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Evaluation of fish oil impacts on hepatic structure in male rats

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Abstract
This study aimed to evaluate the protective impacts of fish oil on lead acetate induced toxicity of liver in rats. Forty male rats were randomly divided to four sets, each of them contained of ten rats. Control male rats received normal saline solution, whilst treated rats received lead acetate (150 mg/kg body weight) three times a week, fish oil and lead acetate (650 mg/kg body weight/day), and the last group received fish oil (650 mg/kg body weight/day) for six weeks by sterile tube. The results illustrated an insignificant increase in average liver weight with greatest and least liver weight gained was of 10.49±1.51g and 9.76±2.09 g respectively. on the other hands, the hepatic histology of rats treated with fish oil showed slight alteration such as; congestion around central vein. Also, the results of present study revealed that fish oil have been starting the positive effect on hepatic normal structure in lead acetate treated male rats which could be attributed to presence of antioxidants that reduce oxidative stress.

Keywords: Fish oil, lead acetate, liver histology, hepatic structure, male rats

Introduction

The multiple negative impacts on human health recently are caused by heavy metal exposure in the environment, and it is regarded as one of the most critical topics of concern across the world. Lead is one of the most common hazardous metals and industrial contaminants on the earth. Lead is naturally distributed throughout the Earth's crust [1]. However, the majority of the high concentrations seen in the environment are the result of human activities. Volcanic emissions, forest fires, and other erosion processes are examples of natural processes that might occur. Similarly, some of the human activities causes concentration and dispersion, notably in the mining of metallic ores [2]. Heavy metals are an environmental contaminant that is a big concern in the majority of the world's countries. These poisonous chemicals can build in crucial organs over time in bio-organs [3]. Metallic compounds found on land and in water can have a negative impact on the health of all living organisms, including cattle and wild animals where these complexes make up a significant component of industrial waste [4]. Lead (Pb) is a heavy highly poisonous metal found in various concentrations in rocks and it can leak out to soil and water sources by different ways. In addition, Pb can find its way to animals and human tissues resulting in a threat to human and animal organs when exposure it reaches at high levels [5]. Pb is considered as one of the most dangerous environmental pollutants and the main risk factors of liver toxicity in human and animals. Therefore, it has been a great concern on human health. In the last years, there is a big increasing in the levels of lead in the environment compared to those recommendations of the World Health Organization due to human activities [6].

Fish oil have several healthy benefits where contains long-chain poly unsaturated fatty acids (n-3 PUFA) most important are the omega-3 fatty acid. Docosahexaenoic acid, Eicosatetraenoic acid (EPA) and Alpha-linoleic acid (ALA) which believed to play an important role in the development of human body systems [7]. Numerous studies in rats have supported these beneficial compounds when fish oil consumption. Briefly, fish oil may have protective effects on arthritis, cardiac arrhythmias, cancer, and bone mass in aging stage [8]. Recently, there is interesting increase in the utilizing of fish oil for medicinal purposes. Also, the researchers have revealed that fish oil may have crucial health benefits for the human body and controlling of some diseases. Therefore, it is wide range used in therapeutic for its general tonic, immunomodulatory, antiallergic and anti-inflammatory [9]. liver is one of the most body organs systems largely account for lead (Pb) toxic effect which the major site of Pb accumulations rapidly after absorption through the intestine resulting in huge damage in the liver and their functions. Although, the fish oil has myriad of medicinal features, there is a scarcity of studies its regulatory role in the heavy metals' toxicity and effect on hepatic structure [10]. This study aimed to evaluation of the potential antioxidant and protective effects of fish oil against changes in liver of male rats exposed to lead acetate using of some biochemical parameters and histological studies.

Materials and methods

Ethical approval

This practical research has been approved by the Ethical Committee of the Animal Care and King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia.

Experimental animals and housing

The study was conducted using forty male albino rats of the Wistar strain (*Rattus norvegicus*), which their initial body weight ranged from 200gm to 250gm. These experimental animals were obtained from the Animal Unit of King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. The experimental rats were bred in four cages with average ten rats in each cage with a controlled temperature of (20±1 ºC) and 65% humidity percent. Moreover, the Rats were kept under a 12 hours light: 12 hours dark as well as had their access to distilled water and commercial food (ad libitum).

