التأثير الوقائى لمادة الديفروكسامين
من السمية الكبدية المحدثة بالكونكانفالين - أ فى الجرذان

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تمهيد: يعتبر الحديد الزائد عن حاجة الجسم عامل معروف فى سمية الكبد وتليفه. وتم فى هذه الدراسة فحص تأثير جرعات مختلفة من الديفيروكسامين على الكبد، المزيل الرئيسى للحديد، فى دراسة السمية الكبدية الحادة.

الطرق: تم تقسيم عدد من الجرذان الذكور البيضاء بصورة عشوائية إلى ٦ مجموعات (٦ حيوانات فى كل مجموعة)، المجموعة ١: هى مجموعة ضابطة من الجرذان السليمة وتم حقنها فقط بمحلول ملحى، والمجموعة ٢: تلقت جرعة واحدة من الكونكانافالين - أ (٢٠ ملج/كج فى الوريد)، والمجموعات (٣ و ٤ و ٥ و ٦) تم حقنهم بجرعات مختلفة من الديفيروكسامين (٧٥ ، ١٥٠، ٦٠٠،٣٠٠ ملج / كج داخل التجويف البريتونى على التوالى)، ثم تم حقنهم بالكونكانافالين - أ بعد ساعة واحدة. وقد تم جمع عينات مصل الدم من كل مجموعة بعد ٢٤ ساعة من حقن الكونكانافالين - أ، ثم تم ذبح الحيوانات وفصل أنسجة الكبد.

النتائج: أظهرت النتائج أن حقنة واحدة من الكونكانافالين - أ نتج عنها تسمم كبدى حاد من خلال الزيادة الكبيرة فى وزن الكبد وإنزيماته والتى تم تأكيدها من قبل فحص أنسجة الكبد. وقد أظهر فحص جرعات مختلفة من الديفيروكسامين أن المعالجة بجرعة ٣٠٠ ملج/ كج توفر أفضل حماية للكبد. حيث اتضح ذلك من التحسن الكبير فى مؤشرات الكبد وأيضاً من الحفاظ على البنية الطبيعية لخلاياه بالمقارنة مع مجموعة الكونكانافالين - أ.

الخلاصة: تقدم هذه الدراسة أدلة عن الحماية القوية التى يوفرها الديفيروكسامين للكبد. كما أن المعالجة المسبقة بالديفيروكسامين (٣٠٠ ملج/ كلج) بساعة واحدة قبل الكونكانافالين - أ تعتبر الأكثر فعالية فى خفض سمية الكبد خلال ٢٤ ساعة أكثر من أى جرعة أخرى. وبالتالى قد يوفر الديفيروكسامين حماية واعدة للكبد.

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Fig. 4. Photomicrographs of liver sections stained by H&E (×400). Control: normal rats showed normal histological structure of central vein (cv) with normal surrounding hepatocytes, Con- A: nuclear vaculation (arrow), massive inflammatory infiltration (circle), congested central vein and distortion of normal architecture. DFO (75): no significant changes from Con- A group, DFO (150): less degenerative changes and less congestion in portal tract, DFO(300): preservation of hepatocytes normal architecture with normal nucleus, DFO(600): inflammatory cells around portal area and mild congestion in central vein.

