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Original Review Article

ANTIOXIDANT SYSTEM OF LIVER AND KIDNEY OF *DUTTAPHRYNUS MELANOSTICTUS* IN DIFFERENT SEASON

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Abstract

Objective

Body condition a reliable indicator of energetic condition, has an important fitness consequence. The 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.

Methods

*Duttaphrynus melanostictus*were collected from the 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 the Asian common toad *Duttaphrynus melanostictus*in different statistical methods. The lipid peroxidation (LPX), ascorbic acid (ASA), lipid, reduced glutathione (GSH), protein content superoxide dismutase (SOD), catalase (CAT) activity in liver and kidney tissue were measured indifferent season.

Results

Results of the present study show both tissue's protein content highest in winter, GSH content highest in rainy, LPX content highest in rainy, ASA content highest in summer, lipid content highest in rainy, SOD content highest in summer, and catalase content highest at winter season.

Conclusion:

The current study sought to investigate eco-physiological interactions, as well as to assess environmental impact or risk in the natural population of the poikilotherms*Duttaphrynus melanostictus* using OS physiology parameters as markers. The current study found that seasonal variation provided significant information in the liver and kidney tissue of Duttaphrynus melanostictus.

Keywords: ASA, CAT,*Duttaphrynus melanostictus*, GSH, Lipid, LPX, Protein, SOD

INTRODUCTION

Amphibians may signal environmental stress, including pollution, earlier than most organisms and may serve as critical bio-indicators to study environmental physiology and the health of ecosystems. Although they are considered good bioindicators (due to their poikilothermic nature) to study the physiologic effects of

habitat changes, few data are available concerning the fluctuation of their biochemical pathways in natural populations. [1] Recently, much attention has been paid to find out suitable physiological biomarkers related to the 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 the environmental physiology of these organisms.[2] Amphibians are important components of aquatic habitats, especially in tropical regions of the world.[6] One of the non-target biological groups that are mostly affected by pesticides is amphibians.[3,4,7] 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.[5, 8,9]

MATERIALS AND METHODS

Duttaphrynus melanostictus (70 g to 120 g) were collected during the night and early morning time locally atNorth Orissa University in Baripada, Mayurbhanj in a different season (2016 to 2019). The animals were kept in a bottle and sacrificed during different seasons (summer, rainy, and winter) and different parameters were measured.The bodyweight of *D. melanostictus* was measured by digital monopan balance (Shimadzu; ELB 300) and tissues (liver and kidney) were also measured. The tissues are dissected 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^{o}C. The homogenate was centrifuged at 4000 rpm (1000Xg) for 10 minutes at 4^{o}C in Cooling Centrifuge (Remi). The supernatant was taken for biochemical assay.

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

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

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

Lipid: Lipid estimation of samples was measured according to the method of Folch *et al., (*1957).

Ascorbic acid (ASA): Ascorbic acid estimation of samples was measured according to the method of Jagota and Dani (1982).

Superoxide dismutase (SOD) activity: Superoxide dismutase(SOD; EC 1.15.1.1) activity was measured according to the method of Das *et al.,* (2000). SOD activity was expressed as units/mg of protein.

Catalase(CAT)activity:

Catalase (CAT; EC 1.11.1.6) activity was estimated according to Beers and Sizer (1952). The activity of catalase was expressed as nkat/mg protein (1nkat=1mole of substrate converted to product per sec, 1U=16.67 nkat).

RESULTS AND DISCUSSIONS

Liver

Protein content

Protein content (mg/g tissue) in the liver of *D. melanostictus*in summer was 33.7570 ± 2.26896, rainy35.4020 ± 3.54648, and in winter 45.2210 ± 3.20174 respectively. Protein content (mg/g tissue) decreased in summer in comparison to the rainy and winter season. (Fig 1). One-way ANOVA was performed 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 the liver of toads 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 in summer, winter, and rainy ($P < 0.05$; LSD).

Reduced Glutathione (GSH)

Reduced Glutathione (mg of GSH/g tissue) level in the liver of *D. melanostictus* in summer 0.05310 ± 0.00252, rainy0.06032 ± 0.028093, and winter 0.03639 ± 0.002311 respectively. Reduced Glutathione (mg of GSH/g tissue) decreased in winter.

