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Effect of cadmium on body weight and liver histopathological changes of *Rana ridibunda ridibunda*

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ABSTRACT

Cadmium is ubiquitous metals in the environment that induces a broad range of physiological dysfunctions. The purpose of this study was to investigate the chronic effect of Cadmium (Cd) on body weight, size and histopathology of the liver of *Rana ridibunda ridibunda*. Animals were divided at random groups into a control and 3 experimental groups exposed to different concentration of Cadmium chloride ($CdCl_2$) (2.5, 5.0 and 10µg / L) for (28 days). The animal body weight, total body length and width were measured before and at the end of experiments,then animals sacrificed. Liver was dissected out for histological examination. Cd -exposure had an obvious negative effect on the rate of overall growth of the test animals. A number of morphological change were observed in the liver, including the disturbed arrangement of the hepatic cords, dilatation of sinusoids, the presence of binucleated hepatocytes, dissociation of the hepatic cord and the presence of pigment aggregations as well swelling of hepatocytes, crowding of hepatocytes plus the presence of area of necrosis of hepatocytes at the three cadmium treatments, the more severe was at 10 µg /L cadmium. It can be concluded that the Cd exposure increase tissue damage in liver. In summary, this study provides data on toxic effect in amphibian exposed to different concentration sub lethal of heavy metals in particular cadmium. *Rana ridibunda ridibunda* Develops regressive alterations in the liver when exposed to Cd which may partially be a result of the oxidative stress induced by metal toxicant. The frog liver has a strong regenerative capability but severe liver injury do not develop even after 28 days of exposure to relatively high doses of Cadmium.

KEYWORDS

Rana ridibunda ridibunda;
Cadmium;
Vacuolization;
Necrosis.

INTRODUCTION

In recent years, increasing attention has been drawn to the effects of heavy metals present ubiquitously in the environment, on human and animal health. Heavy metals intoxication especially by cadmium constitutes threat to animal and human health[1] Cadmium (Cd) is a toxic substance that widely distributed in the environment, with wide range of organ toxicity, along elimination half–life, in aquatic environments, Cd is generally adsorbed to organic materials and easily accumulates in blood and stored primarily in the liver and excreted through glomerular filtration in the kidney and may have toxic effect on several organs e.g liver and kidney[2-4].

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Cd has been reported as a possible cause of various histopathological lesions in the liver, such as periportal liver cell necrosis and bile duct hyperplasia in rats[5], cell death and regeneration[6], dilatation of sinusoids, and an increase in the number of Kupffer cells[7] as well as Kupffer cell enlargement[8,9]. Metal toxic cities might be associated with oxidative tissue damage and has been shown to stimulate the production of intracellular reaction oxygen species (ROS)[10]. Elucidation of Cd effect is important for mechanistic interpretation of metal toxic effects. Xenobiotic is an influential factors, which may have direct or indirect effect by generation bioactive molecules intracellularly (free radicals) causing functional and structural alteration at tissue levels[11]. This work is to report the toxic effects of different chronic dose of cadmium on the rate of growth and histopathology of the liver of *R.r.r*

MATERIAL AND METHODS

frogs, *R.r.r* were collected from Chira region North Mosul / Iraq in June 2012. Twenty adult female frogs (weight rang 26–30g) were placed for 10 days in plastic aquariums (120x65x60 cm) for acclimatization. Frogs divided randomly to four groups (Five frogs for each group), the first group as controls, were placed in clean tap water, and the rest of them were the experimental groups, exposed to different concentrations of Cadmium chloride ($CdCl_2$)(2.5,5and 10 µg/L Cd)as for chronic exposure. This exposure concentration based on preliminary experiments showed that these concentrations are a sublethal for *R.r.r* and on corresponded previous study[12]. Thus, to avoided death of animals due to Cd, which exposed to relatively high concentrations of Cd, to achieve acute effects. After being assigned to a respective treatment groups (2.5.5and 10 µg/L),each group of frog was maintained in an individual container (volume 2.0L)for (28 days). The frog were fed boiled spinach throughout the 28-day test. At the end of 28th day of the exposure experiment, frogs were anaesthetized. Body weight, total body length and width were measured before and by end of experiments. Body length from snout to tip of tail was recorded using electronic calipers and values represented in millimeters, during each recording of body weight, the frog was taken from the media, and excess water was removed using a soft tissue. therefore, stress (if any) induced by this process remained same for all treated and control frog.

