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Protective effect of ellagic acid and pumpkin seed oil against methotrexate-induced small intestine damage in rats

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Gastrointestinal toxicity is one of the most serious side effects in the methotrexate (MTX) treatment. This study was designed to investigate whether ellagic acid (EA) and/or pumpkin seed oil (PSO) had a protective effect on MTX-induced small intestine damage. Forty albino rats were randomized into five groups of 8 rats each. Group I served as a normal control group. In Group II, MTX was administered as a single dose (20 mg/kg) intraperitoneally. Groups III, IV and V were pre-treated respectively with either PSO (40 mg/kg), EA (10 mg/kg) or 0.2% DMSO (vehicle control) orally every day by gavage for 5 days and then they received MTX. All animals were sacrificed 5 days after the intraperitoneal injection of MTX for histopathological examination, estimation of serum prostaglandin E_2 (PGE_2) level, assay of tissue malondialdehyde (MDA), reduced glutathione (GSH) and nitric oxide (NO) levels and myloperoxidase (MPO), xanthine oxidase (XO) and adenosine deaminase (AD) activities. Administration of EA and/or PSO decreased the intestinal damage, PGE_2, MDA and NO levels and MPO, XO and AD activities and increased GSH level. These results suggest that EA and PSO protect the small intestine of rats from MTX-induced damage through their antioxidant and anti-inflammatory effects and thus have potential as a promising drug in the prevention of undesired side effects of MTX.

Keywords: Methotrexate, Oxidative stress, Ellagic acid, Pumpkin seed oil, Purine catabolizing enzymes, Prostaglandin E_2.

Methotrexate (MTX), a folic acid antagonist is widely used as a cytotoxic chemotherapeutic agent for leukemia and other malignancies. Over the past five decades, low-dose MTX has also been used for the treatment of various inflammatory diseases, such as psoriatic and rheumatoid arthritis[1]. However, the efficacy of this agent is often limited by severe side-effects and toxic sequelae. Since cytotoxic effect of MTX is not selective for cancer cells, it also affects normal tissues that have a high rate of proliferation, including the haematopoietic cells of bone marrow and actively-dividing cells of gut mucosa. Thus, one of the major toxic effects of MTX is intestinal injury and enterocolitis[2]. The small intestinal damage induced by MTX treatment results in malabsorption and diarrhea. The malabsorption results in weight loss and disturbs the cancer chemotherapy of the patients[3].

Being a high affinity inhibitor of dihydrofolate reductase, MTX is a pro-oxidant compound that causes depletion of the dihydrofolate pool and directly affects the synthesis of thymidilate, suppressing DNA synthesis[4]. Moreover, cytosolic NAD(P)-dependent dehydrogenases[5] and NADP malic enzyme are inhibited by MTX, suggesting that the drug could decrease the availability of NADPH in the cells by inhibiting the pentose cycle enzymes[6]. Under normal conditions, NADPH is used by glutathione reductase to maintain the reduced state of cellular glutathione (GSH), which is an important cytosolic antioxidant that protects against reactive oxygen species (ROS). Considering the deleterious effects of MTX on both GSH and DNA synthesis, interest has been focused on the compounds that act as antioxidants, are capable of stimulating GSH and DNA synthesis or that have trophic effects on gut mucosa.

Polyphenolic compounds are widely distributed in the vegetable kingdom and are often encountered in our daily lives, being found in tea, red wine, fruits, etc[7]. Ellagic acid (EA, Fig. 1) is a phenolic compound

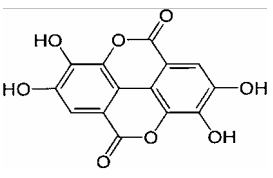

Fig. 1— Structure of ellagic acid

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Abbreviations: AD, adenosine deaminase; DMSO, dimethyl sulphoxide; EA, ellagic acid; MDA, malondialdehyde; MPO, myloperoxidase; MTX, methotrexate; NO, nitric oxide; PGE_2: prostaglandin E_2; PSO, pumpkin seed oil; ROS, reactive oxygen species; XO, xanthine oxidase.

