Original Article

Vitamin-E: Anti-Ulcer Activity; Beyond the Antioxidant Functions

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ABSTRACT

Objective: To evaluate the anti-ulcer activity of vitamin-E on caecal ulcerogenicity of Diclofenac sodium in Albino rats.

Study Design: A prospective experimental study

Place and Duration of Study: Department of Anatomy, Basic Medical Sciences Institute, Jinnah Post Graduate Medical Centre Karachi during 2003.

Materials and Methods: Diclofenac sodium and Vitamin-E were administered to Albino Rats separately and simultaneously at a dose of 2 mg/kg body weight (for each drug) orally once daily for two weeks. These animals were sacrificed, Caeca were identified and removed, opened along mesenteric border and examined under dissecting microscope for dilatation of the blood vessels, blood streaks and hemorrhagic areas. The caeca were fixed in 10% formalin, Embedded in paraplast, 4 um thick sections were cut on rotary microtome, stained with Haematoxylin and eosin stains. The histomorphological Features of caecal mucosa were compared with those in the control animals and analyzed statistically.

Results: The study revealed that simultaneous use of vitamin-E administration with (NSAIDs) produced Anti-ulcer activity in caecal ulcers of albino Rats.

Conclusion: The results suggest that vitamin-E produced anti-ulcer activity in Caecal ulcers of albino rats.

Key Words: Vitamin-E, Diclofenac sodium, Antiulcer activity, Caecal ulcer, Albino rat.

INTRODUCTION

Although vitamin-E was discovered nearly 85 years ago[1], the search for its biological function continues. Whereas the antioxidant function of Vitamin-E in vivo is no doubt crucial, there is a growing body of evidence that RRR-a-tocopherol may exert non-antioxidant effects on various aspects of cell metabolism. Vitamin-E, a potent peroxyl radical scavenger, is a chain breaking antioxidant that prevents the propagation of free radical damage in biological membranes[2,3]. Vitamin-E is the collective name for eight naturally occurring molecules, four tocopherols and four tocotrienols. Tocotrienols differ from tocopherols in that they have an unsaturated phytyl side chain; the four forms of tocopherols and tocotrenols differ in the number of methyl groups on the chromanol nucleus(α-has 3,β- and γ-have 2,whereas δ-has1).The biological activity of the various vitamin-E forms roughly correlate with their anti-oxidant activities; the order of relative peroxyl radical scavenging reactivities of α-, β-, γ-, and δ-tocopherols (100, 60, 25, and 27 respectively[4] is the same as the relative order of their biological activities (1.5, 0.75, 0.15, and 0.05 mg/iu respectively, determined by using the classic fetal resorption assay in rats.[5]

Upon closer scrutiny, the relation between antioxidant and biological activities breaks down. α-tocotrenols has only one third the biological activity of α-tocopherol[5,6] yet it has higher[7]or equivalent[8] antioxidant activity. A vitamin-E analog [2,4,6,7-tetramethyl-2-(4', 8', 12'-trimethyltridecyl)-5-hydroxy-3,4-dihydrobenzofuran] with equivalent biological activity to RRR-a-tocopherol[9] has 1.5 times the antioxidant activity[10]. Furthermore, synthetic vitamin-E (all-rac-a-tocopherol) contains equal amounts of eight different sterioisomers of α-tocopherol that have equivalent antioxidant activity, but each of which has a different biological activity[11].The biological activities of the 2-S forms are generally lower than the 2-R forms[11].Overall, the highest biological activity is found in molecules with three methyl groups and a free hydroxyl on the chromanol ring and the phytyl tail meeting the ring in the R-orientation. This specific requirement suggests specific interactions of vitamin-E with proteins and perhaps with other molecules, such as DNA.

This study was designed to evaluate the anti-ulcer activity of Vitamin-E on caecal ulcers, induced by diclofenac sodium (NSAIDs) in albino rats.

