Protective effect of turmeric extract on ethotrexate-induced intestinal damage and oxidative stress

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[ABSTRACT] AIM: The most important side effect of methotrexate (MTX) is mucositis. The purpose of this study was to evaluate the effect of turmeric extract on intestinal damage and oxidative stress in rats receiving methotrexate. METHODS: Experiments were performed on male Wistar albino rats divided into six groups. First group received normal saline orally, the second group received turmeric extract (100 mg·kg⁻¹) orally for 30 days, the third group received turmeric extract (200 mg·kg⁻¹) orally for 30 days, the fourth group received a single dose of methotrexate (20 mg·kg⁻¹) i.p. at day 30, the fifth group received turmeric extract (100 mg·kg⁻¹) orally for 30 days and a single dose of methotrexate (20 mg·kg⁻¹) i.p. at day 30, and the sixth group received turmeric extract (200 mg·kg⁻¹) orally for 30 days and single dose of methotrexate (20 mg·kg⁻¹) i.p. at day 30. Four days after methotrexate injection, animals were anesthetized, blood samples were taken to determine total antioxidant status (TAS) and jejunum samples were taken for glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), aldehyde malondialdehyde (MDA), and histopathological assessment. RESULTS: Microscopic evaluation from intestinal tissues of the MTX treated group, showed severe villus shortening and blunting, inflammatory cell infiltration and hemorrhage in lamina propria, along with epithelial cell necrosis. Levels of SOD, GSH-Px and CAT decreased in the MTX received group, but increased significantly (P < 0.05) in the turmeric + MTX groups. MTX increased lipid peroxidation, however, turmeric decreased peroxidation significantly (P < 0.05). CONCLUSION: These results suggest that turmeric extract may protect the small intestine of rats from methotrexate-induced damage. Turmeric effects could result from its antioxidant properties.

[KEY WORDS] Turmeric; Methotrexate; Intestinal damage; Oxidative stress


1 Introduction

Cancer chemotherapy is a complicated process with lots of difficulties and a prolonged period. Unlike cancer surgical treatment, it incurs multiple side effects such as hair loss, nausea, vomiting and diarrhea. Methotrexate (MTX), an analogue of folic acid, is widely using as a cancer chemotherapeutic agent in the treatment of various malignancies [1]. However, in addition to cancer cells being affected by MTX, rapid proliferating cells such as bone marrow and gastrointestinal cells are also affected. One of the most important side effects of MTX is related to the gastrointestinal tract. Mucositis, nausea, diarrhea, vomiting and enterocolitis are the most important side effects observed following the use of MTX [1]. Recently, it has been demonstrated that MTX reduces the levels of oxidative enzymes and causes cells to be susceptible to react with oxygen [2]. Because of the MTX side effects, recent studies have focused on antioxidants. Some reports indicate a positive impact of MTX used in combination with antioxidants such as: vitamin A, garlic extract, N-acetyl cysteine, sodium tungstate and proanthocyanidins [3-5], but still...
the MTX side effects on the gastrointestinal system are challenging for clinicians.

Nowadays traditional natural medicine treatments, including the use of supplements and total extracts is common around the world. Increasing numbers of patients use medicinal plants or seek the advice of their physician regarding their use. Previous studies have shown that plant medications could be useful in ameliorating cancer chemotherapy side effects [6-8].

Turmeric is one of very powerful antioxidants that are found in abundance everywhere. It can be used against various diseases from cancer to extensive fibrosis [9]. The active ingredient of turmeric is called curcumin. Several studies indicated useful effects on different diseases related to oxidative stress [9-10, 28].

The purpose of this study was to evaluate the effect of turmeric extract on intestinal damage and oxidative stress in rats receiving methotrexate.

## 2 Materials and Methods

### 2.1 Animals

Experiments were performed on male Wistar albino rats weighing between 250 and 300 g, purchased from Razi Institute. Animals were housed in a temperature and humidity controlled environment under a 12-h light/dark cycle (lights on at 7 AM). Food and water were available ad libitum. The National Institutes of Health Guidelines for the Care and Use of Laboratory Animals were followed. All efforts were made to minimize the number of animals used and their suffering degree. The methods used in this investigation were approved by Ardabil University of Medical Sciences (approval number: 90374).

