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Hepatoprotective effect of flaxseed oil and alpha lipoic acid against cisplatin-induced oxidative stress and apoptosis in rats

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ABSTRACT

Cisplatin (CP) is a widely used chemotherapy agent. The study's goal was to assess the antioxidant and antiapoptoptic effects of flaxseed oil (FXO) and/or alpha lipoic acid (ALA) against cisplatin-induced hepatic toxicity. Seven groups (7 rats/group) were assigned; control (saline) group, FXO group; ALA; CP toxic group; CP+FXO, CP+ALA, and CP+FXO+ALA groups. Saline, FXO, and ALA were administered for 21 days. After CP injection, serum AST and ALT activities were decreased and serum albumin level was deceased. Malondialdehyde (MDA) in the liver tissues was significantly increased and reduced glutathione and catalase levels were decreased. Additionally, CP toxicity induced hepatocellular damage and elevation of caspase-3 expression in the liver tissues. Furthermore, rats intoxicated with CP and given FXO and ALA alone or in combination showed significant reductions in oxidative stress indicators and biochemical parameters, as well as improved hepatic tissue architecture, when compared to the CP group. So, concurrent administration of FXO and/or ALA during CP therapy is advisable.

1. INTRODUCTION

One of the widely used chemotherapeutic drugs is cisplatin (CP). Although cisplatin has antineoplastic activity against numerous malignancies, its administration is restricted by side effects, primarily because of increased apoptosis and oxidative effects in various tissues (Elsayed et al., 2021). The generation of reactive oxygen species (ROS) increases lipid peroxidation and cell damage, is what causes CP toxicity. Cells undergo cell arrest and apoptosis in response to excessive ROS (Anbar et al., 2022). Numerous studies have documented liver damage carried on by CP (Elkomy et al., 2020; Elsayed et al., 2021; Aboraya et al., 2022; Anbar et al., 2022; Ibrahim et al., 2022). Also CP induced toxicity in different organs as kidney, testes and heart (Sallam et al., 2021; Elsayed et al., 2022).

Linaceae, also known as flaxseed (FXO) or linseed, has been cultivated for the production of essential oils (Madhusudhan 2009). Flaxseed constitutes oil (40%), ash (4%), fiber (30%), moisture (6%), and protein (20%) (Wang et al., 2008). The flaxseed thought to be the highest source of alpha-linolenic acid and omega-3 fatty acids (Hendawi et al., 2016). Omega 3 fatty acids had cardioprotective, anti-diabetic, and immunomodulatory effects (Sekine et al. 2008). Flaxseed contains substances that lower cholesterol and prevent atherosclerosis (Lee and Prasad 2003). Because of its probiotic properties, FXO may be used to treat neurological and hormonal disorders, coronary heart disease, and other diseases (Hendawi et al., 2016).

Alpha-lipoic acid (ALA) is a well-known disulfide derivative of octanoic acid having powerful antioxidant, anti-inflammatory, and anticancer properties. ALA is a naturally occurring substance that can be obtained from a variety of food sources or produced internally (El-Mancy et al., 2022). It participates in the metabolism of fats and carbohydrates, primarily by serving as a cofactor for numerous mitochondrial enzyme complexes (Pibiri et al., 2020).

Furthermore, ALA has a metal chelating activity and ability to restore a reduced form of intracellular antioxidants such as GSH, vitamin C, and vitamin E against environmental pollutants. It is said to reduce inflammation and oxidative stress in a variety of diseases (Hossain et al., 2021). In fact, it was suggested that ALA would provide more defense against oxidative damage than the body's natural reduced/oxidized glutathione system. Its high redox potential might be to blame for this. Furthermore, its unique amphiphilic properties may facilitate improved distribution between intracellular and extracellular compartments (Rochette et al., 2015). Commercially, ALA is offered as dietary, anti-aging, and multivitamin supplements. ALA was found to inhibit the activation of hepatic stellate cells and guard against hepatotoxicity brought on by a variety of toxins (Deore et al., 2021). ALA secures against fatty liver caused by a high-fat diet (Yang et al., 2014), concanavalin A-induced hepatitis (Fei et al., 2016), acetaminophen-induced liver damage (Mahmoud et al., 2015), lipopolysaccharide induced-acute liver injury (Tanaka et al., 2015), carbon–tetrachloride-induced liver cirrhosis (Liu et al., 2019), and cyclosporine A-induced hepatic toxicity (El-Mancy et al., 2022). The present work was carried out to assess the antioxidant and antiapoptoptic effects of FXO and/or ALA against CP hepatotoxicity.

