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EFFECT OF COLD SHOCK ON LIPID PEROXIDATION AND REDUCED GLUTATHIONE LEVEL OF THE KIDNEY OF *Hemidactylus flaviviridis*

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Abstract: In the present work, cold shock was given to *Hemidactylus* for 30 minute and 60 minute to study the change in antioxitant parameters (lipid peroxidation and reduced glutathione) in the kidney of *Hemidactylus* in comparison to the control.
Keywords: *Hemidactylus*, lipid peroxidation, reduced glutathione, cold shock.

Introduction

Organisms that endure frequent anoxic or ischemic episodes in nature are typically prepared with high constitutive activities of antioxidant enzymes (e.g., superoxide dismutase, catalase, glutathione peroxidase, glutathione S-transferase, and peroxiredoxin), proteins (e.g., thioredoxin), and metabolites (e.g. ascorbate, glutathione) (Hermes-Lima and Zenteno-Savin, 2002; Hermes-Lima, 2001). Adult freshwater turtles (*T. s. elegans*) that are excellent facultative anaerobes and able to survive for as long as 3 months in deoxygenated water at low temperature (Jackson, 2002) have the highest antioxidant enzyme activities among cold-blooded vertebrates that have been examined (Willmore and Storey' 1997a; Willmore and Storey, 1997b). Catalase activity increased strongly in kidney of hatchlings of several turtle species in response to either anoxia or freezing exposure (Dinkelacker *et al*., 2005). Freezing exposure of garter snakes (*T. sirtalis*), resulted in significant increases in the activities of catalase and glutathione peroxidase in skeletal muscle whereas anoxia exposure strongly increased superoxide dismutase in kidney (Hermes-Lima and Storey, 1993).

Species of lizards with different preferred body temperature provide excellent models for testing the hypothesis that tissue becomes specialized to the body temperature maintain by thermoregulation (Hill *et al*., 2008). In the present work, cold shock was given to *Hemidactylus* for 30 minute and 60 minute to study the change in antioxitant parameters (lipid peroxidation and reduced glutathione) in the kidney of *Hemidactylus* in comparison to the control.

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Materials and Methods

Animal

Hemidactylus flaviviridis were catched from different houses of Baripada, Mayurbhanj, Odisha and used for the experiment. *H. flaviviridis* were acclimatized for seven days in the laboratory condition prior to the experiment.

Control (C)

About 6-7g body weight (size about 12-13cm in length) of *H. flaviviridis* were segregated from the collected sample and kept at room temperature.

Experimental (E)

About 6-7 gm body weight and 12-13 cm of *H. flaviviridis* were segregated from the collected sample and was exposed to cold shock by putting it into the upper chamber of refrigerator (-7^0C) for 30 minutes.

Simillarly, about 6-7 gm body weight and 12-13 cm of *H. flaviviridis* were segregated from the collected sample and was exposed to cold shock by putting it into the upper chamber of refrigerator (-7^0C) for 60 minutes.

Preparation of tissue sample

The Kidney was dissected out (both from control and experimental) and kept at 0˚C. the weight of kidney tissue was taken by the help of Monopan digital machine (Shimadzu). Then the kidney tissue of *H. flaviviridis* was homogenize with phosphate buffer (pH 7.4). The tissue homogenate was centrifuged at 4000 rpm for 10 minutes by cold centrifuge machine (Remi).

Protein estimation

Protein estimation of the sample was made according to the method of Lowry (1951). To 0.1 ml suitably homogenizes of tissue 0.4 ml of distilled water was added. Then 5 ml of biuret reagent (containing alkaline Na_2CO_3, 0.5% $CuSO_4$ solution and 1% sodium potassium tartarate solution in the ratio 100:2:2) was added and properly mixed up.

After 10 Minutes of incubation at room temperature, 0.5 ml of folin ciocalteau phenol reagent was added and incubated at 37˚C for 30 minutes at room temperature. Absorbance was measured at 700 nm against an appropriate blank by the help of UV-Visible spectrophotometer (Varian).

