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ORIGINAL PAPER

Erdosteine Prevents Colonic Inflammation Through Its Antioxidant and Free Radical Scavenging Activities

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Abstract After intracolonic administration of trinitrobenzene sulphonic acid (TNBS), Sprague-Dawley rats were treated orally either with saline or erdosteine (100 mg/kg per day), a sulfhydryl-containing antioxidant, for 3 days. On the 4th day, rats were decapitated and distal colon was removed for the macroscopic and microscopic damage scoring, for the measurement of malondialdehyde (MDA), glutathione (GSH) and collagen levels, myeloperoxidase (MPO) activity, luminol and lucigenin chemiluminescences (CL) and DNA fragmentation. Lactate dehydrogenase (LDH) activity, tumor necrosis factor-α, interleukin (IL)-1β, IL-6, and antioxidant capacity were assayed in blood samples. Colitis caused significant increases in the colonic CL values, macroscopic and microscopic damage scores, MDA and collagen levels, MPO activity and DNA fragmentation, along with a significant decrease in tissue GSH level. Similarly, serum cytokines and LDH were elevated in the saline-treated colitis group as compared with the control group. On the other hand, erdosteine treatment reversed all these biochemical indices, and histopathologic alterations induced by TNBS, suggesting that erdosteine protects the colonic tissue via its radical scavenging and antioxidant activities.

Keywords Erdosteine · Colitis · Oxidative damage · Inflammation · TNBS

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Inflammatory bowel diseases (IBD), encompassing ulcerative colitis (UC) and Crohn's disease, are idiopathic chronic inflammation in gut with diffuse inflammation of the colon and rectum, characterized by cycles of acute inflammation, ulceration, and bleeding of the mucosa [1]. Trinitrobenzene sulphonic acid (TNBS)-induced colitis model, applied by intracolonic administration of diluted TNBS solutions [2–4], produces a diffuse colonic inflammation, characterized by increased leukocyte infiltration, edema, and ulceration. There is substantial evidence that excessive production of reactive oxygen species (ROS) by the inflamed mucosa contribute significantly to the development of tissue injury in UC [5], where activated neutrophils and macrophages are the major components of active lesions [6, 7]. Large numbers

of neutrophils and macrophages pass out of the circulation and enter the inflamed mucosa and submucosa of the large intestine during acute inflammation, leading to overproduction of oxygen free radicals [8, 9], which appear to be associated with the increased lipid peroxidation observed in the mucosa of UC in experimental animals and humans [10, 11].

Erdosteine is a thiol derivative that has recently been introduced into clinical practice as a well-tolerated mucoactive drug, used for its mucolytic and antioxidant effects [12, 13]. Its molecule contains 2 sulphur atoms; 1 is present in an aliphatic side chain and the other is enclosed in the heterocyclic ring [14]. These chemically blocked sulfhydryl groups are liberated following hepatic metabolism and thereby the molecule subsequently exerts its free radical scavenging and antioxidant properties [15]. Based on its free radical scavenging activity, its protective effects against oxidant-induced tissue damage have been demonstrated in various inflammation models [16–19]. However, most of the studies have focused on the beneficial effects of erdosteine on chemotherapeutic drug-induced toxicity of the renal, pulmonary, and cardiac tissues, but the protective effect of erdosteine on the gastrointestinal tissue was not evaluated yet.

On the basis of this background, using biochemical and histologic examination, we sought to study the putative protective effects of erdosteine on the colonic tissue in a rat model of colitis.

Materials and methods

Animals

Adult female Sprague-Dawley rats (250–300 g) were kept in a light- and temperature-controlled room with 12:12-hour light–dark cycles, where the temperature (22 $\pm$ 0.5°C) and relative humidity (65–70%) were kept constant. The animals were fed a standard pellet and food was withdrawn overnight before colitis induction. Access to water was allowed ad libitum. Experiments were approved by the Marmara University School of Medicine Animal Care and Use Committee.

Induction of colitis and drug administration

Animals were fasted for 18 hours before the induction of colitis. Under light ether anesthesia, a polyethylene catheter (PE-60) was inserted into the colon with its tip positioned 8 cm from the anus. To induce colitis ($n = 8$), a single solution of 1 mL of a 30 mg/mL TNBS solution, dissolved in 40% ethanol in saline was instilled. The rats in the control group ($n = 8$) were subjected to the same procedure with the exception that an equal volume of isotonic saline was substituted for TNBS. Erdosteine (100 mg/kg; Sandoz Ilac, Istanbul, Turkey) or saline (1 mL per rat) were given orally 5 minutes after induction of colitis and the treatment was continued for the following 3 days. On the 4th day of colitis induction, rats were decapitated and trunk blood was collected for the assessment of lactate dehydrogenase (LDH) activity, as a marker of tissue injury, and the levels of the pro-inflammatory cytokines, tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6. Distal 8 cm of the colon obtained from each animal were initially examined for recording macroscopic damage scores and tissue wet weight index (WWI), and then stored at -80°C until the determination of collagen content (a fibrosis marker), myeloperoxidase (MPO) activity (an indirect evidence of neutrophil infiltration), malondialdehyde (MDA; an index of lipid peroxidation), and glutathione (GSH; a key antioxidant) levels in the colonic tissue. Formation of reactive oxygen species in the colonic tissue samples was monitored by the chemiluminescence (CL) technique using luminol and lucigenin probes. For the histologic analysis, extra 1-cm^2 samples were obtained from each animal at 8 cm from the anus to be fixed in formaldehyde.

