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Research Paper

EFFECT OF NICKEL ON ANTIOXIDANT ENZYME (SUPEROXIDE DISMUTASE ANEY OF DUTTAPND CATALASE) OF KIDHRYNUS MELANOSTICTUS

Puspanjali Parida || P. G. Department of Zoology
North Orissa University
Baripada
Odisha
757003 India

ABSTRACT **Duttaphrynus melanostictus 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**

) 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. 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 kidney at different time intervals were measured and compared.*

KEYWORDS :Duttaphrynus melanostictus, Nickel, kidney, superoxide dismutase and

INTRODUCTION

A wide range of contaminants are continuously introduced into the aquatic environment mainly due to increased industrialization, technological development, growing human population, oil exploration and exploitation, agricultural and domestic wastes run-off (Lima *et al.,*2008). Among these contaminants, heavy metals constitute one of the most dangerous groups because of their persistent nature, toxicity, tendency to accumulate in organisms and undergo food chain amplification and more still, they are nondegradable (Fufeyin and Egborge, 1998). Antioxidant defense enzymes (ADS) play a crucial role in maintaining cell homeostasis. ADS may be induced after exposure to pollutants, this response reflecting an adaptation of the species to their environment. This system may also be inhibited, which may lead to antioxidantmediated toxicities. Some heavy metals are hepatotoxic agents causing liver disorders, largely due to their active metabolites and free radicals. In small quantities, certain heavy metals are nutritionally essential for a healthy life. Some of these are trace elements (e.g., iron, copper, manganese, and zinc). These elements, or some form of them, are commonly found naturally in foodstuffs, fruits and vegetables, and in commercially available multivitamin products. Diagnostic medical applications include direct injection of gallium during radiological procedures, dosing with chromium in parenteral nutrition mixtures, and the use of lead as a radiation shield around xray equipment (Roberts, 1999). Animal studies have reported reproductive and developmental effects, such as a decreased number of live pups per litter, increased pup mortality, and reduction in fatal body weight, and effects to the dam from oral exposure to soluble salts of nickel (U.S. EPA IRIS 1999) In the present work generation of ROS in response to nickel were estimated by measuring Protein content, Superoxide dismutase (SOD) and Catalase (CAT) activity in kidney of *Duttaphrynus maelanostictus* at different time intervals (24 hr, 48hr and 72hr) and compared with the control.

MATERIALS AND METHODS Animal (*Duttaphrynus melanostictus*)

Duttaphrynus melanostictus (80 g to 110) g were collected during night and early morning time locally in Baripada from August 2014 to November 2014.They were acclimatized for seven 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 and experimental.

Treatment process

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

Preparation of tissue sample

Body weight of *Duttaphrynus melanostictus* (both control and experimental) was measured by digital monopan balance (Shimadzu; ELB 300) and were sacrified at 0 hr, 24 hr, 48hr and 72 hr of time interval. The kidney 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 Cool- ing Centrifuge (Remi). The supernatant was taken for biochemical assay.

Measurement of protein content

Protein estimation of samples was made according to the method of Lowry *et al*., (1961). The data were expressed in mg/g tissue.

Estimation of superoxide dismutase (SOD) activity

Superoxide dismutase (SOD; EC 1.15.1.1) activity was deter- mined 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 prod- uct 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 *Duttaphrynus melanostictus* treated with nickel 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 pack age 16.0.

RESULTS Body weight and colour of kidney

Body weight of *Duttaphyrnus melanostitus* exposed to nickel were 96.6±14.33 before dose (BD) and 94.6±14.33 after dose (AD) in 24 hour, 95.4±16.42before dose and 92.2±16.08 after dose in 48 hour , 95.6 ± 27.46 before dose and 90.6 ± 27.46 after dose in 72 hour. In other words body weight of *Duttaphrynus melanostitus* after dose decreased maximum in 72 hour (Fig.5). The colour of kidney varies at different time intervals (Figs 1, 2, 3,4).

