

Amelioration of Acetic Acid-Induced Colitis in Rats by Oral Administration of Ginger Extract

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ABSTRACT

Ulcerative colitis (UC) is a chronically recurrent inflammatory bowel disease of unknown origin. The aim of the present study is to evaluate possible protective effects of ginger extract (GE) on the extent and severity of UC caused by intracolonic administration of acetic acid in rats. Animals received either GE (100, 200 and 400 mg/kg) or sulphasalazine (500 mg/kg), for 3 consecutive days before intra-rectal acetic acid administration (1 ml, 4% v/v), and continued for another 7 days after the induction. The degree of tissue injuries was assessed by macroscopical and histopathological scores of the colonic mucosa. The biochemical studies involve the redox state including colon mucosal content of malondialdehyde (MDA) as an index of lipid peroxidation, glutathione (GSH) and protein carbonyl content (PCO) as indexes of protein oxidation as well as the activity of catalase and superoxide dismutase (SOD) enzymes in addition to some indicators of the inflammatory response myeloperoxidase (MPO) activity, index of neutrophilic infiltration, and the tissue contents of tumor necrosis factor (TNF- α), and prostaglandin E₂ (PGE₂). Oral pretreatment with ginger extract and sulphasalazine were able to correct altered parameters significantly. Moreover, ginger extract attenuated the macroscopic colonic damage and the histopathological changes-induced by acetic acid. These results suggest a beneficial protective effect of ginger extract against acetic acid-induced colitis possibly by its antioxidant and anti-inflammatory effects.

Key words: ulcerative colitis, ginger extract, TNF- α , PGE₂, MPO, oxidative stress, rats.

INTRODUCTION

Ulcerative colitis and Crohn's disease are chronic, relapsing, immunologically mediated disorders that are collectively referred to as inflammatory bowel diseases (IBD). The prevalence of IBD rapidly increased in Europe and North America in the second half of the twentieth century and is becoming

more common in the rest of the world as different countries adopt a Western lifestyle^[1]. Etiology and pathogenesis of inflammatory bowel disease, however, remain obscure, although environmental factors in combination with genetic factors are suggested to be involved in its pathogenesis^[2]. Even with this knowledge, the etiologies of these diseases remain an enigma. In the past few years,

however, work in animal models, human genetics, basic science and clinical trials have provided new insights into the pathogenesis of these diseases^[3].

The role of oxidative stress in the pathogenesis of ulcerative colitis has been investigated, where oxygen-centered radicals, such as superoxide and hydroxyl radicals generated by phagocytes, have been suggested to be involved in the pathogenesis of chronic inflammations of the bowel, such as Crohn's disease and colitis ulcerosa^{[4]; [5]}, due to its major tissue-destructive force.

Generation of reactive oxygen species (ROS) by inflammatory cells is a major microbiocidal mechanism and may mediate important components of the inflammatory response^[6]. Prolonged or inadequate activation of the intestinal immune system plays an important role in the pathophysiology of chronic mucosal inflammation^[7], and infiltration of neutrophils, macrophages, lymphocytes and mast cells, ultimately give rise to mucosal disruption and ulceration^[2]. The infiltrated neutrophils represent an important source of reactive oxygen species (ROS), which are cytotoxic agents, inducing cellular oxidative stress by cross-linking proteins, lipids, and nucleic acids, leading to cellular dysfunction and damage^[8]. The disturbed redox system can be reflected by the elevation of protein carbonyls levels (PCO)^[9], and lipid peroxidation^{[10], [11]}.

Oxidative stress occurs when there is an imbalance between generation of ROS and inadequate antioxidant defense systems. This fact

holds true in IBD, since both levels of non-enzymatic endogenous antioxidants, such as reduced glutathione (GSH), and the activity of the enzymatic ones, for example, catalase (CAT) and superoxide dismutase (SOD), are all significantly decreased in these patients, thus augmenting the deleterious effect of ROS^{[12]; [13]}. Most currently used therapeutic drugs for IBD, in particular sulphasalazine and its active moiety 5-aminosalicylic acid, control the disease partly by their property as potent ROS scavengers^{[14]; [15]}.

In addition to free radicals, neutrophils can also release proteases and lipid mediators that can contribute to intestinal injury^[16]. Inflammatory bowel disease (IBD) is associated with increased activation of intestinal immune cells, whose overproduction of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-alpha) and interleukin-1 beta (IL-1 beta) is implicated in mediating the sustained inflammatory response^[17]. TNF- α level is often increased^[18] both in animal models^[19] and patients with ulcerative colitis^[20]. This cytokine is a key immunoregulatory cytokine that amplifies the inflammatory response by activating a cascade of immune cells. TNF- α , secreted by activated macrophages, stimulate production of cytokines, arachidonic acid metabolites, and proteases by intestinal macrophages, neutrophils, smooth muscle cells, fibroblast, and epithelial cells^[21]. Moreover, myeloperoxidase (MPO), which is an enzyme mainly found in azurophilic granules of neutrophils, is a good marker of neutrophil infiltration^{[22]; [5]}.

