The Possible Protective Effect of Ginger Against Intestinal Damage Induced by Methotrexate in Rats

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Abstract

Methotrexate (MTX) is widely used in treatment of malignant tumors and autoimmune diseases. Gastrointestinal toxicity is an important factor limiting its use. This study was performed to assess the possible protective effect of ginger on MTX-induced intestinal damage and the mechanisms involved. Forty adult rats were randomly assigned into 4 Groups: Control group, Ginger group (200mg/kg/d, orally), MTX group (single dose, 20mg/kg/i.p.) and MTX+ ginger group (pretreated with ginger 3 days before and after MTX administration). At the end of the treatment period, rats were sacrificed, and jejunal tissue samples were taken for biochemical, histological and immunohistochemical assessment. Data showed that ginger produced significant decrease in lipid peroxidation product malondialdehyde (MDA) with significant increase in glutathione content (GSH), and antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) levels compared to MTX group. Also, pretreatment with ginger resulted in significant decrease in intestinal levels of myeloperoxidase (MPO) and pro-inflammatory cytokines (TNF-α and IL-1β) levels. The total microscopic damage score of MTX+ ginger-treated rats was found to be significantly reduced compared to the MTX group. Additionally, a significant decrease in enterocyte apoptosis in the jejunum of MTX+ ginger rats was accompanied by decreased Bax protein and increased Bcl-2 protein expression. The results of this study demonstrated that administration of ginger powder protects the jejunal mucosa from damage caused by MTX. Ginger was shown to reduce oxidative stress and lessen inflammation and apoptosis in the jejunal tissues of rats with MTX-induced mucositis.

Key Words: Ginger – MTX – Mucositis – Intestinal damage – Oxidative stress – Apoptosis.

Introduction

INTESTINAL mucositis is a dose limiting side-effect of cancer chemotherapy, which leads to decreased absorption of nutrients, increased epithelial permeability, recurrent diarrhea, and weight loss [1]. Methotrexate (MTX), a folate antagonist agent, is mainly used in the treatment of malignant tumors; it has also been found to have a major therapeutic role in non-neoplastic diseases as an anti-inflammatory and immunosuppressive agent. MTX is a well-known cause of intestinal mucositis, which impairs rapidly dividing cells, such as epithelial stem cells within intestinal crypts, thereby causing diminished enterocyte replacement [2]. It has previously demonstrated that MTX administration in rats causes villus atrophy with consequent reduction of the overall mucosal absorptive surface area [3].

MTX induced small intestinal damage is characterized by marked inflammation and increased production of reactive oxygen species and therefore increased oxidative stress [4]. Huang et al., [5] demonstrated that MTX-induced apoptosis in the small intestine which is reactive oxygen species (ROS)-dependent and occurs along a mitochondria-mediated pathway. Based on this result, numerous studies aiming to prevent MTX-induced damage by using antioxidant agents such as N-Acetylcyesteine [6], lactoferrin [7], melatonin [8], prostaglandin E1 [9] and garlic extract [10] revealed beneficial effects.

Plant derived products have been used for medicinal purposes for centuries and also being used in our daily food intake. Focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems [11].

Ginger (Zingiber officinale Rosc.) has been cultivated for thousands of years as a spice and for medicinal purposes [12]. It has been used as an ingredient of Chinese traditional medicine for
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thousands of years [13]. The major pungent constituents of ginger, 6-gingerol and 6-shogaol, have been shown to have many interesting pharmacological effects, such as antithrombotic [14], antioxidant, antitumor promoting and anti-inflammatory effects [15,16]. Ginger has staring potential for treating a number of ailments including degenerative disorders (arthritis and rheumatism), cardiovascular disorders (atherosclerosis and hypertension), vomiting, diabetes mellitus, and cancer. Also, it has antimicrobial potential as well which can help in treating infectious diseases [17-19]. Furthermore, it has been used to treat a number of medical conditions, affecting the digestive tract such as dyspepsia, flatulence, nausea and abdominal pain [20]. Aromatic, spasmylytic, carminative and absorbent properties of ginger are probably responsible for the therapeutic applications in digestive tract ailments [21]. Ginger is generally considered a safe herbal medicine with only few and insignificant adverse/side effects [22].