Assessment of colitis severity

The distal 8 cm of the colons were opened longitudinally down their mesenteric borders, cleansed of luminal contents, gently rinsed in saline, and dried on filter paper. The severity of colitis was assessed using macroscopic and microscopic damage scoring, WWI, and tissue collagen content.

For macroscopic scoring of colonic damage, the 8-cm colonic segments were scored according to the following criteria: 0, no damage; 1, localized hyperemia, no ulcers; 2, ulceration without hyperemia or bowel wall thickening; 3, ulceration with inflammation at 1 site; 4, $\geq$2 sites of ulceration/inflammation; 5, major sites of damage extending $>$1 cm along the length of colon; and 6–10, damage extending $>$2 cm along the length of colon, where the score is increased by 1 for each additional 1 cm [20]. The scoring of colonic damage was performed by an observer who was unaware of the treatments received by the rats. After scoring, tissue weights were recorded, corrected for body weight, and expressed as tissue WWI (g/100 g body weight).

For light microscopic analysis, samples from distal colon were fixed in 10% buffered formalin for 48 hours, dehydrated in ascending alcohol series, and embedded in paraffin wax. Approximately 7-μm-thick sections were stained with hematoxylin and eosin for general morphology and with 1% acidified toluidine blue (pH 2.5) for the mucus accumulation in goblet cells. Stained sections were observed under an Olympus BX50 photomicroscope (Tokyo, Japan). All tissue sections were examined by an experienced histologist who was unaware of the treatments. Assessment of the colonic

injury was performed by using the previously described criteria: damage/necrosis *(0: none, 1: localized, 2: moderate, 3: severe)*, submucosal edema *(0: none, 1: mild, 2: moderate, 3: severe)*, inflammatory cell infiltration *(0: none, 1: mild, 2: moderate, 3: severe)*, vasculitis *(0: none, 1: mild, 2: moderate, 3: severe)*, and perforation *(0: absent, 1: present)*, with a maximum score of 13 [21].

For scanning electron microscopic investigation, the samples were fixed in 4% phosphate buffered glutaraldehyde (0.13 mol and pH 7.4) for 4 hours and postfixed with 1% OsO_4 for 1 hour, dehydrated in graded alcohol series, put into amyl acetate, dried with liquid CO_2 under pressure with critical point dryer (Bio-Rad E 3000; Hercules, CA) and covered with gold particles (Bio-Rad SC502). These samples were observed under a Jeol 1200 JSM (Tokyo, Japan) scanning electron microscope.

For collagen measurement, tissue samples were cut with a razor blade, immediately fixed in 10% formaldehyde, and then embedded in 0.1 mol paraffin to obtain approximately 15-μm-thick sections. The method of collagen measurement is based on the selective binding of Sirius red and Fast Green to collagen and noncollagenous components, respectively, when the sections are stained with both dyes dissolved in aqueous saturated picric acid [22]. Both dyes were eluted readily and simultaneously with NaOH-methanol, and the absorbances obtained at 540 and 605 nm were used to determine the amount of the collagen and protein, respectively.

Biochemical analyses

Measurement of serum LDH activity and cytokine levels

Serum LDH was determined spectrophotometrically using an automated analyzer [23], and levels of TNF-α, IL-1β, and IL-6 were quantified using enzyme-linked immunosorbent assay kits specific for the previously mentioned rat cytokines according to the manufacturer's instructions and guidelines (Biosource International, Camarillo, CA). These particular assay kits were selected because of their high degree of sensitivity, specificity, inter- and intraassay precision, and small amount of plasma sample required to conduct the assay. The total antioxidant capacity (AOC) in plasma were measured by using colorimetric test system (ImAnOx, catalog no. KC5200; Immunodiagnostic AG, Bensheim, Germany), according to the instructions provided by the manufacturer.

Tissue MPO activity

The activity of tissue-associated MPO, a natural constituent of primary granules of neutrophils, was determined in the colonic samples. Because a direct relationship between the tissue MPO activity and the number of neutrophils was previously shown [24], MPO activity was regarded as an indication of neutrophil accumulation. All reagents for MPO assay were obtained from Sigma (St. Louis, MO). The tissue samples (0.2–0.3 g) were homogenized in 10 volumes of ice-cold potassium phosphate buffer (50 mmol K_2HPO_4, pH 6.0) containing hexadecyltrimethylammonium bromide (0.5%, w/v). The homogenate was centrifuged at 41.000 g for 10 minutes at 4°C, and the supernatant was discarded. The pellet was then rehomogenized with an equivalent volume of 50 mmol K_2HPO_4 containing 0.5% (w/v) hexadecyltrimethylammonium bromide and 10 mmol ethylenediaminetetraacetic acid (EDTA; Sigma). MPO activity was assessed by measuring the H_2O_2-dependent oxidation of o-dianizidine.2 HCl. One unit (U) of enzyme activity was defined as the amount of the MPO present per gram of tissue weight that caused a change in absorbance of 1.0/minutes at 460 nm and 37°C.

CL assay

To assess the role of ROS in TNBS-induced colonic damage, luminol and lucigenin chemiluminescences were measured as indicators of radical formation. Lucigenin (bis-*N*-methylacridiniumnitrate) and luminol (5- amino-2,3-dihydro-1,4-phthalazinedione) were obtained from Sigma. Measurements were made at room temperature using Junior LB 9509 luminometer (EG&G, Berthold, Germany). Specimens were put into vials containing phosphate-buffered saline (PBS)-HEPES buffer (0.5 mol PBS containing 20 mmol HEPES, pH 7.2). ROS were quantitated after the addition of the enhancers, lucigenin or luminal, for a final concentration of 0.2 mmol. Luminol detects a group of reactive species, namely, H_2O_2, hypochlorous acid (HOCl) and $^{\bullet}OH$ radical whereas lucigenin is selective for $O^{-\bullet}{}_2$ radical [25, 26]. Counts were obtained at 1-minute intervals and the results were given as the area under curve for a counting period of 5 minutes. Counts was corrected for wet tissue weight [relative light unit (rlu)/mg tissue)] [27].

