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Biology

EFFECT OF FURADAN ON PROTEIN, LIPID PEROXIDATION AND REDUCED GLUTATHIONE LEVEL OF LIVER OF *Gallus gallus domesticus in vitro*

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ABSTRACT Liver of chicken, *Gallus gallus domesticus* were dissected out, kept at 0°C and homogenized. The homogenate was treated with furadan (0.05 mg) and incubate (*in vitro*) for 1-3 hours. The protein, reduced glutathione (GSH), lipid peroxidation (LPX) content of liver of the animal were measured at different time intervals and compared with the control (untreated).

KEYWORDS : *Gallus gallus domesticus,* furadan, liver, lipid peroxidation, reduced glutathione

INTRODUCTION

Carbofuran is widely used as an insecticide, nematicide and acaricide to protect the agricultural and industrial products (Osten *et al.*, 2005; Gera *et al.*, 2011). It accumulates in the fat depots and may cause toxicity to different vital organs such as brain, liver, skeletal muscles and heart (Rai *et al*., 2009) also exhibit neurotoxic, neurobehavioral and neuropsychological consequences in non-target subjects (Kamel and Hoppin, 2004; Rai and Sharma, 2007). Birds may encounter carbofuran through respiratory, dermal, and oral routes. Depending on the dietary requirements of particular species, ingestion of contaminated vegetables and poisoned invertebrates may be important exposure routes (Finlayson et al. 1979). This study was designed to see he toxic effects of furadan on liver of *Gallus gallus domesticus in vitro* by measuring GSH, LPX, content at different time intervals of 1h, 2h and 3h as experimental condition in comparison to that of the control.

MATERIAL AND METHODS

Animal

Chicken, *Gallus gallus domesticus* of various size (body weight ranging from 2.5-2.7 kg) were selected at the butchery shop. Immediately after the animal were sacrificed for sale, the liver were collected, wrapped with aluminium foil and kept in the ice box (4°C). Chicken livers were taken out from the ice box (4°C), was measured by digital monopan balance (Shimadzu; ELB 300) and tissue homogenate were prepared in ice-cold 50 mM phosphate buffer (pH 7.4) using pre-chilled porcelain mortar and pestle by up and down strokes at 4°C., The homogenate was divided into 4 test tubes: 1 test tube (control) and rest 3 test tubes (experimental). 50µl (0.05 mg furadan/ ml) was added to the experimental test tubes and incubate for 1-3 hous. Then centrifuge at 4000 rpm (1000Xg) for 10 minutes at 4°C in Cooling Centrifuge (Remi). The supernatant was taken for biochemical assay.

Protein estimation

Protein estimation of the samples were made according to the method of Lowry *et al*. (1951). To 0.1ml suitably homogenate of tissue 0.4ml 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 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. Absorbance was measured at 700 nm against an appropriate blank. Protein content was expressed as mg/g wet weight of the tissue and aqueous BSA (Bovine Serum Albumin) was taken as standard protein.

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 were centrifuged at 4000 rpm for 10 minutes. The absorbance of the supernatant was measured at 532 nm against a appropriate blank.

Statistical methods

One-way ANOVA and Post Hoc analysis was carried out to find out the level of significance between *Eudrilus eugeniae* treated with nickel chloride over a period of 24 h, 48 h, 72 h and in control. A difference was taken as significant when P was less than 0.05. Statistical analysis was done with the help of software SPSS package 16.0.

RESULTS AND DISCUSSION

Protien content

Protien content (mg/g tissue) in the liver of *Gallus gallus domesticus* teated with furadan were 29.98±0.0737mg/g tissue, 21.58±2.31mg/g tissue, 35.7±3.57mg/g tissue and 57.63±5.92mg/g tissue at 0 h, 1h, 2h and 3h respectively. The protein content of liver tissue of Gallus gallus domesticus treated with furadan is highest at 3 h from treatment and lowest at 0 hr.

Table-1: One way ANOVA of protein content at different time intervals in *Gallus gallus domesticus* exposed to Furadan (0.05 mg/ml).

PROTEIN	ANOVA				
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1575.595	3	525.198	39.430	0.000
Within Groups	106.557	8	13.320		
Total	1682.152	11			

One way ANOVA revealed that the protein content at different time intervals in the liver of *Gallus gallus domesticus* is significant at 0.05 Post Hoc analysis revealed that the protein content in the liver of *Gallus gallus domesticus* exposed to furadan at different time interval were significant with respect to control.