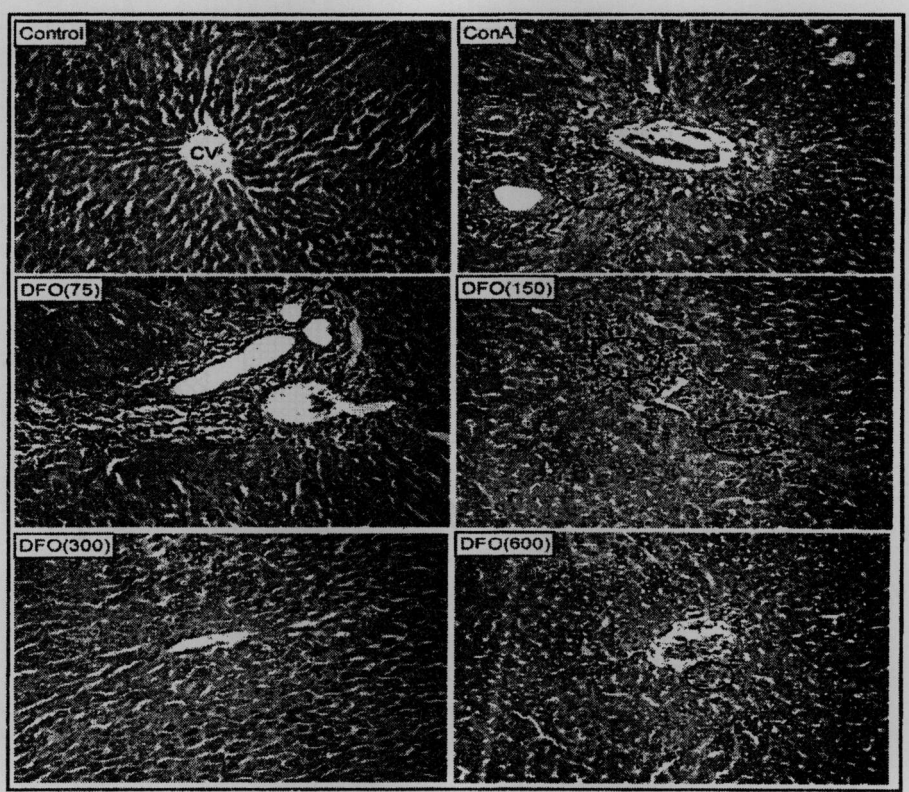

Fig.3. Effects of different doses of DFO on serum AST activity in rats subjected to acute Con A hepatotoxicity

Rats were treated with DFO (75, 150, 300 or 600 mg/kg, i.p.), then Con- A (20 mg/kg i.v.) was injected after 1 h.After 24 h, animals were sacrificed, serum samples stored at -80°C and liver tissues were collected. Data are represented as mean (n=6), dotted line represents control group mean.

a: significantly different from the corresponding (Control) group,

b: significantly different from (Con- A) group, at $P<0.05$ using one-way ANOVA followed by Tukey-Kramer multiple comparison test.

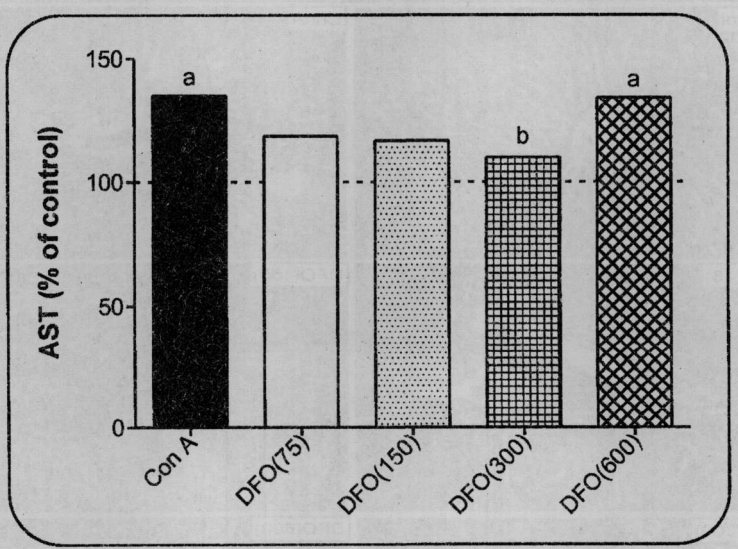

Fig.2. Effects of different doses of DFO on serum ALT activity in rats subjected to acute Con A hepatotoxicity

Rats were treated with DFO (75, 150, 300 or 600 mg/kg, i.p.), then Con A (20 mg/kg i.v.) was injected after 1 h.After 24 h, animals were sacrificed, serum samples stored at -80°C and liver tissues were collected.Data are represented as mean (n=6), dotted line represents control group mean.

a: significantly different from the corresponding (Control) group,

b: significantly different from (Con- A) group, at $P<0.05$ using one-way ANOVA followed by Tukey-Kramer multiple comparison test.