Experimental models in which colitis is induced by chemicals or haptens have been widely used to understand the mechanisms involved in the disease state with the goal of discovering new therapeutic agents^{[23];[24]}. Among these experimental models, intrarectal administration of organic acids (acetic acid) is known to create initial injurious effects to the colonic mucosa, a process that produces acute colitis^{[25];[5]}.

Ginger (*Zingiber officinale Roscoe*), is a well-known herbaceous species, which is consumed in most parts of the world and are rich in gingerols, shogaols, and other constituents, which exhibit antioxidant^[26], antihyperlipidemic^[27;28] antiproliferative^[29], and anticarcinogenic proprieties under “*in vitro*” and “*in vivo*” systems^{[30];[31]}. Furthermore, in folk medicine, ginger has been used traditionally as a carminative, diaphoretic, anti-spasmodic and anti-emetic agent against motion sickness^[32].

However, the possible role of ginger in modulating colon inflammation has not been verified yet, thus we aimed in the current investigation to evaluate the effect of ginger extract on acetic acid-induced ulcerative colitis in rats and its possible mechanism(s) of action.

MATERIALS & METHODS

Chemicals

Acetic acid, sodium sulfate, Triton X-100, 2-thiobarbituric acid (TBA), trichloroacetic acid (TCA), Tris base, phosphate buffer, n-butanol were purchased from Merck

(Germany), 1,1,3,3-tetraethoxypropane, sulphasalazine, 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB), reduced glutathione (GSH), adenosine diphosphate, agents for myeloperoxidase assay were procured from Sigma-Aldrich (St Louis, MO, USA). TNF- α and PGE₂ ELISA kits were obtained from Amersham (Pharmacia Biotech, Little Chalfont, UK) and R&D Systems Inc. (Minneapolis, USA), respectively. All other chemicals used were of analytical grade.

Animals

Male Sprague-Dawley rats (190 - 210 g) were exposed to the same environmental conditions and were maintained on a proper diet chow and water *ad libitum*. Animals were randomly divided into six groups (8 animals each) and the study was conducted in accordance with ethical procedures and policies approved by the Animal Care and Use Committee of Faculty of Pharmacy, Al-Azhar University Cairo, Egypt.

Preparation of the extract

Fresh rhizome of ginger (*Zingiber officinale Roscoe*) was purchased from a local market in Egypt and authenticated by a pharmacognosist (Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Cairo) according to Langner et al.^[32]. The plant was dried in the shade and the dried rhizome was powdered mechanically and extracted by cold percolation with 95% ethanol in a soxhlet apparatus for 72 h. The process was repeated three times and the three extracts were pooled together and the combined extract was concentrated under reduced pressure (22–26 mmHg) at 45–60°C. The

solvent was removed under reduced pressure to give a dry extract, 9.5% yield, w/w (with respect to the crude material) and doses equivalent to 100, 200 and 400 mg of the crude drug per kg body weight, were calculated and suspended in 0.5 % carboxy methyl cellulose (CMC) solution for the experiment.

Induction of experimental colitis in rats

Colitis was induced using the method previously described by Millar et al.,^[23], where animals fasted for 24 h with free access to water ad libitum before induction of colitis, and each rat was sedated by phenobarbitone (35 mg/kg, i.p). One milliliter of acetic acid (4 %, v/v, in 0.9 % saline) was infused for 30s using a polyethylene tube (2 mm in diameter), which was inserted through the rectum into the colon to a distance of 8 cm. The acetic acid was retained in the colon for another 30s after which the fluid was withdrawn and animals' head were kept in a downward position for 30s then returned to cages. Animals were divided in 6 groups, where group 1 served as normal control receiving 0.9% saline, group 2 were subjected to ulcerative colitis model and used as positive control, groups 3, 4, and 5 received ginger extract in doses of 100, 200 and 400 mg/kg, respectively, and the last group was used as reference group, where animals received sulphasalazine (500 mg/kg). Drugs were given once daily (0.5 ml/100 g body weight, p.o) for 3 days before the induction of colitis and continued for another 7 consecutive days. Drugs were suspended in 0.5% carboxymethyl cellulose (CMC),

which does not affect the severity of acetic acid-induced ulcerative colitis^[24]. Following completion of the experiment, rats were ether anesthetized and colonic biopsies were taken for macroscopic scoring, histopathological examination and biochemical studies.

