DOI: https://doi.org/10.53350/pjmhs22161516

ORIGINAL ARTICLE

Evaluation of Protective Action of Selenium Against Hepatotoxicity Caused by Methotrexate In Mice

AAMNA KHOKHAR[1], BILAL KARIM[2], ABDUL AZEEM[3], SADIA LODHI[4], RUKHSANA MUNAWAR[5], YASIR SADIQ[6],TALHA LAIQUE[7]

[1]*Department of Pharmacology, Islamabad Medical and Dental College, Islamabad-Pakistan*
[2]*Department of Biochemistry, Islamabad Medical and Dental College, Islamabad-Pakistan*
[3]*Department of Pharmacology, Watim Medical and Dental College, Rawalpindi-Pakistan*
[4]*Department of Pharmacology, Shifa College of Medicine, Islamabad-Pakistan*
[5]*Department of Pharmacology, Poonch Medical College, Rawalakot-Pakistan*
[6]*Department of Community Medicine, Islam Medical College, Sialkot-Pakistan*
[7]*Department of Pharmacology, Allama Iqbal Medical College, Lahore-Pakistan*
Correspondence to: Talha Laique, Email: talhalaique51@gmail.com: Cell:+92-331-0346682

ABSTRACT

Drug induced liver injury has been regarded as the main cause of acute liver injury in many countries.
Objectives: To evaluate the protective action of selenium against hepatotoxicity caused by methotrexate in mice.
Study Design: Randomized, single blinded study.
Methodology: Present study was done on 18 mice (n = 18) after taking approval from Ethical Review Committee. Administration of methotrexate induced hepatotoxicity. Selenium was administered along with methotrexate in order to evaluate the protective effects. The extent of hepatotoxicity and protective effects were analyzed by measuring levels of serum liver enzymes and histological examination of liver samples.
Statistical analysis: One way ANOVA test was applied for comparison of biochemical markers between groups. Chi Square test was also applied for histopathological results.
Results: In present study, results showed that significant hepatotoxicity was observed ($p < 0.05$) with elevated levels of serum liver enzymes with methotrexate. Selenium was found to significantly reduce the hepatotoxicity ($p < 0.05$). Histological examination revealed steatosis, which was found to be markedly decreased after treatment with selenium along with methotrexate. **Conclusion:** We concluded that selenium helped to protect against hepatotoxicity induced by methotrexate in mice.
Keywords: Drug induced liver injury, Hepatotoxicity, Methotrexate and Selenium.

INTRODUCTION

Drug induced liver injury has been regarded as the main cause of acute liver injury in many countries[1,2]. It is generally classified into two main types i.e. intrinsic and idiosyncratic[2,3]. Commonly, intrinsic hepatic injury occurs among most individuals due to exposure to some drugs that usually occurs in short interval of time while other type happens in lesser number of exposed individuals with onset of days to weeks[3]. Most drug induced liver injuries depict de-arranged liver function tests (raised serum transaminase enzymes)[4].

Methotrexate is an antimetabolite that antagonizes the action of folic acid, making the cells unable to divide[5]. Therefore, it has been employed as a chemotherapeutic agent. Adverse effects of methotrexate include nausea, headache, fatigue, and hair loss[6]. However, excessive administration has been found to result in injury to bone marrow, lungs and liver with hepatotoxicity ranging from mild steatosis to severe fibrosis and necrosis[7].

Selenium is an essential component of glutathione peroxidase and plays an important role in inhibition of free oxygen radicals and hence acts as an antioxidant[8]. Selenium increases the activity of glutathione peroxidase, thus preventing damage caused by free oxygen radicals[9]. Selenium deficiency has been found to be associated with various diseases[10]. Supplementation with selenium has been found to protect against various immunological and neurodegenerative diseases[11]. A study conducted on cadmium exposed mice revealed that selenium supplementation has been found to reduce apoptosis protein factors in the liver and thus offering protection against hepatotoxicity[12]. Selenium, also through its antioxidant potential has been found to regenerate liver cells, thus reducing hepatotoxicity[10]. Other than hepatoprotective effects, selenium has also been found to be beneficial against neurodegenerative diseases through its antioxidant and antiapoptotic properties[12].
Objectives: To evaluate the protective action of selenium against hepatotoxicity caused by methotrexate in mice.

METHODOLOGY

Present study was done on 18 mice (n = 18) after taking approval from Ethical Review Committee. Administration of methotrexate induced hepatotoxicity. Selenium was administered along with methotrexate in order to evaluate the protective effects.
Group-A (control group): It was given intraperitoneal injection of 0.2 ml normal saline.
Group-B: It was given intraperitoneal injection of 20 mg/ kg methotrexate (MTX) and was labelled as MTX group[13].
Group-C: It was given Selenium 0.1 mg/kg oral for 7 days with 20 mg/ kg methotrexate at day 4 and was labelled as MTX + Selenium group[14].

After 24 hours of treatment of each group blood samples were taken in order to determine serum Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) levels in U/ L. Liver samples were also obtained for histopathological examination.

Statistical Analysis: Data was analyzed by using SPSS software v23. One way ANOVA test was applied for comparison of biochemical markers between groups. Chi Square test was also applied for histopathological results with $p < 0.05$ as significant.

