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Effect of Selenium and Olive Oil on Hepatotoxicity Induced by Cadmium chloride in Adult Male Albino Rats (Structural, biochemical and immunohistochemical study).

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Abstract

Background: Cadmium (Cd) is one of the non-essential toxic heavy metals disturbing the body tissues and producing many diseases due to excessive production of reactive oxygen species. Selenium and Olive Oil are antioxidants that could help to counteract the damage produced by reactive oxygen species.

Aim of the study: This study intended to explore the potential effect of Selenium or Olive Oil in alleviating the hepatotoxicity induced by oral administration of Cadmium in albino rats.

Material and methods: fourty adult male albino rats were chosen and randomly divided into four groups (n=10) included: Group 1: control group; Group 2: Cd-exposed group: Rats orally received Cd (50 mg/kg), Group 3: Rats orally received Cd (50 mg/kg) plus (2 mg/ kg b.wt) of Selenium and Group 4: Rats orally received Cd (50 mg/kg) plus (2 mg/ kg b.wt) of Olive Oil. Blood samples were taken for analysis of Serum liver enzymes, tissue level of SOD, GPX and CAT, MDA and H2O2 levels were investigated. Histopathological changes of the liver, percentage area density of collagen fibers and immunohistochemical expression of caspase 3 were also assessed.

Results: Oral intake of Cd to rats for 28 days caused a structural hepatotoxic changes, a considerable increase in collagen fibers deposition and apoptotic changes in liver. Combined oral intake of either Selenium or Olive oil with cadmium exposure for 28 days resulted in significant alleviation of all the hepatotoxic changes.

Conclusion: Long-term exposure to Cadmium lead to degenerative changes in the liver structures which could alleviated by Selenium or Olive Oil through their antioxidant, antifibrotic and antiapoptotic effects.

Keywords: Cadmium; Liver, Oxidative stress, Selenium, Olive Oil.

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Introduction

Cadmium (Cd) and other heavy metals represent a major risk to human and animal health causing many pathological disorders as they are excessively accumulated in the body through contamination of food, drinking water, and air (Khafaga et al., 2019). Cadmium has long biological half-life (10–30 years), causing many hazardous health effects, including kidney diseases, cardiovascular diseases, osteoporosis, hepatotoxicity, alterations in pancreatic functions, and even malignancies in various body organs (Amamou et al., 2015). The liver is the most exposed organ to Cd toxicity, regardless of the route of Cd exposure as the intestine absorbs the majority of heavy metals, which then travel to be accumulated in the liver (Sanjeev et al., 2019).

The cadmium causes hepatotoxicity through increasing the production of reactive oxygen species in cells, resulting in oxidative damages in many tissues. The modifications in the cellular antioxidant defense system caused by Cd toxicity, results in decrease of cellular antioxidants and alteration of lipid peroxidation (Mallya et al., 2017). Also, Cd is known to affect endocrine function, causing oxidative stress inflammation, disturb the regulation of cell cycle, and change apoptotic signaling (Everson et al., 2018).

Natural antioxidants have received more attention due to their defensive benefits against heavy metal-induced toxicities, particularly when reactive oxygen species are involved.Trace elements like zinc, iron, calcium, and selenium may counteract Cd-toxicity by competitive binding to Cd-bound proteins which have been proven to decrease the Cd-toxicity (Fan et al., 2018). Selenium has an antioxidant, and anti-cancerous properties as it represents the catalytic center of various selenoproteins, including glutathione peroxidase (GPx), thioredoxin reductase, and catalase (CAT). So, it is considered a free radical scavenger and enhancing the immune system (Gupta & Gupta, 2017).

However, the efficacy of Selenium is still doubtful as selenium can have nutritious or potentially harmful effects depending on the amount consumed. High dose of selenium is poisonous as it causes cytotoxicity through disrupting the mTOR/Akt pathway, resulting in autophagic cell death (Hossain et al., 2018). But, several researchers have reported that low doses of Selenium have a beneficial effect more than other plants (Hasanuzzaman et al., 2011; Saidi et al., 2014).

Olive oil (OO) is one of the Mediterranean diets, its consumption lowers the risk of heart diseases, neurological diseases, breast and colon cancer. The bioactive molecules in OO are Polyphenols which act as free radical scavengers providing a good protection against the auto-oxidation of unsaturated fatty acid (Mohammadian et al., 2018). Several studies were conducted on OO because of its anti-apoptotic, anti-inflammatory, and anti-oxidative effects against the hepatotoxicity caused by oxidative stress induced by different heavy metals as deltamethrin, cadmium, acrylamide, and aluminum (Amamou et al., 2015; Ghorbel et al., 2015, 2017; Khalatbary et al., 2017).

