Protective Effect of Selenium against Methotrexate Induced Hepatotoxicity

Dr. Hafiz Arslan Ul Hassan¹, Dr. Usman Waleed², Dr. Abdul Mannan³

¹ MO at 129/15L Mian Channu
² MO at BHU 90/F Hasilpur
³ MO at RHC 56 WB Vehari

ABSTRACT

Introduction: The liver is the largest internal organ by percent weight in the human body and has crucial functions, including cholesterol production, intermediary metabolism, hormone synthesis, bile and urea production and drug detoxification.

Objectives: The main objective of the study is to analyse the Protective effect of selenium against methotrexate induced hepatotoxicity in mice.

Material and methods: This descriptive study was conducted in Jinnah Hospital Lahore during 2020. Selenium and all chemical reagents of analytical grades were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). The dose of MTX diluted in water (50% 1ml/kg of body weight of Mice were applied twice a week for six weeks to induced hepatocellular injury determined by liver function tests. The mice were maintained under pathogen-free conditions with air conditioning, a 12-hr light/12-hr dark cycle, and 55% humidity.

Results: Results shows that a significant increase in serum enzymes (ALT, AST, ALP) and significant decrease in total protein was observed in all groups receiving MTX for the induction of hepatic injury. Mice receiving combination therapy of selenium along with MTX shows a time course recovery towards normalcy. The highest value of ALT (94.83 IU/L), AST (73.21 IU/L) and ALP (157 IU/L) were recorded in Mice receiving MTX for hepatic injury. The lowest values (63.67, 51.49 and 139.95 IU/L) was recorded in group treated with MTX+ Se (200mg/kg b.w) but both the groups differed non significantly and shows the same trend but a decreasing trend in serum enzymes (ALT 32.85%), (AST 29.67%) and (ALP 11.40%).

Conclusion: It is concluded that selenium supplementation in MTX treated rats elicited a reduction in the toxic effects of the pesticide by improving the studied parameters, which was confirmed by the biochemical analysis of serum.

KEY WORDS: Human, Injury, LFT, Liver.

INTRODUCTION

The liver is the largest internal organ by percent weight in the human body and has crucial functions, including cholesterol production, intermediary metabolism, hormone synthesis, bile and urea production and drug detoxification. The functional cells of the liver are eosinophilic cells alluded to as hepatocytes [1]. The liver is coordinated into underlying units called lobules. Every lobule is fixed on a terminal hepatic venule. Emanating from the focal venule are plates of hepatocytes isolated by wide vascular channels called sinusoids. Sinusoids convey blood from the terminal parts of the entry vein and hepatic supply route, which bring supplement rich blood from the gastrointestinal lot and oxygen rich-blood from the lungs, respectively [2].

Methotrexate (MTX), a folic corrosive antimetabolite, is utilized as antineoplastic medication acting in the therapy of many circumstances like different diseases, rheumatoid joint pain and psoriatic arthritis [3]. Methotrexate has numerous application fields as a restorative specialist at high portions in numerous malignancies and at low dosages in immune system infections. Notwithstanding these signs of wide use, MTX has drawn consideration with a scope of secondary effects like nephrotoxicity and hepatotoxicity. The fundamental system of MTX-interceded liver harmfulness has not yet been explained fully [4].

Selenium is a minor component that is essential for people and other living life forms. It is engaged with a few natural capacities since it is a part of numerous selenoproteins and selenoenzymes. Selenium is plentiful in soils. Its focuses in soils change generally from area to region [5]. Selenium is found in various structures. Inorganic selenium compounds, selenite and selenate, overwhelm in soil and water, though natural selenium structures, selenomethionine and selenocysteine, rule in plants. Selenium harmfulness in food sources relies upon its synthetic structures. By and large, natural selenium is less harmful than the inorganic selenium [6].
Nonetheless, there are various logical investigations on the theory that oxidative pressure brings about a lot of free extreme arrangement as one of the fundamental reasons. In this way, it has been seen that MTX causes the oxidative injury of the DNA and triggers lipid peroxidation. Overproduction of receptive oxygen species (ROS) involved in MTX hepatotoxicity drains cell enzymatic and non-enzymatic cancer prevention agent guard systems [7].