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2. MATERIAL AND METHODS

2.1. Chemicals

EIMC Pharmaceuticals Company produced CP (50 mg/ml parenteral administration) (Cairo, Egypt). FXO was obtained from El-Captain, Company (El-Obour City, Cairo, Egypt). ALA was brought from the EVA Company (Cairo, Egypt). The analytical kits were purchased from Bio-diagnostics Company (Giza, Egypt)

2.2. Experimental rats and design

Forty nine Wister Albino male rats weighing 190±20 g were obtained from the Egyptian Organization for Biological Products and Vaccines. Rats they kept in wire cages at 25 2 °C with a 12:12 h light/dark cycle, free access to water, and commercial pellets. After one week of adaptation, rats were randomly assigned to seven groups with seven rats each as follows: saline was only administered orally to the vehicle control group once daily; FXO group received 0.3 ml orally once daily at a dose of 1.000 mg/kg (Bhatia et al., 2007); ALA group received 100 mg/kg ALA orally once daily (El-Mancy et al., 2022); On the 15th day of the experiment, a single 7.5 mg/kg IP dose of CP was administered to the CP toxic group along with saline orally once daily (Elsayed et al., 2021); CP+FXO, CP+ALA, and CP+FXO+ALA groups. Saline, FXO, and ALA were orally administered for 21 days.

2.3. Sampling and processing

One day after the final treatment, isoflurane was used to sedate rats. For separation of serum, blood samples were collected from the retro-orbital plexus (centrifugation for 15 min at 1200 g). For biochemical analysis, sera were kept at -20°C. ALT, AST (Reitman and Frankel, 1957), and albumin (Doumas et al., 1971) were the biochemical parameters.

The livers were quickly removed, washed in saline, and then perfused with ice-cold 50 mmol/L sodium phosphate-buffered saline (100 mmol/L Na_2HPO_4/NaH_2PO_4, pH 7.4) containing 0.1 mmol/L EDTA. The tissue samples were kept at -80^{o}C until used. Tissues were homogenized on ice using an electrical homogenizer, and N-ethylmaleimide was added right away to stop GSH from being oxidized. 1g of tissue was homogenized with 5 ml of phosphate buffer pH 7.4. The homogenates were centrifuged at 1200 x g for 20 min at 4^{o}C to separate the supernatants after homogenization. Reduced glutathione (GSH; Beutler et al., 1963), catalase (CAT; Aebi, 1984), and malondialdehyde (MDA; Ohkawa et al., 1979) were assessed in rat liver using kits. The remaining liver tissues were immediately fixed with 10% neutral buffered formalin for 24 hours for histological and immunohistochemical analysis. Tissue specimens were then washed with tap water and dehydrated in ascending strength of ethyl alcohol then cleared in xylol and embedded in paraffin. Thin paraffin sections were routinely prepared and stained with hematoxylin and eosin sections for histopathological analysis under a light microscope according to Bancroft and Gamble (2008). Sections of the liver tissues were prepared and stained for immunostaining in accordance with Elsayed et al., (2021).

2.4. Statistical analysis

Data from different groups were represented as the mean ± SE. using the statistical program SPSS for Windows (Version 21.0; SPSS Inc., Chicago, IL, USA), one-way ANOVA and Duncan's post hoc test for multiple group comparisons were used to analyze the data. Differences were considered statistically significant at $P \leq 0.05$

3. RESULTS

Cisplatin induced hepatotoxicity, which was indicated by elevated serum levels of liver biomarkers (Figure 1). In comparison to the control rats, AST and ALT activities were markedly elevated after CP toxicity. On the other hand, pretreatment with FXO, ALA, or in combination (FXO and ALA), was followed by lowering of these serum biomarkers when compared to the CP group. Additionally, CP decreased the levels of serum albumin. Notably, compared to CP group, albumin was significantly elevated in rat models of CP intoxication and pretreated with FXO and ALA was administered. The combination of FXO and ALA provided better hepatic protection than either substance alone.

