Role of Flaxseed Oil Against Lead Toxicity in Liver of Male Rats

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Abstract

The present study aimed to prove the protective role of flaxseed oil (FSO) against lead (Pb) in male rats by measuring the enzyme activities as well as the concentration of some parameters and the histological changes in liver tissues. Forty adult male rats weighing 150-200 g were divided into four groups, control group received distilled water, group two orally administered 60 mg/kg b.w. lead acetate by using the stomach tube day after day, group three orally administered 600 mg/kg b.w. FSO using stomach tube day after day, group four orally administered 600 mg/kg b.w. FSO then 60 mg/kg b.w. lead acetate day after day for four weeks. The results showed an increase in the activity of ALT, AST, ALP and GGT enzymes in group administered lead acetate compared with control group, and convergence in results was observed in the group administered FSO compared with control group. While, in the group administered both FSO and lead acetate, the levels of these enzymes decreased compared to Group II, which was administered lead acetate only. Rats administered lead acetate showed an increase in the values of TBIL, TP, GLU, and ALB. It was observed that there was a convergence in all their values in the group administered FSO compared with control group. While these values were decreased in the group administered both FSO and lead acetate compared to Group II administered lead acetate only. The results of the histological examination, after administration of lead acetate, changes were appeared as swollen, necrotic and apoptotic hepatocytes. Also, many of the portal areas were completely masked by accumulation of inflammatory mononuclear cells and bile ducts were difficult to be seen. Administration of flaxseed oil before lead acetate in the 3rd group showed a significant enhance in the histopathology of the liver cells. The results showed an effect of lead on biochemical measurements, as well as pathological effects on liver tissues in rats. On the contrary, the results showed the protective role of flaxseed oil in maintaining the biochemical parameters and histological tests with their levels and status in the control group. These results confirmed the effective role of FSO against lead and by raising the efficiency of the body to protect or treat it against toxins that affect the important organ of the body the liver.

Keywords: Flaxseed Oil; Lead Acetate; Physiology; Histology; Liver; Rats

Introduction

Lead (Pb) is a toxic substance that has different effects on the human body. It is found in many industrial products such as pipes, paints and automobile industries. Inhalation is one of the most important ways to enter the body, it causes many biochemical, physiological and neurological risks in humans. Therefore, exposure to Pb causes harmful effects on the liver, due it performs important

functions such as storage, remove toxins and biotransformation [6]. Pb enters the body by air inhalation contaminated by vehicle exhaust or by food such as fish due to contamination of the marine environment, canned foods and plants. Children younger than six years of age are more susceptible to Pb poisoning than adults, because they are not fully developed, thus becoming more sensitive to Pb poisoning [32].

Flax (*Linum unisatissimum*) is an annual plant, grow to length 60 cm, its flowers are light blue color [30]. Flaxseeds (FS) are glossy elliptical smooth surface, while the color is brown to [17]. It was used by humans early in history as a medicine in Egypt and Greece as well as a food source of energy [30].

Liver is one of the most important organs in the body involved in metabolism and is a major target of toxins entering the body. Hepatic toxicity occurs when the liver is exposed to contaminants such as heavy metals or drugs. Increasing the concentration of these toxic substances causes health risks to humans. Exposure to Pb causes necrosis in the liver tissue, cell dissolution and dilation in blood vessels [6]. According to [10] evidences showed that acute exposure to lead caused hepatocyte necrosis and increases in ALT and malondialdehyde (MDA) levels.

A study was conducted to investigate the protective role of ω -3 polyunsaturated fatty acids (125 and 260 mg/kg body weight) against lead acetate (25 mg/kg body weight) induced toxicity in liver and kidney of female rats. Pb acetate was found to increase oxidative stress through elevation MDA associated with decrease in antioxidant enzymes activities in the tissues. Also, elevation of serum enzymes activities of ALT, AST, alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and the level of TP were decreased. In addition, lead acetate induced hyperlipidemia via increasing lipid profiles associated with decline in high density lipoprotein cholesterol (HDL-c) level. The biochemical alterations of lead acetate were confirmed by histopathological changes and DNA damage. The administration of ω -3 provided significant protection against lead acetate toxicity [5].