The Body weight increasing percentage was calculated according to Diniz et al[13] formulae with modification:

Initial body weight was measured using an electronic balance (Germany analytical balance precision 0.001 g).

Somatic indices (SI) = (total body weight / Initial body weight) X 100

Animals were then sacrificed and liver dissected out for tissues preparation for light microscopy. Small sections of liver were collected and were fixed in buffered neutral formalin according Luna[14] dehydrated in ethanol series and xylene and stained with hematoxylin and eosin (H&E). Sections (5-7µ thick) were obtained and stained with hematoxylin-eosin for light microscopic examination. Observations and photographs were made using zeils axiophotomicroscope equipped with an automatic photomcrographic system.

Statistical analysis

The data were checked for normal distribution, and then significant variations in Body weight, total body length and width between treatment and control were analyzed by analysis of variance (ANOVA) using SPSS software (version 10.5).

RESULT

There were no mortality during the 28th days experiment and no significant differences in mean body size (lengths and width) were detected as compared with control (TABLE and Figure 1).

TABLE 1 : Effect of Cadmium on body size.

size	Control	2. 5 µg /L Cd-treated	5 µg /L Cd-treated	10 µg /L Cd-treated
Body length/cm	6.840±0.626	6.750±0.880	6.500±0.500	6.100±0.822
Body width/cm	3.900±0.651	3.383±0.873	3.200±0.570	3.100±0.418

The results obtained are presented in TABLE 2 and well as in Figure 2and Cd exposure had an obvious negative effect on the rate of overall growth of the test animals. The final weight of the frog treated with the highest Cd dose (10 µg /L /28days) was significantly lower than in those of control, 2.5and 5 µg /L Cd treated / 28days). Therefore, there is a trend with regard to the effect of different doses of cadmium on growth of the animals, although it was chronic exposure.

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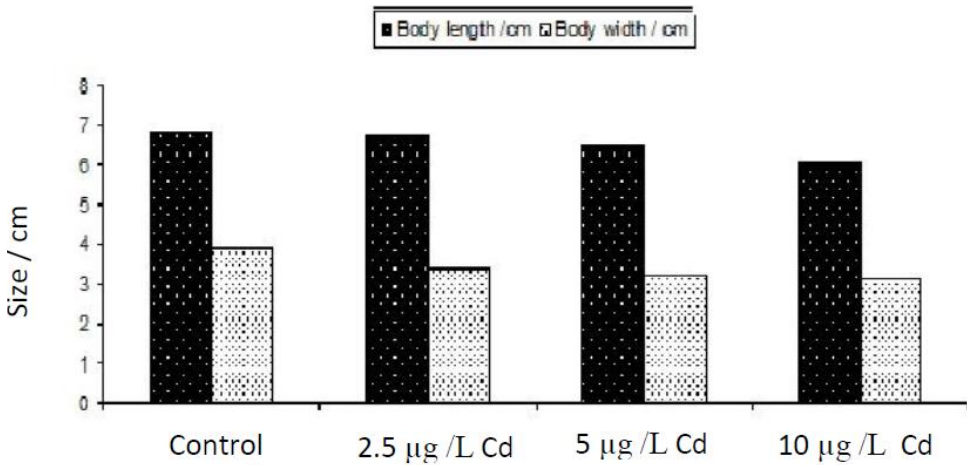

Figure 1 : Body length and width [cm] of the control animals and of the animals treated with different doses of cadmium chloride (2.5, 5 and 10 µg /L Cd / 28 days)

TABLE 2 : Effect of Cadmium on body weight

Treatments	Initial BW /g	Final BW /g	Somatic indices (SI)
Control	29.3±2	32.9 ± 3	(112.2)
2.5mg cadmium chloride-treated	29.460±4.79	20.660±2.96	(70.1)*
5mg cadmium chloride-treated	29.840±4.80	20.780±1.63	(69.6)*
10mg cadmium Chloridetreated	26.783±6.19	18.833±3.55	(68.3)*