Therefore, we aimed to assess the comparative effect of Selenium and Olive Oil in case of combined oral intake with Cadmium to improve the Cadmium-induced hepatotoxicity in albino rats via histopathological, immunohistochemical and biochemical parameters.

Materials and methods:

Chemicals

Sodium selenite (Na2SeO3) and cadmium chloride anhydrous (CdCl2) were purchased from Sigma –Aldrich company (USA) and dissolved in distilled water before administration.

Olive Oil:

Olive Oil was purchased from National Research Center, Dokki, Giza, Egypt. the extract supplied to groups of animals as a single dose (2 ml/kg b.wt.) via oral gavages daily for 4 weeks (Mahmoud et al., 2015).

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Animals

In this study, 40 adult male albino rats (six-week old, weighing 120-160 g) were prepared. Rats were kept in polypropylene cages with 12 hours of light and dark cycles, at room temperature 22±2°C and 10% humidity and they received water and standard pellet diet ad libitum.

Experimental design and drug administration

Animals were randomly divided into four groups (10 rats each- 5/cage) after one week of acclimatization. All groups received the following treatment by oral gavage daily for four weeks (28 days):

Group I (control), rats received normal feeding and distilled water ad libitum; Group 2, Cd-group, rats received CdCl2 dissolved in distilled water and given orally in a daily dose of (50 mg/Kg) and fed with normal diet; Group 3 rats orally received Cd (50 mg/kg) and Selenium (2 ml/kg b.wt.); Group 4 rats orally received Cd (50 mg/kg) and olive oil (2 ml/kg b.wt.).

Blood collection, serum, and tissue preparations

At the end of the experiment (on day 28), blood samples were collected from the retro-orbital plexus, and then, serum was separated by centrifugation at 1200 g for 15 min, collected and kept at 20 °C for further biochemical analyses.

Serum biochemical analyses

The serum concentrations of liver enzymes were examined by an automated hematology analyzer.

Determination of oxidative/Antioxidative status

The liver tissue was rapidly removed, rinsed in ice cold saline buffer (20 mM Tris–HCl, 0.14 M NaCl buffer, pH 7.4), weighed, minced in the same solution, and homogenized by a homogenizer (10 percent, w/v). The homogenized tissue and plasma were utilized for an initial lipid peroxidation experiment, and aliquots of the homogenate were kept for additional biochemical analysis. The levels of tissue malondialdehyde (MDA) and hydrogen peroxide (H2O2), Superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) activities were determined according to the manufacturer's instructions.

Assessment of histological and immunohistochemical changes:

The liver specimens were fixed in 10% formalin for at least 24 h. The collected liver tissues were processed and sectioned at a thickness of 5 μm and stained with, Hx.&E for detection of structural changes, Masson trichrome for detection of fibrosis and immunostaining of caspase-3 for detection of apoptosis. The prepared slides were examined under light microscopy. The images were photographed & assessment of the percentage area density of collagen fibers and immune expression of caspase 3 using an Raywild E5 microscope with an Raywild M-300 digital camera with image-analyzing system (Mvi-mage program v12).

Statistical analysis:

Data are represented as the mean ± SE. Data were analyzed by the two-tailed Student's-test and one-way ANOVA using the statistical software package SPSS for Windows (Version 21.0; SPSS Inc., Chicago, IL, USA), followed by Duncan's post hoc test for multiple group comparison. Statistical significance was accepted at $P < 0.05$.

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Results:

Serum liver enzymes levels in the different studied groups

Cadmium chloride treated rats showed a significant increase of liver enzymes (ALT, AST and ALP) compared to control group ($p < 0.05$). The combined administration of CdCl2 with either Selenium or Olive oil showed a significant decrease of ALT and AST levels in comparison to CdCl2-treated group ($p < 0.05$), however, the liver enzymes are significantly increased in comparison to the control group (Table 1).