Tissue MDA and GSH assays

Colonic samples were homogenized in ice-cold 150 mM KCl for the determination of MDA and GSH levels. The MDA levels were assayed for products of lipid peroxidation [28]. Results are expressed as nmol MDA g^{-1} tissue. GSH was determined by the spectrephotometric method using Ellman's reagent [29] and the results are expressed as μmol GSH g^{-1} tissue.

DNA fragmentation assay

Mucosal samples from colonic tissues were homogenized in 10 volumes of a lysis buffer (5 mmol Tris HCL, 20 mmol EDTA, 0.5% [v/v] *t*-octylphenoxypolyethoxyethanol [Triton-X 100]; pH, 8.0). Two separate samples of 1 mL

were taken from the mucosal samples and centrifuged at 25,000g for 30 minutes to separate the intact chromatin in the pellet from the fragmented DNA in the supernatant [30]. The supernatant was taken out to be saved and the pellet was resuspended in 1 mL of Tri-EDTA buffer (pH, 8,0), 10 mmol:1 mmol, respectively. Both the supernatant and the resuspended pellet were assayed then for the DNA content by diphenylamine reaction described by Burton [31].

Statistical analysis

All data are expressed as mean values ± SEM. Statistical analysis was carried out using Instat statistical package (GraphPad Software, San Diego, CA). Following the assurance of normal distribution of data, groups of data were compared with 1-way ANOVA followed by Tukey–Kramer post hoc test for multiple comparisons. Values of $P < .05$ were regarded as significant.

Results

Serum LDH activity, TNF-α, IL-1β, and IL-6 Levels and AOC

As shown in Table 1, in TNBS-applied rats, plasma LDH activity showed a significant increase in saline-treated ($P < .01$) group with respect to control animals, whereas erdosteine administration abolished this response ($P < .01$). In the saline-treated rats with colitis, the levels of all cytokines, TNF-α, IL-1β, and IL-6 were significantly increased when compared with the control group ($P < .001$), and this colitis-induced rise in serum cytokine levels was reduced significantly by erdosteine treatment ($P < .001$). On the other hand, the total AOC was decreased significantly ($P < .001$) owing to colitis, whereas erdosteine treatment in the rats with colitis increased this parameter significantly ($P < .01$).

Table 1 Serum LDH Activity, TNF-α, IL-1β, and IL-6 levels and AOC

	Control	Colitis	
		Saline-treated	Erdosteine-treated
LDH (U/L)	1881 ± 116	3078 ± 288**	2067 ± 115++
TNF-α (pg/mL)	3.7 ± 0.7	23.8 ± 2.1***	10.1 ± 0.7+++
IL-1β (pg/mL)	42. 2 ± 0.9	117.1 ± 2.6***	54.3 ± 3.7+++
IL-6 (pg/mL)	35.8 ± 4.2	87.7 ± 1.3***	45.6 ± 3.7+++
AOC (μmol/L)	505 ± 51	255 ± 14***	405 ± 33++

AOC antioxidant capacity, *IL* interleukin, *LDH* Lactate dehydrogenase, *TNF* tumor necrosis factor.

**$P < .01$; ***$P < .001$ compared with the control group.

++$P < .01$; +++$P < .001$ compared with the saline-treated colitis group.

Severity of colonic injury

When compared with the intact colonic tissue of the control group (0.3 ± 0.2), intracolonic administration of TNBS increased the macroscopic damage score (11. 5 ± 0.8; $P < .001$), whereas erdosteine reduced the score significantly (5.6 ± 1.5; $P < .01$; Fig. 1a).

Similarly, colitis-induced increase in tissue WWI (0.99 ± 0.1 g/100 g), as compared with the control group (0.43 ± 0.1 g/100 g; $P < .001$), was reduced in rats that received erdosteine treatment (0.7 ± 0.1 g/100 g; Fig. 1b).

In contrast to the nearly normal appearance of the control group with a minute microscopic damage score (0.83 ± 0.5), light microscopic observation in the colitis group showed severe epithelial and glandular damage accompanied by epithelial degeneration, severe submucosal edema, vasculitis, and inflammatory cell infiltration with high microscopic damage score (11.8 ± 0.1; $P < .001$; Fig. 1c). Histologic damage score was reduced by erdosteine nearly back to control level (2.6 ± 0.5; $P < .001$).

As an indicator of enhanced tissue fibrotic activity caused by oxidants, the collagen content in the colonic tissue of saline-treated colitis group was markedly increased (31.8 ± 2.1 μg/mg protein) with respect to the control group (13.8 ± 1.2 μg/mg protein; $P < .001$). On the other hand, erdosteine treatment suppressed the fibrotic activity significantly (18.8 ± 2.7 μg/mg protein; $P < .001$; Fig. 1d).

