Journal of Medicinal Plants Research Vol. 6(24), pp. 4128-4134, 28 June, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR12.658
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Effect of corn oil, flaxseed oil and black seed oil on lead acetate-induced hepatic tissue damage: A histological study

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Accepted 22 May, 2012

Lead is considered as one of the major environmental pollutants that continued to pose health hazards in animal and man in many parts of the world. The current study aimed to investigate the histological effects of corn oil, flaxseed oil and black seed oil against lead acetate-induced hepatic tissue damage in mice. Animals were divided into five groups. The first group served as a control group. The second group was inoculated with 20 mg/kg lead acetate. The third, fourth and fifth groups were inoculated with lead acetate then, treated with 1000 mg/kg of corn oil, flaxseed oil and black seed oil, respectively. At day 5 post-treatment, the liver sections were prepared for the histological study. Lead acetate induced severe hepatic tissue damage mainly in the form of inflammatory cellular infiltration, hepatocytic vacuolation and sinusoidal dilatation. Mice treated with corn oil for 5 days still have histopathological lesions. Both flaxseed oil and black seed oil could reduce the lead acetate-induced hepatic tissue damage.

Key words: Lead acetate, corn oil, flaxseed oil, black seed oil, liver.

INTRODUCTION

Liver is an important organ and is actively involved in many metabolic functions and is the frequent target for a number of toxicants (Meyer and Kulkarni, 2001). Hepatic damage is associated with distortion of these metabolic functions (Wolf, 1999). Hepatotoxicity is defined as injury to the liver that is associated with impaired liver function caused by exposure to a drug or another noninfectious agent (Navarro and Senior, 2006). Many metals play important roles in the functioning of enzymes, cell-signaling processes, and gene regulation. Increasing concern has been expressed about the rapidly rising level of chemicals in the environment, particularly lead, which has well-known hazardous effects (Courtois et al., 2003). Lead is a toxic metal that induces a broad range of physiological, biochemical and neurological dysfunctions in humans (Skerfving and Bergdahl, 2007). Lead is considered as one of the major environmental pollutants. Although, lead is eliminated from petrol in many countries, it may have other origins such as industrial pollution (Ghorbe et al., 2001; Patel et al., 2001). Lead is a dangerous heavy metal and harmful even in small amounts. Nevertheless, humans get exposed to lead through their environment and diet (Gidlow, 2004). All sources of lead contribute to an increase in permissible exposure limit for metallic lead, lead oxide, and lead salts and soaps that has been set by WHO and other health organizations (Harbison,1998; Patterson,1965). The manifestations of lead poisoning in humans are non-specific. They may include weight loss, anemia (Khalil-Manesh et al., 1994; Waldron, 1966), memory loss (Hopkins, 1970), nephropathy, infertility, liver, testis and heart damages' (Patocka and Cerný, 2003; Gurer-Orhan et al., 2004).

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Corn oil is composed of 99% triacylglycerols with polyunsaturated fatty acid (PUFA) 59%, mono-unsaturated fatty acid 24%, and saturated fatty acid (SFA) 13%. The PUFA is linoleic acid (C18:2n-6) primarily, with a small amount of linolenic acid (C18:3n-3) giving an n-6/n-3 ratio of 83. Corn oil contains a significant amount of ubiquinone and high amounts of alpha- and gamma-tocopherols (vitamin E) that protect it from oxidative rancidity. It has good sensory qualities for use as a salad and cooking oil. Corn oil is highly digestible and provides energy and essential fatty acids. Linoleic acid is a dietary essential that is necessary for integrity of the skin, cell membranes, the immune system, and for synthesis of icosanoids. Icosanoids are necessary for reproductive, cardiovascular, renal, and gastrointestinal functions and resistance to disease (Dupont et al., 1990).

