Original Research Paper

Volume-8 | Issue-5 | May-2018 | PRINT ISSN No 2249-555X

Biology

EFFECT OF PROTEIN, LIPID, REDUCED GLUTATHIONE, ASCORBIC ACID AND LIPID PEROXIDATION LEVEL OF THE EARTHWORM, *Eudrilus eugeniae* EXPOSED TO FURADAN, CURCUMIN AND VITAMIN A

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ABSTRACT Earthworms *(Eudrilus eugeniae*, n=40) were segregated from the vermiculture stock maintained by the laboratory into 4 different bucket filled with 750 g of soil and were divided into two groups: Control (Group A) and Experimental (Group B, C and D). 15mg of furadan was mixed thoroughly with the soil of Bucket B, C and D. 1mg of curcumin and 50µl of vitamin A were added to the experimental group C and D respectively. The animals were sacrificed after different time intervals (24hr, 48hr and 72hr) and processed for the biochemical analysis. The lipid peroxidation (LPX), ascorbic acid (ASA), lipid, reduced glutathione (GSH) and protein content of both control and experimental group were measured and compared.

KEYWORDS : Furadan, curcumin, vitaminA, protein, LPX, GSH, ASA, lipid, Eudrilus eugeniae

INTRODUCTION

Earthworms constitute a major component in soil functioning and play a major role in chemical with which they sense chemicals in the soil (Haque and Ebing, 1983). They also ingest soil particles together with the microscopic organisms on which they feed. They are therefore in very close contact with whatever substance are present in the soil. This makes them very suitable as models for testing of toxic effects of chemicals on soil organisms. (Reineke S.A. *et al.* 2005).Earthworms are often preferred subject in soil ecotoxological research because they are quite easy to handle and measure their different life cycle parameters, in accumulation and excretion of metals and biochemical responses, earthworms ingest large amount of soil and are exposed to pesticides through their intestine as well as through the skin, where fore concentrating heavy metals from the soil in their body (Morgan, 1999). Contamination of soil by pesticide can change the functioning of soil ecosystem qualitatively and quantitatively by disturbing the activities of soil fauna (Cortet *et al.,* 1999).

In the present work earthworm (bioindicator of soil) were exposed to four different condition: (i) No furadan, (ii) furadan; (iii) furadan+curcumin; (iv) furadan+vitamin A. After different time interval different biochemical parameters were measured and compared.

Materials and methods

Earthworms (*Eudrilus eugeniae)* were collected from the soil chemist office, Markona, Balasore and acclatamised for 10 days in the laboratory prior to the experiment. The earthworms were segregated into four different bucket filled with 750 g of soil and were divided into two groups: Control (Group A) and Experimental (Group B, C and D). 15mg of furadan was mixed thoroughly with the soil of Bucket B, C and D. 1mg of curcumin and 50µl of vitamin A were added to the experimental group C and D respectively. The animals were sacrificed at different time intervals and the whole body tissue homogenate were prepared with phosphate buffer (pH 7.4) for GSH, LPX, protein, ethanol for lipid, metaphosphoric acid for ASA and then centrifuged at 4000 rpm for 10 minutes in a cold centrifuged machine.

Protein: Protein estimation of samples were measured according to the method of Lowry *et al*., (1961).

Lipid Peroxidation (LPX): Lipid peroxidation estimation of samples were measured according to the method of Ohkawa *et al*., (1979).

Reduced Glutathione (GSH): Reduced glutathione estimation of samples were measured according to the method of Ellman (1959).

Lipid : Lipid estimation of samples were measured according to the method of Folch *et al., (*1957).

Ascorbic acid (ASA): Ascorbic acid estimation of samples were measured according to the method of Jagota and Dani (1982).