Table 1: Assay of serum liver enzymes levels in the different studied groups

Groups / Parameters	Control	Cadmium	Cd +SEL	Cd +O.O
ALT (U/L)	**32.71±5.37**	**97.44 ± 10.67***	**66.71 ± 3.27#**	**60.4±9.21#**
AST (U/L)	**48.18±8.17**	**101.52±13.41***	**68.12±7.62#**	**60.32±6.47#**
ALP (mg/dl)	**341.41±24.21**	**641.91±65.51***	**414.81±14.07#**	**394.66±37.25#**

Alanine transaminase (ALT), Aspartate transaminase(AST), alkaline phosphatase (ALP),

* Significant differences between the Cd and the control group; # significant differences between Cd plus Se or O.O-treated groups than the Cd group**.**

Tissue levels of oxidative/antioxidative stress parameters in the different studied groups

Rats exposed to Cd showed significant decrease of liver tissue levels of SOD, GPX and CAT in comparison to control group ($p < 0.05$). while combined oral intake of either Selenium or Olive oil with Cd-administration resulted in significant increase of SOD, GPx and CAT levels when compared to Cd-exposed group ($p < 0.05$). But, the levels of MDA and H2O2 were increased Cd-exposed group in comparison to control group ($p < 0.05$). The concomitant oral intake of Cd with either Selenium or Olive Oil revealed a significant decrease of those levels in comparison to Cd-exposed group ($p < 0.05$) (Table 2).

Table 2: Assay of tissue levels of oxidative/antioxidative stress parameters different groups:

Groups / Parameters	Control	Cd	Cd +Se	Cd + O.O
SOD (U/ mg protein)	**7.57±0.81**	**4.14±0.67***	**6.22±0.49#**	**6.90±0.75#**
GPx (U/ mg protein)	**5.73±0.28**	**3.07±0.64***	**4.69±0.57#**	**4.93±0.66#**
CAT (U/ mg protein)	**75.14±9.62**	**54.47±6.43***	**61.07±9.71#**	**64.37±7.85#**
MDA (Umol/g protein)	**7.91±0.72**	**11.62± 0.48***	**9.83 ± 0.29#**	**9.49± 0.55#**
H2O2 (U/ mg protein)	**1.38 ± 0.27#**	**4.69 ± 0.80#**	**2.64 ± 0.34#**	**2.04 ± 0.41#**

SOD: Superoxide dismutase, GPx: glutathione peroxidase, CAT: catalase, MDA, malondialdehyde, H2O2: hydrogen peroxide.

* Significant differences between the Cd and the control group; # significant differences between Cd plus Se or O.O-treated groups than the Cd group**.**

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Assessment of Liver fibrosis and apoptosis markers in the different studied groups

Morphometric analysis of Liver sections from the different studied groups revealed significant increase of percentage area density of both collagen fibers content and the immune expression of the hepatic pro-apoptotic protein caspase-3 in Cd-exposed group when compared to the control group. While, those levels were significantly decreased in both groups exposed to Cd associated with either Selenium or Olive Oil in comparison to Cd-exposed group (Table 3).

Table: 4 Assessment of Liver fibrosis and apoptosis markers in the different studied groups

Groups / Parameters	Control	Cd	Cd +Se	Cd +O.O
Collagen $(\mu m)^2$	**1.72±0.27**	**5.38±0.71***	**2..69±0.84#**	**2.44±0.52#**
Caspase-3 $(\mu m)^2$	**0.90±0.39**	**3.17±0.33***	**1.07±0.49#**	**1.02±0.36#**

* Significant differences between the Cd and the control group; # significant differences between Cd plus Se or O.O-treated groups than the Cd group**.**

Light microscopic results in the liver:

Hematoxylin and Eosin-stained sections results

Liver section of Cd-intoxicated rats showed marked dilatation, congestion of both central and portal veins, the hepatocytes appeared degenerated with pyknotic nuclei and vacuolar cytoplasm, dilated congested blood sinusoids with many kupffer cells. While the liver sections of both rat groups exposed to co-administration of CdCl2 with selenium or Olive Oil showing more or less restoration of normal liver structure with less dilated, congested central & portal veins (Figure 1).

Masson trichrome stained sections:

Liver section of Cd intoxicated rats in comparison to control group showed excessive collagen deposition ($p < 0.005$) around the portal vein, in comparison to control group. While the liver sections of both rat groups exposed to co-administration of CdCl2 with either Selenium or Olive Oil showing less deposition of collagen in comparison to Cd-exposed group (Figure 2).

Immunohistochemical assessment of caspase-3:

The rat liver showed marked immune expression of caspase 3 in Cd-exposed rats in comparison to control group while concomitant administration of selenium or Olive Oil decreased immune expression of caspase 3 in comparison to Cd-exposed rats (Figure 3).