MATERIALS AND METHODS

Seventy-five albino rats were used in this study, which were obtained from Animal House of Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi. All were male, 20 weeks of age, weighing 180—200 grams, looking active and healthy. These animals were housed in the experimental room of Animal House maintained on balanced laboratory diet and water ad libitum with 12 hours light and dark cycle. Seventy-five animals were divided into three equal Groups; A, B and C, each comprising of 25 animals. Group 'A' animals were given Diclofenac sodium

(developed in Novartis Pharma Pakistan Ltd) at a therapeutic dose of 2 mg/kg/ body weight orally once daily for 2 weeks[12]. Group 'B' animals were given simultaneously Vitamin-E at a dose of 2mg/kg/body weight orally once daily, 30 minutes before administration of Diclofenac sodium (2 mg/kg/body weight) orally once daily for 2weeks[13]. Group 'C' animals acted as control and were given normal saline (equal volume of dose given to groups 'A' and 'B') orally Once daily for 2 weeks.

All the rats were sacrificed on day 15th of the experiment by giving deep ether Anesthesia and were operated to obtain their caeca, which were fixed in 10% Formalin, embedded in paraplast and 4 um thick sections were cut on rotary microtome. These sections were stained with Haematoxylin and eosin reagents. The histomor-phological features of caeca in all the groups were observed with respect to total epithelial cell count per unit area (0.0324 mm^2/field) and mucosal thickness was measured by micrometry and the data was subjected to statistical analysis. Student "t" test was employed to see the significance of results.[14]

RESULTS

The animals in Group 'A' looked slow and weak during last 2-3 days of experimental period. They appeared lethargic, their response to stimuli was sluggish and food intake was decreased as compared to animals of Group 'B' and 'C'.

Table No.1: Comparison of Mucosal thickness (µm) and Total epithelial cell count per unit area between Groups – A, B and C during experimental period.

Groups	Mucosal thickness (µm)	Total epithelial cell count	
		NFO	
A(n=25)	52.562 ± 1.436*	25	441.655±1.963*
B(n=25)	149.062±0.439**	25	576.256±1.130**
C(n=25)	151.375±1.126	25	578.851±1.753

Statistical Comparison

Groups	P Value	Significant / Non-significant
A vs B	< 0.001*	Highly Significant ↓
A vs C	< 0.001	Highly Significant ↓
B vs C	> 0.05**	Non-significant change

Note: Values are given as mean ± standard error of mean.

* P ‹ 0.001 (Highly significant)

** P › 0.05 (Non-significant)

Key: NFO = No; of field observed (0.0324 mm^2)

µm = Micrometer

Under light microscope the animals of Group 'A' showed epithelial mucous secreting cells in mucosa disrupted and exfoliated at places with moderate degree of pyknotic nuclei. Inflammatory exudates including numerous lymphocytes, plasma cells, neutrophils and degenerated cells were observed in abundance within and around the erosions/ulcers, as shown in Figure -1.

The Group 'B' animals showed almost intact histological structure without any change in caecal mucosa with decreased lymphocyte infiltration and degenerating cells, as shown in Figure-2. While Group 'C' control animals showed intact histological structure without any change in caecal mucosa, as shown in figure – 3.

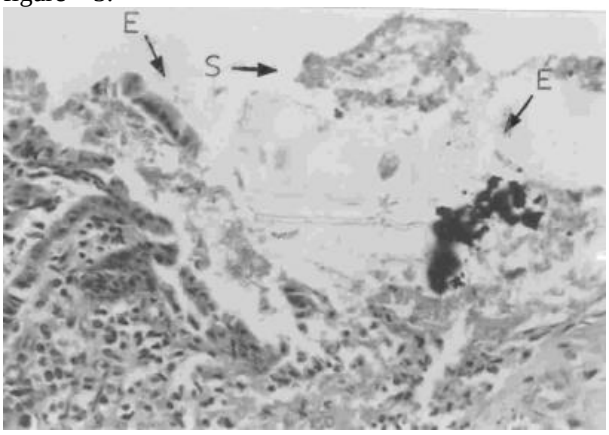

Figure No.1: Photomicrograph of 4 um thick paraplast section of caecal mucosa stained with H & E in Diclofenac sodium treated (Group 'A') albino rat, showing an erosion, marked against (E→) with inflammatory exudates and sloughing of surface epithelial cells (S→) under high power. Objective, x416