Forty-two rats were randomly divided into six groups, the first group was a control group and received normal saline orally for 30 days, the second group received turmeric extract (100 mg·kg⁻¹) orally by gastric gavage for 30 days [11], the third group received turmeric extract (200 mg·kg⁻¹) orally for 30 days, the fourth group single dose of methotrexate (20 mg·kg⁻¹) i.p. at day 30, the fifth group received turmeric extract (100 mg·kg⁻¹) orally for 30 days, and a single dose of methotrexate (20 mg·kg⁻¹) i.p. at day 30. Four days after methotrexate injection animals were anesthetized, blood samples were taken to determine total antioxidant status (TAS), and jejunal samples were taken to determine glutathione peroxidase (GPx), superoxide dismutase (SOD), aldehyde malondialdehyde (MDA), and histopathological assessment.

### 2.2 Chemicals

Methotrexate was purchased from EBEWE Pharma Ges, Unterrach am Atters, Austria. All chemical kits were utilized Randox® kits (Cat No. NX2332, Randox Laboratories, Ltd, Crumlin, UK). Dimethyl sulfoxide (DMSO), ethylene diamine tetracetic acid (EDTA) 3-(4, 5-dimethylthiazol-2-yl), ethanol, ethyl acetate, Tris HCl, 2-thiobarbituric acid (TBA) and other chemicals were obtained from Sigma Chemical Co. (St Louis, MO, USA). All chemicals used were of analytical grade.

### 2.3 Extraction method

*Curcuma longa* L. (Zingiberaceae) was purchased from a local market. The plant was identified by Ali Namvaran, herbal plants expert in Department of Pharmacology at Shiraz University, Iran and the voucher specimen has been deposited in the Herbarium of Ardabil University of Medical Sciences. Extract preparation was used according to the method [12] presented by Katiyar et al in 1996. The plant was thoroughly washed, air-dried and pulverized in a grinding mill to a powder. A sample (10 g) of the plant was weighed and macerated with 90% ethanol (100 mL) for 3 h at room temperature. The mixture was filtered frequently and centrifuged. The supernatant was evaporated by employing rotary under reduced pressure at 40 °C. The yield was 5% (% weight of turmeric powder). Finally, the extract was dissolved in PBS at different concentrations for turmeric (100 mg/0.2 mL, 200 mg/0.2 mL) [12].

### 2.4 Measurement of antioxidant status

#### 2.4.1 Measurement of total antioxidant status (TAS) level

Blood samples were centrifuged at 4 000 r·min⁻¹ for 10 min. Plasma total antioxidative level (TAS) was measured using an automated colorimetric version of the total antioxidant response (TAR) method described by Erel [13].

#### 2.4.2 Measurements of superoxide dismutase (SOD)

Obtained tissues were immediately frozen in liquid nitro-

### Table 1  Schematic diagram of the study protocol

<table>
<thead>
<tr>
<th>Groups</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of rats</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Treated protocol (for 30 days)</td>
<td>Saline</td>
<td>TUR</td>
<td>TUR</td>
<td>Saline</td>
<td>TUR</td>
<td>TUR</td>
</tr>
<tr>
<td>Drugs received (on day 30)</td>
<td>NaCl</td>
<td>NaCl</td>
<td>NaCl</td>
<td>MTX</td>
<td>MTX</td>
<td>MTX</td>
</tr>
<tr>
<td>MTX dose (mg·kg⁻¹·d⁻¹)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Turmeric dose (mg·kg⁻¹·d⁻¹)</td>
<td>–</td>
<td>100</td>
<td>200</td>
<td>–</td>
<td>100</td>
<td>200</td>
</tr>
</tbody>
</table>

Abbreviations: MTX: Methotrexate; TUR: Turmeric; NaCl: Isotonic saline solution (ISS)
gen and stored at –70 °C until laboratory tests were performed. The tissues were homogenized in 1.15% KCl solution and a 20% (W/V) homogenate was prepared. Homogenized intestinal tissues used to measure SOD activity were assayed by a spectrophotometric method based on the inhibition of a superoxide-induced reduced nicotinamide adenine dinucleotide (NADH) oxidation according to Paoletti et al [14].

2.4.4 Glutathione peroxidase (GSH-Px) activities

GSH-Px activity was determined in intestine tissues according to Paglia et al [15].

2.4.5 Catalase (CAT) activities

The method presented by Claiborne was used for CAT measurement [16].

