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Effect of ferric chloride on reduced glutathione and lipid peroxidation level in muscle of *Hemidactylus leschenaultii*

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Abstract

Hemidactylus leschenaulultii were caught locally from North Orissa University, campus Baripada. They were divided into four groups and treated with ferric chloride at different time interval (24 h, 48 h and 72 h) against the control. The protein content, reduced glutathione level (GSH) and lipid peroxidation (LPX) level were measured in the muscle of *Hemidactylus* in both control and experimental (treated) group. It is observed that, GSH and LPX level of muscle were varied at different time intervals. It is found that ferric chloride (0.01µg), even at low dose altered the biochemical parameters and induces oxidative stress.

Key words: Muscle, Ferric chloride, Reduced glutathione and lipid peroxidation, *Hemidactylus leschenaulultii*

Introduction

In 2010, 28% of the reptiles evaluated by the International Union for the Conservation of Nature (IUCN) were listed as Critically Endangered (CR), Endangered (EN) or Vulnerable (VU) (IUCN, 2010) and environmental pollution has been recognized as one of the main contributing factors (Lange *et al.*, 2009; Todd *et al.*, 2010). Despite consistent calls for greater emphasis on reptile ecotoxicology research, there is still a lack of knowledge regarding the responses of reptiles to contaminants (Sparling *et al.*, 2010).

Iron is an absolute requirement for all forms of life. Importance of iron is especially notable in biogeochemical processes because of its unique ability to serve as both an electron donor and acceptor and thus can play an important role in metabolic processes of many organisms. Iron can also be potentially toxic at high concentrations. Iron's ability to donate and accept electrons means that if iron is free within the cell, it can catalyze the conversion of hydrogen peroxide into free radicals. Free radicals can cause damage to a wide variety of cellular structures, and ultimately kill the cell (Crichton *et al.* 2002).

In the present paper, Ferric Chloride was given orally to *Hemidactylus leschenaulultii* and oxidative stress

paramaters (lipid peroxidation and reduced glutathione) in muscle were measured and compared at different time intervals.

Materials and Methods

Hemidactylus leschenaulultii of various size (body weight ranging from 15-18g) for the experiment were caught locally from North Orissa University, campus Baripada, Mayurbhanj, Odisha from the month of October to March. The lizards were kept inside the labeled plastic jars with small holes to allow air to pass into it. They are acclimatized for 2 days in laboratory condition before the experiment.

Hemidactylus leschenaulultii (n=12) were divided into four groups, each group has 3 animals. The stock solution was prepared by dissolving 1mg of ferric chloride in 1ml of distilled water (stock solution). The stock solution diluted 1000 times. Then 100 µl (0.01µg) of working solution was taken in pipette and given orally to the animal, irrespective of body weight.

Group I (control) animals received distilled water; Group II-IV (experimental) treated orally with 0.01µg ferric Chloride (dissolved in distilled water). The treated animals were sacrificed after the time intervals of 24 hour, 48 hour and 72 hour (Group II-IV) whereas the control animals were sacrificed immediately (0h). The muscle of both control and experimental group were dissected out quickly and kept at 0^0C. A 20% homogenate was prepared with phosphate buffer (pH 7.4). The muscle homogenate was centrifuged at 4000 rpm for 10 minutes.

Protein estimation

Protein estimation of the samples were made according to the method of Lowry *et al.* (1951). To 0.1ml suitably homogenate of tissue 0.4ml 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 0.5ml of Folin Ciocalteau phenol reagent (the commercial reagent diluted three times with distilled water) was added and incubated at 37^0c for 30 minutes at room temperature. Absorbance was measured at 700 nm against an appropriate blank. Absorbance was measured at 700 nm against an appropriate blank. Protein content was expressed as mg/g wet weight of the tissue and aqueous BSA (Bovine Serum Albumin) was taken as standard protein.

Reduced Glutathione

Reduced glutathione of the sample were estimated by Ellman (1959) method. 0.7ml of the tissue homogenate was added to 0.7ml of TCA. Then the substances in the tubes were centrifuged at 4000 rpm for 10 minutes. 0.5ml supernatant was added to 2.5ml of DNTB (DNTB 30 mM) was diluted in PO_4 buffer 100 times. The absorbance was taken at 412 nm with in between 5 to 30 minutes against an appropriate blank.

Lipid Peroxidation

Lipid peroxidation of the sample is estimated as thiobarbiturate acid reacting

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

Results and Discussion

Protein content (mg/g tissue) in the muscle of *H. leschenaulultii* treated with ferric chloride were 26.209±1.164mg/g tissue , 27.653±7.115mg/g tissue, 35.248±5.144mg/g tissue and 28.162±3.461mg/g tissue at 0 hr,24 hr, 48 hr and 72 hr respectively (Fig 1).

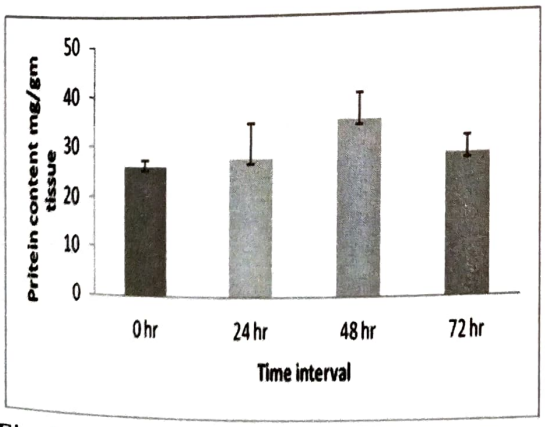

Fig. 1 Comparisons of protein content in muscle tissue of *Hemidactylus leschenaultii* treated with ferric chloride at different time intervals.

