Effect of cadmium chloride on the liver of albino rat and the possible protective role of selenium combined with other antioxidants

Thesis
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Abstract

Cadmium is a toxic trace metal which arises from batteries, electroplating, plastic and fertilizer industries. The aim of the present work was to study the effect of cadmium on the liver of adult albino rats and to demonstrate the protective role of combined antioxidants (selenium, vitamins A, C and E). For this study, thirty adult male albino Wistar rats were divided into three groups: control group, cadmium-received group and cadmium supplemented by combined antioxidants (selenium, vitamins A, C and E) received group. Structurally, cadmium-received rats demonstrated markedly disturbed hepatic architecture, widespread dilatation of the central veins as well as hepatic sinusoids, mononuclear cellular infiltration and significant increase in both the kupffer cells as well as the amount of collagen fibers (using Masson's trichrome stain). The hepatocytes displayed hydropic cytoplasmic degeneration and variable nuclear degenerative changes. Concomitantly, there was a decrease in the carbohydrate content of these hepatocytes (using PAS stain) and significant elevation in serum liver enzymes. Electron microscopic study of the hepatocytes of cadmium-received group revealed marked rarefaction and vacuolation of the cytoplasm with loss of most cell organelles. In liver specimens of animals received cadmium supplemented by antioxidants, there was a rectifying effect on the studied parameters to variable degrees. **Conclusion,** cadmium had produced injurious effects to the liver and the use of a combination of antioxidants (selenium, vitamins A, C and E) has a protective effect against these injurious effects.

Key words: cadmium-liver-selenium-antioxidants

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Introduction and aim of the work

Introduction

Cadmium is one of the most toxic heavy metals. It has many sources in our environment. It is produced as a discharge from the industries of electroplating, plastic production, pigment and battery manufacturing, sewage treatment plants, gas from the municipal incinerators and pesticides **(Friberg, 1984).**

Cadmium is more widespread, dangerous than lead and has many potential hazards (**Clarkson et al., 1988; Nordberg and Nordberg, 1988; Skerfving, 1988; Pleasants et al., 1993**). It has an extremely long biological half-life (10–30 years) that essentially makes it a cumulative toxin **(Stohs et al., 2001).** Also, it is proved to be multitarget toxin which causes damage to many organs, such as liver, kidney, lung, brain, testis, bone and placenta **(Patrick, 2003).**

After exposure to cadmium, the metal rapidly accumulates in the liver and produces vaculation, degeneration, increased density of nuclear chromatin with blurred trabecular structure, mono-nuclear cell infiltration and necrosis of the hepatocytes **(Klaassen et al., 1998; Brzóska et al., 2003)**.

A number of studies had demonstrated the protective role of antioxidants against cadmium-induced toxicity. **Beytut et al. (2003)** investigated the role of dietary vitamin (E) in cadmium-induced oxidative damage in rabbit's blood, liver and kidneys. They concluded that vitamin (E) administration is effective in reducing the oxidative stress in cadmium-treated rabbits. In addition, it has been reported that selenium may minimize cadmium-induced oxidative stress by decreasing lipid peroxidation and altering the antioxidant

defense system in rat liver and kidneys **(Ognjanović et al., 2008).** Furthermore, the combined antioxidants (vit C, vit E and selenium) had been shown to protect against cadmium-induced renal damage **(Karabulut-Bulan et al., 2008).** However, the protective role of combined antioxidants (selenium, vitamins; A, C and E**)** on cadmium-induced hepatic damage was not fully elucidated by the previous workers.

AIM OF THE WORK

The aim of the present work was to study the effect of oral cadmium administration on the liver of adult albino rats and to demonstrate the possible protective role of combined antioxidants (selenium, vitamins; A, C and E) in inhibiting cadmium induced liver changes.

Review of literature

Normal structure of the liver

The liver is an essential organ for maintenance of life. It is formed of complex network of hepatocytes intervened by supportive connective tissue and permeated by great number of blood vessels that perfuse the liver with a rich flow of blood. The liver has two sources of blood supply; the hepatic artery which provides nutrition and the portal vein which delivers substances absorbed by the gastrointestinal tract **(Popp and Cattley, 1991).** Hepatocytes, which carry out the major metabolic activities of this organ, are assisted by additional classes of cells which have storage, phagocytic and mechanically supportive functions **(Sherlock and Doodley, 1989)**.