Figure 1 depicted effect of CP toxicity and using FXO, ALA, and their mixture on MDA, CAT, and GSH in the hepatic tissues. When compared to control rats, CP-intoxicated rats showed significantly higher MDA levels and significantly lower CAT and GSH levels in the liver. FXO and ALA treatments reduced the effects of CP on MDA, CAT, and GSH in the liver tissue; however, there was a significant difference between these parameters and the control values. Compared to the FXO or ALA treatments alone, the combined FXO and ALA treatment significantly reduced the oxidative damage induced by CP in the hepatic tissues.

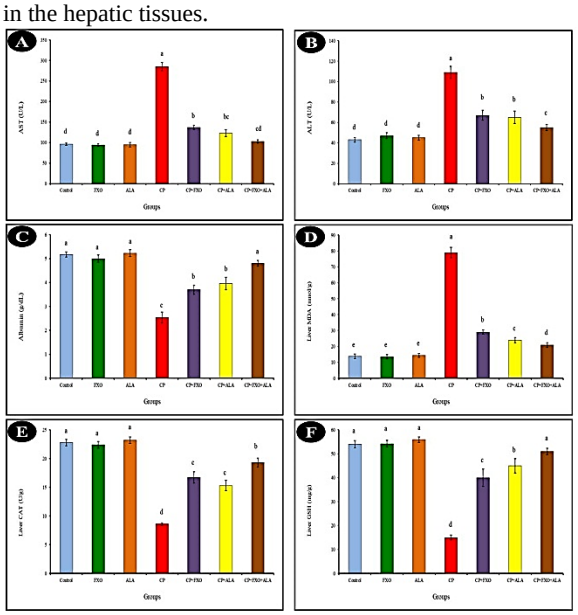

Figure (1): Effects of FXO, ALA and/or CP on serum hepatic biochemical parameters (AST, ALT, and albumin) and antioxidant parameters (MDA, CAT, and GSH) in hepatic tissues (n=7)

Microscopically, the examined liver of control, FXO, and ALA groups showed normal architecture of hepatic cords separated by blood sinusoids (Figure 2 A-F). However, CP group; severe hepatic damage was recorded and evidenced by disrupted organization of hepatic cords, centrilobular areas of necrosis, vacuolar and hydropic degeneration of hepatocytes (Figure 3 A-B). In FXO+CP treated group, the liver damage was attenuated, where only mild vacuolar and hydropic degeneration of hepatocytes and congestion of central veins were found (Figure 3 C-D). Despite fewer necrosis and damaged areas in the liver, its overall structure appeared normal. Some sections, however, showed eosinophilic cytoplasm and pyknotic nuclei of the hepatocytes. The portal veins were also affected by a lesser extent of inflammation, although there was some fibrous

tissue growth were recorded in CP+ALP group (Figure 3 E-F). The examined liver of rats in CP+FXO+ALA treated group demonstrated a significant reduction of the detrimental effects of CP on hepatic tissue where few inflammatory cellular infiltrations with more or less intact hepatic cells were detected (Figure 3 G-H).

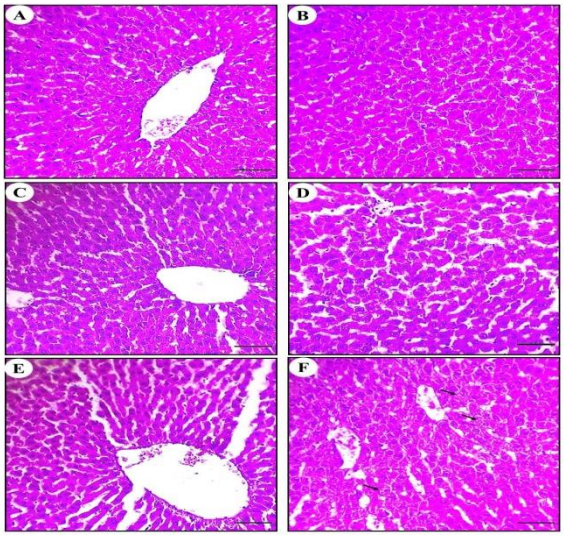

Figure (2): Histological sections of livers from control, FXO, and ALA groups. Histological examination of control group showing normal hepatic cells (H), central vein (CV), and hepatic sinusoid (S) with Kupffer cell (K) (Figure 2 A-B). FXO-treated group displaying normal architecture of hepatic cords separated by slightly dilated blood sinusoids with minor Kupffer cells activation (Figure 2 C-D). ALA treated group displayed slight hydropic degeneration of hepatocytes (black arrow) (Figure 2 E-F). H&E stain, scale bars=50µm