OBJECTIVES
The main objective of the study is:
- To analyse the Protective effect of selenium against methotrexate induced hepatotoxicity in mice.

MATERIAL AND METHODS
This descriptive study was conducted in Jinnah Hospital Lahore during 2020. Selenium and all chemical reagents of analytical grades were purchased from Sigma Chemical Co. (St. Louis, Mo, USA).

INDUCTION OF HEPATIC DAMAGE
The dose of MTX diluted in water (50% 1ml/kg of body weight of Mice were applied twice a week for six weeks to induced hepatocellular injury determined by liver function tests. The mice were maintained under pathogen-free conditions with air conditioning, a 12-hr light/12-hr dark cycle, and %55 humidity. In addition, all of the mice had free access to food and water during the experiments. In our study, we used 36 Wistar Albino male mice (age; 9 weeks, weight; 210-230 g).

EXPERIMENTAL DESIGN

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Control</td>
</tr>
<tr>
<td>B</td>
<td>MTX (1ml/kg body weight)</td>
</tr>
<tr>
<td>C</td>
<td>MTX (1ml /kg body weight) + Selenium (50mg/kg/body weight)</td>
</tr>
<tr>
<td>D</td>
<td>MTX (1ml/kg body weight) + Selenium (100mg/kg/body weight)</td>
</tr>
</tbody>
</table>

1.0 ml blood sample was taken from coccygeal vein of the rat. Blood was subjected to centrifuge at 3000-4000 rpm for 10-15 minutes for the separation of serum. The estimation of AST, ALT ALP and antioxidants was estimated by following principle by using commercially available Bio Merux and Randox kits.

STATISTICAL ANALYSIS
Statistical analysis were performed using Instat-3 computer program (Graph pad software Inc, San Diego, CA, USA). The level of significance was set at p = 0.05.

Results
Results shows that a significant increase in serum enzymes (ALT, AST, ALP) and significant decrease in total protein was observed in all groups receiving MTX for the induction of hepatic injury. Mice receiving combination therapy of selenium along with MTX shows a time course recovery towards normalcy. The highest value of ALT (94.83 IU/L), AST (73.21 IU/L) and ALP (157 IU/L) were recorded in Mice receiving MTX for hepatic injury. The lowest values (63.67, 51.49 and 139.95 IU/L) was recorded in group treated with MTX+ Se (200mg/kg b.w) but both the groups differed non significantly and shows the same trend but a decreasing trend in serum enzymes.

Minimum value (4.12 IU/L) for total protein was recorded in Mice receiving MTX with 33.65% decrease as compared to normal control. Second highest value (5.57 IU/L) of TP was recorded in rat group treated with MTX+Se (200mg/kg b.w) but differed non significantly with Mice group receiving MTX+Se (50mg/kg b.w).

An increasing trend (25.63% and 26.19%) in TP as compared to MTX control near to normal control was noted in a group administered with Se. The time course studies revealed that the effect of Se significantly correlate with time span or healing process by directly proportional to a specific time period for serum enzymes and TP.
The present study investigated the oxidative damage and hepatotoxicity of acute oral administrations of MTX and the protective role of selenium [8]. Lipid peroxidation level and cell reinforcement catalyst exercises were estimated in serum and plasma, individually, to inspect the entire wellbeing and oxidative status of creatures because of abundance admission of MTX. The noticed side effects in the test mice because of the given dosages of MTX demonstrate its poisonousness when taken in overabundance. Selenium-instigated oxidative pressure mirrors the poisonousness and forestall harm of the body organs remembering liver for the current review [9].

Our outcomes showed that persistent treatment with 2,4-D brought about hepatotoxicity, as uncovered by an expansion in liver capacity markers Aminotransferases (ALT, AST), antacid phosphatase (ALP), lactate dehydrogenase (LDH) and absolute bilirubin (TB), alongside diminished all out protein content and egg whites [10]. A general favorable to oxidant impact was related with a decline in the diminished glutathione (GSH) content and the enzymatic action of glutathione-S-transferase (GST), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx), and an expansion in malondialdehyde (MDA) and protein carbonyl levels [11].