Taking into consideration the potential clinical use of MTX and the numerous health benefits of ginger, the present work was planned to explore whether ginger has a possible protective effect on MTX- induced intestinal injury.

Material and Methods

Animals:

Adult male Sprague-Dawley rats weighing 200-250g were purchased from Helwan farm (VAC-SERA), Egypt. The animals were housed (4 per cage) in the animal facility of the Pharmacology Department, Faculty of Medicine, Benha University, Egypt, during June 2013 and left for one week before beginning the experiment for acclimatization. Rats were kept under the standard laboratory conditions (12h light/dark cycles at 25 ±2°C) with free access to standard balanced diet and freshwater supply.

Drugs:

Methotrexate (Ebewe Pharma, Austria), 50mg in 5ml was dissolved in normal saline. Ginger pure powder (Sigma), was suspended in 0.5% carboxymethylcellulose (CMC) in distilled water (vehicle) so that 1ml of the vehicle contained the desired dose. All drugs were freshly prepared immediately just before administration.

Experimental design:

A total of 40 rats were randomly assigned into four groups, 10 rats in each. The total duration of the experiment was 6 days. MTX was injected intraperitoneally (i.p.) at a single dose on day 3 of the experiment. Ginger was administered for 6 days to the second and forth groups, 3 days before and after MTX injection.

Control group: Received 1ml of saline (the vehicle of MTX) intraperitoneally once and 1ml of 0.5% CMC in water (the vehicle of ginger) by gavage for 6 days.

Ginger group: Received ginger powder (200 mg/kg, orally) [23], suspended in 1ml of 0.5% CMC in water for 6 days. Rats also received single dose of saline (1ml, i.p.) on day 3 of the experiment.

MTX group: Received a single dose of MTX (20mg/kg, i.p.). Dosage and route of administration of MTX were determined according to a model previously described [24]. Rats also received 1ml of 0.5% CMC in water orally for 6 days.

MTX+ ginger group: Received a single dose of MTX (20mg/kg, i.p.), and also received ginger (200mg/kg, orally) 3 days before and after MTX administration.

At the end of the experiment, all rats were anesthetized using urethane (0.6ml/100g body weight of 25% solution i.p.), and sacrificed by decapitation. The tissue samples of jejunum were taken from each animal for biochemical, histopathological and immunohistochemical studies.

Determination of body weight:

Body weight of all animals in each group was recorded using an electronic balance which is considered the initial body weight. At the end of the experiment, body weight was again recorded for all animals in each group.

Biochemical study:

The first portion of jejunum was washed two times with cold saline solution and homogenized using a tissue homogenizer in phosphate buffer saline (10% w/v). The homogenate was centrifuged for 10 minutes at 4°C to remove the cell debris. The clear supernatants were separated and used for determination of malondialdehyde (MDA, a biomarker of oxidative damage) according to the method of Uchiyama and Mihara [25], and reduced glutathione (GSH, a biomarker of protective oxidative injury) was also determined [26].

In addition, superoxide dismutase (SOD) was determined according to the method of Sun et al., [27] and catalase (CAT) activity was assayed using the method of Cohen et al., [28].
myeloperoxidase activity (MPO, an index of the degree of neutrophil accumulation and inflammation) was measured using a procedure similar to that documented by Hillegass et al., [29]. Evaluation of proinflammatory cytokines, tumor necrosis factor- alpha (TNF-α) and interleukin-1 beta (IL-1β) in rat small intestine cells homogenate were evaluated by ELISA assay according to manufacturer's instructions and as previously described [30,31].