Light microscopic evaluation of the control group, demonstrated well-designated, regular epithelial lining with abundant goblet cells containing mucus (Fig. 2A). However, the saline-treated colitis group showed a severe mucosal damage with hemorrhage in the lamina propria, distortion in the epithelium with hemorrhage, accumulation of inflammatory cells, and reduced mucus production from goblet cells (Fig. 2B). In the erdosteine-treated colitis group, the epithelial alignment and lamina propria showed a prominent recovery, as well as a significant re-production of mucus from the goblet cells (Fig. 2C). Reduced mucus production following epithelial degeneration was increased in the regenerative phase, becoming more abundant than the control group.

Examination by scanning electron microscopy revealed regular colon epithelium and crypts in the control group (Fig. 3A), and in the saline-treated colitis group, degenerated epithelium with denuded lamina propria in some regions, and severe inflammatory cell infiltration were observed (Fig. 3B). Reversal of epithelial degeneration was noted in the erdosteine-treated group, with the crypts in normal appearance as in the control group (Fig. 3C).

Colonic MDA and GSH levels and MPO activity

Colitis induction followed by saline treatment significantly increased MDA level in the colonic tissue ($P < .001$; Fig. 4a)

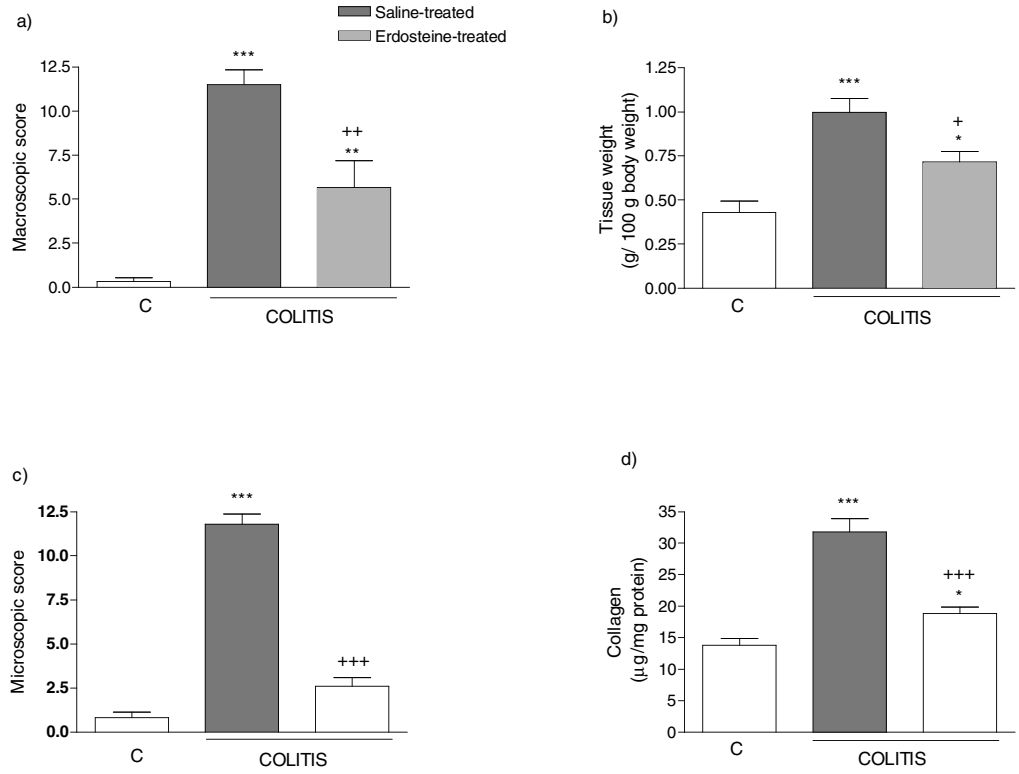

Fig. 1 (**a**) Macroscopic scores, (**b**) Wet weight index (WWI) (**c**) microscopic scores, and (**d**) collagen contents in the colonic tissues of control (C) and saline- or erdosteine-treated rats decapitated on the 4th day of TNBS instillation ($n = 8$ per group). $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$ compared with the control group. $^{+}P < .05$; $^{++}P < .01$; $^{+++}P < .001$ compared with saline-treated colitis groups

compared with the control group, but erdosteine treatment abolished colitis-induced elevation in MDA level ($P < .01$). On the other hand, colitis induction depleted colonic GSH level to a significantly lower level than that of the control group ($P < .001$), and erdosteine administration prevented the reduction in GSH and replenished it back to that of the control tissues ($P < .001$; Fig. 4b).

Intracolonic instillation of TNBS, as assessed by elevated MPO activity in the colonic tissues of the saline-treated group, caused a significant increase in neutrophil infiltration when compared with the control group ($P < .001$; Fig. 4c). On the other hand, erdosteine administration following colitis induction abolished the colonic MPO activity back to the control level ($P < .001$).

Luminol and lucigenin CL levels

Luminol and lucigenin CL levels in the saline-treated colitis group were increased dramatically ($P < .01$–0.001) compared with those in the control group; erdosteine treatment after colitis induction prevented radical formation ($P < .01$–0.001; Fig. 5).

DNA Fragmentation

DNA fragmentation (%) in the colonic mucosa was analyzed as an indicator of cell death, including apoptosis. In the colonic mucosa of the colitis group treated with saline, DNA fragmentation was elevated significantly compared with the control group ($P < .001$; Fig. 6), whereas erdosteine treatment significantly prevented the DNA damage of the colonic mucosa ($P < .001$).

Discussion

As confirmed by the macroscopic and microscopic scores, the results of the present study demonstrate that treatment with erdosteine markedly improves the colonic damage of rats with TNBS-induced colitis. Moreover, all the parameters indicating the presence of oxidative injury were markedly reversed by erdosteine treatment, suggesting that erdosteine has a potent anti-inflammatory effect on the inflamed colonic tissue.