Lipid Peroxidation Assay

Lipid peroxidation of the samples were estimated as thiobarbiturate acid reacting substance (TBARS) by thiobarbituric acid (TBA) according to the method of Ohkawa *et al*.1979)
3.8 ml of TBA reagent contain (2 ml of 8.1% SDS,1.5 ml of 20% acetic acid of pH 3.5,1.5 ml of 0.8% aqueous solution of TBA, 5 ml of distilled water and 1 ml of BHT) was added to 0.2 ml of suitably diluted post nuclear supernatant. After mixing thoroughly, the test tubes (closed with glass bead) were boiled in water bath for 1 hour. The tubes were cooled down to the room temperature. Then the test tubes were centrifuged at 4000 rpm for 10 minutes by table top centrifuge (Remi). The absorbance of the supernatant was measured at 532 nm against an appropriate blank.

Glutathione Assay (GSH)

Glutathione of the sample was estimated by Ellman (1959) method. 0.7 ml of the tissue homogenate was added to 0.7 ml of TCA. Then the substances in the tubes were centrifuged at 4000 rpm for 10 minutes. 0.5 ml of supernatant was added to 2.5 ml of DTNB (DTNB 30 mm) was diluted in phosphate buffer 100 times. The absorbance was taken at 412 nm within between 5-30 minutes against an appropriate blank.

Results and Discussion

Protein synthesis is one of the greatest energy consuming activities in all cells (e.g., using about 36% of total ATP turnover in normoxic turtle hepatocytes) (Hochachka *et al*., 1996) for it requires over 4 ATP equivalents per peptide bond formed (Nelson and Cox, 2005). Cell systems under stress typically suppress protein synthesis as an early response to energy limitation and our studies of freezing and/ or anoxia tolerant organisms have shown that global suppression of transcription and translation is a critical part of the metabolic rate depression that supports anoxia/freezing survival (Storey, 2004a; Storey, 2004b; Storey and Storey, 2004).
It is found that the protein content (Fig 1) of control *Hemidactylus flaviviridis* was 98.1 ± 0.424 and in experimental group such as cold shock (30 minutes) and cold shock (60 minutes), the protein content were 54.14 ± 0.891 and 55.58 ± 0.240 respectively. It indicates that protein content decreases when duration of cold shock increases. The t-test between control and cold shock (30 minutes) is significant at $P > 0.05$ but not significant at $P > 0.01$, and $P > 0.001$. The t-test between control and cold shock (60 minutes) is significant at $P >$

0.05 but not significant at P > 0.01 and P < 0.001. The t-test between cold shock (30 minutes) and cold shock (60 minutes) is significant at P > 0.05, P > 0.01 but not significant at P > 0.001.

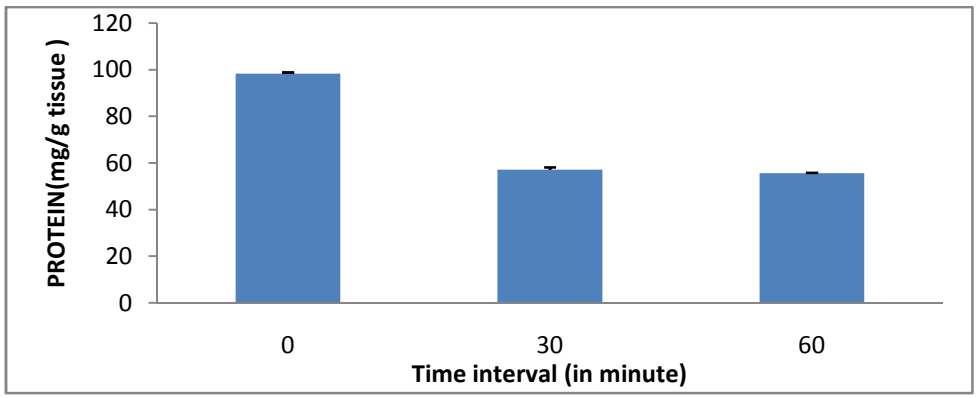

Fig 1: Comparison of protein content(mg/g) of kidney of *H. flaviviridis* in different time intervals(0 min, 30 min and 60 min)

