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Effect of lead on haematological parameters of Duttaphrynus melanostictus

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ABSTRACT

Background

Amphibians are one of the non-target animals mostly affected by heavy metals. The present study evaluated the effect of lead on hematological parameters of Duttaphrynus melanostictus at different time intervals.

Methods

The animals were randomly divided into control (n=10) and experimental groups (n=10 for each group). The experimental animals were treated orally with lead solution(0.2 microlitre/100g body weight). Total erythrocyte count, total leukocyte count, differential leukocyte count, haemoglobin content and micronucleus test were done in the present study.

Results

The total erythrocyte count and haemoglobin content decreased where as total leukocyte count increased after lead exposure. Increased number of micronuclei were also observed in lead treated animals as compared to control. Number of differential leukocytes also increased as compared to control.

Keywords: Lead, haematological parameters, Duttaphrynus melanostictus, micronucleus test

I. INTRODUCTION

Amphibians are much more susceptible to environmental contaminants due to their biphasic life cycle, non amniotic egg and semi-permeable skin (Blaustein et al., 2003) than that of other vertebrates. Presence of heavy metals in the environment poses significant toxicological risks to a number of non-target organisms and finds its way to the food chain, threatening the ecological balance and biodiversity of nature. Heavy metals specifically lead is a major contributory environmental contaminant which may cause decline of amphibian population (Davidson et al., 2001). In this study the effect of lead on hematological parameters ofDuttaphrynus melanostictuswas investigated.

II. MATERIALS AND METHODS

Adult Duttaphrynus melanostictus (body weight 100g-120g) were captured by hand net from 2016-19 in and around Baripada, Odisha, India. The animals were acclimatized in laboratory condition for 7 days prior to experiment. Then the animals were randomly divided into control (C_o) and experimental (E_{24}, E_{48}, E_{72}) groups. As the natural assimilation pathways of environmental components are the oral and dermal routes, the experimental group of animals were treated with lead solution (0.2micro litre/100g body weight) orally. Blood samples of control and treated animals were collected by cardiac puncture. Total erythrocyte count and total leukocyte count was performed in a Neubauer's chamber. Haemoglobin estimation was done by Sahli's haemoglobinometer.

Two sets (five number of slides were taken) of clean and numbered glass slide one for morphology of RBC and another for differential count were taken. A drop of blood was placed on each slide. All slides were air-dried, fixed with methanol and then stained with Leishman's stain. The above process was repeated five times for each animal. Counting procedures generally followed Davis and Maerz (2008a, b). Erythrocytes and their variations were identified following Hadji-Azimi et al. (1987).

The leukocytes were identified as lymphocytes, monocytes, eosinophils, neutrophils and basophils, following Hadji-Azimi et al. (1987). Slides were viewed in zigzag pattern, covering all parts of the blood smear and all leukocytes were counted in each field of view until 100 cells were counted.

For micronuclei analysis five peripheral blood smears for each animal were prepared on clean slides, fixed and stained by 10% Giemsa stain. The micronucleus frequency was determined in 1000

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erythrocytes from each slide using 100X magnification (Campana et al., 2003). Statistical analysis was done by SPSS 23.0.

III. RESULTS AND DISCUSSION

Total erythrocyte count of lead treated animals were found to be 0.793±0.049227, 0.436 ± 0.027968, 0.434 ± 0.038064 and 0.371 ± 0.03178 $x10^6$ mm^3at 0 hour, 24 hours, 48 hours and 72 hours, respectively (Fig- 2). Short term exposure to lead resulted a significant variation [F (3, 36) = 260.462, P = 0.000] in total erythrocyte count. Total leukocyte count of lead treated animals were found to be 21.55 ± 1.569501, 24.82 ± 2.274154, 28.71± 2.192639, 35.005 ± 1.770193 x 10^3 mm at 0 hour, 24 hours, 48 hours and 72 hours, respectively (Fig- 3). A significant variation [F (3, 36) = 85.918, P = 0.000] in total leukocyte count was observed in lead treated animals. Haemoglobin content of lead treated animals were found to be 10.4 ± 0.625389, 5.8 ± 0.49888, 5.38 ± 0.332666 and 4.66 ± 0.462361 at 0 hour, 24 hours, 48 hours and 72 hours, respectively (Fig- 4). Haemoglobin content showed a significant variation [F (3, 36) = 280.999, P = 0.000] in lead treated animals. Normal mature erythrocyte shape is either oval or circular in shape. Abnormalities in the morphology of erythrocytes were observed (fig-1) in the present study.

