Histo-morphometric changes in Methotrexate induced Hepatotoxicity in Albino rat

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ABSTRACT

Objective: To observe the toxic effects of Methotrexate on the Histomorphometry of liver in rat model.
Study Design: An Experimental, Observational Study.
Place and Duration: At Department of Anatomy, Baqai Medical University, Karachi from 1st July 2017 to 31st August 2017.
Methodology: Male adult albino Wistar rats (n=30), aged 10-12 weeks and weighing 180-200g of weight were divided in two equal groups. Group-A (control), received no intervention and Group-B received single dose of 20mg/kg of methotrexate (MTX) intraperitoneally. Liver was harvested and its architecture was assessed microscopically. Mean hepatocyte count, hepatocyte diameter and hepatocyte nuclear diameter were calculated with micrometer.
Results: Liver sections from Methotrexate treated group B exhibited a distortion in normal hepatic architecture. Deranged hepatic cords, large areas of parenchymal degeneration and hemorrhage, and dilation of sinusoids and central vein were observed. The hepatocytes showed advanced hydropic degeneration, fragmentation and pyknosis of nuclei. Mean hepatocyte count and mean hepatocyte nuclear diameter was decreased while hepatocyte diameter was increased significantly in Methotrexate group.
Conclusion: Methotrexate produces toxic cellular changes as seen in the histomorphometry of liver tissue in rat model.
Keywords: Albino rat, Methotrexate, Hepatotoxicity, Histomorphometry, Hepatocyte count, Hepatocyte diameter, Nuclear diameter.

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INTRODUCTION

The liver is the largest organ which provides major exocrine and endocrine functions, including production of bile, metabolism of dietary composites, detoxification, maintenance of glucose levels and the blood homeostasis control[1,2]. Metabolism of drugs takes place in the liver and therefore it is the target site for modulating biotransformation of chemicals[3]. Drugs and other chemicals cause 5% of liver injuries[4].

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Methotrexate (MTX) is an anti-folate and antimetabolite, which is extensively used as a chemotherapy agent and immune system suppressant. It has long been used in the therapy of cancer and for medical abortions but is now most commonly used for treating autoimmune diseases like rheumatoid arthritis and psoriasis[5]. MTX inhibits cellular growth by inhibiting DNA, RNA, thymidylate and protein synthesis. It increases oxidative stress and also has association with increase in lipid peroxidation,[6,7] resulting in MTX causing hepatotoxicity in 10% of its users[8].

To the best of our knowledge, no data has been published regarding quantitative measurements of hepatic parenchyma and hepatocytes in methotrexate-induced toxicity. Therefore, this study was conducted with an objective to observe the toxic changes caused by methotrexate on the histomorphometry of liver in rat model.

METHODOLOGY

This experimental, observational study designed to illustrate the histological changes in methotrexate induced hepato-toxicity in albino rats. The study was approved and accepted by the Board of Advance Research and Studies (BASR) and Ethics committee of Baqai Medical University. This study was performed at animal house of Baqai Medical University and Department of Anatomy (Baqai Medical University), Karachi from 1st July 2017 to 31st August 2017.

Study included 30 adult albino rats of age 10 - 12 weeks and weighing 180 - 200gm. Animals were kept in transparent plastic

cages with bedding of soft wood-chip (5 animals per cage) at a temperature of 25-30°C and humidity ranging between 40-70%. Natural 12 hours day and night cycle and laboratory pellet diet and water ad libitum were provided to them.

They were first acclimatized then were divided randomly in 2 groups of 15 each. Group A served as control group; received no intervention; kept on standard diet for 10 days. While Group B received 20mg/kg dose of methotrexate (injection Unitraxate) on 4th day of study intraperitoneally.

On the 8th day, rats were given ether anesthesia in a glass container. They were fixed on dissection board and a midline incision from interclavicle fossa to lower pelvic region was given to completely uncover the organs.

Liver was dissected out and tissue was fixed in 10% formalin for 24-48 hours, then processed to make tissue blocks. Six micron thick sections of tissue was made with rotatory microtome and stained with Hematoxylin and eosin to observe under microscope for histomorphology and micrometry. Approximately ten (8-12) microscopic fields were randomly observed in each liver section.

Data Analysis: SPSS (Statistical Packager for Social Sciences) version 23.0 was used to store and analyze all data. All quantitative variables were calculated through means with standard deviation error, expressed as mean ± standard error (Mean ± SE). Statistical analysis was performed using student's t test between 2 groups. P - Value of 0.05 or less was considered significant at 95 % confidence interval.

RESULTS

The morphological examination of the H & E stained liver section of albino rats in control group A showed normal architecture of hepatic lobule, characterized by a radial arrangement of hepatocytes around the central vein in each lobule. The cell cords are separated by narrow blood sinusoids. The sinusoids are shown to be lined by endothelial cells and Kupffer cells. Large polyhedral hepatocytes with homogenously distributed acidophilic cytoplasm with darkly stained nuclei are seen. Few bi-nucleated cells are also seen. The portal triad showed portal vein, hepatic artery and 1-2 bile ducts.

The examination of liver section of rats in MTX treated group B showed loss of normal hepatic architecture and marked distortion of the hepatic cell. Large areas of apoptosis in parenchyma and degeneration were seen. The central vein was distorted, dilated and congested. Moderate sinusoidal dilation was also visualized. The hepatocytes showed advance hydropic degeneration and fragmentation and pyknosis of nuclei. There was sparse lymphocytic infiltration around the central vein.

