The protective role of silymarin against methotrexate-induced gastrointestinal injury

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ABSTRACT

The protective effect of silymarin was investigated against methotrexate-induced GI injury. Forty Swiss-Albino mice were divided into 4 groups as control (Normal saline), methotrexate (15 mg/kg), silymarin (100 mg/kg) and methotrexate+silymarin (100 mg/kg + 15 mg/kg respectively). Blood and tissue samples were collected from the animals for biochemical and pathological examinations. Plasma, intestinal and gastric tissue malondialdehyde and TSA levels in methotrexate group were significantly higher than in control whereas whole blood glutathione concentration was lower in methotraxe group than in control. Plasma, intestinal and gastric TSA levels in methotrexate group were significantly increased compared to control and methotrexate+silymarin groups. Gastric tissue of methotrexate treated mice showed degeneration, necrosis, desquamation and widespread edema. However, these alterations were less severe in methotrexate+silymarin group. In conclusion, silymarin could be of therapeutic value against methotrexa-induced GI injury.

Key words: GI injury, GSH, MDA, Methotrexate, Sialic acid, Silymarin.

Abbreviations: GI: Gastrointestinal injury, TSA: Total sialic acid, GSH: Glutathione, MDA: Malondialdeyde.

Methotrexate is an antineoplastic drug which is widely used against various types of cancers including leukemia, lymphoma, osteosarcoma, head and neck tumors, lung and breast cancer. It is also used in the treatment of psoriatic arthritis and rheumatoid arthritis (RA) due to its anti-inflammatory effects (Jolivet et al., 1983; Kane et al., 2004). However, clinical use of methotrexate may be limited due to frequent and long-term gastrointestinal toxic effects which occur during the treatment (Dadhania et al., 2010; Jahovic et al., 2004). One of the major toxic effects during methotrexate treatment is associated with intestinal damage and enterocolitis (Nagakubo et al., 2001). A number of studies indicated that toxic effect of methotrexate in various organs might be associated with oxidative damage. It has been reported that methotrexate is capable of inducing oxidative stress in the rat intestine (Dadhania et al., 2010). Other organs including liver, kidney and heart are also affected adversely by methotrexate in which oxidative stress via excessive production of reactive oxygen species (ROS) is reported to be one of the important mechanisms for the toxic effects (Devrim et al., 2005; Jahovic et al., 2004; Uz et al., 2005). Several studies indicated that methotrexate induced toxic effects in various organs could be also related to altered antioxidant status contributing to oxidative stress, and agents with antioxidant properties may be of therapeutic value (Dadhania et al., 2010; Sener et al., 2006).

Silymarin (milk thistle) is a compound which is obtained from the seeds of Silybum marianum plant. Silymarin contains at least 7 flavonolignans including isosilybin, silichristin, silydianin, 2,3-dehydroisobolin and flavonoids taxifolin and quercetin, while silybin constitutes the major component of silymarin (Ramasamy and Agarwal, 2008). Historically, Silybum marianum has been mainly used against liver and gastrointestinal diseases. The extract of Silybum marianum is used in cirrhosis, chronic hepatitis, alcohol-related liver disease and various environmental toxicants (Gazak et al., 2007; Pliskova et al., 2005). Silymarin is a substance having strong antioxidant and free radical scavenging activities, and these effects have been shown in several studies (Asghar and Masood, 2008; Koksal et al., 2009). Anthracycline drug-induced cardiotoxicity due to the generation of free radicals and oxidative stress was reported to be prevented by silymarin (Chlopcikova et al., 2004). Silymarin has also stabilizing effect on the cell membranes. The cellular protection by silymarin has been attributed to its antioxidant and free radical scavenging activities (Asghar and Masood, 2008; Basiglio et al., 2009; Koksal et al., 2009).

In this study, it was aimed to investigate the protective role and therapeutic potential of silymarin against methotrexate-induced gastro-intestinal damage in Swiss-Albino mice by evaluating some biochemical and histopathological parameters.