Short term exposure to lead resulted in marked decline in total erythrocyte count. Decrease in RBC is accompanied by a significant decrease in haemoglobin content. The decrease in erythrocytes may have been due to haemolysis which is caused by bio-concentration of lead. It might be decreased due to destruction of mature RBCs or inhibition of erythropoiesis due to degeneration erythropoietic tissues in kidney and spleen (Hota, 1995). The results of the study showed that lead induces morphological abnormalitiescould be found due to oxidative damage. Alteration in shape of nuclei could result from aneuploidy which may lead to chromosomal abnormalities. Studies by Ezemony and Enuneku (2011) also suggested declined haematological parameters in Bufo maculatus exposed to cadmium.

Total leukocyte count increased which shows lead induced inflammation (Yagminas et al., 1990). An increase in WBC count was also found in fishes by Sastry and Sharma (1980), Kumar et al., (2017).

Number of lymphocytes of lead treated animals were found to be 35.9±0.99442, 38.6±0.96609, 41.2±0.91893, 41.8±0.63245 after 0 hour, 24 hours, 48 hours and 72 hours, respectively. Adult D. melanostictus exposed to sublethal levels of lead elicited time dependent significant (ANOVA: P<0.05) increase in number of lymphocytes at 24, 48 and 72 hours of exposure period. It is revealed that number of lymphocytes increased significantly [F (3, 36) = 92.158; P = 0.000] from each other at different time intervals after lead treatment.

Number of monocytes of lead treated animals were found to be 8.5±0.52704, 8.7±0.48304, 9.2±0.42163, 11.5±0.70710 after 0 hour, 24 hours, 48 hours and 72 hours, respectively. Adult D. melanostictus exposed to sublethal levels of lead elicited time dependent significant (ANOVA: P<0.05) increase in number of monocytes at 24, 48 and 72 hours of exposure period. It is revealed that number of monocytes increased significantly [F (3, 36) = 64.234; P = 0.000] from each other at different time intervals after lead treatment.

Number of neutrophils of lead treated animals were found to be 31.4±0.69920, 33.8±0.78881, 36.4±0.51639, 36±2.49443 after 0 hour, 24 hours, 48 hours and 72 hours, respectively. Adult D. melanostictus exposed to sublethal levels of lead elicited time dependent significant (ANOVA: P<0.05) increase in number of neutrophils at 24, 48 and 72 hours of exposure period. It is revealed that number of neutrophils increased significantly [F (3, 36) = 27.930; P = 0.000] from each other at different time intervals after lead treatment.

Number of eosinophils of lead treated animals were found to be 15.5±0.84983, 16.9±0.73786, 16.2±0.78881, 12±1.63299 after 0 hour, 24 hours, 48 hours and 72 hours, respectively. Adult D. melanostictus exposed to sublethal levels of lead elicited time dependent significant (ANOVA: P<0.05) increase in number of eosinophils at 24, 48 and 72 hours of exposure period. It is revealed that number of eosinophils increased significantly [F (3, 36) = 41.590; P = 0.000] from each other at different time intervals after lead treatment.

Number of basophils of lead treated animals were found to be 5.7±0.48304, 5.4±0.51639, 5.9±0.31622, 5.1±0.31622 after 0 hour, 24 hours, 48 hours and 72 hours, respectively. Adult D. melanostictus exposed to sublethal levels of lead elicited time dependent significant (ANOVA: P<0.05) increase in number of basophils at 24, 48 and 72 hours of exposure period. It is

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revealed that number of basophils increased significantly [F (3, 36) = 7.000; P = 0.001] from each other at different time intervals after lead treatment.

Besides haematological alteration lead also induce genotoxic effect in amphibians. As a result micronuclei were observed in the present study in an increased number in lead treated animals. Micronuclei may be originated as a result of break down of a chromosome or during the formation of mitotic spindle apparatus (Heddle, 1973). Micronuclei detect chromosomal and genomic mutations after repair.

