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

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Abstract: Cold shock (stress) is given to *Hemidactylus* and then oxidative stress parameters (reduced glutathione and lipid peroxidation) were measured. It is found that Protein content (mg/g tissue) of liver of *H.flaviviridis* is significantly decreased when the animal is exposed to cold shock (30minutes or 60 minutes). Lipid peroxidation (LPX) level (nM TBA/mg) of liver of *H.flaviviridis* is significantly increased for 30 minutes of cold shock but significantly decreased for 60 minutes of cold shock. Reduced Glutathione (GSH) level (mg/g tissue) of liver of *H.flaviviridis* is not changed significantly or almost equal when the animal is treated with cold shock (30minutes or 60 minutes) in comparison to the control.
Keywords: *Hemidactylus*, Lipid peroxidation, Reduced glutathione, Cold shock.

Introduction

Under natural conditions, freezing within cells (intracellular freezing) almost always kills the cells in which it occurs. Intracellular freezing is thus fatal for animals unless they can survive without the cells that are frozen. On the other hand, many animals are remarkable tolerant of wide spread ice formation in their extracellular body fluid (Hill *et al*., 2008). The poikilotherms are typically thermal generalist: they must be capable of at a variety of different body temperature. A plausible hypothesis is that, when a species thermo regulate, its tissues and cells can improve their performance by becoming thermally specialised to function relatively optimally at the body temperature maintained. In the complete absence of thermoregulations, tissues are equally likely to be at almost any temperature accordingly, specialisation to function at particular temperatures might be disadvantages, and tissues might do best by being "jack-of-all-treads, master of none" (Hill *et al.*, 2008).

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.The genus *Hemidactylus* is distributed over large parts of tropical Asia, Africa, Mediterranean Europe and the Americas. It has been observed that a great majority of representatives of *Hemidactylus* geckos are restricted in distribution and confined to southern Asia and Africa, with only eight species namely *H. brookii*, *H. bowringii*, *H. flaviviridis*, *H.*

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frenatus, *H. garnotii*, *H. persicus*, *H. mabouia* and *H. turcicus* colonizing most of the geographical extent of this genus (Kluge 1969; Carranza and Arnold 2006). In India, *Hemidactylus* is represented by 24 recognized species (Smith 1935; Sharma 1981; Shukla 1983; Bauer and Russell 1995; Giri 2008; Giri and Bauer 2008;; Mahony 2009; Vyas *et al*. 2006). In the present work cold shock (stress) is given to *Hemidactylus* and then oxidative stress parameters (reduced glutathione and lipid peroxidation) were measured.

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 one day in the laboratory condition prior to the experiment. Five number of (6-7g body weight and 12-13cm in length) of *H. flaviviridis* were segregated from the collected sample and kept at room temperature as control group (C).

Similarly, same size of ten number of *H. flaviviridis* were taken for each experimental group: E1 (cold shock for 30 minutes), E2 (cold shock for 60 minutes). The animals were exposed to cold shock by putting it into the upper chamber of refrigerator (-7^0C) for 30 and 60 minutes.

Preparation of tissue sample

The liver of *H .flaviviridis* was dissected out (both from control and experimental) and kept at 0˚C. the weight of liver was taken by the help of Monopan digital machine (Shimadzu).Then the liver 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 thiobarbituric 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 was measured by UV-Visible spectrophotometer (Varian) 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 UV-Visible spectrophotometer (Varian) at 412 nm within between 5-30 minutes against an appropriate blank.

Results and Discussion

Natural freeze tolerance is a key part of winter cold hardiness for a variety of reptile species that live in seasonally cold climates. However, freezing survival is achieved without the accumulation of high concentrations of colligative cryoprotectants. Instead, reptiles appear to emphasize high anoxia tolerance and well-developed antioxidant defences to allow endurance of ischemia–reperfusion stress associated with cycles of freeze–thaw (Storey, 2006).

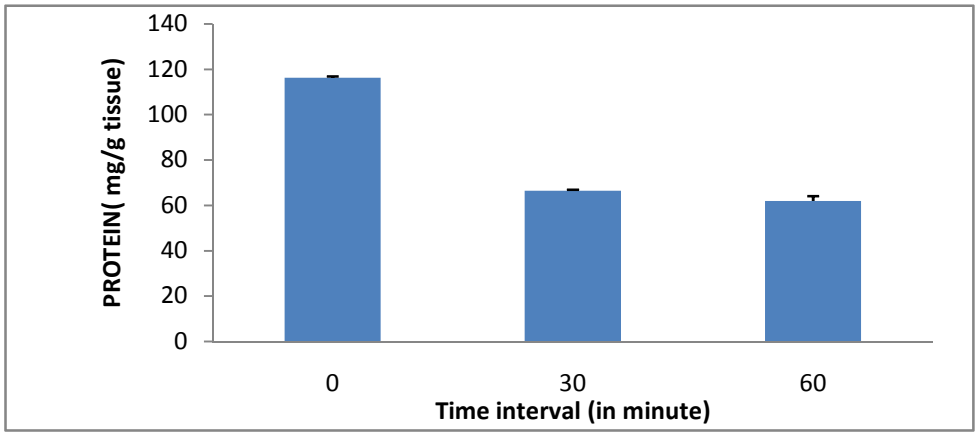

Fig 1: Comparison of protein content(mg/g) of liver of *H. flaviviridis* in different time intervals(0 min, 30 min and 60 min)

