RESEARCH PAPER | Biology | Volume : 5 | Issue : 9 | September 2015 | ISSN - 2249-555X

Comparison of Reduced Glutathione And Lipid Peroxidation Level in Kidney and Liver of *Duttaphrynus Melanostictus* Treated with Nickel Chloride

KEYWORDS: Nickel chloride, liver. kidney, *Duttaphrynus melanostictus*

Puspanjali Parida	Aeman Naaz	Priyambada Panda
P. G. Department of Zoology, North Orissa University, Baripada, Odisha, 757003 India Corresponding author	P. G. Department of Zoology, North Orissa University, Baripada, Odisha, 757003 India.	P. G. Department of Zoology, North Orissa University, Baripada, Odisha, 757003 India.

ABSTRACT *Duttaphrynus melanostictus was treated orally with 50µg nickel chloride (dissolved in 50 µl water). After 24 hour the animals were sacrificed. The reduced glutathione and lipid peroxidation level of liver and kidney were compared with that of control. In both the cases, protein content reduced while the level of reduced glutathione and lipid peroxidation level increased in case of treated when compared with that of untreated (control) animal.*

INTRODUCTION

Nickel is a naturally occurring element that is present in soil, water, air and biological materials. It is a natural component of earth's crust and is present in igneous rocks (Chauhan *et al*., 2008). Natural sources of nickel include dusts from volcanic emissions and the weathering of rocks and soils (Kasprzak *et al*., 2003). Inorganic fertilizers particularly phosphate fertilizers have variable levels of nickel depending on their resources (Sharma and Agarwal, 2005).

In humans, nickel is known to cause liver, kidney, spleen, brain and tissue damage, vesicular eczema, lung and nasal cancer on acute exposure (IPCS, 1992). Nickel induces embryotoxic and nephrotoxic effects, allergic reactions and contact dermatitis (EPA, 2002). Nickel sensitization also occurs in general population from exposure to coins, jewellery, watchcases, and clothing. It causes conjunctivitis, eosinophilic pneumonites, asthma and local or system reactions to nickel containing prostheses such as joint replacement, cardiac valve replacements, cardiac pacemaker wires and dental inlays (Hostynek and Maibach, 2002).

In the present work, *Duttaphrynus* (amphibia) is taken as model system. The toxic effect nickel chloride were observed in liver and kidneybin response to liver and kidney.

Animal

D.melanostictus (female) of various size (body weight ranging from 90-100g) were collected locally from January to April 2014. They were acclimatized for seven days prior to the experiment. The animals were divided into two group: i) Control group and ii) Experimental group. The animals were treated orally with 50µg nickel chloride (dissolved in water).

Preparation of tissue sample

For preparation of sample, the toads (both control and experimental) sacrificed at 0 hour (control), 24 hour (experimental). Liver and Kidney were dissected out quickly and kept at 0^0C. For both the tissue, different 20% homogenate were prepared with phosphate buffer (pH 7 .4). The tissue homogenate was centrifuged at 4000rpm for 10 minutes.

Protein Estimation

Protein estimation was done by Lowry *et al*. (1961). To 0.1 ml suitably homogenate of tissue 0.4 ml of distilled water was added .Then 5 ml of biuret reagent (containing alkaline Na_2CO_3 0.5% $CuSo_4$ solution and 1% sodium potassium tartarate solution in the ratio 100:2:2) was added and properly mixed up. After 10minutes of incubation at room temperature 0.5ml of Folin ciocalteau phenol reagent (the commercial reagent diluted three times was distilled water) was added and incubated at 37^0c for 30 minutes at room temperature. Absorbance was measured at 700 nm against an appropriate blank.

Reduced Glutathione (GSH) Estimation

Glutathione of the sample were estimated by Ellman (1958) method .0.7ml of tissue homogenate was added to 0.7ml of TCA. Then the substances in the tubes were centrifuged at 4000rpm for 10 minutes 0.5ml supernatant was added to 2.5 ml of DTMB (DTMB 30Mm) was diluted in PO_4 buffer 100 times. The absorbance was taken at 412nm with in between 5 to 30 minutes against a appropriate blank.

Lipid Peroxidation Assay (LPX)

Lipid Peroxidation of sample is estimated as thiobarbiturate acid reacting substance (TRA-RS) by thiobarbituric acid (TBA) according to the method of (Ohkawa *et.al*., (1979).3.8ml of TBA reagent contain (2ml of 8.1%SDS,1.5 ml of acetic acid of pH 3.5, 1.5ml of 0.8% aqueous solution of TBA ,5ml of distilled water and 1 ml of BST) was added to 0.2ml of suitably diluted post nuclear supernatant. After mixing thoroughly ,the test tube substance was boiled in water bath for 1hour.The tubes were cooled down to the room temperature .Then the tube substances were centrifuged at 4000 rpm for 10minutes.The absorbance of the supernatant was measured at 532 nm against a appropriate blank.