The organization of liver parenchyma is traditionally described in three different ways: *the classic hepatic lobule, the portal lobule, and the liver acinus*. **Cormack (1987)** reported that *the classic hepatic lobule* description is based on the distribution of the branches of the portal vein and hepatic artery within the organ and the pathway of blood in them. He also added that the classic hepatic lobule is a roughly hexagonal mass of tissue consists of stacks of anastomosing plates of hepatocytes, one cell thick, separated by the anastomosing system of sinusoids that perfuse the cells with the mixed portal and arterial blood. At the center of the lobule, there is a relatively large venule termed the terminal hepatic venule (central vein), from which the plates of cells and the sinusoids radiate to the periphery of the lobule. At the angles of the hexagon, there are the portal areas (portal canals) which are loose stromal connective tissue characterized by the presence of the portal triad (portal vein, hepatic artery and bile duct). This connective tissue is ultimately continuous with the fibrous capsule of the liver. The portal canal is bordered by the outermost hepatocytes of the lobule **(Cormack, 1987).**

The portal lobule is described as a triangular block of tissue bordered by three imaginary lines drawn between the central veins of adjacent classic lobules that are closest to that portal triad **(Fawcet and Jensih, 1997)**.

The liver acinus is the structural unit that provides the best correlation between blood perfusion, metabolic activity, and liver pathology. It is lozenge-shaped and represents the smallest functional unit of the hepatic parenchyma. The short axis of the acinus is defined by the terminal branches of the portal triad that lie along the border between two classic lobules. The long axis is a line drawn between the two central veins closest to the short axis. Therefore, in a two-dimensional view the liver acinus occupies parts of adjacent classic lobules **(Janquera et al., 1998).**

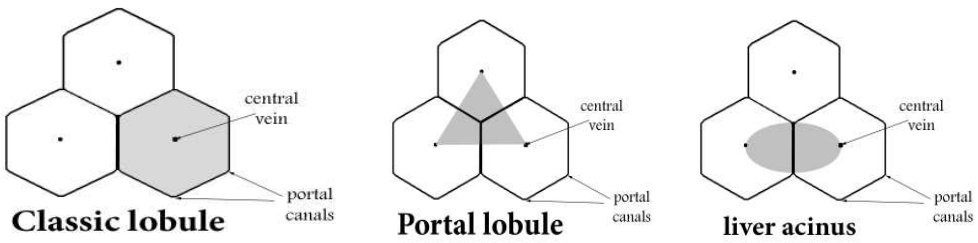

Diagram (A): Comparison between the classic lobule, portal lobule and liver acinus. The area indicated in grey shows the territory of each of the three units relating to liver organization and function. The classic lobule has the central vein at the center of the lobule and the portal canals containing portal triads at the peripheral angles of the lobule. The portal lobule has a portal canal at the center of the lobule and the central veins at the peripheral angles of the lobule. The liver acinus has distributing vessels at the equator and the central veins at each pole (Schematic representation after **Ross and Pawlina, 2006**).

Jungermann (1986) reported that the hepatocytes in each *liver acinus* are described as being arranged in three concentric elliptical zones surrounding the short axis of the liver acinus:

- *Zone one is* closest to the short axis and the blood supply from penetrating branches of the portal vein and hepatic artery. This zone corresponds to the periphery of the classic lobules and is also called periportal zone.
- *Zone two* lies between zone 1 and zone 3 with no sharp boundaries in-between.
- *Zone three* is the farthest from the short axis and closest to the central vein. This zone corresponds to the most central part of the classic lobule that surrounds the central vein and it is called pericentral zone.

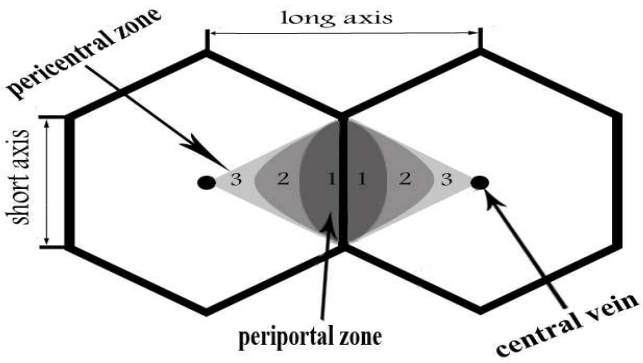

Diagram (B): The zones of liver acinus. It consists of adjacent sectors of neighboring hexagonal fields of classic lobules partially separated by distributing blood vessels. The zones, marked 1, 2 and 3 are supplied with blood that is most oxygenated and richest in nutrients in zone 1 and least so in zone 3. The central veins are at the edges of the acinus instead of being at the center, as in classical lobule (Schematic representation after **Ross and Pawlina, 2006**).

