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EFFECT OF ROUNDUP® HERBICIDE ON PHYSIOLOGICAL INDICES IN MARSH FROG *PELOPHYLAX RIDIBUNDUS*

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Abstract

This study was carried out to evaluate the effect of Roundup® on physiological indices (number of erythrocytes, leukocytes, glycaemia, hepatosomatic index, and cholesterol and trygliceride value) in marsh frog Pelophylax ridibundus. Adult male and female frogs were exposed to 0.138x10^{-3} ml Roundup®/g of body weight administrated by intraperitoneal injection, 1 injection at 2 days in a scheme of 3 weeks. The animals were kept at 4-6˚C, respectively at 22-24˚C in tap water tank. We observed a decreased number of erythrocytes and glycaemia, increased plasma cholesterol and triglyceride, increased number of leukocytes, and hepatosomatic index value. These changes were more powerful at 22-24ºC than at 4-6ºC.

INTRODUCTION

In human-dominated ecosystems, organisms can be confronted with multiple environmental stressors, of both natural and anthropogenic origins [12]. In this regard, considerable research efforts have focused on the stress factors contributing to the global decline of amphibian populations across the globe. Potential causes include habitat destruction or fragmentation, climate change, introduced predators or competitors, diseases, and the presence of chemical contaminants [3, 5, 6, 8, 14].

The indiscriminate use of herbicide, careless handling, accidental spillage, or discharges of treated effluents into natural waterways have harmful effects on the fish population and other forms of aquatic life and may contribute long term effects in the environment [2].

Herbicides are actively used in terrestrial and aquatic ecosystems to control weeds, and their use has generated serious concerns about the potential adverse effects of these chemicals on the environment and human health [11].

The formulation of Roundup® consists of the herbicide glyphosate as the active ingredient with polyethoxylene amine added as a surfactant. Glyphosate is a non-selective, systemic herbicide that can control most annual and perennial plants. It controls weeds by inhibiting the synthesis of aromatic amino acids necessary for protein formation in susceptible plants.

The acute toxicity of Roundup® (particularly of glyphosate) to animals is considered to be low according to the World Health Organization [15], but the extensive use of Roundup® may still cause environmental problems with negative impact on wildlife, particularly in an aquatic environment where chemicals may persist for a long time. Some surfactants that are included in some formulations of glyphosate, however, are highly toxic to aquatic organisms.

Therefore, in this paper we studied the effects of Roundup® on some hematological and biochemical parameters in marsh frog (*Pelophylax ridibundus*) at two thermic level (4-6ºC and 22-24ºC).

MATERIAL AND METHODS

In present study we used of *Pelophylax ridibundus*, adults of both sexes, captured in spring (April-May) from the surrounding areas of the city Piteşti (South Romania). The animals were kept in laboratory condition in aquaterrarios filled with tap water for five days to test their health and accommodate them for the experiment. The water was changed daily to avoid the accumulation of toxic substances.

After 10 days of adaptation in the lab, the frogs were separated in lots, which were used separately for the following experiments: two lots of control individuals, containing animals kept in laboratory at 4-6^{o}C, respectively at 22-24ºC with no treatment, in running water which was changed everyday, (1) one lot containing animals which were subjected to treatment with Roundup® herbicide in a dose of 0.138 x 10^{-3} ml/g of body weight and kept at 4-6ºC, (2) a second lot containing animals which were subjected to treatment with Roundup® herbicide in a dose of 0.138 x 10^{-3} ml/g of body weight and kept at 22-24ºC in a thermostatic chamber. Ten animals were used for each lot.

The toxic was administered by intraperitoneal shots, one shot every two days, in a scheme of 3 weeks. The administered dosage of toxic was not lethal as none of the subjects died through the experiment.

At the end of treatment blood specimens were withdrawn from the frogs by cardiac puncture after chloroform anesthesia. The values of operational factors under discussion were determined by using standard automated method: number of erythrocytes and leukocytes was microscopically determined with a Thoma cells numbering chamber [13]; the glycaemia, cholesterol and triglycerides level has been determinate using an Accutrend GCT.