On micrometric examination, mean hepatocyte count of group A was found to be 16.12±1.51 which was decreased significantly (P=0.01) in group B to the value of 7.18±1.36. Mean hepatocyte diameter of group A was 13.34±0.66 which increased significantly (P=0.01) in group B to 17.6±1.51. Mean nuclear diameter of group A was 7.28±1.51 which was decreased significantly (P=0.01) to 5.29±1.41 in group B.

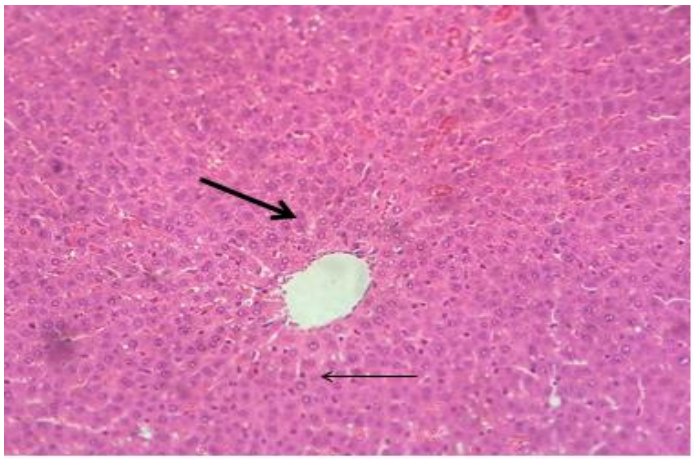

Figure-1 Photomicrograph of liver section of MTX group B showing degenerative hepatocytes (thick arrow) with condense nuclei (thin arrow) H & E ×100

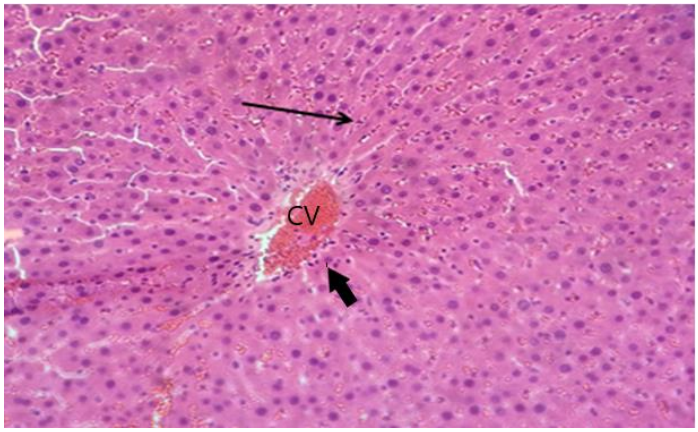

Figure-2: Photomicrograph of liver section of MTX treated group B showing congestion of central vein (CV) and hepatic sinusoid with necrotic cells and scattered degenerating hepatocytes (thin arrow). Lymphocytes are also seen around central vein (Thick arrow) H & E × 100

Table-I: Mean hepatocyte count per reticule, mean hepatocyte diameter and mean nuclear diameter of rats liver in different groups (N=30)

Group	Treatment	Hepatocyte Count Mean ± SD	Hepatocytes Diameter Mean ± SD	Nuclear Diameter Mean ± SD
A (n=15)	Control	16.12±1.51	13.34±0.66	7.28±1.51
B (n=15)	Methotrexate	7.18±1.36*	17.6±1.51*	5.29±1.41*
*p<0.05 statistically significant				

DISCUSSION

Methotrexate has been associated with structural and histo-morphological alterations in the liver[9]. In the present study, acute hepatotoxicity was induced by 20mg/kg dose of MTX, administered through intra-peritoneal route. The amount of dose was similar to previous studies of Abo-Haded et al[10] and Khokhar et al[11].

In current study, morphological examination of liver sections of

MTX group B exhibited significant histological changes in comparison to control group A. This is in consistence with the study of Sabiu and colleagues[12] in which drug treated liver sections showed changes in liver architecture. A distortion in normal hepatic architecture and hepatic cell chords were observed with large areas of degeneration and hemorrhage in hepatic parenchyma. Central vein, sinusoids and portal vein were dilated and congested with accumulation of red blood cells and mononuclear cell infiltration within and around the portal triad. The study conducted by Abo-Haded and colleague[10] also showed same changes in the liver of mice when treated with MTX.

The mean hepatocyte count per reticule of liver sections of animals treated with MTX was lower than that of animals in the control group. This is in accordance with the study of Maheshwari et al et al[13] and Semenov et al[14] with other hepatotoxic agents like carbamazepine and tri-terpene derivatives.

There was a significant increase in hepatocyte diameter in MTX treated groups in comparison to control group A. The cellular enlargement is due to ballooning degeneration, with irregularly clumped cytoplasm and large clear spaces, secondary to damage of cytoskeleton by MTX induced oxidative stress. This is in agreement with the results obtained by Rasheed et al[15] and Bhadoria and colleagues[16] in their respective studies.

The nuclear diameter in MTX treated group B was significantly decreased than that in control group A. This is similar to the observations made by Omotso et al[17]. On the contrary Chaudhary[18] reported increase in nuclear span in doxorubicin caused liver toxicity.

CONCLUSION

Methotrexate is an effectual treatment for different diseases but its use is restricted due to its potential hepatotoxic nature. Our results showed the toxic cellular changes induced by MTX in the histomorphometry of liver tissue in rat model.

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AUTHOR'S CONTRIBUTION

Kazmi T: Main investigator and analysis, interpretation of data.
Younus N: Drafting the work and revising it critically for important intellectual content
Ali AB: Literature search and Interpretation of data.
Sultana K: Designed research methodology.

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