Mean ± SD, N=10, p<0.05 (ANOVA followed by Duncan-comparison-test)

The impact of Cd chronic exposure on frog, reflected deterioration effect of Cd on the liver histoarchitecturely and Histopathologicaly. Light microscope observation revealed that treatment with 2.5,5 and 10 mg/L Cd induced a gradual morphological changes in the liver according to dose level. These changes are at 2.5 µg /L Cd treatment there disturbed arrangement of the hepatic cords, dilatation of sinusoids, the presence of binucleated hepatocytes. Figure 3b moreover a dissociation of the hepatic cords noticed and the presence of larger number and dense pigment aggregations. Figure 3c, whereas at 5 µg /L Cd acute swelling of hepatocytes, crowding of hepatocytes. dilated sinusoids, and the presence of multiple and dense aggregation of pigment were seen Figure 3d plus the presence of an area of necrosis of hepatocytes vacuolar degeneration. Figure 3e. At 10 µg /L Cd, cholangiol proliferation, and diffuse infiltration of mononuclear cells in the interstitium of the liver as well within branches of the bile duct. Figure 3f, beside a sever hyperplasia of hepatocytes and dense aggregations of pigment could be seen Figure 3g.

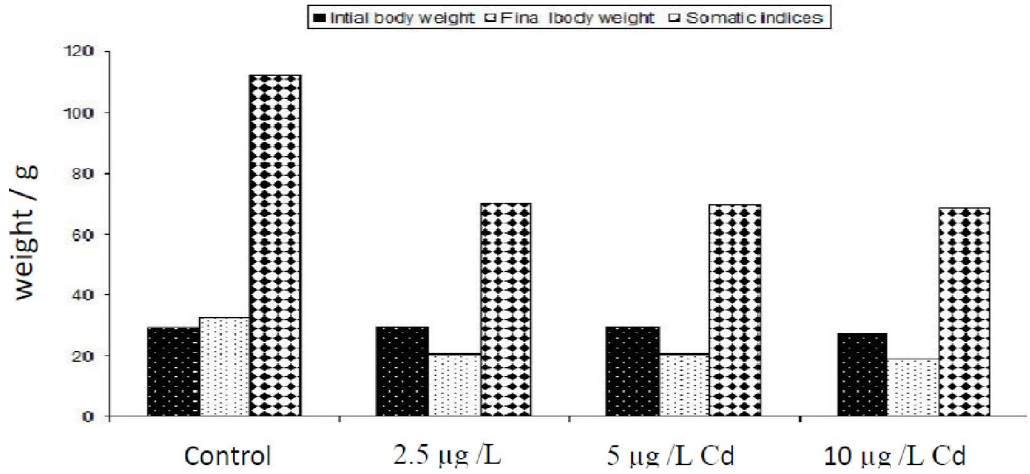

Figure 2 : Initial and final weights[g] of the control animals and of the animals treated with different doses of cadmium chloride (2.5.5 and 10 µg /L Cd /28 days)

DISCUSSION

The major challenge in experimental ecotoxicological studies is to cope with the relative paucity of literature