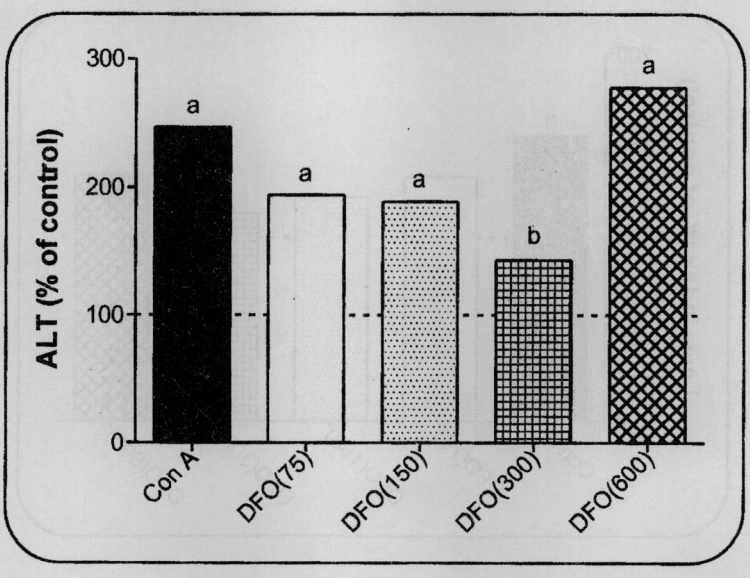

Fig.1. Effects of different doses of DFO on liver index in rats subjected to acute Con- A hepatotoxicity

Rats were treated with DFO (75, 150, 300 or 600 mg/kg, i.p.), then Con A (20 mg/kg i.v.) was injected after 1 h.After 24 h, animals were sacrificed, serum samples stored at -80°C and liver tissues were collected.Data are represented as mean (n=6), dotted line represents control group mean.

a: significantly different from the corresponding (Control) group,

b: significantly different from (Con- A) group, at $P<0.05$ using one-way ANOVA followed by Tukey-Kramer multiple comparison test.

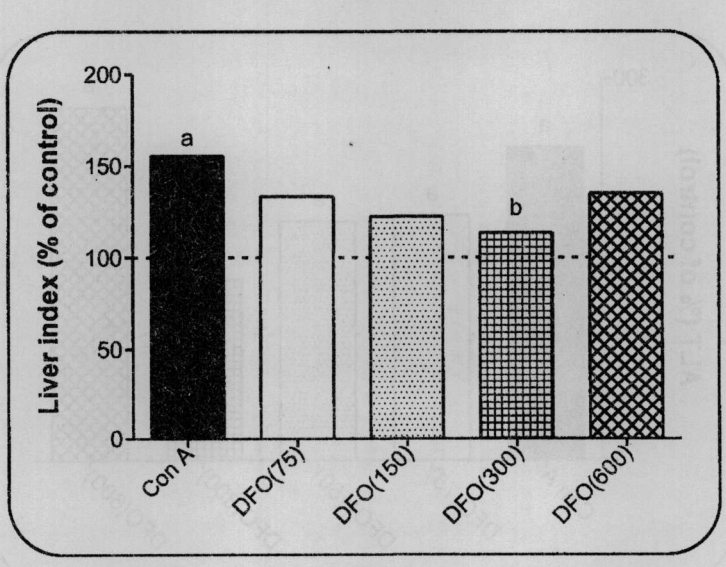

Table (1): Effects of different doses of DFO on liver index and serum activities of liver enzymes in rats subjected to acute Con A hepatotoxicity:

Groups	Liver index (%)	ALT (U/L)	AST (U/L)
Control	3.6 ± 0.3	7.0 ± 0.6	19.9 ± 1.8
ConA	5.6 ± 0.5[a]	17.3 ± 1.1[a]	26.9 ± 2.5[a]
DFO(75)	4.8 ± 0.4	13.6 ± 1.3[a]	23.6 ± 2.4
DFO(150)	4.4 ± 0.4	13.2 ± 0.9[a]	23.2 ± 2.3
DFO(300)	4.1 ± 0.2[b]	10.0 ± 0.6[b]	21.9 ± 0.4[b]
DFO(600)	4.8 ± 0.1	19.5 ± 1.3[a]	26.7 ± 1.0[a]

- Rats were treated with DFO (75, 150, 300 or 600 mg/kg, i.p.), then Con A (20 mg/kg i.v.) was injected after 1 h.
- After 24 h, animals were sacrificed, serum samples stored at -80°C and liver tissues were collected.
- Data are represented as mean ± SD (n=6).
  a: significantly different from the corresponding (Control) group, b: significantly different from (ConA) group, at $P<0.05$ using one-way ANOVA followed by Tukey-Kramer multiple comparison test.

of Con-A, as explained in previous studies **(Schnellmann *et al.*, 1999)**.