present in fruits and nuts, including blueberries, blackberries, raspberries, strawberries and walnuts[8]. It has been found to have antimutagenic, antiviral and antioxidative properties[9]. It contains four hydroxyl groups and two lactone groups in which hydroxyl group is known to increase antioxidant activity in lipid peroxidation and protect cells from oxidative damage[10]. Although the molecular mechanism of EA is unknown, its potent scavenging action against hydroxyl (OH^-) and superoxide radicals (O_2^-) might be responsible for these effects[9]. Recent reports have shown that oral administration of EA exerts a protective influence against cisplatin[11] and cyclosporine-induced toxicity[12].

Pumpkin seed oil (PSO) is a natural product commonly used in folk medicine. It has been shown in several countries that incidence of hypertension, atherosclerosis and prostatic hypertrophy is reduced in people regularly consuming the seed oil[13]. PSO is rich in many antioxidants and beneficial nutritional supplements such as essential fatty acids, β-carotenes, lutein, γ and β-tocopherols, phytosterols, chlorophyll and selenium[14].

In the present study, the level of oxidative stress and possible protective effects of EA and PSO have been evaluated in MTX-induced intestinal damage in rats.

Materials and Methods

Chemicals

Methotrexate was obtained from Ebewe Pharma, Austria. Ellagic acid (EA) was procured from Sigma-Aldrich Chemicals Co., St. Louis, USA. PSO (Pepon capsules) was obtained from MEPACO Co., Cairo, Egypt. All other chemicals and reagents were of the highest purity commercially available.

Animals

Male Wistar rats weighing 170-200 g were obtained from the animal house of Faculty of Medicine, Cairo University, Egypt. They were kept at a constant temperature (22 ± 1°C) with 12 h light and dark cycles, fed a standard rat chow and allowed to accommodate for 1 week before any experimental manipulation.

Experimental design

Rats were randomly divided into five groups of 8 rats in each. Group I (control group) was injected with saline. Group II (MTX group) was injected with a single dose of MTX in saline (20 mg/kg; intraperitoneal)[2]. Group III (MTX + PSO) rats orally received PSO (40 mg/kg)[15] dissolved in 0.2% dimethyl sulphoxide (DMSO) for 5 days using intragastric intubation. Group IV (MTX + EA) rats received EA (10 mg/kg)[16] orally dissolved in 0.2% DMSO for 5 days using intragastric intubation. Group V (MTX + DMSO) rats orally treated with 0.2% DMSO for 5 days using intragastric intubation. Following a single dose of MTX on the fifth day of pre-treatment, either PSO, EA and DMSO were administered for the next 5 days. Then the animals were sacrificed by decapitation, blood was collected and tissue samples from the small intestine were taken for biochemical analysis and histopathology.

Biochemical analysis

Blood samples were allowed to clot and serum was separated for the determination of prostaglandin E_2 (PGE_2) level using ELISA kit (Neogen, USA). The separated intestine was weighed, homogenized in ice cold saline (20% w/v) using potter-Elvejhem glass homogenizer and the homogenate was divided into six portions.

The first portion was mixed with ice cold 2.3% KCl solution and centrifuged at 600 × *g* for 15 min at 4°C. The supernatant was used for determination of malondialdehyde (MDA) identified as the product of lipid peroxidation that reacts with thiobarbituric acid to give a pink color. The absorbance was recorded at 535 nm and 520 nm using tetramethoxypropane as a standard[17]. The difference between the two determinations was calculated as TBA value and expressed as nmol/mg protein.

The second portion was treated with 7.5% sulfosalicylic acid and centrifuged at 600 x *g* at 4°C for 10 min. The protein-free supernatant was used for the estimation of reduced glutathione (GSH) levels based on the reaction of GSH with 5,5-dithiobis-2-nitrobenzoic acid forming a product that has a maximal absorbance at 412 nm. The results were expressed as μmol/g wet tissue[18].