In a study, blood samples were taken after 2, 4 and 6 weeks of treatment with lead acetate in diet (500 mg/kg). It was found that Pb significantly induce elevations in serum ALT, AST, GGT and ALP enzyme activities, while serum low-density lipoprotein cholesterol (LDL-c) was decreased. Pb also induced hepatocyte proliferation, portal inflammatory infiltrate with disruption of the limiting plates, steatosis, apoptosis and mild fibrosis were detected especially by sixth week of lead administration. Administration of vitamin C and silymarin to animals exposed to lead showed marked improvement of the biochemical and histopathological findings. These experimental results strongly indicate the protective effect of Vitamin C and silymarin against toxic effects of Pb on liver tissue [31].

The possible hepatoprotective role of sesame oil against lead acetate induced hepatotoxicity in albino mice from the histological and biochemical aspects was investigated by [9]. The results indicated that, in lead treated animals (500 mg /kg diet), there were severe structural damage in the liver. Also, decreased body weight and increased liver weight were observed. In addition, AST, ALT, ALP, and GGT enzymes' activities were increased and serum total proteins and albumin were decreased. Administration of sesame oil (5 ml/kg body wt.) significantly improved the structural changes in the liver and also the serum AST, ALT, ALP, and Gamma Glutamyl Transferase (GGT) activities were significantly declined and serum TP and ALB were elevated. In conclusion, sesame oil showed effective hepatoprotective action against lead acetate induced hepatotoxicity in albino mice.

Pb is a toxic heavy metal of global concern, induces toxicity in various organs via oxidative stress. Thereby, the protective role of curcumin (400 mg/kg bw) against lead acetate (100 mg/kg bw) induced toxicity in male albino rats was studied by [1]. Their results revealed that lead acetate toxicity induced normocytic normochromic anemia with significant leukocytosis and lymphocytosis. Moreover, lead acetate-intoxicated rats showed a marked elevation in the liver enzyme activities, serum CHOL and TG levels. In contrast, sero-immunological parameters, TP, ALB, globulin, and testosterone levels were significantly reduced compared to the control rats. Additionally, lead acetate-induced hepatic and testicular oxidative damage revealed by marked increased MDA level with prominent reduction in the antioxidant system. Curcumin treatment could partially improve the hematological, biochemical, and histopathological alterations induced by lead acetate. Also, it was observed that curcumin significantly restored hepatic pro-inflammatory markers and testicular steroidogenic enzymes.

The possible protective role of *N. sativa* oil (700 mg/kg/day) in female rats exposed to lead acetate (150 mg/kg BW) was studied, the results showed that oral administration of lead acetate exhibited highly significant increase in the activities of the enzymes ALT, AST, ALP, GGT and total bilirubin (TBIL). Histological examinations of liver sections from rats exposed to lead acetate showed severe alterations. Then, administration of *N. sativa* oil attenuated the biochemical and histological changes induced by lead intoxication [8].

[22] investigated the protective effect of FSO against hepatorenal toxicity induced by methotrexate, a drug used to treat certain types of cancer. Rats treated with methotrexate exhibited elevations in the levels of AST, ALT, ALP, LDH, urea, CR, uric acid, and TBIL. Furthermore, the levels of MDA, and NO were significantly increased, accompanied by a decrease in the TP, SOD, GPx and GSH content. Alterations in the lipid profile and hepatorenal histology were observed in rats treated with methotrexate. However, oral treatment of FSO exhibited a protective effect against methotrexate toxicity in rats that could be attributed to its potent antioxidant, anti-inflammatory, and anti-apoptotic activities.

Materials and Methods

Experimental animals

Forty adult male albino rats of the Wistar strain (*Rattus norvegicus*) weighing 150–220 g were used in the present study. Rats were housed in standard plastic cages and maintained under controlled laboratory conditions of humidity (65%), temperature ($20 \pm 1^\circ\text{C}$) and 12:12 h light: dark cycle. Rats were fed on normal commercial chow and had free access to water ad libitum. The experimental treatments were conducted following the ethical guidelines of the Animal Care and Use Committee of King Abdulaziz University.

Experimental design: A total of 40 adult male rats were randomly distributed into 4 experimental groups, 10 rats each and the experimental groups were treated as follows:

- **Group I: Control group (GI):** Rats received distilled water through gastric intubations day after day for four weeks.
- **Group II:** Rats were orally administered 60 mg/kg b.w. of lead acetate dose ($1/10\text{LD}_{50}$) by using the stomach tube day after day for four weeks.
- **Group III:** Rats were orally administered 600 mg/kg b.w. of flaxseed oil by using the stomach tube day after day for four weeks.
- **Group IV:** Rats were orally be administered 600 mg/kg b.w. of flaxseed oil then given 60 mg/kg b.w. of lead acetate dose ($1/10\text{LD}_{50}$) by using the stomach tube day after day for four weeks.

