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Biology

EFFECT OF NICKEL CHLORIDE ON REDUCED GLUTATHIONE AND LIPID PEROXIDATION LEVEL OF 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 nickel 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. Change of different parameters of muscle were observed at different time intervals. On the basis of the results, it is concluded that nickel chloride even at low dose altered the biochemical parameters and induces oxidative stress.

KEYWORDS : Nickel chloride, Muscle, 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). In the present paper, Nickel Chloride was given orally to *Hemidactylus leschenaulultii* and oxidative stress paramaters (lipid peroxidation and reduced glutathione) were measured and compared at different time intervals.

Nickel and nickel compounds have many industrial and commercial uses, and the progress of industrialization has led to increased emission of pollutants into ecosystems. Although Ni is omnipresent and is vital for the function of many organisms, concentrations in some areas from both anthropogenic release and naturally varying levels may be toxic to living organisms (Scott-Fordsmand, 1997; Haber *et al.*, 2000 and Diagomanol, 2004). In the present paper, Nickel Chloride was given orally to *Hemidactylus leschenaulultii* and lipid peroxidation and reduced glutathione level of muscle were measured spectrophotometrically and compared at different time intervals.

MATERIALS AND METHODS

Animal

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 of 3 animals each. Group I (control) animals received distilled water; Group II-IV (experimental) treated orally with 5µg nickel 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 immediatetly (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 a 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 a appropriate blank.

Statistics

One way ANOVA and Post Hoc analysis was carried out to find out the level of significance between *Hemidactylus leschenaultii* treated with nickel chloride over a period of 24 hr, 48 hr, 72 hr and in control. A difference was taken as significant when P was less than 0.05. Statistics is done with the help of software SPSS package 16.0.

RESULTS AND DISCUSSION

Protein content (mg/g tissue) in the muscle of *H. leschenaulultii* treated with nickel chloride(1µl/g body weight) were 26.542± 0.909mg/g tissue , 31.745± 5.782 mg/g tissue , 33.522 ± 2.195mg/g tissue and 28.073 ± 1.269mg/g tissue at 0hr,24hr,48hr and 72 hr respectively. The protein content of muscle tissue of *H. leschenaulultii* treated with nickel chloride is highest at 48 hour from treatment and lowest at 0 hour (control).

One way ANOVA was performed to analyse the effect of nickel chloride on the protein content at different time intervals in the muscle of *H. leschenaultii*. One way ANOVA revealed that the protein content at different time intervals in the muscle of H. leschenaultii is significant [F (3, 11) =1.160,P=0.383]. Post Hoc analysis revealed that the protein content in the muscle of *Hemidactylus* exposed to nickel chloride (0.001µg/ ml) at different time intervals were significant with respect to control(P<0.05;LSD)

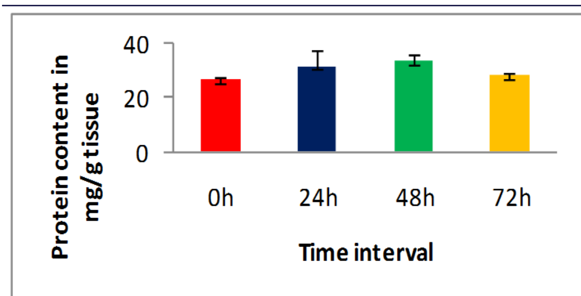

Fig. 1 Comparisons of protein content in muscle tissue of *Hemidactylus leschenaultii* treated with Nickel chloride at different time intervals.

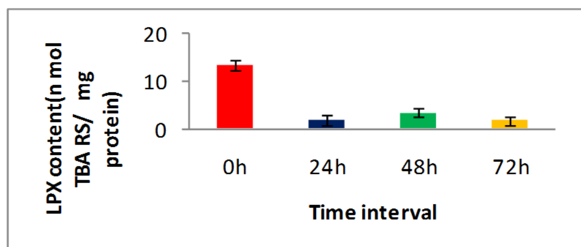

Fig. 2 Comparisons of LPX in muscle tissue of *Hemidactylus leschenaultii* treated with Nickel chloride at different time intervals.

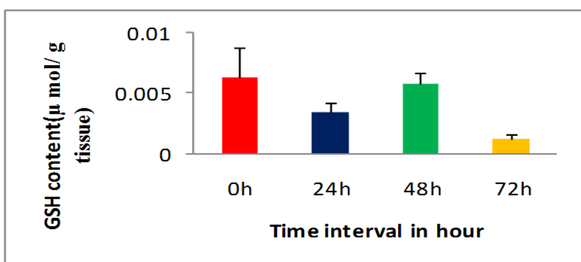

Fig. 3 Comparisons of GSH content in muscle tissue of *Hemidactylus leschenaultii* treated with Nickel chloride at different time intervals.