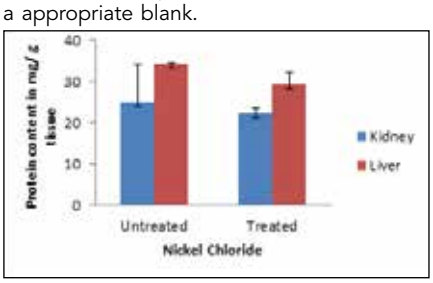

Fig 1.Comparison of protein content of kidney and liver in *D.melanostictus*

Volume : 5 | Issue : 9 | September 2015 | ISSN - 2249-555X

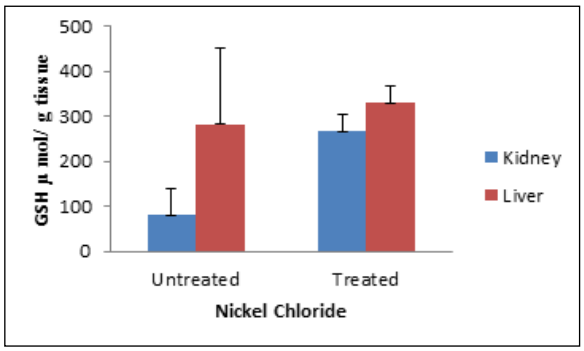

Fig 2.Comparison of GSH content of kidney and liver in *D.melanostictus*

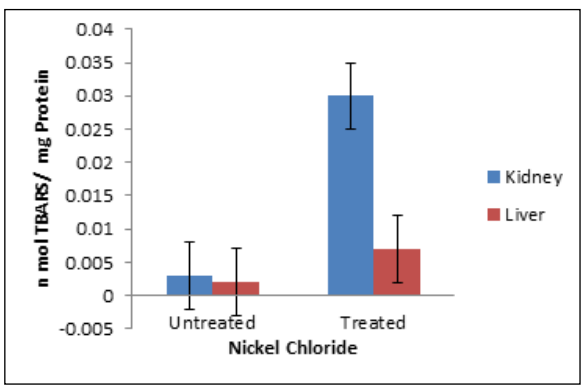

Fig 3.Comparison of lipid peroxidation level of kidney and liver in *D.melanostictus*

RESULTS AND DISCUSSIONS

When the *D. melanostictus* was treated with nickel, it was observed that the weight of toad was decreased in 24 hour from its normal body weight.

When the different parameters were done the protein content in *D.melanostictus* at control group was 24.80±9.46 mg/g and in experimental group such as in 24 hour it was 22.32±1.39 mg/g tissue. It shows that the protein content in control group was more and it gradually decreased at 24 hour after treatment (fig.1).

The GSH level of *D.melanostictus* at control group was 81.77±57.65 mg/g tissue and at 24 hour was 266.82±38.34 mg/g tissue. It indicates that GSH level was higher in treated group of *D.melanostictus* (fig.2).

The LPX level in control group of *D.melanostictus* was 0.003±0.001 TBA-RS/mg. In experimental group such as in 24 hour it was 0.030±0.042 TBA-RS/mg. It indicates that LPX level was higher in treated group of *D.melanostictus* (fig.3).

When the different parameters were done the protein content in *D.melanostictus* at control group was 34.27±0.18 mg/g and in experimental group such as in 24 hour it was 29.31±2.76 mg/g tissue. It shows that the protein content in control group was more and it gradually decreased at 24 hour after treatment (fig.1).

The GSH level of *D.melanostictus* at control group was 283.3±167.75 mg/g tissue and at 24 hour was 331.7±37.38 mg/g tissue. It indicates that GSH level was higher in treated group of *D.melanostictus* (fig.2).

The LPX level in control group of *D.melanostictus* was 0.002±0.0005 TBA-RS/mg. In experimental group such as in 24 hour it was 0.007±0.0005 TBA-RS/mg. It indicates that LPX level was higher in treated group of *D.melanostictus* (fig.3).

REFERENCE Chauhan, S. S., Thakur, R. and Sharma, G. D. (2008). Nickel:its availability and reactions in soil. J. Indl. Polln. Ctrl. 24(1) : 57 – 62. | | | Ellman, G.L., 1959. Tissue sulphydryl groups. Arch. Biochem. Biophy. pp 82:70-77. | | EPA, (2002). Nickel and nickel compound. Poll. Prevent. Fact Sheet, 96: 1 – 2 | | | Hostynek, J. J. and Maibach, H. I. (2002). Nickel and the skin, CRC Press, Boca Raton, 1 – 249. | | International Programme on Chemical Safety (IPCS), (1991). Environmental Health Criteria Series #108, Nickel, World Health Organization, Geneva, 383. | | Kasprzak, S., Sunderman, F. W. and Salnikow, K. (2003). Nickel carcinogenesis, Mut. Res. 533: 67 – 97 | | Lowry, O.H., Resbrough, N.J., Farr, A.L. and Randoll, R.J., (1951). Protein measurement with the Folin-phenol reagent. J.Biol. Chem. 19: 265-275. | | Ohkawa H., Ohishi, N. and Yagi, K. 1979. Assay of LPX in animal tissues by thiobarbituric acid reaction. Anal Biochem. Physiol.118C (1):33-37. | | Sharma, R. K. and Agarwal, M. (2005). Biological effects of heavy metals: An overview, J. Environ. Biol. 26(2): 301 – 313 |