Protein content

Protein content (mg/g tissue) in *Duttaphrynus melanostictus* treated with nickel were 22.33 ± 1.59 mg/g tissue , 22.03 ± 1.80 mg/g tissue , 28.60 ± 1.24 mg/g tissue , 13.28 ± 0.90 mg/g tissue IJSR INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH 409 Volume : 5 | Issue : 5 | May 2016 • ISSN No 2277 - 8179 | IF : 3.508 |

IC Value : 69.48 after 0 hour ,24 hour , 48 hour and 72 hour respectively. Protein content (mg/g tissue) gradually decreased at 0 hour, 24 hour and 72 hours. It was lower in 72 hour in comparision to *Duttaphrynus melanostictus* exposed to nickel at different time intervals. The protein content was highest at 48 hours (Fig.6).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(3,18) = 135.212, 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).

Superoxide dismutase (SOD) activity

Superoxide dismutase activity (unit/mg protein) in *Duttaphrynus melanostictus* treated with nickel we were 34.09 ± 47.46 unit/ mg protein at 0 hour,109.73 ± 124.55 unit/mg protein after 24 hours, 51.31 ± 56.51 unit/mg protein after 48 hours, 38.47 ± 42.30 unit/mg protein after 72 hours. 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 (Fig. 7).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 .

Fig 1. Untreated kidney of Duttaphrynus melanostictus

Fig 2. Kidney colour became more dark at 24 hour in nickel treated

Duttaphrynus melanostictus

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Fig 3. Kidney colour became slightly reddish at 48 hour in nickel treated *Duttaphrynus melanostictus*

Fig 4. Kidney colour normal at 72 hour in nickel treated Duttaphrynus melanostictus

Fig.5: Comparison of body weight in Duttaphrynus melanostictus treated with Nickel at different time interval.

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Fig.6:Comparison of Protein content activity (mg/g tissue) in Duttaphrynus melanostictus treated with Nickel at different time interval.

Fig.7: Comparison of SOD activity (unit/mg protein) in Duttaphrynus melanostictus treated with Nickel at different time interval.

Fig.8:Comparison of CAT activity (nkat/mg protein) in Duttaphrynus melanostictus treated with Nickel at different time interval.

Catalase (CAT) activity

Catalase activity (nkat/ mg protein) in *Duttaphrynus melanostictus* treated with nickel were 4.02 ± 0.55 nkat/mg protein,4.46 ± 1.015 nkat/ mg protein, 4.42 ± 0.59 nkat/ mg protein,3.99 ± 0.45 nkat/ mg protein after 0 hour, 24 hours. 48 hours and 72 hours respectively. The CAT level (nkat/ mg protein) was lower in 0 hour in comparison to 24 hour and then gradually decreased at 48 hours and 72 hours. The CAT level (nkat/ mg protein) of Duttaphrynus *melanostictus* exposed to nickel was Volume : 5 | Issue : 5 | May 2016 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 69.48 highest at 24 hours (Fig. 8).One way ANOVA revealed that the CAT activity (nkat/mg protein)in *Duttaphrynus melanostictus* exposed to nickel at different time intervals is significant [F(3, 19)= .665, P= .585]. 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). However, not significant at 72 hour with respect to the control.

DISCUSSION

It has been demonstrated that the organophosphates diazinon and chlorpyrifos both reduce rates of metamorphosis in tadpoles of the Asian common toad *Duttaphrynus melanostictus* (Sumanadasa *et al.,* 2008). In our study we found the body weight of *Duttaphrynus melanostictus* exposed to nickel significantly decreased than that of controls. At 24 hour, 48 hour and 72 hours time intervals of nickel exposure, the body weight of toads were decreasing with increase of time and also change in the colour of kidney.The two important antioxidant enzyme SOD and CAT increases at 24 hour indicate efficient mechanism of kidney in response to oxidative stress generated in response to Nickel Chloride. At 72 h the animal almost neutralize the level of SOD and CAT.

CONCLUSION

From this work it is known that the animal has well equipped with two important antioxidant enzyme SOD and CAT to neutralize the environmental oxidative stress for its survival.

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