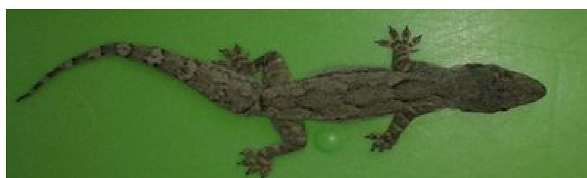

Fig. 4. *Hemidactulus leschenaultii* with normal body and patches (Untreated)

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

Fig. 6. *Hemidactulus leschenaultii* treated with FeCl2 at (48 h) patches are not visible clearly.

Fig. 7. *Hemidactulus leschenaultii* treated with $FeCl_2$ at (72 h) showing uniform colour (no patche mark).

The LPX level (n mol TBARS/mg protein) in muscle tissue of *H. leschenaulultii* treated with nickel is 13.472±0.537n mol TBARS/mg protein , 1.876 ±0.366n mol TBARS/mg protein, 3.545± 0.259n mol TBARS/mg protein, 1.834± 0.047 n mol TBARS/mg protein at 0hr,24hr,48hr and 72 hr respectively. The LPX level of muscle tissue of *H. leschenaulultii* treated with nickel varies a great from 0 hr (control) to 24 hr, 48 hr and 72 hr. The LPX level is highest at 0 hr (control) less than 24 hr, 48 hr and 72 hr.

One way ANOVA revealed that the LPX level at different time intervals in the liver of *H. leschenaulultii* is significant [F (3, 10) = 636.001, P=000]. Post Hoc analysis revealed that the protein content in the muscle of *Hemidactylus* exposed to nickel chloride (0.001µg/ ml) at different time intervals were significant with respect to control(P<0.05; LSD).

GSH content (µmol/g) tissue in muscle tissue of *H. leschenaulultii* treated with nickel (1µl/g body weight) were 0.0063 ± 0.0025µ mol/g tissue, 0.0034± 0.0008 µ mol/g tissue, 0.0057± 0.0009 µ mol/g tissue and 0.0012± 0.0004µ mol/g tissue at 0hr, 24hr, 48hr and 72 hr respectively. The GSH level is highest at 0 hr and lowest at 72hr of muscle tissue of *H. leschenaulultii* treated with nickel chloride.

One way ANOVA was performed to analyse the effect of nickel chloride on the GSH content at different time intervals in the muscle of *Hemidactylus leschenaultii*. One way ANOVA revealed that the LPX level at different time intervals in the liver of Hemidactylus leschenaultii is significant [F (3,11) =7.846, P=0.009]. Post Hoc analysis revealed that the protein content in the muscle of *Hemidactylus* exposed to nickel chloride (0.001µg/ ml) at different time intervals were significant with respect to control(P<0.05;LSD)

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REFERENCES
1. Blokhina O, Virolainen, E, Fagerstedt, KV (2003) Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann. Bot. 91, 179–194
2. Diagomanolin V., Farhang M., Ghazi-Khansari M., Jafarzadeh N. (2004) Heavy metals (Ni, Cr, Cu) in the Karoon waterway river, Iran. Toxicol. Lett. 151 (1), 63.
3. Ellman GL (1959). Tissue sulphydryl groups. Arch. Biochem. Biophy. pp 82:70-77.
4. Haber L.T., Erdreicht L., Diamond G.L., Maier A.M., Ratney R., Zhao Q., Dourson M.L. (2000) Hazard identification and dose response of inhaled nickel-soluble salts. Regul. Toxicol. Pharmacol. 31, 210.
5. IUCN (2010) IUCN Red List of Threatened Species.
6. Lange HJD, Lahr, J, Van der Pol, JJ., Wessels Y, Faber H (2009) Ecological vulnerability in wildlife: an expert judgment and multicriteria analysis tool using ecological traits to assess relative impact of pollutants. Environ. Toxicol. Chem. 28, 2233–2240
7. Lowry OH, Resbrough NJ, Farr AL and Randoll RJ (1951) Protein measurement with the Folin-phenol reagent. J.Biol. Chem.19: 265-275.
8. Ohkawa H, Ohishi N and Yagi K (1979) Assay of Lipid Peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. Physiol.118C (1):33-37.
9. Scott-Fordsmand J.J.(1997) Toxicity of nickel to soil organisms in Denmark. Rev. Environ. Contam. Toxicol. 148, 1.
10. Sparling DW, Linder G, Bishop CA, Krest SK (2010) Recent advancements in amphibian and reptile ecotoxicology. In: Sparling, D.W., Linder, G., Bishop, C.A., Krest, S.K. (Eds.), Ecotoxicology of Amphibians and Reptiles. Taylor and Francis, New York, pp. 1–14.
11. Todd, BD, Wilson, JD, Gibbons, JW, 2010. The global status of reptiles and causes of their decline. In: Sparling, D.W., Linder, G., Bishop, C.A., Krest, S.K. (Eds.), Ecotoxicology of Amphibians and Reptiles. CRC Press, New York, pp. 69–104.