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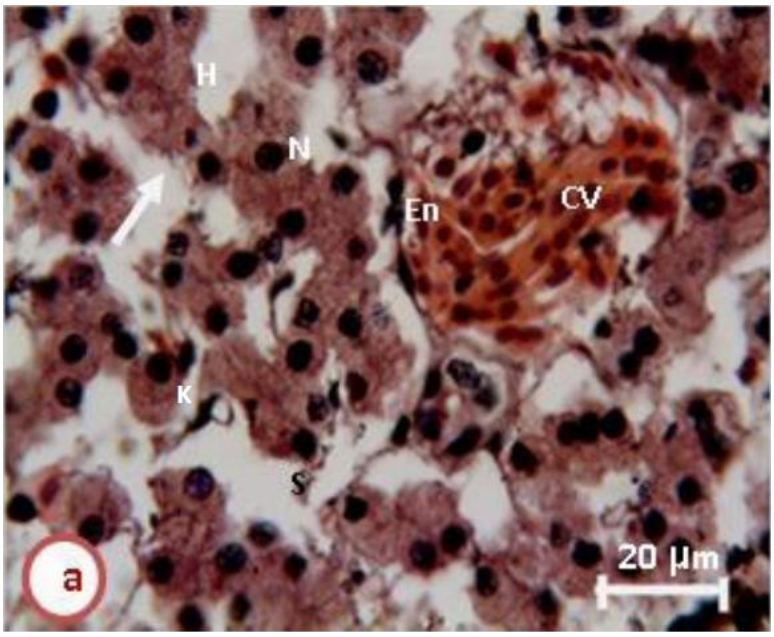
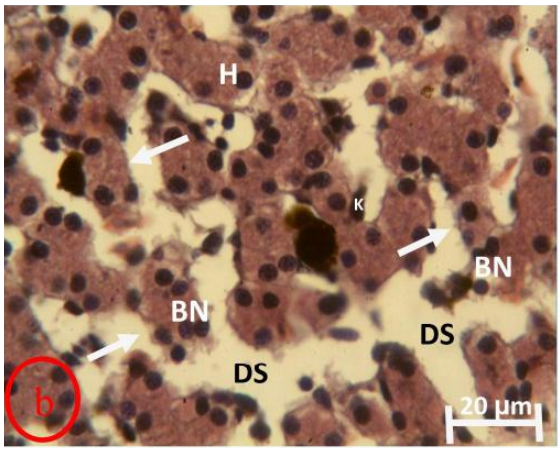
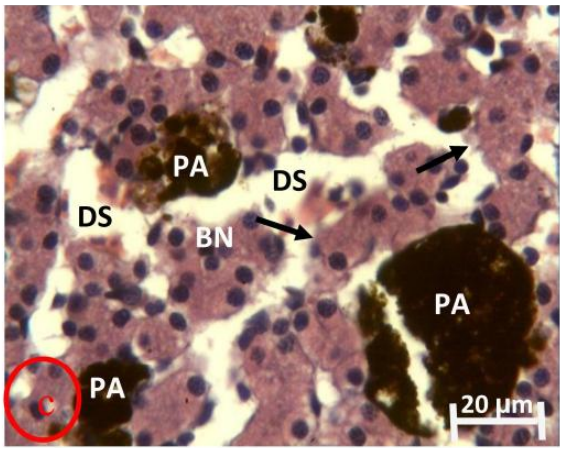
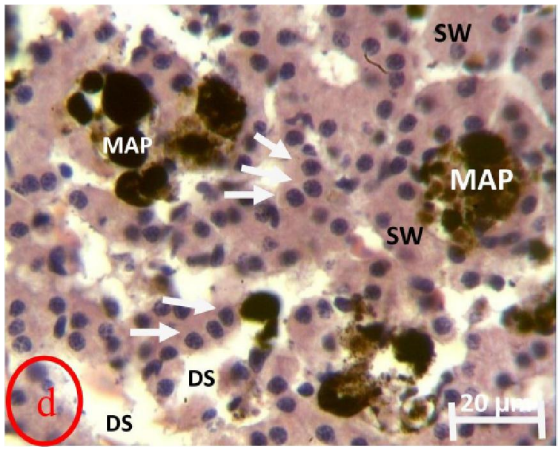


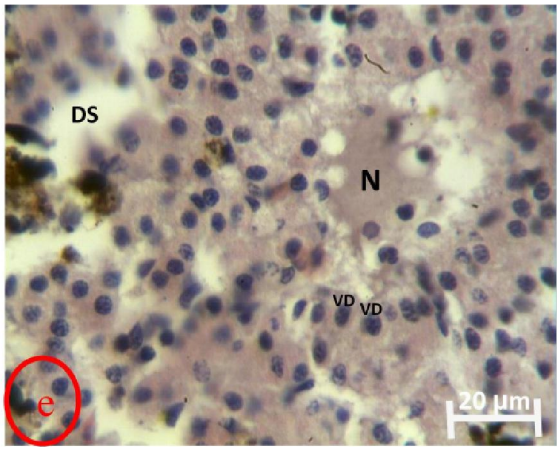
a) histologically normal control liver showing hepatocytes with clear cytoplasm, H- hepatocytes ,N-nucleus, S-sinousoid, cv-central vein ,En- Endothelium, K : Kuppfer cell arrow: hepatic cord





(b)&(c) treated with2.5 µg /L Cd , H- hepatocytes, arrows – hepatic cords, DS – dilation sinusoids, BN – binucleated, PA – pigment aggregations, K- kuppfer cell.