Assessment of colitis

- Macroscopic scoring

At post-mortem laparotomy, 6 cm of colon extending proximally for 2 cm above the anal orifice was removed. The tissue was first split longitudinally, pinned out onto card, and the macroscopic appearance of the colonic mucosa was scored by an independent observer according to Millar et al.^[23], a scale ranging from 0 to 4 as follows: 0 =No macroscopic changes, 1 =Mucosal erythema only, 2 =Mild mucosal edema, slight bleeding or small erosions, 3 =Moderate edema, bleeding ulcers or erosions, 4 = Severe ulceration, erosions, edema and tissue necrosis

- Histopathological study

Sections of colon were obtained from the same areas of the large intestine from four representative animals in each of the treatment groups during autopsy. They were fixed in 8phosphate buffered formaldehyde, embedded in paraffin, and routine 5 µm sections were prepared. Tissues were routinely stained with haematoxylin and eosin and were evaluated by light microscopy by a pathologist .

-Biochemical studies

Myeloperoxidase (MPO, EC 1.11.1.7) activity was determined as an index of neutrophil accumulation^[33], and the rate of change in absorbance was measured

spectrophotometrically at 650 nm. MPO activity was defined as the quantity of enzyme degrading 1 mmol of peroxide/min at 37°C and expressed in units per gram weight of wet tissue. Catalase (CAT) (EC 1.11.1.6) activity was measured according to the Aebi^[34], where one unit of CAT activity was defined as the amount of enzyme required to decompose 1 μmol of H₂O₂ in 1 min. The rate of decomposition of H₂O₂ was measured spectrophotometrically at 240 nm for 1 min. Superoxide dismutase (SOD) (EC 1.15.1.1) activity was determined according to Marklund and Marklund^[35]. A colorimetric assay involving generation of superoxide by pyrogallol auto-oxidation was measured at 570 nm. Protein content was measured according to Lowry et al.^[36].

Lipid peroxidation in colon tissue homogenate was evaluated by measuring MDA using thiobarbituric method described by Ohkawa et al.^[37], and the absorbance of the supernatant was measured spectrophotometrically at 532 nm. PCO content in colon homogenates were determined spectrophotometrically by the method of Levine et al.^[38], based on the reaction of the carbonyl group with 2,4-dinitrophenylhydrazine to form 2,4 dinitrophenylhydrazone. GSH content was determined as previously described by Owens and Belcher^[39] based on the reaction of 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) with the GSH present and the absorbance was measured by a double beam spectrophotometer at 412 nm.

TNF- α was assessed according to the method of Reinecker et al.^[40], and was quantified by enzyme-linked

immunoabsorbent assay [ELISA] (Amersham Pharmacia Biotech, Little Chalfont, UK) and the results were expressed as picograms /gram of wet tissue. PGE₂ was also measured by ELISA kits (R&D Systems, USA)^[41].

Statistical analysis

All data are expressed as mean \pm standard error of the mean (SEM) for eight rats per experimental group. Statistical analysis was performed with SPSS 10.0 statistical software. Parametric one-way analysis of variance (ANOVA) followed by Tukey-Kramer Multiple Comparisons test was used to compare the mean values of quantitative variables among the groups. The minimal level of significance was identified at $p < 0.05$. Correlation coefficient was determined by linear regression analysis. The non-parametric ANOVA, followed by Kruskal-Wallis Test was used for analyzing the macroscopical test.

RESULTS

Table 1 depicts the macroscopic changes related to the 3 dose levels of ginger extract and the reference drug, sulfasalazine, in comparison with vehicle-treated rats. Acetic acid caused severe macroscopic edematous inflammation in the colon, 24 h after rectal administration, as assessed by the high score of colonic damage and the increased colon wet weight. Ginger extract ameliorated the acetic acid effect as reflected by the significant dose dependant anti-edematous effect and the percentage reduction in the lesion scores. Treatment with sulphasalazine showed similar effect. However,

ginger extract (100 mg/kg) did not show any improvement regarding score difference from acetic acid. These findings were emphasized by the histopathological studies present in figure 1, where acetic acid treated rats showed transmural necrosis, edema and diffuse inflammatory cell infiltration in the mucosa. There was focal ulceration of the colonic mucosa extending through the muscularis mucosa, desquamated areas with loss of the epithelium. The architecture of the crypts was distorted and the lamina propria was thickened in peripheral areas of distorted crypts especially in basal areas. An infiltrate consisting of mixed inflammatory cells was observed (Fig. 1B). Treatment of rats with sulphasalazine (Fig. 1C) or GE (Fig. 1D) significantly attenuated the extent and severity of the histological signs of cell damage. Histological studies confirmed the intestinal anti-inflammatory effect exerted by the two drugs used. This effect was mirrored by a 4 fold increase in mucosal MPO activity following acetic acid administration. Pre-treatment with either GE (100, 200 and 400 mg/kg) or sulphasalazine produced a significant reduction in MPO activity, however, none was able to reach the control value (Figure 3). Ulcerated non-treated group showed a vast elevation in the colonic TNF- α , an effect which leveled off in

