Original Research Paper

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Biology

EFFECT OF NICKEL CHLORIDE ON ANTIOXIDANT ENZYMES (SUPEROXIDE DISMUTASE AND CATALASE) IN LIVER OF *Duttaphrynus melanostictus*

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ABSTRACT *Duttaphrynus melanostictus* (80 g to 110 g) were divided into four groups as A, B, C and D. Eeach group comprising of five animals. Animals of group A (control) were given orally 50 µl of distilled water. The experimental animals of group B, C and D were administered orally with 50 µl (50 µg) of nickel chloride. The animals were sacrificed after different time intervals such as 0h (group A), 24h (group B), 48h (group C) and 72h (group D). The superoxide dismutase and catalase of liver at different time intervals were measured and compared. It is observed that the protein content was highest at 48 h, superoxide dismutase (SOD) level was highest at 24 h and then gradually decreases at 48 h and 72 h. The catalase (CAT) level was lower at 0 hour in comparison to 24 hour and then gradually decreased at 48 hours and 72 hours. The CAT level highest at 24 hours

KEYWORDS : *Duttaphrynus melanostictus,* Nickel chloride, liver, superoxide dismutase and catalase

INTRODUCTION

Living organisms constitute a vast diversity of taxonomy, life history, physiology, morphology, behavior, and geographical distribution. Thus different species have different sensitivities to compounds. Sensitivity is the ability of an organism to respond and react to external stimuli. In the field, a multitude of species can be exposed to numerous toxicants. Therefore, to predict the effects of toxicants and to understand changes in species composition within communities, it is desirable to know how sensitive individual species are to specific toxicants (Von Der Ohe and Liess, 2004)

In ecosystem, amphibians acts as trophic link between insects and other vertebrates (Sparling et al., 2000). Decrease in the number of amphibian due to several factor indirectly affect the balance of ecosystem.

This study was designed to see the toxic effects of nickel chloride on antioxidant enzyme (superoxide dismutase and catalase) in liver of *Duttaphrynus melanostictus* at different time intervals (0h, 24h,48h and 72h).

MATERIALS AND METHODS

Duttaphrynus melanostictus (70 g to 120) g were collected locally around the North Orissa University Campus. They were acclimatized for two days prior to the experiment.

The stock solution was prepared by dissolving 1mg of nickel in 1ml of distilled water. From this stock solution 50µl (50µg) of nickel chloride were given orally to 15 numbers of *Duttaphrynus.* The animals were sacrificed at 0hour, 24 hours, 48 hours and 72 hours and different parameters were measured.

Jar No.	No. of toad	Nickel dose	Time interval (Hours)
$C_{Ni\ (Group\ A)}$	5	Nil	0 h
$J_{Ni1\ (Group\ B)}$	5	50µg	24 h
$J_{Ni2\ (Group\ C)}$	5	50 µg	48 h
$J_{Ni3(Group\ D)}$	5	50 µg	72 h

Preparation of supernatant

Body weight of *Duttaphrynus melanostictus* (both control and experimental) was measured by digital monopan balance (Shimadzu; ELB 300) and were sacrified at 0 hour, 24 hour, 48 hour and 72 hour 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 4oC. The homogenate was centrifuged at 4000 rpm (1000Xg) for 10 minutes at 4oC in Cooling Centrifuge (Remi). The supernatant was taken for biochemical assay.

Protein estimation

Protein estimation of the samples was made according to the method of Lowry *et al.*, (1951). Protein content was expressed as mg/g wet weight of the tissue and aqueous BSA (Bovine Serum Albumin) was taken as standard protein.

Estimation of superoxide dismutase

Superoxide dismutase (SOD; EC 1.15.1.1) activity was determined according to the method of Das *et al.,* (2000). SOD activity was expressed as units/mg protein

Estimation of catalase (CAT)

Catalase (CAT; EC 1.11.1.6) activity was estimated according to Beers and Sizer (1952). The activity of catalase was expressed as nkat/mg protein (1nkat=1mole of substrate converted to product per sec, 1U=16.67 nkat).

Statistical methods

One-way ANOVA and Post Hoc analysis was carried out to find out the level of significance between *Eudrilus eugeniae* treated with nickel chloride 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. Statistical analysis was done with the help of software SPSS package 16.0.

RESULTS AND DISCUSSIONS

In response to nickel chloride distinct red spots appear on the skin of D. melanostictus (Fig 2) and gradually decreases at/after 72h. The colour of liver becomes more deep at 24 hour, slightly reddish at 48h and then at 72h in comparison to that of the control.

It is found that weight of treated animals were less in comparison to control and gradually decreases with increase of time intervals (Fig. 7)

Table1: Comparison of protein content (mg/g tissue), Superoxide dismutase activity (SOD) in Unit/mg protein and Catalase (CAT) level in liver nkat/mg protein of D. melanostictus after treatment of nickel chloride at different time interval. The value are expressed in Mean± S.D.

Duration after treatment with furadan	Protein content (mg/g tissue)	SOD (Unit/mg protein)	CAT (nkat/mg protein)
C_{Ni} (0h)	36.61± 2.07	41.65 ± 52.96	3.94 ± 0.29
J_{Ni1} (24h)	35.43 ± 3.77	85.80± 125.67	4.29± 0.69
J_{Ni1} (48h)	50.46 ± 2.01	56.47 ± 69.91	4.12 ± 0.69
J_{Ni1} (72h)	13.92 ± 0.97	50.82 ± 37.45	3.63 ± 1.46

Protein content (mg/g tissue) gradually decreased at 0 hour and 24 hour and then it was increased in 48 hours. It was lower in 72 hour in comparison to *Duttaphrynus melanostictus* exposed to nickel at different time intervals. The protein content was highest at 48 hours (Table 1 and Fig.8).

