

ROTECTIVE ROLE OF ANTI-OXIDANT AGAINST CAECAL ULCEROGENICITY BY DICLOFENAC SODIUM IN ALBINO RATS

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ABSTRACT

Objective: To evaluate the protective role of vitamin-E (α -tocopherol) on caecal ulcerogenicity of diclofenac sodium administration in albino rats.

Design: An animal study carried on experimental albino rats.

Setting: The study was carried out in the department of Anatomy, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi, from 2001 to 2002.

Method: Diclofenac sodium and vitamin-E were administered to male 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, stretched and examined under dissecting microscope.

The severity of erosions and ulcers was rated according to an arbitrary scale of Bonta. The caecum was then fixed in 10% formalin, embedded in paraplast. 4 μ m thick sections were cut on rotary microtome and stained with haematoxylin and eosin (H&E). The histomorphological features of caecal mucosa were compared with those in the control animals and analyzed statistically.

Results: The study revealed that diclofenac sodium administration in albino rats produced ulcerative changes in caecal mucosa and simultaneously vitamin-E protected caecal ulcerogenicity.

Conclusion: These results suggest that diclofenac sodium causes severe caecal mucosal damage in albino rats, however, could be protected by simultaneous administration of vitamin-E in albino rats.

Keywords: Diclofenac sodium, Caecal ulcer, Vitamin-E

INTRODUCTION

The anti-inflammatory, analgesic and antipyretic drugs are a heterogeneous group of compounds often chemically unrelated (although most of them are organic acids) which nevertheless share certain therapeutic actions and side-effects. The prototype is aspirin, hence these compounds are often referred to as aspirin-like drugs. They also are frequently called non-steroidal anti-inflammatory drugs (NSAIDs). Diclofenac sodium is the first drug of a series of phenyl acetic acid derivatives. It is an anti-

inflammatory agent approved for several uses in the United States.

There has been substantial progress in elucidating the mechanism of action of NSAIDs. Inhibition of cyclooxygenase (COX), the enzyme responsible for the biosynthesis of the prostaglandins and certain related autoids is generally thought to be a major fact of the mechanism of NSAIDs (Gillman *et al.*, 2001).

Vitamin-E is the collective name for eight naturally occurring molecules, four

tocopherols (α , β , γ and δ) and four tocotrienols (α , β , γ and δ) (Traber and Packer, 1995). It is a low molecular weight lipid soluble free radical scavenger that preferentially partitions into membrane functions by reducing lipophilic free radical species to a less toxic form. Tocopherol can react with free radicals and possibly with other oxidizing intermediates, prevents the chain of events (Freeman and Crapo, 1982; Bieri *et al.*, 1983).

This study was designed to evaluate the effects of diclofenac sodium and vitamin-E administration separately and simultaneously on morphology of caecal mucosa of laboratory animals.

MATERIALS AND METHODS

Forty-eight albino rats were used in this study, which were obtained from Animal House of Basic Medical Sciences Institute, JPMC, Karachi. All were males, 20 weeks of age, weighing 180-200 gms, looking active and healthy. These animals were housed in experimental room in the Animal House and

maintained on balanced laboratory diet and water ad libitum with 12 hours light and dark cycle.

Forty-eight animals were divided into three equal groups; A, B and C, each comprising of 16 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 (Manocha and Venkataraman, 2000).

Group B animals were given simultaneously vitamin-E at a dose of 2 mg/kg/body weight orally once daily, 30 minutes before administration of diclofenac sodium (2 mg/kg/body weight) orally once daily for 2 weeks (Martindale, 1994).

Group C animals used 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-15 of the experiment by giving deep ether

Table
Comparison of Body weight (gm), Mucosal thickness (μ m), and Total epithelial cell count per unit area between Groups A, B and C during Experimental Period

Groups	Body Weight (gm)				Mucosal Thickness (μ m)	Total Epithelial Cell Count	
	D1	D7	D14	G/L		NFO(0.0324mm ²)	
A (n=16)	191.5 $\pm$ 0.55	200.4 $\pm$ 0.63	210.9 $\pm$ 0.60	19.4 $\pm$ 0.60	100.9 $\pm$ 2.21	8	301.1 $\pm$ 2.03
B (n=16)	184.9 $\pm$ 0.92	199.8 $\pm$ 0.68	211.9 $\pm$ 0.86	27.0 $\pm$ 0.29	338.8 $\pm$ 0.53	8	520.8 $\pm$ 0.58
C (n=16)	188.0 $\pm$ 1.60	195.5 $\pm$ 0.80	218.4 $\pm$ 3.87	30.4 $\pm$ 3.20	340.8 $\pm$ 1.19	8	523.1 $\pm$ 2.20

Note: Values are given as mean $\pm$ standard error of mean.

