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Effect of Malathion on Protein Content, Reduced Glutathione and Lipid Peroxidation Level on Muscle of Bufo melanostictus Schneider, 1799

KEYWORDS: *Bufo melanostictus*, malathion, reduced glutathione, lipid peroxidation, muscle

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ABSTRACT *Bufo melanostictus (n=20) of various sizes (body weight: 90g- 120g) were divided into four groups of 5 animals each. Group I (control) animals received distilled water; Group II-IV (experimental) treated orally with 0.1µl malathion /g body weight. The treated animals were sacrificed after the time intervals of 24 hour, 48 hour and 72 hour (Group II-IV) whereas the control animal was sacrificed immediatetly (0h) and the muscle was dissected out and kept at 00C. The protein content, reduced glutathione level (GSH) and lipid peroxidation (LPX) level were measured in the liver of B. melanostictus in both control and experimental group. Variation of different parameteres of muscle were observed at different time intervals. On the basis of the results, It is concluded that malathion even at low dose altered the biochemical parameters and induces oxidative stress.*

INTRODUCTION

Pesticides may affect amphibian populations in a number of ways (Carey and Bryant, 1995); they may kill individual amphibians directly (Kirk, 1988) or indirectly through alterations in immune or neurological function (Cooke, 1971). Pesticides may also affect recruitment in amphibian populations by disrupting normal growth and development of the young or by impairing adult reproduction (Carey and Bryant, 1995). It has been reported that pesticide ingestion either by direct or indirect exposure may lead to generation of reactive oxygen species (ROS), which are detrimental to the health of humans and non-target organisms (Otitoju and Onwurah, 2007). In the present study generation of ROS in muscle of *Bufo melanostictus* in response to malathion were estimated by measuring reduced glutathione and lipid peroxidation level after different time intervals of 24 hour, 48 hour and 72 hour and compared against the control (0 hour).

MATERIALS AND METHODS

B. melanostictus were collected locally near the North Orissa University campus, during night and early morning time. They were acclimatized for seven days prior to the experiment. *Bufo melanostictus* (n=20) of various sizes (body weight: 90g- 120g) were divided into four groups of 5 animals each. Group I (control) animals received distilled water; Group II-IV (experimental) treated orally with 0.1µl malathion /g body weight. The treated animals were sacrificed after the time intervals of 24 hour, 48 hour and 72 hour (Group II-IV) whereas the control animal was sacrificed immediatetly (0h).

The muscle of both control and experimental group 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 4000 rpm for 10 minutes. The supernatant was used for various assay.

Protein

Protein estimation of the sample were made according to the method of Lowry *et. al.*,(1961). To 0.1ml suitably homogenate of tissue 0.4ml 0f 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 10 minutes of incubation at room temperature 0.5ml of Folin Ciocalteau phenol reagent (the commercial reagent diluted three times with 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

Reduced glutathione of the sample were estimated by Ellman (1959) method. 0.7ml of the tissue homogenate was added to 0.7ml of TCA. Then the substances in the tubes were centrifuged at 4000 rpm for 10 minutes. 0.5ml supernatant was added to 2.5ml of DNTB (DNTB 30 mM) was diluted in PO_4 buffer 100 times. The absorbance was taken at 412 nm with in between 5 to 30 minutes against a appropriate blank.

Lipid Peroxidation

Lipid peroxidation of the sample is estimated as thiobarbiturate acid reacting substance (TBARS) by thiobarbituric acid (TBA) according to the method of Ohkawa *et. al.* (1979). 3.8ml of TBA reagent contain (2ml of 8.1% SDS , 1.5ml of 20% acetic acid of pH 3.5, 1.5ml of 0.8% aqueous solution of TBA, 5ml of distilled water and 1ml of BHT) was added to 0.2ml of suitably diluted post nuclear supernatant. After mixing thoroughly, the test tube's substance was boiled in water bath for 1 hour. The tubes were cooled down to the room temperature. Then the tube substances ware centrifuged at 4000 rpm for 10 minutes. The absorbance of the supernatant was measured at 532 nm against a appropriate blank.

Table1: Comparison of protein content (mg/g tissue), GSH level (µ mol/g tissue), LPX level(n mol TBARS/mg protein)of muscle in Bufo melanostictus after treatment of malathion (0.1µl/g body weight) at different time interval. The value are expressed in Mean± S.D.