groups treated with either GE (100, 200 and 400 mg/kg) or sulphasalazine (Figure 4). Effect of acetic acid alone or with either GE (100, 200 and 400 mg/kg) or sulphasalazine show the same pattern as illustrated in figure 5, and none of the treatment agents could normalize the PGE₂ level. Using pair-wise comparisons among the groups. Ginger extract in a dose of 400 mg kg⁻¹ was the most potent at reducing PGE₂ level. Regarding the redox state, acetic acid group caused a significant decrease in colonic non-enzymatic (figure 2) and enzymatic (table 3) defense systems, however, these effects were altered in animals pre-treated with either treatment regimen, to reach nearly the normal values with the highest dose of GE. The overwhelmed defense systems result in a significant increase in concentration of both MDA (5.9 fold) and PCO (2.6 fold) in acetic acid non-treated animals (table 2). Pretreatment with GE produced a marked decrease in MDA and PCO levels in a dose-dependant manner, and so did sulphasalazine (table 2). Analysis of the correlation coefficients between gross lesion score and PGE₂, TNF- α , and MPO is shown in Fig. 6a-c. A significant positive correlation was observed between gross lesion score and colonic PGE₂, TNF- α and MPO ($r^2 = 0.994$, 0.987 and 0.968 , respectively, $P < 0.001$).

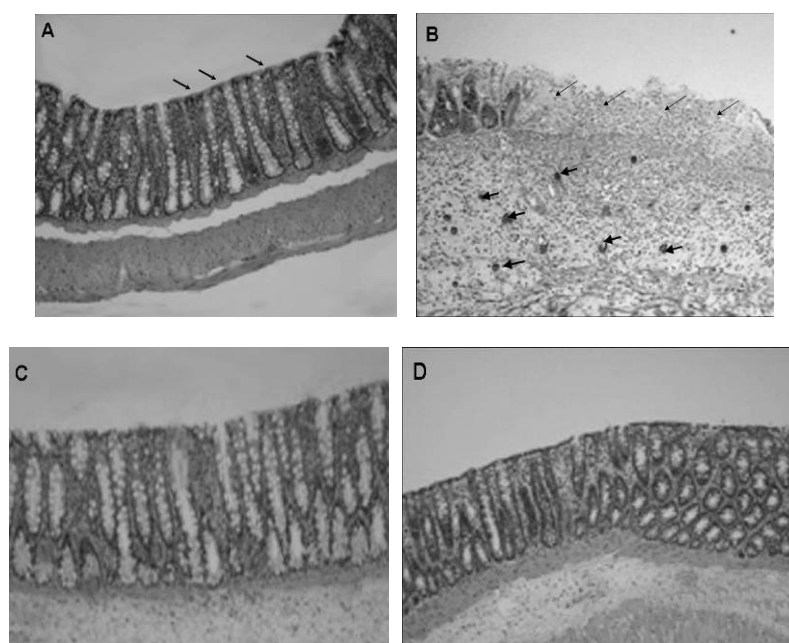


Fig.1. Photomicrographs of haematoxylin and eosin stained paraffin sections of rat colonic tissues. (A) Normal intact mucosa from normal control animals showed intact epithelial surface. (B) Acetic acid-induced colitis showing massive necrotic destruction of epithelium and infiltrated neutrophils. (C) Pre-treatment with Ginger extract (400 mg/kg) and (D) sulfasalazine (500 mg/kg), attenuated the extent and severity of the histological signs of cell damage. Magnification X100.

Table 1. Effects of different doses of ginger extract (100, 200, 400 mg/kg, p.o) and sulphasalazine (500 mg/kg, p.o) on score lesion and wet weight of colonic segments from rats with acetic-acid-induced ulcerative colitis.

Groups	% Reduction	Gross lesion score	Colon weight/Length (mg/cm)
Normal Control	-	-	68.7± 2.4
Acetic acid (4%)	0	3.88 ± 0.03	162 ± 9.2*
+ Sulphasalazine (500 mg/kg)	48	2.02 ± 0.03 ^ϕ	136 ± 5.8 ^{*,ϕ}
+ Ginger extract (100 mg/kg)	40	2.31 ± 0.01 ^ϕ	142 ± 6.8 ^{*,ϕ}
+ Ginger extract (200 mg/kg)	51	1.90 ± 0.05 ^ϕ	127 ± 6.3 ^{*,ϕ}
+ Ginger extract (400 mg/kg)	59	1.60 ± 0.02 ^ϕ	103 ± 5.1 ^{*,ϕ}

Treatments were administered for 3 days before acetic acid-induced UC and for 7 days after. Values are means of 8 animals ± SEM. As compared with normal control (*), acetic acid

treated (^ϕ) groups (one-way ANOVA followed by Tukey-Kramer Multiple Comparisons Test), P<0.05 (Parametric analysis).

Table 2. Effects of different doses of ginger extract (100, 200 and 400 mg/kg, p.o) and sulphasalazine (500 mg/kg, p.o) on colonic levels of protein oxidation (PCO) and lipid peroxidation (MDA) in rats with acetic acid-induced ulcerative colitis.