Statistical Analysis of Body weight, Mucosal thickness and Total epithelial cell count per unit area

Groups	P value	Significant / Non-significant / High/Significant
A vs B	< 0.001	Highly Significant $\downarrow$
A vs C	< 0.001	Highly Significant $\downarrow$
B vs C	> 0.05	Non-significant --

Key: D = Day; G/L = Gain/Loss; NFO = No. of Field Observed; μ m Micrometer; gm = Gram

anaesthesia and were operated to obtain their caeca, which were fixed in 10% formalin, embedded in paraplast and 4 μm thick sections were cut on rotary microtome, These sections were stained with haematoxylin and eosin (H&E). The histomorphological features of caeca in all the groups were observed with respect to total epithelial cell count per unit area ($0.0324 \text{ mm}^2/\text{field}$) 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 (Bland, 1987).

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 groups B and C.

Mean Gain and Loss in Body Weight

The mean gain and loss in body weight in groups A, B and C was recorded as 19.4 ± 0.26 gm, 27.0 ± 0.29 gms, and 30.4 ± 3.20 gms respectively, as shown in Table.

A statistically significant less gain in body weight in group A was observed when compared with groups B and C ($P < 0.001$) while no significant difference was observed between groups B and C ($P > 0.05$).

Mean Mucosal Thickness

The mean value of the mucosal thickness in groups A, B and C was recorded as 100.9 ± 2.21 μm , 338.8 ± 0.53 μm , and 340.8 ± 1.19 μm respectively, as shown in Table. A significant less in mucosal thickness in group A was observed when compared with groups B and C ($P < 0.001$) while no significant change was observed between group B and control group C ($P > 0.05$).

Mean Total Epithelial Cell Count per unit area

The mean value of total epithelial cell count per unit area in groups A, B and C was recorded as 301.1 ± 2.03 , 520.8 ± 0.58 , and 523.1 ± 2.20 respectively, as shown in Table. A

significant less number of total epithelial cell count per unit area in group A was observed when compared with groups B and C ($P < 0.001$) while no significant change was observed between group B and control group C ($P > 0.05$).

Gross and Microscopic Examination

In group A animals on naked eye examination, 3-6 dark brownish to black patches were observed mainly on the mesenteric border in the basal portion of each caecum, under Spencer stereoscope. These appeared as circumscribed elongated erosions and ulcers. Numerous spider-web like tortuous dilated blood vessels in eight animals along with bleeding spots in four animals and bleeding streaks only in four animals were observed on the internal surface surrounding the erosions and ulcers. Under light microscope the mucosa showed exfoliation of the epithelium at places, moderate degree of pyknosis of nuclei and markedly dilated blood vessels were observed close to the muscularis mucosae in all the animals. The surface epithelium as well as epithelium of the glands was necrosed. Epithelial cells showed pyknotic nuclei within the erosive area, lymphocytes, plasma cells, neutrophil cells and degenerated cells were observed in abundance within and around the ulcers, as shown in Figure-1.

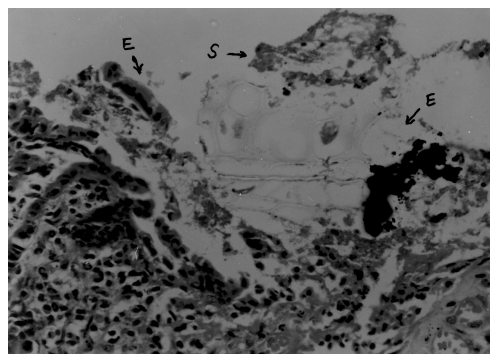


Figure-1: Photomicrograph of μm thick paraplast section of caecal mucosa stained with H&E in diclofenac sodium treated (group-A) albino rat, showing an erosion, marked against (E→) with Inflammatory exudates and sloughing of surface epithelial cells (S→) under high power objective. x416

In group B animals showed almost intact histological structure without any change in caecal mucosa and showed decreased lymphocytic infiltration and degenerating cells, as shown in Figure-2. Similarly group C animals showed intact histological structure without any change in caecal mucosa, as shown in Figure-3.

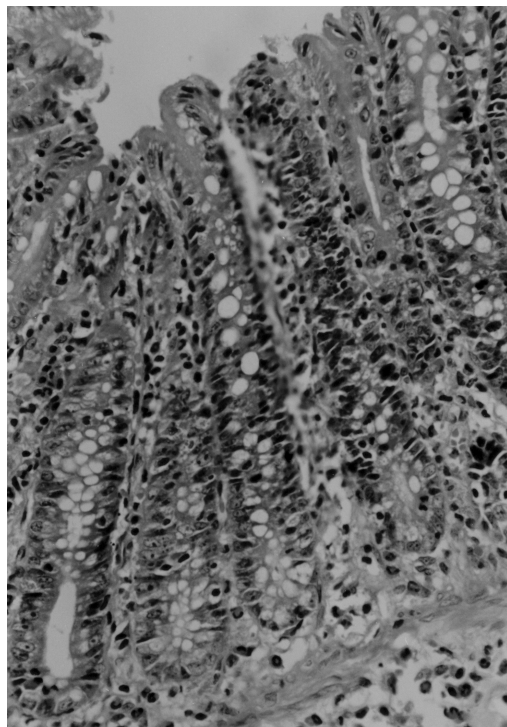


Figure-2: Photomicrograph of μm thick paraplast section of caecal mucosa stained with H&E in diclofenac sodium and vitamin-E treated (group-B) albino rat, showing surface epithelial cells comparable with normal control under high power objective. x416

