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ORIGINAL ARTICLE

Hepatic Alterations in the Amphibian *Sclerophrys regularis* as Biomarkers for Habitat Destruction

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KEYWORDS

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ABSTRACT: Heavy metals contamination may have negative impacts on the wetland biota, particularly on amphibians, and result in a severe decline globally. Because they cannot be degraded biologically, heavy metals are difficult to remove from the environment. To assess the histopathological biomarkers, some heavy metals Fe, Mn, Pb, Cd, and Cr were analyzed in the livers of Egyptian toads (*Sclerophrys regularis*) from North Giza (Abu Rawash) and El Wahat Regions (El Bawiti) as well as histological changes. The findings demonstrated that the heavy metals concentrations in the Egyptian toad liver at El Wahat were lower than those recorded in the El Giza region. In accordance with heavy metals burden, hepatic alterations were demonstrated and described. Because the liver is the organ where all types of toxins and chemicals are detoxified, the severity of the hepatic alterations observed in this study may be related to the damaging effects of environmental pollutants on hepatocytes.

INTRODUCTION

Multiple factors combine to significantly contribute to the global loss of amphibians rather than being alone responsible for it. Climate change, which is the main worldwide problem, alters the matter- and energy-cycles in a variety of ways that have an impact on water, soils, and ecosystems [1]. Threats outside of significant incidents include invading species, pollution, disease, and habitat degradation [2]. Some Egyptian amphibians are endangered, while others have declined or disappeared in different locations due to habitat degradation, excessive pesticide application, and traffic fatalities [3]. One of the main causes of the decline in amphibian specimens in natural environments is chemical contamination [4]. However, the most important adaptation for anuran amphibians to survive in heavy metal-polluted habitat is their capacity to prevent excessive loads of heavy metals in their bodies [5]. Heavy metals contaminations have long been a focus of research due to its distinctive effects on ecosystems and associated organism in different routes [1]. Heavy metals have the capacity to enter food webs and chains, interfere with biomolecules, and cause oxidative stress, which has the ability to inflict injury or death in addition to biochemical and histological changes [6-8]. Amphibians take metals either orally or through their skin, which is also highly permeable, during the adult and larval phases of their development. Histopathological anomalies in numerous organs, including the liver, were frequently utilized in ecotoxicological studies as biomarkers for environmental effects on many aquatic and terrestrial vertebrates [9, 10]. Therefore, the aim of the current study is an

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extension of our previous works on the effects of habitat destruction and environmental pollution on the amphibia in Egypt [9, 11-13]. Here, we attempt to evaluate the effect of some heavy metals, we assessed previously in habitat and tissues [13] on the liver of Egyptian toad (*Sclerophrys regularis*) collected from two different localities. Since the liver is the site of detoxification, liver histopathology studies are a promising approach to reveal the sensitivity of this tissue to stressors. Numerous studies have used liver histopathology as biomarkers for xenobiotic impacts and environmental pollution, both in situ and in vitro [9, 14-16].

MATERIALS AND METHODS

Study area

For the current study, two regions were chosen (Figure 1); Abu Rawash receives a range of pollutants from point and non-point sources, such as municipal wastes, fertilizers, chemical wastes, as well as wastewater treatment plants. The second site is El Bawiti Region, located in the Western Desert in the middle of 370 km southwest of Cairo. The Egyptian toad (*Sclerophrys regularis*) were freshly examined and transported to the laboratory of Zoology and Entomology, Zoology Department, Faculty of Science (Girls), Al-Azhar University, Nasr City, Cairo, Egypt. In the laboratory, toads were anesthetized, dissected and the liver was removed.

Figure 1. Map showing the two studied regions, site 1 (El Giza) and site 2 (El Bawiti).

Determination of heavy metals in the liver

Five heavy metals; Iron (Fe), manganese (Mn), lead (Pb), cadmium (Cd) and chromium (Cr) were determined in the liver of the toads. Liver tissues were placed in plastic bags in a freezer after animals were dissected. The digestion of frozen tissues was carried out in accordance with [17] using ultra-pure analytical grade nitric acid HNO_3 and H_2O_2. Then, digestate was diluted with distilled water and analyzed via atomic absorption spectrophotometer .data were treated with the statistical package SPSS (V 20) and expressed as Mean ± SD.