The present results are in agreement with previous studies that confirmed the hepatoprotective effect of DFO against different models of acute hepatotoxicity other than Con- A. For example, it was indicated that the protective effect of DFO against Acetaminophen-induced liver injury may be attributable to the chelation of iron, which can catalyze the generation of active oxygen species, in hepatocytes **(Sakaida *et al.*, 1995, Schnellmann *et al.*, 1999)**. In recent study, DFO was found to decrease the elevations of liver enzymes and improve liver histopathology in acetaminophen hepatotoxicity **(Najafzadeh *et al.*, 2011)**. In another acute hepatotoxicity model, DFO also showed hapatoprotective effect against CCl_4, by reducing mortality rate, oxidative stress, and limiting inflammatory infiltration and hepatocyte necrosis induced by CCl4 in the rat **(Ritter *et al.*, 2004)**. In addition, DFO was found to be superior to silymarin hepatoprotective effect in decreasing ALT and AST activities, and reducing hepatocyte necrosis induced by iron over-load hepatotoxicity model **(Najafzadeh *et al.*, 2010)**. However, none of all these previous studies screened different doses of DFO to show the most effective one. In addition, Con- A induced hepatotoxicity model is superior to all these previous models, by resembling the pathological changes in autoimmune and viral hepatitis in human **(Hayashi and Sakai, 2011)**.

5 - Conclusion:

The present study provides evidences for the potent hepatoprotective effects of DFO against Con- A induced hepatotoxicity. Therefore, this study may open a new scenario for the clinical usefulness of the use of iron chelators as hepatoprotective agents in liver toxicity associated conditions in the future.

6 - Acknowledgments:

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with the complexed iron center, and hampering the production of reactive oxygen species **(Kalinowski and Richardson, 2005)**. For the past five decades, DFO has been the only iron-chelating agent generally available for clinical use. This ligand has validated the iron chelation therapy as an effective form of treatment by showing an improvement in the survival and well-being of iron-overloaded patients **(Hershko *et al.*, 2003)**.

Herein, we showed that iron chelation has the potential to protect against acute liver damage in rats induced by Con- A in a dose dependent manner. In the present study, we screened the hepatoprotective effects of different doses of DFO against acute hepatotoxicity model. Low doses of DFO (75 and 150mg/kg) induced a little improvement in liver index and liver enzymes, but it wasn't statistically significant as compared to Con- A group. Regarding histopathological examination, rats pre-treated with 75 mg /kg of DFO before Con-A showed no significant changes as compared to Con- A treated rats. While rats pre-treated with 150 mg/kg of DFO showed less degenerative changes and less congestion in portal tract as compared to Con- A treated group, but inflammatory cells were still found. Increasing the dose of DFO provided greater protection against Con- A hepatotoxicity as in case of pre-treatment with 300mg/kg of DFO. This dose showed a significant improvement in liver index and liver enzymes, in addition to the remarkable improvement regarding histopathological examination as compared to Con- A group. It preserved the normal architecture of hepatocytes with normal nucleus without any degenerative or inflammatory cells observed around both portal and central area. Interestingly, pre-treatment with higher doses of DFO (600mg/kg) didn't improve the Con- A induced increase in liver index and liver enzymes, but it showed little improvement by histopathological examination, through observing little inflammatory cells around portal area and mild congestion in central vein. The finding that the higher dose of DFO (600 mg/kg) showed high liver enzymes in spite of the histological improvement, could possibly be explained by the fact that high doses of DFO may cause hypotension, leading to hepatic ischemia, thus augmenting the hepatotoxic effects

which closely mimics the pathogenesis mechanisms of viral and autoimmune hepatitis in humans **(Hayashi and Sakai, 2011)**. Con- A is a kind of lectin, which is purified from *Canavaliabrasiliensis* **(Soares *et al.*, 2011)**. After Con- A intravenous injection, hepatic $CD4^+$ T-cells recognize the Con- A modified major histocompatibility complex structures of Kupffer cells and become activated, followed by an inflammation reaction and the release of inflammatory mediators in the blood **(Carambia and Herkel, 2010)**. Bolus injection of Con- A induces acute hepatitis, while repeated administrations induce a chronic form **(Kimura *et al.*, 1999, Louis *et al.*, 2000)**.