RESULTS AND DISCUSSIONS

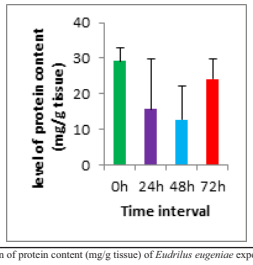

Fig.1: Comparison of protein content (mg/g tissue) of *Eudrilus eugeniae* exposed to Furadan at different time interval

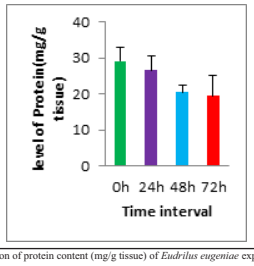

Fig-6; Comparison of protein content (mg/g tissue) of *Eudrilus eugeniae* exposed to Furadan with curcumin at different time interval

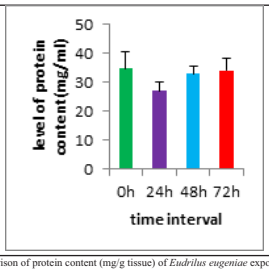

Fig-11; Comparison of protein content (mg/g tissue) of *Eudrilus eugeniae* exposed to Furadan with vitamin A at different time interval

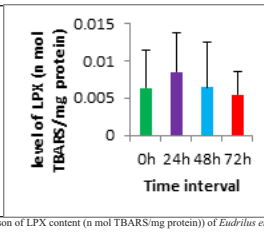

Fig-2; Comparison of LPX content (n mol TBARS/mg protein)) of *Eudrilus eugeniae* exposed to Furadan at different time interval

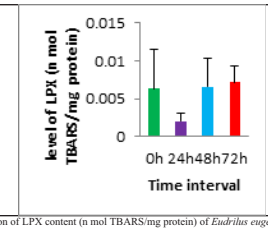

Fig-7; Comparison of LPX content (n mol TBARS/mg protein) of *Eudrilus eugeniae* exposed to Furadan with curcumin at different time interval

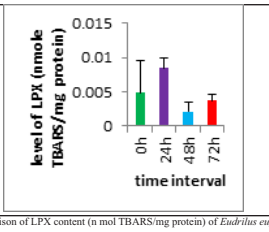

Fig-12; Comparison of LPX content (n mol TBARS/mg protein) of *Eudrilus eugeniae* exposed to Furadan with vitamin A at different time interval

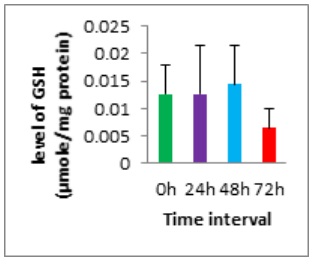

Fig-3; Comparison of GSH content (µmole/mg protein) of *Eudrilus eugeniae* exposed to Furadan at different time interval

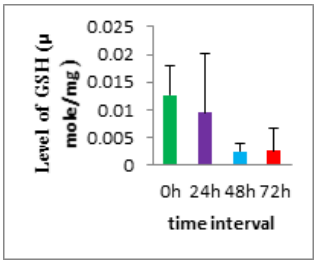

Fig-8; Comparison of GSH content (µmole/mg) of *Eudrilus eugeniae*exposed to Furadan with curcumin at different time interval

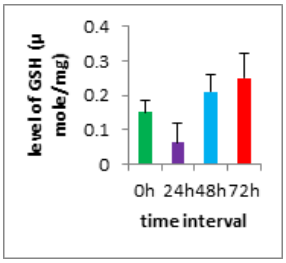

Fig-13. Comparison of GSH content (µmole/mg) of *Eudrilus eugeniae* exposed to Furadan with vitamin A at different time interval

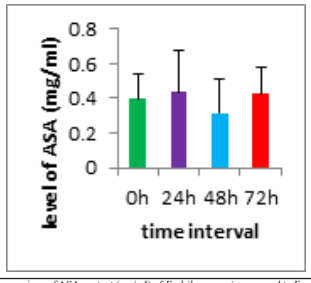

Fig-4; Comparison of ASA content (mg/ml) of *Eudrilus eugeniae* exposed to Furadan at different time interval

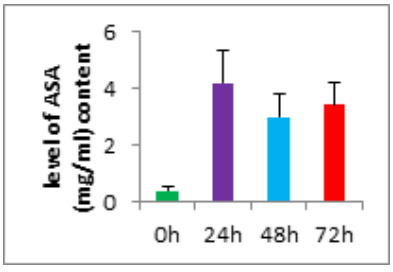

Fig-9; Comparison of ASA content (mg/ml) of *Eudrilus eugeniae* exposed to Furadan with curcumin at different time interval

