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

KEYWORDS: Bufo melanostictus, furadan, reduced glutathione, lipid peroxidation, liver

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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 3µl per 1gm of body weight of furadan (0.005mg of furadan dissolved in 1ml of acetone). 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 liver 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 liver were observed at different time intervals. On the basis of the results, It is concluded that furadan even at low dose altered the biochemical parameters and induces oxidative stress.*

INTRODUCTION

According to Farber et al., (1990) toxic action of pesticides may include the induction of oxidative stress and accumulation of free radicals in the cell. A major form of cellular oxidation damage is lipid peroxidation, which is initiated by hydroxyl free radical through the extraction of hydrogen atom from unsaturated fatty acids of membrane phospholipids. Reactive oxygen species (ROS) such as superoxide anions (O^{2-}), hydroxyl radicals ($\cdot OH$) and H_2O_2 enhance oxidative process and produce lipid peroxidative damage to cell membranes. The $\cdot OH$ radical has been proposed as an initiator of lipid peroxidation (LPX) through an iron-catalysed Fenton reaction (Halliwell and Gutteridge, 1986, 1989). Other antioxidants such as reduced glutathione (GSH) required to reduce H_2O_2 via glutathione peroxidase may also have an important function in mitigating the toxic effects of ROS.

There is an increasing concern about the ecological effect of the use of pesticides. 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). A good bioindicator should have a well-known taxonomy and ecology, be distributed over a broad geographic area, have a high degree of ecological specialization as specialized species are far more vulnerable to environmental perturbations compared to generalists, be cost-effective and relatively easy to survey (Rainio and Niemela 2003; Brischoux et al. 2009).

Although pesticides have long been suggested as a possible cause of amphibian declines (Carey and Bryant 1995, Stebbins and Cohen 1995, Drost and Fellers 1996, Lips 1998), there have been few toxicological studies on declines. Carbofuran or furadan (2, 3-dihydro -2, 2-dimethyl-7-benzofuranyl-N-methylcarbamate) has been reported to have relatively high mammalian toxicity and very toxic to invertebrates and birds and should therefore be handled with a lot of care (Hodgson et al., 1991).

In the present study generation of ROS in liver of Bufo melanostictus in response to furadan 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 3µl per 1gm of body weight of furadan (0.005mg of furadan dissolved in 1ml of acetone). 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 liver 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.

Protein Estimation

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 Assay

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 Assay

Lipid peroxidation of the sample is estimated as thiobarbiturate acid reacting substance (TBA-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 , 1.5ml 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

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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 liver in Bufo melanostictus after treatment of furadan (3µl/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)	63.89±0.389	0.16±0.001	62.46±1.1469
24h (Group-II)	72.24±1.783	0.10±0.001	13.94±0.265
48h (Group-III)	88.50± 0.260	0.21±0.001	17.09±0.120
72h (Group-IV)	93.64±0.371	0.32±0.001	21.64±0.145

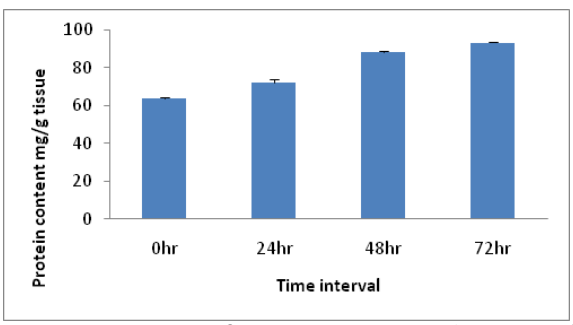

Fig : 1 Comparison of protein content (mg/g tissue) of liver in Bufo melanostictus treated with furadan (3µl/g body weight) at different time interval.

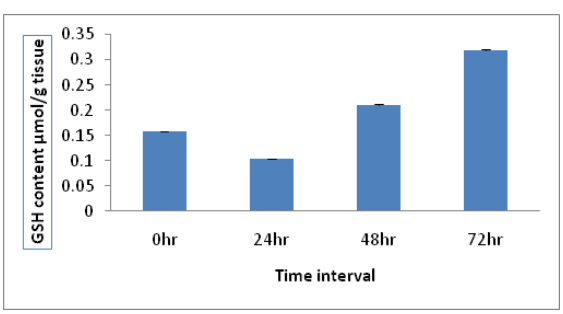

Fig : 2 Comparison of GSH level (µ mol/g tissue) of liver in Bufo melanostictus treated with furadan (3µl/g body weight) at different time interval.

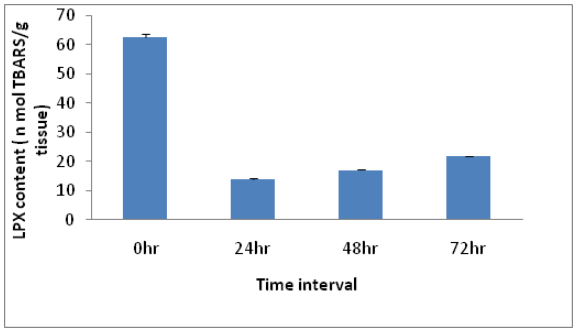

Fig : 3 Comparison of LPX level (n mol TBARS/mg protein) of liver in Bufo melanostictus treated with furadan (3µl/g body weight) at different time interval.

RESULTS AND DISCUSSION

Protein content (mg/g tissue) in the liver of B. melanostictus treated with furadan were 63.888 ± 0.378mg/g tissue, , 72.24 ± 1.782 mg/g tissue , 88.50 ± 0.260 mg/g tissue and 93.64 ± 0.371 mg/g tissue at 0h, 24h, 48h and 72 h respectively (table 1 and fig.1). In other words the protein content of liver of B. melanostictus increases with increase of time intervals means more protein is synthesized to balance the oxidative stress..

GSH content (µ mol/g tissue) in liver tissue of B. melanostictus treated with furadan were 0.1584 ± 0.001356 µ mol/g tissue, 0.1046 ± 0.00102 µ mol/g tissue, 0.2116 ± 0.0012 µ mol/g tissue and 0.3198 ± 0.001166 at 0h, 24h, 48h and 72 h respectively (table 1 and fig. 2). In other words the GSH content of liver of B. melanostictus increases with increase of time intervals means more protein is synthesized to balance the oxidative stress..

The LPX level (n mol TBARS/mg protein) in liver tissue of B. melanostictus treated with furadan is 62.457 ± 1.1469 n mol TBA-RS/mg protein, 13.940 ± 0.2648 n mol TBARS/mg protein, 17.087 ± 1.202 n mol TBA-RS/mg protein and 21.641 ± 0.1453 n mol TBARS/mg protein at 0h, 24h, 48h and 72 hour (table 1 and fig. 3).The LPX content decreases immediately at 24h but gradually increases with increase of time intervals.

From the above it is concluded that Bufo has well developed antioxidant defence mechanism to resist the pesticide insult.

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