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Effect of Nickel Chloride Onreduced Glutathione And Lipid Peroxidation Level of Liver of *Hemidactylus Leschenaultii*

KEYWORDS: Nickel chloride, Liver, *Hemidactylus leschenaulultii*

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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 (0.01µg dissolved in water) at different time intervals (24 h, 48 h and 72 h) against the control (0 h). The protein content, reduced glutathione level (GSH) and lipid peroxidation (LPX) level were measured in the liver of Hemidactylus in both control and experimental (treated) group. Variation of different parameteres of liver were observed at different time intervals. Besides that the change in behavior, colour of liver and weight loss were also observed. On the basis of the results, It is concluded that nickel chloride affect the physiology of the animal even at low dose*

INTRODUCTION

Environmental stress (considered here as those environmental changes disrupting homeostasis and, ultimately, leading to decline in fitness of an individual) caused by human activities can have significant detrimental effects on animal populations (Pimm *et al.*, 1995; Hughes *et al.*, 1997). Anthropogenic pressures have been increasing in the past decades and they are affecting wildlife at all levels of biological organization, often leading to population decline and even to the extinction of entire species (Bohm *et al.*, 2013).

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. Besides that the change in behavior, change in colour of liver and weight loss were also observed.

MATERIALS AND METHODS

Animal

Hemidactylus leschenaultii (8g to 14g) were collected during night and early morning time locally in Baripada from November 2014 to March 2014. They were acclimatized for two days prior to the experiment. The animals were kept in plastic perforated jar for experiment. The animals were divided in to two groups, i.e.,) control (Group A) and ii) experimental (Group B,C,D).

Treatment Process

The stock solution was prepared by dissolving 1mg of nickel 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.

Immediately 3 numbers of animals (Group A) were taken out and sacrificed for biochemical assay. After 24 (Group B), 48 (Group C) and 72 (Group D)hour three numbers of animals were taken out at each time intervals and repeat the same process as control group,

Preparation of Supernatant

Body weight of *Hemidactylus leschenaultii* (both control and experimental) was measured by digital monopan balance (Shimadzu; ELB 300) and were scarified at 0 h, 24 h, 48 h and 72 h of time interval. The liver was dissected out 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°C. The homogenate was centrifuged at 4000 rpm (1000Xg) for 10 minutes at 4°C in Cooling Centrifuge (Remi). The supernatant was taken for biochemical assay.

Protein Estimation

Protein estimation of the samples were made according to the method of Lowry *et al.*, (1951). To 0.1 ml of 20% homogenate, 0.4 ml of distilled water, 5 ml of biuret reagent containing 2% Na_2CO_3 (sodium carbonate) in 0.1N NaOH, 1% $KNaC_4H_6O_6$ (Potassium-sodium tartarate tetrahydrate) and 0.5% CuSO4 (copper II sulphate pentahydrate) in the ratio 100:2:2) was added and vortexed. After 10 minutes of incubation, 0.5 ml of Folin-Ciocalteau's reagent (1 part of commercially available reagent diluted with 2 parts of distilled water) was added and vortexed. After 30 minutes of incubation at room temperature absorbance was measured at 700 nm by spectrophotometer (Systronics Visiscan 167), against an appropriate blank. Protein content was expressed as mg/g weight of the tissue and aqueous BSA (Bovine Serum Albumin) was taken as standard protein.

Assay of Lipid Peroxidation

Lipid Peroxidation assay was determined by Thiobarbituric acid test (TBA test) according to Okhawa *et al*, (1979). 1.9 ml. of TBA reagent containing 8.1%(w/v) Sodium Dodecyl Sulphate (SDS), 20% (V/V) acetic acid pH (3.5), 0.5 ml. of 0.8%(w/v) aqueous solution of Butylated hydrotoluene (BHT), Thiobarbituric acid (TBA) and distilled water was added to 0.1ml of the supernatant and mixed thoroughly. Test substance was then boiled in a water bath for 1 hour, using glass balls as condenses. Then the test tubes were cooled to room temperature and centrifuged at 4500 rpm (1000×g) for 10 minutes at room temperature in Table Top Laboratory Centrifuge (Remi). Absorbance of supernatant

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was measured at 532nm. The concentration of TBARS was calculated from extinction coefficient of 156mM¯1 cm¯1(Wills,1969) and expressed as n mole equivalent of MDA formed per mg protein.