**Histopathological study, including apoptotic scoring:**

The tissue samples of jejunum were fixed in 10% neutral buffered formalin and were embedded in paraffin. Sections of tissue were cut at 5-6 µm, mounted on slides, stained with hematoxylin and eosin (H & E) [32]. An overall score of intestinal damage severity was assessed in stained tissue sections as follows by scoring each of the following histological observations: A- Villus shortening and fusion. B- Epithelial atrophy. C- Crypt loss. D- Inflammatory infiltrate in the lamina propria and E- Goblet cell loss as 0, none; 1, mild; 2, moderate; 3, severe. Thus, the maximum total score was 15, [6]. A total of 10 fields of section were examined per animal.

In hematoxylin and eosin-stained sections, apoptotic cells were identified under light microscopy based on its morphology including nuclear fragmentation (karyorrhexis) and cell shrinkage with condensed nuclei (pyknosis) [33]. The apoptotic cells were counted as the mean of cells in 3 visual fields of one section.

**Immunohistochemical study:**

The jejunum sections were fixed in neutral buffered formalin and processed for preparation of 5 µm paraffin section slides. Immunohistochemical technique for Bcl2 and Bax expression were performed using labeled streptavidin biotin technique according to the manufacturer’s guidelines. Bax and Bcl2 staining was cytoplasmic and shown as brown granules. Monoclonal Bcl-2 (Biovision, USA) and monoclonal bax (Santa Cruz Biotechnology, USA) antibodies were employed in immunohistochemical staining. The positive cells were counted in 100 adjacent epithelial cells and repeated in three high power fields, and the total number of positive cells was expressed as a percentage of 300. The staining was scored as 0, negative; 1, 1-5%; 2, 6-15%; 3, 16-100% [34].

**Statistical analysis:**

All data were expressed as Mean±Standard error of mean (S.E.M.). The statistical analysis of data was done by using SPSS (SPSS, Inc, Chicago, IL) program statistical package for social science version 16. One-way analysis of variance (ANOVA) was used to determine statistically significant differences among the groups, and means of every two different groups were compared with Student's t-test. p<0.05 was considered statistically significant.

**Results**

**Body weight changes:**

The means of initial and final body weight in all groups are summarized in Table (1). The results showed that there was no statistical difference in initial body weight between all groups (p>0.05). Comparison of the final body weight with the initial body weight in all groups revealed a significant (p<0.05) increase in control and Ginger groups and significant (p<0.05) decrease in MTX- treated rats while MTX+ ginger-treated rats showed a non-significant decrease in final body weight (p>0.05).

The data also revealed that the animals treated with MTX showed significant decrease in final body weight in comparison with control or ginger-treated rats (p<0.001). On the other hand, the final body weight of MTX+ ginger group was significantly increased (p<0.05) when compared to MTX-treated rats and significantly decreased (p<0.05) when compared to control group.

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<tr>
<td></td>
<td>Body weight (g)</td>
<td>Groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control group</td>
<td>Ginger group</td>
<td>MTX group</td>
</tr>
<tr>
<td>Initial</td>
<td>245.73±5.32</td>
<td>241.77±4.35</td>
<td>246.28±7.35</td>
<td>244.40±5.4</td>
</tr>
<tr>
<td>Final</td>
<td>266.76±8.4</td>
<td>261.24±6.78</td>
<td>224.20±6.2*</td>
<td>243.63±5.9†</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SE, number 10 rats for each group.

* Significant compared to Control group.

† Significant compared to MTX group.
Biochemical results:

Tissue malondialdehyde (MDA) level:

The level of MDA was found to be significantly ($p<0.0001$) higher in the MTX- treated group when compared with control group. MTX+ ginger group showed a significant ($p<0.05$) decrease in MDA level when compared to MTX group and non-significant ($p>0.05$) difference when compared to control rats. Ginger treatment alone had no effect on MDA (Table 2).