Forty 3 month-old male Swiss-Albino mice were procured from Kafrkas University Faculty of Veterinary Medicine Research and Application Farm. Permission was
Mice were allotted to 10 mice in each group as control, methotrexate, methotrexate + silymarin and silymarin groups. Mice in the control group received the vehicle 0.9% NaCl prepared in propylene glycol 75/25 (v/v) for 7 days via i.p. route. Mice in methotrexate group were intraperitoneally injected with methotrexate (dissolved in 0.9% NaCl and propylene glycol 75/25 (v/v) at a dose of 15 mg / kg for 5 days. In silymarin group, mice were given 100 mg / kg dose of silymarin (prepared in 0.9% NaCl propylene glycol 75/25 (v/v) was administered for 7 days by i.p injection. In silymarin + methotrexate group, mice were injected with silymarin (100 mg/kg, b.w., via i.p. route) for 7 days starting 2 days before methotrexate injection, and on the 2nd day of silymarin injection methotrexate (15 mg/kg b.w., via i.p. route) was administered together with silymarine for 5 days. Following the drug treatments, blood samples were collected under pentobarbital anesthesia, and then the mice were euthanized with an overdose of pentobarbital for biochemical and pathological assessments.

Samples of intestinal and gastric tissues were also collected and stored at -70 °C for biochemical analysis until the analyses. Stomach, intestinal tissue and blood glutathione (GSH) (Beutler, 1986), malondialdehide (MDA) (Yoshioka et al., 1979) and total sialic acid (TSA) levels(Sydow, 1985) was determined by spectrophotometry in accordance with the previously described methods.

All animals were necropsied and gastric and intestinal tissue samples were fixed in 10% buffered formaldehyde solution. Then paraffin blocks were prepared routinely from the tissues. Parafin blocks were sectioned at 5 micrometers in thickness and stained with hematoxylin and eosin (H&E). Sections from the groups were evaluated for degeneration, necrosis and inflammatory cell infiltration.

The data obtained were expressed as mean ± standard deviation. Statistical analyses of data were done using SPSS 12.0 software. Data were initially tested for normality by Kolmogrov Smirnov test. Then the data was tested with ANOVA which is followed by Posthoc Tukey test. P values less than 0.05 (p < 0.05) were considered statistically significant.

The blood, intestine and stomach tissue GSH, MDA and TSA concentrations were presented in Tables 1-3, respectively. Administration of methotrexate in methotrexate group (MTX) reduced the level of blood GSH compared to the control group (p<0.05), while it increased plasma TSA and MDA levels compared to control (p<0.05). Blood GSH, MDA and TSA levels in methotrexate+silymarine (MTX+SLY) group were not significantly different from those of control group. The level of blood GSH in silymarin group was significantly higher than in control group (p<0.05). TSA level of silymarin-treated mice was significantly reduced compared to control (p<0.05). Intestinal and gastric tissue MDA and TSA levels of MTX group were significantly elevated compared with the control group (p<0.05). TSA and MDA levels in MTX+SLY group were lower than in MTX group but higher than in control. Tissue GSH level in silymarin group were significantly increased compared to the other 3 groups. However, no difference was present among control, MTX and MTX+SLY in terms of tissue GSH level.

In the histopathological examinations, structure of intestinal and gastric tissues appeared normal in control (Figure 1) and silymarin groups (Figure 2). Gastric tissue of mice receiving methotrexate for 5 days showed degeneration, necrosis and desquamation in the lamina epithelialis of tunica mucosa (severe in 4 cases, moderate in 2 cases) (Figure 3). Degeneration and necrosis were also observed in the parietal cells close to the mucosal surface. In 1 gastric tissue sample from metotrexate group, widespread necrosis was encountered in the lamina propria (Figure 4). In severely affected animals, widespread edema and moderate mononuclear cell infiltration were detected between tunica mucosa and tunica muscularis. In samples taken from MTX+SLY group, degeneration and necrosis were severe in 1 case, mild in 2 cases and light in 3 cases (Figure 5). Except for the case showing severe degeneration and necrosis, no inflammatory cell infiltration was observed in the gastric tissue of MTX+SLY group.

In this study, MDA level in the plasma and intestinal tissue of the methotrexate group was significantly higher compared to the control and MTX+SLY groups. Oxidation of polyunsaturated fatty acids of cellular membranes by ROS result in the formation of aldehyde and carbonyl compounds giving rise to MDA. Therefore, increased MDA level is an indication of lipid peroxidation. Following the administration...
Figure 2: The appearance of gastric tissue of a mouse from silymarin group (H&E, x90).

Figure 3: Gastric tissue of a mouse from the methotrexate group, degeneration (D) in the lamina epithelialis, necrosis (N), severe edema (E) and mononuclear cell infiltration (M), (H&E, x90).