Histopathology

Animals were gently anesthetized and dissected. The liver was fixed in a 10% neutral formalin solution for 24 hours. The tissues were then transported in 70% ethyl alcohol. To prepare tissues for light microscopy, they were first dehydrated in a succession of ethanol concentrations, cleaned with xylene, and then embedded in paraffin wax [18]. Tissues were sectioned at 5 µ thick and stained with hematoxylin and eosin (H&E). The histological changes in the liver and kidney were examined in the randomly selected sections from each toad.

RESULTS

Heavy metals in liver tissues

According to the findings in Table 1, the levels of heavy metals (Fe, Mn, Pb, Cd, and Cr) in the liver of Egyptian toads at El Wahat were lower than those found in the El Giza region (0.05 >p ≤0.01), except for iron (insignificant). Additionally, the five metals were ranked in both regions as Fe> Mn>Pb> Cd> Cr.

Table 1. Mean ± SD of heavy metals concentrations (µg g^{-1}) in the liver of *Sclerophrys regularis,* collected from the investigated sites.

Heavy metals	El Giza	El Wahat
Fe	2132.818±732.61[a]	2107.510±67.432[a]
Mn	65.820±16.053[a]	3.780±7.453[b]
Pb	10.034±1.111[a]	1.532±0.368b[b]
Cd	4.652±1.821[a]	1.210±0.446[b]
Cr	3.130±1.541[a]	0.892±0.545[b]

Hepatic histopathology

At El-Giza, Abu Rawash (site 1), histological examinations of the Egyptian toad's liver, *Sclerophrys regularis*, revealed some alterations compared to those of from El-Wahat (El-Bawiti). Dilation of sinusoids, centrilobular vein with congested erythrocytes, dilated congested vascular structure. In addition, the magnified view (400x) showed a great deformation of hepatic sinusoids, centrilobular vein and vascular structure, great necrosis of hepatic acini with accumulation of melanomacrophage cells and degeneration and dilation of centrilobular vein have been observed and erythrocytes congestion (Figures 2, 3).

On the other hand, the histological observations of the liver sections of the toads collected from the second site, El-Wahat (El-Bawiti), showed normal hepatic architecture, including normal hepatic sinusoids, normal centrilobular vein, normal hepatic sinusoids, normal nuclei of hepatic acini, and centrilobular vein with moderate congestion, bile secretion appears to be poor. The hepatocytes that aggregate in masses separated by blood sinusoids and arranged in rings around a central vein. Each hepatocyte is polygonal or spherical in shape with well-defined boundaries and contains a large, rounded nucleus (Figures 4, 5).

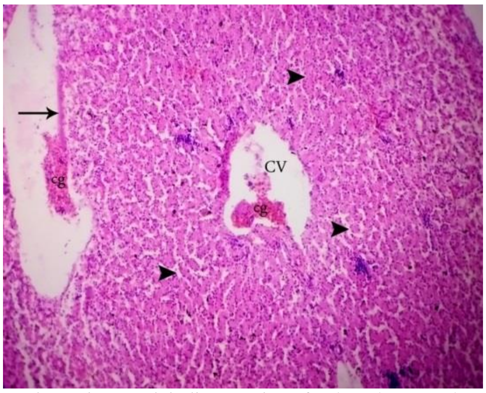

Figure 2. Photomicrograph in liver section of *Sclerophrys regularis* at Abu Rawash (site 1) and showing dilation of sinusoids (arrowhead), centrilobular vein (CV) with erythrocytes congestion (cg) and dilated congested vascular structure (arrow) with erythrocytes (H&E100x).

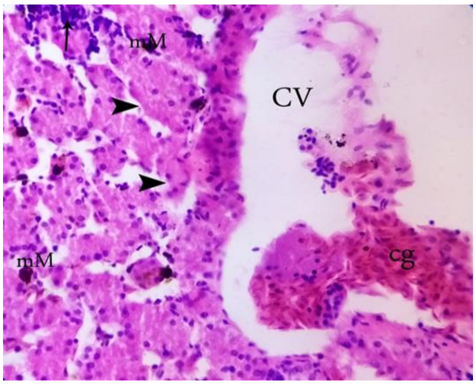

Figure 3. Photomicrograph in liver section of *Sclerophrys regularis* at Abu Rawash (site 1) and showing clear dilation of sinusoids (arrowhead), aggregation of melanomacrophage cells in between the hepatocytes (mM), leucocytes infiltration (arrow), dilated centrilobular vein and congested with erythrocytes (H&E, 400x).