Cytokine-driven inflammation and tissue destruction is a common theme of chronic inflammatory diseases such as rheumatoid arthritis, IBD, psoriasis, chronic obstructive pulmonary disease, and atherosclerosis. In these noninfectious

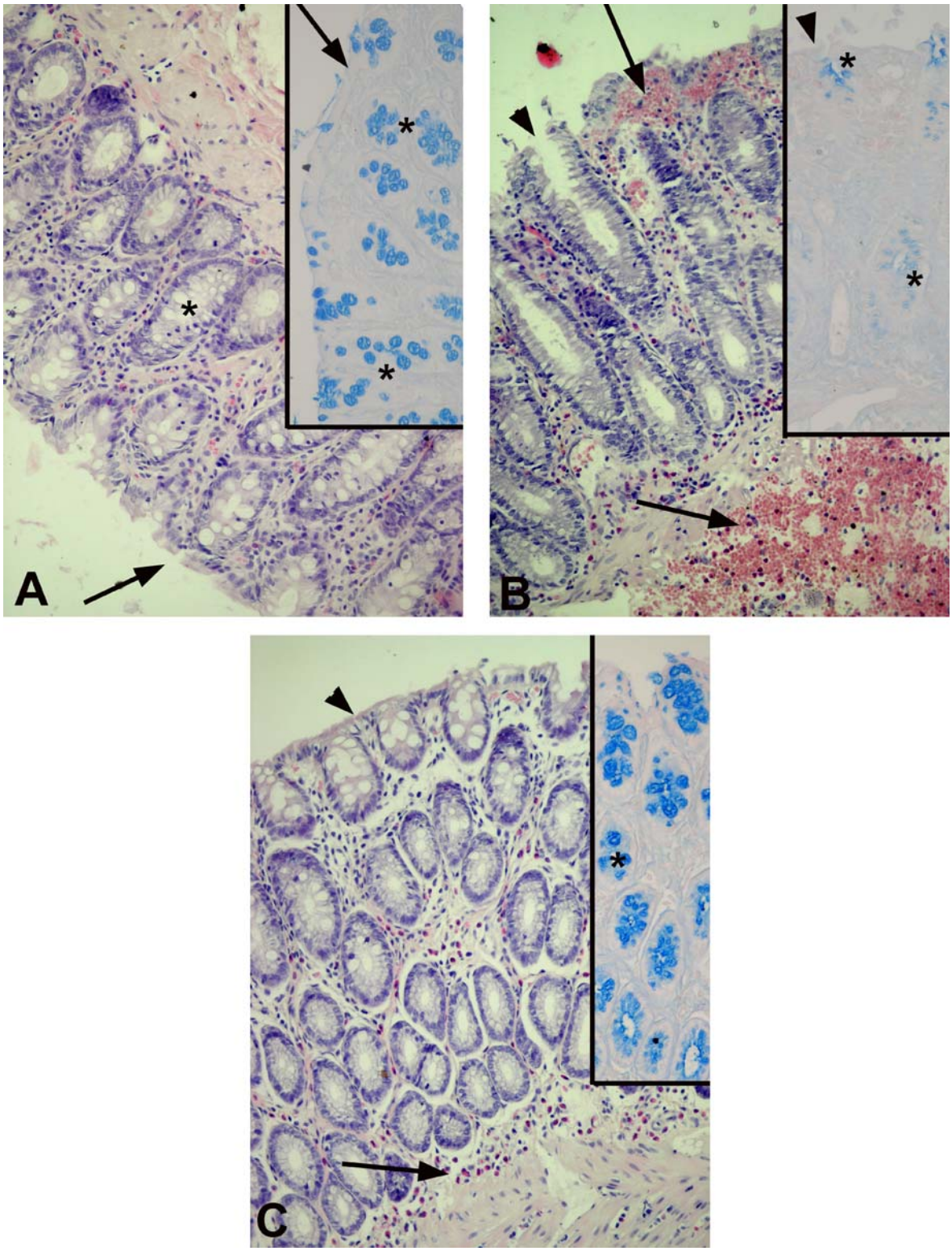

Fig. 2 Light microscopy. (**A**) Control group, regular colon mucosa with surface epithelium (*arrow*). Note the density of mucus cells (*inset*, ∗). (**B**) Colitis group, localized degenerated epithelium (*arrowhead*), hemorrhage in both epithelium and lamina propria and severe inflammatory cell infiltration (*arrows*), prominent decrease in mucus production (inset, ∗) (**C**) Colitis + erdosteine group, regenerated epithelium (*arrowhead*), abundant mucus production (*inset* ∗). Note the presence of inflammatory cells in the lamina propria (*arrow*; H&E × 200 and inset TBX 200)