Blood serum analyses

At the end of the experimental period, rats fasted for 12 hours, water was not restricted and then anesthetized with diethyl ether.

Blood samples were collected from orbital venous plexus in non-heparinized tubes, centrifuged at 2500 rpm for 15 minutes and blood sera were then collected and stored at -80°C . Serum levels of ALT and AST were determined using the methods of [28]. The method of [21] was carried out to determine the level of ALP. The serum level of GGT was evaluated using the method of [23]. Serum levels of bilirubin, total protein, albumin, and glucose were determined using the methods of [13,14,27,33], respectively.

Histopathological examinations

At the end of the experiment (30 days) and after blood sampling, liver specimens were isolated from each group, fixed in 10% neutral formalin solution. Fixed tissues were processed, and then embedded in paraffin blocks. Sectioned of 4 μm thickness and stained with Hematoxylin and Eosin. Then liver, kidney and testis were examined using microscope (Olympus Bx-Ucb - USA) and photographed by a camera (Olympus DP72- USA).

Statistical analysis

The data were statistically analyzed with a completely randomized design by using the Statistical Package for Social Sciences (SPSS for Windows, version 12.0). Significant differences between treatments were tested with Student's t-test and ANOVA analysis.

Results

Body weights

The mean values $\pm$ SD of the body weights of rats in different experimental groups from week one till week four are presented in table 1 and figure 1. Rats of the lead acetate group (GII) showed percentage differences from control group by 15.97, 18.66, 23.98 and 21.76% during 1st, 2nd, 3rd and 4th weeks, respectively. Also, rats of FSO group (GIII) showed a percentage differences from control group by 7.64, 11.94, 13.47 and 16%, during 1st, 2nd, 3rd and 4th weeks, respectively. On the other hand, rats of FSO + lead acetate group (GIV) showed percentage differences from lead acetate group by -6.33%, -5%, -5.56% and 0.7%, during 1st, 2nd, 3rd and 4th weeks, respectively.

Biochemical results

Results of the activities of the enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and Gamma Glutamyl Transferase (GGT)

The results of the activities of the enzymes AST, ALT, ALP and GGT in different groups studied are presented in table 2 and figures

Groups	Weight/g				
	0 Week	1 st Week	2 nd Week	3 rd Week	4 th Week
Control (GI)	123.57 ± 4.28	144.00 ± 4.28	163.86 ± 2.54	178.14 ± 11.01	200.86 ± 13.39
Lead acetate (GII)	135.57 ± 4.24	167.00 ± 5.97 (15.97%)	194.43 ± 9.74 (18.66%)	220.86 ± 13.01 (23.98%)	244.57 ± 17.08 (21.76%)
Flaxseed oil (GIII)	136.14 ± 3.58	155.00 ± 6.88 (7.64%)	183.43 ± 8.96 (11.94%)	202.14 ± 15.66 (13.47%)	233.00 ± 20.72 (16%)
Flaxseed oil + lead acetate (GIV)	135.71 ± 5.16	156.43 ± 7.59 [-6.33%]	184.71 ± 13.26 [-5%]	208.57 ± 12.93 [-5.56%]	246.29 ± 15.47 [0.7%]

Table 1: Mean values ± SD of the body weights of rats in different experimental groups from week one till week four.

() % of change from control group, [] % of change from group GII.

Figure 1: Percentage of Change of Body Weights among groups.

2, 3, 4 and 5. In the control group (GI), the mean values ± SD of the enzymes AST, ALT, ALP and GGT activities were 20.40 ± 1.82 , 18.40 ± 2.07 , 62.00 ± 6.32 and 19.20 ± 3.35 IU/L, respectively. While in lead treated group (GII), the activities of AST, ALT, ALP and GGT enzymes significantly increased ($P < 0.05$) when compared with the control group (GI). In flaxseed oil treated group (GIII), the activities of the enzymes AST, ALT, ALP and GGT were slightly higher when

compared with the control group (GI). While on using flaxseed oil + lead acetate (GIV), the mean values for the enzyme's activities were significantly decreased ($P < 0.05$) for AST, ALT and GGT when compared with lead acetate group (GII), respectively, but for ALP enzyme, it was insignificantly decreased. Flaxseed oil + lead acetate group (GIV) showed a percentage difference from lead acetate group (GII) in the activities of the enzymes AST, ALT, ALP and GGT of -45.62, -33.73, -40.68 and -18.24%, respectively.