The third portion was mixed with 10 vols of ice-cold Tris-EDTA buffer, pH 7.6 (100 mM Tris and 0.2 mM EDTA) and centrifuged at 105,000 × *g* at 4°C for 40 min using Dupont Sorvall ultracentrifuge (USA). The resulting cytosolic fraction was used for the determination of xanthine oxidase (XO) activity. This was measured spectrophotometrically by the formation of uric acid from xanthine through the increase in absorbance at 293 nm[19]. One unit of activity was defined as 1 μmol urate formed per min

at 37°C in phosphate buffer (pH 7.5, 50 mM) and results were expressed as U/g protein.

The fourth portion was mixed with 0.25 M sucrose buffer pH 7.4 (10% w/v), centrifuged at 105,000 × *g* at 4°C for 40 min and the resultant supernatant was used for estimating intestinal adenosine deaminase (AD) activity. This was estimated spectrophotometrically by the method of Giusti[20], which is based on the direct measurement of the ammonia produced when AD acts in excess of adenosine and results were expressed as U/g protein.

The fifth portion was mixed with ice-cold 50 mM phosphate buffer containing 0.5% hexadecyltrimethyl ammonium bromide (pH 6). After three freeze and thaw cycles with sonication between cycles, the samples were centrifuged at 41,400 × *g* at 4°C for 10 min and the supernatant was used for the determination of myeloperoxidase (MPO) activity. This was measured spectrophotometrically using *o*-dianisidine dihydrochloride as a substrate for MPO-mediated oxidation by H_2O_2. The change in absorbance at 460 nm was recorded and expressed in U/mg protein[21].

The last portion of the homogenate was centrifuged at 17.000 × *g* at 4°C for 15 min and the supernatant was used for the determination of nitric oxide (NO) level measured as total nitrites with the spectrophotometric Greiss reaction. Results were expressed as nmol/mg protein[22]. The protein content of the different fractions, resulting from ultracentrifugation of intestine homogenate was determined by the method of Lowry et al.[23] using bovine serum albumin as standard.

Histopathological examination

Intestine specimens were fixed with 10% formaldehyde and processed routinely for embedding in paraffin. Sections of 4 μm were stained with hematoxylin and eosin (H & E) and examined under the light microscope[24].

Statistical analysis

The results were expressed as mean ± standard error of the mean (SEM). Groups were compared with an analysis of variance (ANOVA), followed by Tukey's multiple comparisons tests. Values of $p < 0.05$ were considered as significant.

Results

Biochemical results

To examine the oxidative stress, DNA catabolism and inflammation in MTX-induced intestinal damage, the levels of MDA, GSH and NO, the activities of MPO, AD and XO in the small intestinal tissue homogenates and serum PGE_2 level were determined.

MPO activity showed about seven-fold increase, following MTX injection. This rise was ameliorated by treatment with EA and/or PSO (Fig. 2). A significant increase of NO level in intestinal tissue of MTX-treated rats was observed in the present study. This marked rise was decreased by both antioxidant therapies. PSO was found to be more effective than EA in reducing NO level (Fig. 3). A considerable elevation of lipid peroxides (MDA) in intestinal tissue was demonstrated, following MTX injection which was completely normalized by treatment with EA and/or PSO (Fig. 4). The MTX-induced toxicity in the intestinal tissue was further revealed by the significant reduction of the antioxidant defense mechanisms, as shown by the significant decrease in GSH content. EA and/or PSO significantly ameliorated the GSH level (Fig. 5).

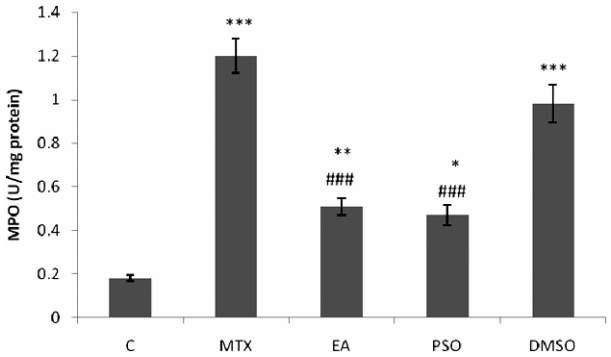

Fig. 2—MPO activity in intestinal tissue of rats after MTX and/or EA, PSO, DMSO treatment [*p<0.05, **p<0.01, ***p<0.001 when compared with the control (saline) group. #p<0.05, ##p<0.01, ###p<0.001 as compared to MTX group]