Flaxseed, *Linum usitatissimum*, has been the focus of increased interest in the field of diet and disease research due to the potential health benefits (Abdel-Moneim et al., 2010) associated with some of its biologically active components. It contains 32 to 45% of its mass as oil of which 51 to 55% is alpha-linolenic acid (18:3 n-3 omega-3 fatty acid), a precursor to eicosapentanoic and docosahexanoic acid, and it may have beneficial effects on health and in control of chronic diseases (Mantzioris et al.,1994), as well as being a good source of dietary fiber and lignan.

The last two decades witnessed an enormous research rush to reveal the pharmacological actions of an annual spicy delicate and beautiful herb known by the Latin name *Nigella sativa* Linnaeus variety hispidula (brachyloba) that belongs to the botanical family Ranunculaceae. It was first identified and described by Linnaeus in 1753 (Jansen, 1981). The plant is known to all Arabian and Islamic countries and carries various colloquial names. It is known generally by the names Habbat Albarakah, Alhabahat Alsawda and Alkamoun Alaswad. In some countries, it is known by the names Shuniz and Khodhira. *N. sativa* seeds contain 36 to 38% fixed oils, proteins, alkaloids, saponin and 0.4 to 2.5% essential oil (Ali and Blunden, 2003). Black seed components display a remarkable array of biochemical, immunological and pharmacological actions (Agarwal et al., 1979; Boulos 1983; Al-Hader et al., 1993; Houghton et al., 1995; Haq et al., 1999). The current study aimed to investigate the histological effects of corn oil, flaxseed oil and black seed oil against lead acetate-induced hepatic tissue damage in mice.

MATERIALS AND METHODS

Animals

Thirty adult male albino mice weighing 20 to 25 g were used for this study. They were obtained from the animal facility of King Saud University. The animals were kept in wire bottomed cages under standard condition of illumination with a 12 h light-dark cycle at 25 ± 1°C. They were provided with tap water and balanced diet *ad libitum*. The experiments were approved by the state authorities and followed Saudi Arabian rules of animal protection.

Experimental design

Animals were randomly allocated to five groups of six mice each. Group 1 served as a control group. Each mouse orally received 0.1 ml saline and after 1 h, 100 µl of saline was injected intraperitoneally (i.p.). Group 2 daily received i.p. injection of lead acetate (20 mg/kg body wt) (Ito et al., 1985; Abdelmoneim et al., 2010), for 5 days. Every day and for 5 days, the animals of groups 3, 4 and 5 were orally inoculated with 1000 mg/kg corn oil (Wadi Al-Nahil, Riyadh , Saudi Arabia), 1000 mg/kg flaxseed oil (Wadi Al-Nahil, Saudi Arabia) and 1000 mg/kg black seed oil (Wadi Al-Nahil, Saudi Arabia), respectively. An-hour after the treatment, the groups III, IV and V were injected intrapritoneally with 100 µl of 20 mg/kg lead acetate for 5 days. After 24 h of the last i.p. injection of lead acetate, the animals of all groups were cervically dislocated and liver samples were collected.

Histology of the liver

Livers were collected and cut into small pieces, fixed in 10% neutral buffered formalin. Following fixation, specimens were dehydrated, embedded in wax, and then sectioned to 5 µm thickness. Sections were stained with hematoxylin and eosin. Also, other sections were stained with Masson trichrome stain according to Drury and Wallington (1980).

RESULTS

Hepatic tissues of the non-treated control mice were investigated for the purpose of comparison. Liver were seen to be composed of parenchymal cells arranged in lobules. Each lobule appeared with a central vein at its center and portal canals at the periphery. The portal canal comprises branches of the portal vein, hepatic artery and bile duct, often also with a lymphatic vessel lying in a small amount of connective tissue. The hepatic cells are arranged in series of branching plates between which are the sinusoidal plates (Figure 1a and b).

Livers of mice received 20 mg/kg/b.w. of i.p. injection of lead acetate for 5 days showed severe histopathological changes remarked by distorted liver sections in which necrotic foci were abundant in the liver tissue; degeneration in the cells with complete digestion of the nuclei, cytoplasmic vacuolization appeared besides the appearance of kupfer cells (Figure 2a). Binucleated cells with necrotic foci in between appeared in the tissue and aggregations of lymphocytic infiltration (Figure 2b). Large necrotic areas appeared in Figure 3b with great dilatation in sinusoids. Dilatation in blood vessels with congestion was present in addition to bundles of collagenous fibers (Figure 2d).