Experimental design and treatment

After the acclimatization period, the experiment was performed for six weeks for all four experimental rats' groups. The first group (n = 10) were used as control and received only normal saline solution (0.9 ml) daily orally during the experiment period. The second group (n = 10) were received lead acetate at concentration of 150 mg/kg body weight of 1% solution using tubular tube, that is three times weekly. The third group (n = 10) were administrated lead acetate at the same concentration which was given of the first group (150 mg/kg) and fish oil (650 mg/kg body weight/day) utilizing sterile tube for six weeks. The last group was administrated only fish oil orally with a concentration of 650 mg/kg body weight/ day.

Organ weight measurement

At the end of the experiment, tested male rats were euthanized and their organs were dissected. Then, liver was removed and weighed for each rat in all treatments and control group.

Histological analysis

The liver tissues were kept in formalin (concentration of 10%) immediately after dissection of rats and removal from their bodies. After that, the liver tissues were dehydrated across ascending degrees of isopropyl alcohol by immersing in concentration of 80% isopropanol overnight and in concentration of 100% isopropyl alcohol, that is for one hour. The dehydrated liver tissues were filtered twice of xylene for one hour to each of tissue. The tissues were impregnated in paraffin block which were cut using rotary microtome at four-micron thickness. The paraffin was removed from treated tissues by immersing in xylene for ten minutes in horizontal staining bottle. After that, the specimens were washed in isopropyl alcohol (concentration of 100%) and stained in Ehrlich's hematoxylin for eight minutes in horizontal staining jar. The next step, the staining samples were washed with tap water and immersed in acid alcohol (8.3% HCl in 70% alcohol). Then the treated tissues were put under running tap water for ten minutes for pick up the color blue (slow alkalization), then the tissues were stained in (1%) aqueous eosin solution for one minute, the extra stain was washed by tap water. After that the samples were incubated for five minutes in incubator at 60ºC. Finally, the tissues samples were cooled and fixed in DPX mount of glass optical index, then wetted in xylene and inverted on the DPX mount and placed on the coverslip [11]. The samples were examined in the microscopic unit at King Fahd Medical Research using light microscope (Olympus BX61- USA) with motorized controller unit (Olympus bx-ucb- USA) and photographed by a digital camera (Olympus DP72- USA).

Statistical analysis

Statistical Package of Social Science (SPP version 21.0) was used for data management and analysis. The obtained data were analyzed by one-way ANOVA to compare the average of the studied parameters in all tested groups. The value of $p<0.05$ was considered as significant.

Results

Body weight gain

After six weeks of treatment, the obtained results of SD male rats from all experimental animal groups revealed a significant increasing pattern in average daily of body weight (Figure 1). Where they were their weighing ranged from 200gm to 250 gm, but after treatments, it was found slight enhancements in the weight measures of body at the end of the experiment. In addition, the highest increasing was observed in rats' weights of control group which was 103.8 gm. While, the lowest body weight was exhibited by fish oil group with increasing of only 64.6 gm. On the other side, the greatest increment in liver weight of the experimental animals was spotted by lead acetate group which was 10.49 gm. Whilst, the least liver weight in rats' weights was observed in lead acetate & fish oil group with 9.76 gm (Figure 2).

