Original Article

Hepatocytes in Methotrexate Induced Hepatotoxicity

Morphology of Hepatocytes in Methotrexate Induced Hepatotoxicity and Evaluate the Preventive Role of Antioxidant Agents in Rats: A Comparative Study

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ABSTRACT

Objective: Compare the oxidative role of β-carotene (BC) and Resveratrol (RSV) in Methotrexate (MTX) induced liver toxicity based on histopathology.

Study Design: A comparative study

Place and Duration of Study: This study was conducted at the Pharmacology, Al-Tibri Medical College, Isra University Karachi Campus from January 2021 for a period of October 2021.

Materials and Methods: 48 healthy Wistar albino rats were included in the study and divided into 6 groups each consisting of 8 subjects, Group I(Control group) was given Normal Saline, Group II was given BC, Group III was given RSV, Group IV MTX, Group V BC+MTX, Group VI RSV+MTX for a period of 24 days. On the 25th day, subjects were euthanized and the liver organ was extracted and sectioned to be studied under a light microscope with H&E staining being performed. For glycogen study, staining was carried out using PAS and for collagen fibers it was carried out using Trichome staining.

Results: Normal Morphology, glycogen content, and deposition around the central vein was shown in Group I, II, and III. In Group IV central architecture was lost, congestion was seen around central vein, glycogen content was depleted, and deposition in collagen was present. In the both intervention Group V and VI, hepatic lobular architecture and hepatocytes were normal, uneven distribution of glycogen along with restoration was present, and no collagen was present.

Conclusion: Beta-carotene and Resveratrol showed hepatoprotective effects in methotrexate induced hepatotoxicity in rats.

Key Words: Methotrexate, Beta-carotene, Resveratrol, Hepatotoxicity

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INTRODUCTION

The liver is the second largest organ of the human body, and is considered to be one the most remarkable organ that protects the human from various insults from xenobiotic compounds. Unfortunately, as it protects the body and performs other myriad of functions, it is still susceptible to injuries caused by other chemical compounds[1]. Loss of liver activity is deemed to be detrimental not just to it, but to other multiple organs as well because of the release of many detrimental toxic factors that causes injury to the liver[2]. In medicine there are many drugs that through scientific data have shown to cause hepatotoxicity. These includes Anti-epileptic, Anti-tuberculosis drugs, and NSAIDs to name a few [3-5]. One of these drugs is Methotrexate, a key drug in the treatment of rheumatoid arthritis and other rheumatic diseases. The folate antagonism is known to be a contributing factor to the effects that are required to treat against malignant diseases in methotrexate[6]. Although methotrexate is a widely used drug in clinical medicine, the most common side effect associated with its use is also hepatic injury. Use of repeatedly high doses of methotrexate or chronic use of methotrexate is associated with damaged to the liver [7]. Beta-carotene is a lipid soluble carotenoid, which is a naturally

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occurring antioxidant that can boost up the antioxidant defense of the body by inhibiting the single oxygen and fighting off oxygen radicals [8]. Presence of beta-carotene in nanoparticles substantially improves its biological activity in aqueous media[9]. Similarly, another antioxidant compounds Resveratrol, a bioactive compound found in plants such as plums, grapes, and peanuts, is also said to have anti-inflammatory and antioxidant effects [10-11]. If antioxidant effects can be initiated by these elements, then the possibility of reducing the hepatotoxic effects caused by methotrexate can also be reduced. With that in mind a study was conducted to compare the oxidative role of Bea-carotene and Resveratrol in methotrexate induced liver toxicity on the basis of histopathology.

MATERIALS AND METHODS

We conducted an experimental study on 48 healthy Wistar albino rats after ethical approval was granted from the institutional review board of the university. The length of the study spanned from January 2021 for a period of October 2021. The subjects were acquired by the animal house of the college with each albino rats weighting 210-310 grams that were measured using a weight balance. The animals that were acquired were also kept in a well-ventilated room with adequate light and ambient temperature (23-25◦C) inside clean cages. Food and water were supplied ad libitum. From a local pharmacy shop near the institute, we purchased Methotrexate, Beta-Carotene, and Resveratrol. The subjects were then divided into 6 different each having 8 subjects with the following interventions taking place in each group.

- Group I (control) rats received equivalent volumes of saline `
- Group II (β-carotene) rats received β-carotene (10mg/kg/ day intraperitoneal) for 24 days.
- Group III (Resveratrol) rats received resveratrol (10mg/kg/ day intraperitoneal) for 24 days.
- Group IV (MTX) rats were given MTX as a single intraperitoneally dose (in saline, 20mg/kg) on day 21 of the experiment.
- Group V (β-carotene + MTX) rats were given β-carotene by intraperitoneal injection in vehicle (saline) 10 mg/kg/day for 24 days and then further administered MTX at a dose level of 20 mg/kg on day 21 of the experiment.

