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Reduced Glutathione and Lipid Peroxidation Level in Skeletal Muscle and Heart Muscle of Duttaphrynus Melanostictus Treated with Nickel Chloride

KEYWORDS: Nickel chloride, reduced glutathione, lipid peroxidation, *Duttaphrynus melanostictus*

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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 skeletal muscle and heart muscle were compared with that of control.*

In heart muscle, there is decrease in both GSH and LPX level and reverse situation occur in case of skeletal muscle in response to Nickel Chloride in comparison to control.

INTRODUCTION

During the past decades the ecology and ecotoxicology of amphibians started to get attention (Sparling *et al.,* 2000) because of global amphibian population declines (Houlahan *et al,.* 2000). Based on the lists of the International Union for the Conservation of Nature (IUCN) there are 787 rare or endangered amphibian species (Frost *et al,* 2006) and about 1,900 species known to be threatened (Stuart *et al.*, 2008). Frogs and toads are about 90% of all amphibians (McDiarmid and Micthell, 2000). Therefore, they are an important link between human and ecosystem health (Hayes *et al.*, 2002) and they are main components of aquatic and terrestrial ecosystems (Unrine *et al.* 2007). Most adult frogs and toads feed on invertebrates, so they are important, energy-efficient trophic link between insects and other vertebrates (Sparling *et al.*, 2000). They are sensitive to environmental changes both in terrestrial and aquatic habitats because they have highly semi-permeable skins and different life cycle stages (Alford and Richards, 1999). In the present work *D.melanostictus* were treated with nickel chloride to observe the change in reduced glutathione and lipid peroxidation level in skeletal muscle and heart muscle.

MATERIALS AND METHODS

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). The skeletal muscle and cardiac muscle (heart) were dissected out quickly and kept at 0^0C. A 20% homogenate was 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 o.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_4buffer 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.

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RESULTS AND DISCUSSIONS

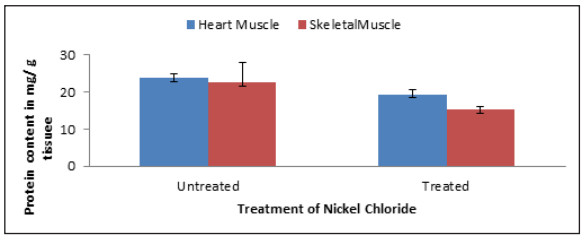

Fig 1.Comparison of protein content of heart muscle and skeletal muscle in *D.melanostictus*

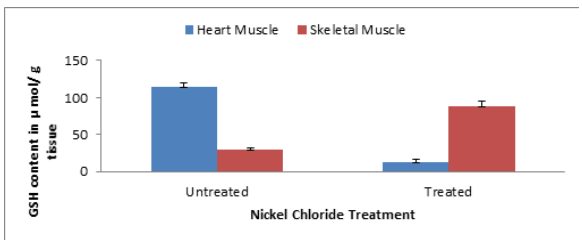

Fig 2.Comparison of Reduced Glutathione of heart muscle and skeletal muscle in *D.melanostictus*

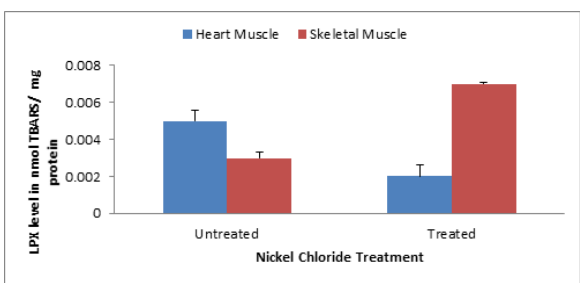

Fig 3.Comparison of Lipid Peroxidation of heart muscle and skeletal muscle in *D.melanostictus*

The protein content of heart muscle and skeletal muscle were 23.81±1.12mg/g tissue and 22.59±5.36 mg/g tissue in control group (untreated) and 19.41±1.13mg/g tissue and 15.36±0.8 mg/g tissue in experimental (treated) group respectively (Fig 1).

The GSH content of heart muscle and skeletal muscle were 114.26 ±5.54 µmol/g tissue and 29.22± 3.3 µmol /g tissue in control group (untreated) and 13.06± 3.74 µmol /g tissue and 3.74±7.93 µmol /g tissue in experimental (treated) group respectively (Fig 2).

The LPX content of heart muscle and skeletal muscle were 0.005±0.0006 nmol TBARS/ mg protein and 0.003±0.0003 nmol TBARS/ mg protein in control group (untreated) and 0.002±0.0006 nmol TBARS/ mg protein and 0.007±0.0001 nmol TBARS/ mg protein in experimental (treated) group respectively (Fig 3).

From the above data it is found that, protein content of both the tissue decreases. However in heart muscle, there is decrease in both GSH and LPX level and reverse situation occur in case of skeletal muscle in response to Nickel Chloride. It indicate, skeletal muscle are more vulnerable than heart muscle in comparison to control.

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