2.4.6 Estimation of lipid peroxidation

Malondialdehyde (MDA) levels were assessed utilizing the thiobarbituric acid reactive substances (TBARS) method [17].

2.5 Histopathological analysis

Tissue samples of the jejunum (0.5 cm) from each animal were taken from the proximal end of the jejunum, fixed by 10% neutral formalin, embedded in paraffin and cut with a microtome set at a thickness of 5–6 mm. The tissue sections were stained with hematoxylin and eosin (H&E) for histopathological analysis and examined with a light microscope. The degree of jejunal tissue injury was evaluated semi-quantitatively by an expert pathologist blinded to the experiment, according to the method reported by Gulgun et al. (2010) [1]. Villus height shortening of the jejunum from each animal was scored as follows: 0, normal; 1, mild shortening; 2, moderate shortening; 3, severe shortening; and 4, villus absent. Ulceration was scored as follows: 0, normal; 1, epithelial desquamation; 2, total loss of epithelium; 3, total loss of villus; and 4, destruction of the muscle layer. Inflammation was scored as follows 0, normal; 1, focal infiltration of inflammatory cells; 2, slight inflammation only in the lamina propria; 3, intense inflammation in the lamina propria; and 4, severe inflammation extended to the muscle layer. For each specimen, a total of 10 fields of section were examined per animal.

2.6 Data Analysis

The results were analyzed using IBM version of SPSS software 17. The quantitative data obtained were expressed as mean ± SD, and statistical comparison among groups were performed by one way analysis of variance (ANOVA) followed by Tukey's test. The results of the histopathological scoring were compared with the Kruskal–Wallis test. When differences between the groups were detected, group means were compared using the Mann–Whitney U test. \( P < 0.05 \) was considered of statistical significance.

3 Results

Microscopically, the histologic structure of the jejunal tissue from the normal healthy control group was normal, and there was no histopathologic change in the bowel tissue of this group (Fig. 1). In the turmeric extract (100 mg·kg⁻¹) treated group, intestinal tissue was normal, and there was no sign of considerable treatment-related injury in the intestinal tissue of this group (Fig. 2). In histopathologic observations of the intestinal tissue of the turmeric (200 mg·kg⁻¹) treatment group, the intestinal tissue was intact, and there was no considerable pathologic change (Fig. 3). However, there were no statistically significant differences in inflammatory cell infiltration, ulceration and villus height.

Fig. 1  Microscopic view of the intestinal tissue belonged to control group. Histological structure of the intestinal tissue is normal (H&E)

Fig. 2  Microscopic view of the intestinal tissue belonged to turmeric (100 mg·kg⁻¹) treatment group. There is no treatment related damage in this section (H&E)

Fig. 3  Microscopic view of the intestinal tissue belonged to turmeric (200 mg·kg⁻¹) treatment group. Intestinal tissue is normal. No histopathologic change in intestinal tissue (H&E)
shortening scores between the turmeric (100 and 200 mg·kg$^{-1}$) treatment groups and the control group.

Microscopic evaluation of the intestinal tissues of the MTX treated group showed severe villus shortening and blunting, inflammatory cell infiltration and hemorrhage in lamina propria along with epithelial cells necrosis (Fig. 4). The total inflammation, ulceration and villus height shortening scores in the MTX-treated group were significantly increased when compared with other groups ($P < 0.01$). Microscopic view of the intestinal tissues of the MTX + turmeric (100 mg·kg$^{-1}$) treatment group illustrated mild inflammation of the lamina propria, as well as epithelial cell necrosis and desquamation in the upper portions of the villi. The intestinal lumens were filled with the epithelial debris (Fig. 5). In the MTX + turmeric (200 mg·kg$^{-1}$) treatment group, treatment with turmeric had reversed the pathologic changes induced by MTX, and histologic appearance of the intestine was near to normalcy (Fig. 6). The total scores of villus shortening, inflammation and ulceration in the MTX + turmeric (100 and 200 mg·kg$^{-1}$) groups were significantly ($P < 0.05$ and $P < 0.01$, respectively) less than in the MTX treated group. The results of the histopathological scoring in all groups are summarized in Table 2.