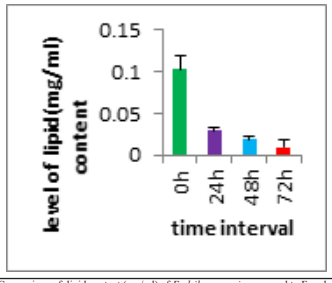

Fig-14; Comparison of lipid content (mg/ml) of *Eudrilus eugeniae* exposed to Furadan with vitamin A at different time interval

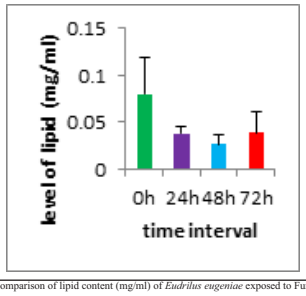

Fig-5; Comparison of lipid content (mg/ml) of *Eudrilus eugeniae* exposed to Furadan at different time interval

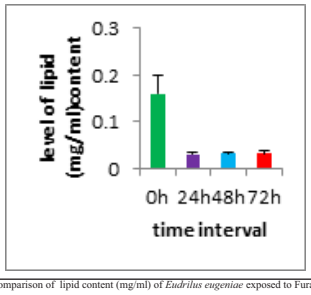

Fig-10; Comparison of lipid content (mg/ml) of *Eudrilus eugeniae* exposed to Furadan with curcumin at different time interval

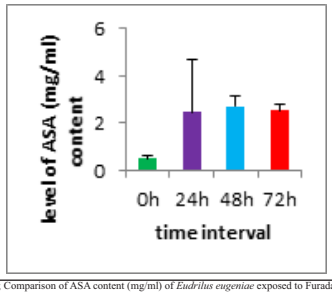

Fig-15; Comparison of ASA content (mg/ml) of *Eudrilus eugeniae* exposed to Furadan with vitamin A at different time interval

Protein content

Protein content (mg/g tissue) in *Eudrilus eugeniae* exposed with Furadan (15 mg/kg soil) were 29.176± 3.754917 mg/g tissue, 15.644 ± 14.12724 mg/g tissue, 12.8053 ± 9.545127 mg/g tissue, 24.13 ± 5.844528 mg/g tissue after 0 hour, 24 hours, 48 hours and 72 hours respectively .

The protein content (mg/g tissue) gradually decreased at 24 hour and 48 hours and then it was increased at 72 hours. It was lower in 48 hours in comparison to *Eudrilus eugeniae* exposed to Furadan at different time intervals. The protein content was highest at 0 hours (Fig.1).

One way ANOVA reveals that the protein content at different time intervals in *Eudrilus eugeniae* is significant (F (3, 39) =11.144, P=0.000). Post Hoc analysis revealed that the protein content at different time intervals when exposed with Furadan in *Eudrilus eugeniae* was significant (P<0.05; LSD) at 0.05 level of significance.

Protein content (mg/g tissue) in *Eudrilus eugeniae* exposed with furadan (15 mg/kg soil) and curcumin (mg/ml) were 29.176± 3.754917 mg/g tissue, 26.646 ± 3.904277 mg/g tissue, 20.47 ± 2.020896 mg/g tissue, 19.494 ± 5.691439 mg/g tissue after 0 hour, 24 hours, 48 hours and 72 hours respectively .

The protein content (mg/g tissue) gradually decreased at 24 hour, 48 hours and 72 hours. It was lower in 72 hours in comparison to *Eudrilus eugeniae* exposed to furadan with curcumin at different time intervals. The protein content was highest at 0 hours (Fig.6).

One way ANOVA reveals that the protein content at different time intervals in *Eudrilus eugeniae* is significant (F (3, 39) =33.050, P=0.000). Post Hoc analysis revealed that the protein content at different time intervals when exposed with Furadan with curcumin in *Eudrilus eugeniae* was significant (P<0.05; LSD) at 0.05 level of significance.

Protein content (mg/g tissue) in *Eudrilus eugeniae* exposed with Furadan (15 mg/kg soil) and vitamin A (mg/ml) were 34.7307± 5.715569 mg/g tissue, 27.0196 ± 2.952909 mg/g tissue, 33.0205 ± 2.712821 mg/g tissue, 34.0251 ± 4.099929 mg/g tissue after 0 hour, 24 hours, 48 hours and 72 hours respectively .