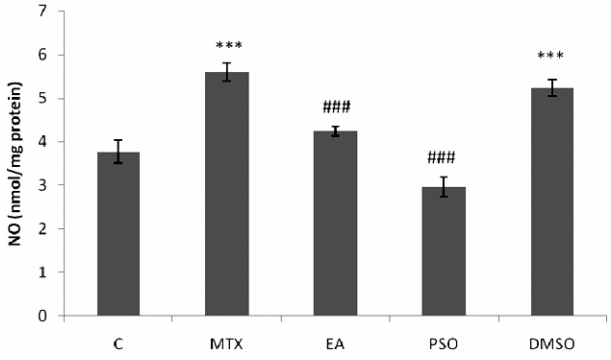

Fig. 3—NO level in intestinal tissue of rats after MTX and/or EA, PSO and DMSO treatment [*p<0.05, **p<0.01, ***p<0.001 when compared with the control (saline) group. #p<0.05, ##p<0.01, ###p<0.001 as compared to MTX group]

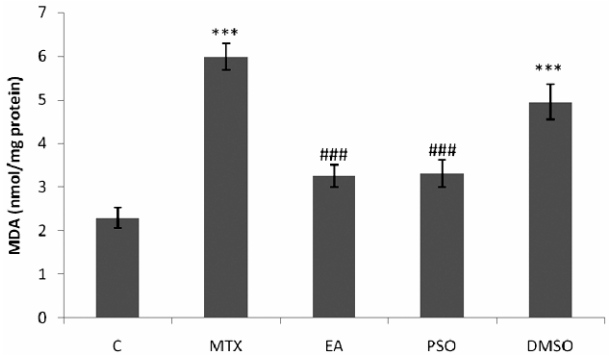

Fig. 4—MDA level in intestinal tissue of rats after MTX and/or EA, PSO and DMSO treatment [*p<0.05, **p<0.01, ***p<0.001 when compared with the control (saline) group. #p<0.05, ##p<0.01, ###p<0.001 as compared to MTX group]

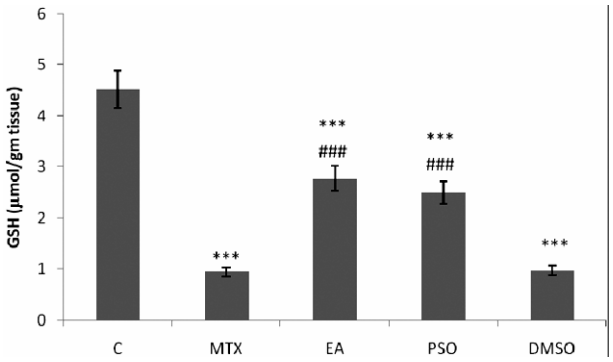

Fig. 5—GSH level in intestinal tissue of rats after MTX and/or EA, PSO and DMSO treatment [*p<0.05, **p<0.01, ***p<0.001 when compared with the control (saline) group. #p<0.05, ##p<0.01, ###p<0.001 as compared to MTX group]

XO and AD activities showed about 1.7-fold increase following MTX injection. This rise was completely normalized by both the therapies (Figs 6 & 7). Similarly, MTX injection in normal rats evoked a two-fold elevation in serum PGE_2 level and pre-treatment with either EA or PSO resulted in a beneficial restoration of normal level of this mediator in treated rats (Fig. 8).

Thus, EA and/or PSO administration ameliorated the oxidative stress by decreasing MPO activity, MDA and NO levels and increasing the GSH level. Moreover, they decreased the activities of purine catabolizing enzymes (XO and AD) and reduced the inflammation by decreasing PGE_2 level in the intestinal tissue.