Parameters	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	GGT (IU/L)
Groups				
Control (GI)	20.40 ± 1.82	18.40 ± 2.07	62.00 ± 6.32	19.20 ± 3.35
Lead acetate (GII)	75.40 ± 5.03* (375.8%)	51.00 ± 9.00* (177.17%)	188.80 ± 13.29* (204.52%)	65.80 ± 4.66* (242.71%)
Flaxseed oil (GIII)	21.00 ± 2.74 (-78.43%)	19.60 ± 3.58 (6.52%)	73.60 ± 9.91 (18.71%)	24.60 ± 3.78 (28.13%)
Flaxseed oil + lead acetate (GIV)	41.00 ± 3.93** [-45.62%]	33.80 ± 6.53** [-33.73%]	112.00 ± 10.37 [-40.68%]	53.80 ± 6.58** [-18.24%]

Table 2: Mean values ± SD of the activities of the enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) in different groups.

* : Significantly increased when compared with control group (GI)

** : Significantly increased or decreased when compared with lead acetate group (GII) and control group (GI)

() % of change from control group; [] % of change from group GII.

Figure 2: Mean values ± SD of the activities of the enzyme aspartate aminotransferase (AST) in different treated groups.

Figure 4: Mean values ± SD of the activities of the enzyme alkaline phosphatase (ALP) in different treated groups.

Figure 3: Mean values ± SD of the activities of the enzyme alanine aminotransferase (ALT) in different treated groups.

Figure 5: Mean values ± SD of the activities of the enzyme Gamma Glutamyl Transferase (GGT) in different treated groups.

Results of total bilirubin (TBIL), albumin (ALB), total protein (TP) and glucose (GLU)

Table 3 and figures 6,7, 8 and 9 illustrate the results of total bilirubin (TBIL), albumin (ALB), glucose (GLU) and total protein (TP) in different groups studied. In the control group (GI), the mean values $\pm$ SD for TBIL, ALB, GLU and TP were (0.63 ± 0.16 , 4.08 ± 0.51 , 100.60 ± 7.70 mg/dl and 6.87 ± 0.49 g/dl respectively). When comparing the mean values in lead acetate treated group (GII) with

control group (GI) TBIL, ALP, GLU and TP were increased. On the other hand, the values in flaxseed oil treated group (GIII), the mean values for TBIL increased when compared with GI, while for ALB, GLU and TP were decreased. While on using flaxseed oil + lead acetate (GIV), the mean values for TBIL, ALB, GLU and TP were decreased when compared with lead acetate group (GII). The values of TBIL, ALB, GLU and TP of lead acetate + flaxseed oil group (GIV), showed a percentage difference from lead acetate group GII values of -42.76%, -20.83%, -40.68% and -16.11%, respectively.

Parameters	TBIL (mg/dl)	ALB (mg/dl)	GLU (mg/dl)	TP (g/dl)
Groups				
Control (GI)	0.63 ± 0.16	4.08 ± 0.51	62.00 ± 6.32	100.60 ± 7.70
Lead acetate (GII)	$1.45 \pm 0.11^*$ (130.16%)	5.81 ± 0.15 (42.4%)	$188.80 \pm 13.29^*$ (204.52%)	$149.00 \pm 17.35^*$ (48.11%)
Flaxseed oil (GIII)	0.73 ± 0.17 (15.87%)	3.92 ± 0.31 (-3.92%)	73.60 ± 9.91 (18.71%)	99.40 ± 10.01 (-1.19%)
Flaxseed oil + Lead acetate (GIV)	0.83 ± 0.08 [-42.76%]	4.60 ± 0.64 [-20.83%]	$112.00 \pm 10.37^{**}$ [-40.68%]	125.00 ± 7.78 [-16.11%]

Table 3: Mean values $\pm$ SD of total bilirubin (TBIL), albumin (ALB), glucose (GLU) and total protein (TP) in different treated groups.

*: Significantly increased when compared with control group (GI).