(d)&(e) treated with 5 µg /L Cd , DS – dilation sinusoids, MAP – multiple aggregation of pigment, SW – swelling, arrows – crowding, N – necrosis, VD – vacuolar degeneration.

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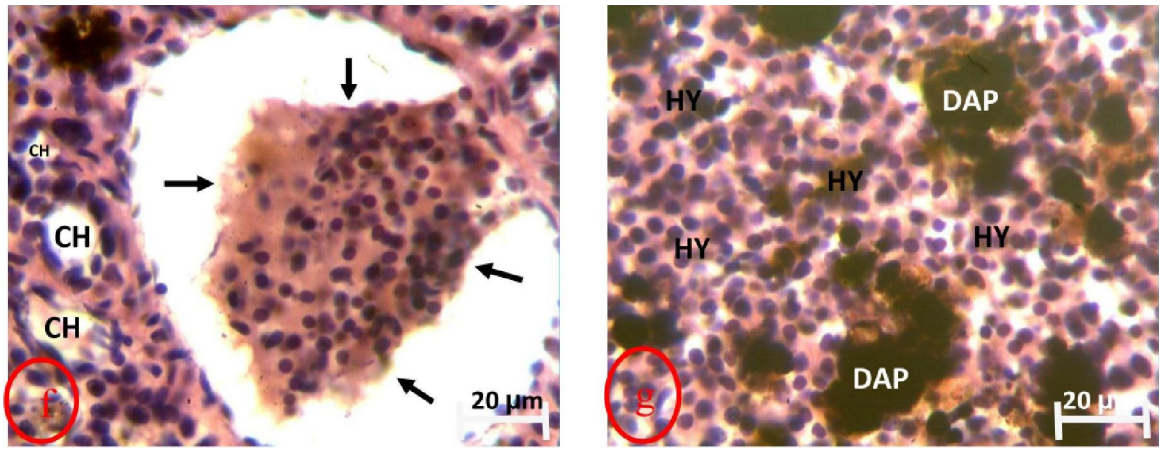

(f)&(g) treated with 10 µg /L Cd, arrows – diffuse infiltration of mononuclear cells, CH – cholangiole, HY – hyperplasia of hepatocytes, DAP – desnse aggregations of pigment.

Figure 3 : Transverse section of liver frog

on toxicity induced by environmental pollution may elicit both adaptive and adverse response in animals at different structural levels, i.e, cells, tissues and organs. These reactions depend on a variety of factors, such as the type of contaminant and its concentration, the rate of exposure and the susceptibility, of the organisms[15]. The present study investigated the effect of different sub lethal dose of Cd on growth indices and liver tissue architecture of Rana ridibunda. Amphibians typically do not drink water and obtain water through the ventral skin, but accidental drinking of water could not be excluded. The frogs in our experiment were constantly kept in 2-3 cm of water, thus always remaining exposed to cadmium. Test animals continuously exposed to three Cd-concentrations (2.5,5 and 10 µg /L), It was found a remarkable decrease in body weight in comparison with the control values. Thus, it seems likely that Cd-uptake takes place first across the skin, this is implied by the negative correlation between Cd- concentration and growth rate observed in different Cd- exposure. Results of the present study (TABLE 1 and 2) as well (Figure 1 and 2), demonstrate the adverse effect of cadmium- treatment. Body weight gain was altered significantly in the three treatments and more pronounced at 10 µg /L Cd- treated frogs in comparison to the control, the deleterious effect of cadmium on the different body growth indices maybe due to the toxic effect of cadmium itself on specific body, and not to the bad health of animals as pointed out in previous studies on rats[16,17]. This observation suggests that Cd- treatment induced significant metabolic alterations in the body of frog, Hence, these body weight and size decline observed in the Cd-treated frogs may be due to direct interaction of ROS with the body cell membranes. The present finding is also consistent with previous studies conducted on rats[18,19].