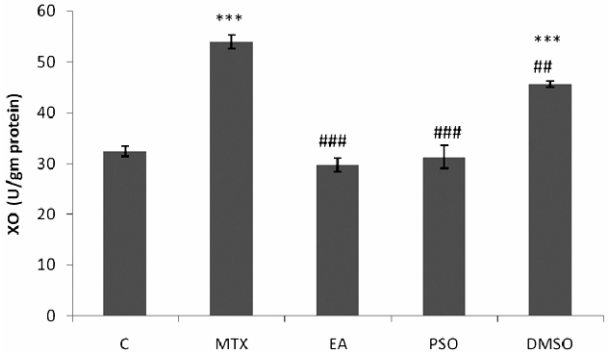

Fig. 6—XO activiy in intestinal tissue of rats after MTX and/or EA, PSO and DMSO treatment [*p<0.05, **p<0.01, ***p<0.001 when compared with the control (saline) group. #p<0.05, ##p<0.01, ###p<0.001 as compared to MTX group]

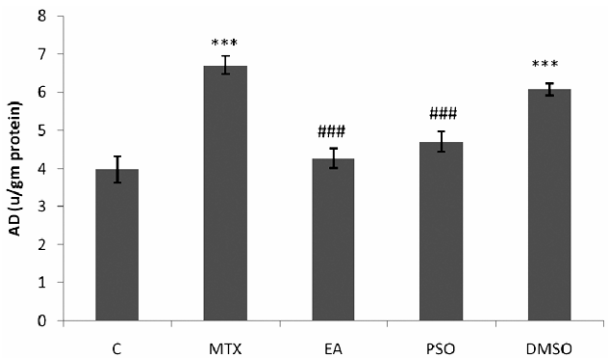

Fig. 7—AD activity in intestinal tissue of rats after MTX and/or EA, PSO and DMSO treatment [*p<0.05, **p<0.01, ***p<0.001 when compared with the control (saline) group. #p<0.05, ##p<0.01, ###p<0.001 as compared to MTX group]

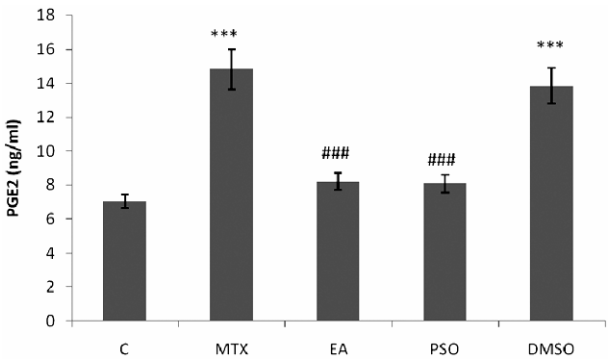

Fig. 8—PGE_2 level in serum of rats after MTX and/or EA, PSO and DMSO treatment [*p<0.05, **p<0.01, ***p<0.001 when compared with the control (saline) group. #p<0.05, ##p<0.01, ###p<0.001 as compared to MTX group]

Histopathological examination

As shown in Fig. 9, the intestine of normal control rats revealed no histological alteration. Normal histological structure of mucosal lining epithelium, lamina propria, muscularis and serosa was recorded (Fig. 9A). In contrast, intestine sections from rats receiving MTX showed sloughing in the lining epithelial cells of the mucosa of the thick intestinal folds associated with inflammatory cells infiltration in the underlying lamina propria. Oedema was noticed in the submucosa and serosal layers (Fig. 9B & C). Pre-treatment with EA (Fig. 9D) and PSO (Fig. 9E)