Reduced Glutathione

GSH content of the tissue samples was determined according to the method of Ellman (1959) with slight modification. 0.7ml. of the supernatant was precipitated in ice cold Tri-carboxylic acid (10% concentration) and centrifuged at 4000 rpm (1000×g) for 10 minute at 4°C and the supernatant was used for GSH assay. 0.5ml. supernatant was mixed with 2.5 ml of DTNB (5,5-Dithiobis 2 Nitro benzoic acid) and incubated at room temperature.

Absorbance at 412 nm was recorded against a blank containing only DTNB within 5-30 minutes. The GSH content of the tissue was expressed as mg/g tissue.

Statistical Methods

One-way ANOVA and Post Hoc analysis was carried out to find out the level of significance between *Hemidactylus leschenaultii* treated with nickel over a period of 24 h, 48 h, 72 h 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

The toxic effect of nickel against *Hemidactylus leschenaultii* was recorded at 0 hour (control), 24 hour 48 hour and 72 hour. The body colour become lighter in 24 hour of exposure which is gradually changes in 48 hour and 72 hour. The behavioral responses of the *Hemidactylus* also changed in response to nickel. It is also observed that the colour of liver becomes darker, slightly reddish and then chocolate colour after 24h, 48h and 72h of treatment of Nickel,

Body weight of nickel treated *Hemidactylus* were 11.75± 0.45 before dose (BD) and 12.58 ± 0.44 after dose (AD) at 24 hour, 11.24± 3.10 before dose and 11.08 ± 3.10 after dose in 48 hour, 10.86 ± 1.16 ± 1.16 before dose and 12.52 ± 1.41 after dose in 72 hour. In other words body weight of *Hemidactylus* after dose decreased at 48 hour and increased at 24 and 72 hour.

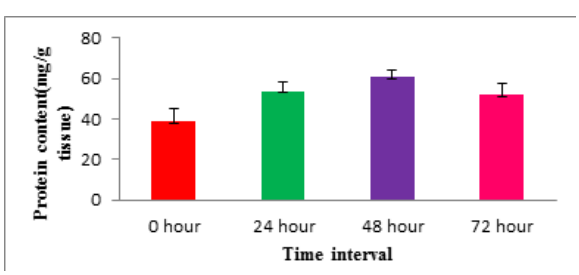

Fig. 1. Comparison of protein content (mg/g tissue) in *Hemidactylus* treated with nickel chloride at different time intervals.

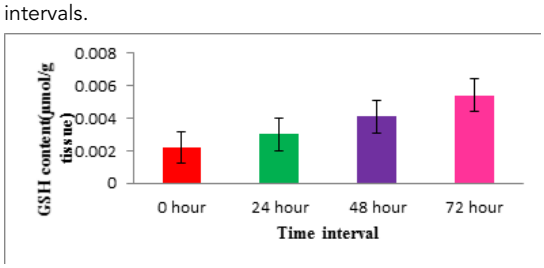

Fig. 2. Comparison of GSHcontent (µ mol/g tissue) in *Hemidactylus* treated with nickel chloride at different time intervals

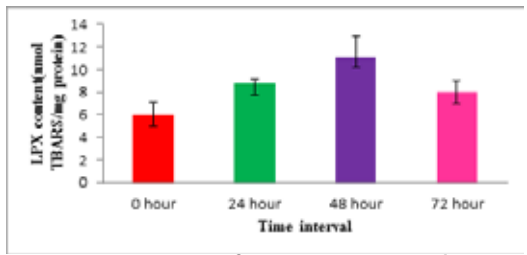

Fig. 3. Comparison of LPX content (nmol TBARS/ mg protein) in *Hemidactylus* treated with nickel chloride at different time intervals

One way ANOVA was performed in order to analyze the effect of nickel on the body weight before at different time intervals in *Hemidactylus leschenaultii*. One way ANOVA revealed that the body weight before at different time intervals in *Hemidactylus leschenaultii* is significant [F (2, 7) = 0.30, P = 0.74]

Post Hoc analysis revealed that the body weight before at different time intervals when treated *Hemidactylus leschenaultii* with nickel in was significant at 24 hour, 48 hour and 72 hour ($p < 0.05$; LSD).

