HEPATOPROTECTIVE EFFECT OF DEFERASIROX IN CONCANAVALIN A-INDUCED ACUTE LIVER INJURY IN RATS

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Abstract

Hepatitis poses a significant health issue worldwide as it may progress to cirrhosis and hepatocellular carcinoma. The crucial role of liver in different metabolic and synthetic functions of the body stimulates researchers to continually explore and develop different hepatoprotective drugs. The present study was designed to assess the potential hepatoprotective effect of deferasirox (DFX) in a rat model of acute liver injury induced by concanavalin A (Con A) at a dose of 20 mg/kg intravenously dissolved in normal saline solution. The hepatoprotective effect of DFX was screened at the doses (25, 50, and 100 mg/kg) via assessing the hepatotoxicity indices and histopathological examination. DFX at a dose of 100 mg/kg was the most effective in preventing the rise in activities of hepatotoxicity serum markers; alanine aminotransferase (ALT), and aspartate aminotransferase (AST) enzymes and histopathologic changes induced by con A.

Keywords: Deferasirox; Concanavalin A; Hepatoprotective; Liver injury.

1. INTRODUCTION

Liver diseases represent a critical public health problem globally because of the significant morbidity and mortality associated with it, which negatively affects the quality of life of the individuals.

Concanavalin A (Con A)-induced liver injury is the most reliable animal model available nowadays for the study of viral and autoimmune hepatitis. Con A injection in rats results in the apoptosis and necrosis of hepatocytes with the subsequent release of one of the very specific markers for liver damage, alanine transaminase (ALT) **(Yin et al., 2010)**. Acute liver injury induced by con A also depends on the activation of both adaptive and innate immune responses, specifically $CD4^+$ T-cells and natural killer cells **(Tiegs et al., 1992)**, and thus it can resemble the immunologically-mediated liver damage in humans.

Deferasirox (DFX) is an orally-active, tri-dentate iron chelator that is used to treat iron overload conditions caused by repeated blood transfusions or genetic blood disorders in children and adults **(Lindsey and Olin, 2007; Nisbet-Brown et al., 2003)** that was clinically proved to be well tolerated in patients **(Nisbet-Brown et al., 2003)**.

Deferoxamine has been the iron chelator of choice since the 1970s **(Neufeld, 2006)**, but the requirement for subcutaneous infusion of deferoxamine over 8 to 12 hours, 5 to 7 days a week and its associated side effects made it less compliant for patients **(Cappellini, 2005; Modell et al., 2000; Olivieri and Brittenham, 1997)**. DFX showed anti-inflammatory, anti-oxidant, and hepatoprotective and cardioprotective effects in previous studies **(Al-Rousan et al., 2011, 2009; Messa et al., 2010)** which make it a promising candidate in our study.

In the present study, we aimed to investigate the potential hepatoprotective effect of DFX at three different doses; 25, 50, and 100 mg/kg in concanavalin A-induced acute liver injury in rats.

2. MATERIALS AND METHODS

2.1. Materials

Deferasirox was purchased from Royal Pharms Co. (China). It was dissolved in a mixture of 10% DMSO in corn oil at concentration (25 mg/ml). Concanavalin A was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA), and was dissolved in normal saline solution at concentration (10 mg/ml). All other chemicals and solvents were of highest grade commercially available.

2.2. Animals and experimental protocol

The study design was approved by the Ethics Committee of Faculty of Pharmacy, Ain Shams University, Egypt. Male Wistar rats weighing 150-200 g were used. They were kept in the animal house facility in air-conditioned atmosphere and were provided with rodent chow and water ad libitum. Diet pellets contained not less than 20% protein, 5% fiber, 3.5% fat, 6.5% ash, and a vitamin mixture).

Fifty rats were randomly divided into five different groups (10 rats/group); Group A was considered the control rats receiving the vehicle only once. Group B was the disease group where rats received single intravenous injection of Con A (20 mg/kg). Groups C, D, E received a single dose of DFX orally (25, 50, 100 mg/kg, respectively) followed by single intravenous injection of Con A (20 mg/kg) 2 hours later.

