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Research Article

Cadmium Accumulation in Some Organs of *Rana Ridibunda Ridibunda* Affect Erythrocytic Nuclear Abnormalities

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Abstract— In the current research, accumulation of cadmium was investigated in various organs kidney, liver and the testis of Ranaridibunda exposed to diverse experimental levels of cadmium and erythrocytic abnormalities. *Rana ridibunda* species inhabits small shallow streams located in North Mosul / Iraq. Although cadmium level was not detectable in source water and expected to be with quality water guideline levels to protect the life in fresh freshwater proposed by the Agency of environmental protection (EPA), therefore the noticeable Cd level detected in experimental animals could pose danger to aquatic organisms. The Cd concentration in the different frog tissues was variable, the highest concentration was found in testis and the lowest in the liver. Frogs from treated groups had significantly higher hepatic (1.3232 2.1800, 3.5130 µg / g), renal (3.4556, 4.2850, 4.9992 µg /g) and testicular (3.5812, 4.8170, 5.5556 µg / g) for 2.5, 5 and 10 Cd / µg /L respectively than those from the control group (1.0718, 1.9678, 3.2290 µg / g). There was erythrocytic nuclear abnormalities which significantly higher in number of Micronuclei (MN), notched nuclei (N), Lobed nuclei (L) and nuclei with kidney shaped (K) respectively were noted in the groups for 2.5 and 5 Cd / µg /L treated groups than that from natural sites, also with higher frequency significantly of immature erythrocyte with deleterious effect and totally erythrocytes demolished at 10 Cd / µg /g. This research found that cadmium accumulation of the different treatments frogs have higher in testicular, renal, hepatic and found a higher number of erythrocytic nuclear abnormalities when compared to frogs from natural habitat. Therefore, the accumulation of cadmium in the tissues that are used with concentrations and sampling periods is taken into consideration.

Keywords— Ranaridibunda, erythrocytic abnormalities, Genotoxicity, Cadmium.

1. Introduction

Recently, great attentions would paid to of the heavy metals damages scattered throughout the earth, to the health and safety of plants and animals as well as to humans, and cause the so-called poisoning of heavy metals, especially cadmium [1]. As for the vertebrates that live in water, amphibians are among these animals that are sensitive particularly to changes of environment, because their life has terrestrial and aquaticstages, and their skin is semi-permeable to external materials [2]. That is why amphibians are among the animals that are an important bio indicator of pollution in water [3]. As mentioned above, this is mainly because the skin it is in is highly permeable and absorbs substances from the environment [4, 5]. Which may also be responsible for the further decline in the numbers of these animals in all environments of the world [6].Therefore, these animals are widely used to identify the health of the ecosystem in which they live, as they are known to accumulate minerals in their bodies [7].

The liver in animals is the main organ for detecting any change in the representation of nutrients, as it plays a major role in absorption and accumulation, as well as removing the toxic effect [8, 9, 10]. This is supported by it as well [11]. And that the levels of metal accumulation in the liver and the rest of the tissues reflect the levels of these metals in the environment as well as, the accumulation of these materials varies in different tissues of the body [12, 13], which is what happens in the cupping of Ranaredibunda.

Cadmium (Cd) is a widely distributed toxic element in the environment, with a large group of toxic elements, with a long elimination half-life, especially in the aquatic environment [14]. This element is generally absorbed and accumulated with organic matter in the blood, then it will be stored mainly in the liver [15, 16]. Cadmium is one of the most toxic substances that cause liver toxicity and is excreted to the outside through the glomerular filtration of the kidneys, so it has a toxic effect on many organs, the most important of which are the liver, blood and kidneys [17], in addition to the histological changes it causes, Cd may appear genetically toxic as well [16, 18].

The studies of heavy metals determined their effects and its genotoxic effect of cadmium on the frog Ranaredibunda, and these studies confirmed the acute toxicity of this element in young frogs [19]. To achieve the objectives of this study, the accumulation of this element in the liver of these animals was evaluated, in addition to the measurement of erythrocyte nuclear abnormality (ENA) and its genetic toxicity. and sensitivity to environmental influences [20]. Although most studies of its toxicity in amphibians focused on its effects on larval growth, development and behaviour [3, 16], only very few studies have been published on the bioaccumulation of these elements under natural conditions [12].Cadmium is one of the most important pollutants, although it is used on a large scale in industrial processes and products. Cadmium due to human input is the source of pollution in cadmium to the aquatic environment. Aquatic animals tend to absorb this cadmium from the water and accumulate it in the tissues of these animals. This uptake depends on many factors, such as the route of exposure, the amount of dose, the time of exposure, the physiological state of the animal and environmental parameters. The tissue accumulation of these substances in animals is the result of absorption rates, and their concentration changes according to the distribution patterns of these substances in different animal tissues [21].

