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Effect of Furadan on Reduced Glutathione, Lipid Peroxidation and Ascorbic Acid Content of Liver of *Psammophilus blanfordanus*

KEYWORDS: Psammophilus blanfordanus, furadan, liver, ascorbic acid, lipid peroxidation, reduced glutathione

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ABSTRACT *Psammophilus blanfordanus were divided into four groups as A, B, C and D. Eeach group comprising of five animals. Animals of group A (control) were given orally 3 µl of acetone/g body weight. The experimental animals of group B, C and D were administered orally with 3 µl of furadan (0.005gm of furadan dissolved in 1ml of acetone)/g body weight. The animals were sacrificed after different time intervals such as 0h (group A), 24h (group B), 48h (group C) and 72h (group D). The protein, reduced glutathione (GSH), lipid peroxidation (LPX) and ascorbic acid (ASA) content of liver of the animal were measured and compared at different time intervals.*

INTRODUCTION

Carbofuran (2, 3-dihydro-2, 2-dimethyl-7-benzofuranol methyl carbamate), which is an organocarbamate pesticide and commonly known as furadan, has broad spectrum of action and short half life in the environment. It is therefore widely used as an insecticide, nematicide and acaricide to protect the agricultural and industrial products (Gupta, 1994; Osten *et al*., 2005; Gera *et al*., 2011). The application of carbofuran is preferred over the organophosphates (OPs) and organochlorines (OCls) due to its lower toxicity in comparison to OPs and OCls (Agrawal and Sharma, 2010). However, due to indiscriminate application and lack of selectivity, it also exerts toxicity on non-target mammals. The presence of carbofuran has been detected in different environmental components such as soil and water (Otieno *et al*., 2010), as well as in the maternal plasma, umbilical cord and blood of African-American women and new-born babies, respectively (Whyatt *et al*., 2003). The accumulation of carbofuran in animals and humans may take place via ingestion of pesticide-contaminated food and water as well as accidental or occupational poisoning (Kumari *et al*., 2002). Carbofuran accumulates in the fat depots and may cause toxicity to different vital organs such as brain, liver, skeletal muscles and heart (Gupta, 1994; Kaur and Sandhir, 2006; Rai *et al*., 2009). Carbofuran has been reported to exhibit neurotoxic, neurobehavioral and neuropsychological consequences in non-target subjects (Fahmg *et al*., 1970; Kamel and Hoppin, 2004; Rai and Sharma, 2007). This study was designed to see the toxic effects of carbofuran on liver of *Psammophilus blanfordanus* by measuring GSH, LPX, Ascorbic acid content of liver at different time intervals of (control) 0h and experimental (24h,48h,72h) .

MATERIALS AND METHODS

Psammophilus were caught locally from Baripada, Mayurbhanj, Odisha from the month of January 2012 to August 2012. The animals were divided into four groups as A, B, C and D. Eeach group comprising of five animals. Five animals of each group were kept in four different terrarium. They are acclimatized for 7 days in laboratory condition before the experiment. Animals of group A (control) were given orally 3 µl of acetone/g body weight. The experimental animals of group B, C and D were administered orally with 3 µl of furadan (0.005gm of furadan dissolved in 1ml of acetone)/g body weight. The animal after administered orally with acetone or furadan were separated into labeled, perforated plastic bottle. The animals of control group (0hr) were sacrificed immediately, whereas the animals of experimental group B, C and D were sacrificed after 24hr, 48hr and 72hr of treatment. Immediately liver was dissected out and kept at 0°C. The tissue homogenate was prepared with phosphate buffer (pH 7.4) and then centrifuged at 4000 rpm for 10 minutes in a cold centrifuge machine.

Measurement of protein content

Protein estimation of samples was made according to the method of Lowry *et al*. (1961). The data were expressed in mg/g tissue.

Measurement of Lipid Peroxidation

Lipid peroxidation of the sample was estimated as thiobarbituric acid reacting substance (TBARS) by thiobarbituric acid (TBA) according to the method of Ohkawa *et. al*. (1979). The data were expressed as nmoles of TBARS/mg protein.

Measurement of reduced glutathione (GSH)

Glutathione content (GSH) was estimated by the method of Ellman (1959) and the amount of glutathione is expressed as mg/ g tissue.