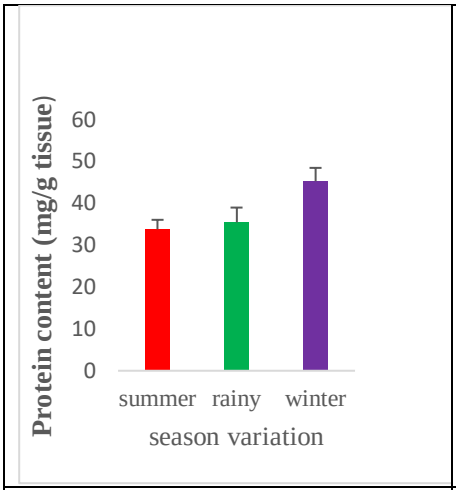

Fig.1: Comparison of protein content (mg/g tissue) of the *D.melanostictus* in liver at seasonal variation

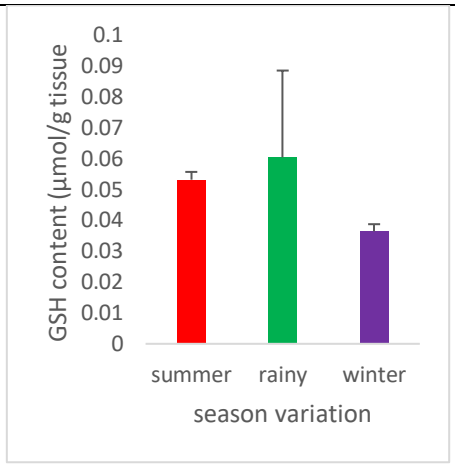

Fig 2: Comparison of reduced glutathione content (µmol/mg protein) of the *D.melanostictus* in liver at seasonal variation.

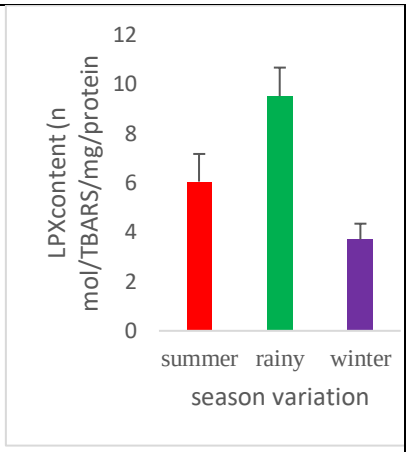

Fig 3: Comparison of lipid peroxidation content (nmol/TBARS/mg protein) of the *D.melanostictus* in liver at seasonal variation.

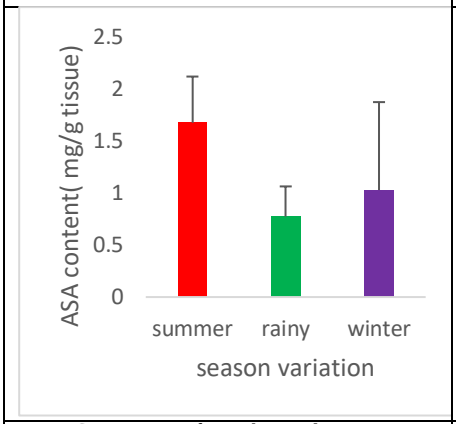

Fig 4: Comparison of ascorbic acid content (mg/ml) of the *D.melanostictus* in liver at seasonal variation.

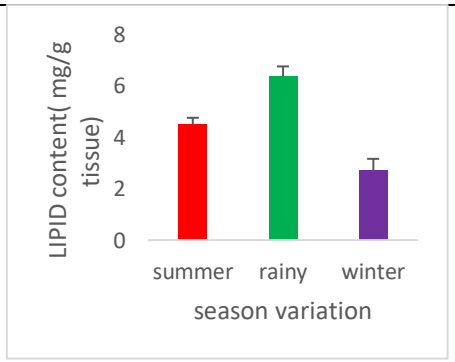

Fig 5: Comparison of lipid content (mg/g) of the *D.melanostictus* in liver at seasonal variation.