3. Materials and Methods

This study was conducted in the north of the city of Mosul during the month of January (2021). There are many small shallow streams, and the reference source chosen for this study is the Chira region where the presence of Ranaridibunda frogs was recorded. And these streams have a water volume that changes according to the rise of water to these springs from the neighbouring mountains. Also, no egg masses were recorded on the water in these springs. Live frogs were collected from different sites of this area using hand net, transported to the laboratory and left for acclimatisation for 10 days in a water-filled rearing tank (60 cm x 40 cm x 35 cm). Then it was presented to three chemical tablets with concentrations (2.5,5 and 10 µg/l) of cadmium and these doses were selected based on previous studies [22].After defining the study groups, 10 frogs per group were placed in an isolated container (volume of 2.0 litres) for the duration of the study. The frogs were fed boiled spinach for 28 days.

3.1 Chemical analyses

Samples of water collected from spots from which the frogs were taken for chemical analysis using 0.5 liter containers containing (65%) nitric acid, with a pH of less than 2, to prevent the transfer of cadmium.

3.2 Metal quantification

The samples that were taken were treated for extraction and quantification of cadmium using the applied analytical procedure [23]. The animals were autopsied and the liver, kidneys and testicles were removed from them. A weight of 0.1 mg was taken from it to estimate the amount of cadmium in the liver. The digestion was done and the tissues were then dried in an oven at 105 °C for 24 hours until a solid mass was obtained. Next, the tissue weight was recorded to 0.1 mg. the samples then were digested into a 2:1 (volume/volume) mixture of nitric (Merck, Ultrapur) and Merck perchloric acid, Suprapur). 0.1g was placed in the digestion tube and 2 ml of concentrated nitric acid was add gradually, heated at 150 °C for 1 h, after which 1 ml of perchloric acid was added after heating at 150C°. As a final point, 1 ml of oxygenated water (Merck, Ultrapur) was added. Disappear of the rise of fumes and the solution became free of solid fragments indicate completing of process. After that, 0.5 ml aliquots of hydrogen peroxide (30%) MERCK were added after lysis, the volume of the final solution was balanced to 5 ml in a volumetric flask with Milli-Q® water. Following the same steps, blanks samples were obtained, the solution was quantitatively conveyed to a 5 ml detection limit that was about 0.01 µg metal/g dry weight. Heavy metals concentrations tested in tissues were expressed in micrograms per gram of dry mass.

3.3 Estimation of sub lethal concentration

Determination of immature erythrocyte (IE) frequency and erythrocyte nuclear abnormalities ENA. To evaluate Cd toxic effect, the ENA was achieved in mature peripheral erythrocytes . Therefore, from each animal, three blood samples were taken. These swabs were fixed by methanol for 10 minutes and stained with Giemsa for 30 minute. One thousand red blood cell were counted per slide at 1000x× magnification power in order to identify deformities in the nucleus of red blood cells. A classification was adopted for [24], these nuclear changes were divided into several categories: the overall shape of the cell, the serration of the nucleus, and the lobe of the nucleus. The number of immature red blood cells (IE) per slide (1000 red blood cells) was also determined using following equation [25].

$$\text{IE frequency (\%)} = \frac{IE}{ME + IE} \times 100 \quad (1)$$

IE = the immature erythrocyte
ME = the mature erythrocyte

3.4 Statistical analyses

The results of this study were analysed using one-way analysis of variance (ANOVA).

To test the statistical significance of each of the metal content in the studied organs, nuclear abnormalities in the erythrocytes, the frequency of IE for animals and according to the sites from which they were taken, as well as exposure to cadmium.