Livers of mice received 1000 mg/kg/b.w. of corn oil orally for 5 days after i.p. injection of 20 mg/kg/b.w. of lead acetate appeared with no marked improvement that vacuolar degeneration appeared in the cytoplasm of hepatocytes, in addition to lymphocytic infiltration area

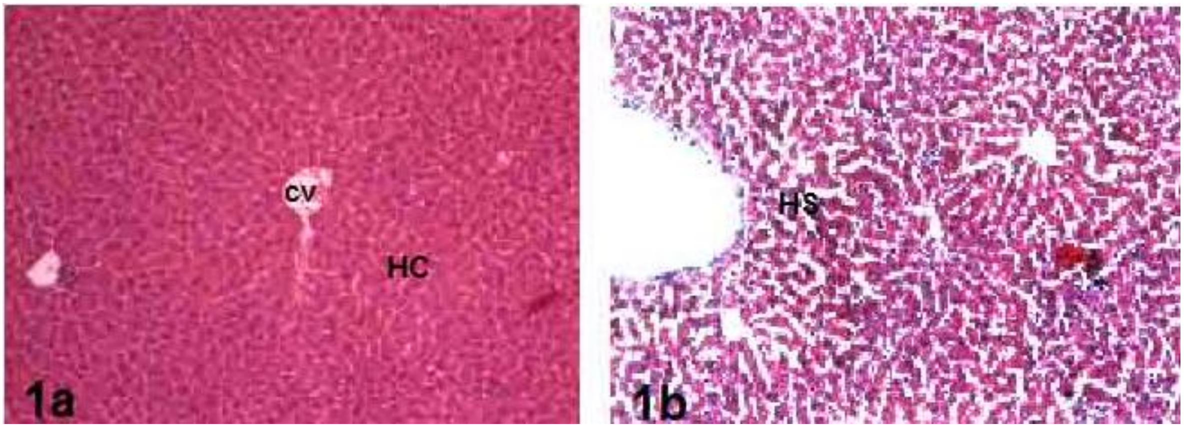

Figure 1. Non-treated control liver sections with normal hepatic architectures. (a, b): Control liver with central vein (CV) and surrounding hepatocytes, sinusoids (HS) lined with Kupffer cells. Section (a) was stained with hematoxylin-eosin, Magnification, X100. Section (b) was stained with Masson trichome stain, Magnification, X100.

