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Comparative Study of Lipid Peroxidation and Reduced Glutathione in Pre-Clitellar, Clitellar and Post-Clitellar Region of *Eudrilus Eugeniae* Exposed to Furadan

KEYWORDS: furadan, Eudrilus eugeniae, clitellar, reduced glutathione, lipid peroxidation

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ABSTRACT *Protein content, lipid peroxidation (LPX) and reduced glutathione (GSH) level in pre-clitellar, clitellar and post-clitellar region of Eudrilus eugeniae exposed to the soil, spiked with furadan @ of 20mg/kg (at different time interval of 24 h, 48 h, 72h) were measured and compared with that of the control (0 h).*

INTRODUCTION

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). 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. Extensive field and lab investigations have indicated that earthworms are sensitive to a wide range of contaminants, including pesticides (Capowiz *et al.,* 2003; O'Halloran *et al.,* 1999). Edwards and Bohlen (1992) concluded that the susceptibility of different species of earthworms does not appear to differ greatly since the species' behavioural pattern as it relates to exposure is more important. Bioindicators are a good way to monitor the effects of toxic materials on organisms when this might be difficult to assess through direct toxicity level assessment in nature (Avgin and Luff, 2010).

Carbofuran or furadan (2, 3-dihydro -2, 2-dimethyl-7-benzofuranyl-N-methylcarbamate) is a widely used systemic and contact insecticide, acaricide and nematicide which has broad spectrum of activity against many agricultural pests. It 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). Bioaccumulation of pesticides in the food chain can lead to potentially adverse effect in humans and useful animal (Palmeira, 1999). In the present work generation of ROS in response to furadan were estimated by measuring reduced glutathione and lipid peroxidation level in different region (precliteller, clitellar, postclitellar) of (soil dwelling) earthworm, *Eudrilus eugeniae* and compared it with that of the control.

MATERIALS AND METHODS

Animal

Eudrilus eugeniae were purchased from soil conservation office, Baripada, Mayurbhanj and kept in plastic tray (30cm x 25cm x 6.5cm) in the laboratory. Each tray contains soil covered with net and moist gunny cloth (maintain temperature and darkness). *Eudrilus* were acclimatized for seven days in the laboratory condition prior to the experiment.

Treatment process

Four trays were taken and labeled as C (for control or untreated) and E24, E48 and E72 (for experimental or treated). Each tray containing 20 matured earthworm per 1.5 Kg soil and. The furadan (dose @ of 20mg/kg soil) was added to the soil of E24, E48 and E72 numbered tray.

Preparation of tissue samples

For preparation of sample a pool of 2 numbers of *Eudrilus eugeniae* were taken from each experimental tray (E24, E48 and E72) at different time intervals and also from the control (C). The Body of the earthworms was cut into 3 parts with the help of a sharp blade i.e. pre-clitellar, clitellar, and post-clitellar. The tissues (pre-clitellar, clitellar and post-clitellar) were kept at 4^0C in ice box.

The weights of the different parts were taken by the help of monopan digital weight machine (Shimandzu). Homogenate of tissues (different parts of the earthworm) were prepared with phosphate buffer (pH 7.4) and then centrifuged at 4°C in 4000 rpm for 10 minutes with high speed cold centrifuge (Remi).

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.

All the solution were prepared by using Millipore distilled water. The above experiments were repeated for 10 times.

RESULTS AND DISCUSSION

The protein content (mg/ g tissue) of pre clitellar region of treated *Eudrilus* at 24 h and 72 h were slightly higher in comparison to the untreated *Eudrilus* (0 h), however the protein content of treated (48h) is almost same to that of the untreated *Eudrilus* (Fig 1). In clitellar region, the protein content were always (after 24h, 48h and 72h of treatment) higher in comparison to untreated *Eudrilus* (Fig2). In post clitellar region the protein content increases at 24 h and decreases at 72 h and almost same at 48 h when compared with that of untreated *Eudrilus* (Fig 3).

GSH content of treated *Eudrilus* were found less than that of the untreated *Eudrilus* in all experimental tests in pre clitellar region of *Eudrilus eugeniae*. GSH content was almost same in 48 hour tests as compared to control, at 24 h it increased

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and 72 h GSH content was decreased in experimental group (fig 4). In clitellar region the concentration of GSH decreases more in 72 h as compared to 24 h and 48 h in experimental group (Fig 5). In post clitellar region the GSH content same in 24 h and at 48 h as compared to the GSH content in 72 h in experimental group (Fig 6).