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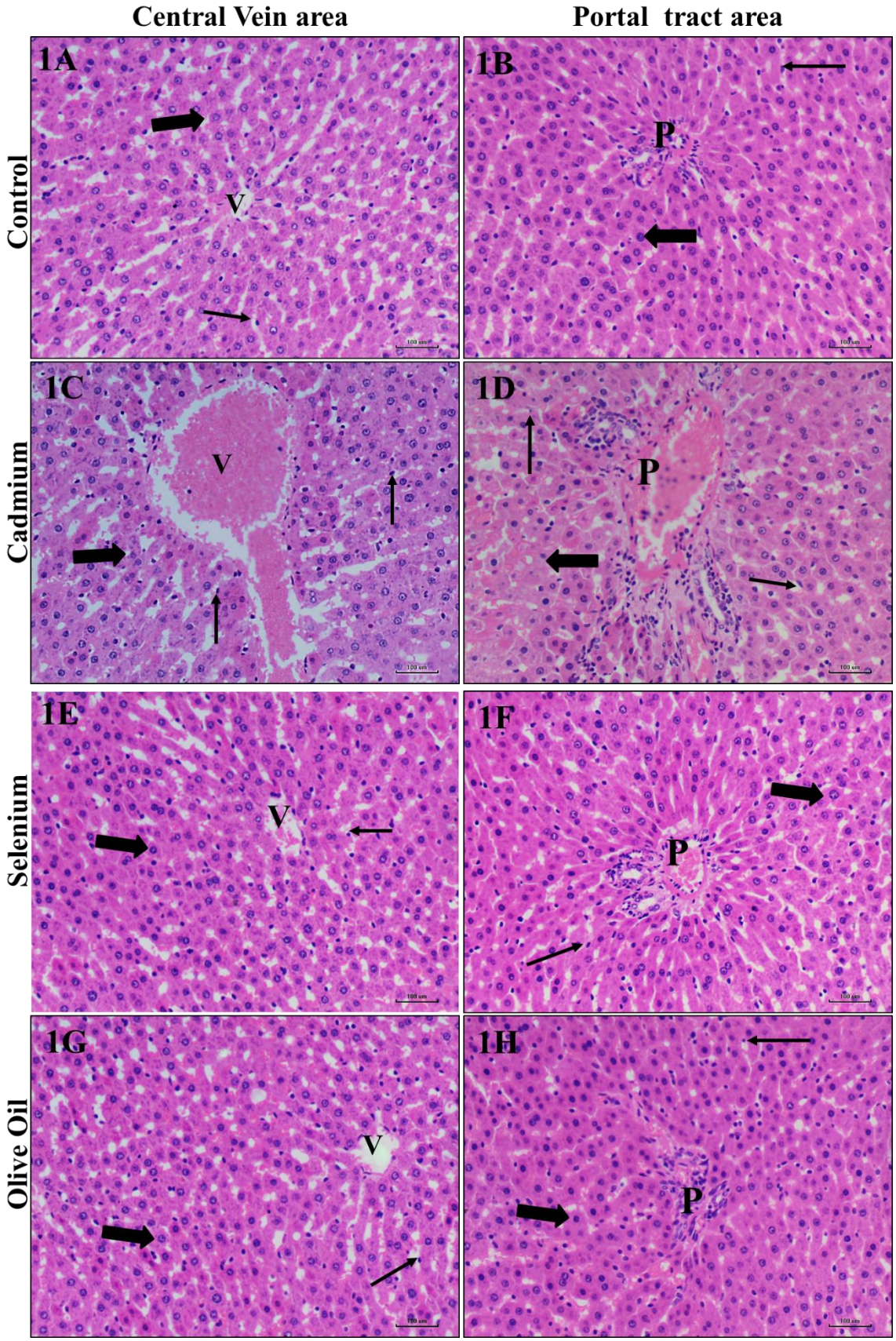

Figure (1). A: liver sections of central vein area of the control group, showed normal hepatic architecture, normal central vein (V) with branching cords of hepatocytes separated by blood sinusoids (thin arrow), the hepatocytes showed centrally