Number of micronuclei of lead treated animals were found to be 2.3±0.6749486, 8.8±0.6324555, 14.3±0.8232726, 21±0.6666667 after 0 hour, 24 hours, 48 hours and 72 hours, respectively (Fig- 6). Adult D. melanostictus exposed to sublethal levels of lead elicited time dependent significant (ANOVA: $P<0.05$) increase in number of micronuclei at 24, 48 and 72 hours of exposure period. It is revealed that number of micronuclei increased significantly [F (3, 36) = 1280.764; P = 0.000] from each other at different time intervals after lead treatment. Increased micronuclei has been observed in the present study which is in agreement with Mouchet et al (2007) for tadpoles of Xenopus laevis.

IV. CONCLUSION

Haematological analysis help in assessment of toxic effect of heavy metals as well as other contaminants. Biomagnification of heavy metals in amphibians due to heavy metals can serve as a link between terrestrial and aquatic food chains. Sub lethal exposure of amphibians to heavy metals may help in assessing sensitivity than lethal exposure.

REFERENCES

[1]. Blaustein A R, Keisecker J M (2002) Complexity in Conservation: Lessons from the global decline of amphibian populations. Ecology Letters**5**: 597-608.

[2]. Campana M A, Panzeri A M, Moreno V J, Dulout F N (2003) Micronuclei induction in Rana catesbeiana tadpoles by the pyrethroid insecticide lambda-cyhalothrin, Genetics and Molecular Biology**26**: 99–103

[3]. Davidson C H, Shafer B, Jennings M R (2001) Decline of the California red-legged frog: Climate, UV-B, habitat and pesticides hypothesis. Applied Ecology**11:** 464-479.

[4]. Davis, A. K., Maerz J. C. (2008b) Sex-related differences in hematological stress indicesof breeding, paedomorphic mole salamanders. Journal of Herpetology,**42**: 197– 201.

[5]. Davis, A. K., Maerz, J. C. (2008a) Comparison of haematological stress indicators in recently captured and captive paedomorphic mole salamanders, Ambystomatalpoideum. Copeia, 613– 617.

[6]. Ezemonye L I, Enuneku A A (2011) Haematological changes in Bufo maculatus treated with sublethal concentrations of cadmium. Journal of Xenobiotics **1** (1): 7.

[7]. Hadji-Azimi, I., Coosemans, V. and Canicatti, C. (1987) Atlas of adult Xenopus laevis laevis hematology. Developmental and Comparative Immunology,**11**: 807– 874.

[8]. Heddle J (1973) A rapid in vivo test for chromosomal damage. Mutational Research **18** (2): 187-190.

[9]. Hota S (1995) Toxic effect of Arsenic on haemato-biochemical abnormalities in Channa punctatus. Journal of Ecotoxicology and Environmental Monitoring **5:** 249- 255.