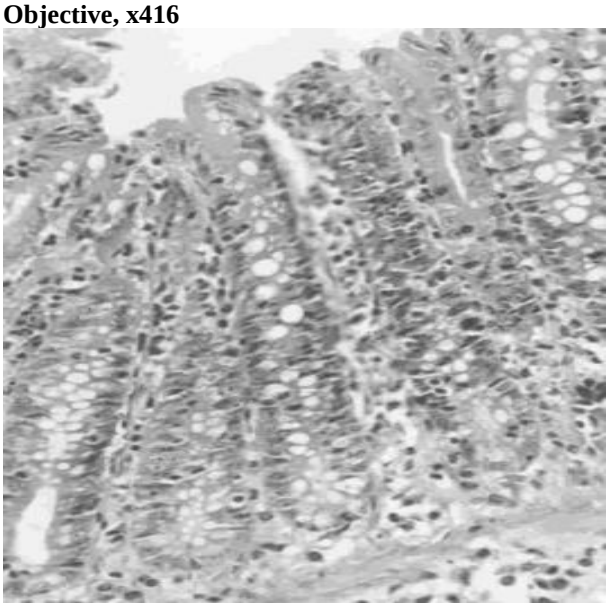

Figure No.2: Photomicrograph of 4 um thick paraplast section of caecal mucosa Stained with H & E in Diclofenac sodium and Vitamin – E (group-B) treated albino rat, showing surface epithelial cells comparable with normal control under high power Objective, x416.

The mean values of the mucosal thickness in group 'A', 'B' and 'C' were recorded as 52.562±1.436, 149.062±

0.439 and 151.375±1.126 µm respectively, as shown in the Table-1. A remarkable highly significant (P <0.001) decrease in mucosal thickness in Group 'A' was observed when the difference of mean was compared with Group 'B' and 'C' while no significant (P>0.05) change was observed when the difference of mean in Group 'B' was compared with animals in control Group 'C'.

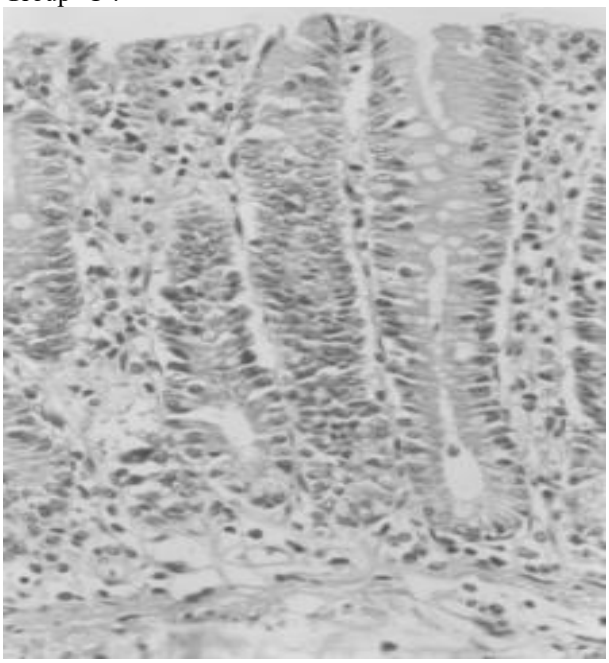

Figure No.3: Photomicrograph of 4 um thick paraplast section of caecal mucosa Stained with H & E in normal control (group-C) treated albino rat, showing entire mucosal thickness under high power Objective, x416.