In pre clitellar region lipid peroxidation of *Eudrilus eugeniae* decrease at 24 h and 72 h in experimental group and at 48 h it increases as compare to control test (Fig 7). Its decrease in different time intervals (24 h, 48 h and 72 h). In clitellar region LPX was decreased in experimental samples of *Eudrilus eugeniae* as compared to the control samples

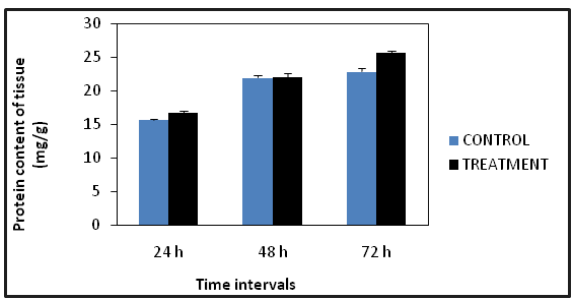

Fig-1: Comparison of protein content (mg/g) of pre-clitellar region of Eudrilus eugeniae at different time intervals.

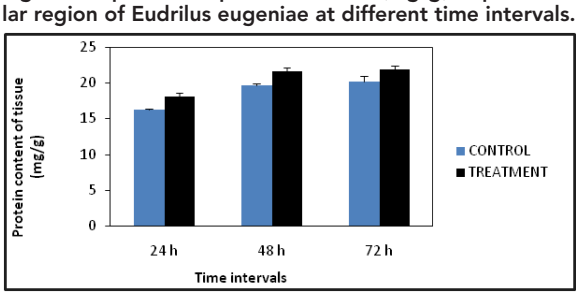

Fig-2: Comparison of protein content (mg/g) of clitellar region of Eudrilus eugeniae at different time intervals.

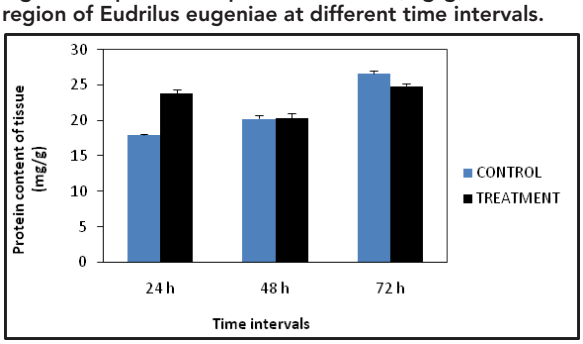

Fig-3: Comparison of protein content (mg/g) of post-clitellar region of Eudrilus eugeniae at different time interval.

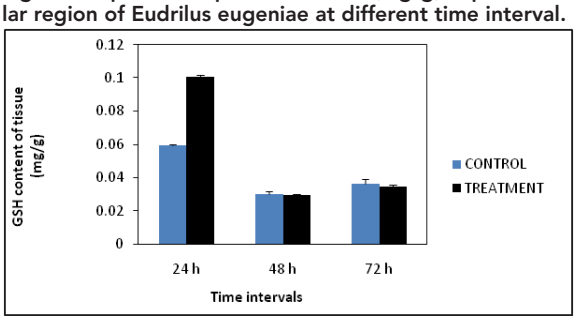

Fig-4: Comparison of reduced glutathione (mg/g) of pre-clitellar region of Eudrilus eugeniae at different time intervals.

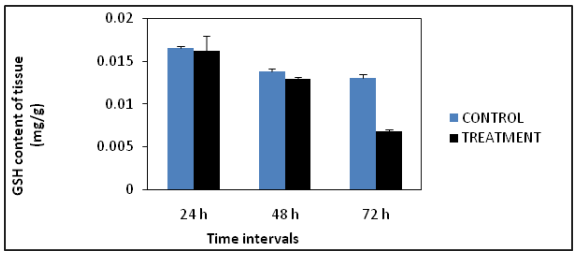

Fig-5: Comparison of reduced glutathione (mg/g) of clitellar region of Eudrilus eugeniae at different time intervals.