located vesicular nucleus with acidophilic cytoplasm (thick arrow). B: The portal area of the control group, showed normal portal triad (P) containing a large portal venule, a hepatic arteriole and bile ductule with branching cords of hepatocytes separated by blood sinusoids (thin arrow), the hepatocytes showed centrally located vesicular nucleus with acidophilic cytoplasm (thick arrow). C: Central region of the liver of Cd-exposed group showed; markedly dilated and congested central vein (V), degenerated hepatocytes with vacuolated cytoplasm and deeply stained shrunken nuclei (thick arrow) and dilated congested blood sinusoids with many kupffer cells (thin arrows). D: The portal area region of the liver sections Cd group showed; degenerated hepatocytes with vacuolated cytoplasm and deeply stained shrunken nuclei (thick arrow) and dilated congested blood sinusoids with many kupffer cells (thin arrow), dilated congested bile duct, hepatic arteriole and portal vein (P). E&G: Central region of the liver of Cd-exposed groups with co-administration of either Selenium or Olive Oil respectively, showed less dilated central vein (V) that the hepatocytes restore their normal shape (thick arrow), Less dilated and no or minimally congested blood sinusoids with less kuppfer cells (thin arrow). F&H: The portal area of the liver of Cd-exposed groups with co-administration of either Selenium or Olive Oil respectively, showed more or less normal portal triad (P) containing a large portal venule, a hepatic arteriole and bile ductule with branching cords of hepatocytes separated by less dilated blood sinusoids with less kupffer cells(thin arrow), the hepatocytes showed centrally located vesicular nucleus with acidophilic cytoplasm (thick arrow). A: Control group; B: Cd-exposed group; C: Cd + Selenium group; and D: Cd + Olive Oil group. (Hx.&E. X400) Scale bars, 100 um.