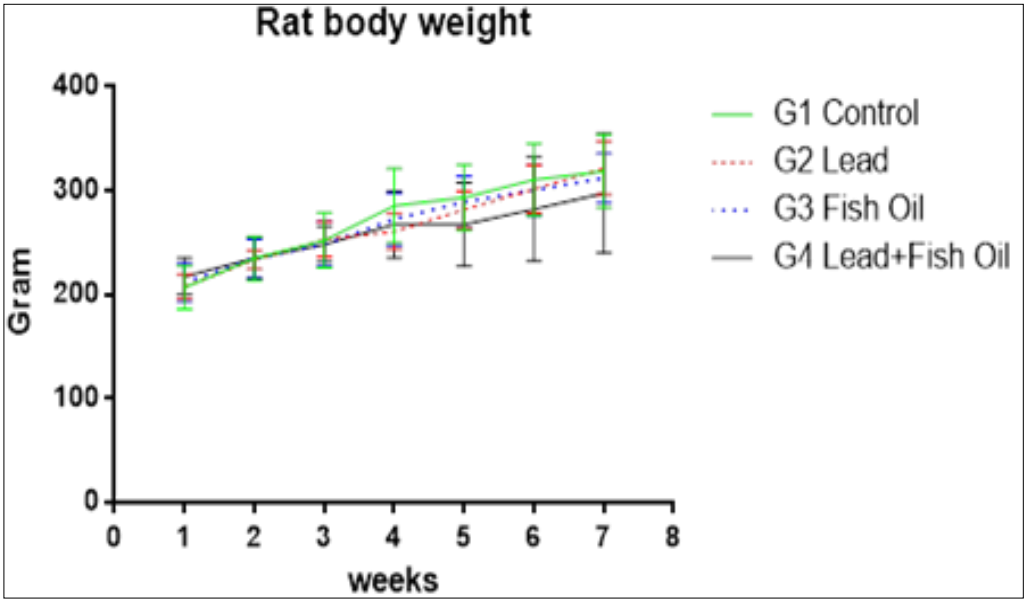

Fig 1: Trend of increase in body weights (gm) of the tested animals' groups. Net body weight increment in each experimental group is as follows; control group (G:1) (103.8±13.88 gm), lead acetate rats' group (G:2) (94.1±11.57 gm), lead acetate & fish oil rats' group (G:3) (88.7±6.03 gm), fish oil rats' group (G:4) (64.06±32.41 gm).

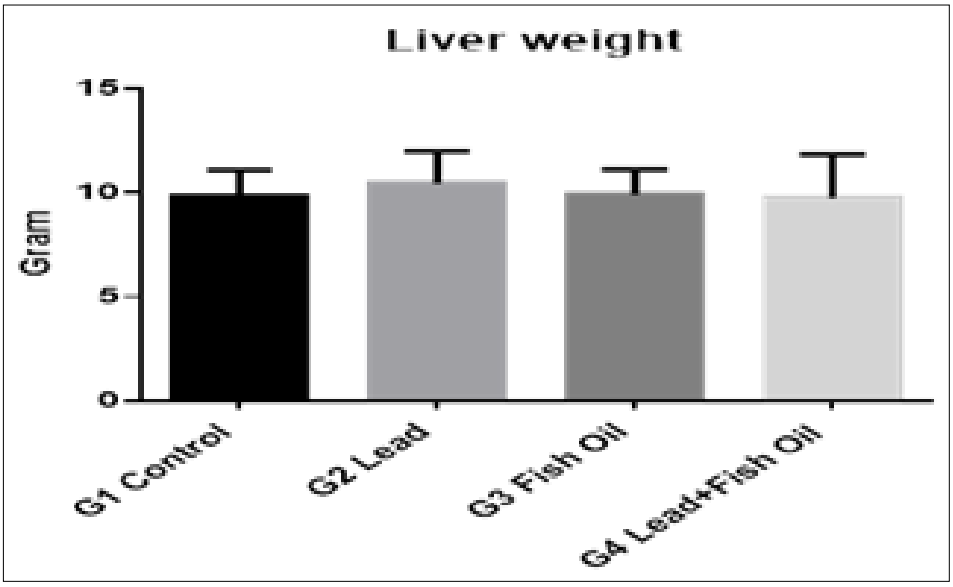

Fig 2: Trend of liver weight (gm) of the animals' experimental groups. The net liver weight increment in all experimental groups were as follows; control group (G:1) (9.84±1.28 gm), lead acetate group (G:2) (10.49±1.51gm), fish oil group (G:3) (9.92±1.22 gm) a lead acetate & fish oil group (G:4) (9.76±2.09 gm).

Histopathological examination of liver

The liver tissues of control rats' group (G1) showed normal structure which composed of hepatocyte cell cords that extended from the central vein to the portal areas. Hepatocyte cells were separated cords by sinusoids lined with endothelial cells. Additionally, Kupffer cells were also present along the sinusoidal lumen. Each hepatocyte is a polygonal cell with a large centrally located spheroid nucleus having dispersed chromatin distribution and a distinct nucleolus. Moreover, the cytoplasm of hepatocytes is faintly basophilic and granular (Figure 3.G1).

In lead treated rats' group (G2), liver histopathological examination revealed degenerated changes in numerous hepatocytes and necrosis, with increased cytoplasmic eosinophilic granularity with dilated and congested veins. Beside that the enlarged cytoplasmic vacuoles, peri-portal necrosis of hepatocytes that surround the portal area and infiltrating leukocytes. In addition, the degenerative changes such as diffuse disorganization of hepatic cords, vascular congestion and dilatation of sinusoids. Numerous Kupffer cells was were shown around sinusoids. Mild lymphoid and mononuclear infiltration was observed within the portal areas and central vein. Nuclei chromatin was fragmented and Pyknotic nuclei formations (Fig. 3.G2 and Fig. 4.G2).