Duration after treatment with furadan	Protein content (mg/g tissue)	GSH level (µ mol/ g tissue)	LPX level (n mol TBARS/ mg protein)
0h (Group-I)	48.33±0.31	2.83±0.30	28.29±0.27
24h (Group-II)	25.13±0.77	0.49± 0.03	42.28±3.08
48h (Group-III)	34.95± 1.05	0.21±001	14.14±0.93
72h (Group-IV)	45.75±1.31	0.04±0.03	19.05±0.11

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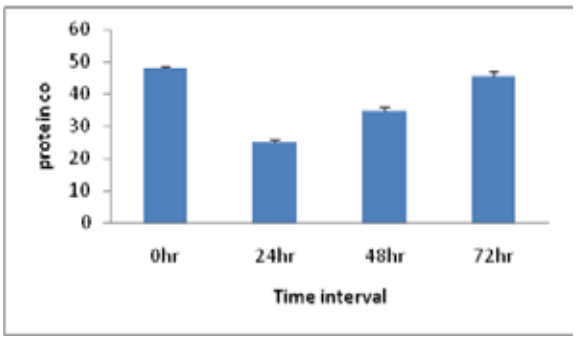

Fig 1: Comparison of protein content (mg/g tissue) of muscle in *Bufo melanostictus* treated with malathion (0.1µl/g body weight) at different time interval.

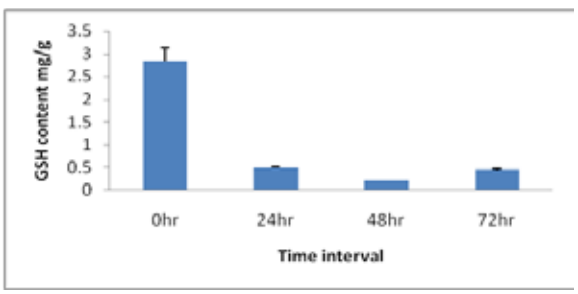

Fig 2: Comparison of GSH level (µ mol/g tissue) of muscle in *Bufo melanostictus* treated with malathion (0.1µl/g body weight) at different time interval.

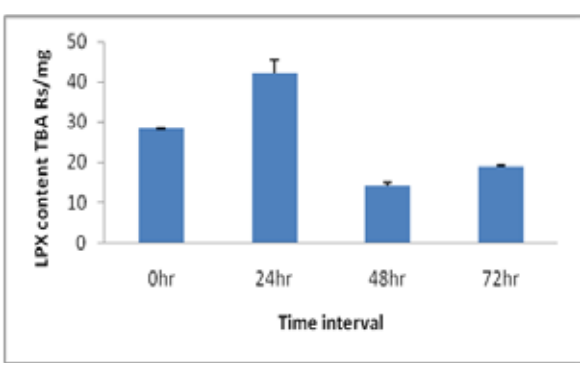

Fig 3: Comparison of LPX level (n mol TBARS/mg protein) of muscle in *Bufo melanostictus* treated with malathion (0.1µl/g body weight) at different time interval.

RESULTS AND DISCUSSION

Protein content in *B. melanostictus* were 48.33±0.31 mg/g, 25.13±0.77 mg/g tissue, 34.95±1.05and 45.75±1.31 mg/g tissue at 0h, 24h, 48h and 72h respectively. Protein content decreases at 24h but gradually increases at 48h and 72h (Table 1 and figure 1).

The GSH level of *B. melanostictus* were 2.83±0.30 µ mol/g tissue, 0.49±0.03 µ mol/g tissue, 0.21±0.01 µ mol/g tissue and 0.44±0.03 µ mol/g tissue at 0h, 24h, 48h and 72h respectively. It indicates that the GSH level decreases in response to malathion (Table 1 and figure2).

The LPX level of *B. melanostictus* were 28.29±0.27, 42.28±3.08, 14.14±0.93 and19.05±0.11 n mol TBARS/mg protein at 0h, 24h, 48h and 72h respectively. The LPX level increases in response to malathion but gradually decreases as the antioxidant system of body act quickly and efficiently (Table 1 and figure 3).

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