Oxidative pressure and aggravation occasions are two head instruments to cause apoptosis of liver cells16. Many examinations have demonstrated the enactment of proapoptotic proteins in liver harmful harm instigated by MTX [12]. Apoptosis begins by various

**DISCUSSION**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD</td>
<td>0.32</td>
<td>0.026</td>
<td>0.036</td>
<td>0.031</td>
<td>0.02</td>
<td>0.099</td>
</tr>
<tr>
<td></td>
<td>(0.028–0.049)</td>
<td>(0.02–0.044)</td>
<td>(0.024–0.046)</td>
<td>(0.02–0.049)</td>
<td>(0.010–0.026)</td>
<td></td>
</tr>
<tr>
<td>CAT</td>
<td>0.22</td>
<td>0.50</td>
<td>0.32</td>
<td>0.41</td>
<td>0.22</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>(0.11–0.30)</td>
<td>(0.22–0.94)</td>
<td>(0.21–0.74)</td>
<td>(0.06–0.86)</td>
<td>(0.07–4.38)</td>
<td></td>
</tr>
<tr>
<td>GSH-Px</td>
<td>0.83</td>
<td>4.69</td>
<td>1.24</td>
<td>1.52</td>
<td>0.96</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(0.62–1.50)</td>
<td>(2.20–7.90)</td>
<td>(0.66–5.84)</td>
<td>(0.90–2.64)</td>
<td>(0.36–5.86)</td>
<td></td>
</tr>
<tr>
<td>MDA</td>
<td>3.27</td>
<td>6.98</td>
<td>11.18</td>
<td>8.86</td>
<td>13.97</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GSH</td>
<td>2.07</td>
<td>2.46</td>
<td>1.97</td>
<td>2.37</td>
<td>1.58</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>(0.5–3.09)</td>
<td>(1.9–2.76)</td>
<td>(1.26–2.87)</td>
<td>(2.09–2.73)</td>
<td>(1.05–1.90)</td>
<td></td>
</tr>
<tr>
<td>MPO</td>
<td>12.75</td>
<td>13.76</td>
<td>13.49</td>
<td>13.86</td>
<td>14.65</td>
<td>0.259</td>
</tr>
</tbody>
</table>

**Table 03:** The level of the tissue antioxidants and oxidants measured in mice

**Table 02:** The level of serum transaminases measured in the mice

**Table 01:** Analysis of variance for the effect of standardized extracts of Se in MTX treated mice
occasions that lead to the actuation of a group of caspases or proteases. Caspases are answerable for the morphologic and biochemical qualities of apoptotic cells. Caspases are huge flagging particles of apoptosis, discovery of caspases determinative for an essential stage in apoptosis instrument. It was accounted for that caspase-3 is one of the most significant proteases that start both the extraneous and natural apoptosis pathways and furthermore a marker of the irreversible phase of the apoptosis. MTX prompts mitochondrial expanding and film harm initiating the caspase course. Perhaps the most secure method for deciding MTX-incited liver poisonousness is liver biopsy and histopathological examination [13].

CONCLUSION
It is presumed that selenium supplementation in MTX treated rodents inspired a decrease in the harmful impacts of the pesticide by working on the concentrated on boundaries, which was affirmed by the biochemical investigation of serum. Selenium seems to have a promising prophylactic impact through its successful enemy of revolutionary activity against the hepatotoxic impacts of MTX.

REFERENCES
1. Mahmood Rasool, Javed Iqbal, Arif Malik, Hafiza Sobia Ramzan, Muhammad Saed Qureshi, Muhammad Asif, Mahmood Husain Qazi, Mohammad Amjad Kamal, Adeel Gulzar Ahmed Chaudhary, Mohammed Hussain Al-Tahtani, Siew Hua Gan, Sajjad Karim. Hepatoprotective Effects of Silybum marianum (Silymarin) and Glycyrrhiza glabra (Glycyrrhizina) in Combination: A Possible Synergy. Evidence-Based Complementary and Alternative Medicine 2014; 2014: 1 doi: 10.1155/2014/641597