Lipid peroxidation level

The LPX level (n mol TBRS/mg potien) in the liver of *Gallus gallus domesticus* treated with furadan is 0.0013±0.002 n mol TBRS/mg protein, 0.0011±.0005 n mol TBRS/mg protein 0.0009±.0003 n mol TBARS/mg protein and 0.0023±.0007 n mol TBARS/mg protein at 0 h, 1 h, 2 h and 3 h respectively. The LPX level of liver of *Gallus gallus domesticus* treated with furadan varies from 0 h (control) to 1, 2 and 3 h. The LPX level is highest at 3 h and lowest at 0 h.

Table-2: One way ANOVA of LPX content at different time intervals in *Gallus gallus domesticus* exposed to Furadan (0.05mg/ml).

LPX	ANOVA				
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8.328	3	2.776	3.604	0.065
Within Groups	6.161	8	0.770		
Total	14.490	11			

One way ANOVA revealed that the LPX level at different time intervals in the liver of *Gallus gallus domesticus* is significant at 0.05. Post Hoc analysis revealed that the LPX level in the liver of *Gallus gallus domesticus* treated with furadan at different time intervals were significant with respect to control.

Reduced glutathione level

Reduced glutathione (GSH) content liver tissue of *Gallus gallus domesticus* treated with furadan were 0.3389±0.0807, 0.0405±0.0205, 0.1502±0.0041 and 0.1057±0.0597 mg/ g tissue at 0 hr, 1 hr, 2 hr and 3 hr respectively. The GSH level is highest at 0 hour and lowest at 1 hour of liver tissue of Gallus gallus domesticus treated with furadan.

Table-3: One way ANOVA of GSH level at different time intervals in *Gallus gallus domesticus* exposed to Furadan (0.05 mg/ml).

GSH					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.148	3	0.049	18.733	0.001
Within Groups	0.021	8	0.003		
Total	0.170	11			

One way ANOVA revealed that the GSH level at different time intervals in the liver of *Gallus gallus domesticus* is significant 0.05. Post Hoc analysis revealed that the GSH content in the liver of *Gallus gallus domesticus* exposed to furadan at different time intervals were al significant with respect to control is at 0.05

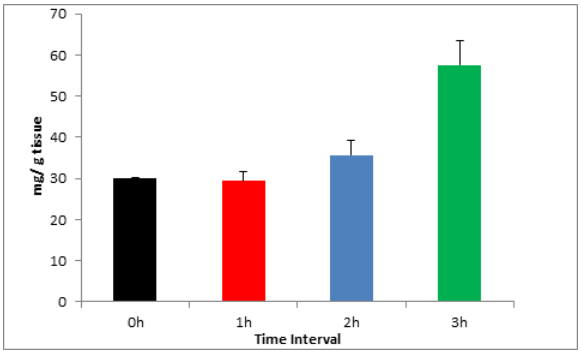

Fig1-: Comparision of protein content in liver of *Gallus gallus domesticus* treated with furadan at different time intervals.

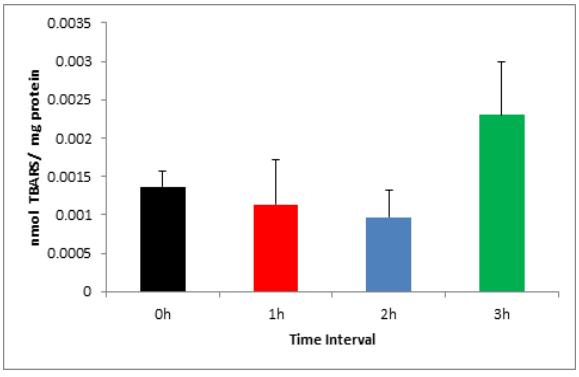

Fig 2:- Comparision of LPX in liver of *Gallus gallus domesticus* treated with furadan at different time intervals.

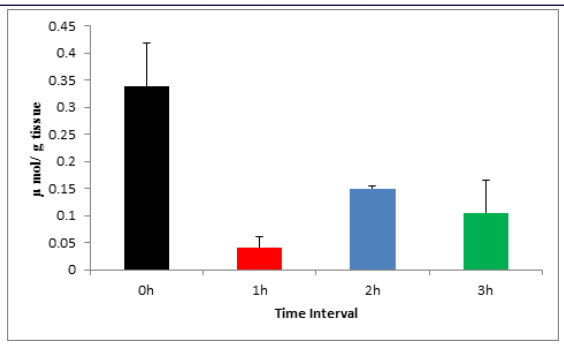

Fig 3- Comparision of GSH in liver of *Gallus gallus domesticus* treated with furadan at different time intervals.

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