Reduced glutathione content (GSH) and antioxidant enzymes level (superoxide dismutase (SOD) and catalase (CAT)):

As shown in Table (2), administration of MTX significantly ($p<0.05$) reduced the GSH level in the jejunum tissue as compared to control group. However, reduced GSH content was significantly ($p<0.05$) increased in the group pretreated with ginger as compared to MTX group. At the same time, MTX+ ginger group showed a non significant difference ($p>0.05$) when compared to control group.

On measuring the enzyme activity of SOD; the data recorded a significant ($p<0.05$) decrease in MTX- treated rats compared with control group. Also, there was a significant ($p<0.05$) improvement in MTX+ ginger group as compared to MTX group and non-statistically ($p>0.05$) difference when compared to control rats.

The current work revealed that CAT activity was significantly ($p<0.01$) attenuated in MTX- treated rats compared to control rats. However, a significant ($p<0.05$) increase in CAT activity was observed in MTX+ ginger-treated rats as compared to MTX group. At the same time, MTX+ ginger-treated rats showed non-significant difference ($p>0.05$) when compared to control group. Ginger alone did not cause any significant ($p>0.05$) alteration in GSH, SOD or CAT compared to control group.

Tissue myeloperoxidase (MPO) activity:

As depicted in Fig. (1), tissue MPO activity was found to be significantly ($p<0.0001$) increased in MTX- treated rats when compared to the control group. However, pretreatment with ginger to MTX- treated rats produced a significant ($p<0.001$) reduction in MPO activity as compared to MTX group but still significantly ($p<0.001$) higher than the control value.

Table (2): Effect of ginger pretreatment on malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) in the jejunum segment of rats exposed to MTX- induced intestinal mucositis.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MDA (nmol/mg)</td>
</tr>
<tr>
<td>Control group</td>
<td>0.5±0.1</td>
</tr>
<tr>
<td>Ginger group</td>
<td>0.4±0.3</td>
</tr>
<tr>
<td>MTX group</td>
<td>1.71±0.2*</td>
</tr>
<tr>
<td>MTX+ ginger group</td>
<td>0.8±0.3†</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ±SE, number 10 rats for each group.

* Significant compared to Control group.
† Significant compared to MTX group.

Results are expressed as Mean ±SE (n=10 rats/group).

* Significant compared to Control group.
† Significant compared to MTX group.

Fig. (1): Effect of Ginger pretreatment on myeloperoxidase in jejunal tissue of MTX- induced intestinal injury.
Tumor necrosis factor-alpha (TNF-α) concentration:

Fig. (2A) demonstrates that MTX-treated rats showed a significant \((p<0.001)\) increase in intestinal TNF-α level compared to control group. Pretreatment of the MTX-treated rats with Ginger abolished this increase and resulted in a significant reduction \((p<0.001)\) in the level of intestinal TNF-α as compared to those in MTX-treated rats only. There was a significant difference \((p<0.001)\) between MTX+ ginger treated rats and control group.

Interlukin-1 beta (IL-1β) level:

The concentration of IL-1β was significantly \((p<0.0001)\) increased after the administration of MTX compared to those seen in the control rats. At the same time, pretreatment with ginger to MTX-treated rats attenuated this increase significantly \((p<0.01)\) when compared to MTX group with a significant \((p<0.0001)\) difference when compared to control group (Fig. 2B).

Histopathological results:

As shown in Fig. (3A), jejunal sections of control group revealed no significant pathological changes, where the jejunal mucosa showed long slender, finger like villi. Each villus had a core of loose connective tissue (extending from the lamina propria) and a covering of tall columnar epithelium with goblet cells. The lamina propria, forming the villus core contained a small lacteal vessel and was separated from the epithelium by a thin well defined basement membrane. Intestinal crypts of Lieberkühn extended from the bases of villi into the lamina propria. The tall columnar epithelial cells (enterocytes) exhibited eosinophilic cytoplasm and basophilic basal oval nuclei. The luminal surface of the villi was covered by striated (brush) border. The crypt epithelium demonstrated paneth cells among the enterocytes, which appeared pyramidal with basal rounded nuclei and apical eosinophilic granules. Light microscopic examination of the jejunum in ginger group showed normal morphology similar to that of the control group (Fig. 3B).