Biochemical results showed that the SOD levels were significantly ($P < 0.05$) higher in the TUR group (group III) and significantly ($P < 0.05$) lower in the MTX group compared with the control group, whereas the SOD level was significantly ($P < 0.05$) improved in the TUR + MTX group (group VI) when compared with the MTX group. In the TUR (100 mg·kg$^{-1}$) + MTX (group V) the levels of SOD were increased compared with the MTX group, but were not statistically significant. Similar significant results were seen in the CAT levels. Administration of MTX significantly ($P < 0.05$) decreased the levels of GSH-Px compared with the control group. But, it was increased significantly in the TUR + MTX (group VI) compared with the MTX group (Table 3).

Levels of MDA were increased in the MTX group compared with the control group, but were decreased in the TUR + MTX (group VI) group compared with the MTX group, in which both were meaningful ($P < 0.05$). Administration of turmeric increased the levels of TAS, but showed a significant ($P < 0.05$) decrease following administration of methotrexate compared with control group. Turmeric and methotrexate (group VI) administrations together caused significant ($P < 0.05$) increase in TAS levels compared with the MTX group (Table 4).
Table 2  Comparison of the effect of turmeric on jejunal injury scores among the experimental groups (mean ± SEM)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Degree of jejunal tissue injury</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Villus height shortening</td>
</tr>
<tr>
<td>Control</td>
<td>0.32 ± 0.14</td>
</tr>
<tr>
<td>TUR (100 mg·kg⁻¹)</td>
<td>0.29 ± 0.05</td>
</tr>
<tr>
<td>TUR (200 mg·kg⁻¹)</td>
<td>0.24 ± 0.03</td>
</tr>
<tr>
<td>MTX (20 mg·kg⁻¹)</td>
<td>2.38 ± 0.25&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>TUR (100 mg·kg⁻¹) + MTX</td>
<td>1.42 ± 0.16&lt;sup&gt;ac&lt;/sup&gt;</td>
</tr>
<tr>
<td>TUR (200 mg·kg⁻¹) + MTX</td>
<td>0.38 ± 0.08&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> P < 0.05; <sup>b</sup> P < 0.01 vs the control group. <sup>c</sup> P < 0.05; <sup>d</sup> P < 0.01 vs the MTX-treated group.

Table 3  Effect of turmeric on the antioxidant status in rat intestine (mean ± SD)

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD/(U/mL/tissue)</th>
<th>GSH-Px/(U/mL/tissue)</th>
<th>CAT/(U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.86 ± 0.25</td>
<td>4 ± 0.31</td>
<td>2.43 ± 0.20</td>
</tr>
<tr>
<td>TUR (100 mg·kg⁻¹)</td>
<td>5.3 ± 0.21</td>
<td>4.17 ± 0.34</td>
<td>2.69 ± 0.27</td>
</tr>
<tr>
<td>TUR (200 mg·kg⁻¹)</td>
<td>5.9 ± 0.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.56 ± 0.36</td>
<td>3.31 ± 0.35&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MTX (20 mg·kg⁻¹)</td>
<td>3.12 ± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.75 ± 0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.35 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TUR (100 mg·kg⁻¹) + MTX</td>
<td>3.74 ± 0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.22 ± 0.25</td>
<td>1.6 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TUR (200 mg·kg⁻¹) + MTX</td>
<td>4.68 ± 0.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.83 ± 0.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.1 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> P < 0.05 vs the control group; <sup>b</sup> P < 0.05 vs with the MTX group

Table 4  Effect of turmeric extract on lipid peroxidation and total antioxidant status in rat intestine (mean ± SD)

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA/(U/mL/tissue)</th>
<th>TAS/(mmol Trolox Eq/L)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>3.87 ± 0.43</td>
<td>1.8 ± 0.60</td>
</tr>
<tr>
<td>TUR (100 mg·kg⁻¹)</td>
<td>3.62 ± 0.30</td>
<td>2.13 ± 0.22</td>
</tr>
<tr>
<td>TUR (200 mg·kg⁻¹)</td>
<td>3.45 ± 0.28</td>
<td>2.71 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MTX (20 mg·kg⁻¹)</td>
<td>5.36 ± 0.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.73 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TUR (100 mg·kg⁻¹) + MTX</td>
<td>4.68 ± 0.34</td>
<td>1.16 ± 0.13</td>
</tr>
<tr>
<td>TUR (200 mg·kg⁻¹) + MTX</td>
<td>4.1 ± 0.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.62 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> P < 0.05 vs the control group; <sup>b</sup> P < 0.05 vs with the MTX group

4 Discussion

The epithelial barrier of the intestine operates as an abiotic and mechanical barrier and is an important functional section in the gastrointestinal system. Any interruption in its normal function can lead to malnutrition, infection, and other critical problems [21]. Using cancer therapeutic agents such as MTX could have harmful effects on the gastrointestinal system function that could lead to decreasing life quality of people who are suffering from malignances.

