Biology



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

KEYWORDS	Bufo melanostictus, malathion, reduced glutathione, lipid peroxidation, kidney				
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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 0.1µI 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 immediately (0h) and the kidney 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 kidney 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

There is growing evidence that pesticides are responsible for amphibian declines (Sparling et al., 2001; Blaustein and Keisecker, 2002). Biochemical changes induced by pesticidal stress lead to metabolic disturbances, inhibition of important enzymes, retardation of growth and reduction in the fecundity and longevity of the organism (Murty, 1986). 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 kidney of Bufo melanostictus in response to malathion were estimated by measuring reduced glutathione (GSH) and lipid peroxidation (LPX) 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 kidney of both control and experimental group were dissected out quickly and kept at 0°C. A 20% homogenate was prepared with phosphate buffer (pH 7.4). The tissue homogenate was centrifuged at 4000 rpm for 10 minutes.

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% CuSO₄ 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°C 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 kidney in Bufo melanostictus after treatment of malathion (0.1 μ l/g body weight) at different time interval. The value are expressed in Mean± S.D.

Duration after treatment with furadan		Protein con- tent (mg/g tissue)	GSH level (µ mol/ g tissue)	LPX level (n mol TBARS/ mg protein)
0h	(Group-I)	73.78±0.92	0.16 ± 0.001	12.78±0.28
24h	(Group-II)	16.05±0.36	0.34±0.002	60.14±1.77
48h	(Group-III)	59.56±2.36	0.17±0.002	20.75±0.24
72h	(Group-IV)	29.57±0.55	0.11±0.004	55.01±1.26

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Protein ⁸⁰ ⁴⁰ ²⁰ ⁰ ⁰ hour 24 hours 48 hours 72 hours Time interval

Comparison of protein content (mg/g tissue) of kidney in Bufo melanostictus treated with malathion $(0.1\mu l/g body weight)$ at different time interval.



Comparison of GSH level (μ mol/g tissue) of kidney in Bufo melanostictus treated with malathion (0.1 μ l/g body weight) at different time interval



Comparison of LPX level (n mol TBARS/mg protein) of kidney in Bufo melanostictus treated with malathion (0.1μ l/g body weight) at different time interval

RESULTS AND DISCUSSION Protein content (mg/g tissue) in the kidney of *B. melanostictus* treated with malathion were 73.78 ± 0.92 mg/g tissue at 0 hour (control), $16.05.\pm0.36$ mg/g tissue at 24 hour, 59.56 ± 2.36 mg/g tissue at 48 hour and 29.57 ± 0.55 mg/g tissue at 72 hour.(table 1 and fig 1)

In other words the protein content of kidney of *B. melanostictus* treated with malathion is highest at 0 hour (control) and lowest at 24 than 48 and 72 hour.

GSH content (μ mol/g tissue) in kidney tissue of B.melanostictus treated with malathion were 0.16 ± 0.001 μ mol/g tissue at 0 hour (control), 0.34 ± 0.002 μ mol/g tissue at 24 hour, 0.17 ± 0.002 μ mol/g tissue at 48 hour and 0.11 ± 0.004 at 72 hour. (table 1 and fig 2)

The GSH level is highest at 24 hour (malathion treated) and lowest at 72 (malathion treated) hour.

The LPX level (n mol TBARS/mg protein) in kidney tissue of *B. melanostictus* treated with malathion is 12.78 ± 0.28 n mol TBA-RS/mg protein at 0 hour (control), 60.14 ± 1.77 n mol TBA-RS/mg protein at 24 hour, 20.75 ± 0.24 n mol TBA-RS/mg protein at 48 hour and 55.01 ± 1.26 n mol TBA-RS/mg protein at 72 hour. The LPX level of liver tissue of *B. melanostictus* treated with malathion is highest at 24 hour and lowest at 0 hour. (table 1and fig 3)

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