Hepatotoxicity and Nephrotoxicity of Lead Nitrate in Toad *Bufo viridis*

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**A R T I C L E  I N F O**

**ABSTRACT**

The present investigation dealt with the study of the effect of two doses of lead nitrate (40mg/kg and 80mg/kg) for three weeks on the liver and kidney of the male toad, *Bufo viridis*. Lead nitrate caused several histological alterations in the studied organs in a dose dependent pattern. The histological alterations included degeneration of hepatocytes, dilation of blood sinusoids, leucocytes infiltration in the liver and kidney, degeneration of kidney tubule epithelial lining cells. The most important finding was the significant dose dependent increase in number of melanomacrophage centers (MMGs) in the liver compared to control. The later results can be used as an important marker for water pollution by this heavy metal.

**Keywords:**
Liver; kidney; lead nitrate; melanomacrophage center

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1. INTRODUCTION

Lead (Pb) is a ubiquitous environmental pollutant, widely distributed, representing a high toxicological and ecotoxicological risk. Lead is in frighteningly large array of consumer products, from art supplies and automobile components to specialty paints, some hair dyes, and even candy especially the local Kirkuk oil refineries (Al-Dabbas et al., 2014, Al-Dabbas et al., 2012). Lead (Pb) exposure is also considered to be a major public health problem; therefore(Chiesa et al., 2006).

Lead has been found to induce a wide range of behavioral, biochemical and physiological effects. The liver, kidneys, and brain are considered to be the target organs for the toxic effects of lead (Jackie et al., 2011). Lead affects the metabolism of other minerals and has affinity for bone, where it acts by replacing calcium. Thus, the highest concentrations of lead are usually found in bone, kidney and liver (Gurer and Ercal, 2000, Al Zadjali et al., 2015).

Oxidative stress with subsequent lipid peroxidation (LP) induced by production of reactive oxygen species (ROS) has been reported to be one of the important
mechanisms involved in toxic effects of lead (Yin et al., 2008).

Amphibians are of interest, because during their development they move from aquatic to terrestrial habitats, which may be polluted by the metal since they are receptors of products generated by anthropogenic activities (Arrieta et al., 2004). The most previous researches concerning the effect of lead ions on the liver or/and kidney included the species Rana (Vogiatzis and Loumbourdis, 2001, Loumbourdis, 2003, Fenoglio et al., 2006, Jayawardena et al., 2017) and few were included Bufo (Enuneku and Ezemonye, 2012).

Melanomacrophage centers (MMGs) of the spleen, liver, and kidney as part of the defense system of fish, amphibians, and reptile (Steinel and Bolnick, 2017, Vaissi et al., 2017) are more and more often used as an object of micropathomorphological and toxicological studies (Pronina et al., 2014). A functional differences among MMGs of liver, kidney and spleen of fishes were determined (Ribeiro et al., 2011). The main functions of MMGs are the iron capture and storage in haemolytic diseases storage, antigen trapping and presentation to lymphocytes, sequestration of products of cellular degradation and potentially toxic tissue materials, such as melanins, free radicals and catabolic breakdown products, destruction and detoxification of endogenous and exogenous materials (Agius and Roberts, 2003). They are thought to be the site of primary melanogenesis rather than mere storage (Agius and Roberts, 2003). There is evidence that liver MMGs represent a metabolically (melanin synthesis/degradation) and cytokinetically (proliferation/death) active cell population during the annual cycle of the frog (Barni et al., 2002).

The aim of the present work was to investigate the hepatotoxicity and nephrotoxicity of lead giving as lead acetate in

*Bufo viridis* (Amphibia: Anura: Bufonidae) with special attention to the effect on the MMGs.

2. MATERIALS AND METHODS

In this study, male toad, *Bufo viridis*, weighing 25-27 g collected from certain pond in Erbil city, were used. The experiments were conducted at College of science, Salahaddin University, Erbil-Iraq. The animals were kept in convenient plastic boxes in an environment mimic the natural pond. The fifteen toads were randomly and equally divided to three groups: control group (G1) given 1mL distilled water by gavage, lead nitrate (40mg/kg) as group 2 and lead nitrate (80mg/kg) as group 3. All the animals were treated for 3 weeks and they have been sacrificed 24 hours after the last oral dose. The liver and kidney of all animals were removed and processed for the histological study.

2.1. Histological studies

For histological study, fresh removed organs pieces were fixed in 10% buffered formalin, dehydrated in ethanol and embedded in paraffin. Serial sections with 5 μm thickness were obtained using miritome (Bright Co.) and stained according to hematoxylin and eosin procedure (Kiernan, 1981). Certain tissue samples (size ≤ 1mm3) were fixed in 3% glutaraldehyde in cacodylate buffer then postfixed in 1% OsO4, dehydration, clear and then embedded in araldite mixture for preparing plastic blocks. Semithin sections were stained by 1% toluidine blue in 1% borax (Burns, 1978). Counting cell number and photography were undertaken by special digital camera microscope (Olympus) per mm2.
3. RESULTS AND DISCUSSION

Amphibians living in ponds and rivers may be exposed daily to environmental pollutants which may accumulate in their tissues and induce various histopathological alterations (Seixas Filho et al., 2017). In the present investigation, lead nitrate was used as pollutant to evaluate its hepatotoxicity and nephrotoxicity in toad, Bufo viridis collected in autumn from local ponds in Erbil city.