The protein content (mg/g tissue) decreased at 24 hour and gradually increased at 48 hours and 72 hours. It was lower in 24 hours in comparison to *Eudrilus eugeniae* exposed to Furadan with vitaminA at different time intervals. The protein content was highest at 0 hours (Fig.11).

One way ANOVA reveals that the protein content at different time intervals in *Eudrilus eugeniae* is significant (F (3, 39) =7.575, P=0.000). Post Hoc analysis revealed that the protein content at different time intervals when exposed with Furadan and vitamin A in *Eudrilus eugeniae* was significant (P<0.05; LSD) at 0.05 level of significance.

LPX content

Lipid peroxidation content (mg/g tissue) in *Eudrilus eugeniae* exposed with Furadan (15 mg/kg soil) were 0.00634± 0.0052 mg/g tissue, 0.00849 ± 0.005266 mg/g tissue, 0.00645 ± 0.005997 mg/g tissue, 0.00545 ± 0.003172 mg/g tissue after 0 hour, 24 hours, 48 hours and 72 hours respectively .

Lipid peroxidation content (mg/g tissue) increased at 24 hour and then gradually decreased at 48 hours and 72 hours. It was lower in 72 hours in comparison to *Eudrilus eugeniae* exposed to Furadan at different time intervals. Lipid peroxidation content was highest at 24hours (Fig.2).

One way ANOVA reveals that the protein content at different time intervals in *Eudrilus eugeniae* is significant (F (3, 39) =1.068,

P=0.375). Post Hoc analysis revealed that the protein content at different time intervals when exposed with Furadan in *Eudrilus eugeniae* was not significant (P<0.05; LSD) at 0.05 level of significance.

Lipid peroxidation content (mg/g tissue) in *Eudrilus eugeniae* exposed with Furadan (15 mg/kg soil) and curcumin (mg/ml) were 0.00634± 0.0052 mg/g tissue, 0.00203 ± 0.001098 mg/g tissue, 0.00655 ± 0.003775 mg/g tissue, 0.00714 ± 0.00221 mg/g tissue after 0 hour, 24 hours, 48 hours and 72 hours respectively .

Lipid peroxidation content (mg/g tissue) decreased at 24 hour and 48 hours and then it was gradually increased at 48 hours and 72 hours. It was lower in 24 hours in comparison to *Eudrilus eugeniae* exposed to Furadan with curcumin at different time intervals. Lipid peroxidation content was highest at 72 hours (Fig.7).

One way ANOVA reveals that the protein content at different time intervals in *Eudrilus eugeniae* is significant (F (3, 39) =4.903, P=0.006). Post Hoc analysis revealed that the protein content at different time intervals when exposed with Furadan with curcumin in *Eudrilus eugeniae* was significant (P<0.05; LSD) at 0.05 level of significance.

Lipid peroxidation (mg/g tissue) in *Eudrilus eugeniae* exposed with Furadan (15 mg/kg soil) and vitamin A (mg/ml) were 0.00482± 0.004746 mg/g tissue, 0.0085 ± 0.001434 mg/g tissue, 0.00206 ± 0.001408 mg/g tissue, 0.00368 ± 0.000935 mg/g tissue after 0 hour, 24 hours, 48 hours and 72 hours respectively .

Lipid peroxidation content (mg/g tissue) increased at 24 hour and decreased at 48 hours and then again it was increased at 72 hours. It was lower in 48 hours in comparison to *Eudrilus eugeniae* exposed to Furadan and vitamin A at different time intervals. Lipid peroxidation content was highest at 24 hours (Fig.12).

One way ANOVA reveals that the protein content at different time intervals in *Eudrilus eugeniae* is significant (F (3, 39) =10.908, P=0.000). Post Hoc analysis revealed that the protein content at different time intervals when exposed with Furadan with vitamin A in *Eudrilus eugeniae* was significant (P<0.05; LSD) at 0.05 level of significance.

GSH content

Reduced glutathione content (mg/g tissue) in *Eudrilus eugeniae* exposed with Furadan (15 mg/kg soil) were 0.01268± 0.005209 mg/g tissue, 0.01263 ± 0.008755 mg/g tissue, 0.01446 ± 0.007069 mg/g tissue, 0.00649 ± 0.003408 mg/g tissue after 0 hour, 24 hours, 48 hours and 72 hours respectively .