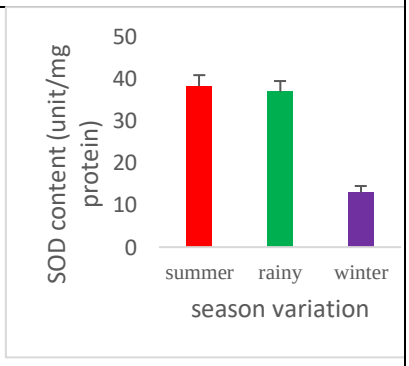

Fig 6: Comparison of superoxide dismutase activity (unit/mg protein) of the *D.melanostictus* in liver at seasonal variation.

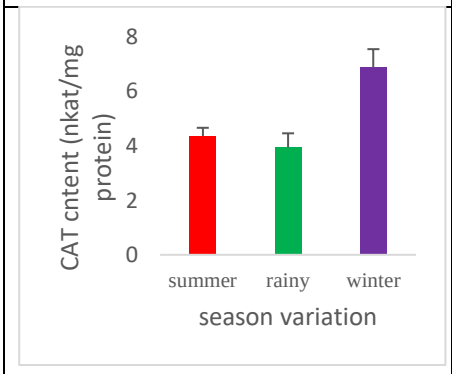

Fig 7: Comparison of catalase activity (nkat/mg protein) of the *D.melanostictus* in liver at seasonal variation.

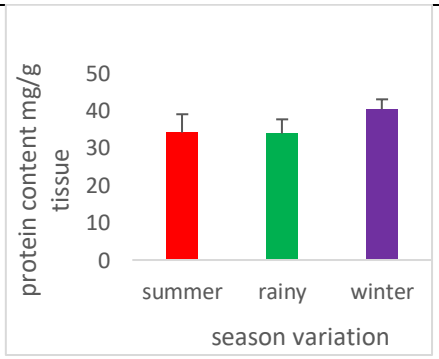

Fig 8: Comparison of protein content (mg/g tissue) of the *D.melanostictus* in kidney at seasonal variation.

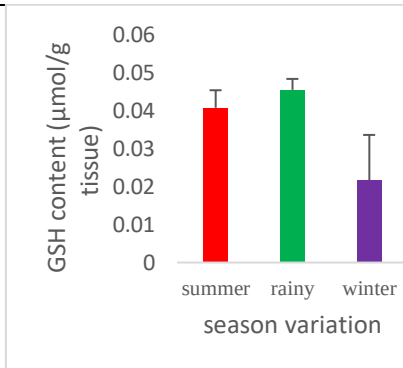

Fig 9: Comparison of reduced glutathione content (µmol/mg protein) of the *D.melanostictus* in kidney at seasonal variation.

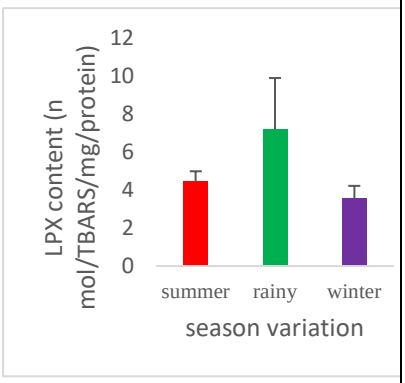

Fig 10: Comparison of lipid peroxidation content (nmol/TBARS/mg protein) of the *D.melanostictus* in kidney at seasonal variation.

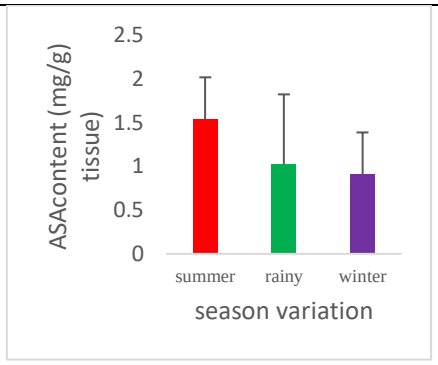

Fig11:Comparison of ascorbic acid content (mg/g) of the *D.melanostictus* in kidney at seasonal variation.