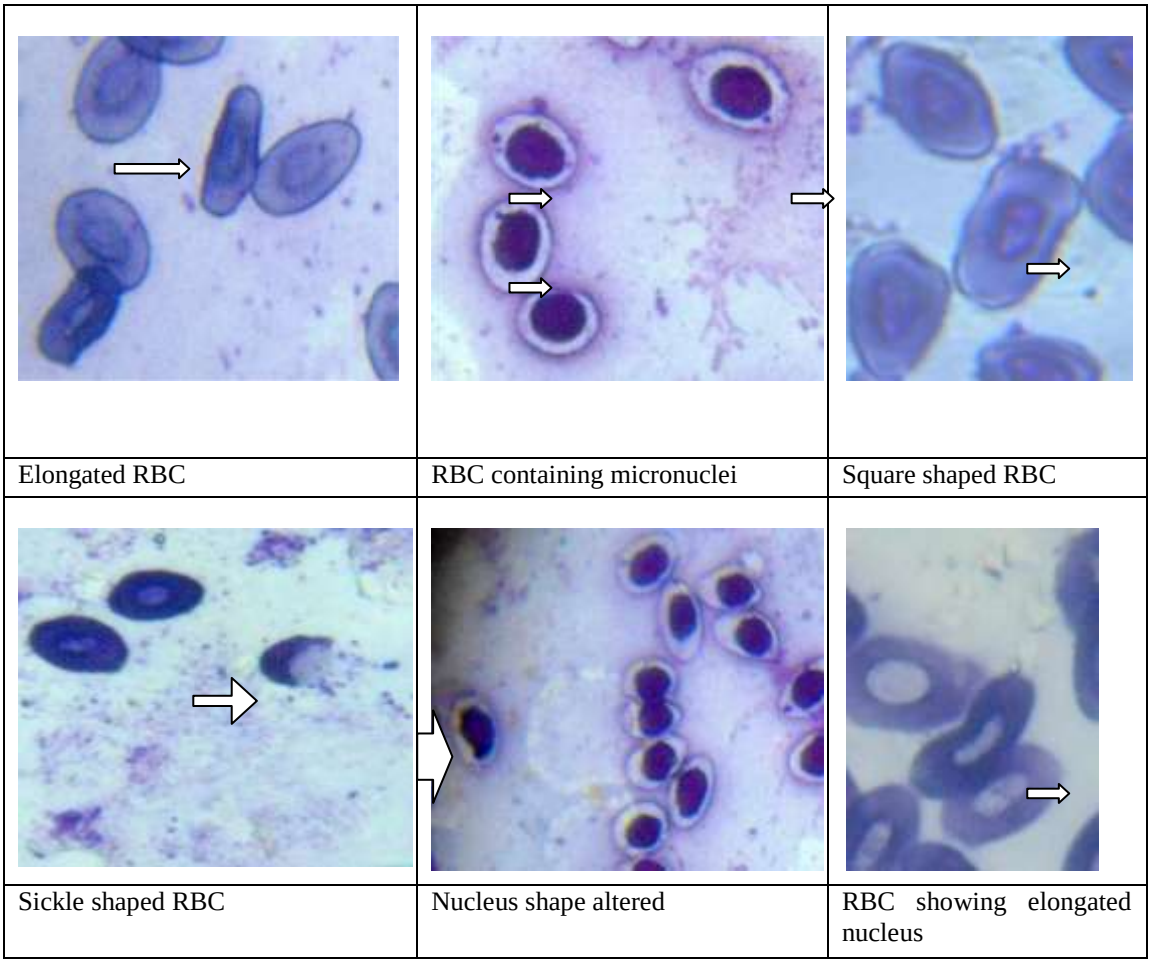
[10]. Krauter P W (1993) Micronucleus incidence and hematologicaleffects in bullfrog tadpoles (Rana catesbeiana) exposed to 2-acetylaminofluorene and 2-aminofluorene, Archives of Environmental Contamination and Toxicology**24**: 487–493.

[11]. Kumar M, Kumar D, Kumar R (2017) Sublethal effects of cadmium and copper on the blood characteristics of cat fish Clarias batrachus (Linn.) International Journal of Advanced Research in Biological science **4**(1): 123-128.

[12]. Mouchet F, Gauthier L, Baudrimont M, Gonzalez P, Mailhes C, Ferrier V, Devaux A (2007) Comparative evaluation of the toxicity and genotoxicity ofcadmium in amphibian larvae (Xenopus laevis and Pleurodeles waltl) using the comet assay and the micronucleus test. Environmental Toxicology**22**: 422–435.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 1113-1117 www.ijprajournal.com ISSN: 2249-7781

[13]. Sastry K V, Sharma K (1980) Mercury induced haematological anomalies in Ophiocephalus (Channa) punctatus. Toxicology letters **5**(3-4): 245-249.
[14]. Yagminas A P, Franklin C A, Villeneuve D C, Gilman A P, Little P B, Valli V E (1990) Subchronic oral toxicity of triethyl lead in the male weanling rat Clinical, biochemical, haematological and histopathological effects. Fundamentals of Applied Toxicology **15**: 580-596.



Elongated RBC	RBC containing micronuclei	Square shaped RBC
Sickle shaped RBC	Nucleus shape altered	RBC showing elongated nucleus

Fig-1: Morphological alteration in cell and nuclei of RBC of lead treated adult Duttaphrynus melanostictus

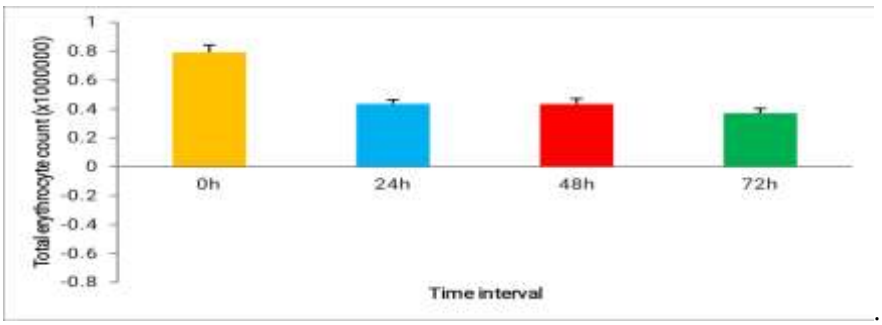


Fig- 2: Total erythrocyte count in lead treated adult D. melanostictus at different time intervals

