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Effect of Nickel Chloride on Protein, Superoxide Dismutase, Catalase And Morphology on Tail of Tadpole Larva of *Hoplobatrachus Tigerinus*

KEYWORDS: Nickel chloride, catalase, superoxide dismutase, Hoplobatrachus tigerinus

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ABSTRACT *Hoplobatrachus tigerinus was exposed to nickel chloride for different time interval (0 h, 24 h, 48 h and 72 h) and the change of antioxidant enzyme (superoxide dismutase and catalase) between exposed and un-exposed were measured spectrophotometrically. It is observed that both superoxide dismutase and catalase increases significantly at 24 h and then decreases at 48h. and then slightly increases at 72 h.*

INTRODUCTION

Habitats of many frog and toad populations are small, temporary ponds and the surrounding forested area, which are usually suffered by many stressors such as UV-radiation (Cummins 2003, Hatch and Blaustein 2003), the use of pesticides (Gendron et al. 2006, Fellers et al. 2004) and industrial chemicals (Bishop and Gendron 1998, Sower et al. 2000), urbanization (Barrett et al. 2010), climate change (Corn 2005). Since frogs and toads are sensitive to the alterations of their environment, they could be used as bioindicator organisms to follow changes in their habitats and in ecotoxicological studies (Henry 2000). As their populations usually contains high numbers of individuals and they are good representatives of freshwater environments, they are good model organisms for pollution studies (Burger and Snodgrass 1998). This study was designed to see the toxic effects of Nickel chloride on tadpole tail of Hoplobatrachus tigerinus by measuring superoxide dismutase and catalase activity at different time intervals.

MATERIALS AND METHODS

Animal

Egg masses of Hoplobatrachus tigerinus were collected from various sites in and around Mayurbhanj District of Odisha from July 2013 to May, 2014 and kept in the aquarium for hatching and then up to the feeding stage of the tadpoles (characteristics of this stage is developed mouth but limbs are absent) (Stage 23, Gosner,1960).

Fourty (n=40)number of tadpoles with approximately same size (3.1cm to 3.4cm) and weight (0.28g to 0.32g) were taken from the aquarium (stock) and then transferred to the four number of small aquarium such as C for control group and E_1, E_2, and E_3 for experimental groups. The water of E_1, E_2, and E_3 were mixed with Nickel chloride solution (concentration = 0.0001 mg/ml).

Table 1:Experimental Set-Up

Aquarium No.	No. of Tadpole	Nickel chloride dose/ml water	Time interval
C	10	Nil	0 hr
E_1	10	0.0001 mg/ml	24 hr
E_2	10	0.0001 mg/ml	48 hr
E_3	10	0.0001 mg/ml	72 hr

Preparation of supernatant

Two number of tadpole were picked up from each aquarium and their tail were cut by a sharp blade. The pooled weight of tails were measured in digital monopan balance (Shimadzu; ELB 300). A 20% homogenate was prepared in ice-cold 50mM 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 4500 rpm (1000 Xg) for 10 minutes at 4°C in Cooling Centrifuge (Remi). The supernatant (sample) was taken for biochemical assay. The process was repeated at least for five tims.

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 (SOD) activity

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 Hoplobatrachus tigerinus tadpoles exposed to Nickel chloride over a period of 24 hr, 48 hr, and 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

Morphological abnormalities like tail abnormalities, such as narrow margins, bent or drooped, edema and stunted growth, were observed in Hoplobatrachus tigerinus tadpoles exposed to Nickel chloride (Fig 1) in comparison to the untreated (control).

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Table2: Comparison of protein content, Superoxide dismutase activity (SOD) in and Catalase (CAT) level of *Hoplobatrachus tigerinus* after exposed to Nickel chloride at different time interval. The value are expressed in Mean± S.D.

Duration after exposed to Nickel chloride	Protein content (mg/g tissue)	SOD activity (Unit/ mg protein)	CAT level (nkat/mg protein)
C (0h)	9.92 ± 0.24	0.22 ± 0.11	0.07 ± 0.012
E_1 (24h)	8.43 ± 0.16	2.45 ± 0.37	0.24 ± 0.02
E_2 (48h)	10.68±0.20	1.19 ± 0.40	0.10 ± 0.02
E_3 (72h)	12.18±0.75	0.81 ± 0.66	0.07 ± 0.02

The protein content (mg/g tissue) gradually increases from 24 hour to 72 hours. It was lower in 24 hour in comparison to the tail of Hoplobatrachus tigerinus tadpoles exposed to nickel chloride (0.0001 mg/ml) at different time intervals. The protein content was highest at 72 hours (Table 2 and Fig. 2). One way ANOVA was performed in order to analyse the effect of nickel chloride on the protein content at different time intervals in the tail of Hoplobatrachus tigerinus tadpoles. One way ANOVA revealed that the protein content at different time intervals in the tail of Hoplobatrachus tigerinus tadpoles is significant [F (3, 15) = 42.692, P=0.000]. Post Hoc analysis revealed that the protein content in the tail of Hoplobatrachus tigerinus tadpoles exposed to nickel chloride (0.0001 mg/ml) at different time intervals were all significant with respect to control (P<0.05; LSD).