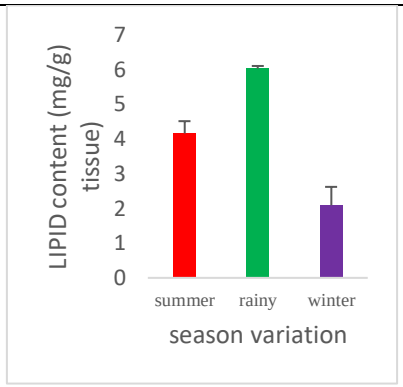

Fig 12: Comparison of lipid content(mg/g)of the *D.melanostictus* in kidney at seasonal variation.

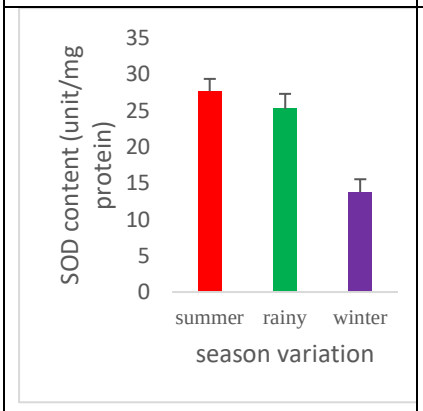

Fig 13: Comparison of superoxide dismutase activity (unit/mg protein) of the *D.melanostictus* in kidney at seasonal variation.

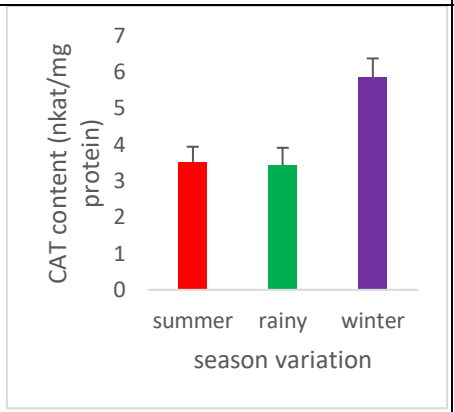

Fig 14:Comparison of catalase activity (nkat/mg protein) of the *D.melanostictus* in kidney at seasonal variation.

It was higher in the rainy season in comparison to the liver of toads with reduced glutathione levels in the winter and summer seasons.(Fig2). One-way ANOVA was performed 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*was 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 in summer, winter, and rainy ($P < 0.05$; LSD).

Lipid Peroxidation

Lipid Peroxidation (nmol of TBARS/mg tissue) in the liver of *D. melanostictus* in summer 6.04884 ± 1.119893, rainy9.5135 ± 1.155979, and in winter 3.70446 ± 0.638586 respectively. Lipid Peroxidation (nmol of TBA-RS/mg tissue) decreased in winter in comparison to liver of *D. melanostictus* lipid peroxidation content in summer and rainy. The lipid peroxidation content was highest in the rainy season(Fig3). One-way ANOVA was performed 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 the 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 in summer, winter, and rainy (P < 0.05; LSD).

Ascorbic acid content

Ascorbic acid content (mg/g tissue) in liver of *Duttaphrynus melanostictus* in summer 1.67196 ± 0.0443129, rainy0.77116 ± 0.290251 and in winter 1.02666± 0.843293 respectively. Ascorbic acid content (mg/g tissue) decreased in the rainy seasonin comparison to winter and summer.The ascorbic acid content was highest in summer season (Fig4). 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 ascorbic acid content at different seasons in liver of *Duttaphrynus melanostictus*is significant [F(2,29) = 47.319, P =0.000]. Post Hoc analysis revealedthat the ascorbic acid content at different seasons in *Duttaphrynus melanostictus* was significant in summer, winter, and rainy (P < 0.05; LSD).