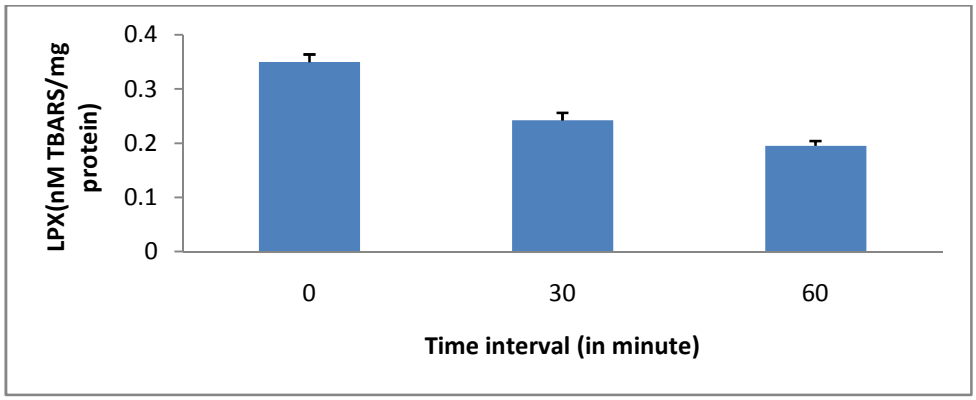

Fig 2: Comparison of LPX(nM TBA/mg protein) of kidney of *H. flaviviridis* in different time intervals(0 min, 30 min and 60 min)

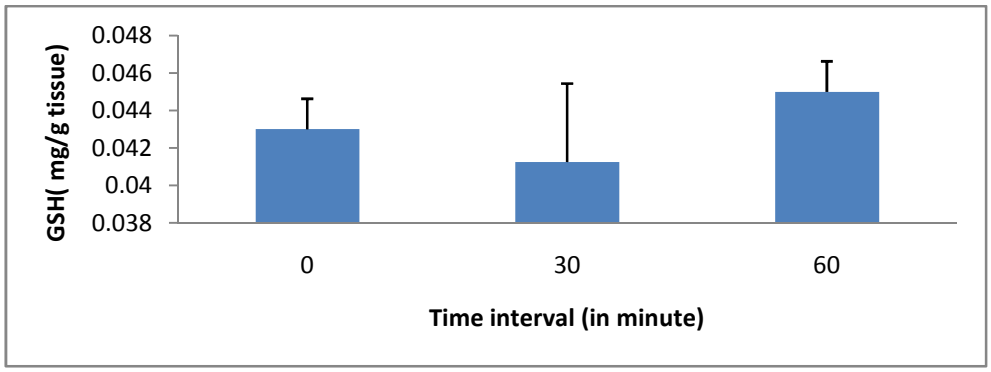

Fig 3: Comparison of reduced glutathione(GSH)(mg/g tissue) of kidney of *H. flaviviridis* in different time intervals(0 min, 30 min and 60 min)

It is found that (Fig. 2) the lipid peroxidation assay (LPX) level of control *Hemidactylus flaviviridis* was 0.349 ± 0.110 and in experimental group such as cold shock (30 minutes) and cold shock (60 minutes), the lipid peroxidation level were 0.241 ± 0.107 and 0.195 ± 0.086 respectively. So it indicates that LPX level decreases both at cold shock (30 minutes) and cold shock (60 minutes). The t-test between control and cold shock (30 minutes) is significant at $P > 0.05$ and $P > 0.001$, but not significant at $P > 0.01$. The t-test between control and cold shock (60 minutes) is significant at $P > 0.05$ but not significant at $P > 0.01$ and $P > 0.001$. The t-test between cold shock (30 minutes) and cold shock (60 minutes) is significant at $P > 0.05$, $P > 0.01$ but not significant at $P > 0.001$.

It is found that (Fig. 3) the reduced glutathione (GSH) level of control *Hemidactylus flaviviridis* was 0.043 ± 0.014 and in experimental group such as cold shock (30 minutes) and cold shock (60 minutes), the glutathione level were 0.041 ± 0.0605 and 0.045 ± 0.037 respectively. It indicates the GSH level increases when the duration of cold shock increases. The t-test between control and cold shock (30 minutes) is not significant at $P < 0.05$, but, significant at $P > 0.01$ and $P > 0.001$. The t-test between control and cold shock (60 minutes) is not significant at $P < 0.05$ but significant at $P > 0.01$ and $P > 0.001$. The t-test between cold shock (30 minutes) and cold shock (60 minutes) is not significant at $P > 0.05$, but not significant at $P > 0.01$ and $P > 0.001$.

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