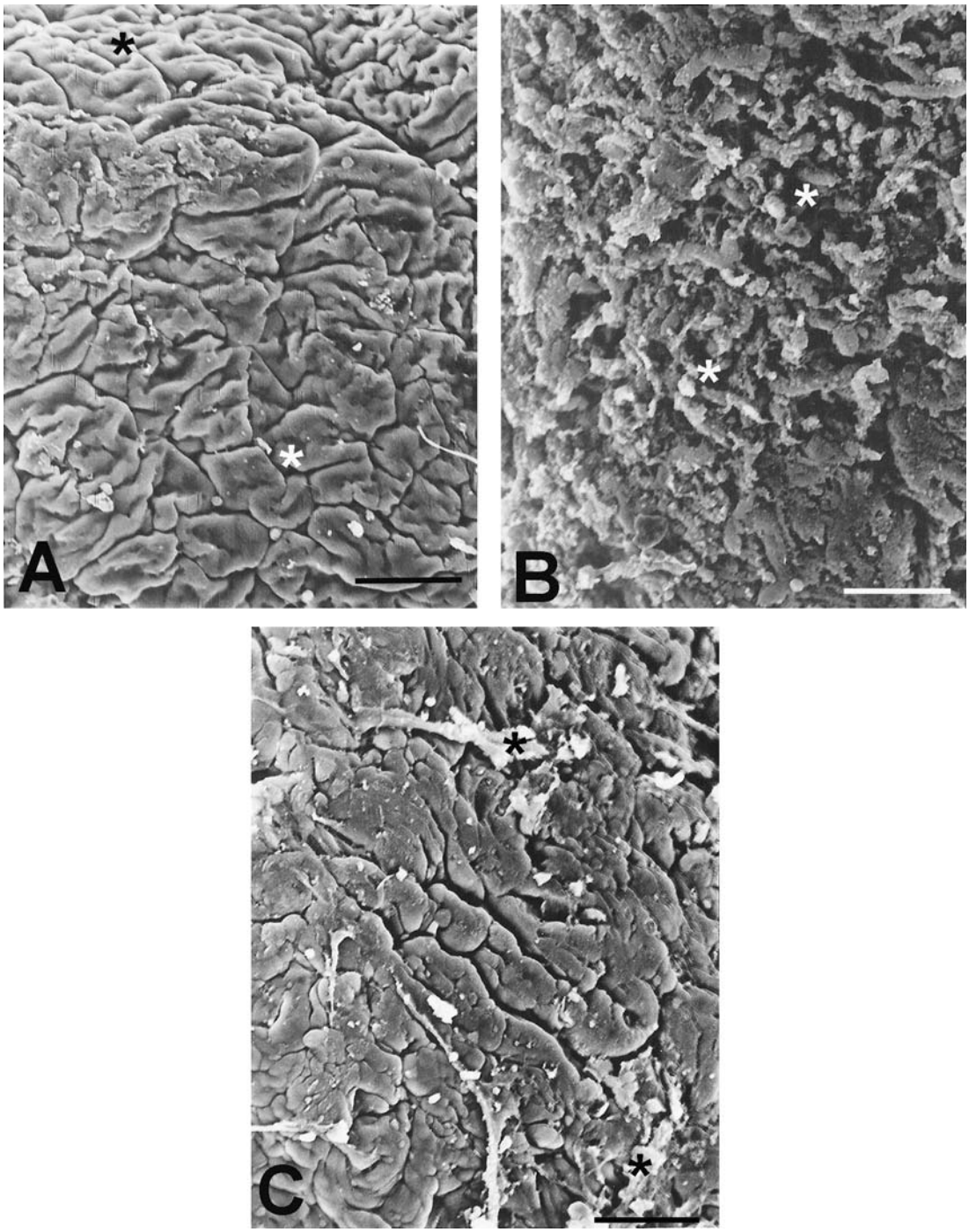

Fig. 3 Scanning electron microscopy. (**A**) Control group with regular epithelial alignment (∗). (**B**) Colitis group, denuded mucosa with mucus and cellular debris on the surface (∗). (**C**) Colitis + erdosteine group, regenerated surface epithelium with increase in mucus production (∗). Bar, 500 μm

inflammatory human diseases, the extravasal recruitment of neutrophils, a major source of reactive oxygen radicals, plays a crucial role in the development of tissue damage, which, when persistent, can lead to irreversible organ dysfunction [32]. Targeting oxidative stress with antioxidants or boosting the endogenous levels of antioxidants is likely to be beneficial in the treatment of these chronic inflammatory diseases. Thus, it is possible that compounds implicated in the pathogenesis of either inflammatory disease may also be effective in the treatment of other chronic inflammatory processes. Among various approaches to enhance lung AOC, erdosteine was used in patients with chronic obstructive lung disease [12]. As an expectorant agent with effects on bacterial adhesiveness, as well as antioxidant properties, erdosteine was

