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

KEYWORDS: ferric chloride, catalase, superoxide dismutase, *Hoplobatrachus tigerinus*

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ABSTRACT *Hoplobatrachus tigerinus was exposed to ferric 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

Amphibians are more threatened and are declining more rapidly than either birds or mammals (Stuart, *et al.*, 2004). Amphibians are important components of many ecosystems, acting as prey, predators or herbivores (Blaustein *et al.*, 1994). Because of their contribution to trophic dynamics, loss of amphibian populations will probably affect other organisms (Blaustein *et al.*, 1994).

Amphibia maintain both aquatic and terrestrial life. Its larval stage is purely aquatic. So they are exposed to pollutant in water. The tail of the tadpole plays a vital role in its development period. It gradually decrease at its growth stage and finally vanishes when the limbs of the animal develops. This study was designed to see the toxic effects of ferric 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 ferric chloride solution (concentration = 0.0001 mg/ml).

Table 1:Experimental Set-Up

Aquarium No.	No. of Tadpole	ferric 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 tail 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 Ferric 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

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 ferric chloride (0.0001 mg/ml) at different time intervals. The protein content was highest at 48 and 72 hours (Table 2 and Fig.2). One way ANOVA revealed that the protein content at different time intervals in the tail of *Hoplobatrachus ti-*

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gerinus tadpoles is significant [F (3, 15) = 49.382, P=0.000]. Post Hoc analysis revealed that the protein content in the tail of *Hoplobatrachus tigerinus* tadpoles exposed to ferric chloride (0.0001 mg/ml) at different time intervals were all significant with respect to control (P<0.05; LSD)

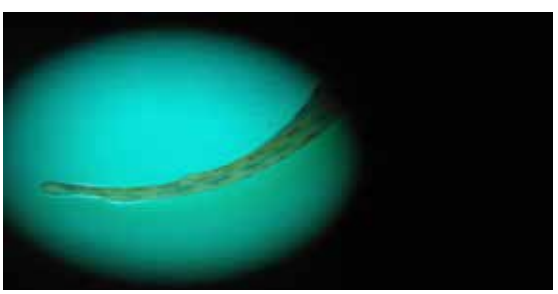
Table2: Comparison of protein content, Superoxide dismutase activity (SOD) in and Catalase (CAT) level of *Hoplobatrachus tigerinus* after exposed to ferric chloride at different time interval. The value are expressed in Mean± S.D.

Duration after exposed to ferric chloride	Protein content (mg/g tissue)	SOD activity (Unit/ mg protein)	CAT level (nkat/mg protein)
C (0h)	11.18 ± 0.00	0.36 ± 0.14	0.07 ± 0.02
E_1 (24h)	8.31 ± 0.02	1.13 ± 0.25	0.26 ± 0.026
E_2 (48h)	12.35 ± 0.81	0.25 ± 0.25	0.06 ± 0.01
E_3 (72h)	12.17 ± 0.43	0.51 ± 0.04	0.08 ± 0.01

The SOD level (unit/mg protein) in the tail of *Hoplobatrachus tigerinus* tadpoles exposed to ferric chloride (0.0001 mg/ml) at different time intervals was highest at 24 hour and then decreased at 48 hours and again increases at 72 hours. The SOD level was very low at 48 hours (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 ferric chloride (0.0001 mg/ml) at different time intervals was significant at [F (3, 15) = 12.679, P=0.000]. Post Hoc analysis revealed that the SOD activity (unit/mg protein) at different time intervals when treated with ferric chloride in the tail of *Hoplobatrachus tigerinus* tadpoles at different time intervals was only significant at 24 hours (P<0.05; LSD). While, 48 and 72 hours was not significant with respect to the control.

The CAT level (nkat/mg protein) was highest at 24 hours and then gradually decreased at 48 hours and again increases at 72 hours with respect to control (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 ferric chloride (0.0001 mg/ml) at different time intervals is significant [F (3, 15) =92.890, P=0.000]. Post Hoc analysis revealed that the CAT activity (nkat/mg protein) in the tail of *Hoplobatrachus tigerinus* tadpoles exposed to ferric chloride (0.0001 mg/ml) at different time intervals was only significant only at 24 hours (P<0.05; LSD). While, 48 and 72 hours are not significant with respect to control

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



Ventral view of swollen larvae exposed to ferric chloride

Ventral view of bifurcated tail and swollen outings larvae exposed to ferric chloridet

Ventral view of bifurcated tail in larvae exposed to ferric chloride

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

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

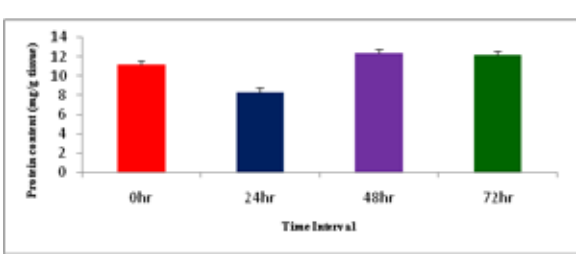


Fig.2: Comparison of protein content (mg/g tissue) in

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the tail of *Hoplobatrachus tigerinus* tadpoles exposed to ferric chloride (0.0001 mg/ml) at different time intervals.

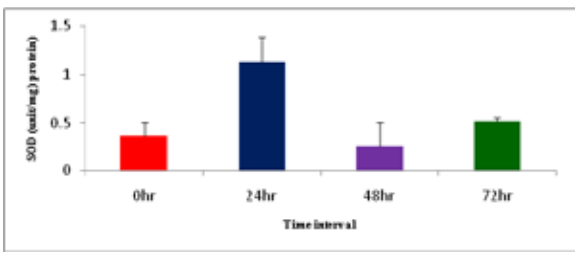

Fig.3: Comparison of SOD activity (unit/mg protein) in the tail of *Hoplobatrachus tigerinus* tadpoles exposed to ferric chloride (0.0001 mg/ml) at different time intervals.

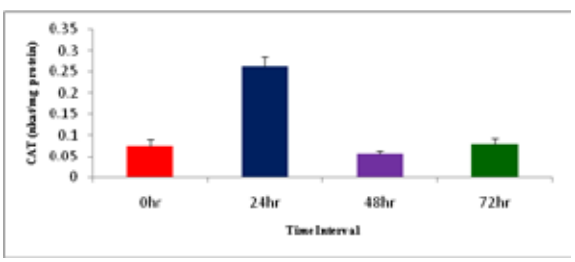

Fig.4: Comparison of CAT activity (nkat/mg protein) in the tail of *Hoplobatrachus tigerinus* tadpoles exposed to ferric chloride (0.0001 mg/ml) at different time intervals.

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