Twenty-four hours after Con A injection, blood samples were collected from the retro-orbital plexus and allowed to clot. Serum was separated by centrifugation of blood at 4000 rpm for 15 minutes at 4 °C then stored at -20 °C for further biochemical analyses. The rats were then sacrificed, liver tissues were dissected and washed with ice-cold saline. Tissue sections were fixed in 10% formalin for histopathological analysis.

2.3. Assessment of hepatotoxicity indices

The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes were measured colorimetrically in serum using commercially-available kits (Spectrum Diagnostics, Cairo, Egypt) according to the manufacturer's instructions.

2.4. Histopathological examination

Liver specimens were fixed in 10% formalin, and embedded in paraffin blocks. Sections of 4 μm thickness were cut and stained using hematoxylin and eosin (H & E) stain and examined using light microscope.

2.5. Statistical analysis

Data are presented as mean ± SD. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey-Kramer as a post hoc test. The 0.05 level of probability was used as the criterion for significance. All statistical analyses were performed using Instat software package (version 3.06). Graphs were sketched using GraphPad Prism (ISI® software, USA) version 5.0.

3. RESULTS

3.1. Effect of DFX on hepatotoxicity indices:

Con A administration induced a significant increase in ALT and AST activities by 111% and 33% respectively as compared to the control group. Pretreatment with DFX at a dose of 25 mg/kg did not show any significant difference in ALT and AST levels as compared to Con A group. DFX at a dose of 50 mg/kg showed a significant decrease in ALT level by 30% but no significant decrease in AST level as compared to Con A group. Only DFX at the dose 100 mg/kg was able to significantly decrease both ALT and AST levels by 29% as compared to Con A group (Table 1).

Table 1. Effect of different doses of Deferasirox on serum ALT and AST levels.

Groups	ALT (U/L)	AST (U/L)
Control	11.59 ± 4.1^{b}	66.84 ± 1.11^{b}
Con A	24.43 ± 4.93^{a}	88.63 ± 15.89^{a}
Con A + DFX (25 mg/kg)	22.85 ± 4.2^{a}	73.36 ± 16.28
Con A + DFX (50 mg/kg)	17.03 ± 3.03^{b}	79.05 ± 5.21
Con A + DFX (100 mg/kg)	17.25 ± 2.7^{b}	62.53 ± 6.4^{b}

Data are represented as mean ± S.D. (n=10). a or b: significantly different from the control or con A group respectively at P-value < 0.05 using ANOVA followed by Tukey-Kramer multiple comparisons test as post-hoc test.

3.2. Histopathological examination:

Liver sections from control rats showed no histopathological alteration with normal histological structure of the central vein and surrounding hepatocytes in the

hepatic parenchyma (Fig. 1A). Con A group showed severe congestion in both central and portal veins associated with degeneration in the adjacent surrounding hepatocytes as well as inflammatory cells infiltration in the portal area (Fig. 1B). Pretreatment of intoxicated rats with DFX at the doses 25 mg/kg and 50 mg/kg DFX still showed dilatation and some congestion in the central and portal veins associated with inflammatory cell infiltration in the portal area (Fig. 1C, 1D). On the other hand, pretreatment of intoxicated rats with DFX at a dose of 100 mg/kg could almost restore the normal hepatic architecture (Fig. 1E). Scoring of these histopathological findings was done by a histopathologist (Table 2).

Table 2. Histopathological grading

Groups	Histopathological Alteration		
	Centrilobular necrosis	**Ballooning degeneration**	**Dilatation of central vein**
Control	-	-	-
Con A	+++	+++	+++
Con A+ DFX (25 mg/kg)	+++	++	++
Con A+ DFX (50 mg/kg)	++	++	++
Con A+ DFX (100 mg/kg)	-	-	-