The Mean values of Total epithelial cell count per Unit area in Groups 'A', 'B' and 'C' were recorded as 441.655±1.963, 576.256±1.130 and 578.851±1.753 respectively, as shown in the Table-1. A highly significant P <0.001) decrease in total epithelial cell count per unit area in Group 'A' was observed when the difference of mean was compared with Group 'B' and 'C'. While no significant (P>0.05) change was observed when the difference of mean in Group 'B' was compared with animals in Group 'C'.

DISCUSSION

The present study was designed to observe the anti-ulcer activity of vitamin-E when used simultaneously with the Diclofenac sodium (NSAID) in caecal mucosa of albino rats. The diclofenac sodium administered in a normal therapeutic dose of 2 mg/kg body weight, once daily orally for 2 weeks.[12] After treatment with diclofenac sodium (NSAID) in animals of Group 'A', general behavior changed to ill, sluggish and decreased food intake which may be attributed to unwanted effects of diclofenac sodium toxicity. In this context our results are in agreement with Gabriel et al[15], Bjarnason et al[16], and graham et al[17] who stated that administration of Diclofenac sodium was associated with increased gastrointestinal toxicity include mild dyspepsia or cachexia as well as more serious gastrointestinal reactions such as ulceration, bleeding, perforation and other events leading to hospitalization or death.

On microscopic examination of caecum revealed decreased mucosal thickness with decreased total epithelial cell count per unit area. These changes are in conformity with the Studies by Van-kolfshoten[18], Kaufman[19], Graham et al[17] and Manocha[12]. A highly significant (P <0.001) decrease in mucosal thickness was observed which may be attributed to the injurious effect caused by Diclofenac sodium (NSAID) which might have resulted into onset of the demolition with extensive exfoliation of surface epithelial cells and ulceration. At places, mucosal lining of caecum showed necrosis which according to Kumar et al[20] resulted most commonly from sudden severe ischemia due to irreversible injury to cells.

A non-significant (P>0.05) change in mucosal thickness in Group 'B' was observed, which may be attributed to anti-ulcer activity of vitamin-E, as vitamin-E reduces the damaging effect of NSAIDs on the gastro-duodenal mucosa of rat with ulcers thus normalizing the phospho-lipid contents by decreasing lipid per-oxidation (LPO) markedly. It is suggested that LPO may be involved in the pathogenesis of ulcer and that factors attenuating the process of LPO may prevent ulcerogenesis.[21,22]

Our results are in complete agreement with Tariq[23] who found that pretreatment of animals with vitamin-E produces a significant inhibition of gastric lesions induced by NSAIDs. An increase in synthesis of prostaglandins and a high level of glutathione in tissues of vitamin-E treated animals have been suggested as a possible mechanism of anti-ulcer activity of vitamin-E.

A highly significant (P <0.001) decrease in total epithelial cell count per unit area in Group 'A' was observed, which may be attributed to decrease in secretory activity and flattening of cells due to ulcerogenic effect on cell morphology, the mucin content in goblet cells become depleted. Our results are in complete agreement with Lee[24] who found that the crypts showed substantial goblet cell depletion with diclofenac sodium, the inflammatory changes in which both plasma cells and lymphocytes participated were accompanied by more severe reaction and even crypt dissolution.

A non-significant (P>0.05) change in total epithelial cell count per unit area in group-B was observed, which may be attributed to anti-ulcer activity of vitamin-E, which when administered simultaneously produced these changes.

On microscopic examination of the caecal wall of Group 'C' animals, mucosal thickness and the total epithelial cell count per unit area were normal.

CONCLUSION

These results strongly suggest that Diclofenac sodium causes severe caecal ulcers, which could be prevented (as an anti-ulcer activity) by simultaneous administration of vitamin-E in albino rat.

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