Groups	Protein carbonyl content (nmol/mg protein)	Lipid peroxidation (nmol/g wet tissue)
Normal Control	2.21 ± 0.08	9.71 ± 0.22
Acetic acid (4%)	5.78 ± 0.09*	57.6 ± 3.3*
+ Sulphasalazine	3.41 ± 0.07*, ^φ	31.5 ± 1.3*, ^φ
+ Ginger extract (100 mg/kg)	3.93 ± 0.03*, ^φ	34.6 ± 1.2*, ^φ
+ Ginger extract (200 mg/kg)	3.10 ± 0.05*, ^φ	25.2 ± 0.38*, ^φ
+ Ginger extract (400 mg/kg)	2.78 ± 0.02*, ^φ	18.7 ± 0.23*, ^φ

Treatments were administered for 3 days before acetic acid-induced UC and for 7 days after. Values are means of 8 animals ± SEM. As compared with normal control (*), and acetic acid

treated (^φ) groups (one-way ANOVA followed by Tukey-Kramer Multiple Comparisons Test), P<0.05.

Table 3. Effects of different doses of ginger extract (100, 200 and 400 mg/kg) and sulphasalazine (500 mg/kg, p.o) on the activity of colonic catalase and SOD of rats with acetic acid-induced ulcerative colitis.

Groups	Catalase (U/mg protein)	SOD (U/mg protein)
Normal Control	21.6 ± 0.23	6.23 ± 0.06
Acetic acid (4%)	10.23 ± 0.12*	3.52 ± 0.02*
+ Sulphasalazine	14.75 ± 0.18*, ^φ	4.20 ± 0.05*, ^φ
+ Ginger extract (100 mg/kg)	14.0 ± 0.25*, ^φ	4.41 ± 0.03*, ^φ
+ Ginger extract (200 mg/kg)	17.2 ± 0.28*, ^φ	6.22 ± 0.08 ^φ
+ Ginger extract (400 mg/kg)	20.8 ± 0.31 ^φ	8.23 ± 0.04*, ^φ

Treatments were administered for 3 days before acetic acid-induced UC and for 7 days after. Values are means of 8 animals ± SEM. As compared with normal control (*), and acetic

acid treated (^φ) groups (one-way ANOVA followed by Tukey-Kramer Multiple Comparisons Test), P<0.05.

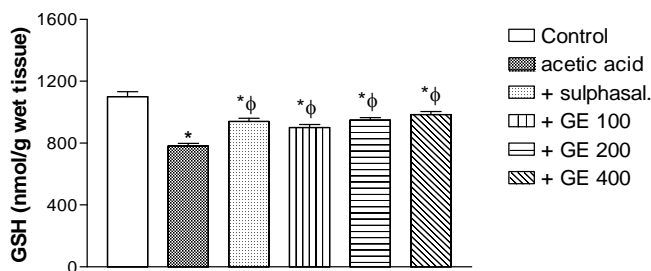


Figure 2: Effects of different doses of ginger extract (100, 200 and 400 mg/kg, p.o) and sulphasalazine (500 mg/kg, p.o) on colonic reduced glutathione content of rats with acetic acid-induced ulcerative colitis (mean of 8 animals \pm SEM.). Treatments were administered once daily for 3 days before induction of colitis and for 7 consecutive days after. As compared with normal control ($\bar{}$), and acetic acid treated (ϕ) groups (one-way ANOVA followed by Tukey-Kramer Multiple Comparisons Test), $P < 0.05$.

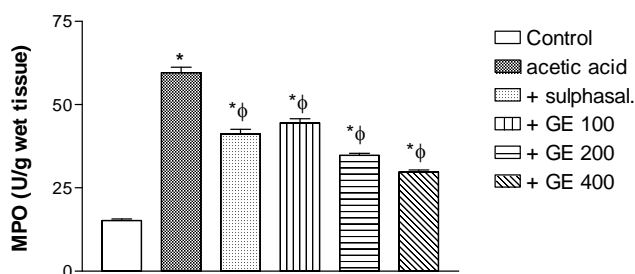


Figure 3: Effects of different doses of ginger extract (100, 200 and 400 mg/kg, p.o) and sulphasalazine (500 mg/kg, p.o) on colonic myeloperoxidase (MPO) activity of rats with acetic acid-induced ulcerative colitis (mean of 8 animals \pm SEM.). Treatments were administered once daily for 3 days before induction of colitis and for 7 consecutive days after. As compared with normal control ($\bar{}$), and acetic acid treated (ϕ) groups (one-way ANOVA followed by Tukey-Kramer Multiple Comparisons Test), $P < 0.05$.