Reduced glutathione content (mg/g tissue) gradually decreased at 24 hour and increased 48 hours and then it was again decreased at 72 hours. It was lower in 72 hours in comparison to *Eudrilus eugeniae* exposed to Furadan at different time intervals. Reduced glutathione content was highest at 48 hours (Fig.3).

One way ANOVA reveals that the protein content at different time intervals in *Eudrilus eugeniae* is significant (F (3, 39) =5.103, P=0.005). Post Hoc analysis revealed that the protein content at different time intervals when exposed with Furadan in *Eudrilus eugeniae* was significant (P<0.05; LSD) at 0.05 level of significance.

Reduced glutathione content (mg/g tissue) in *Eudrilus eugeniae* exposed with Furadan (15 mg/kg soil) and curcumin (mg/ml) were 0.01268± 0.005209 mg/g tissue, 0.00956 ± 0.010663 mg/g tissue, 0.00257 ± 0.001437 mg/g tissue, 0.00268 ± 0.004014 mg/g tissue after 0 hour, 24 hours, 48 hours and 72 hours respectively .

Reduced glutathione content (mg/g tissue) gradually decreased at 24 hour and 48 hours and then it was increased at 72 hours. It was lower in 48 hours in comparison to *Eudrilus eugeniae* exposed to Furadan with curcumin at different time intervals. Reduced glutathione content was highest at 0 hours (Fig.8).

One way ANOVA reveals that the protein content at different time intervals in *Eudrilus eugeniae* is significant (F (3, 39) =9.089, P=0.000). Post Hoc analysis revealed that the protein content at different time intervals when exposed with Furadan with curcumin in *Eudrilus eugeniae* was significant (P<0.05; LSD) at 0.05 level of significance.

Reduced glutathione (mg/g tissue) in *Eudrilus eugeniae* exposed with Furadan (15 mg/kg soil) and vitamin A (mg/ml) were 0.1504± 0.036525 mg/g tissue, 0.0167 ± 0.058638 mg/g tissue, 0.2105 ± 0.049214 mg/g tissue, 0.2493 ± 0.071793 mg/g tissue after 0 hour, 24 hours, 48 hours and 72 hours respectively .

Reduced glutathione content (mg/g tissue) decreased at 24 hour and gradually increased 48 hours and 72 hours. It was lower in 24 hours in comparison to *Eudrilus eugeniae* exposed to Furadan and vitamin A at different time intervals. Reduced glutathione content was highest at 72 hours (Fig.13).

One way ANOVA reveals that the protein content at different time intervals in *Eudrilus eugeniae* is significant (F (3, 39) =21.622, P=0.000). Post Hoc analysis revealed that the protein content at different time intervals when exposed with Furadan and vitamin A in *Eudrilus eugeniae* was significant (P<0.05; LSD) at 0.05 level of significance.

Lipid content

Lipid content (mg/g tissue) in *Eudrilus eugeniae* exposed with Furadan (15 mg/kg soil) were 0.0797± 0.038976 mg/g tissue, 0.0384 ± 0.007905 mg/g tissue, 0.027 ± 0.010467 mg/g tissue, 0.0393 ± 0.022031 mg/g tissue after 0 hour, 24 hours, 48 hours and 72 hours respectively .

Lipid content (mg/g tissue) gradually decreased at 24 hour and 48 hours and then it was increased at 72 hours. It was lower in 48 hours in comparison to *Eudrilus eugeniae* exposed to Furadan at different time intervals. The lipid content was highest at 0 hours (Fig.5).

One wayANOVA reveals that the protein content at different time intervals in *Eudrilus eugeniae* is significant (F (3, 39) =9.797, P=0.000). Post Hoc analysis revealed that the protein content at different time intervals when exposed with Furadan in *Eudrilus eugeniae* was significant (P<0.05; LSD) at 0.05 level of significance.