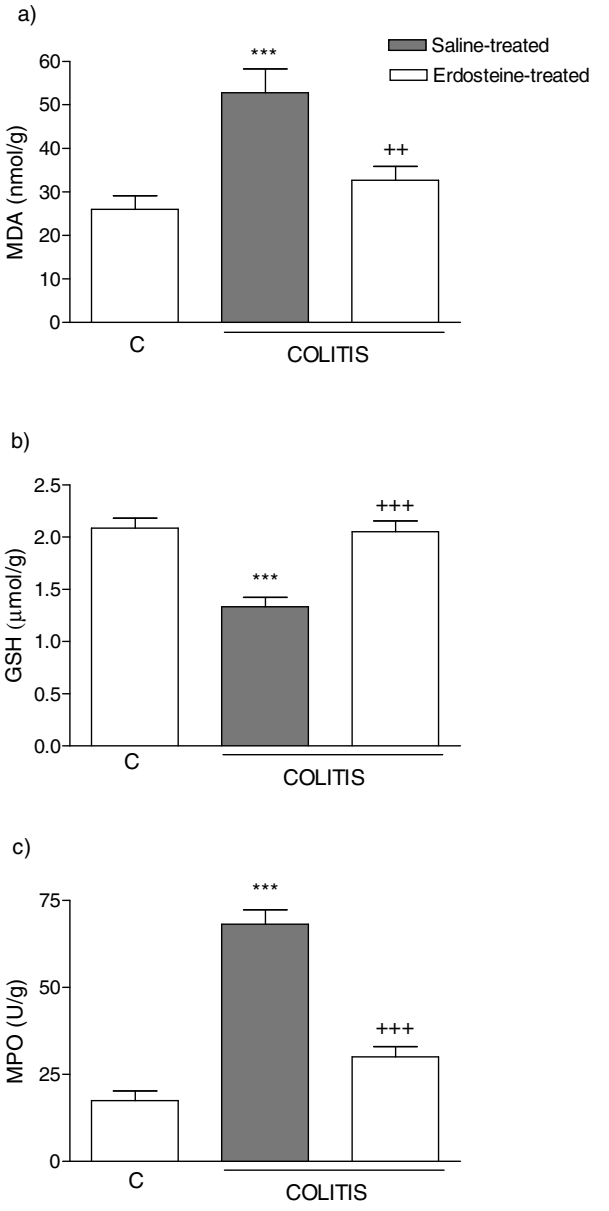

Fig. 4 (**a**) MDA, (**b**) GSH levels, and (**c**) MPO activity in the colonic tissues of control (C), saline-treated, or erdosteine-treated rats decapitated on the 4th day of TNBS instillation ($n = 8$ per group). $^{***}P < .001$ compared with the control group. $^{++}P < 0.01$; $^{+++}P < .001$ compared with the saline-treated colitis group

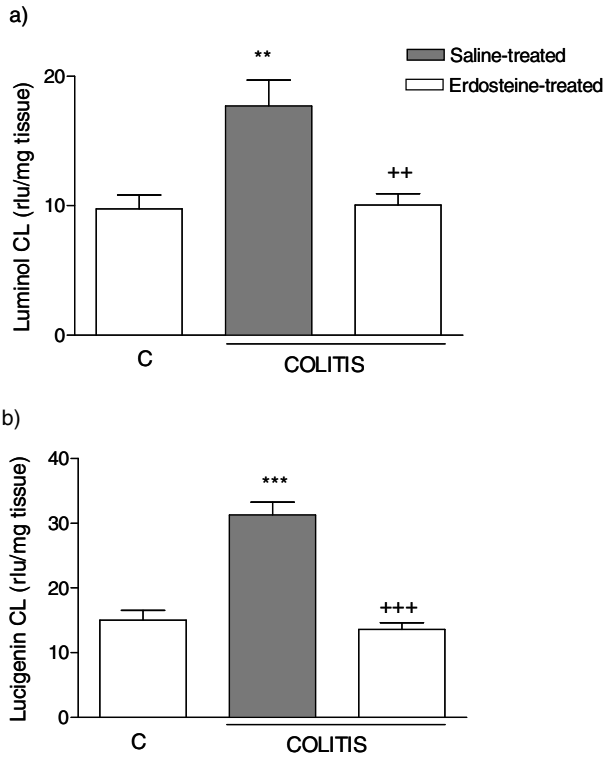

Fig. 5 (**a**) Luminol and (**b**) lucigenin chemiluminescence levels in the colonic tissues of control (C), saline-treated, or erdosteine-treated rats decapitated on the 4th day of TNBS instillation ($n = 8$ per group). $^{**}P < .01$; $^{***}P < .001$ compared with the control group. $^{++}P < .01$; $^{+++}P < .001$ compared with the saline-treated colitis group (rlu: relative light unit)

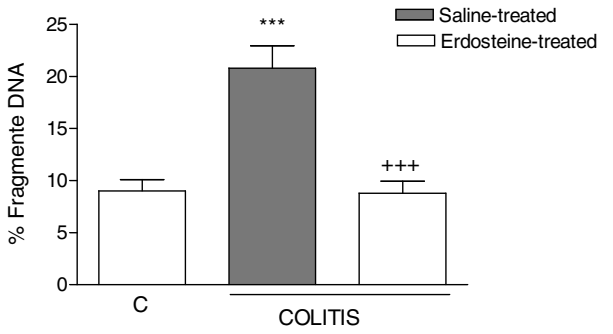

Fig. 6 DNA fragmentation in the colonic tissues of control (C), saline-treated, or erdosteine-treated rats decapitated on the 4th day of TNBS instillation ($n = 8$ per group). $^{***}P < .001$ compared with the control group. $^{+++}P < .001$ compared with the saline-treated colitis group

shown to modulate mucus production and viscosity and to increase mucociliary transport. Moreover, the active metabolites with sulfhydryl groups, released following first-pass metabolism, were reported to exhibit an inhibitory activity against the effects of free radicals produced by cigarette smoke. Based on these findings, the anti-inflammatory effect of erdosteine on colonic inflammation was evaluated using an experimental model of IBD.