In the present study, single injection of Con- A induced hepatocellular damage, by showing significant increase in liver enzymes (ALT and AST), indicating dysfunction of hepatocytes, as well as the significant increase in relative liver weight, thus confirming liver hypertrophy. These results had been confirmed by histopathological examination, in which single injection of Con A showed degenerative changes in hepatocytes and nuclear vaculation, in addition to massive inflammatory infiltration, congested central vein and distortion of normal architecture. These results were in agreement with other previous studies, which confirmed that single injection of Con- A induced acute model of hepatitis in rats. Nakano *et al.*, confirmed that the injection of 20 mg/kg of Con- A induced acute hepatitis in rats with significant elevation of liver enzymes and accompanied with mononuclear cellinfiltration and histopathological alterations in liver **(Nakano *et al.*, 2010)**. Other study by Murayama *et al.*, also confirmed the acute hepatotoxicity induced by single injection of 20 mg/kg of Con- A through the significant increase in liver enzymes and significant hitopathological alterations in liver **(Murayama *et al.*, 2008)**.

DFO (or DFO-B mesylate) is a bacterial hexadentate siderophore derived from ferrioxamine B, the iron-bearing metabolite isolated in 1960 from *Streptomyces pilosus* **(Keberle, 1964)**. With such hexadentate ligand, the coordination sphere of iron is fully occupied, resulting in 1:1 complex, which is metabolically inactive and redox-stable, thus preventing direct access of H_2O_2 or oxygen

group, while inflammatory cells were still found. Furthermore, liver sections of rats treated with 300 mg/kg of DFO preserved the normal architecture of hepatocytes with normal nucleus and any degenerative or inflammatory cells were not observed around both portal and central area. Interestingly, treatment with 600 mg/kg of DFO showed inflammatory cells around portal area and mild congestion in central vein (Figure 4).

4 - Discussion:

The hemostasis of iron inthe body is strictly regulated **(Aisen *et al.*, 2001)**. Most of the other iron is stored in the liver as a component of iron-containing enzymessuch as cytochrome P-450. However, the presence of free ironand deposition of iron in cells often occurs under some pathologicalconditions, triggering oxidative stress and inflammation **(Papanikolaou and Pantopoulos, 2005)**. For instance, hemochromatosis and iron-overload due to the excessive intake of iron-containing medicine andsupplements frequently induce liver cell damage and, in somecases, cirrhosis **(Pietrangelo, 2004)**.

It has been shown that the deposition of iron occurs frequentlyin some liver diseases, including viral hepatitis **(Bonkovsky, 2002)**, alcoholic liver injury **(Purohit *et al.*, 2003)**, and nonalcoholic steatohepatitis (NASH) **(Fargion *et al.*, 2001)**. Thus, iron is considered to play a role in theonset of liver cell damage. Free iron induces the production of proinflammatory and fibrogenic mediators such as TNF-α and transforming growth factor-beta (TGF-β) and nuclear factor-κB (NF-κB) activation in hepatic macrophages **(She *et al.*, 2002, Ishizaka *et al.*, 2005)**. Free iron ions are known to be the most potent generatorof free radicals, which induce lipid peroxidation, DNA breakage, resulting in tissue damage and DNA mutagenesis **(Papanikolaou and Pantopoulos, 2005, Fujita *et al.*, 2007)**. Although it has been clarified that iron overload may be associated with hepatitis, cirrhosis, or liver cancer, it isstill unclear whether iron chelator could be used as protective agents against acute liver injury and fibrosis.

The Con- A model is a typical and well established model for investigating T-cell and macrophage dependent liver injury in rodents,

150 mg/kg of DFO didn't show significant change from Con- A group. On the other hand, pre-treatment with 300 mg/kg of DFO significantly reduced liver index by 27% as compared to Con- A group. Interestingly, pre-treatment with 600 mg/kg of DFO didn't show any significant change from Con- A group.