Figure 5: Gastric tissue of a mouse from the methotrexate+silymarin group, moderate degeneration (D) and necrosis (N) in the lamina epithelialis, mild mononuclear cell infiltration (M), (H&E, x185).

Table 1: Whole blood reduced glutathione (GSH), plasma malondialdehyde (MDA) and total sialic acid (TSA) levels (mean±SD) in Swiss-Albino mice.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Methotrexate</th>
<th>Methotrexate+Silymarin</th>
<th>Silymarin</th>
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<tbody>
<tr>
<td>GSH (mg/L)</td>
<td>20.09±2.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.92±2.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.66±1.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.96±1.08&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>MDA(µmol/L)</td>
<td>13.04±0.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.01±1.79&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.62±5.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.17±1.15&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>TSA (mg/L)</td>
<td>58.55±1.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>61.19±1.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.08±1.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td>47.03±1.13&lt;sup&gt;c&lt;/sup&gt;</td>
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<sup>a, b, c</sup>: Different letters within the same row indicate statistical significance (P<0.05).

Table 2: Intestinal tissue reduced glutathione (GSH), malondialdehyde (MDA) and total sialic acid (TSA) levels (mean±SD) in Swiss-Albino mice.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Methotrexate</th>
<th>Methotrexate+Silymarin</th>
<th>Silymarin</th>
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<tbody>
<tr>
<td>GSH (mg/g)</td>
<td>2.84±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.91±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.63±0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.52±0.27&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MDA(µmol/g)</td>
<td>1.06±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.15±0.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.51±0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.05±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TSA (mg/g)</td>
<td>2.36±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.43±0.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.45±0.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.39±0.47&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
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<sup>a, b, c</sup>: Different letters within the same row indicate statistical significance (P<0.05).
of MTX, increased level of MDA has been shown in previous studies (Jahovic et al., 2004; Uz et al., 2005). It has been reported that antioxidants play an important role in the regulation of the gastrointestinal tract. Glutathione and glutathione related enzymes which might have a protective role on the inflammatory diseases of GIS have been reported in experimental studies (Jube et al., 2005; Siegers et al., 1988). In the present study, blood GSH level in MTX+SLY group was significantly increased compared to MTX group. However, except for SLY group, no significant difference in GSH levels was determined in the gastric and intestinal tissues among control, MTX and MTX+SLY. Miyazano et al. (2004) reported that MTX reduced GSH and other antioxidant enzymes in the small intestine of rats. In contrast, Kuralay et al. (2003) reported that some antioxidant enzymes could be increased in response oxidative stress in the experimentally induced colit model.

In the current study, TSA levels in plasma, intestinal and stomach tissues of MTX group were significantly higher compared to control and MTX+SLY group. The amount of TSA in inflammatory diseases such as arthritis, renal disease, diabetes, central nervous system disease, Behcet’s disease, bacterial infections, crohn’s disease, psoriasis is increased significantly (Sillanaukee et al., 1999a; Sillanaukee et al., 1999b). It has been reported that plasma sialic acid levels are increased in aged rat due to increased free radical formation (Uslu, 1995). TSA levels may increase as a result of the release of sialic acids from cellular membranes due to lipid peroxidation-induced damage in cellular membranes (Uslu, 1995). Silymarin treatment in the current study reduced the level of TSA and MDA levels in MTX+SLY group compared to control and MTX groups. According to the results of this study, silymarin is protective against oxidative stress in the GI tissue, and the protection could be due its antioxidant activity. Silymarin has strong antioxidant, free radical scavenging activity and cell membrane stabilizing effects (Asghar and Masood, 2008; Basiglio et al., 2009; Koksal et al., 2009). In addition, silymarin has an anti-inflammatory, antiproliferative and immunomodulating effects. It was reported that silymarin prevents oxidative stress in cisplatin-induced nephrotoxicity and hepatotoxicity in rats (Mansour et al., 2006).

The histopathological findings indicate that silymarin can reduce the severity of MTX-induced pathological lesions and supported the biochemical results. Severe degeneration, necrosis and edema were observed in the epithelial layer of gastric tissue of MTX-treated group. However, moderate level of degeneration and necrosis were present in MTX+SLY group without edema.

It can be concluded that silymarin could be protective against MTX-induced gastrointestinal damage. Although the protective effect of silymarin may be due to different mechanisms, in the perspective of this study, silymarin could ameliorate methotrexate-induced gastrointestinal damage via antioxidant mechanism and may be of therapeutic value during the methotrexate treatment.

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REFERENCES