Lipid content (mg/g tissue) in *Eudrilus eugeniae* exposed with Furadan (15 mg/kg soil) and curcumin were 0.1594± 0.38976 mg/g tissue, 0.0306 ± 0.002836 mg/g tissue, 0.0318 ± 0.003327 mg/g tissue, 0.0326 ± 0.004827 mg/g tissue after 0 hour, 24 hours, 48 hours and 72 hours respectively .

Lipid content (mg/g tissue) decreased at 24 hour and gradually increased at 48 hours and 72 hours. It was lower in 24 hours in comparison to *Eudrilus eugeniae* exposed to Furadan and curcumin at different time intervals. The lipid content was highest at 0 hours (Fig.10).

One way ANOVA reveals that the protein content at different time intervals in *Eudrilus eugeniae* is significant (F (3, 34) =12.325, P=0.000). Post Hoc analysis revealed that the protein content at different time intervals when exposed with Furadan and curcumin in *Eudrilus eugeniae* was significant (P<0.05; LSD) at 0.05 level of significance.

Lipid content (mg/g tissue) in *Eudrilus eugeniae* exposed with Furadan (15 mg/kg soil) and vitamin A were 0.1032± 0.016346 mg/g tissue, 0.0296 ± 0.002966 mg/g tissue, 0.0192 ± 0.003701 mg/g tissue, 0.0092 ± .000968 mg/g tissue after 0 hour, 24 hours, 48 hours and 72 hours respectively .

Lipid content (mg/g tissue) gradually decreased at 24 hour and 48 hours and 72 hours. It was lower in 72 hours in comparison to *Eudrilus eugeniae* exposed to Furadan and vitamin A at different time intervals. The lipid content was highest at 0 hours (Fig.14).

One way ANOVA reveals that the protein content at different time intervals in *Eudrilus eugeniae* is significant (F (3, 37) =5.403, P=0.004). Post Hoc analysis revealed that the protein content at different time intervals when exposed with Furadan and vitamin A in *Eudrilus eugeniae* was significant (P<0.05; LSD) at 0.05 level of significance.

ASA content

Ascorbic acid content (mg/g tissue) in *Eudrilus eugeniae* exposed with Furadan (15 mg/kg soil) were 0.3987± 0.138996 mg/g tissue, 0.436 ± 0.243956 mg/g tissue, 0.3145 ± 0.195753 mg/g tissue, 0.4247 ± 0.150515 mg/g tissue after 0 hour, 24 hours, 48 hours and 72 hours respectively .

Ascorbic acid content (mg/g tissue) gradually increased at 24 hour and decreased at 48 hours and then it was again increased at 72 hours. It was lower in 48 hours in comparison to *Eudrilus eugeniae* exposed to Furadan at different time intervals. The Ascorbic acid was highest at 24 hours (Fig.4).

One way ANOVA reveals that the protein content at different time intervals in *Eudrilus eugeniae* is significant (F (3, 39) =0.863, P=0.469). Post Hoc analysis revealed that the protein content at different time intervals when exposed with Furadan in *Eudrilus eugeniae* was significant (P<0.05; LSD) at 0.05 level of significance.

Ascorbic acid content (mg/g tissue) in *Eudrilus eugeniae* exposed with Furadan and curcumin were 0.3987± 0.138996 mg/g tissue, 4.1944 ± 1.136559 mg/g tissue, 2.9916 ± 0.842732 mg/g tissue, 3.4186 ± 0.861968 mg/g tissue after 0 hour, 24 hours, 48 hours and 72 hours respectively .

Ascorbic acid content (mg/g tissue) gradually increased at 24 hour and decreased at 48 hours and then it was again increased at 72 hours. It was lower in 48 hours in comparison to *Eudrilus eugeniae* exposed to Furadan and curcumin at different time intervals. Ascorbic acid content was highest at 24 hours (Fig.9).

One way ANOVA reveals that the protein content at different time intervals in *Eudrilus eugeniae* is significant (F (3, 34) =39.143, P=0.000). Post Hoc analysis revealed that the protein content at different time intervals when exposed with Furadan in *Eudrilus eugeniae* was significant (P<0.05; LSD) at 0.05 level of significance.

Ascorbic acid content (mg/g tissue) in *Eudrilus eugeniae* exposed with Furadan and vitamin A (15 mg/kg soil) were 0.5046± 0.099673 mg/g tissue, 2.4712 ± 2.223077 mg/g tissue, 2.707 ± 0.465925 mg/g tissue, 2.5424 ± 0.248412 mg/g tissue after 0 hour, 24 hours, 48 hours and 72 hours respectively .