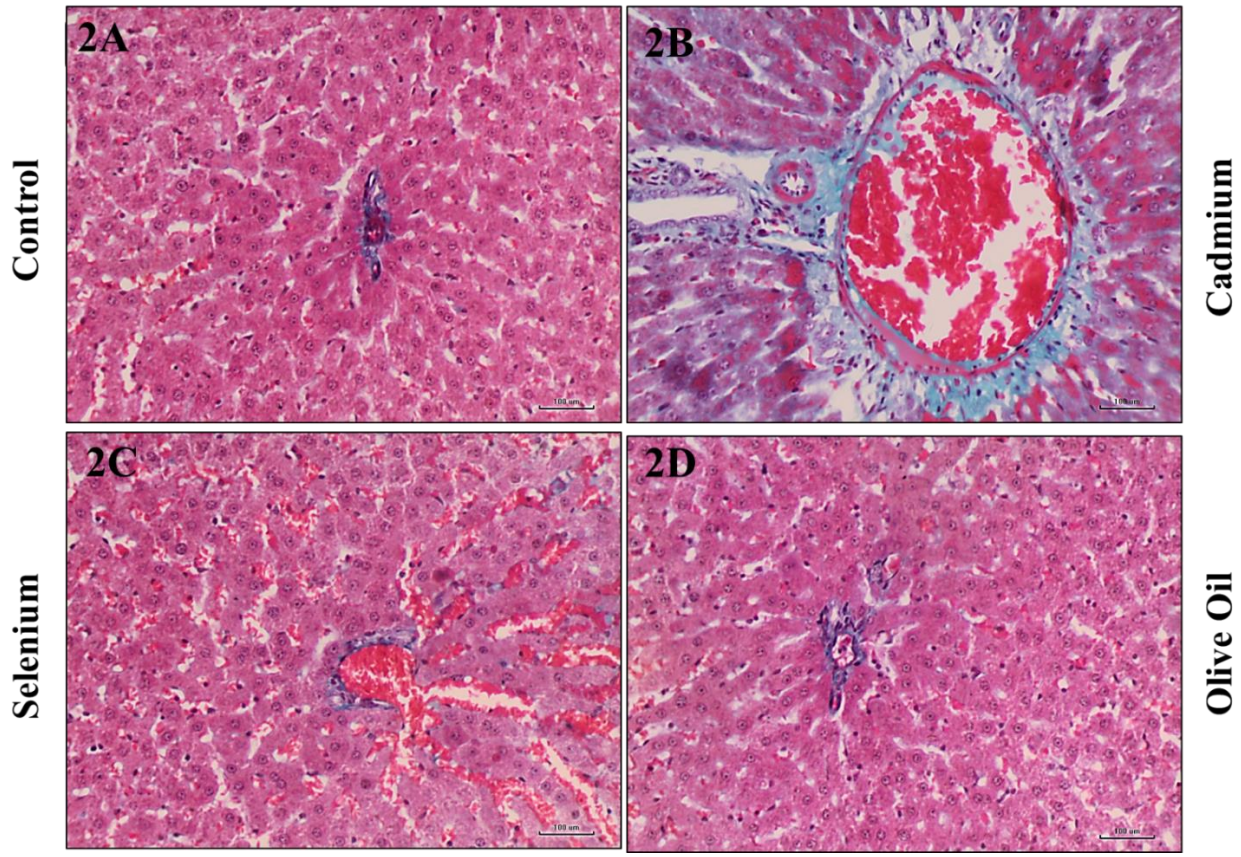

Figure (2): Photomicrographs of masson trichrome stained liver sections of the portal area. A: control group showing least deposition of collagen fibers. B: marked deposition of collagen fibers in Cd-exposed group. C &D: weak deposition of collagen fibers in treated groups with concomitant administration of selenium or Olive Oil with Cd-exposure. A: Control group; B: Cd-exposed group; C: Cd + Selenium group; and D: Cd + Olive Oil group. (Masson trichrome stain X400) Scale bars, 100 um.

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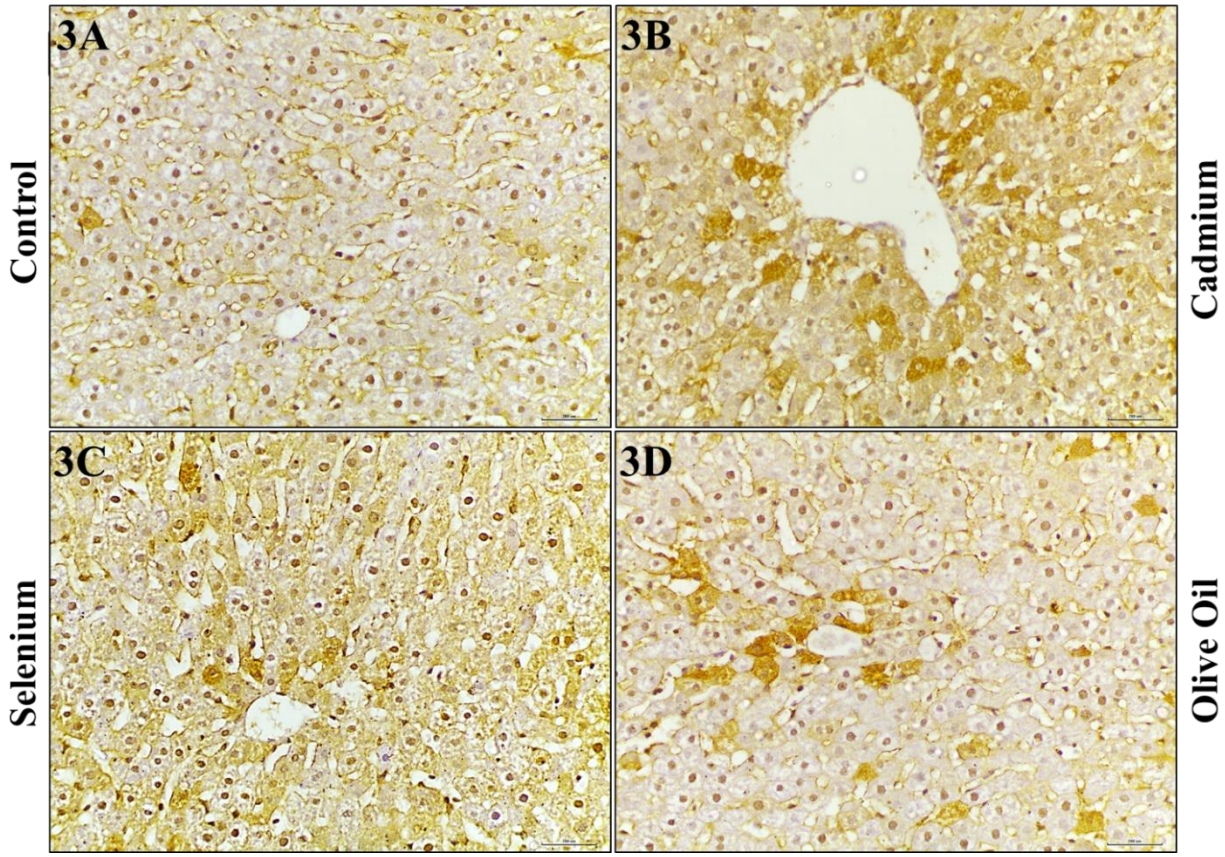

Figure (3): Photomicrographs of Immunostained liver sections for caspase-3 . A: control group showing weakest expression of caspase-3. B: highest expression of caspase-3 in Cd-exposed group. C&D: weak expression of caspase-3 in treated groups with concomitant administration of either selenium or Olive Oil with Cd-exposure. A: Control group; B: Cd-exposed group; C: Cd + Selenium group; and D: Cd + Olive Oil group. (Caspase 3 immune stain. X400) Scale bars, 200 pixel.