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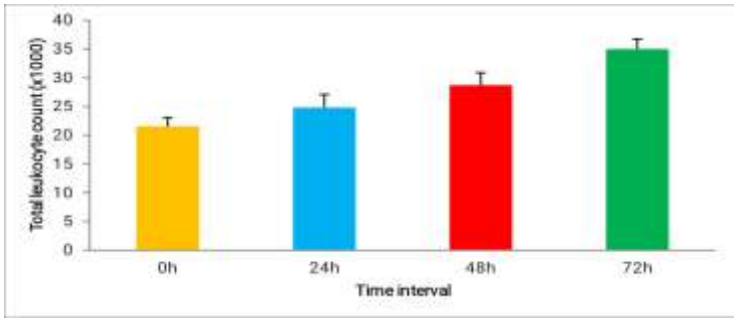

Fig-3: Total leukocyte count in lead treated adult D. melanostictus at different time intervals

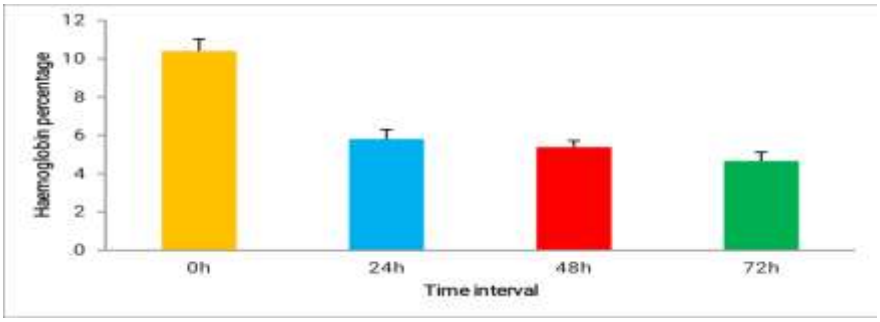

Fig-4: Haemoglobin percentage in lead treated adult D. melanostictus at different time intervals

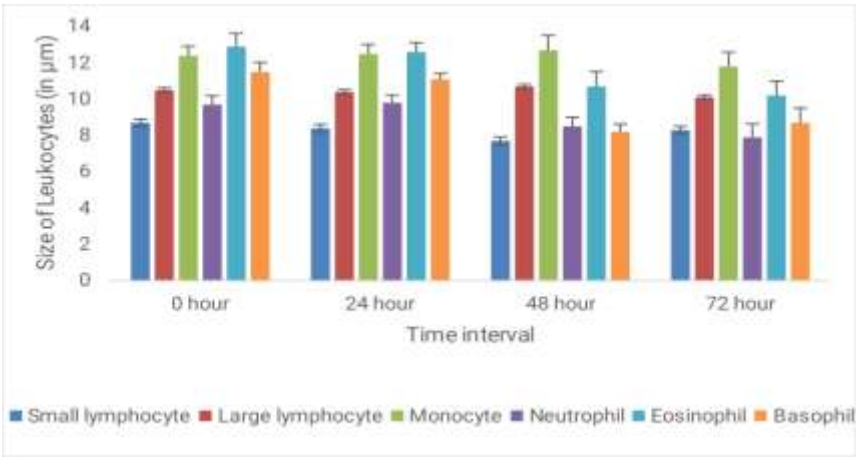

Fig-5: Differential leukocyte count in lead treated adult D. melanostictus at different time intervals

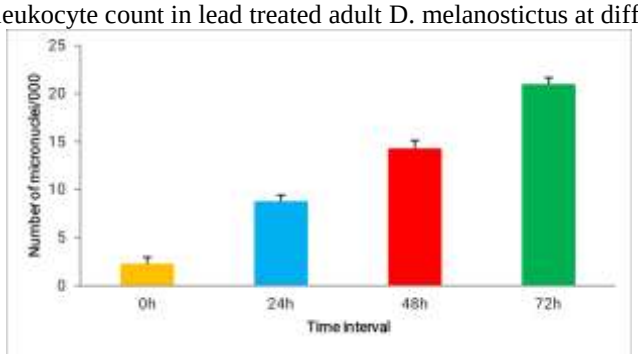

Fig- 6: Number of micronuclei in lead treated adult D. melanostictus at different time intervals