RESULTS

In group B that is MTX group, significant hepatotoxicity was observed ($p < 0.05$) with elevated levels of serum liver enzymes with methotrexate (Table-1).

Table 1: Serum AST, ALT, ALP levels among Different Groups

Parameters	ALT (U/L)	AST (U/L)	ALP (U/L)
Group A (Control)	31.33 ± 3.28	87.83 ± 3.57	94.67 ± 4.9
Group B (MTX)	73.67 ± 3.66 [a]	128.50 ± 7.77[a]	315.33 ± 12.44[a]
Group C (MTX + Selenium)	46.67 ± 4.33 [b]	96.00 ± 2.65[b]	163.50 ± 3.41[b]

[a] $p < 0.05$ versus control group, [b] $p < 0.05$ versus MTX group.

In group B, liver histopathological examination revealed steatosis (Figure-1).

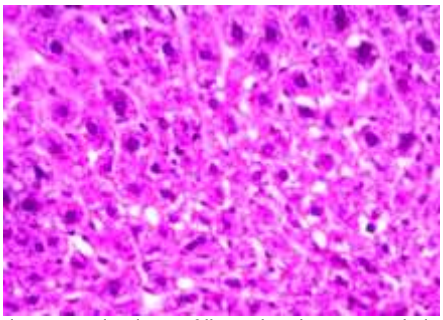

Figure 1: Histology of liver showing steatosis in Group-B

However, steatosis was found to be markedly decreased in group-C animals who were treated with selenium along with methotrexate (Figure -2 & Figure – 3).

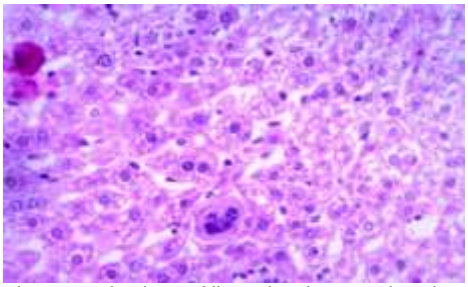

Figure 2: Histology of liver showing reactive changes in Group-C

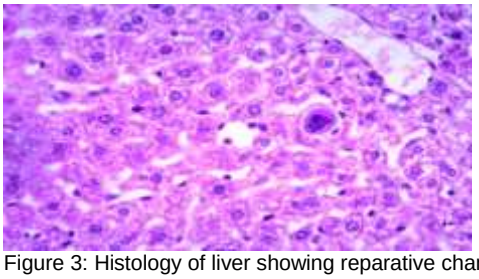

Figure 3: Histology of liver showing reparative changes in Group C

DISCUSSION

Liver is the chief organ that is involved in metabolism of a large number of drugs[15]. Drug induced liver injury has always been the major challenge faced by health care professional[16]. The mechanism usually involves a toxic metabolite which may be a chemical or free radical which either evokes an immune response or directly affects the cell[15,16].

Many drugs have been found to be the cause of hepatotoxicity. A single injection of methotrexate has been found to result in elevated levels of serum transaminases and abnormalities in liver histology[17]. Although the precise mechanism of methotrexate induced liver injury is unclear, however different mechanisms have been reported. Several studies have reported oxidative stress to be the possible cause of methotrexate induced liver injury, resulting in production of free oxygen radicals such as superoxide anions, hydrogen peroxide radicals, promoting lipid peroxidation of membranes[17-19]. At low doses, methotrexate has the potential to induce T-cell apoptosis, proliferation of cells and production of inflammatory cytokines[18]. Since methotrexate causes reduction in folic acid, decrease in folic acid stores in liver may be responsible for hepatotoxicity but it has not been experimentally confirmed. However, supplementation with folic acid has been found to result in reduction in transaminase levels[6].

In our study, administration of methotrexate resulted in elevation in serum ALT, AST and ALP levels. This is in line with the studies conducted by Samdanci et al, Al-Rashidyet al and Santhakumar et al[20-22]. Histopathological analysis revealed steatosis which is also in line with these studies.

After administration of selenium along with methotrexate, serum transaminase levels were reduced and reparative changes were seen in liver parenchymal tissues on histological examinations which indicated protective effects on liver. These results were in line with the experimental studies conducted by Hadi et al and Gadallah et al[9,23]. Selenium helps to prevent lipid peroxidation of membranes by enhancing the activity of glutathione peroxidase enzyme and hence maintains the integrity of tissues[9]. Therefore, after hepatotoxicity, this antioxidant action of selenium dependent glutathione peroxidase helps in regeneration of liver cells[24]. Selenium also has anti-inflammatory effects on the liver as it is found to reduce proinflammatory cytokines of the liver as well as serum C-reactive protein[9].

Limitations: Our study had limitations like financial constraints, lack of resources and small sample size.

CONCLUSION

We concluded that selenium helped to protect against hepatotoxicity induced by methotrexate in mice.

Authors' Contribution: AK&BK: Conceptualized the study, analyzed the data, and formulated the initial draft.

AA&SL: Contributed to the histomorphological evaluation.

RM&YS: Contributed to the analysis of data and proofread the draft.

TL: Contributed to the proofreading the manuscript for intellectual content.

Acknowledgement: All authors are thankful to Allah SubhanaoTaála.

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