**: Significantly increased or decreased when compared with lead acetate group (GII) and control group (GI).

() % of change from control group; [] % of change from Lead acetate group GII.

Figure 6: Mean values $\pm$ SD of Total Bilirubin (TBIL) in different treated groups.

Figure 7: Mean values $\pm$ SD of Albumin in different treated groups.

Figure 8: Mean values $\pm$ SD of Glucose (GLU) in different treated groups.

Figure 9: Mean values $\pm$ SD of Total Protein (TP) in different treated groups.

Histological results of the liver

The Liver of Control group (G1)

Examination of the liver tissue sections from the control group (G1) revealed normal structure of the liver. The hepatic lobules appeared as polygonal lobules showing plates of epithelial cells called hepatocytes radiating around the central vein (CV). Hepatocytes arranged as the interconnected plate like bricks of a wall around the

central veins. The central vein of each lobule was indicated by its location in the middle of each lobule, where portal areas appeared at its peripheral corners. The portal areas displayed branches of hepatic artery, bile ducts and portal vein. Hepatocytes have a basophilic cytoplasm with central rounded vesicular nuclei. Some of the liver cells were binucleated. Blood sinusoids (liver sinusoids) lied between the hepatic cords. Its endothelial cell lining appeared as squamous cells with flattened nuclei (Figure 10 a, b).

Figure 10: Photomicrographs of the rat liver of normal control group (G1).

- Central vein region (CV): showing the normal hepatocyte cells (black arrows) arranged in plates radiating from the central vein (CV). They have central rounded light vesicular nuclei. Narrow blood sinusoids (white arrows) could be seen between hepatocytes plates. (H & E X 100).
- Portal area region (PA): showing portal area content, branches of hepatic artery (HA), bile ducts (BD) and portal vein. Hepatocytes around the area (black arrows) and blood sinusoids (white arrows) showed normal histological. (H & E X 400).

The Liver of Lead acetate treated group (GII):

Histological examination of the liver in lead acetate group (GII) showed signs of degeneration. The hepatocyte cells appeared swollen and others were necrotic with loss of their cell outlines showing the piecemeal appearance. The cytoplasm of hepatocytes had weak basophilic staining. The nuclei had signs of necrosis ranged from pyknosis to karyolysis. Many apoptotic hepatocytes were seen with dark acidophilic stained cytoplasm and pyknotic (small dark) nuclei. Blood sinusoids compressed by the swollen cells and difficulty demonstrated between hepatocytic plates. Mononuclear inflammatory cells were seen at the site of necrotic cells. In addition, many of the portal areas were completely masked by accumulation of inflammatory mononuclear cells and bile ducts were difficult to be seen. Hepatocytes around the area showed apoptotic features as the hepatocytes appeared with dark acidophilic cytoplasm and nuclear changes from pyknosis to karyolysis. The blood sinusoids displayed increased in the hypertrophic phagocytic Kupffer cells (Figure 11 a, b).

The Liver of Flaxseed oil treated group (GIII)

The examined liver sections of the flaxseed oil group (GIII) revealed normal structure like control group (GI). The liver cells (hepatocytes) appeared like control group with absence of any features of necrosis or apoptosis as that had seen in the lead group. They had active central rounded basophilic vesicular nuclei. Some cells were binucleated having 2 nuclei. Blood sinusoids looked normal and not compressed between plates of the hepatocytes. Most of the examined sections displayed a control histological structures of the portal areas with no features of inflammatory cells. Branches of hepatic artery and bile ducts looked normal. Hepatocytes around the area had a basophilic cytoplasm with few cells showed degenerative changes (Figure 12 a, b).

The Liver of Lead acetate and flaxseed oil (FSO) treated group (GIV)

Examination of the right liver lobe exposed to the FSO before lead acetate (GVI) revealed a marked preservation of the normal hepatocyte structure which looked similar to control group with absence of any features of necrosis or apoptosis as seen in lead acetate group. Few cells still appeared dark acidophilic, but their nuclei were active. Blood sinusoids looked normal and not com-

Figure 11: Photomicrographs of rat liver section of the lead acetate group (GII).

a. Central vein region (CV), showing swollen and necrotic hepatocyte cells where cell outline is lost with weak stained cytoplasm of the hepatocytes (red arrows). The nuclei become small with karyolysis and karyolysis of some nuclei. Other hepatocytes appeared shrunken with dark stained cytoplasm and small pyknotic dark nuclei (black arrows). Blood sinusoids are compressed by swollen cells and could not be seen between hepatocytes plates. Mononuclear inflammatory cells are seen at the site of necrotic cells (white star) (H&E x100).

b. Portal area region (PA), showing portal area content completely masked by accumulation of inflammatory mononuclear cells (white star). Bile ducts (BD, dotted arrows) are difficult to be seen. Hepatocytes around the area (black arrows) have deep acidophilic cytoplasm and pyknotic nuclei. The blood sinusoids display hypertrophic phagocytic Kupffer cells (white arrows) (H&E X 400).