Measurement of Ascorbic Acid (ASA)

Ascorbic acid of the sample were estimated by Jagota and Dani (1982) method.

All the solution were prepared by using Millipore distilled water. The above experiments were repeated for 5 times.

RESULTS AND DISCUSSION

Protein content (mg/g tissue) in liver of *Psammophilus blanfordanus* treated with furadan (0.005mg/ml of acetone) were 60.488±0.002 mg/g at 0 hr (control), 56.112±0.001 mg/g tissue at 24 hr, 77.525±0.025 mg/g tissue at 48 hr and 68.388±1.016 mg/g tissue at 72 hr. The protein content (mg/g tissue) of *Psammophilus blanfordanus* exposed to furadan was highest at 48hr then decreased at 72 hr but higher than control. The protein content was lowest at 24 hr in comparison to control (Fig1). The correlation analysis was used for the measurement of the linear association between variables. Pearson's correlation coefficients (r^2) among the analytical variables showed that the time interval is significant with protein content in liver tissue of *Psammophilus blanfordanus*. [Time interval – protein content (0.618; $P \leq 0.05$)]. One way ANOVA showed that incubation period has significant effect on protein content of liver tissue. Post-hoc analysis revealed that only 48 hr is significant [$F(3,8)=2.070$, $P< 0.001$)]. All the combination are different from each other ($P< 0.001$, LSD).

Volume : 4 | Issue : 3 | Mar 2014 | ISSN - 2249-555X

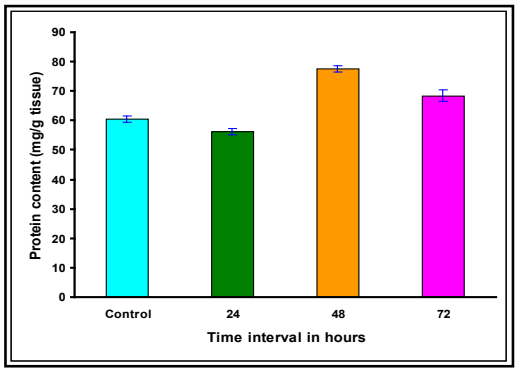

Fig. 1: Comparison of protein content in liver (mg/g tissue) of *Psammophilus* treated with furadan

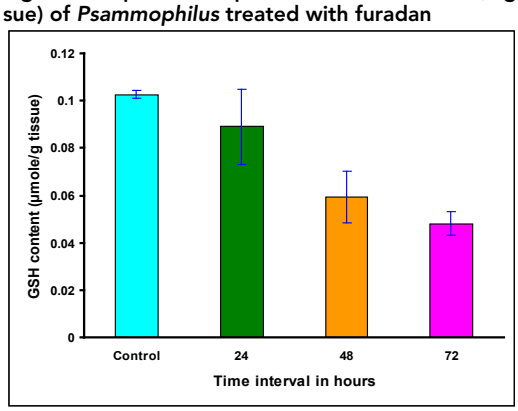

Fig.2: Comparison of GSH content in liver µ mol/g tissue) of *Psammophilus* treated with furadan

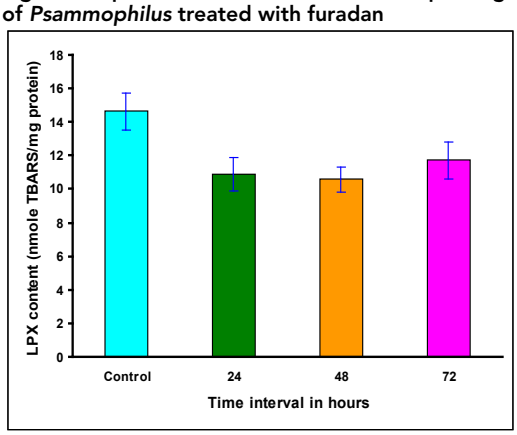

Fig. 3: Comparison of LPX content in liver (nmol TBARS/ mg protein) of *Psammophilus* treated with furadan