For up to 28 days Cd exposure developed progressive liver alterations characterized by the disturbed arrangement of the hepatic cords, dilatation of sinusoids, the presence of binucleated hepatocytes. Figure 3b moreover a dissociation of the hepatic cords noticed and the presence of larger number and dense pigment aggregations. Figure 3c, whereas at 5 µg /L Cd acute swelling of hepatocytes, crowding of hepatocytes. dilated sinusoids, and the presence of multiple and dense aggregation of pigment were seen Figure 3d plus the presence of an area of necrosis of hepatocytes vacuolar degeneration. Figure 3e. At 10 µg /L Cd, cholangiol proliferation, and diffuse infiltration of mononuclear cells in the interstitium of the liver as well within branches of the bile duct. Figure 3f, beside a sever hyperplasia of hepatocytes and dense aggregations of pigment could be seen Figure 3g, has been noted in surviving animals given different sub lethal dose of Cd.

Cadmium is an extremely toxic environmental contaminant that causes the production of reactive oxygen system (ROS). The oxyradicals such as Superoxide anion radical (O2), hydrogen peroxide (H2O2) and hydroxyl radical (OH). Thus ROS can cause cytotoxic alteration including enzyme inactivation, lipid peroxidation and protein degradation as well as DNA damage and ultimately cell death[20]. Liver and kidney are critical organs used to describe and document the effect of pollutants, during exposure, Cd induced tox-

icity in the liver and kidneys is dependent on hepatic and renal Cd concentrations[21]. Based on results of this study and recent experiments (5,24), the selected dose of Cd (5.98 mg/kg b.w) appeared to be toxic to liver. Cadmium is transported in the blood and widely distributed in the body but accumulates primarily in the liver and kidneys[22]. Hepatic and renal toxicity may occurred if toxic Cd level is attained in these organs regardless to exposure periods[23]. Results in Figure 3,b,c and d of the present study demonstrates that, frog treated with Cadmium developed progressive liver alteration characterized by obvious lesion. disarrangements and Scattered of hepatocytes, enlargement of Kuppfer cell, cytoplasmic vacuolation. Multinucleated hepatocytes,aggregation of pigment, necrosis, regenerative and eventually followed by cell death. Liver fibrosis and the ''cirrhosis''-like morphological picture observed in the present study Figure 3g have also been reported in surviving bullfrog exposed to aflatoxins[24], male rats exposed chronically to cadmium (19) and mice exposed to lethal dose of cadmium chloride[25].

The liver damage has been reported in other mammalian and non mammalian species, in addition necrosis strongly associated with oxidative stress[22-26]. Beside direct damage of proteins, another underlying mechanism for toxicant induce cell injury may have influential factors which may have direct or indirect effect by generation bioactive molecules intracellularly (e.g. free radicals), causing functional and structural alteration at tissue levels due to the ability of oxidant substances to cause oxidative stress in the liver[11]. Therefore, the present findings indicate that the susceptibility of the Rana ridibunda liver to Cd is similar to most mammalian species already studied. On the other hand, increased production of reactive oxygen species (ROS) is one of the responses observed in the livers of wild frogs exposed to environmental pollution[15]. Cd-exposed frogs in the present study could be under some oxidative stress. The abundance and enlargement of Pigmented macrophages as well Kuppfer cells (Figure 3, b) and (Figure 4, c,d), may be attributable to the antioxidant apparatus of the amphibians liver, which includes hepatocytes, Kuppfer cells and the Kuppfer cells-derived melanomacrophage centers (MMCs), all them interrelated and intensely involved in xenobiotic detoxification through a combination of enzymatic biotransformation and melanin scavenger activity[15,26-28].

In conclusion, as in other mammalian and non-mammalian species already studied, Rana ridibunda Develops regressive alterations in the liver when exposed to Cd which may partially be a result of the oxidative stress induced by metal toxicant. The frog liver has a strong regenerative capability but severe liver injury do not develop even after 28 days of exposure to relatively high doses of Cd (10 μg /L).

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