of ulcers in D1 and D2 was 1.00 ± 1.55 and 0.67 ± 1.03 respectively (Table 1, Graph 1). The data presented in our experiment showed that vitamin E protected the stomach in a manner similar to that previously reported by Jan C becker. In animal studies, vitamin E has a key role in the preservation of

gastric mucosal integrity by inhibition of lipid peroxidation and accumulation of activated neutrophils.¹² It was suggested that administered vitamin E defends gastric mucosal lesions in rats through its anti-oxidant and anti-inflammatory effects.⁹

In our study, ulcers were either irregular, pin-dot, linear, and punched out in C and D groups (Table 2, Figure 1). Only punched out and flat-shaped ulcers with irregular margins were found in the study carried out in 2009.¹³ Shape of ulcers were observed under dissecting microscope.

In our study, the mean linear dimension of ulcer in C1 was $262.50\mu\text{m}$ and in C2 was $232.50\mu\text{m}$ (Table 3, Figure 2, Graph 2). In the previous study by Maqbool et al in 2006 the mean diameter of ulcers in rats was $362.5\mu\text{m}$.⁶ We attribute this difference due to the difference in both dose and route of administration of the drug. The mechanism of ulcer formation was the destruction of mitochondria at the site of high levels which caused damage to the gastric mucosa and results in ulceration.¹¹ The mean of ulcer in group D1 was $56.50\mu\text{m}$ and in D2 was $50.00\mu\text{m}$ (Table 3, Figure 3, Graph 2). It is concluded that oxygen reactive species and lipid peroxidation is has a key role in the formation of gastric injuries produced by stress and if given, antioxidant vitamin E can prevent the occurrence of gastric injuries by decreasing the product of lipid peroxidation and hence reduces damage to gastric mucosal integrity.¹⁴ Cetin also concluded in his study that area of mucosal injury was significantly less in the groups given both early and late vitamin E prophylaxis on stress-induced gastric lesions. Mean ulcer areas were significantly less in rats treated with vitamin E ($p < 0.05$).¹⁵

Conclusion

The current study proves that vitamin E does protect the gastric mucosa from damage produced by indomethacin. The ulcerogenic effects of Indomethacin are a well-known fact. When the use of this drug is inevitable, precautions should be taken to prevent gastric ulcers. Our findings suggest that Vitamin E supplementation strengthens the gastric mucosa and reduces the severity and prevents gastric mucosal lesions. Vitamin E can be used as a natural anti-ulcerogenic agent as a prophylactic measure before indomethacin use.

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