Figure 12: Photomicrographs of the rat liver of flaxseed oil treated group (GIII).

a. Central vein region (CV), showing preservation of normal hepatocyte structure (black arrows). The hepatocytes have basophilic vesicular central rounded nuclei. Some cells are binucleated. Blood sinusoids (white arrows) are seen between the hepatocytes (H&E X100).

b. Portal area region (PA), showing the portal area contents with no features of inflammatory cells as seen in lead group. Most of the hepatocytes around the portal area (black arrows) have a control appearance with focal cells with necrotic changes (circle). Branches of hepatic artery (HA) and bile ducts (BD) (H&E X 400).

pressed hepatocytes plates. The portal areas showed absence of the mononuclear inflammatory cells compared to the examined portal area of the lead group (G2). Branches of portal vessels and small bile ducts looked normal. Hepatocytes around the area were

normal with few cells showed necrotic or apoptotic changes. Few blood sinusoids looked normal, but others still compressed (Figure 13 a, b).

Figure 13: Photomicrographs of the rat liver of (FSO + lead acetate) group (GIV).

a. Central vein region (CV): showing a potential marked preservation of normal hepatocyte structure (black arrows) which looked like control group with absence of any features of necrosis or apoptosis. Few hepatocytes still have an acidophilic cytoplasm, but their nuclei are active (dotted arrows) Blood sinusoids (white arrows) looked normal and not compressed hepatocytes plates (H&E X100).

b. Portal area region (PA): showing absence of inflammatory cells (star) seen portal area of lead group, Branches of portal vessels (PV) and small bile ducts (BD) looked normal. Hepatocytes around the area (black arrows) are normal with few cells showed necrotic or apoptotic changes (dotted arrows). Few blood sinusoids (white arrows) looked normal, but others still compressed (H&E X400).

Discussion and Conclusion

The present work was designed to study the possible protective effect of FSO against the toxicity of lead acetate on the serum biochemical parameters and histology of liver tissues of adult male rats. Rats were divided into 4 groups the 1st group served as control (GI), the 2nd received 60 mg/kg b.w. lead acetate (GII), the 3rd group received 600 mg/kg b.w. FSO (GIII) and the 4th group received both 600 mg/kg b.w. FSO, and 60 mg/kg lead acetate (GIV).

The results of the present study showed that lead acetate causes toxicities and damages in the biochemical parameters and in the histological sections of liver tissues. On studying the activities of AST, ALT, ALP and GGT, they were increased after treatment with lead acetate when compared with control group. These results come in accordance with the results of [12], who found that lead exposure caused a significant increase ($p < 0.05$) in serum ALP, AST, ALT and TB. This might be due to the toxicity induced by lead acetate that resulted from the generation of free radicals [3,29] which lead to lipid peroxidation (LPO), causing damage and changing in many organs in their functions and structure [18]. Exposure to lead acetate causes decrease in enzymatic activities of ALP and GGT [29]. [3] investigated the effect of various doses of aqueous FSO, they found significant increase in the activities of the studied serum enzymes; AST, ALT and ALP in rats. Also [25], examined the influence of chronic Pb toxicity on the activity of serum enzymes AST and ALT, and reported that increased AST and ALT activities in serum is most likely the consequence of lead hepatotoxicity. Lead acetate induced damage of the liver obvious by an increase in AST and ALT in the blood [12].

In the present study, increase in the levels of plasma AST, ALT, ALP and GGT indicates liver damage. Generally, the liver is one of the critical target organs of Pb toxicity. Exposure to lead acetate is well known to induce a variety of toxicity symptoms, lead acetate induced necrosis in the liver that can cause release of abnormal amounts of AST into the blood [19].