3.1. Hepatotoxicity

Lead is a widespread constituent of earth's crust (Needleman, 1999). It can cause hypertension, developmental defects, neurological problems, renal dysfunction, and anemia. The most important feature of lead hepatotoxicity in the toad was the dose dependent significant increase in the number of MMGs (Fig 1&2). As shown in Fig.3, this heavy metal was caused hepatocellular changes in the toad as compared with the normal histological structures in the control group. The low and high doses of the lead were found to induce several histological changes such as degeneration of the hepatocytes, dilation of blood sinusoids, congestion of blood vessels and the appearance of inflammatory infiltrated leukocytes.

Melanomacrophage centers are Melanophores exist mainly in the liver, kidney and spleen of fish (Agius and Roberts, 2003), frog and toad (Steinel and Bolnick, 2017). They are phagocytes that synthesize melanin (Gutierre et al., 2018). These cells respond to catabolism processes (Kalashnikova, 2000, Steinel and Bolnick, 2017), immunological disorders (Pronina et al., 2014, Steinel and Bolnick, 2017), Uv Uv exposure (Franco-Belussi et al., 2016) and hibernation (Barni et al., 2002). Preliminary histological analyses suggested that MMGs are structurally similar to the mammalian germinal center (GC), leading to the hypothesis that the MMGs plays a role in the humoral adaptive immune response (Steinel and Bolnick, 2017). Different sizes of these cells were detected in the liver (Fig 2) and although such size different has been detected in normal environmental condition, such increase in size or frequency was detected in conditions of environmental stress and have been suggested as reliable biomarkers for water quality in terms of both deoxygenation and iatrogenic chemical pollution (Agius and Roberts, 2003). An increase in their number was detected in response of the frog Rana (Pelophylax) ridibunda to insecticide exposure (Paunescu et al., 2010) and water polluted with fluoride (Bo et al., 2018). An increase in the number of these MMGs was also recently detected in the liver of carp fish in response to mercury chloride exposure (Tjahjaningsih et al., 2017) and liver, kidney and spleen of catfish, Clarias gariepinus, exposed to silver nanoparticles (Sayed and Younes, 2017) and this may rise the hypothesis of metal chelation by these cells and this should be confirmed by further investigations.

The degeneration of hepatocytes as a response to lead toxicity as revealed by the present investigation may be due to the oxidative stress which was considered as the main mechanism of lead induced toxicity in biological system (Flora et al., 2012).

3.2 Nephrotoxicity

As with the liver, the lead has been found to cause nephrotoxic effect on toad kidney (Fig 4). The features of the nephrotoxicity were the degeneration of the epithelial cells lining the kidney tubules especially in the cortex region and infiltrated inflammatory leukocytes near
the glomeruli and in the interstitial tissue between the renal tubules.

Similar to lead induced hepatotoxicity, it has been found that lead toxicity leads to kidney damage via two separate pathways: (1) the generation of reactive oxygen species (ROS) including hydroperoxides, singlet oxygen, and hydrogen peroxide and (2) the direct depletion of antioxidant reserves (Ercal et al., 2001).

The increase in MMGs noticed in liver couldn’t be observed in kidney and this indicates that functional differences among MMGs of liver, kidney and spleen of toads are exist (Ribeiro et al., 2011).

Fig (1): Number of MMGs in the liver of Bufo viridis exposed to lead nitrate Note: Columns superscript with different letters are significantly different at ($P \leq 0.05$)
Fig. (2): Sections in liver of toad after exposure to lead nitrate showing MMGs (arrows). a) Control group, b) 40mg/kg lead nitrate treated group shown higher number of MMGs (arrows), c) 80mg/kg lead nitrate treated group showing higher number of MMGs compared to both groups, notice the different sizes of MMGs. H&E.

All scale bars=20µm.
Fig. (3): Sections in the liver of toad after exposure to lead nitrate showing various histopathological alterations. a) a lot of MMGs (white arrows), dilated blood sinusoids(S), inverted section stained by toluidine blue, b) Some MMGs (arrows), portal vein (V) congested with blood cells and dilated blood sinusoids (S), paraffin section stained by H&E.

All scale bars=20µm.
Fig. (4): Sections in the kidney of lead nitrate treated toads. a) Control group showing normal histological structure in the cortex region with glomerulus (G) and renal tubules (T). b) 40mg/kg lead nitrate treated group showing renal tubules (T) lined by degenerated epithelial cells (arrows), plastic sections stained by toluidine blue. c) 80mg/kg lead nitrate treated group showing inflammatory infiltrated leukocytes (IF), normal (T) and abnormal renal tubules which are lined by degenerated cells (arrows), paraffin section stained by H&E. All scale bars= 20µm.
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