In the jejunal specimens of the MTX-treated rats, shortening of villus or fusion of villi and atrophy were noticed. The surface epithelium of some villi was severely distorted and those of others was markedly detached and completely separated from the underlying lamina propria. Some areas of lamina propria showed cellular inflammatory infiltration, interstitial edema, crypt loss and marked absence of goblet cells (Figs. 4-6). Apoptotic cells were also detected (Fig. 7).

The pathological changes of the jejunum of MTX+ ginger group were markedly attenuated and showed more or less normal appearance when compared to MTX-treated rats. The surface epithelium of some villi was intact and continuous but others still showed slight epithelial detachment. Some cellular infiltration and small areas of hemorrhage were detected (Fig. 8). The total microscopic score of MTX+ ginger-treated rats was found to be significantly \((p<0.05)\) reduced \((6.83 \pm 1.04)\) when compared to the MTX group \((13.29 \pm 2.43)\). Histological intestinal damage scores of all groups are summarized in Table (3).

Apoptosis assessment revealed that the count of apoptotic cells in control and Ginger groups
was low. Compared to the control group, MTX-treated rats tended to have an elevated number of apoptotic cells with a significant difference \((p<0.0001)\). Pretreatment of rats with ginger resulted in a significant \((p<0.0001)\) decrease in apoptotic cells when compared to MTX group and a significant \((p<0.0001)\) increase when compared to control group (Table 3).

Table (3): Scoring values of the jejunal damage in the histopathological examination.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group</th>
<th>Ginger group</th>
<th>MTX group</th>
<th>MTX+ ginger group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Villus damage</td>
<td>0.0±0.0</td>
<td>0.1±0.0</td>
<td>2.75±0.26*</td>
<td>1.10±0.18*†</td>
</tr>
<tr>
<td>Crypt damage</td>
<td>0.2±0.0</td>
<td>0.1±0.0</td>
<td>2.63±0.38*</td>
<td>1.15±0.22*†</td>
</tr>
<tr>
<td>Epithelial atrophy</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>2.38±0.11*</td>
<td>1.7±0.15*†</td>
</tr>
<tr>
<td>Cellular infiltration</td>
<td>0.3±0.0</td>
<td>0.4±0.0</td>
<td>2.95±0.27*</td>
<td>1.25±0.16*†</td>
</tr>
<tr>
<td>Goblet cell depletion</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>2.58±0.25*</td>
<td>1.63±0.26*†</td>
</tr>
<tr>
<td>Total score of damage</td>
<td>0.5±0.0</td>
<td>0.6±0.0</td>
<td>13.29±2.43*</td>
<td>6.83±1.04*†</td>
</tr>
<tr>
<td>Apoptotic cell count</td>
<td>41.97±1.1</td>
<td>40.15±0.6</td>
<td>82.76±2.6*</td>
<td>57.15±0.8*†</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ±SE, (n=10/group).
* Significant compared to Control group.
† Significant compared to MTX group.
Fig. (6): Photomicrograph of section of jejunum of MTX-treated rats showing mucosal sloughing, loss of normal villus structure and small areas of hemorrhage (H & E x200).

Fig. (7): Photomicrograph of section of jejunum of MTX-treated rats showing apoptotic cells (arrows) and inflammatory infiltrate in lamina propria (H & E x400).

Fig. (8): Photomicrograph of section of jejunum of MTX+ Ginger treated rats showing preservation of normal height of the villi with small areas of hemorrhage in lamina propria (H & E x200).