On the 25th day of the studies all the rats were euthanized under anesthesia after being weighted. Midline longitudinal incision was given to expose the organs and the liver was extracted from the subjects. Histopathological study was carried out at the institution. The liver was thoroughly washed with normal saline and then cut into small sections, fixed into 10% formalin for PAS for 36 hours. H&E staining for histological studies were carried out on the liver samples. For glycogen content, the tissue sections were stained using Periodic acid Schiff (PAS) and for collagen fibres, sections were stained using Masson's trichome stain.

RESULTS

Microscopic Observation in Group I: The morphological examination of H& E stained section of liver of Control group revealed normal architecture of parenchyma with plates of hepatocytes (Figure 1, 2 & 3)**.**

PAS stained section showed glycogen was evenly distributed throughout the lobule (Figure 2). Massons' Trichrome stained section of liver showed deposition of collagen around central vein grade I (Figure 3).

Group II

Deposition of Glycogen: PAS stained section showed that the glycogen was evenly distributed (Figure 5). Massons' Trichrome stained section revealed deposition of collagen fibers around portal triad grade I (Figure 6).

Microscopic Observation in Group III: The morphological examination of H &E stained section of liver in group III showed normal architecture of liver parenchyma (Figure 7). PAS stained section showed even distribution of glycogen (Figure 8). Massons' Trichrome stained section revealed deposition of collagen around central vein grade I (Figure 9).

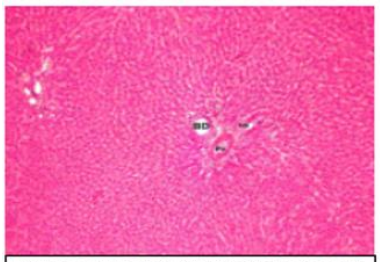

Figure 1: H&E Stained section 5 µm thick section of control (Saline) Group I of liver showing sinusoids (HS), bile duct (BD), Portal vein(PV) and hepatic artery (HA).(Photomicrograph X 100)

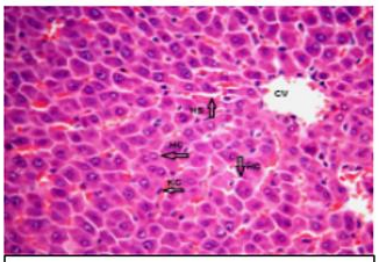

Figure 2: PAS stained section 5 µm thick section of liver of Control Group I showing central vein (CV), hepatocytes(HC), Sinusoids(HS) and kupffer cells (KC). PHOTOMICROGRAPH X 400.

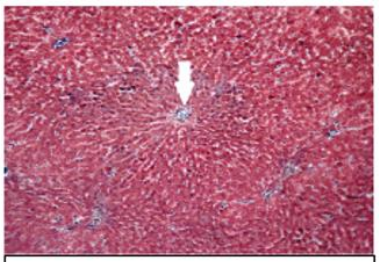

Figure 3: Massons Trichrome stained 5 µm thick section of liver of Control Group I showing Collagen Fibers around central vein (white arrow) Photomicroghraph X 100.

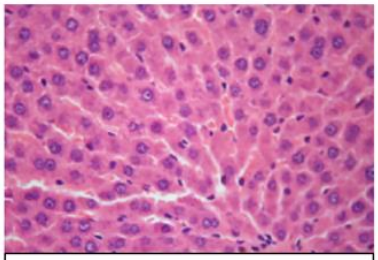

Figure 4: H & E stained 5 µm thick section of β-CAROTENE treated group II liver showing normal architecture Photomicroghraph X 400.

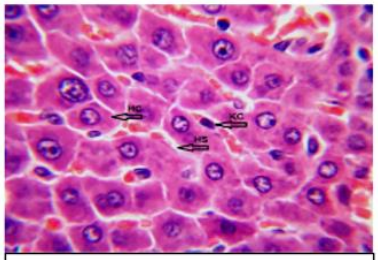

Figure 5:PAS stained 5 µm thick section of β-CAROTENE treated group II liver showing hepatocytes (HC), sinusoids (HS) and kupffer cells (KC). PHOTOMICROGRAPH X 1000.