Ascorbic acid content (mg/g tissue) gradually increased at 24 hour and 48 hours and then it was decreased at 72 hours. It was lower in 48 hours in comparison to *Eudrilus eugeniae* exposed to Furadan and curcumin at different time intervals. Ascorbic acid content was highest at 0 hours (Fig.15).

One way ANOVA reveals that the protein content at different time intervals in *Eudrilus eugeniae* is significant (F (3, 37) =5.347, P=0.004). Post Hoc analysis revealed that the protein content at different time intervals when exposed with Furadan in *Eudrilus eugeniae* was significant (P<0.05; LSD) at 0.05 level of significance.

CONCLUSION

The results of present investigation showed that exposure of furadan promotes the generation of free radicals in *Eudrilus eugeniae* and it varies at different time intervals.

In addition, the results also gave significant information of furadan tolerance capabilities of *Eudrilus eugeniae* after long time exposure to contaminated soil. It has been demonstrated that the toxicological effect of Furadan in soil has different defence responses in affected *Eudrilus eugeniae* tissues and is time dependent. The reverse effects was seen when the antioxidant was exposed with Furadan .the level of protein, LPX, ASA and GSH was increased after some time duration but a little effect was seen in case of lipid.

REFERENCES

1. Abdul Rida,A.M.M., Bouche,M.B.,(1994).A method to assess chemical biorisks in terrestrial ecosystem. In: Donker, M.H., Eijsackers, H., Heimbach, F. (Eds.), Ecotoxicology of soil Organisms, Lewis, Boca Raton, pp.383-394.
2. Booth,L.H.and O Halloran,K.,(2001).Acomparison ofbiomarker responses in the earthworm Aporrectodea caliginosato the organophosphorous insecticidesdiazinon and chlorpyrifos.Environ.Toxicol.Chem.,20:2494-2502.
3. Cortet,J.,Vauflery,A.G.D.,Balaguer,N.P.,Gomot,L.,Texier,Ch.,Cluzeau,D(1999).The use of invertebrate soil fauna in monitoring pollutant effects.European Journal of Soil Biology 35, 115-134.
4. Ellman, G. D., 1959. Tissue sulphydryl groups. Arch. Biochem. Biophys. 82-70.
5. Folch, J., Lees, M. and Sloane Stanley,G.H.,(1957).A Simple Method for isolation and purification of total lipids from animal tissue.J.Biol.Chem.226:497-509
6. Haque, A., and Ebing, W. 1983. Toxicity determination of pesticides to earthewoms in soil substrate. J. Plant Dis. Prot. 90:395-408.
7. Jagota, S. K. and Dani, H. M., 1982. A new colorimetric Technique for the Estimation of Vitamin C using Folin Phenol Reagent. Analytical Biochemistry. 127:178-182.
8. Lee, K. E, 1985. Earthworms, their ecology and relationships with soils and land use. Academic press. London. pp 411.
9. Lowry, O.H., Resbrough, N.J., Farr, A.L. and Randoll, R.J., (1951). Protein measurement with the Folin-phenol reagent. J.Biol. Chem. 19: 265-275.
10. Lee, K. E, 1985. Earthworms, their ecology and relationships with soils and land use. Academic press. London. pp 411.
11. Lavelee,P.,(1997).Faunalactivities and soil processes:adaptive strategies that determine ecosystem function.Advance in ecological research 27,93-132.
12. Morgan, A. J., Sturzenbaum S. R., Winters, C. and Kille, P. 1999. Cellular and Molecular aspects of metal sequestration and toxicity in earthworms. Invertebr Reprod Dev. 6:17-24.
13. Ohkawa H., Ohishi, N. and Yagi, K., (1979). Assay of LPX in animal tissues by thiobarbituric acid reaction. Anal Biochem. Physiol.118C (1):33-37.
14. Stephenson, G.L.,Wren,C.D.,et al.,(1997)."Exposerof earthworm, Lubricus terrestris, to diazinon and the relative risk to passerine birds".Soil Biology and Biochemistry,29:717-720.