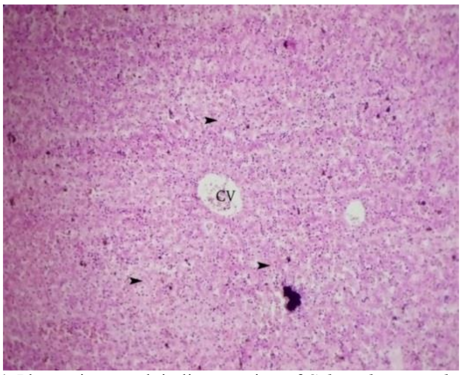

Figure 4. Photomicrograph in liver section of *Sclerophrys regularis* at El-Bawiti and showing normal hepatic architecture including normal hepatic sinusoids (arrowhead) and normal centrilobular vein (CV) (H&E, 100x).

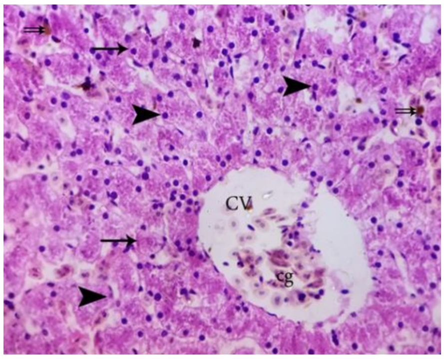

Figure 5. Photomicrograph in liver section of *Sclerophrys regularis* at El-Bawiti and showing normal hepatic sinusoids (arrow), normal nuclei of hepatic acini (arrowhead), centrilobular vein (CV) with moderate congestion. Note weak appearance of bile secretion (double arrows) (H&E, 400x).

DISCUSSION

Many toxins including heavy metals may directly harm toads and cause their death, or they may accumulate in their tissue and spread to other animals in the food chain through predation [9, 12, 19, 20]. Many earlier studies on liver histopathology concentrated on agricultural chemicals and insecticides, which endanger anurans both in their terrestrial and aquatic stages through agricultural runoff into rivers [9, 12]. Since it is well known that heavy metals cannot be naturally broken down and can persist for a very long period in biotopes, the current study concentrated on how they affect histopathology. The results of the present study are in line with those of Sánchez-Chardi, Ribeiro [21], who found higher concentrations of Fe, Mg, Pb, Hg, Cd, Zn, Cu, Mn, Mo, Co, and Cr in the liver of *Crocidura russula* collected from mine wastes spillage receiving site when compared to another from reference site. Also, the studies by Ward, Hassan [22] indicated that tissues revealed greater concentrations of Cd, Pb, Cu, Cr, and Fe in the tissues of *Bufo terrestris* exposed to coal combustion waste when compared to non-polluted habitat. The liver changes observed in this study can be regarded as a reliable biomarker for a persistent environmental effect. The liver was chosen for this type of monitoring because it has a better sensitivity due to its biological functions and because metals accumulate in tissue indicate both earlier and recent exposure. It must be kept in mind that various other toxicants, in addition to heavy metals, may also contribute to the liver lesion observed here. Similar to the current investigation, histological examinations of *Hoplobatrachus occipitalis* exposed to cadmium indicated abnormalities in the liver tissues including hepatocyte ballooning and hyperplasia of Kupffer cells [23]. Also, pigmentation, inflammatory cell infiltrates, extramedullary hematopoiesis, and hepatic inflammation, were present in the liver tissue of a *Euphlyctis hexadactylus* from a heavy metals-polluted site [24]. Abu Rawash region is polluted from both point and non-point sources, such as fertilizers, chemical wastes, and municipal garbage, in addition to wastewater treatment plants. The El Bawiti region, on the other hand, is arid and relies primarily on wells and springs for its groundwater. El Bawiti is thus still far from human activity, and rocks are the primary source of heavy metals. Even though *Sclerophrys regularis* decline is not addressed in this study, we must take into consideration that there are some obvious causal factors that should be considered in an addition to this study because it has become a serious threat to amphibians in general and anurans in particular.

CONCLUSIONS

The findings of the current study suggested that *Sclerophrys regularis* animals have a high sensitivity to hazardous contamination and a propensity to bioaccumulate heavy metals in a contaminated environment. The liver has displayed varying degrees of degradation and injury as a site of detoxification. As a result, the histological alterations in the liver observed here showed that the investigated animals had a significant capacity for accumulation.

Conflict of interests

The all authors declare that there is no conflict of interest.

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