The hepatosomatic index was calculated using formula:

$$HSI = \frac{liverweight}{bodyweight} \times 100$$

Hematological, biochemical and HIS results were expressed as means ± standard deviation (SD). Statistical analysis was performed as control lot versus treated lot using the Student's t-test. The chosen level of significance is $p<0.05$.

RESULTS AND DISCUSSION

Hematological and biochemical parameters are suitable tools for assessing environmental influences and stress effects of anthropogenic origin on the condition and health of aquatic vertebrates [7].

The action of Roundup® on the liver occurs primarily by increasing the value of hepatosomatic index for the two groups studied (Figure 1). In animals treated with these pesticide and kept at a temperature of 4-6ºC, the index value increased by 16.27% compared to the control value, while in animals kept at a temperature of 22-24ºC and treated with the same concentration of pesticide, the index value increased by 25%.

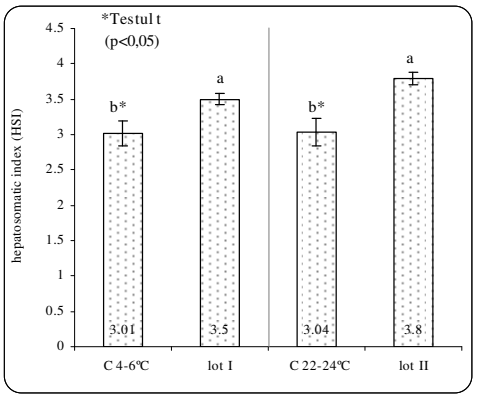

Fig. 1. The influence of Roundup® upon hepatosomatic index in marsh frog

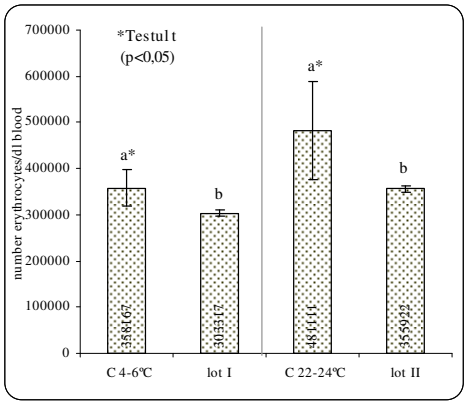

Fig. 2. The influence of Roundup® upon number of erythrocytes in marsh frog

(b* p<0.05 vs control, a p<0.05 vs treated lot)

A slight increase in the HSI of exposed frog indicates that the liver cells were affected possibly causing an increase in the rate of production of endoplasmic reticulum for the synthesis of protein in liver tissue [4]. The liver is responsible for enzymatic decontamination process, production and storage of glycogen as energy reserves. Therefore, in the presence of stressors, these qualities are altered resulting in deleterious effect on the frog [1, 10].

Similar changes in the values of organosomatic indices (hepatosomatic index - HIS, cardiosomatic index - CSI, renatosomatic index - RSI and spleenosomatic index - SSI) was observed by Gabriel et al. [9] in *Clarias gariepinus* after administreted of aqueous extracts of leaves of *Lepidagathis alopecuroides.*

The hematological picture shows anemia associated with Roundup® administration in both groups of animals. Thus, there is a decreased number of red cells by 15.31% compared to control value for animals kept at a temperature of 4-6ºC, and 26.02% compared to control value for animals kept at 22-24ºC (Figure 2).

Increase in number of leukocytes (leukocytosis) may have resulted from the excitation of the defense mechanism to counter the effect of the toxicant (Figure 3). The analysis of this figure shows an increase by 18.86% in the number of white blood cells compared to control value in animals treated with Roundup® and kept at a temperature of 4-6ºC, while for animals treated with the same concentration of toxic and kept a temperature of 22-24ºC, the increase is 25.96% compared to the control value.