Immunohistochemical results:

Immunohistochemistry was used to examine distribution of Bcl-2 family members in the jejunum of experimental rats. The Bax and Bcl-2 positive staining was located in the cytoplasm of intestinal epithelial cells. Goblet cells did not stain positively for any protein investigated. Bcl-2 staining was observed in the cytoplasm of villus epithelial cells and in the lamina propria but was almost negligible in the crypts. Bax staining was observed in the cytoplasm of villus and crypts. Sections of jejunal tissues of control and Ginger groups stained immunohistochemically for Bax and Bcl-2 delineated the normal distribution of Bax and Bcl-2 (Figs. 9A, 10A).

As shown in Table (4), Bax expression was up-regulated in jejunum ($p<0.001$) of MTX-treated rats compared to control animals. Pretreatment of MTX-treated rats with ginger attenuated the pro-apoptotic effects of MTX. MTX+ ginger rats showed a significant ($p<0.001$) decrease in Bax expression in the jejunum compared to MTX group with a significant ($p<0.05$) increase when compared to control rats (Figs. 9B-D). On the other hand, treatment with MTX resulted in a significant ($p<0.001$) down-regulation of Bcl-2 in jejunum compared to control group. MTX+ ginger rats showed a significant ($p<0.05$) increase in Bcl-2 expression in jejunum compared to MTX- animals with a significant ($p<0.001$) decrease when compared to control rats (Fig. 10B-D).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control group</th>
<th>Ginger group</th>
<th>MTX group</th>
<th>MTX+ ginger group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bax</td>
<td>0.43±0.5</td>
<td>0.48±0.7</td>
<td>2.75±0.19*</td>
<td>1.85±0.11 *†</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>1.93±0.23</td>
<td>1.86±0.17</td>
<td>0.47±0.20*</td>
<td>0.95±0.11 *†</td>
</tr>
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</table>

Values are expressed as Mean ± SE, (n=10/group).

* Significant compared to Control group.
† Significant compared to MTX group.
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**Fig. (9):** Immunohistochemical staining of Bax in jejunum of the experimental rats from (A) Control group and (B) Ginger group; (C) MTX group showing a significant increase in immunoreactivity in the cytoplasm of the cells when compared to control or ginger group; (D) MTX + ginger group demonstrating a significant reduction in Bax immunostaining when compared to MTX group. Brown color indicates Bax positivity (Immunostaining x400).

**Fig. (10):** Immunohistochemical staining of Bcl2 in jejunum of the experimental rats from (A) Control group and (B) Ginger group; (C) MTX group showing a significant decrease in immunoreactivity in the cytoplasm of the cells when compared to control or ginger- treated rats; (D) MTX + Ginger group demonstrating a significant increase in Bcl-2 immunostaining when compared to MTX group. Brown color indicates Bcl-2 positivity (Immunostaining x400).
Discussion

Methotrexate (MTX) is widely used as a chemotherapeutic agent. It is currently the most common anti-rheumatic drug prescribed for the treatment of rheumatoid arthritis and other rheumatic disorders. However, one of the major toxic effects of MTX is intestinal injury and enterocolitis [35]. The small intestinal damage induced by MTX treatment results in malabsorption and diarrhea [36].