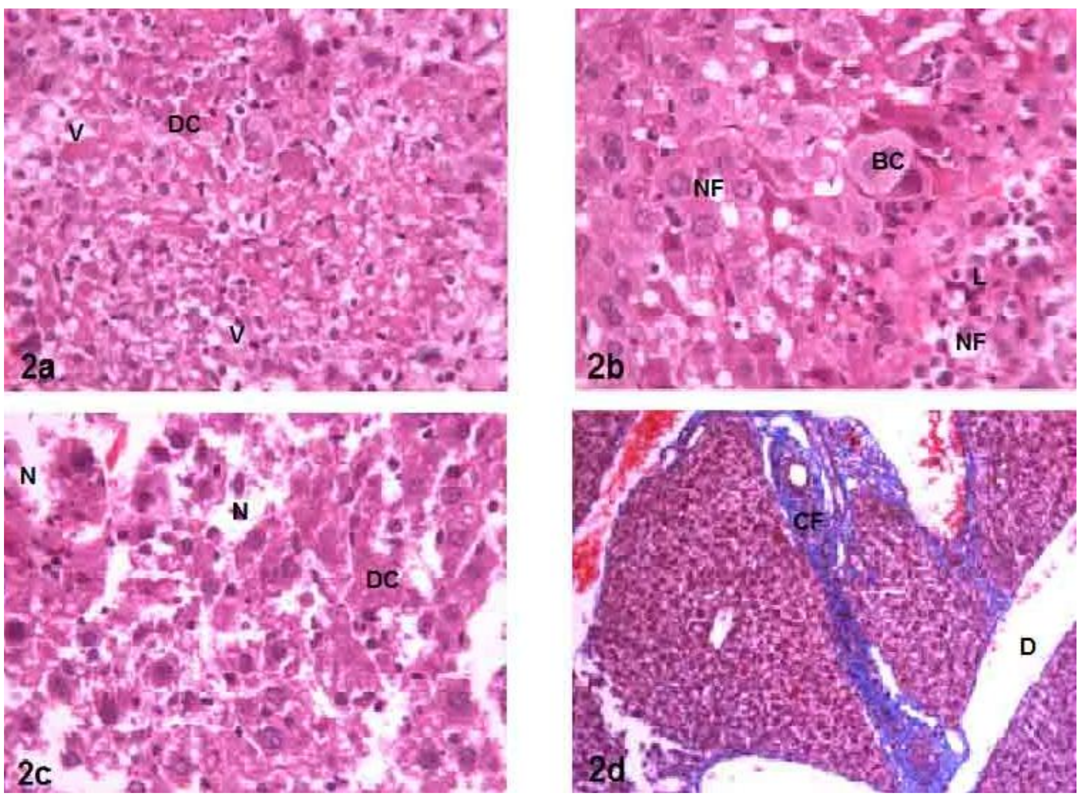

Figure 2. Lead acetate induced hepatic tissue damage. (a) Damaged liver with hepatocytic vacuolation (v) and degenerated cells (DC). (b) Liver sections appeared with prominent lymphocytic infiltration (L), hepatocytes containing binucleated cells (BC) and necrotic foci (NF). (c) Liver section with large necrotic areas (N) and degenerated cells (DC). (d) Liver sections containing dilatation in portal vein (D) and accumulation of collagenous fibers (CF). Sections a, b and c were stained with hematoxylin-eosin, (Magnification, X400). Section d was stained with Masson trichome stain, (Magnification, X100).

(Figure 3a), the liver sections displayed necrotic foci. Moreover, wide and branched portal vein dilatation with blood congestion was abundant (Figure 3b). Destruction in the wall of portal vein appeared, and complete degeneration of hepatocytes nuclei (Figure 3c), wide dilatation in blood sinusoids and destruction in portal vein