3.2 - Effect on liver enzymes:

The effects of different doses of DFO on serum activities of liver enzymes in rats subjected to acute Con- A hepatotoxicity, are shown in Table (1) and Figures (2,3).Comparison between different groups showed that Con- A administration significantly increased the activities of both ALT and AST enzymes by 147% and 35%, respectively as compared to control rats. However, pre-treatment with either 75 or 150 mg/kg of DFO still showed significant increase in ALT by 94% and 89%, respectively as compared to control group, while they didn't show any significant change from Con- A group in either ALT or AST activities. In contrast, pre-treatment with 300 mg/kg of DFO significantly reduced the activities of both ALT and AST enzymes by 42% and 19%, respectively as compared to Con- A group. Interestingly, pre-treatment with 600 mg/kg of DFO still showed significant increase in both ALT and AST activities by 179% and 34%, respectively as compared to control group, while it didn't show any significant change from Con- A group in either ALT or AST activities.

3.3 - Histopathology of liver:

Histological findings of liver tissues in control rats showed normal histological structure of the central vein with normal surrounding hepatocytes. On the other hand, Con- A treated rats showed degenerative changes in hepatocytes and nuclear vaculation, in addition to massive inflammatory infiltration, congested central vein and distortion of normal architecture. However, rats treated with 75 mg /kg of DFO before Con- A showed no significant changes as compared to Con- A treated rats. On the contrary, liver sections from rats treated with 150 mg/kg of DFO showed less degenerative changes and less congestion in portal tract as compared to Con- A treated

from the retro-orbital plexus and allowed to clot, separated by centrifugation at 1000 g for 10 min and stored at -80°C. Then, rats were sacrificed and liver tissues were dissected, weighted and washed with ice-cold saline. Specimens from the liver were fixed in 10% formalin for histopathological assessment.

2.3.1 - Assessment of liver index:
Liver index was calculated according to the formula: (liver weight/body weight) × 100 **(Jones *et al.*, 1992)**.

2.2.1 - Assessment of hepatotoxicity indices:
Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated using colorimetric method **(Reitman and Frankel, 1957)**.

2.3.5 - Histopathological examination:
Liver sections from different groups were fixed in 10% formalin, embedded in paraffin blocks, from which 3 micron-thick sections were obtained, stained with hematoxylin and eosin (H&E), and then evaluated under light microscope **(Avti *et al.*, 2006)**.

2.4 - Statistical analysis:
Data are represented as means ± SD. Multiple group comparisons were carried out using one-way analysis of variance (ANOVA) followed by the Tukey-Kramer post-hoc test. Statistical significance was accepted at a level of $P<0.05$. Analysis of data and presentation of graphs were performed using GraphPad Prism software version 5 (ISI® software, USA).

3 - Results:

3.1 - Effect on liver index:
The effects of different doses of DFO on liver index in rats subjected to acute Con- A hepatotoxicity are shown in Table (1) and Figure (1). Comparison between different groups revealed that Con- A administration significantly increased relative liver weight by 56% as compared to control rats. However, pre-treatment with either 75 or

induced liver injury in both rats and mice **(Sakaida *et al.*, 1995, Schnellmann *et al.*, 1999)**, while co-treatment with N-acetylcysteine reduced mortality rate and hepatocyte necrosis induced by CCl_4 in the rat **(Ritter *et al.*, 2004)**.

Accordingly, the present study aimed to elucidate the possible hepatoprotective effect of DFO against Con- A induced hepatotoxicity model through screening different doses of DFO and studying their effect on liver toxicity parameters.

2 - Material and Methods:

2.1 - Drugs and Chemicals:

DFO was purchased as Desferal® vial from Novartis Pharmaceuticals (Australia). Con- A was purchased from Sigma-Aldrich Co. (St Louis, MO, USA). Stanbio AST and ALT kits (Liqui-UV®) were purchased fromStanibo laboratory (Texas, USA).

2.2 - Animals:

The study was approved by the ethical guidelines of the Research Ethics Committee of Faculty of Medicine, Ain Shams University (FMASU-REC). FMASU-REC operates under Fedral Wide Assurance No. FWA 00006444.36 male albino rats (Sprague Dawley) weighing 150-200 g were obtained from Nile Co. for Pharmaceutical and Chemical Industries, Egypt. Rats were housed in an air-conditioned atmosphere, at a temperature of 25°C with alternatively 12 hour light and dark cycles, and kept on a standard diet and water *ad libitum*. Rats were allowed to acclimate for two weeks before any experimentation.

