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SPECIAL TOPIC

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P-08

INVESTIGATION OF THE PROTECTIVE EFFECTS OF PETROSELINUM CRISPUM (PARSLEY) EXTRACT AGAINST METHOTREXATE-INDUCED HEPATOTOXICITY

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INTRODUCTION

Methotrexate (MTX) is a drug that antagonizes folate metabolism, inhibits the synthesis of thymidine and purine which are in the structure of the nucleic acid, thereby inhibiting the proliferation of rapidly dividing cells such as cancer cells by suppressing DNA synthesis. It is also used in the treatment of arthritis, systemic lupus erythematosus, psoriasis and refractory inflammatory bowel disease [1]. However, as occur with all cytotoxic drugs, due to MTX use causes side effects in multiple organ systems including liver and these side effects lead to dose restriction in the use of the drug [2]. When the mechanism of side effects is examined, it is understood that MTX metabolism-derived oxidant molecules are responsible of the organ damage and toxicity [3]. In this study, we examined whether the *petroselinum crispum* extracts (PCr; parsley) which the antioxidant properties has been previously shown, was protective against MTX induced liver damage.

MATERIALS AND METHODS

Experimental Design

All experimental protocols were approved by the Marmara University School of Medicine Animal Care and Use Committee. Both sexes of Sprague Dawley rats (200-250 g) were kept at a constant temperature (22 + 1o C) with 12 h light and dark cycles.

MTX was injected intraperitoneally and PCr extract was given orally. Following a single dose of methotrexate (20 mg/kg), either saline (MTX group) or PCr (2 g/kg, MTX + PCr group) was administered for 5 days. In other rats, physiological saline (control group) or PCr (2 g/kg, PCr group) was given for 5 days, following a single dose of saline injection. On the 6th day, animals were killed by decapitation and liver tissue samples were taken to analyze malondialdehyde (MDA) and glutathione (GSH) levels, and myeloperoxidase (MPO) activity.

Biochemical Methods

The MDA levels were assayed for products of lipid peroxidation [4]. Results are expressed as nmol MDA/g tissue. GSH was determined by the spectrophotometric method using the Ellman's reagent [5]. Results are expressed g GSH/g tissue.Tissue associated MPO activity was measured using a procedure similar to that documented by Hillegas et al. [6].

Statistics

Statistical analysis was carried out using GraphPad Prism 3.0 (GraphPad Software, San Diego; CA; USA). All data are expressed as means ± SEM. Groups of data were compared with an analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Values of $p<0.05$ were regarded as significant.

RESULTS AND DISCUSSION

Tissue Malondialdehyde and Glutathione levels

The levels of MDA, as products of lipid peroxidation, were increased and GSH levels were decreased after treatment with MTX to levels that are found to be significantly different than the control. On the other hand, PCr treatment following MTX challenge

reversed these parameters back to the control levels (Fig.1).

Tissue Myeloperoxidase Activity

Tissue MPO activity was measured as an indirect evidence of neutrophil infiltration. MTX treatment increased the liver MPO activity, which was reversed back to control levels in MTX + PCr group; Fig. 2).

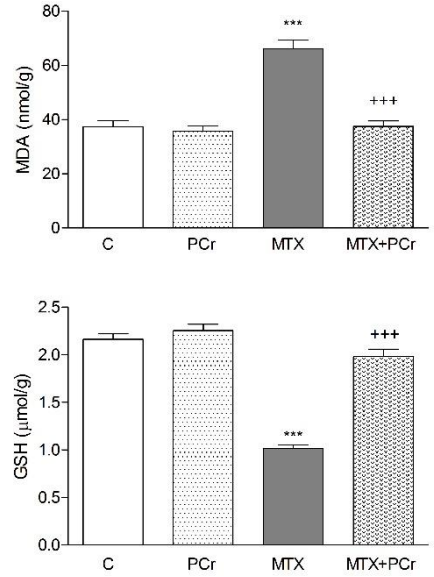


Figure 1. a) Malondialdehyde (MDA) and b) glutathione (GSH) levels the liver tissues of groups. *** p<0.001: vs control group. +++p<0.001: vs saline-treated MTX group.

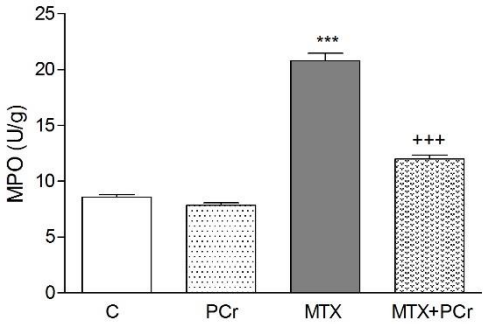


Figure 2. Myeloperoxidase activity in the liver tissues of groups. *** p<0.001: vs control group. +++p<0.01; vs saline-treated MTX group.

There is substantial evidence for a role of reactive oxygen metabolites (ROM) in mediating the hepatic toxicity of some drugs and the pathogenesis of organ failure [7]. Since oxidative stress has been implicated a common link between chronic liver damage and hepatic fibrosis, free radicals may play a role in MTX-induced hepatic toxicity.

As shown in Fig. 1 ROM causes lipid peroxidation in liver tissues since MDA levels were found to be increased. On the other hand MTX also caused tissue antioxidant, GSH.

Besides their direct damaging effects on tissues, ROMs seem to trigger the accumulation of inflammatory cells in the tissues, and as a result aggravate tissue injury. It has been shown that activated neutrophils secrete enzymes (e.g., myeloperoxidase, elastase, proteases) and liberate oxygen radicals [8]. In our observation, elevated MPO levels in liver tissues indicate that neutrophil accumulation contributes to MTX-induced oxidative organ injury. Furthermore, our results also suggest that PCr provided an important protection through the inhibition of neutrophil infiltration.

CONCLUSION

It appears that administration of PCr to MTX treated rats eliminated accumulation of lipid peroxides and returned GSH levels through its antioxidant properties. PCr treatment also inhibited inflammatory reactions. In conclusion, the findings of the current study suggest that PCr may be of therapeutic potential in alleviating the systemic side effects of chemotherapeutics.

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