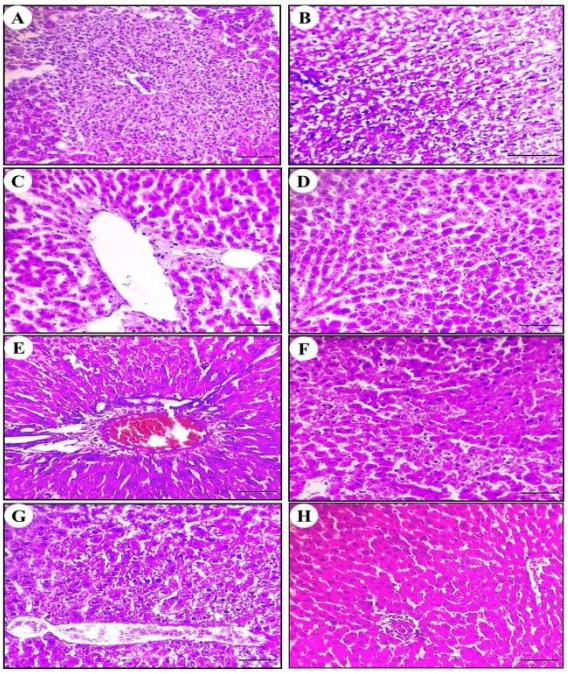

Figure (3): Histological sections of livers from CP, CP+FXO, CP+ALA, and CP+FXO+ALA groups. CP treated group showing disrupted organization of hepatic cords, centrilobular areas of necrosis, vacuolar and hydropic degeneration of hepatocytes (Figure 3 A-B). CP+FXO treated group showing an improvement of the hepatic architecture with moderate degeneration of hepatocytes (Figure 3 C-D). CP+ALA treated group displayed slight fibrotic reaction around portal vein. Small necrotic areas with some eosinophilic cytoplasm and pyknotic nuclei of the hepatocytes (Figure 3 E-F). CP+FXO+ALA treated group displaying few inflammatory cellular infiltrations with more or less intact hepatic cells (Figure 3 G-H). H&E stain, scale bars=50µm

Regarding results of the immunohistochemical study, there were faint or no caspase-3 immunoreaction in hepatic tissues of the control, FXO, and ALA treated groups (Figure 4 A-C). In contrast, the examined liver of rats in CP intoxicated group showed a significant increase in Caspase-3 immunoreactivity, as indicated by strongly brown stained hepatic cells (Figure 4 D-E). Compared with CP group, Caspase-3 protein expression in hepatic tissues was significantly reduced in CP+FXO and CP+ALA groups (Figure 4 F and G) Whereas in CP+FXO+ALA group only few immunoreactive hepatic cells were recorded in the hepatic tissues (Figure 4 H).