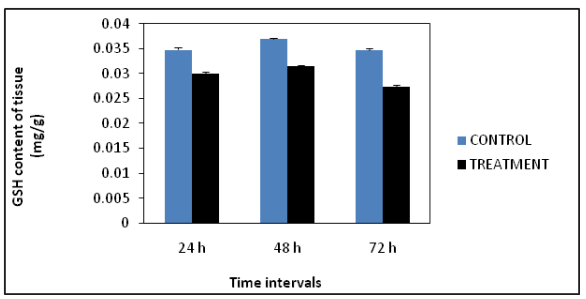

Fig-6: Comparison of reduced glutathione (mg/g) of post-clitellar region of Eudrilus eugeniae at different time interval.

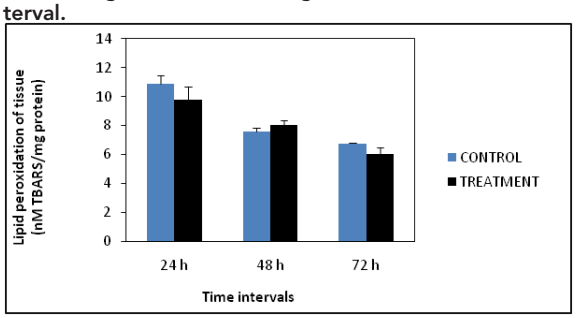

Fig-7: Comparison of lipid peroxidation (nM TBARS/mg protein) of pre-clitellar region of Eudrilus eugeniae at different time intervals.

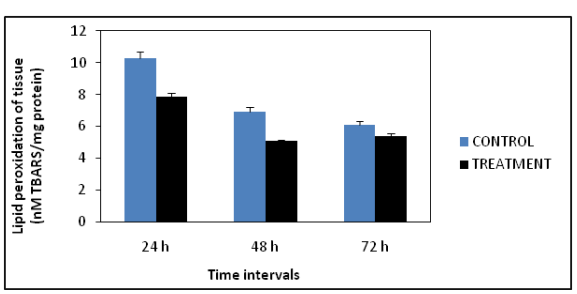

Fig-8: Comparison of lipid peroxidation (nM TBARS/mg protein) of clitellar region of Eudrilus eugeniae at different time intervals.

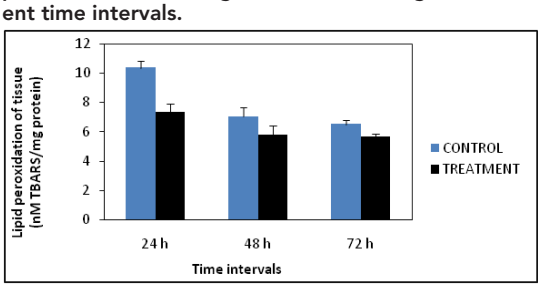

Fig-9: Comparison of lipid peroxidation (nM TBARS/mg protein) of post-clitellar region of Eudrilus eugeniae at different time intervals.

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at 24 h, 48 h and 72 h (Fig 8). In post clitellar region LPX was increased at 48 h and 72 h and decreased at 24 h as compared to control(Fig 9).

STATISTICS

The statistical analysis was done with the help of statistical package SPSS 16.0. Correlation analysis test was carried out to find out the level of significance between *Eudrilus eugeniae* treated with Furadan at different time intervals of 24 h, 48 h, and 72 h along with that of untreated (0 h). A difference was taken as significant when P was less than 0.05.

Correlation analysis of the data revealed that protein content of *Eudrilus eugeniae* of untreated and treated at 24h were varied significantly. Protein content of clitellar and post-clitellar were also highly significant in experimental (treated) group with respect to control(untreated). At 48 h protein content are not significant in any region. At 72 h all regions are significant with respect to control group at the level 0.05(2-tailed).

Correlation analysis showed that GSH content of 24 h treated *Eudrilus* are highly significant in comparison to the corresponding untreated (24h) *Eudrilus* . At 48 h pre clitellar, clitellar and post clitellar are not varied significantly. At 72 h experimental group and control group correlation is highly significant, so it varied significantly at the level 0.05(2-tailed).

LPX content in experimental group at 24 h is not varied significantly but at 48 hour control and experimental group varied significantly. A 72 h also both are highly significant and in all regions the correlation is significant at the 0.05 level (2-tailed).

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