Lipid content

Lipid content (mg/g tissue) in liver of *D. melanostictus* in summer was 4.4904 ± 0.267548, rainy6.37407± 0.382462, and in winter 2.96706 ± 0.464271 respectively. Lipid content (mg/g tissue) decreased in winter in comparison to the liver of *D. melanostictus* lipid content in summer and rainy.The lipid content was highest in the rainy season (Fig5). One-way ANOVA was performed to analyze the effect of season on the ascorbic acid content at different seasons. One-way ANOVA revealed that the lipid content at different seasons in liver of *D. melanostictus*is significant [F(2,29) = 247.073, P =0.000]. Post Hoc analysis revealed that the lipid content at different seasons in *D. melanostictus* was significant in summer, winter, and rainy (P < 0.05; LSD).

Superoxide dismutase activity

SOD activity (unit/mg protein) liver of *Duttaphrynus melanostictus* in summer 38.21433 ± 2.598827, rainy36.91667± 2.497354, and in winter 12.96899± 1.535046 respectively. It was lower in winter in comparison to SOD activity in summer and winter seasons. The Superoxide dismutaseactivitywas highest in summer (Fig6). One-way ANOVA was performed to analyze the effect of season on the SOD activity at different seasons. One-way ANOVA revealed that the SOD activity at different seasons in liver of *Duttaphrynus melanostictus*is significant [F(2,29) = 395.033, P =0.000]. Post Hoc analysis revealed that the SOD activity at different seasons in *Duttaphrynus melanostictus* was significant in summer, winter, and rainy (P < 0.05; LSD).

Catalase activity(CAT)

Catalase activity (nkat/mg protein) liver of *D. melanostictus* in summer 4.33941 ± 0.306325, rainy3.93116± 0.512716, and in winter 6.84927± 0.673141 respectively. It was lower in the rainy season in comparison to catalase activity in the summer and winter seasons. The catalaseactivitywas highest in the winter season (Fig7). One-way ANOVA was performed to analyze the effect of season on the catalase activity at different seasons. One-way ANOVA revealed that the catalase activity at different seasons in the liver of *D. melanostictus*was significant [F(2,29) = 92.497, P =0.000]. Post Hoc analysis revealed that the catalase activity at different seasons in *D. melanostictus* was significant in summer, winter, and rainy (P < 0.05; LSD).

Kidney

Protein content

Protein content (mg/g tissue) in the kidney of *D. melanostictus*in summer was 34.0820± 4.887917, rainy33.8020 ± 3.80786, and in winter 40.3210± 2.637614 respectively. Protein content (mg/g tissue) decreased in summer. It was lower in summer in comparison to protein content in the rainy and winter season. The protein content was highest in winter (fig8).

One-way ANOVA was performed to analyze the effect of season on the protein content at different seasons in *Duttaphrynus melanostictus*. One way ANOVA revealed that the protein content at different seasons in the liver of *Duttaphrynus melanostictus* is

significant [F(2,29) = 8.986, P =0.001]. Post Hoc analysis revealed that the protein content at different seasons in *Duttaphrynus melanostictus* was significant in summer, winter, and rainy (P < 0.05; LSD).

Reduced Glutathione (GSH)

Reduced Glutathione (mg of GSH/g tissue) level in the kidney of *Duttaphrynus melanostictus* in summer was 0.04064 ±0.004613, rainy0.04535 ± 0.002878 and in winter 0.002165 ± 0.011868 respectively. Reduced Glutathione (mg of GSH/g tissue) decreased in winter. It was lower in the winter season in comparison to the liver of toadin reduced glutathione levels in the rainy and summer seasons. The reduced glutathione level was highest in the rainy season (Fig9). One-way ANOVA was performed 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 thekidney of *Duttaphrynus melanostictus* is significant [F(2,29) = 67.544, P =0.000]. Post Hoc analysis revealed that the reduced glutathione level at different seasons in *Duttaphrynus melanostictus* was significant in summer, winter, and rainy (P < 0.05; LSD).