Discussion

The environmental pollution with Cadmium had a worldwide concern as it is extremely hazardous heavy metal that can be ingested or inhaled to cause human and animal poisoning (Seif et al., 2019). Cadmium was proven to induce liver toxicity as the serum liver enzymes, bilirubin and total proteins are important factors which are used to assess liver functions, there disturbance indicate a cellular leakage and a loss of functional integrity of the hepatic membrane structure (Amamou et al., 2015).

In the present study, we investigated the toxic effects of Cadmium on the liver of albino rats and the possible protective effects of concomitant administration of either Selenium or Olive Oil with Cadmium in rats.

As regard the liver functions in our study we found that oral administration of Cadmium to albino rats resulted in significant increase in the serum levels of AST, ALT and ALP and significant decrease in the serum levels of total proteins in comparison to control group. Our results are in agreements with the results of previous researches that have shown that administration of Cd leads to increase in the serum levels of AST, ALT and ALP in comparison to control group (Alharbi et al., 2019; Lavryshyn & Gutyj, 2019). This was in agreement with the findings of a recent study (Seif et al., 2019) exhibited elevation in liver enzymes in Cd-treated group. Also, these findings are consistent with a previous study (Toppo

et al., 2015), who found that Cd poisoning causes hepatic cell destruction, and its enzymes AST, ALP, and ALT are released into circulation, resulting in higher levels of these enzymes in the blood.

The results of this study showed that co-administration of selenium or olive oil with cadmium exposure resulted in enhancement of liver functions. In agreement to our results selenium was found to play a hepatoprotective effects by decreasing the liver enzymatic activity on concomitant exposure to Cd-toxicity (Șlencu et al., 2018). Also Olive oil was proved to alleviate the liver functions by decreasing the liver enzyme level in rats exposed to co-adminstration of olive oil with Cd in comparison to Cd-induced hepatotoxicity (Amamou et al., 2015). Selenium and Olive Oil induced their strong protective potential against Cd-induced liver toxicity by decreasing the liver enzymes via scavenging the free-radicals, protecting the hepatocyte membrane from oxidative damage thus, decreasing the leakage of those enzymes in the blood (Djeffal et al., 2015; Joshi et al., 2014).

One of the mechanisms of Cd poisoning is oxidative stress (Ayaz, 2017). Our study showed a large increase in MDA (a marker of cellular membrane lipid oxidation) in the livers of rats exposed to Cd poisoning, as well as a considerable decrease in antioxidant enzymes such as SOD, CAT, and GST, in comparison to control rats. This result could be a sign of Cd-induced hepatic oxidative stress. Similarly, a previous studies revealed that Cd exposure resulted in decreased SOD, CAT, GR, and GPx activities, as well as an increase in lipid peroxides and GST activities (Adi et al., 2016; Amamou et al., 2015). In this study, Cd-exposed rats had lower plasma levels of SOD and CAT activity, as well as lower hepatic GSH levels. Cd has a strong affinity for the –SH group of GSH, causing it to oxidize to GSSG (oxidized glutathione), which accumulates in the body organs causing cellular toxicity (Kim, 2012).

In our study, Selenium and Olive Oil were found to increase the activity of antioxidant enzymes in the liver on concomitant administration with Cd to the studied rats in comparison to the Cd-intoxicated group. Similar to our results, Several studies reported the protective activities of Se on Cd-induced oxidative stress (Liu et al., 2014; Wan et al., 2018). Because it decreases lipid peroxidation (LPO) and raises the activity of antioxidant enzymes in these tissues, Se may protect the renal and hepatic tissues from Cd toxicity via an antioxidative impact (Newairy et al., 2007). Also, Oleuropein, in glycosylated form, has been identified as the primary natural phenolic antioxidant molecule found in high concentration in olive oil, and its beneficial health outcomes have been linked to the antioxidant characteristics of biological elements found in olive oil (Kasdallah-Grissa et al., 2008). This suggest the antioxidative property of either Selenium or Olive Oil in Cd-intoxicated rats.