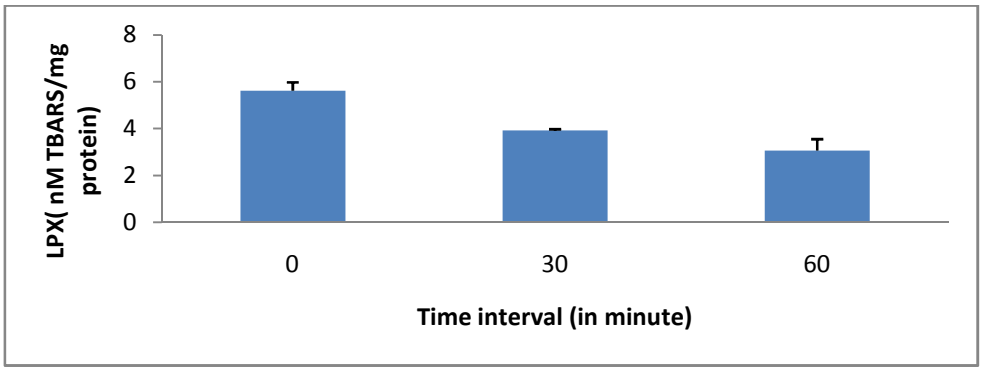

Fig 2: Comparison of LPX(nM TBA/mg protein) of liver of *H. flaviviridis* in different time intervals(0 min, 30 min and 60 min)

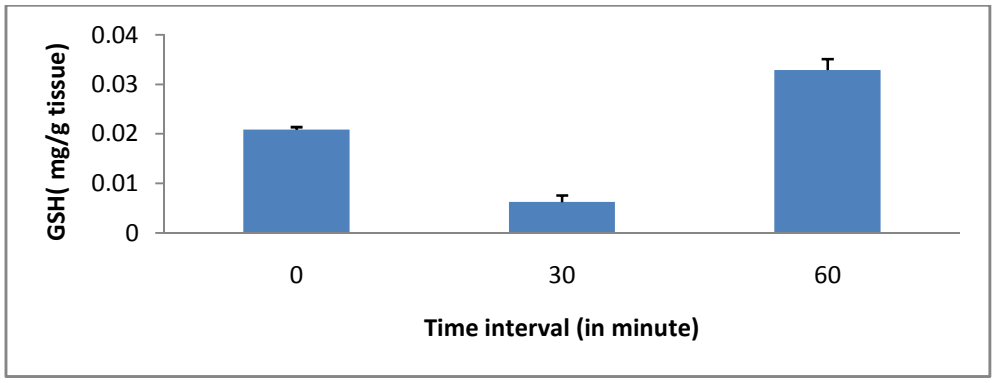

Fig 3: Comparison of reduced glutathione (GSH)(mg/g tissue) of liver of *H. flaviviridis* in different time intervals (0 min, 30 min and 60 min)

It is found that the protein content of control *Hemidactylus flaviviridis* was 116.34±0.417 and in experimental group such as cold shock (30 minutes) and cold shock(60 minutes), the protein content were 66.68±0.478 and 61.95±1.854 respectively. The t-test between control and cold shock (30 minutes) is significant at P>0.05, P>0.01, and P>0.001. The t-test between control and cold shock (60 minutes) is significant at P>0.05 and P>0.01 but not significant at 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, P>0.001 (Fig 1).

It is found that the lipid peroxidation assay(LPX) level of control *Hemidactylus flaviviridis* was 5.623±0.305 and in experimental group such as cold shock (30 minutes) and cold shock (60 minutes), the lipid peroxidation level were 3.929±0.038 and 3.134±0.1148respectively. The t-test between control and cold shock (30 minutes) is significant at P>0.05 and P>0.01, but not significant at P<0.001.The t-test between control and cold shock (60 minutes) is significant at P>0.05 and 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 and P>0.001 (Fig 2).

It is found that the reduced glutathione (GSH) level of control *Hemidactylus flaviviridis* was 0.0208±0.00047 and in experimental group such as cold shock (30 minutes) and cold shock (60 minutes), the glutathione level were 0.0062±0.00115 and 0.0329±0.00147 respectively. The t-test between control and cold shock (30 minutes) is not significant at $P<0.05$, $P<0.01$ and $P<0.001$. The t-test between control and cold shock (60 minutes) is not significant at $P<0.05$, $P<0.01$ and $P<0.001$. The t-test between cold shock (30 minutes) and cold shock (60 minutes) is not significant at $P<0.05$, $P<0.01$ and $P<0.001$ (Fig3).

Conclusion

Protein content (mg/g tissue) of liver of *H.flaviviridis* is significantly decreased when the animal is exposed to cold shock (30minutes or 60 minutes). Lipid peroxidation (LPX) level (nM TBA/mg) of liver of *H.flaviviridis* is significantly increased for 30 minutes of cold shock but significantly decreased for 60 minutes of cold shock. Reduced Glutathione (GSH) level (mg/g tissue) of liver of *H.flaviviridis* is not changed significantly or almost equal when the animal is treated with cold shock (30minutes or 60 minutes) in comparison to control.

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