In the present study MTX treatment in rats resulted in significant weight loss which implies the presence of mucosal injury and malabsorption. In accordance with this, the morphological appearance of the intestinal mucosa revealed marked villous shortening and fusion, epithelial atrophy, crypt loss, inflammatory infiltrate, and goblet cell depletion. Our findings were similar to other studies where MTX has been reported to cause severe damage in the small intestine [37,38]. The inflamed intestine demonstrates the presence of oxidant stress, as evidenced by enhanced lipid peroxidation (as indicated by increase in MDA content and reduction in reduced glutathione). It is well established that depletion of reduced glutathione in tissues promotes oxidative stress and tissue injury [39]. Thus significant decrease in reduced glutathione level promoted by MTX, leads to a reduction of effectiveness of the antioxidant enzyme defense system, thereby sensitizing the cells to reactive oxygen species (ROS) [40]. Many studies have shown a decrease in the level of reduced glutathione in the small intestine following MTX administration [8,41,42]. With respect to the activities of other antioxidant enzymes, superoxide dismutase and catalase, a significant decrease in the activities of both enzymes was observed in the present study. Many studies also support the view that oxidative stress plays a role in MTX-induced small intestinal damage [6,42]. Reactive oxygen species trigger the accumulation of leukocytes in the tissues, and thus aggravate tissue injury indirectly through activated neutrophils. The activated neutrophils secrete myeloperoxidase (MPO) and other proteases [43]. In turn, MPO plays important role in oxidant production by neutrophils. In the current study a marked elevation in MPO activity was observed after MTX treatment of rats, indicating that neutrophil accumulation contributes to MTX-induced small intestinal damage.

In this study, the administration of Ginger powder significantly enhanced intestinal recovery following MTX-induced damage. This is evident from the significant decrease in MTX-induced body weight loss indicated that ginger reserves the mucosal function. Histologically, ginger-treated rats showed more preserved architecture as well as the presence of newly formed crypts and regeneration. In addition, ginger significantly increased the levels of reduced glutathione and the antioxidant enzymes (i.e., catalase and superoxide dismutase) and decreased MDA content and MPO activity. These results were in accordance with other published studies [44-46] in which ginger was demonstrated to be a strong antioxidant. Its antioxidant activity has been attributed to its major active phenolic ingredients (e.g., zingerone, gingerdiol, zingibrene, gingerols and shogaols). In addition, the administration of ginger has been shown to improve oxidative stress by decreasing lipid peroxidation and protein oxidation as free radical-generating sources and elevating the levels of enzymes implicated in the antioxidant defense system. A study by Ahmed et al., [47] showed that Ginger has an equal antioxidant effect to that of ascorbic acid. Animal modeling showed that ginger significantly lowered induced lipid peroxidation and raised the levels of antioxidant enzymes, together with serum glutathione. In one study, ethanol extract of zingiber officinale alone and in combination with vitamin E partially ameliorated cisplatin-induced nephrotoxicity. This protection is mediated by renal antioxidant defense system [48]. In the other study, the protective effect of the ginger extract was examined on carbon tetrachloride (CCl₄) and acetaminophen-induced liver damage and indicated that ginger could be useful in preventing acute liver injury [49].

Cell loss in the small intestine MTX-induced mucositis is mainly regulated by programmed cell death. In addition, oxidative stress is known to induce apoptosis by damaging DNA, oxidizing membrane lipids, and/or directly activating the expression of the genes/proteins responsible for apoptosis [50,51]. Our results showed that the intrinsic pathway, with its regulation by the Bcl-2 family of proteins, was altered by MTX consistent with changes in cell apoptosis: The expression of the pro-apoptotic protein Bax increased, while those of the anti-apoptotic Bcl-2 protein decreased. These changes correlate with the enhanced enterocyte apoptosis during MTX-induced mucositis. In consistent with our results, Verburg et al., [52] found that MTX induced apoptosis at days 1 and 2 after MTX treatment in the proliferative region of small intestinal crypts, as judged by morphological criteria and TUNEL staining.
In this study, results showed that rats treated with Ginger powder had a decrease in apoptosis in the injured intestinal tissues, as the expression of protein levels of the pro-apoptotic Bax decreased, while those of the anti-apoptotic Bcl-2 protein levels increased suggesting increased enterocyte survival. Therefore, the inhibitory effect of ginger on MTX-induced ROS production may be the underlying mechanism for its protective effect against apoptosis, which plays a role in the pathogenesis of MTX-induced mucositis.