One way ANOVA was perfomed in order to analyse the effect of nickel on the protein content at different time intervals in *Duttaphrynus melanostictus* . One way ANOVA revealed that the protein content at different time intervals in *Duttaphrynus melanostictus* is significant [F(3,19)=189.720,P=.000]. Post Hoc analysis revealed that the protein content at different time intervals when treated with nickel in *Duttaphrynus melanostictus* was significant at 24 hour, 48 hour and 72 hour ($P < 0.05$; LSD)

The SOD level (unit/mg protein) lower in control in comparision to *Duttaphrynus melanostictus* exposed to nickel at different time intervals. The SOD level was highest at 24 hours and then gradually decreased at 48 hours and 72 hours (Table 1 and Fig. 9).

One way ANOVA revealed that the SOD activity (unit/mg protein) in *Duttaphrynus melanostictus* exposed to nickel at different time intervals is significant [F(3, 19)= 1.076, P= .387]. Post Hoc analysis revealed that the SOD activity (Unit/mg protein) at different time intervals when treated with nickel in *Duttaphrynus melanostictus* was significant Only at 24 hour ($P < 0.05$; LSD). While 48 hour and 72 hour are not significant with respect to control.

The CAT level (nkat/ mg protein) of *Duttaphrynus melanostictus* exposed to nickel was highest at 24 hours and then gradually decreased at 48 hours and 72 hours. It was lower in 0 hour (control) in comparison to 24 hours (Table 1 and Fig. 10).

One way ANOVA revealed that the CAT activity (nkat/mg protein)in *Duttaphrynus melanostictus* exposed to nickel (0.050 µl) at different time intervals is significant [F(3, 19)= .498, P= .689]. Post Hoc analysis revealed that CAT activity (nkat/mg protein) at different time intervals when treated with nickel in *Duttaphrynus melanostictus* was significant at 24 hour and 48 hours ($P < 0.05$; LSD). While 72 hour are not significant with respect to control.

Fig1.Untreated animal (normal body colour)

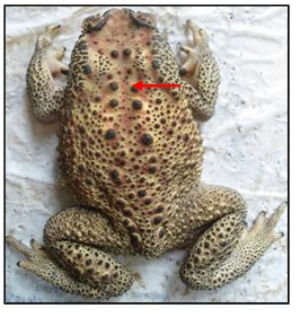

Fig2. Treated at 24 hour (reddish colour body)

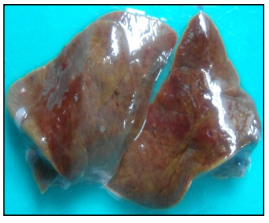

Fig3. Untreated liver of *Duttaphrynus melanostictus*

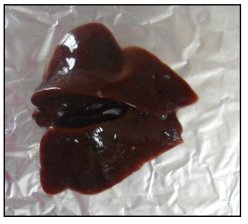

Fig4. Liver colour became more dark at 24 hour in nickel treated *Duttaphrynus melanostictus*

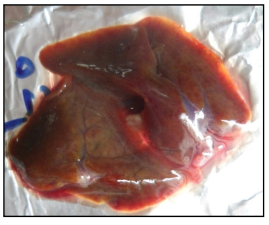

Fig5. Liver colour became slightly reddish at 48 hour in nickel treated *Duttaphrynus melanostictus*

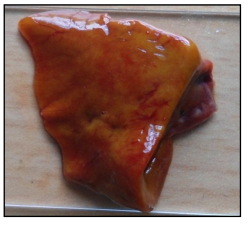

Fig6. Liver colour normal at 72 hour in nickel treated *Duttaphrynus melanostictus*

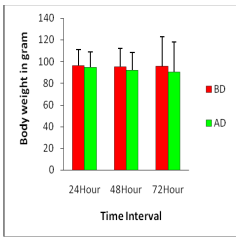

Fig7. Comparison of body weight in *Duttaphrynus melanostictus* treated with Nickel at different time interval (BD=Before dose; AD= after dose)

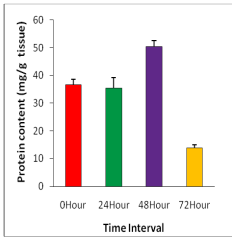

Fig8. Comparison of Protein content activity (mg/g tissue) in *Duttaphrynus melanostictus* treated with Nickel at different time interval

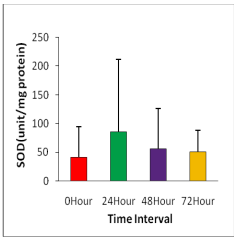

Fig 9. Comparison of SOD activity (unit/mg protein) in *Duttaphrynus melanostictus* treated with Nickel at different time interval

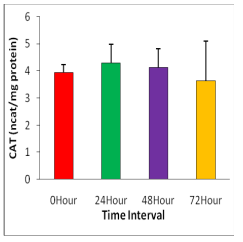

Fig 10. Comparison CAT activity (nkat/mg protein) in *Duttaphrynus melanostictus* treated with Nickel at different time interval

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