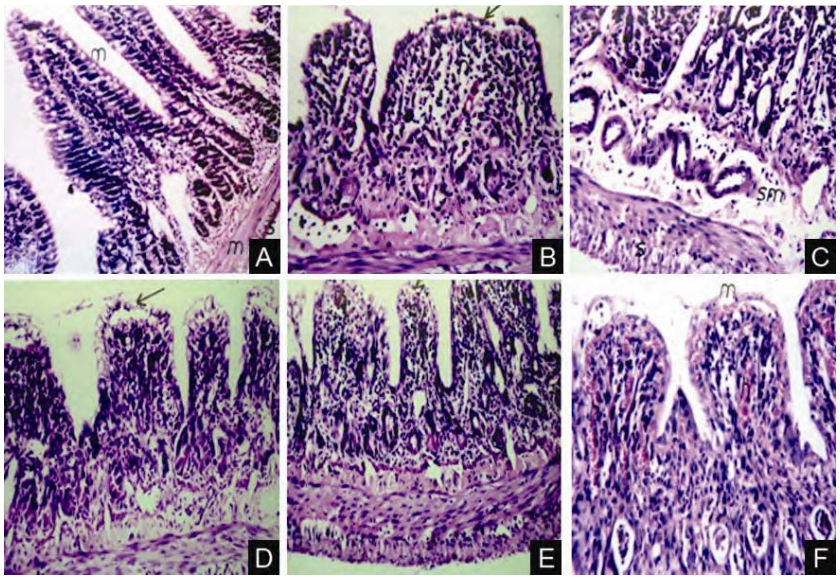

Fig. 9—Photomicrographs of intestine sections from (A) normal control rats (H & E, 40x): the normal histological structure of the mucosa (m), submucosa (L), serosa (S). (B & C) MTX-treated rats (H & E, 64x):thick folds with sloughing lining mucosa (→), inflammatory cells infiltration in the lamine propria (n) and oedema in the submucosa (Sm) and serosal layers (S). (D) MTX + EA (H & E, 40x): thickening in the mucosal folds with globlet cells formation in the sloughing lining mucosal lining epithelium (→). (E) MTX + PSO (H & E, 40x): globlet cells formation in the lining mucosal epithelium (→). (F) MTX + DMSO (H & E, 64x): sloughing of the mucosal lining epithelium (m) with congestion and haemorrages (h) in the lamine propria of the fold (n)

improved the alterations in intestine morphology, as there was thickening in the folds of the intestinal mucosa with sloughing in the lining mucosal epithelium in focal manner and goblet cells formation in other lining epithelium in other areas of the mucosa with congestion in the blood capillaries in the lamina propria. PSO showed more protective effect on intestinal mucosa than EA. On the other hand, the treatment with the vehicle DMSO, followed by MTX challenge showed same changes as MTX group, in addition to focal goblet cells formation all over the lining mucosal epithelium (Fig. 9F).

Discussion

MTX, the widely used drug in cancer therapy or in various forms of arthritis is known to have several side effects. Enterocolitis due to intestinal damage is one of the most frequent and severe side effects of MTX. In this study, MTX was demonstrated to cause a significant increase in XO, AD and NO in rat intestine. These results were in accordance with the previous studies[5,25]. It has been reported that MTX limits the conversion of folic acid to tetrahydrofolate, a molecule necessary for the synthesis of DNA during S-phase and results in improper DNA synthesis and subsequent apoptosis of cells[26]. Moreover, during MTX-induced intestinal toxicity, purines are degraded to hypoxanthine and xanthine dehyrogenase is converted to XO. XO catalyzes the conversion of hypoxanthine to uric acid with superoxide anion production and indirectly hydroxyl radical formation. Thus, it may be suggested that XD/XO can be a source of the MTX-induced ROS[27].

On the other hand, high purine catabolism can be detected by high activity of another enzyme, AD. Parallel to XO activity, AD also showed high activity in MTX-administered rats. These two enzymes may be considered as the indicators of DNA catabolism and their high activities indicated that there might be possible cell and nucleic acid destruction due to the MTX toxicity in intestinal tissue. Furthermore, it has been shown that increased xanthine and adenosine, the substrates for XO and AD, respectively may increase the activity and/or expression of XO and AD in the damaged tissue[28].

NO is a free radical produced from L-arginine by a family of isoenzymes called nitric oxide synthase (NOS). Both constitutive and inducible isoforms of NOS are present within the gastrointestinal tract[29]. Low levels of NO produced by the constitutive isoforms of NOS may play a beneficial or homeostatic role in the gastrointestinal tract[30]. On the other hand, sustained release of NO as a result of iNOS upregulation may lead to cellular injury and gut barrier failure. The biological effects of NO are achieved through chemical interactions with different

targets including oxygen, superoxide and other ROS, transition metals and thiols. It may react with thiol (-SH) groups of amino acids and proteins and form relatively stable nitroso-thiols[31]. The increased NO level in the intestine after MTX administration in the present study suggested that MTX-induced intestinal damage might be contributed by high NO production. This finding was in agreement with earlier report[32]. The concurrent production of superoxide originating from XO activity, and NO leads to the rapid formation of peroxynitrite which may cause further injury in the intestinal tissue and results into lipid peroxidation[33].