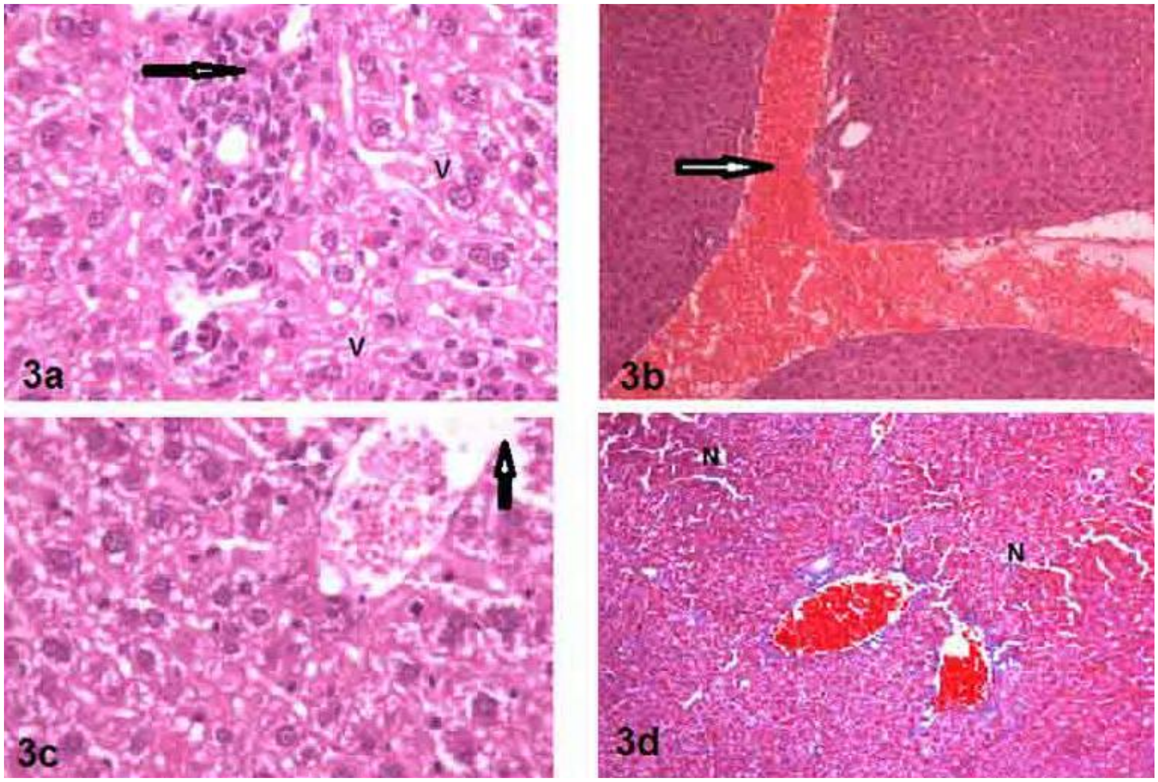

Figure 3. Corn oil induced changes in hepatic tissue of mice inoculated with lead acetate. (a) Hepatic tissue with aggregations of lymphocytes (arrow), cytoplasmic vacuoles in the hepatocytes (V) and dilatation in hepatic sinusoids. (b) Liver appeared with branched and dilated portal vein with blood congestion (arrow). (c) Liver contained dilated central vein (arrow) with destructed wall, and cells with degenerated nuclei. (d) Abnormal liver structure with necrotic foci (N), destructed portal veins with blood congestion surrounded by collagenous fibers. Sections a, b and c were stained with hematoxylin-eosin. Section d was stained with Masson trichome stain (a and c: Magnification, X400; b and d: Magnification, X100).

wall surrounded by some collagenous fibers, were present (Figure 3d).

Liver sections of mice received 1000 mg/kg/b.w. of flaxseed oil orally for 5 days after i.p. injection of 20 mg/kg/b.w. of lead acetate appeared with a marked improvement represented by some healthy hepatocytes, showing regeneration that double nucleated cells were present (Figure 4a). Moreover, aggregations of lymphocytes were still abundant in addition to dilatation in blood sinusoids (Figure 4b), small amounts of collagenous fibers accumulated around dilated portal veins (Figure 4c and d).

Black seed oil was able to reduce the hepatic tissue induced damage due to lead acetate administration. The portal vein appeared surrounded by some extant of healthy hepatocytes with deeply stained nuclei, while others showed faintly stained nuclei (Figure 5a). Figure 5b showed small aggregations of lymphocytes in addition to the appearance of Kupfer cells. Dilatation in portal vein with congestion appeared where the wall of the portal vein was thickened by accumulated collagenous fibers (Figure 5c and d).

From the overall results, it was obvious that corn oil had no protective effect against induced hepatotoxicity lead acetate; whereas, flaxseed oil and black seed oil had protective effect against chemicals that caused hepatotoxicity such as lead acetate.

DISCUSSION

The liver is a very vital organ, and hepatotoxicity ranks one the most frequent causes of acute liver failure. The diagnosis of hepatotoxicity remains a difficult task because of the lack of reliable markers for use in general clinical practice (Andrade et al., 2007). Pb is an immunotoxicant which through human exposure results in immune function changes and has the potential to adversely affect human health. It has many uses in industry including pipes, paints, enamels, glazes, motor industry and others. The major hazard in industry arises from the inhalation of dust and fume but the organic compounds may also be absorbed through the skin. It induces a broad range of physiological, biochemical and neurological dysfunctions in humans (Nordberg et al., 2007). The liver plays a major role in lead metabolism