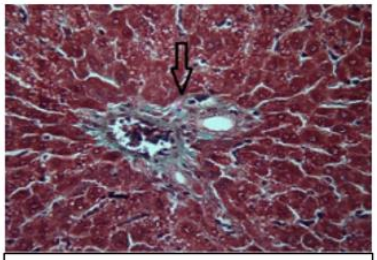

Figure 6: Trichrome Stained, 5 µm thick section of β-carotene treated group II showing deposition of collagen fibers (black arrow showing Portal triad) (photomicrograph x400)

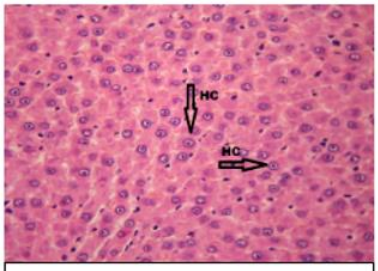

Figure 7: H&E stained 5 µm thick section of liver of Resveratrol treated group III liver showing normal architecture and Hepatocytes (HC-arrows) Photomicrograph X 400.

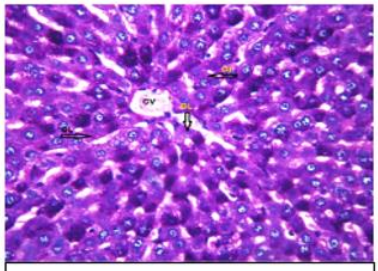

Figure 8: PAS stained 5 µm thick section of Resveratrol treated liver of Group III showing normal glycogen content (GL), Central vein (CV). Photomicroghraph X 400.

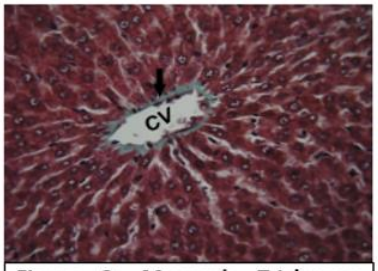

Figure 9: Masson's Trichrome stained 5 µm section of resveratrol treated group III of rat liver showing deposition of collagen fibers around central vein (black arrow) photomicrograph x400

DISCUSSION

Methotrexate is a highly potent drug having anti-folate and anti-metabolite activity. Used in the therapy leukemia, lymphoma, and various other solid tumors. Whereas Beta-carotene and Resveratrol are both potent antioxidant as they have the ability to reduce the oxidative stress, free radical levels, and inflammatory levels in various organs in the body. The study was design to see the hepatoprotective effects of Beta-Carotene and Resveratrol on methotrexate induced hepatic toxicity in wistar rats. The dose used in the study for methotrexate was 20mg/kg of body weight, recommended by Vardi et al (2010)[12]. Changes were seen in the present study that occurred after treatment with methotrexate. These changes include distorted hepatic lobular architecture, congestion of central vein, dilated central vein, ballooning of hepatocytes, mononuclear infiltration, and dilated sinusoids. These findings are similar to another study by Patel et al (2014) who showed similar histopathological changes such as vascular congestion and sinusoidal dilation of hepatocytes with effects on misoprostol on hepatotoxicity caused by methotrexate [13]. When there was administration of Beta-carotene and Resveratrol in Group V and VI respectively, the tissue damage was less and was reported by Firdous et al (2011) that the administration of Beta-carotene had a protective effect against the generation of free radical and oxidative stresses, further reporting an improvement in the architecture of the hepatocytes when Beta-carotene was administered in paracetamol induced hepatotoxicity [14]. The methotrexate treated group showed marked depletion , which can be due to the inhibition of mitochondrial energy metabolism and the lack of liver to store glycogen. In the intervention groups (Group V & VI), restoration of glycogen content was seen due to enhanced protein synthesis. This is in agreement with another study by Kose (2012), who observed restoration of glycogen content after using resveratrol. In our current study, there was also a significant rise in the hepatic tissue collagen content in the methotrexate group, which demonstrated fibrolytic activity also observed by Tunali-Akbay et al (2010) in his own study[15]. The fibrous tissue is created in response to toxic insult and inflammation to liver. In the Beta-carotene and Resveratrol group there was no fibrosis found, a finding comparable to another study. This can be due to the reduction of the oxidative stresses. Future studies can be done on other drugs that cause similar hepatotoxic effects and see if these antioxidant agents provide the same effects in them as well.

CONCLUSION

Based on our study and the evidence from the study, it can be concluded that indeed methotrexate is a hepatotoxic drug, causing liver damage in rats. However, hepatoprotective effects can be seen by both Beta-carotene and Resveratrol. These promising results suggest us that these hepatoprotective effects can be also induced in humans who are chronically using methotrexate.

Author's Contribution:

Concept & Design of Study:	Syed Muhammad Masood Ali
Drafting:	Sara Sughra, Muhammad Iqbal Ahsan
Data Analysis:	Sonia Khan, Fareeda Islam, Qurat-ul-Ain Khan
Revisiting Critically:	Syed Muhammad Masood Ali, Sara Sughra
Final Approval of version:	Syed Muhammad Masood Ali

Conflict of Interest: The study has no conflict of interest to declare by any author.

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