In the present study, increase in lipid peroxidation and MPO activity due to toxic effects of MTX was accompanied by significant reduction in GSH level of the intestinal tissue, indicating the presence of oxidative tissue damage. Lipid peroxidation, mediated by oxygen free radicals is believed to be an important cause of destruction and damage to cell membranes and has been suggested to be a contributing factor to the development of MTX-mediated tissue damage[2], as evidenced by the significant elevated MDA level in the present study. The increased lipid peroxidation is responsible for the formation of lipid hydroperoxides in membrane and would result in damage of membrane bound enzymes. The accumulation of lipid peroxides introduces hydrophilic moieties into hydrophobic phase and thus alters membrane permeability and its functions[34].

In addition to the direct damaging effects on tissues, free radicals trigger the accumulation of leukocytes in the tissues. Especially neutrophils, when suitably stimulated increase their oxygen consumption and cause respiratory or oxidative burst. The activated neutrophils have been demonstrated to secrete enzymes such as MPO, elastase, proteases and liberate more free radicals[2,4]. The significant elevation in MPO activity in the small intestine in the present study indicated that the neutrophil accumulation contributed to MTX-induced injury.

GSH is one of the most important molecules in the cellular defense against chemically reactive toxic compounds or oxidative stress. In its reduced form, GSH is necessary for the detoxification of xenobiotics. Decreased cellular GSH levels and reduced capacity for GSH synthesis sensitize cells to radiation and to certain drugs[35]. It has been reported that GSH could have a role in maintaining the activity of the pentose phosphate cycle at a level that is appropriate for the severity of the oxidative challenge, as well as for the capacity of the cellular antioxidant defenses[36]. The significant reduction in GSH level promoted by MTX represents an alteration in the cellular redox state, suggesting that the cells could be more sensitive to ROS and leads to a reduction of effectiveness of the antioxidant enzyme defense system[5]. In the present study, MTX-induced tissue injury was accompanied by depleted tissue GSH levels. The experimental data have indicated that exaggerated inhibition of glucose-6-phosphate dehydrogenase by MTX will contribute to a decrease of the availability of NADPH and inhibition of glutathione reductase activity and glutathione cycle[5].

Prostaglandins are also involved in the regulation of a variety of physiological and pathological processes in the immune response and inflammation. PGE_2 and other prostanoids are generated through two bifunctional enzymes, cyclooxygenases (COXs)-1 and -2[37]. In general, COX-1 is constitutively expressed in a wide range of tissues, including the gastrointestinal tract and plays a role in the tissue homeostasis, e.g., maintenance of gastrointestinal integrity[38]. The inducible form COX-2, which regulates prostaglandin synthesis is over-expressed in several epithelial cancers and at sites of inflammation[39] and it has been shown that free radicals directly increase COX activity[40]. In the present study, MTX treatment increased PGE_2 production and this was in agreement with earlier observation[41].

Various agents have been attempted for protection and/or prevention of the side effects of many chemotherapeutics. One of these chemopreventive agents is flavonoids, a large group of polyphenolic antioxidants, which are found in almost all food categories with fruits and vegetables being the main source. EA is a naturally occurring plant polyphenol[42] that exhibits antioxidative properties both *in vivo*[43] and *in vitro*[44]. Another chemopreventive agent POS contains linoleic and linolenic acids, β-carotene, lutein, β- and γ-tocopherol, chlorophyll, selenium and ▲[7] phytosterols[14]. The results of present study clearly demonstrated that administration of EA and/or PSO to MTX-treated rats markedly inhibited the inflammation, as indicated by the significant reduction of PGE_2, MDA and NO levels, reduced MPO, XO and AD activities and increased GSH levels in intestinal tissues. These results were in accordance with previous studies[11,15, 45,46].