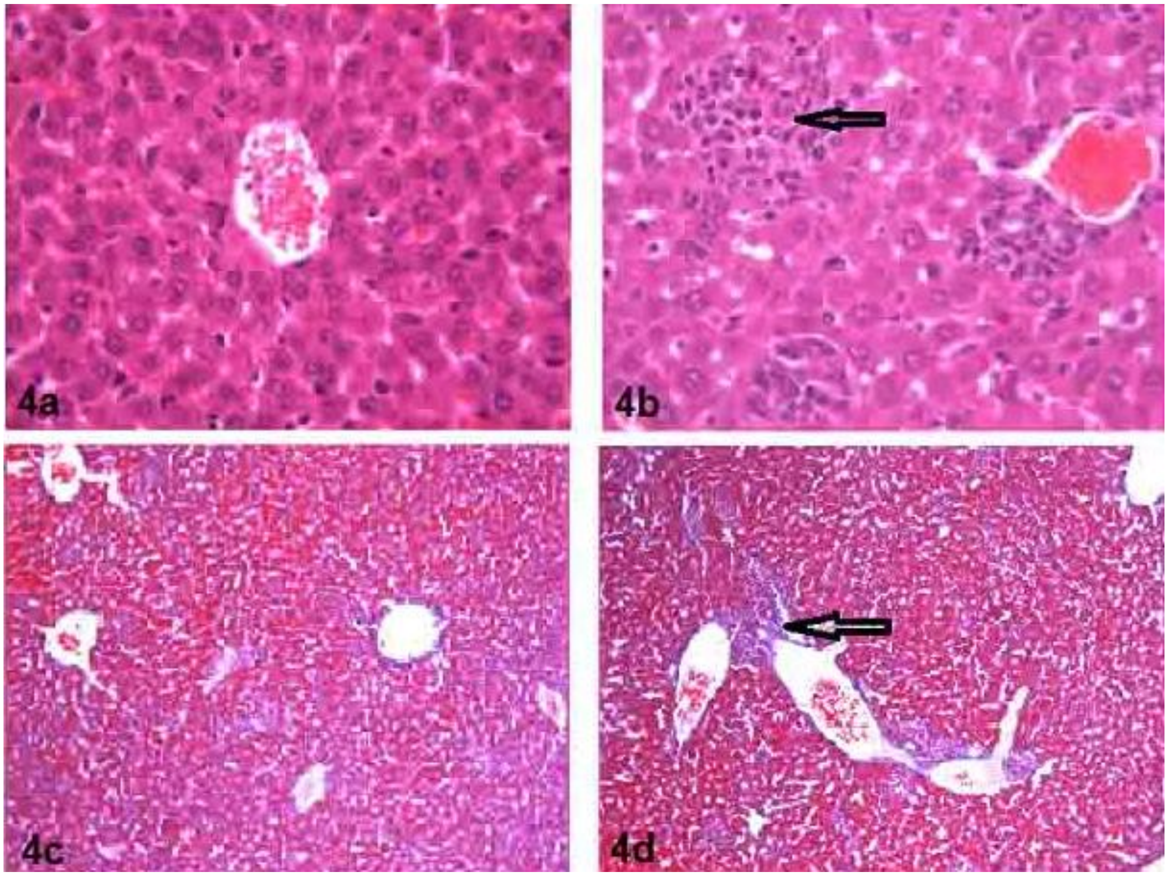

Figure 4. Flaxseed oil reduced the hepatic tissue damage induced by lead acetate. Section (a): liver appeared with regular structure. The central vein is surrounded by healthy hepatocytes. (b): liver contains aggregations of lymphocytes (arrow). (c): Normal hepatic architectures. (c): Liver with small dilatation in portal vein surrounded by an amount of collagenous fibers (arrow). Sections a and b were stained with hematoxylin-eosin, (Magnification, X400). Sections c and d were stained with Masson trichome stain, (Magnification, X100).