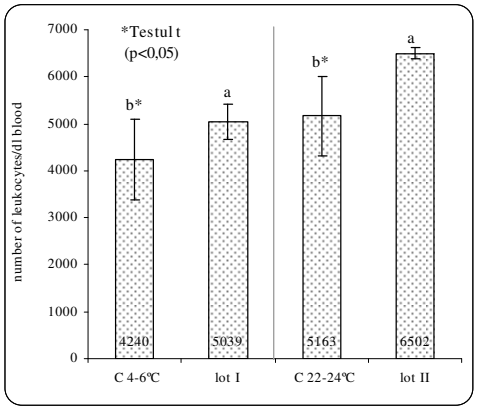

Fig. 3. The influence of Roundup® upon number of leukocytes in marsh frog

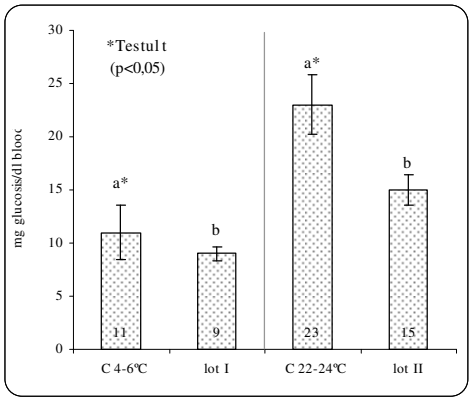

Fig. 4. The influence of Roundup® upon glycaemia in marsh frog

(b* p<0.05 vs control, a p<0.05 vs treated lot)

Biochemical diagnoses provided additional data that have completed the body damage picture induced by this toxic action. Thus, both studied groups show a decrease of blood glucose by 18.18% compared to control group for animals kept at 4-6ºC and 34.78% for animals kept at 22-24ºC (Figure 4).

The toxic substance also works by changing the quantity of plasma cholesterol (Figure 5) and triglycerides (Figure 6). Plasma cholesterol records an increased value by 10.60% compared to control group for animals treated with Roundup® and kept at a temperature of 4-6ºC, and 17.85% compared to control for animals kept at a temperature of 22-24ºC. In terms of triglyceride level, there was an increase of their value in both groups studied by 15.25% compared to control group for animals kept at 4-6ºC, and 25% for animals kept at 22-24ºC.

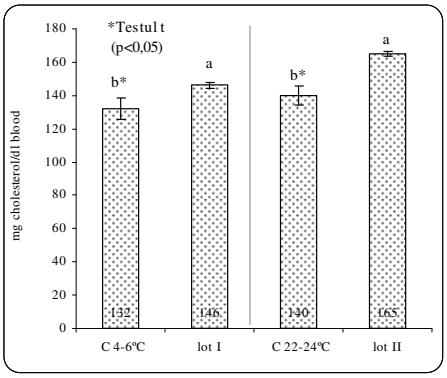

Fig. 5. The influence of Roundup® upon cholesterol level in marsh frog

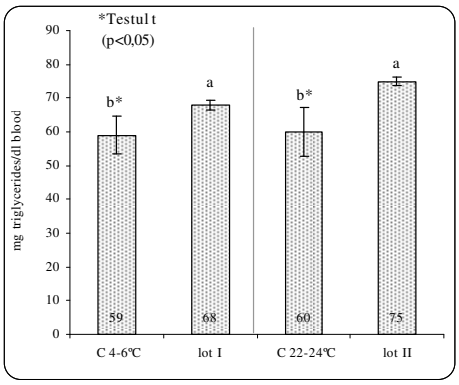

Fig. 6. The influence of Roundup® upon triglycerides level in marsh frog

(b* p<0.05 vs control, a p<0.05 vs treated lot)

CONCLUSIONS

1. After 3 weeks of treatment with sublethal doses of Roundup® herbicide in marsh frog (*Pelophylax ridibundus*) we observed a decreased number of erythrocytes and glycaemia, increased plasma cholesterol and triglyceride, increased number of leukocytes, and hepatosomatic index value.
2. These changes were more powerful in animals treated with toxic substances and kept at 22-24ºC.

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