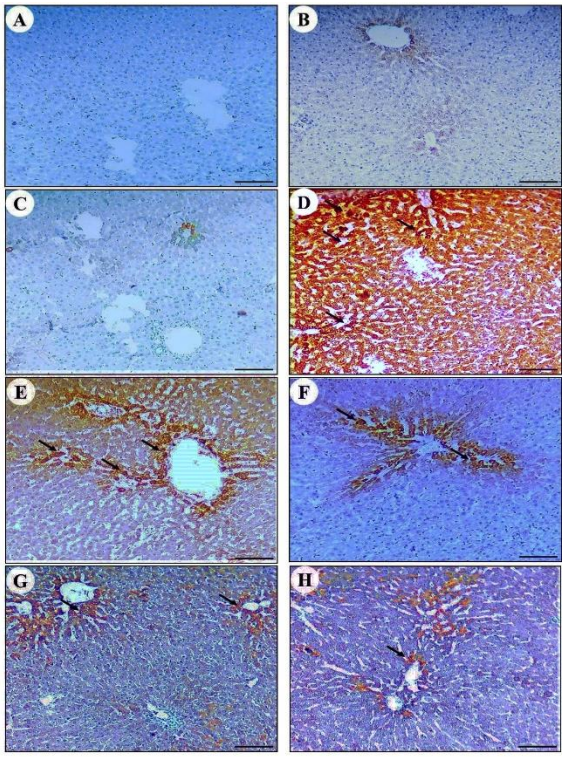

Figure (4): Immunohistochemical staining of caspase3 in hepatic sections from all examined groups. A, B and C; Control, FXO and ALA groups showing no cleaved caspase-3 immunoreactive cells (Figure 4 A) or faint granular staining in the pericentral hepatocytes (Figure 4 B-C). CP group showing marked cytoplasmic and nuclear expression of caspase 3 (Figure 4 D-E). CP+FXO (Figure 4 F) and CP+ALA (Figure 4 G) treated rats showing moderate expression of caspase 3 within the pericentral hepatocytes compared with CP group. CP+FXO+ALA group showing mild caspase3 immunoreaction (Figure 4 H). Scale bars = 50µm

4. DISCUSSION

When plasma membranes of hepatocytes were damaged; AST and ALT released into the blood. The CP-induced hepatotoxicity in this study was demonstrated by notable changes in serum liver enzymes. When CP enters the liver, it builds up in hepatocytes and damages the cells, eventually increasing the levels of circulating liver enzymes (Elsayed et al., 2021). Similar results were recorded by Eid and El-Shitany (2021); Haddar et al., (2021) and Ibrahim et al., (2022).

CP exerted an observable decline in albumin which was recorded also by Elsayed et al., (2021). After liver damage by CP decreases synthesis of protein and the kidney's functional integrity were changed, resulting in proteinuria and, eventually, lower levels of circulating protein (Elsayed et al., 2022).

In the present study, MDA levels were substantially raised and antioxidants (CAT and GSH) were remarkably reduced following CP toxicity. These results were in accordance with Anbar et al., (2022); Ibrahim et al., (2022), and Ferah Okkay *et al*., (2022). The production of ROS, including superoxide anions and hydroxyl radicals, causes oxidative

stress to be mediated as well as the depletion of plasma antioxidants (Elsayed et al., 2021).

ALA is an antioxidant modulator, a free radical scavenger, and an inhibitor of lipid peroxidation and protects against hepatotoxicity (El-Mancy et al., 2022).

Through the capacity of ALA to chelate metals, which prevents hydroxyl radical's formation, its capacity to regenerate endogenous antioxidants and scavenge reactive oxygen species, and its capacity to repair oxidatively damaged protein; alpha-lipoic acid acts as an antioxidant (Abdel-Zaher et al., 2008).

The protective effect of flaxseed oil was due to its high concentration of antioxidant substances like beta-carotene and tocopherols. Also, FXO has a hepatoprotective effect through increasing the activities of CAT, GSH after thiacloprid toxicity (Hendawi et al., 2016).

In CP-intoxicated rats, levels of CAT and GSH were restored by FXO and ALA. We therefore strongly imply that ROS suppression was the mechanism by which FXO and/or ALA-mediated improvements in oxidative stress parameters after CP toxicity.

The pathological findings after CP toxicity showed marked hepatic degenerative changes. Similar results were reported by Elkomy et al., (2020) and Elsayed et al., (2021). CP caused a substantially increase in expression of caspases-3, suggesting apoptosis as mentioned by Elsayed et al., (2021). ALA has been widely studied for its anti-apoptotic impacts in disease models (El-Mancy et al., 2022).

As shown by the enhanced biochemical parameters, oxidative stress markers, histopathology, and caspase-3 expression, pretreatment with FXO and/or ALA offered defense against CP-induced hepatic toxicity. Pretreatment with FXO and/or ALA improved liver function biomarkers, antioxidant status, and liver architecture in the liver of rats intoxicated with CP.

5. CONCLUSION

Finally, it is concluded that CP significantly damages liver tissue, as shown by altered liver function biomarkers and hepatic architecture through oxidative stress and apoptotic mechanisms. FXO and ALA offered significant hepatic protection against CP-induced apoptosis and oxidative stress, both singly and in combination.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest for current data

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