Ross and Pawlina (2006) reported that the zonation is important in the description and interpretation of patterns of degeneration, regeneration, and specific toxic effects in the liver parenchyma. They added that the distribution of liver damage as a result of ischemia and exposure to toxic substances can be explained using this zonal interpretation. Cells in zone one are the first to receive oxygen, nutrients and toxins from the sinusoidal blood and are the first to show morphologic changes after bile duct occlusion (bile stasis). These cells

are also the last to die if circulation is impaired and the first to regenerate. On the other hand, cells in zone three are the first to show ischemic necrosis (centrilobular necrosis) in situations of reduced perfusion and the first to show fat accumulation. They are the last to respond to toxic substances and bile stasis. Normal gradients in enzyme activity, number of cytoplasmic organelles and size of cytoplasmic glycogen deposits are also considered among the three zones. Cells in zone two have intermediate functional and morphologic characteristics between those in zones one and three.

The hepatocytes constitute about 80% of the hepatic cell population. They are irregular polyhedral of variable dimensions and geometric shape. They are arranged in plates of a single cell thickness with intervening sinusoids (**Bioulac-Sage et al., 1999**). The variability in shape and size of the hepatocytes depends on the age, location, metabolic status and regenerative activity of the cells **(Motta et al., 1978)** as well as sinusoidal blood flow and osmotic load (**Dunkelberg et al., 2001).**

The nucleus of the hepatocyte forms five to ten percent of the cell volume. It is spherical in shape and contains one or more prominent nucleoli. Twenty five percent of hepatocytes are binucleated to cope with the extra-ordinary metabolic activity in this organ **(Feldmann, 1992).**

Fawcet and Jenish (1997) describing the ultrastructure of the liver cells, reported that all the hepatocytes are loaded with organelles. *Mitochondria* are numerous and their number may reach 800 per cell. They are elongated, with lamellar or trabecular cristae projecting into their interior. They are smaller in zone one of the liver acinus but nearly twice the size of those in zone two. *The Golgi complex* of the liver cell is not a juxtanuclear organelle but located along both sides of the cell near the bile canaliculati. It

consists of multiple stacks of five to nine cisternae slightly expanded at their ends. *The lysosomes* are membrane bounded dense bodies, usually located in the viscinity of the Golgi body. *The rough endoplasmic reticulum* is abundant in hepatocytes and is responsible for the basophilic bodies in the acidophilic cytoplasm. These bodies are traditionally known as ergastoplasm. *The ribosomes* are either attached to the rough endoplasmic reticulum or lying free in the cytoplasm.

Concerning the hepatic sinusoids, there are four types of cells populate them; *Endothelial cells, von Kupffer cells, Stellate cells and Lymphocytes*. Morphometric analysis indicated that endothelial cells, Kupffer cells and stellate cells account for the vast majority of hepatic non-parenchymal cells **(Ramaoril et al., 1993)**. *Sinusoidal endothelial cells* account for two to five percent of the lobular parenchyma. They have numerous fenestrae and lack a basement membrane **(Arias, 1990)**. *Kupffer cells* account for two percent of the lobular parenchyma and belong to the macrophage monocyte system, representing fixed macrophages of the liver and are the largest population of macrophages any where in the body. They are more numerous in periportal sinusoids but can migrate along the sinusoids into areas of liver injury with and against the blood flow **(Rogoff and Lipsky, 1981; Macphce et al., 1992)**. The perisinusoidal space of Disse which lies between the hepatocytes and the interrupted sinusoidal endothelium contains hepatic *stellate cells* which are also known as *Ito cells*; parasinusoidal cells or fat storing cells as they store vitamin A **(Eng and Friedman, 2000**). They are Stellate in shape, account for 1.4% of lobular parenchyma and lie between hepatocytes in the space of Disse **(Burt, 1999 and Greets, 2001)**. *Sinusoidal lymphocytes* together with several types of lymphocytes *populat the hepatic parenchyma and provide* the liver with inherited immunity **(Wisse et al., 1999)**.