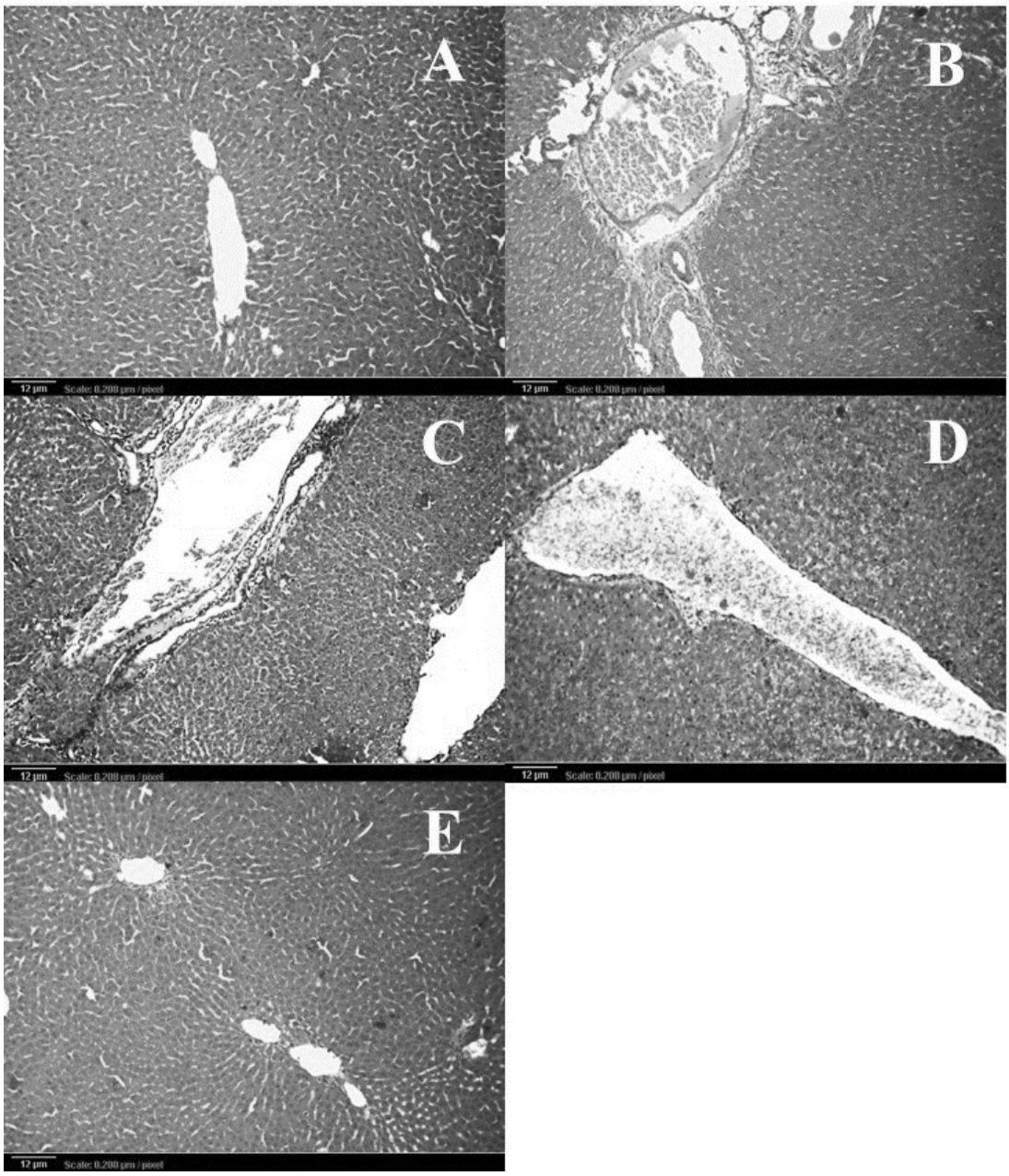

Figure 1. Histopathological analysis of rat liver sections using H & E (× 100). (A): Control group, (B): Con A-treated group, (C): Group pretreated with 25 mg/kg DFX, (D): Group pretreated with 50 mg/kg DFX, (E): Group pretreated with 100 mg/kg DFX.