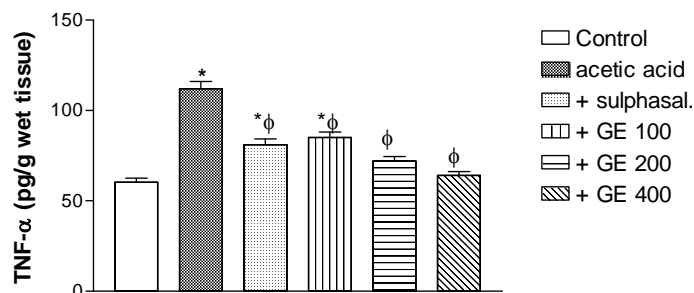


Figure 4: Effects of different doses of ginger extract (100, 200 and 400 mg/kg, p.o) and sulphasalazine (500 mg/kg, p.o) on colonic tumor necrosis factor-alpha (TNF- α) level in rats with acetic acid-induced ulcerative colitis (mean of 8 animals \pm SEM.). Treatments were administered once daily for 3 days before induction of colitis and for 7 consecutive days after. As compared with normal control ($\bar{}$), and acetic acid treated (ϕ) groups (one-way ANOVA followed by Tukey-Kramer Multiple Comparisons Test), $P < 0.05$.

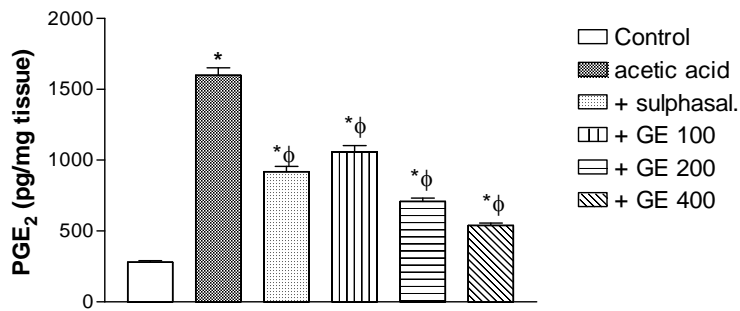


Figure 5: Effects of different doses of ginger extract (100, 200 and 400 mg/kg, p.o) and sulphasalazine (500 mg/kg, p.o) on colonic prostaglandine E₂ (PGE₂) content in rats with acetic acid-induced ulcerative colitis (mean of 8 animals ± SEM.). Treatments were administered once daily for 3 days before induction of colitis and for 7 consecutive days after. As compared with normal control (*), and acetic acid treated (φ) groups (one-way ANOVA followed by Tukey-Kramer Multiple Comparisons Test), P<0.05.

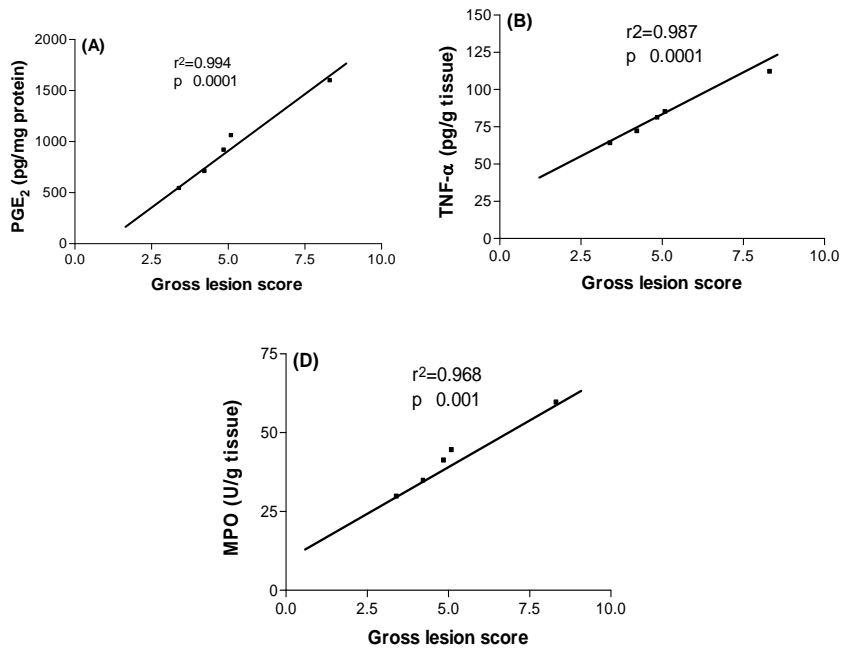


Figure 6: Linear regression analysis of gross lesion score and (a) PGE₂ (r² = 0.994, P < 0.0001), (b) TNF-α (r² = 0.987, P < 0.0001), and (c) MPO (r² = 0.968, P < 0.001).

DISCUSSION

In the present investigation, the beneficial effects observed with ginger extract treatment were assessed based on the improvement of colon weight and length, and the histological preservation of the colon architecture in comparison with the non-treated colitic rats. The biochemical assays performed in the colonic specimens confirmed also the anti-oxidant and anti-inflammatory effects exerted by ginger extract, especially at a dose of 400 mg/kg.