For the fish oil group (G3) and lead acetate & fish oil group (G4), it has been noticed marked preservation of normal liver structure with some little changes such as: congestion around central vein. While, the hepatocytes and blood sinusoids were similar to their counterpart in control groups (Fig.3/G3&G4 and Fig.4/G3&G4).

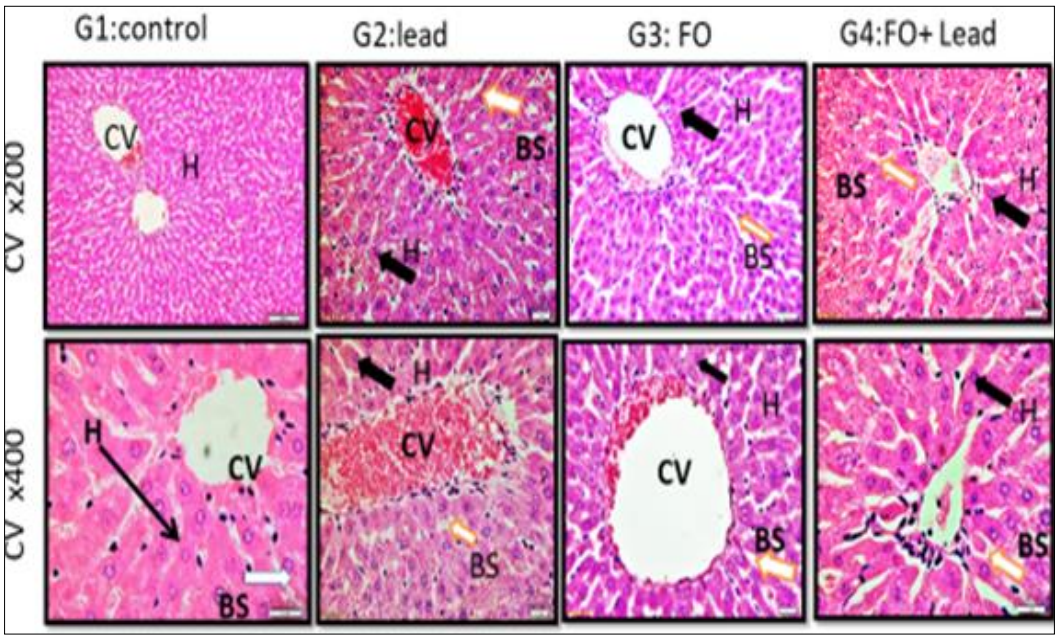

Fig 3: Sections in rat liver Central vein region (CV) stained by H&E to show: G1: Normal hepatocytes (H) & blood sinusoids extended from the central vein (CV). G2: Marked loss of normal structure with features of: cell necrosis and inflammatory cells. Notice the central vein congestion (CV), necrosis of hepatocytes (H. black arrows), prominent Kupffer cells (white arrows). G3+G4: Marked preservation of normal structure with some changes such: congestion around central vein. Hepatocytes (H) and blood sinusoids (arrows) are similar to control.