(lead poisoning cause adverse effects to hepatic cells) because after lead exposure, liver is one of the major organs involved in the storage, biotransformation and detoxification (Sivaprasad et al., 2004). The results of the present study run in full agreement of previous studies that lead is a very toxic agent which affects many vital organs, such as the liver. Abdou and Newairy (2006) showed that histological analysis of livers of rats treated with lead acetate showed sever hepatocytes damage, which was manifested by marked fat vacuolation or empty space, where the hepatic veins were markedly dilated and congested with blood. Furthermore, the hepatocytes cells were disintegrated and necrosed. Also, abnormal localization and infiltration of hepatocytic nuclei were appreciated. Histological examination of livers of Swiss albino mice received oral administration of lead revealed disruption of the normal structural organization of the hepatic lobules and loss of the characteristic cord-like arrangement of the normal liver cells. The central and portal veins were congested. Many hepatic cells were damaged and lost their characteristic appearance while others showed marked cytoplasmic vacuolization. The nuclei of these cells were pyknotic. The central vein and sinusoids between hepatocytes were dilated. Some leukocyte infiltration and fatty deposition were also evident. The present study results agreed with the previous results that dramatically alterations appeared in liver sections of mice that received i.p. injection of 20 mg/kg/b.w.; and that necrotic foci were abundant in the liver tissue, degeneration in the cells with complete digestion of the nuclei, cytoplasmic vacuolization appeared besides the appearance of Kupfer cells, large necrotic areas appeared and great dilatation in sinusoids, dilatation in blood vessels with congestion was present in addition to bundles of collagenous fibers.

Tan et al. (2011) suggested that a combination of poly- and mono-unsaturated fatty acids in corn oil is protective against alcohol and iron induced liver injury. In contrast to the previous results, corn oil had no effect on the hepatotoxicity lead acetate which induced that vacuolar degeneration appeared in the cytoplasm of hepatocytes; in addition to lymphocytic infiltration area, wide dilatation