In the present study we showed a correlation between liver function parameters and structural changes in the liver, we found signs of pathological changes in the liver tissue of Cd-intoxicated rats in the form of dilatation of both central and portal veins, the hepatocytes appeared with pyknotic nuclei, vacuolar cytoplasmic degeneration with hemorrhage in the dilated blood sinusoids. Histopathological examination confirms haptic tissue destruction in rats subjected to Cd poisoning. Some authors have documented similar hepatic histological alterations in experimental animals in response to Cd poisoning including dilated and congested central vein with massive hemorrhage extending to the nearby cells, focal degenerative changes along with dilatation of blood sinusoids with increased number of von Kupffer cells and dilatation of the portal vein. Hepatocytes showed cytoplasmic vacuolization, karyolysis, pyknosis and vacuolar fatty degeneration (Ayaz, 2017; Sakr et al., 2015). The increased number of von Kupffer cells recorded in our study in Cd-exposed group, may be resulted from the cellular defense system as the Kupffer cells support the body defense by eliminating toxins from the portal circulation (Naito et al., 2004).

In this study, Supplementation of Selenium or Olive Oil to Cd-intoxicated rats were found to ameliorate the histopathological changes induced by Cd-exposure in rats. Similarly to our results, the prophylactic role of either selenium

and Olive Oil were recorded on the histological changes of liver of male rabbits exposed to Cadmium (Abu-El-Zahab et al., 2019). This reveal the hepatoprotective activity of either Selenium or Olive Oil in Cd-intoxicated rats.

As regard the detection of liver fibrosis we found increased deposition of collagen fibres in the liver tissue of Cd-exposed rats, this could suggest the fibrotic induced toxic effect of Cd on the liver of the studied rats. Similar results were recorded by a previous study conducted on the liver of rats exposed to a single dose of cadmium at low or medium doses in rats (Cupertino et al., 2013). Moreover that, many previous animal researches showed that Cd exposure for 30 days, 6 weeks, or 12 weeks resulted in varying degrees of collagen fibres deposition in the renal capsule and around the portal area in the liver tissue (Chen et al., 2016; Saleh, 2018). The fibrotic deposition in the liver tissue induced by Cadmium in this study may be explained by the triggering of profibrotic signaling by Cd which promotes myofibroblast differentiation by boosting the expression levels of differentiation marker proteins such -SMA via the SMAD2/3/4 transcription factor (Hu et al., 2017).

Either use of selenium or olive oil with the intake of Cd in rats of the studied group resulted in decrease the fibrotic changes in the liver of both groups in comparison to Cd-intoxicated group. This were in coincidence with the results of previous studies on both selenium or olive oil in the liver and stomach of cd-intoxicated rats (Al-Basher, 2018; Bolkent et al., 2008) This recommend the anti-fibrotic property of either Selenium or Olive Oil in Cd-intoxicated rats .

Apoptosis is triggered by oxidative stress through a variety of signalling mechanisms, including the endoplasmic reticulum (ER) stress response and Cd produces ER stress via increasing reactive oxygen species (ROS), which leads to apoptosis (Jin et al., 2016). In the present study we used the apoptotic marker caspase 3, we found that liver sections in the rats exposed to Cd showed increased expression of caspase-3 activity in comparison to control group. In accordance to our results, increased expression of immune-reaction of caspase-3 in liver, kidney, prostate and various organs, due to cadmium exposure, was previously recorded (Hagar & Al Malki, 2014; Șlencu et al., 2018). This results prove that cadmium causes its toxicity through induction of apoptosis.

The results of the present work also revealed a significant decrease in the expression of caspase 3 immune reaction in both groups of Selenium or Olive oil co-administration with cadmium. This results are in agreements with a recent research which reflected the anti-apoptotic property of either Selenium or Olive Oil in Cd-intoxicated rats (Abu-El-Zahab et al., 2019) .The protective activity of Se and O.O could be due to Several mechanisms concerning the protective role of antioxidants and selenium in Cd-induced tissue damage resulting in changes in Cd absorption or action and distribution in the body and within target organs, for example (Alkhatib et al., 2018).

From the above findings we found a direct association between antioxidants and lipid peroxidation which explain the provided protection of selenium and olive oil against Cd-induced lipid peroxidation.

Conclusion:

Our findings suggest that Cd is a highly toxic heavy metal that can affect liver functions even at low concentrations. Cd exposure causes structural degenerative, fibrotic and apoptotic changes in the hepatic tissue. Oral administration of Selenium or Olive Oil with Cd-exposure could alleviate those changes through the antioxidant, anti-fibrotic, anti-apoptotic activities.

Conflict of interest

The authors do not have any conflict of interest with the content of the paper.

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