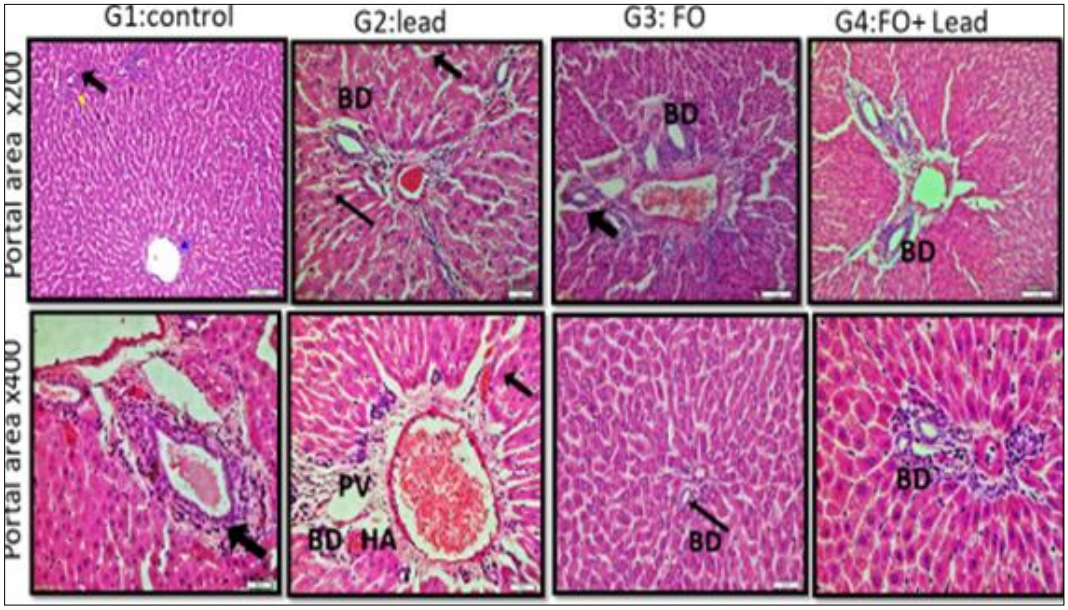

Fig 4: sections in rat liver Portal area region stained by H&E to show: G1, G3and G4: No changes in histological structure. G2: Marked loss of normal structure with features of: cell necrosis, hemorrhage and inflammatory cells, binucleation & bile duct proliferation (BD).

Discussion

Liver tissue is the largest repository of lead-exposed humans among the soft tissues. For this reason, it has been decided to investigate the impact of lead toxicity on the liver and to reflect possible lead effects on histopathological changes during six weeks and evaluate the possible protective effects fish oil against toxicity of liver induced by lead acetate in albino rats. The findings of study revealed liver damage was rats exposed to lead which exhibited by the third group (G3). These histological changes rats of liver include structural damage, hepatocellular necrosis, leukocyte infiltration, and massive hemorrhage where the male rats consumed a solution of lead acetate (100mg/kg) three times weekly markedly hepatic damage which attributed to one of the important mechanisms that caused the mediate death of hepatocytes which is the generation of free radicals induced by heavy metals that caused oxidative stress of liver [12].

In addition, lead is known to cause histological liver damage and possibly disturb the normal biochemical process, resulting in increased liver enzyme levels. Histological studies on humans exposed to lead revealed that liver tissue is the largest repository (33%) of lead to the others soft tissues followed by kidney cortex and medulla [13]. Moreover, in a previous study reported that liver damages caused by lead led to histological changes: necrosis in hepatocytes, vacuolation, congestion within central veins, infiltration of inflammatory cells and binucleation, hemorrhage between hepatic cords [14]. Moreover, the present outcomes were consistent with other studies which showed that the exposure of male rats to lead acetate may causes a decrease in their body weights and liver weight [15]. These decreases were not only a result of decreased food consumption, but also from direct toxicity of the lead acetate which due to the malabsorption of nutrients from toxic impacts on the gastrointestinal tracts and the inhibition of protein biosynthesis process [16]. Additionally, the slight and no significant increase in the total liver weight in lead acetate rats' group with Pb could be attributed to the toxicity which caused hypertrophy of hepatocytes and aggregation of lipid substances as well as to adaptive mechanism to combat of systemic toxicity [17].

On contrary, the examination using light microscope illustrated that the rats were fed of fish oil has enhanced the histological damages induced by Pb toxicity. Moreover, in case of fish oil and lead acetate rats' group, it has been noticed that the level of the adverse tissue's changes was decrement compared to their level in rats group treated only with lead. This reflected the positive effects of fish oil on improving liver rats' tissues against lead toxicity. therefore, Food supplement of fish oil gives an effective impact to reducing of the contusion injury in the muscles. Furthermore, taking of fish oil as a food supplement acts as an anti-coagulant and pro-oxidant. This action indicated to the capability of fish oil in reducing of the negative effect of Pb toxicity which could attributed to that fish oil prevented the oxidative stress that causing by heavy metals where contains high levels of n-3 fatty acids and can used as a preventive agent of some diseases [8]. There is a need to enrich our diets by adding of antioxidant and vital supplements which inhibit cellular disorders that caused from oxidative stress [18].

Conclusions

The current study demonstrated that fish oil had a protective effect on lead induced hepatic negative alterations and oxidative stress which led to damage of liver tissues. Therefore, this study suggested that the fish oil could be beneficial in the prevention and treatment of reduction and damages in hepatic tissues against lead acetate toxicity.

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