One way ANOVA revealed that the protein content at different time intervals in the muscle of *Hemidactylus leschenaultii* is significant [F(3,11)= 1.619, P=0.260]. Post Hoc analysis revealed that the protein content in the muscle of *Hemidactylus leschenaultia* treated with ferric chloride at different time intervals were all significant with respect to control (P<0.05;LSD)

The LPX level (n mol TBARS/mg protein) in muscle tissue of *H. leschenaulultii* treated with ferric is 13.472±0.537n mol TBARS/mg protein , 6.092±1.287 n mol TBARS/mg protein, 4.434±0.604n mol TBARS/mg protein, 6.547±1.754 n mol TBARS/mg protein at 0hr, 24hr, 48hr and 72 hr respectively (Fig 2).

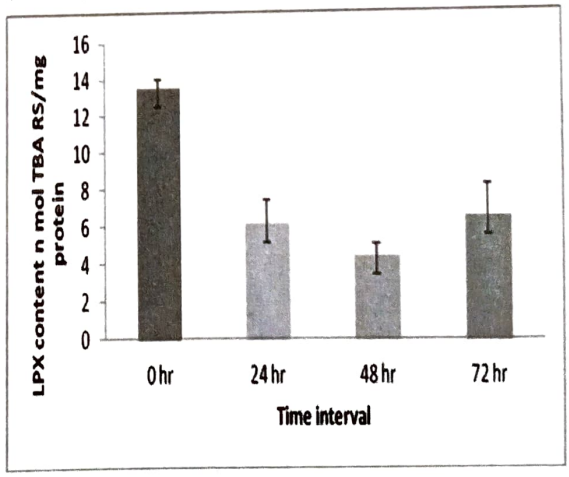

Fig. 2 Comparisons of LPX in muscle tissue of *Hemidactylus leschenaultii* treated with ferric chloride at different time intervals.

One way ANOVA revealed that the LPX level at different time intervals in the muscle of *Hemidactylus leschenaultii* is significant [F(3,11)=35.743, P=0]Post Hoc

analysis revealed that the LPX level in the muscle of *Hemidactylus leschenaultii* treated with ferric chloride at different time intervals were all significant with respect to control($P<0.05$; LSD).

GSH content (µmol/g) tissue in muscle tissue of *H. leschenaulultii* treated with ferric chloride were 0.006±0.003µ mol/g tissue, 0.011±0.007 µ mol/g tissue, 0.0384±0.0269 µ mol/g tissue and 0.006±0.002 µ mol/g tissue at 0 hr, 24 hr, 48 hr and 72 hr respectively (Fig. 3).

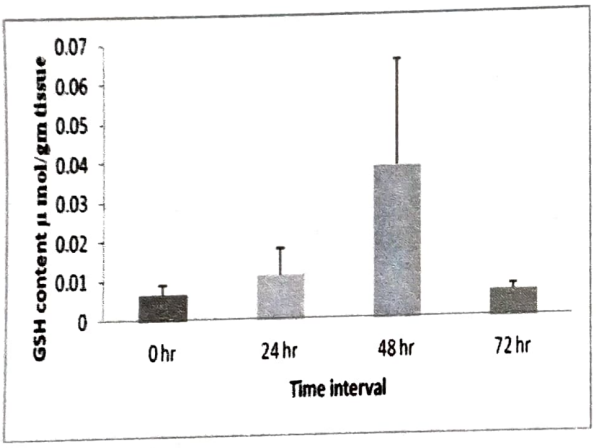

Fig. 3 Comparisons of GSH content in muscle tissue of *Hemidactylus leschenaultii* treated with ferric chloride at different time intervals.

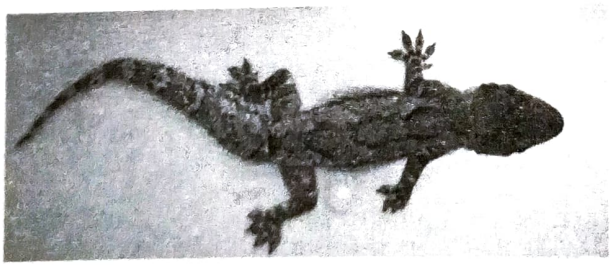

Fig. 4. *Hemidactulus leschenaultii* with normal body and patches.

One way ANOVA revealed that the GSH level at different time intervals in the muscle of *Hemidactylus leschenaultii* is significant [$F(3,11)=3.619, P=0.065$]Post Hoc analysis revealed that the protein content in the muscle of *Hemidactylus leschenaultii* treated with ferric chloride at different time intervals were al significant with respect to control ($P<0.05$;LSD).

Fig. 5. *Hemidactulus leschenaultii* treated with $FeCl_2$ at (24 h) lighter in body colour and patches

Fig. 6. *Hemidactulus leschenaultii* treated with $FeCl_2$ at (48 h) lightening in body colour and patches.

Fig. 7. *Hemidactulus leschenaultii* treated with $FeCl_2$ at (72 h) lightening in body colour and patches.

The skin colour or appearance of *Hemidactylus* also changes at different time intervals in response to ferric chloride and the tail is intact in all cases (Figs. 4 to7).

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