One way ANOVA was performed in order to analyze the effect of nickel on the body weight after at different time intervals in *Hemidactylus leschenaultii*. One way ANOVA revealed that the body weight after at different time intervals in *Hemidactylus leschenaultii* is significant [F (2, 8) = 0.54, P = 0.60]

Post Hoc analysis revealed that the body weight after at different time intervals when treated *Hemidactylus leschenaultii* with nickel in was significant at 24 hour, 48 hour and 72 hour ($p < 0.05$; LSD).

Protein content

Protein content (mg/g tissue) in *Hemidactylus leschenaultii* in untreated at 0 hour is 38.78 ± 6.00 and in treated with nickel were 47.82 ± 0.66, 54.45 ± 2.66, 38.95 ± 3.76 at 24 hour , 48 hour and 72 hour respectively (Fig. 1).

Protein content (mg/g tissue) decreased 72 hours. It was lower in 72 hour in comparison to *Hemidactylus leschenaultii* exposed to nickel at different time intervals. The protein content was highest at 48 hour.

One way ANOVA was performed in order to analyze the effect of nickel on the protein content at different time intervals in *Hemidactylus leschenaultii*. One way ANOVA revealed that the protein content at different time intervals in *Hemidactylus leschenaultii* is significant [F(3,11) = 15.01, P =.001].

Post Hoc analysis revealed that the protein content at different time intervals when treated with nickel in *Hemidactylus leschenaultii* was significant at 24 hour, 48 hour and 72 hour ($P < 0.05$; LSD).

Reduced Glutathione

Reduced Glutathione (mg of GSH/g tissue) in *Hemidactylus leschenaultii* in untreated at 0 hour is 0.002 ± 0.0002 and in treated with nickel were 0.003 ± 0.0003, 0.004 ± 0.0002, 0.005 ± 0.0015 at 24 hour , 48 hour and 72 hour respectively. Reduced Glutathione (mg of GSH/g tissue) increased at 72 hour (Fig2).

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One way ANOVA was performed in order to analyze the effect of nickel on the reduced glutathione at different time intervals in *Hemidactylus leschenaultii*. One way ANOVA revealed that the reduced glutathione at different time intervals in *Hemidactylus leschenaultii* is significant [F(3,10) = 6.13, P =0.023].

Post Hoc analysis revealed that the reduced glutathione at different time intervals when treated with nickel in *Hemidactylus leschenaultii* was significant at 24 hour, 48 hour and 72 hour ($p < 0.05$; LSD).

Lipid Peroxidation

Lipid Peroxidation (n mol of TBA-RS/mg tissue) in *Hemidactylus leschenaultii* in untreated at 0 hour is 5.97 ± 1.17 and in treated with nickel were 8.74 ± 0.44, 11.15 ± 1.79, 8.04 ± 0.92 at 24 hour , 48 hour and 72 hour respectively (Fig. 3)..

Lipid Peroxidation (n mol of TBA-RS/mg) increased at 48 hour. It was decreased in 72 hour in comparison to *Hemidactylus leschenaultii* exposed to nickel at different time intervals. The Lipid Peroxidation was highest at 48 hour.

One way ANOVA was performed in order to analyze the effect of nickel on the lipid Peroxidation at different time intervals in *Hemidactylus leschenaultii*. One way ANOVA revealed that the lipid Peroxidation at different time intervals in *Hemidactylus leschenaultii* is significant [F(3,10) = 8.69, P =0.009].

Post Hoc analysis revealed that the lipid Peroxidation at different time intervals when treated with nickel in *Hemidactylus leschenaultii* was significant at 24 hour, 48 hour and 72 hour ($p < 0.05$; LSD).

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