2.3 - Experimental design:

Rats were divided into 6 groups (n=6) and treated as follows: Group 1: Control rats received i.v. and i.p. injections of normal saline (0.9% w/v), group 2: Rats received single dose of Con- A (20 mg/kg, i.v.) **(Nakano *et al.*, 2010)**. Groups 3, 4, 5, 6: Rats injected i.p. with different doses of the DFO (75, 150, 300, 600 mg/kg., respectively), then after 1h they were injected with Con- A. After 24 h from Con- A injection, rats were anaesthetized and blood samples were collected

1 - Introduction

Iron is essential element for human life. It is present in many food products and dietary supplements. Most of the other iron is stored in the liver, which is the main organ for iron storage and metabolism.However, the liver's physiological iron storage capacity is limited, and excessive iron accumulation can lead to liver damage **(Echeverría *et al.*, 2012)**. Emerging evidence suggests that oxidative stress, mediated by free radicals and reactive oxygen species, may have a role in the pathophysiology of iron-induced liver injury and cell death,followed by the subsequent formation of lipid peroxidation products, which also postulated to be an underlying mechanism of iron-mediated liver injury **(Li *et al.*, 2012)**. In hepatocytes and Kupffer cells, iron catalyses the production of hydroxyl radical ($OH^{\bullet}$) from ROS, superoxide ($O_2^{\bullet}$) and hydrogen peroxide (H_2O_2) via two chemical reactions known as the Fenton and Haber-Weiss reaction **(Liu *et al.*, 2013)**. In addition, iron catalyses the formation of nitrogen dioxide radical (NO_2^{+}) from peroxynitrite ($ONOO^{-}$), which is produced by iNOS **(Videla *et al.*, 2003)**. NO_2^{+} and $OH^{\bullet}$ induce oxidative deterioration of biomolecules (lipid, protein and DNA), leading to tissue injury and cell death **(Papanikolaou and Pantopoulos, 2005)**. The pivotal role of iron in these processes suggests that chelating iron may offer a new approach to arresting or ameliorating liver injury.

Iron chelation therapy involves the use of ligating drugs that avidly bind iron for the treatment of potentially fatal conditions, mainly iron overload disease and cancer **(Kalinowski and Richardson, 2005)**. Based on the number of coordination sites, iron ligands are termed hexadentate, tridentate and bidentate **(Tam *et al.*, 2003)**. These iron chelators may provide new pharmacological means of averting or ameliorating liver damage by binding, inactivating, and eliminating the reactive forms of iron that contribute to oxidative injury of cellular components and/or involved in signal transduction.

For the past five decades, deferoxamine (DFO) has been the only iron-chelating agent available for general clinical use and for the treatment and prevention of iron overload **(Brittenham, 2003)**. *In-vivo* studies showed that DFO protected against acetaminophen-

Hepatoprotective Effect of Deferoxamine in Concanavalin- A Induced Hepatotoxicity in Rats

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Background: Iron overload is a well-known factor of hepatotoxicity and liver fibrosis. Here, we screened the hepatoprotective effect of different doses of deferoxamine (DFO), the main iron chelator, in acute hepatotoxicity study.

Methods: Male albino rats were randomized into 6 groups (6 animals each), group 1: Control rats receivedsaline, group 2: Rats received single dose of Concanvelin A (Con A) (20 mg/kg, i.v.). Groups 3, 4, 5, 6: Rats injected i.p. with different doses of the DFO (75, 150, 300, 600 mg/kg., respectively), then injected after 1h with Con-A. Rats sacrificed after 24 h from Con - A injection. Liver index, liver enzymes (AST and ALT) and histopathology were assessed.

Results: Concanavalin A induced a significant hepatotoxicity with a significant increase in liver index and liver enzymes, confirmed by histopathology. Pre-treatment with 300 mg/kg of DFO was the only dose that significantly reduced liver index and liver enzymes and the most dose that improved the histopathological alterations as compared with Con- A group.

Conclusion: The present study provides evidences for the potent hepatoprotective effects of DFO. Pre-treatment with DFO (300 mg/kg) at 1 h before Con- A was more effective at decreasing the 24 h toxicity than any other dose. Thus, DFO may offer a promising hepatoprotective agent.

Keywords: hepatotoxicity, iron, deferoxamine, concanavalin A.

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