4. Results

Cadmium concentrations were recorded in the studied organs, namely testis, kidney and liver, as shown in (Table 1). The results showed significantly higher values of cadmium in the testis, kidney and liver in the organisms from treated groups respectively as when compared to frogs from control. Duncan's new multiple range test revealed as shown in Table (1) that Cd accumulated significantly in different tested organs in frog exposed to different concentrations of Cd (2.5,5 and 10Cd / µg /L), whereas the among the above Cd concentration medium most the significant effect was observed when animals exposed to 10Cd / µg /L. Cadmium accumulation in the kidneys and testes was greater than in the liver, and it was observed that cadmium accumulation in the testes was the highest of all.

Table 1. Concentrations of cadmium (mean ± standard deviation)

Treatment (Cd / µg /L)	(µg / g)dry weight/ Organs			Effect of Treatment
	Liver	Kidney	Testis	
Control	1.0718 g	1.9678 ef	3.2290 d	2.0895 d
2.5	1.3232 fg	3.4556 cd	3.5812 cd	2.7867 c
5	2.1800 e	4.2850 bc	4.8170 ab	3.7607 b
10	3.5130 cd	4.9992 ab	5.5556 a	4.6893 a
Effect of Organs	2.0220 c	3.6769 b	4.2957 a	

The letter (A) indicates a significant difference in cadmium values that compared with the control group for each member. The different letters a, b, c, d in the table indicate the statistically significant differences between the study criteria between the experimental groups themselves as well as with the control group according to (Duncan's test, $P \leq 0.05$).

4.1 Degree of genotoxicity

Among the changes observed in the nuclei of red blood cells, Five form or types of abnormalities which are recorded in the ENA. These abnormalities included lobulated nuclei as in (Fig. 1a), indentation of nuclei (Fig. 1b), micronucleus (Fig. 1c), nephrotic nuclei (Fig. 1d) and double nuclei (Fig. 1e). The IE frequency was significantlys higher in both 2. 5 µg /L Cd-treated and 5 µg /L Cd-treated as compared to the control. Specially erythrocytes with Micronuclei (MN), notched nuclei (N) and lobed nuclei (L) respectively Table (1) and Fig (2) .Deleterious effect and totally erythrocytes demolished of group treated with at 10 / µg /L Cd was recorded.

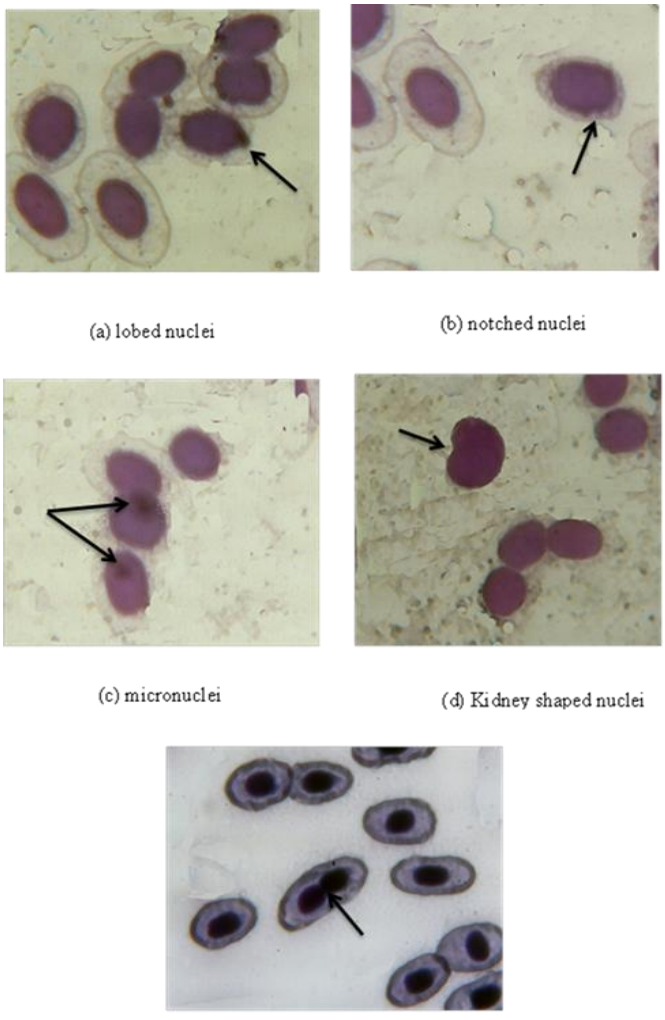

Figure 1. Nuclear changes identified in red blood cells in Ranaridibundaridibunda (a) lobed nuclei; (b) dentate nucleus (c) nucleus small in size micro; (d) The nucleus is renal in shape; (e) duplication of nuclei.

