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Biology

SEASONAL VARIATION ON ANTIOXIDANT SYSTEM OF LIVER OF *DUTTAPHRYNUS MELANOSTICTUS*

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ABSTRACT Body condition a reliable indicator of energetic condition, has an important fitness consequence. Natural population of *Duttaphrynus melanostictus* in response to environmental cues shows several physiologic changes such as reproductive activity, hibernation, aestivation and metabolic depression in different seasons. *Duttaphrynus melanostictus* were collected from local area in North Orissa University in Baripada, Mayurbhanj in different seasons like summer, rainy, winter. We evaluated the seasonal variation of liver and kidney tissue in Asian common toad *Duttaphrynus melanostictus* in different statistical methods. The lipid peroxidation (LPX), reduced glutathione (GSH), protein content activity in liver tissue were measured in different season.

KEYWORDS : Protein, LPX, GSH, *Duttaphrynus melanostictus*

INTRODUCTION

Amphibians may signal environmental stress, including pollution, earlier than most organisms and may serve as critical bio-indicators to study environmental physiology and health of ecosystems. Although they are considered as good bioindicators (due to their poikilothermic nature) to study the physiologic effects of habitat changes, few data are available concerning fluctuation of their biochemical pathways in natural populations (Norris and Jones 2012). Recently, much attention has been paid to find out suitable physiological biomarkers related to redox state in poikilotherms and ectotherms, which can be used to monitor environmental impacts and effects of pollution, and will also improve the understanding of environmental physiology of these organisms (Chainy et al. 2016). Amphibians are important components of aquatic habitats, especially in tropical regions of the world (Mc Diarmid, 1992). One of the non-target biological groups mostly affected by pesticides is amphibians (Fulton and Chambers, 1985; Berrill *et al.*, 1994; Sparling *et al.*, 2001). Amphibians are important organisms in the aquatic and agricultural ecosystems; they are among the most important natural enemies of many agricultural pests. Amphibians have been the focus of attention owing to reports on population declines and species extinctions from many parts of the world. Most amphibians live in two habitats and their sensitivity to changes in environment makes them good indicators of ecosystem's health. The decline of world populations of amphibians is a major environmental issue. Amphibians are an integral part of their ecosystems; affecting nutrient cycling and also serving as high quality prey for many species. The ecological effects of pesticides on amphibian populations are a growing concern (Bishop, 1992; Hall and Henry, 1992 and Phillips, 1994).

In the present work liver tissue of toad were studied in different seasons like summer, rainy,winter. Then different biochemical parameters were measured.

MATERIALS AND METHODS

Duttaphrynus melanostictus (70 g to 120) g were collected during night and early morning time locally in Maharaja Sriram Chandra Bhanja Deo University in Baripada, Mayurbhanj in different season (2016 to 2019).They were kept in laboratory for the experiment. The animals were kept in bottle and sacrificed at different seasons (summer, rainy and winter) and different parameters were measured. Body weight of *Duttaphrynus melanostictus* were measured by digital monopan balance (Shimadzu; ELB 300) and tissue (liver) also measured . The tissues are dissected out quickly and kept at 0°C. A 20% homogenate was prepared in ice-cold 50 mM phosphate buffer (pH 7.4) using pre-chilled porcelain mortar and pestle by up and down strokes at 4°C. The homogenate was centrifuged at 4000 rpm (1000Xg) for 10 minutes at 4°C in Cooling Centrifuge (Remi). The supernatant was taken for biochemical assay.

Protein: Protein estimation of samples were measured according to the method of Lowry *et al*., (1961).

Lipid Peroxidation (LPX): Lipid peroxidation estimation of samples were measured according to the method of Ohkawa *et al*., (1979).

Reduced Glutathione (GSH): Reduced glutathione estimation of samples were measured according to the method of Ellman (1959).

RESULTS AND DISCUSSIONS

Liver

Protein content

Protein content (mg/g tissue) in liver of *D. melanostictus* in summer 33.7570 ± 2.26896, rainy 35.4020 ± 3.54648 and in winter 45.2210 ± 3.20174 respectively. Protein content (mg/g tissue) decreased in summer comparison to rainy and winter season. (Fig 1). One way ANOVA was performed in order to analyze the effect of season on the protein content at different seasons in *D. melanostictus.* One way ANOVA revealed that the protein content at different seasons in liver of toad is significant [F(2,29) = 41.202, P =0.000]. Post Hoc analysis revealed that the protein content at different seasons in *D. melanostictus* was significant at summer, winter and rainy (P < 0.05; LSD).

Reduced Glutathione (GSH)

Reduced Glutathione (mg of GSH/g tissue) level in liver of *D. melanostictus* in summer 0.05310 ± 0.00252, rainy 0.06032 ± 0.028093 and in winter 0.03639 ± 0.002311 respectively. Reduced Glutathione (mg of GSH/g tissue) decreased in winter.

It was higher in rainy season comparison to liver of toad in reduced glutathione level in winter and summer season.(Fig2) . One way ANOVA was performed in order to analyze the effect of season on the reduced glutathione level at different seasons. One way ANOVA revealed that the reduced glutathione level at different seasons in liver of *D. melanostictus* significant [F(2,29) = 5.643, P =0.009].Post Hoc analysis revealed that the reduced glutathione level at different seasons in *D. melanostictus* was significant at summer, winter and rainy (P < 0.05; LSD).

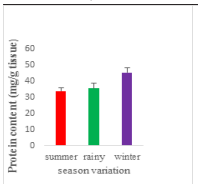

Fig.1: Comparison of protein content (mg/g tissue) of the *D.melanostictus* in liver at seasonal variation

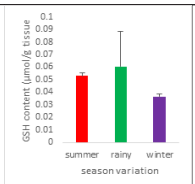

Fig 2: Comparison of reduced glutathione content (µmol/mg protein) of the *D. melanostictus* in liver at seasonal variation.

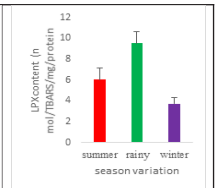

Fig 3: Comparison of lipid peroxidation content (nmol/TBARS/mg protein) of the *D.melanostictus* in liver at seasonal variation.

Lipid Peroxidation

Lipid Peroxidation (n mol of TBARS/mg tissue) in liver of *D. melanostictus* in summer 6.04884 ± 1.119893, rainy 9.5135 ± 1.155979 and in winter 3.70446 ± 0.638586 respectively. Lipid Peroxidation (n mol of TBA-RS/mg tissue) decreased in winter comparison to liver of *D. melanostictus* lipid peroxidation content in summer and rainy. The lipid peroxidation content was highest in rainy

season (Fig3). One way ANOVA was performed in order to analyze the effect of season on the ascorbic acid content at different seasons. One way ANOVA revealed that the lipid peroxidation content at different seasons in liver of *D. melanostictus*is significant [F(2,29) = 85.458, P =0.000]. Post Hoc analysis revealed that the reduced glutathione level at different seasons in *Dmelanostictus* was significant at summer, winter and rainy ($P < 0.05$; LSD).

CONCLUSION

The present study was undertaken with an objective to explore eco-physiological interaction, to study environmental impact or risk assessment in the natural population of a poikilotherm *Duttaphrynus melanostictus* considering OS physiology parameters as markers.The results of present investigation showed that seasonal variation gave significant information in liver and kidney tissue of *Duttaphrynus melanostictus*.

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