Effects of acetic acid-induced ulcerative colitis are supported by previous findings^{[42]; [5]}, where defense systems are impaired (and the contents of oxidized lipids and proteins are elevated. This fact is reflected in patients with IBD, where there is an elevation in lipid peroxides [LPO]^[43] and protein carbonyl contents [PCO], with decrease in SOD^[44], catalase activity^[42] and glutathione level^[45]. Other animal models of IBD also support our data^{[46]; [47]}.

Oxidative stress (OS) occurs when the cellular defense systems are overwhelmed by the excessive generation of ROS. These species can attack cells either directly or through altering signaling pathways, producing a unifying mechanism of injury in many types of disease processes, including gastrointestinal diseases^[10]. In inflammatory bowel disease, OS is believed to constitute a major tissue-destructive force that play a role in disease initiation and progression^[48]. These ROS attack the cellular macromolecules, viz., cell membrane lipids, causing their

peroxidation in a nonstop cycle, cell proteins and DNA, thus rendering it more vulnerable to oxidative tissue damage, hindering recovery of the mucosa and return of epithelial cell layer integrity, especially in case of impaired endogenous defense systems^[45].

Intestine is in a constant state of controlled inflammation, mediated by a balance between aggressive luminal forces and host protective mechanisms. This delicate balance can be deranged by any number of environmental triggering events and is in dysequilibrium in IBD. Amplification of the inflammatory response activates effector cells and infiltration of inflammatory molecules, such as neutrophils, macrophages, lymphocytes and mast cells, which mediate tissue injury and physiologic responses leading to symptoms of IBD^{[49]; [7]}. These infiltrated neutrophils represent an important source of ROS.

In normal bowels, the immune reaction is sophisticatedly regulated while keeping a balance between the effectors and the regulators, and as a result, the homeostasis of the gut is maintained. A lot of evidences indicate that mucosal immunity is dysregulated in the bowel of IBD^[50]. For the development of intestinal inflammation, leukocyte trafficking to the gut is an important step^[2]. Myeloperoxidase is an enzyme found in cells of myeloid origin, and has been used extensively as a biochemical marker of granulocyte (mainly neutrophil) infiltration into gastrointestinal tissues^{[22]; [5]}.

Our study showed that acetic acid raised the levels of colonic MPO, which was ameliorated in ginger

extract and sulfasalazine treated groups. Activation of MPO indicates infiltration of neutrophils and perturbation of the inflammatory system^[33], which is documented both in animal models^{[42];[5]; [51]} and patients with IBD^[43]. The neutrophils may contribute to the production of ROS via activation of their NADPH oxidase and secretion of MPO into extracellular space. Although we did not measure NADPH oxidase activity in our experiment, it was speculated that this enzyme could also be activated by acetic acid enema since NADPH oxidase and MPO are two enzymes that are activated almost simultaneously^[52]. In this study, however, the amount of MDA was associated with catalase activation and neutrophilic myeloperoxidase activity, which suggests a hydrogen peroxide (H₂O₂) - and/or hypochlorous acid (HOCl)-mediated mechanism^[43].

Under normal situations, the intestinal mucosa is in a state of 'controlled' inflammation regulated by a delicate balance of proinflammatory (tumour necrosis factor [TNF]-alpha, interferon [IFN]-gamma, interleukin [IL-1, IL-6, IL-12) and anti-inflammatory cytokines (IL-4, IL-10, IL-11). The mucosal immune system is the central effector of intestinal inflammation and injury, with cytokines playing a central role in modulating inflammation^{[18];[53]}. TNF- α is a proinflammatory cytokine which is abundantly expressed in the gut of IBD patients^[53], this information is evidenced in this work.

Promotion of TNF- α is mediated by a new transcription factor termed lipopolysaccharide-induced TNF-alpha factor (LITAF)^[54]. This factor was shown

to mediate TNF-alpha expression in human macrophages by direct binding to specific sequences in the promoter region of the TNF-alpha gene. LITAF is readily detectable in ileal and colonic tissues from patients with either CD or UC. It is significantly elevated above controls, and is localized to macrophages, a major source of TNF-alpha^[54].

Another inflammatory mediator, viz., PGE₂ was increased in rats with ulcerative colitis caused by acetic acid installation rectally, this goes with the fact that high amounts of this mediator are detected in the inflamed mucosa of patients with inflammatory bowel disease (IBD)^[55]. The increased level of PGE₂ is attributed to enhanced synthesis rather than reduced catabolism as reported by Otani et al.^[55]. They found also that TNF- α affects the expression of 15-hydroxyprostaglandin dehydrogenase (15-PGDH), which plays a major role in the catabolism of PGE₂. TNF- α suppressed the transcription of *15-PGDH* in human colonocytes, resulting in reduced amounts of 15-PGDH mRNA and protein and enzyme activity. In contrast, TNF- α induced two enzymes, cyclooxygenase-2 and microsomal prostaglandin E synthase-1 that contribute to increased synthesis of PGE₂. Overexpressing 15-PGDH blocked the increase in PGE₂ production mediated by TNF- α .^[55] Our results show increased levels of both TNF- α and PGE₂, which are known to produce epithelial cell necrosis, edema, neutrophil infiltration and, global cell depletion, effects which are documented by the histopathological study of this work.