Pathological and biochemical results of this study demonstrate that MTX may induce severe intestinal damage. This severe damage could be recognized as villus shortening and blunting, inflammatory cell infiltration and hemorrhaging in lamina propria, along with epithelial cell necrosis and desquamation, as well as ulceration of the mucosa in the MTX treatment group. Treatment with turmeric reversed the pathologic changes induced by MTX and histologic appearance of intestine was restored nearly to normalcy.

In the normal situation, homeostasis of the intestine is achieved through balance between proliferation and apoptosis. Methotrexate is not specific for tumor cells and inhibits the dihydrofolate reductase enzyme and suppresses DNA synthesis in normal and malignant cells [18-19]. Hematopoietic and gastrointestinal cells that are rapidly proliferating are the most affected cells due to the use of MTX [20]. In the present study, intestinal damage occurred even with one single dose of MTX. This is one of the reasons that intestinal ulcers and damage occurred following the use of MTX. The other mechanism described is formation of reactive oxygen species following neutrophil accumulation, as observed in the pathology figures [5]. Oxidative stress and increasing apoptosis following MTX administration makes the intestinal epithelium and villus susceptible to digestive enzymes [20]. These results showed that turmeric extract could decrease the severity of MTX-induced intestinal inflammation and ulcers when
compared with when MTX was given alone. Different studies have proved turmeric as an antioxidant, anti-prooxidation and having a radical scavenging effect. As there is insufficient evidence for turmeric to prevent apoptosis in normal cells, the main mechanism for the therapeutic effects of turmeric is due to its antioxidant effects.

Previous studies have shown that curcumin, the turmeric active ingredient, could reduce the levels of D-lactate and diamine oxidase in MTX-induced rat enteritis model [21]. This reduction could improve the permeability of the intestinal mucosa, prevent intestinal mucosal damage, and finally protect intestinal mucosal barrier function. The Anti-inflammatory effect of curcumin is due to suppressing of mRNA that expressing intercellular adhesion molecule-1, tumor necrosis factor α and interleukin 1β [21]. On the other hand interleukin 10 that identifies as anti-inflammatory cytokine was up-regulated by curcumin. Curcumin could decrease neutrophil infiltration and oxidative damages due to their over-activation [21].

Reactive oxygen species (ROS) play an important role in the pathophysiology of inflammatory and oxidative damage of intestine [22-23]. Free radical scavenger and antioxidants could be used to treat mucosal damaging diseases such as chronic diarrhea, salmonella infection, and ulcerative colitis [24-25]. SOD, GSH-Px and catalase (CAT) are mucosal anti-oxidant defensive systems and can protect against ROS [3]. ROS plays an important role in gastrointestinal system toxicity and MTX-induced oxidative stress can cause damage in sulfhydryl bonds in proteins, nucleotides in DNA and polyunsaturated fatty acids found in cellular membranes [26]. Decrease in GSH-Px, SOD, CAT, and an increase in lipid peroxidation showed that oxidative stress has caused intestinal damage. Oxidative effects, cytotoxic effects, immunologic effects and allergic effects are the main reasons of the MTX-induced side effects [1, 3, 26, 28].

The same result of a previous study [1], the MDA level was increased in this study. Increasing MDA reveals ROS-induced lipid peroxidation in the MTX-treated rats. Turmeric decreased the level of MDA in the group VI rats, and this is the other protective effect of turmeric. SOD is an anti-oxidant indicator of the body’s ability to eliminate free radicals [27]. As these results show, the increase of SOD following administration of MTX could be controlled by turmeric at a dose of 200 mg·kg⁻¹; the same results have seen in previous study [21]. Also a dose of 100 mg·kg⁻¹ of turmeric increased the level of SOD, but the outcome was not statistically significant compared with the MTX group. Thus, the anti-oxidant effect of turmeric is dose-dependent. Similar results were achieved for GSH-Px and CAT.

5 Conclusion

These results suggest that turmeric extract may protect the small intestine of rats from methotrexate-induced damage. Turmeric effects could result from its antioxidant properties.

6 Acknowledgments

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References