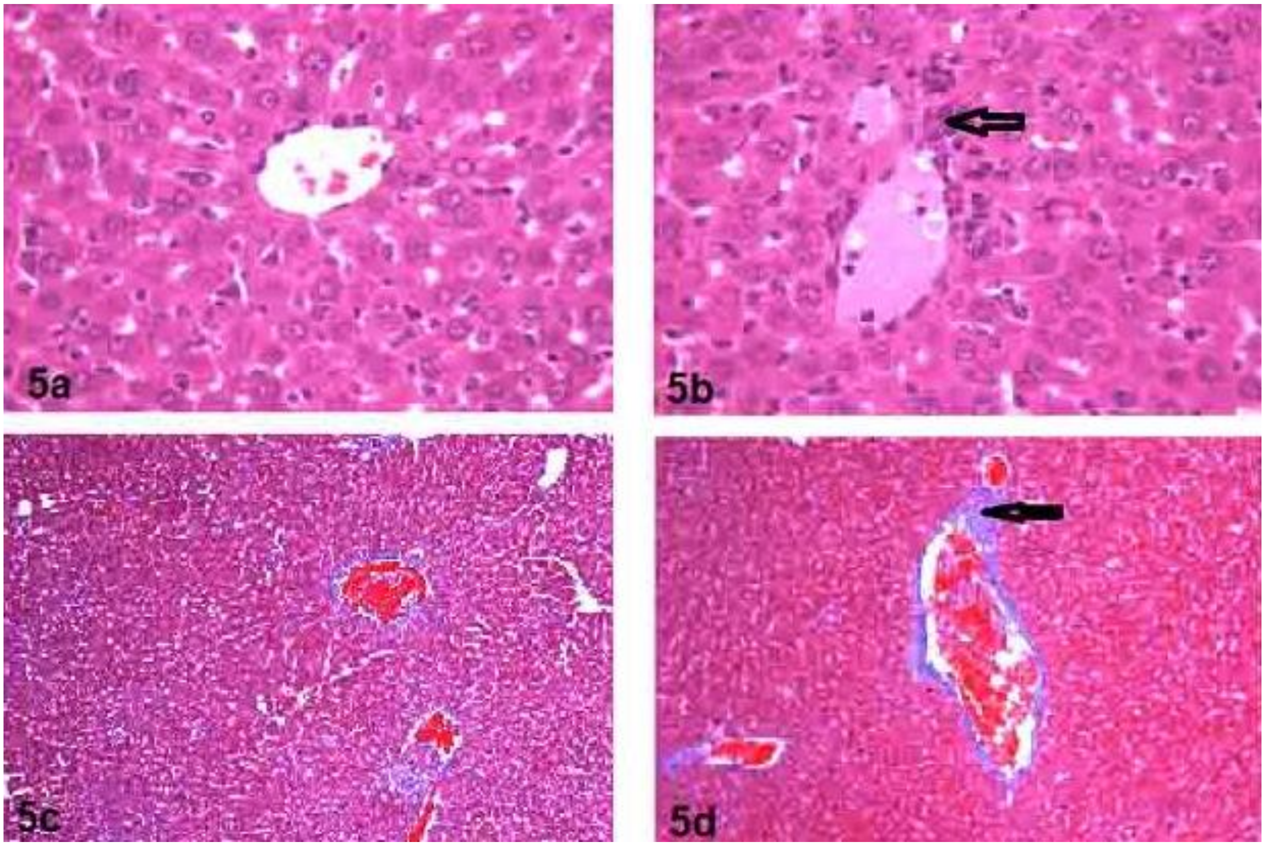

Figure 5. Black seed oil reduced the lead acetate induced hepatic tissue damage. (a) Normal hepatic tissue architectures appeared with central vein surrounded by faintly stained hepatocytic nuclei. (b) Liver appeared with small lymphocytic infiltration (arrow). (c and d) Livers with dilated congested central vein surrounded by layer of collagenous fibers (arrow). Sections a and b were stained with hematoxylin-eosin, (Magnification, X400). Sections c and d were stained with Masson trichome stain, (Magnification, X100).

in blood sinusoids and destruction in portal vein wall surrounded by some collagenous fibers were present.

Histological examination of liver tissue in rats treated with lead acetate plus flaxseed lignans showed minimal alterations with reduced fat deposition as compared to the lead acetate treated rats. These findings confirmed the protective effect of flaxseed lignans against the histological changes in lead acetate hepatotoxicity (Abdou and Newairy, 2006). Moreover, Abdel-Moneim et al. (2010) mentioned that treatment of rats with flaxseed oil before i.p. injection of lead acetate resulted in marked improvement in histopathological feature and reported that flaxseed oil is a nature product that can be protect against lead acetate-mediated hepatotoxicity. In agreement with the previous studies, in the present study, histopathological investigation of mice liver received flaxseed oil at dose of 1000 mg/kg/b.w. orally after i.p. injection of 20 mg/kg/b.w. of lead acetate, resulted in marked improvement in liver structure characterized by normal central vein surrounded by some extent healthy hepatocytes with central nuclei, whereas some cells still showed digestion of the nuclei, aggregations of lymphocytes were abundant in liver tissue.

Farrag et al. (2007) suggested that combined treatment of lead-exposed animals with black seed showed marked improvement in both biochemical and histopathological findings as well as reduction in the damaged areas, and that a normal structure of liver was observed when rats were fed LD_{50} of lead acetate plus *N. sativa* in the diet. El-Dakhakhny et al. (2000) showed that black seed oil was able to give complete protection against d-galactosamine and partial protection against carbon tetrachloride hepatotoxicity. In agreement with the previous studies, black seed oil displayed protective role against hepatotoxicity induced by lead acetate, that marked improvement in liver represented by normal portal vein surrounded by some extant healthy hepatocytes, whereas, small aggregations of lymphocytes were present in addition to the appearance of Kupffer cells.

Collectively, it is clear that both flaxseed oil and black seed oil are natural products of nutritive values that can protect against lead-induced hepatotoxicity.

ACKNOWLEDGEMENT

This project was supported by Deanship of Scientific research, College of Science, Research Center, King Saud University.

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