DISCUSSION

The ulcers were measured in millimeter (mm) under Spencer stereoscope according to arbitrary scale of Bonta (1961). The size of ulcers was large in the present study as compared to previous experimental studies carried on albino rats in stomach and duodenum as different ulcerogenic agents have been used by Okabe *et al.* (1982), Van-

kolschoten *et al.* (1983), and Furukawa *et al.* (1988). Our findings are in agreement with the results of Bjarnason *et al.* (1993) who observed the ulceration of caecum, transverse colon, and sigmoid colon in patients or rats (animals) on NSAID therapy.

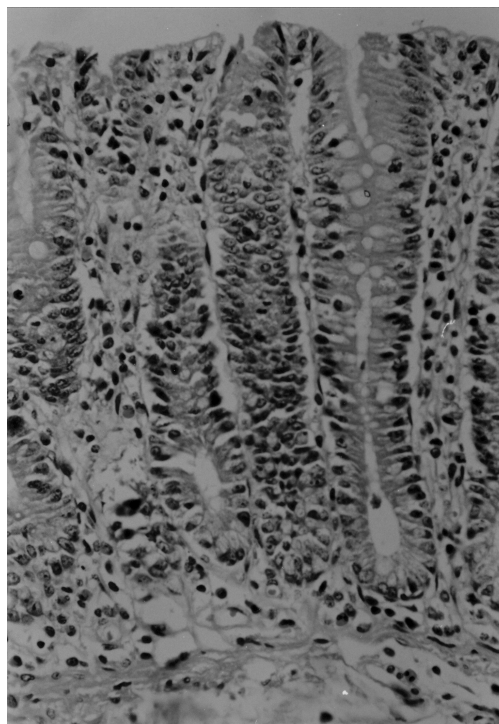


Figure-3: Photomicrograph of μm thick paraplast section of caecal mucosa stained with H&E in normal control (group-C) albino rat, showing entire mucosal thickness under high power objective. x416.

The present study could not be compared with earlier studies carried out by Van-kolschoten *et al.* (1983), Kaufman & Taubin (1987), Graham *et al.* (1993), and Manocha and Venkataraman (2000) these investigators found a common mucosal lesions, i.e. erosions and ulcers found in stomach, small and large intestines except caecum. However, none of these workers have found the caecal ulcer, total epithelial cell count per unit area, and mucosal thickness as well.

A significant less in the body weight in group A was observed which may be

attributed to less food intake and diarrhoea. These observations are in accordance with Tanner and Raghunath (1988) and Reuter et al (1994) who found that treatment with NSAID was presented with diarrhoea and weight loss over a period of time.

The group B animals showed increase in body weight which may be attributed to the protective role of vitamin-E against the unwanted effects of diclofenac sodium on growth. These findings are in agreement with Martin (1936) who found that the administration of vitamin-E was marked by an increase in the growth rate.

A significant less mucosal thickness in group A 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 intestine showed necrosis which according to Kumar et al (1989) resulted most commonly from sudden severe ischaemia due to irreversible injury to cells.

A non-significant change in mucosal thickness in group B was observed, which may be attributed to protective role of vitamin-E, as vitamin-E reduces the damaging effect of NSAIDs on the gastroduodenal mucosa of rat with ulcers thus normalizing the phospholipid contents by decreasing lipid peroxidation (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 (Morenkova *et al.*, 1987 and Stainslavchuk *et al.*, 1995).

Our results are in complete agreement with Tariq (1988) 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 antiulcer activity of α -tocopherol.

A highly significant decrease in total epithelial cell count per unit area in group A was observed. This may be attributed to decrease in secretory activity and flattening of cells due to ulcerogenic effect on cell morphology as the mucin contents in goblet cells become depleted. Our results are in complete agreement with Lee (1993) 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 change in total epithelial cell count per unit area in group B was observed, which may be attributed to protective role of vitamin-E, which when administered produced improvement in growth. These observations are in accordance with Emerson and Evans (1937) and Olcott and Mattill (1937) who found that the addition of vitamin-E permits an improvement in growth rate.

CONCLUSION

This study suggests that severe caecal mucosal damage caused by diclofenac sodium could be prevented by simultaneous administration of vitamin-E in albino rats.

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