The two lactone groups of EA (Fig. 1) can act as a hydrogen bond donor and acceptor[47] and might be involved in the free radical scavenging action and

decreased free radicals-mediated lipid peroxidation. They also inhibit the enzymes responsible for superoxide anion production (XO and protein kinase C) and ROS generation (cyclooxygenase, lipoxygenase and NADH oxidase)[48]. In addition to their antioxidative activity, polyphenols can also chelate metal ions and prevent iron and copper-catalyzed formation of ROS[49]. Moreover, the phenolic compounds have also been demonstrated to effectively inhibit NO production, independent of their antioxidant properties[50] and can also scavenge peroxy radical and peroxynitrite[51]. In the present study, EA being a phenolic compound might have inhibited NO production.

The PSO is found to be rich in unsaturated fatty acids such as linoleic and linolenic acids, which compete with arachidonate for oxidative enzymes, thereby reducing the production of archidonate cycloxygenase products[52]. Earlier, it has been shown that a diet rich in γ-linolenic acid has actions similar to non-steroidal anti-inflammatory agents in reducing the production of PGE_2 and LTB_4 generated during the inflammation[53].

Tocopherols and selenium (Se) present in PSO by their combined antioxidant properties might contribute in the prevention of GSH depletion observed during MTX treatment[54]. Tocopherols and Se function synergistically in animal tissues to constitute an important antioxidant defense mechanism against free-radical-mediated lipid peroxidation of cell membrane. As an essential component of Se-dependent glutathione peroxidase, Se is involved in cellular antioxidant defense by reducing semi-stable hydroperoxides to the less reactive alcohols. Furthermore, tocopherols and Se-glutathione peroxidase could play the more specific role of modulating the enzymatic oxidation of arachidonic acid through inhibition of cycloxygenase and lipoxygenase pathways of PGE_2 and LTB_4 biosynthesis[55].

Carotinoids present in PSO are also currently recognized as cellular antioxidants. They are extremely efficient quenchers of singlet oxygen. In addition, β-carotenes react chemically with peroxyl radicals to produce epoxide and apocarotenol products. Reports have shown that β-carotenes inhibit the oxidation of linoleic acid by lipoxygenase as well as the formation of hydroperoxide products. The carotinoids could protect alone or with other antioxidants in a rather unspecific manner against oxidative damage to membranes, organelles and protein[56].

In this study, histological appearance of intestinal mucosa was characterized by crypt loss, villus fusion and atrophy, gross capillary dilatation and a mixed cellular infiltrate. Our findings were similar to other studies, where MTX has been reported to cause severe damage in the small intestine[57,58]. Pre-treatment of rats with PSO and or EA before MTX administration prevented the increase in intestinal damage and histological lesions appreciably. These results suggested that PSO and EA might have potential protective effect against MTX-induced intestinal toxicity. PSO was found more effective than EA in preventing MTX-induced histological changes.

Dimethyl sulfoxide (DMSO) is widely used as solvent in biological studies and as vehicle for drug administration. It possesses several biological effects, including antioxidant and anti-inflammatory effects. It has also been proposed to be of therapeutic value in several disorders, such as gastrointestinal diseases, rheumatologic diseases, as well as in the treatment of several manifestations of amyloidosis[59]. In the present study, the biochemical changes triggered by MTX were slightly ameliorated by DMSO administration. However, EA and PSO suspended in DMSO were found to be more effective than DMSO alone in reversing the changes due to MTX, suggesting that the offered protection was due to EA and PSO not DMSO.

In conclusion, the present study demonstrated the evidence of intestinal damage by MTX and ability of PSO and EA in preventing the damage by inhibiting the peroxidation of lipids, NO generation, infiltration of neutrophils, improving antioxidant level, reducing purine catabolizing enzymes and preventing intestinal inflammation. However, further studies are warranted to evaluate the efficacy and optimum dosage of PSO and EA to be used as protective agent against the side effects of chemotherapeutics.

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