It is well known that administration of an enema containing the contact-sensitizing allergen TNBS in ethanol produces an acute inflammation, which progresses to a chronic stage and is morphologically similar to Crohn's disease [33]. In the present study, histologic analysis revealed that intracolonic TNBS yields to a massive increase in colonic wall thickness, goblet cell loss, and submucosal infiltration of lymphocytes and neutrophils, which have been suggested to contribute markedly to tissue damage and mucosal dysfunction by releasing proteases, lactoferrin, and lipid mediators

[34]. Activated neutrophils can release superoxide anion and nitric oxide (NO), which subsequently combine to yield peroxynitrite anions, powerful and harmful oxidants that preferentially mediate the oxidation of the thiol groups in proteins and nonprotein molecules. MPO enzyme of the neutrophils catalyzes the formation of such potent cytotoxic oxidants as HOCl from H_2O_2 and chloride ions and *N*-chloramines. These oxidants play a direct role in the inflammatory process in chronic inflammatory processes by increasing the number of neutrophils and macrophages that induce a self-sustaining phlogogenic loop [35]. Infiltration of leukocytes into the mucosa has been suggested to contribute significantly to the tissue necrosis and mucosal dysfunction associated with colitis [34] because they are the producers of pro-inflammatory mediators and a major source of reactive oxygen radicals in the inflamed colon mucosa [36]. On the other hand, the mucolytic erdosteine was shown to reduce neutrophil release of ROS and the peroxynitrite generated by the reaction of superoxide anion with NO, thus disrupting the phlogogenic loop sustained by activated neutrophils in the pulmonary tissue [37]. In our observation, elevated MPO levels in colonic tissues indicate that neutrophil accumulation contributes to the colitis-induced oxidative injury and erdosteine appears to have a preventive effect through the inhibition of neutrophil infiltration. Similarly, in the study by Siddiqui et al. [38], increased MPO activity and pro-inflammatory cytokines in the distal colon of TNBS-treated animals were inhibited by another thiol-containing agent, *N*-acetylcysteine. In our study, the antioxidant action of erdosteine could be related with its inhibitory effect on neutrophil recruitment and subsequent release of pro-inflammatory mediators. Similarly, bleomycin-induced pulmonary injury was previously shown to be prevented by erdosteine via the repression of neutrophil accumulation, resulting in the inhibition of lipid peroxidation and fibrosis [16].

Lipid peroxidation, mediated by oxygen free radicals, is believed to be an important cause of destruction and damage to cell membranes [39] and has been suggested to be a contributing factor in the development of TNBS-mediated colonic damage [40, 41]. Quantitatively, the main free radical in tissues is superoxide anion (O_2^-), produced by both endothelial cells and activated neutrophils. In addition, secondary lipid peroxidation products exert similar toxic effects, prolonging and potentiating the primary free radical-initiated damage In the present study, MDA, which is a good indicator of the degree of lipid peroxidation [41, 42], was increased in the colonic tissues of TNBS-treated rats, indicating the presence of oxidative damage. It is well known that IBD is characterized by an increased expression of inflammatory cytokines (TNF-α, IL-1β, and IFN-γ) and enzymes (inducible NO synthase and cyclooxygenase-2) [43, 44]. Elevated plasma levels of the pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 support the notion that tissue injury induced by TNBS involves inflammatory cytokines. Moreover, increased levels of luminol- and lucigenin-enhanced CL demonstrate that toxic oxygen metabolites were also found to be elevated in the colonic tissue. Because erdosteine treatment prevented elevations in tissue MDA and attenuated the increases in tissue luminol- and lucigenin-enhanced CL and cytokine levels, it seems likely erdosteine ameliorates TNBS-induced oxidative injury, in part, by scavenging the reactive oxygen radicals. Previously it was shown that erdosteine and its active metabolite decrease luminol-dependent CL and possess scavenging activities against H_2O_2 and HOCl in vitro [45]. Similarly, an erdosteine metabolite was reported to react with ROS, NO, and NO-derived peroxynitrite and exert both antioxidant and scavenging activities [46]. Moreover, erdosteine inhibits cytokine synthesis and free radical production in rat alveolar macrophages by effectively decomposing NO [47].

GSH is an important constituent of intracellular protective mechanisms against various noxious stimuli, including oxidative stress. However, reduced GSH as the main component of endogenous nonprotein sulfhydryl pool, is known to be a major low-molecular-weight scavenger of free radicals in the cytoplasm [48]. It was reported that tissue GSH levels and the activities of GSH reductase and GSH peroxidase, which are critical constituents of GSH-redox cycle, were significantly reduced owing to oxidative stress, permitting enhanced free radical-induced tissue damage [49]. In accordance with previous reports, our results also show that depletion of tissue GSH, as observed in the TNBS-induced colonic injury, is one of the major factors that permit lipid peroxidation and subsequent tissue damage. Thus, the decrease in colonic GSH levels may be due to its consumption during TNBS-induced oxidative stress. Furthermore, prevention of colonic GSH depletion by erdosteine may be responsible for the maintenance of AOC in protecting colonic tissue against oxidative stress.

It is well known that the molecule most often reported to be damaged by oxygen radicals and the resultant injury is DNA [50]. In a study Martin et al. [51], DNA fragmentation was significantly increased in rats with TNBS colitis. Similarly in our study, TNBS-induced colitis resulted in increased DNA fragmentation in the colonic tissue. On the other hand, the results indicate that erdosteine treatment reduces free radical generation, subsequent lipid peroxidation, and DNA damage and thereby supports the maintenance of cellular integrity.

Histologic evaluation of the damaged colonic tissue in the erdosteine-treated group revealed that erdosteine may have an additional protective effect by inhibiting the production and deposition of extracellular matrix components that would result in enhanced tissue fibrosis. Furthermore, as it is observed when used as an expectorant agent in the respiratory tract inflammation [12], present results also show that

the protective effect of erdosteine on the colonic epithelium may involve the modulation of mucus production from the colonic mucosa.

In conclusion, erdosteine, by preventing free radical damaging cascades and oxidant radical release and through its mucoregulatory property, supports the maintenance of colonic integrity against chronic inflammatory processes. Thus, further experimental and clinical studies are required to confirm whether erdosteine may provide an important contribution to the treatment of IBD.

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