In the present research, the levels of the enzymes AST, ALT, ALP and GGT activities were increased after administration of Pb and then decreased approximately to the control level when administering FSO. These results could be due to the protective effect of FSO.

Significant decreases in the plasma levels of AST, ALT and ALP activities in the animals pre-treated with FSO prior to the administration of lead acetate may be a reference of protective effect of FSO on liver damage induced by lead acetate [3]. [34] reported that, treatment of rats with FSO showed a significant decrease in the levels of AST, ALT and ALP. Thus, they proposed that pretreatment with FSO changed the levels of the liver enzymes and consequently can improve the liver functions. [20] observed that treatment with FSO in rats significantly decreased AST, ALT and GGT in blood serum, when compared with control groups.

[11] investigated the therapeutic effect of FSO against acute liver injury. They found increase in the activities of liver marker enzymes; AST and ALT in experimental rabbits exposed to chemically induced acute liver injuries. They declared that FSO decreased the activities of these enzymes. They suggested that FSO has a potential role in therapeutic properties against chemically induced acute liver injuries.

[15] evaluated the efficacy of FSO as a hepatoprotective and as an antioxidant against lead acetate induced hepatocellular damage. They found increases in the enzyme activity of ALT which is indicative of hepatocellular damage. Another study showed that treatment with FSO significantly reversed the acute carbon tetrachloride induced alterations in the liver tissue and offered almost complete protection. Their results indicated that FSO showed a good hepatoprotective and antioxidant potential against lead acetate induced hepatocellular damage in rats that might be due to decreased free radical generation.

In the present study, it was found that administration of FSO caused decrease in the levels of GLU, TP, bilirubin and ALB after treatment with lead acetate when compared with lead acetate treated group (Table 3 and Figures 6, 7, 8 and 9). [12] reported that administration of lead acetate produced increases in the level of TP. In another study they showed a significant reduction in ALB level in serum after treatment with lead acetate, this result is not consistent with our experience [4,16]. On the other hand, the results of bilirubin and TP were increased after treatment with lead acetate [24]. [3] reported increases in the level of ALB and TP after treatment with lead acetate, and they showed significant decreases in their levels after treatment with FSO in rats. On the other hand, FSO

was contributed to lower the levels of glucose, TP and ALB which were increased after treatment with lead acetate. They also added that, pre-treatment with FSO in lead acetate exposed rats showed a significant decrease in the levels of TP and TBIL as compared with the lead acetate alone treated rats. The results of the present study showed that lead acetate causes decrease in the values of TP and TBIL after treatment with FSO and lead acetate when compared with control group.

Because the liver is the main drug metabolic organ of the body, it is vulnerable to drug toxicity. In the current study, the hepatic tissues of the rats exposed to lead acetate (group GII) demonstrated histopathological signs of different degrees of inflammation from degeneration to necrosis of focal areas. The compressed blood sinusoids by swollen cells together with mononuclear inflammatory cells are seen at the site of necrotic cells. These findings are in accordance with the description of a previous study of [2] who stated that lead acetate resulted in prominent inflammation and hepatocytic vacuolation. Presence of the accumulated inflammatory cells masking the bile ducts with the apoptotic changes of hepatocytes around the portal areas give a strong evidence that the injurious agent reached the liver through the systemic circulation as it affects the zone I of liver acinus. The induced oxidative stress by lead acetate affected the liver tissues in the form of the lymphocytic mononuclear cellular infiltration. The spread of the areas with apoptotic hepatocytes is explained in another study as a result of induction of hyperplasia followed by apoptosis [26]. The well circumscribed cytoplasmic vacuolation usually is mainly a result of the accumulation of the fat inclusions because of disturbed fat metabolism in hepatocytes. This conclusion is reported in a previous study on mice, where the authors stated that lead acetate induce general disturbance of the liver physiology [10].

The current treated group with FSO (G IV) has marked degree of improvement of the hepatocytes with a nearly similar picture compared to the control group. The features of necrosis or apoptosis is nearly ameliorated with normal appearance of nuclei and scattered binucleated hepatocytes. These positive effects on the hepatocytes under effect of flax seeds are not similar to the effect of dose of 4 ml/kg of the flax seed oil for 4 weeks in rats after of hepatocytic affection by carbon tetrachloride [15]. In several studies, histological assessment of liver of lead acetate-exposed rat, observed hepatic necrosis due to exposure to lead acetate [7,12,15,18].

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