Table 1. Statistical mean of recurrence of immature erythrocytes and mean recurrence (‰) of each type of nuclear change (±SD) in red blood cells in the peripheral blood of cadmium-treated group animals as well as the control group. As * indicates a statistical difference at the level of significance ($p < 0.05$) between 2.5 µg/l cadmium for treatment and control. And ** represents a statistical difference at the level of significance ($p < 0.05$) between 5 µg/l treated with Cd and both were 2.5 µg/l for the cadmium-treated groups and the control group.

parameters	control	2.5µg/L Cd-treated	5µg/L Cd-treated	10 µg/L Cd-treated
IE Frequency (%)	84.58±16.32	107.73±15.21*	202.00±30.33**	…….
Micronuclei(MN)	0.00±0.00	16.8±3.34*	20.8±4.14**	…….
notched nuclei(N)	0.70±1.1	11.2±4.14*	15.4±3.2**	…….
Lobed nuclei (L)	1.5±1.3	9.5±1.87*	14.8±4.43**	…….
Kidney shape nuclei	0.00±0.00	7.2±1.7*	12.2±3.7**	…….
Total of (MN+N+L + KS)	2.2±2.4	44.7±11.11	63.2±15.47**	……

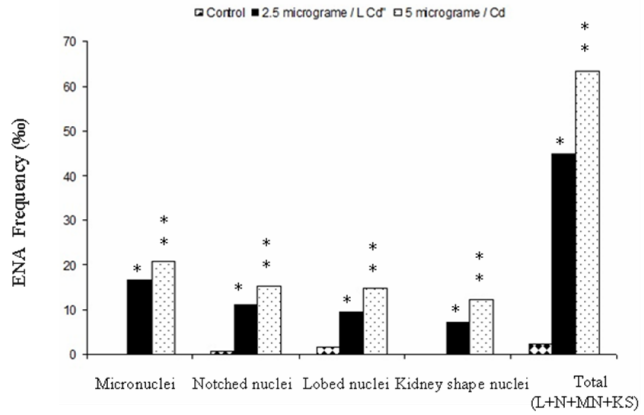

Figure 2. Average frequency of all nuclear changes and total nuclear change in the studied animals. Where * represents a statistically significant difference at ($P < 0.05$) between 2.5 µg/l between the treated animals and the control group, and ** represents a statistical difference at the level ($P < 0.05$) µg/l treated with cadmium and both are 2.5 µg/l. A CD for all studied groups.

5. DISCUSSION

The current study investigated the effects of different sublethal doses of cadmium accumulationin a number of *Ranaridibunda* tissues, namely the liver as well as the kidneys and testis. It is known about amphibians that they do not usually drink water, but rather get it through the abdominal skin, however, it cannot be ruled out that they drink water accidentally. In this study, frogs were kept continuously in 2-3 cm of cadmium-containing water and thus were permanently exposed to the cadmium at three concentrations of cadmium (2.5.5 and 10 µg/L).

As the Cd level in natural water analysed was not detectable, the considerable Cd level detected in negative experimental control animals is presumably due to the diet Cd accumulated in both natural habitat and laboratory culture. Although cadmium level was not detectable in source water and expected to be with quality water guideline levels to protection of freshwater and the aquatic life proposed by the environmental protection Agency (EPA) [26]. Therefore the noticeable Cd level detected in experimental animals could pose danger to aquatic organisms. Undetected cadmium in field water and the presence of a large content of cadmium in negative laboratory control animals (Table 1) This may be due to the animals absorbing these toxic substances through the feeding process, as well as the surface of their bodies, as well as depending on the chemical properties of these toxic substances and the accompanying environmental factors [27].

In other studies, it was found that aquatic animals have the ability to absorb toxic substances through their food contaminated with these substances, as well as possible for these substances to enter through the surface of their bodies [28]. Thus, it can be said that the uptake of cadmium in these aquatic animals occurred first through the diet and then could be through the skin.