4. DISCUSSION

Hepatitis is a growing problem worldwide, occurring due to different etiological factors including viral hepatitis, auto-immune hepatitis, and alcoholic liver disease that may progress to acute liver failure. The crucial role of liver in different metabolic and synthetic functions of the body stimulates researchers to continually explore and develop different hepatoprotective drugs **(Xie et al., 2015)**.

Con A is a bean-derived lectin, that induces liver injury in mice mediated by the activated $CD4^+$ T-cells **(Tiegs et al., 1992)**. Con A binds to sinusoidal endothelial cells and kupffer cells, activating $CD4^+$ T-cells and stimulating the release of tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), and other inflammatory cytokines **(Knolle et al., 1996; Schümann et al., 2000)**. Thus, con A model of T-cell mediated liver injury can resemble auto-immune hepatitis, viral hepatitis, and the related acute liver failure **(Tsutsui and Nishiguchi, 2014; Wang et al., 2012)**.

The current study investigated the potential hepatoprotective effect of DFX against con A-induced acute hepatotoxicity. In our study, intravenous injection of Con A in rats caused a significant increase in serum ALT and AST enzymes' activities as compared to the control group. These enzymes are released from hepatocytes into circulation upon hepatocellular injury leading to an increase in their serum activities indicating loss of cell membrane integrity and cellular leakage **(Rajesh and Latha, 2004)**. These findings were further confirmed by the histopathological examination where con A intoxication caused severe congestion, hepatocyte degeneration, and inflammatory cell infiltration. DFX at the doses 25 mg/kg and 50 mg/kg slightly reduced the activities of both ALT and AST enzymes but with no statistical significance and failed to prevent the histopathological damage to the liver tissue as shown by the presence of marked congestion and inflammatory cell infiltration caused by con A intoxication. On the other hand, DFX pre-treatment at the dose 100 mg/kg significantly reduced the activities of both ALT and AST enzymes. Additionally, it showed protection against Con A-induced histopathological alterations as evidenced by the restoration of the normal hepatic architecture except for few inflammatory cell infiltrations in the portal area. In agreement with our findings, the hepatoprotective effect of another iron chelator; deferoxamine was previously reported against endotoxemia-induced and carbon tetrachloride-induced liver injury **(Cermanova et al., 2014; Mohammed et al., 2016)**.

Based on the aforementioned biochemical analysis and histopathological examination, the most hepatoprotective effect was observed at the 100 mg/kg dose of deferasirox. In conclusion, our results revealed for the first time the potential hepatoprotective effect of DFX against con A-induced acute liver injury.

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تأثير الكبد الوقائي لعقار "ديفرازيروكس" في نموذج تجريبي لإصابة الكبد الحادة الناجمة عن كونكانافالين-أ

للسادة الدكاترة

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يشكل التهاب الكبد مشكلة صحية كبيرة في جميع أنحاء العالم، لأنه قد يتطور إلى تليف و سرطان للكبد. تعد وظيفة الكبد في الجسم ذات أهمية قصوى حيث يشارك في عمليات الأيض المختلفة و تصنيع العديد من البروتينات الضرورية للجسم، مما يدفع العلماء للبحث باستمرار عن أدوية جديدة ذات تأثير وقائي للكبد. صممت هذه الدراسة لتقييم فاعلية عقار "ديفرازيروكس" بجرعات مختلفة في نموذج تجريبي لإصابة الكبد الحادة الناجمة عن حقن الكونكانافالين-أ عن طريق الوريد في الجرذان بجرعة ٢٠ مغ/كغ مذابة في محلول ملحي (٠.٩%). تم حقن عقار "ديفرازيروكس" في الجرذان عن طريق الفم بجرعات ٢٥، ٥٠، و ١٠٠ مغ/كغ مرة واحدة و للتحقق من تأثيره الوقائي للكبد، تم عمل اختبارات وظائف الكبد و التشريح المرضي لأجزاء من نسيج الكبد. و استناداً إلى النتائج، كانت جرعة ١٠٠ مغ/كغ من عقار "ديفرازيروكس" هي الأكفأ في منع زيادة إنزيمات الكبد و استعادة بنية الكبد.