In accordance with our results, Lee et al., [53] reported that (6)-gingerol protected against β-amyloid-induced cytotoxicity and apoptotic cell death, such as DNA fragmentation, disruption of mitochondrial membrane potential, elevated Bax/Bcl-2 ratio, and activation of caspase-3, by inhibition of intracellular accumulation of reactive oxygen species and/or reactive nitrogen species and subsequent oxidative and/or nitrosative damages. Recently, Kim and Kwon [54], suggested that following oxidative stress, (6)-shogaol protects astrocytes from oxidative damage and apoptosis by attenuating the impairment of mitochondrial function proteins such as Bcl-2 and Bcl-xL. Additionally, (6)-shogaol inhibits the expression of the apoptotic proteins Bax and caspase-3 in hydrogen peroxide-treated astrocytes.

In the current study the intestinal inflammatory response appeared higher in MTX-treated rats than in control, since we observed an increase in the inflammatory cellular infiltration histologically. Furthermore, the levels of TNF-α and IL-1β in the jejunal mucosa were significantly increased. It has been suggested that proinflammatory cytokines, e.g., TNF-α and IL-1β, may be involved in the amplification phase of intestinal mucositis [58] but that inflammation may be the functional consequence of the weakened barrier function, i.e., weakened epithelial integrity (atrophy and altered protein metabolism) and altered mucus protection. Alamir et al., [56] found that intramucosal concentration of proinflammatory cytokines, interleukin-β and cytokine-induced neutrophil chemoattractant, was markedly increased in methotrexate-treated rats.

Our results revealed that Ginger decreased the level of TNF-α and IL-1β significantly in the jejunal mucosa. The effect of inhibition of TNF-α production by the ginger extract was earlier reported in synoviocytes by Frondoza et al., [57]. Extensive studies in recent years have displayed that ginger and its pungent ingredients exhibit anti-inflammatory responses. Gingerol, shogaol, and other structurally-related substances in Ginger can inhibit synthesis of pro-inflammatory cytokines such as IL-1, TNF-α, and IL-8 [58]. In another investigation, Pan et al., [59] showed that in macrophages, (6)-shogaol can down-regulate inflammatory inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) gene expression. Jung, et al., [60] indicated that rhizome hexane fraction extract of Z. officinale inhibited the excessive production of NO, PGE2, TNF-α, and IL-1β. In addition, Habib et al., [61] showed that Ginger extract can reduce the elevated expression of NF κB and TNF-α in rats with liver cancer. Ko and Leung, [45] reported that ginger extract mainly inhibits the expression of the chemokines and to some extent TNF-α. It significantly reduces the gastric ulcer area in a dose-dependent manner, with concomitant attenuation of the elevated activities of xanthine oxidase and myeloperoxidase, as well as malondialdehyde level in the ulcerated mucosa. Studies by Nonn et al., [62] have shown that (6)-gingerol inhibited the TNF-α, and IL-1ß-induced increase in the p38-dependent NF-κB activation and expression of pro-inflammatory genes of IL-6 and IL-8 in normal prostatic epithelial cells. Our results are in accordance with these studies and suggest an effect of ginger on inflammatory processes at cell level.

**In conclusion:**

Our findings provide evidence that Ginger may be used to protect against severe intestinal injury induced by MTX. The possible mechanisms of ginger effects in attenuating tissue injury and apoptosis in the intestine involves the anti-inflammatory and anti-oxidant actions of ginger, including reduced proinflammatory cytokines (TNF-α and IL-1β) and increased antioxidant enzyme activities, decreased oxidative stress and lipid peroxidation, additionally, lessened neutrophil activation. Therefore, further studies are required to establish its clinical application.

**Acknowledgement:**

Deepest thanks to Dr. Ghada A. Abd-Elfattah, Lecturer of Pathology, Faculty of Medicine, Benha University for her participation in performing the histopathological part of this study.

**References**


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