The results also showed, through the concentrations of cadmium used (2.5.5 and 10 µg / litre), that cadmium can be transported through the blood and spread in the body widely, but it accumulates mainly in the filtered organs in the body, namely the liver and kidneys. Cadmium is a typical industrial poison, and it is also a very harmful environmental pollutant that poisons the organs and tissues of all kinds of living organisms. Many researches and studies have shown that the accumulation of cadmium in liver and kidneys in particular, as it was discovered that it may reach 75% of the total accumulation of this element in the body in these organs alone [29]. Its effect on the liver is attributed to its importance as an important organ in the body. It plays a significant role in biotransformation processes, Excretory of xenobiotics, and detoxification, as it is the center for their processing within the body[30]. Cadmium causes kidney tissue dysfunction by damaging the renal tubules, disrupting normal reabsorption, and reducing renal tubular phosphate reabsorption [31]. The rate of transference of cadmium, in the form of a cadmium-metallothionein complex, from liver to kidney depends on the time required for metallothionein synthesis [32]. Once the complex reaches the renal tubules, it is degraded by lysosomes, which contain digestive enzymes that release cadmium. This, in turn, leads to the production of renal metallothionein, which accumulates in the kidney at higher levels than in the liver [33]. General comparisons between the results of different treatments with cadmium show that the highest concentration appeared in the testis, which is much higher than in the tissues of both liver and kidney (Table 1), although the liver is one of the main organs targeted by cadmium in this animal and the rest of the other vertebrate animals, but that The results of this study showed the opposite (13). In other studies, it was found [34], that the liver of this frog contains a very high cadmium concentration. That 26% of the absorbed cadmium accumulates in the liver [35]. However, the results in this study showed a different trend, as the cadmium accumulation in the organs (testis, kidney) is more pronounced than its accumulation in the liver [36]. This may be due to the fact that the liver in these animals is the primary cadmium accumulation site while the kidneys and testis are the final cadmium accumulation site. It was also found in this study, that the accumulation of cadmium is high in the testicles compared to the rest of the organs, which is expected because it is known that cadmium is a toxic substance that directly targets the testicles [37].

The results of the ENA revealed that when considering the total number of nuclear abnormalities, the frequency of IE and significant differences there were abnormal morphological changes of erythrocyte nuclei in the treated animals (Figure 2). The micronucleus (MN) assay is a simple and one of the best methods for assessing genotoxic damage because it enables measurement of chromosome loss and refraction [34]. Micronuclei are found in dividing blood cells that contain spacers between chromosomes and/or chromosomes that are unable to move to the spindle poles during mitosis, leading to abnormalities [20, 36, 38, 39]. Also, the chemical factors entering the body can interact with the nuclear components, leading to nuclear changes, which was explained by some researchers [40, 41] .Thus, the nuclear abnormalities that appeared as lobulated nuclei; Serrated nuclei, micronuclei and renal nuclei (1, A, B, C, D) in the

ENA assay are the result of exposure of the genes of these cells to toxic pollutants [24, 40, 42]. Also, the binuclear cells that appeared in the results of this study were also recorded in other studies on other vertebrate animals, which is a degenerative or dystrophic change [43, 44].

As mentioned earlier, cadmium is a highly toxic environmental pollutant that may cause the production and formation of reactive oxygen (ROS), oxygen radicals such as hydrogen peroxide (H_2O_2), superoxide radicals (O_2), and hydroxyl radical (OH), and thus can cause ROS to Serious and cytotoxic changes occur including degradation of protein production as well as DNA damage and alteration and the appearance of mutations [45, 46].

Conclusion

This study concluded that frogs' exposure to cadmium, directly or indirectly, leads to its accumulation in sensitive and important tissues, including the liver, kidneys, and testes. This study found that cadmium accumulation was higher in the kidneys and testes than in the liver. It also leads to abnormalities in the shape of red blood cells, affecting their function and ultimately impacting the animals' health. Therefore, these pollutants must be prevented from entering the natural environment of these frogs to preserve them as an important part of the ecosystem.

Data Availability

None

Conflict of Interest

Authors declare that they do not have any conflict of interest.

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None

Authors' Contributions

Author-1 and 4 researched literature and conceived the study. Author-2 and 3 involved in protocol development, gaining ethical approval, patient recruitment. Author-1 and 4 wrote the first draft of the manuscript and data analysis. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

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