Ginger extract (*Zingiber officinale Roscoe*) used in this study significantly reduced the wet weight of distal colon segments and the gross lesion score, compared with controls that received the vehicle. Further, they effectively reduced the histological signs of inflammation such as leukocyte infiltration, edema, and tissue injury and improved the acetic acid-induced biochemical alterations both in redox parameters and inflammatory ones. Ginger is ranked one of the plants with highest antioxidant values^{[56]; [26]}, thus, one of the mechanisms involved could be its antioxidant properties and its ability to inhibit free radical generation, properties which can guard against cellular macromolecules attack and depletion of the colon antioxidant defense systems.

The antiinflammatory properties of *Z. officinale* present in our study offers another mechanism of action to this plant. This action was reported previously in other inflammatory models^{[57]; [58]; [46]; [59]}. In these studies, ginger extract affected the level of TNF- α and PGE₂^[57], reported that ginger extract significantly inhibited the activation of TNF- α and COX-2 expression in human synoviocytes and suppressed production of TNF- α and PGE-2. Using the *in vitro* assay, these authors proved that ginger extract blocks activation of proinflammatory mediators and its transcriptional regulator suggesting its mode of action.

This study showed that the antioxidant and antiinflammatory properties of ginger extract are comparable to those reported for sulfasalazine^{[15]; [4]; [60]}. These

observations indicate that ginger extract could be beneficial as a complementary agent and offers an alternative approach to modulate the inflammatory process involved in IBD.

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التأثير المحسن لخلاصة الزنجبيل لالتهاب القولون التقرحي المحدث في الجرذان

التهاب القولون التقرحي من الأمراض المزمنة متكررة الحدوث وغير معروفة السبب. تهدف الدراسة تقييم فاعلية خلاصة الزنجبيل للحد من التهابات القولون التقيحية المحدثة بواسطة الحقن الشرجي لحمض الخليك (٤%). اشتملت الدراسة علي ٤٨ من الجرذان تم تقسيمهم إلي ٦ مجموعات، المجموعة الأولى هي المجموعة الضابطة وتم حقنها عن طريق الشرج بمحلول الملح، المجموعة الثانية وتم حقنها بحمض الخليك، والمجموعات من الثالثة إلي السادسة فتم إعطائهم عن طريق الفم خلاصة الزنجبيل (١٠٠، ٢٠٠، ٤٠٠ مللجرام/كج) وحقار السلفاسلازين (٥٠٠ مللجرام/كج) ٣ أيام قبل و ٧ أيام بعد حقنهم بحمض الخليك عن طريق الشرج. تم قياسه المؤشر الالتهابي التقرحي للقولون بواسطة الهيستوباثولوجي وبعض القياسات البيوكيميائية مثل قياس مستوى إنزيم المايلوبيروكسيدياز والإنزيمات المضادة للأكسدة (الكتاليز والسوبر أكسيد ديسميوتاز) ومستوي الجلوتاثيون وفوق أكسدة الدهون ومحتوي الكربونيل للبروتين ومعامل التكرز التورمي ألفا (TNF- α) وبروستاجلاندين-٢٥ (PGE2) في أنسجة القولون. أظهرت النتائج أن حقن الجرذان بحمض الخليك يؤدي الي التهاب تقرحي في القولون أدى إلي زيادة جوهريه في معامل التكرز التورمي ألفا (TNF- α) وبروستاجلاندين-٢٥ (PGE2)، وفوق أكسدة الدهون ومحتوي الكربونيل للبروتين ونشاط إنزيم المايلوبيروكسيدياز والي انخفاض في مستوي الجلوتاثيون والإنزيمات المضادة للأكسدة (الكتاليز والسوبر اكسيد ديسميوتاز). وأثبتت النتائج أن إعطاء الجرذان خلاصة الزنجبيل ٣ أيام قبل و ٧ أيام بعد الحقن بحمض الخليك أدت إلي انخفاضاً جوهرياً في مؤشرات الالتهاب مثل معامل التكرز التورمي ألفا والبروستاجلاندين-٥ وإنزيم المايلوبيروكسيدياز وفي مؤشرات الإجهاد التأكسدي مثل فوق أكسدة الدهون ومحتوي الكربونيل للبروتين. كما أعطت زيادة ذو دلالة إحصائية في

مستوي الجلوتاثيون والإنزيمات المضادة للأكسدة. ولهذا من الممكن أن نستخلص من هذا البحث أن إعطاء خلاصة الزنجبيل عن طريق الفم يؤدي إلي حماية القولون من الالتهابات التقرحية المزمنة وأن هذه الحماية لخلاصة الزنجبيل ترجع إلي احتوائها علي مضادات للأكسدة.