Lipid Peroxidation content

Lipid Peroxidation (nmol of TBARS/mg tissue) in the kidney of *Duttaphrynus melanostictus* in summer 4.43633 ± 0.540032, rainy7.17878 ± 2.692216 and in winter 3.57092 ± 0.639147 respectively. Lipid Peroxidation (nmol of TBA-RS/mg tissue) decreased in winter. It was lower in winter in comparison to the kidney of *Duttaphrynus melanostictus* lipid peroxidation content in summer and winter. The lipid peroxidation content was highest in the rainy season(Fig10). One-way ANOVA was performed to analyze the effect of season on the lipid peroxidation content at different seasons. One-way ANOVA revealed that the lipid peroxidation content at different seasons in the kidney of *Duttaphrynus melanostictus* significant [F(2,29) = 13.391, P =0.000]. Post Hoc analysis revealed that the lipid peroxidation at different seasons in *Duttaphrynus melanostictus* was significant in summer, winter, and rainy (P < 0.05; LSD).

Ascorbic acid content

Ascorbic acid content (mg/g tissue) inthe kidney of *Duttaphrynus melanostictus* in summer 1.53488 ± 0.479432, rainy1.01852 ± 0.801027, and in winter 0.90859± 0.476984 respectively. Ascorbic acid content mg/g tissue) decreased in the winter season. It was lower in comparison to rainy and summer. The ascorbic acid content was highest in the summer season (Fig111). One-way ANOVA was performed to analyze the effect of season on the ascorbic acid content at different seasons. One way ANOVA revealed that the ascorbic acid content at different seasons in the kidney of *Duttaphrynus melanostictus* is significant [F(2,29) = 5.355, P =0.011]. Post Hoc analysis revealed that the reduced glutathione level at different seasons in *Duttaphrynus melanostictus* was significant in summer, winter, and rainy (P < 0.05; LSD).

Lipid content

Lipid content (mg/g tissue) in the kidney of *Duttaphrynus melanostictus* in summer was 4.15779 ± 0.354265, rainy6.0247± 0.07655,1, and in winter 2.08865 ± 0.531543 respectively. Lipid content(mg/g tissue) decreased in winter. It was lower in winter in comparison to the liver of *Duttaphrynus melanostictus* lipid content in summer and winter. The lipid content was highest in the rainy season(Fig12). One-way ANOVA was performed to analyze the effect of season on the lipid content at different seasons. One-way ANOVA revealed that the lipid content at different seasons in the kidney of *Duttaphrynus melanostictus* significant [F(2,29) = 295.196, P =0.000]. Post Hoc analysis revealed that the lipid content at different seasons in the kidney of *Duttaphrynus melanostictus* was significant at summer, winter, and rainy (P < 0.05; LSD).

Superoxide dismutase activity

SOD activity (unit/mg protein) kidney of *Duttaphrynus melanostictus* in summer 27.61103 ± 1.695045, rainy25.17678± 2.069028, and in winter 13.72702 ± 1.77898 respectively. It was lower in

winter in comparison to SOD activity in the summer and rainy seasons. The Superoxide dismutaseactivitywas highest in summer (Fig13). One-way ANOVA was performed to analyze the effect of season on the SOD activity at different seasons. One-way ANOVA revealed that the SOD activity at different seasons in thekidney of *Duttaphrynus melanostictus*is significant [F(2,29) = 169.390, P =0.000]. Post Hoc analysis revealed that the SOD activity at different seasons in the kidney of *Duttaphrynus melanostictus* was significant in summer, winter, and rainy (P < 0.05; LSD).

Catalase activity(CAT)

Catalase activity (nkat/mg protein) kidney of *Duttaphrynus melanostictus* in summer 3.5210 ± 0.415968, rainy3.43476± 0.47366 and in winter 5.84041± 0.527445 respectively. It was lower in the rainy season in comparison to catalase activity in the summer and winter seasons. The catalaseactivitywas highest in the winter season (Fig14). One-way ANOVA was performed to analyze the effect of season on the catalase activity at different seasons. One-way ANOVA revealed that the catalase activity at different seasons in the kidney of *Duttaphrynus melanostictus* is significant [F(2,29) = 83.090, P =0.000]. Post Hoc analysis revealed that the catalase activity at different seasons inthe kidney of *Duttaphrynus melanostictus* was significant in summer, winter, and rainy (P < 0.05; LSD).

CONCLUSION

The present study was undertaken 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 the present investigation showed thatseasonal variation gave significant information in the liver and kidney tissue of *Duttaphrynus melanostictus*.

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