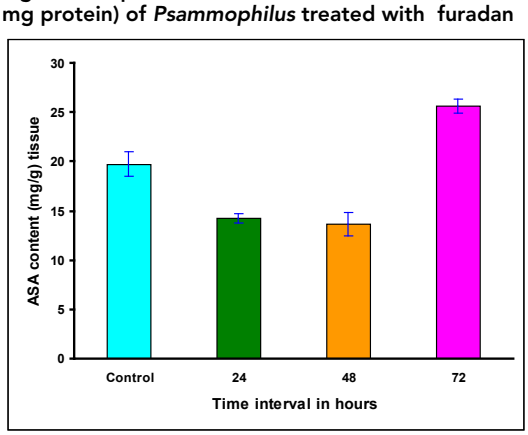

Fig.4:Comparison of ASA content in liver (mg/ g tissue) tissue) of *Psammophilus* treated with furadan

GSH content (µmol/g tissue) in liver tissue of *Psammophilus blanfordanus* treated with furadan (0.005mg/ml of acetone) were 0.1026± 0.0016 µmol/g tissue at 0 hr (control), 0.089±0.016 µmol/g tissue at 24 hr, 0.0592±0.011 µmol/g tissue at 48 hr and 0.048±0.005 µmol/g tissue at 72 hr. It is enumerated that the concentration of GSH was found highest in control groups and gradually decreases from control groups to 72 hr in liver tissue of *Psammophilus blanfordanus*. (Fig 2). The correlation analysis was used for the measurement of the linear association between variables. Pearson's correlation coefficients (r^2) among the analytical variables showed that the time interval is highly significant with GSH content in liver tissue of *Psammophilus blanfordanus*. [Time interval – GSH content (-0.922; $P \leq 0.05$)]. One way ANOVA showed that incubation period has significant effect on GSH content of liver tissue. Post-hoc analysis revealed that only 48 hr was highly significant [$F(3,8) = 19.168$, $P < 0.001$)]. All the combinations are different from each other ($P < 0.001$, LSD).

LPX content (nmol TBARS/mg protein) in liver tissue of *Psammophilus blanfordanus* treated with furadan (0.005mg/ml of acetone) were 14.63±1.12 nmol TBARS/mg protein at 0 hr (control), 10.866±0.999 nmol TBARS/mg protein tissue at 24 hr, 10.578±0.767 nmol TBARS/mg protein tissue at 48 hr and 11.728±1.107 nmol TBARS/mg protein tissue at 72 hr . It was estimated that the concentration of LPX was found highest in 0 hr and decreases from 72 hrs to 24 hr to 48 hr in liver tissue of *Psammophilus blanfordanus* (Fig.3).The correlation analysis was used for the measurement of the linear association between variables. Pearson's correlation coefficients (r^2) among the analytical variables showed that the time interval is significant with LPX content in liver tissue of *Psammophilus blanfordanus*. [Time interval – LPX content (-0.558; $P \leq 0.05$)]. One way ANOVA showed that incubation period has significant effect on LPX content of liver tissue. Post-hoc analysis revealed that only 72 hr is significant [$F(3,8)=10.122$, $P < 0.001$)]. All the combinations are different from each other ($P < 0.001$, LSD).

Ascorbic acid content (mg/g tissue) in liver tissue of *Psammophilus blanfordanus* treated with furadan (0.005mg/ml of acetone) were19.73±1.2 mg/g at 0 hr (control), 14.194 ±0.473 mg/g tissue at 24 hr, 13.61±1.21 mg/g tissue at 48 hr and 25.606±0.715 mg/g tissue at 72 hr. It was estimated that the concentration of Ascorbic acid was found highest in 72 hr and lowest in 48 hr. The ascorbic acid content at 0 hr was more than 24 hr in liver tissue of *Psammophilus blanfordanus*. (Fig-4). The correlation analysis was used for the measurement of the linear association between variables. Pearson's correlation coefficients (r^2) among the analytical variables showed that the time interval is significant with Ascorbic acid content in liver of *Psammophilus blanfordanus*. [Time interval – Ascorbic acid content (0.388; $P \leq 0.05$)]. One way ANOVA showed that incubation period has significant effect on Ascorbic acid content of liver tissue. Post-hoc analysis revealed that only 72 hr is significant [$F(3,8) = 103.628$, $P < 0.001$)]. All the combinations are different from each other. ($P < 0.001$, LSD).

Volume : 4 | Issue : 3 | Mar 2014 | ISSN - 2249-555X

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