The SOD level (unit/mg protein) in the tail of Hoplobatrachus tigerinus tadpoles exposed to nickel chloride (0.0001 mg/ml) at different time intervals was highest at 24 hour and then gradually decreased at 48 hours and 72 hours. The SOD level was very low at 0 hour (control) (Table 2 and Fig 3).One way ANOVA revealed that the SOD activity (unit/mg protein) in the tail of Hoplobatrachus tigerinus tadpoles exposed to nickel chloride (0.0001 mg/ml) at different time intervals was significant at [F (3, 15) = 14.153, P=0.000]. Post Hoc analysis revealed that the SOD activity (unit/mg protein) at different time intervals when treated with nickel chloride in the tail of Hoplobatrachus tigerinus tadpoles at different time intervals was only significant at 24 and 48 hours (P<0.05; LSD). While, 72 hours was not significant with respect to control.

The CAT level (nkat/mg protein) was lower in 0 hour (control) and 72 hour in comparison to 24 and 48 hours. The CAT level (nkat/mg protein) was highest at 24 hours and then gradually decreased at 48 hours and 72 hours (Table 2 and Fig 4). One way ANOVA revealed that the CAT activity (nkat/mg protein) in the tail of Hoplobatrachus tigerinus tadpoles exposed to nickel chloride (0.0001 mg/ml) at different time intervals is significant [F (3, 15) =60.706, P=0.000]. Post Hoc analysis revealed that the CAT activity (nkat/mg protein) in the tail of Hoplobatrachus tigerinus tadpoles exposed to nickel chloride (0.0001 mg/ml) at different time intervals was only significant only at 24 and 48 hours (P<0.05; LSD). While, 72 hours are not significant with respect to control.

Dorso-lateral view of untreated larvae of *Hoplobatrachus tigerinus*

Ventral view of malformed larvae with swellings exposed to Nickel chloride

Dorsal view of malformed larva having bifurcated tail exposed to Nickel chloride

Fig 1. Toxic effect of Nickel chloride on the morphology of larvae of Indian bullfrog (*Hoplobatrachus tigerinus*).

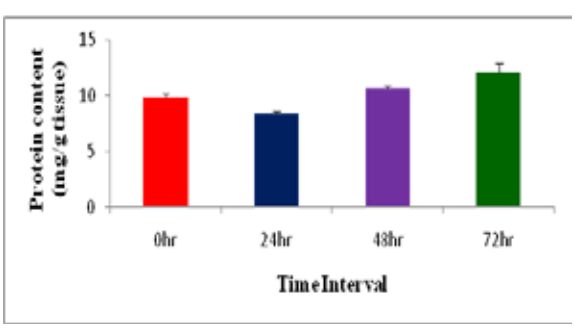

Fig.2: Comparison of protein content (mg/g tissue) in the tail of *Hoplobatrachus tigerinus* tadpoles exposed to Nickel chloride (0.0001 mg/ml) at different time intervals.

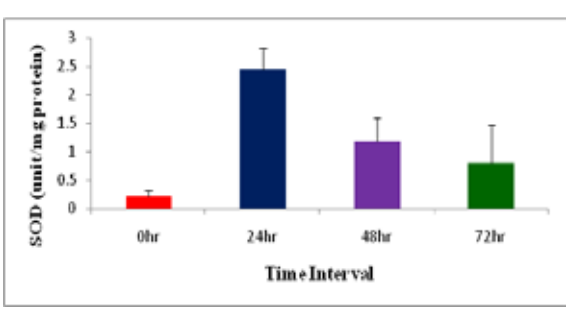

Fig.3: Comparison of SOD activity (unit/mg protein) in the tail of *Hoplobatrachus tigerinus* tadpoles exposed to Nickel chloride (0.0001 mg/ml) at different time intervals.

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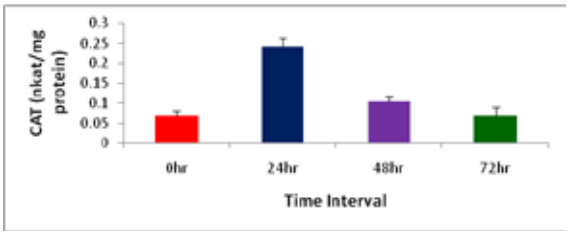

Fig.4: Comparison of CAT activity (nkat/mg protein) in the tail of *Hoplobatrachus tigerinus* tadpoles exposed to Nickel chloride (0.0001 mg/ml) at different time intervals.

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