Original Research Paper

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Biology

EFFECT OF FERRIC CHLORIDE ON PROTEIN, LIPID, REDUCED GLUTATHIONE, LIPID PEROXIDATION, SUPEROXIDE DISMUTASE AND CATALASE LEVEL OF *Eudrilus eugeniae*

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ABSTRACT *Eudrilus eugeniae* (earthworm) cultured in laboratory condition were divided into four groups as C_{E0}, T_{E24}, T_{E48} and T_{E72} Each group placed in culture tray containing 1 kg of soil. 4mg of ferric Chloride were mixed in each tray of experimental earthworms (T_{E24}, T_{E48} and T_{E72}). The earthworms were sacrificed after different time intervals such as 0h (C_{E0}), 24h (T_{E24}), 48h (T_{E48}) and 72h (T_{E72}). The Protein, Lipid, Reduced Glutathione, Lipid Peroxidation, Superoxide Dismutase and Catalase level earthworm at different time intervals were measured and compared.

KEYWORDS : *Eudrilus eugeniae,* Ferric chloride, Protein, Lipid, Reduced Glutathione, Lipid Peroxidation, Superoxide dismutase and Catalase

INTRODUCTION

In biological systems, heavy metals have been reported to affect cellular organelles and components such as cell membrane, mitochondrial, lysosome, endoplasmic reticulum, nuclei, and some enzymes involved in metabolism, detoxification, and damage repair (Wang *et al*., 2001). Earthworms constitute a major component in soil functioning, and they play an important role in chemical element transformations (Lee, 1985). Contamination by heavy metal can change the functioning of soil ecosystems qualitatively and quantitatively by disturbing the activities of soil fauna (Cortet *et al*., 1999). The influence of heavy metals in soils on earthworms and their bioaccumulation has been the subject of many studies for a long time (e.g. Bouche ´, 1972; Kennette *et al*., 2002).This study was designed to see the toxic effects of ferric chloride on *Eudrilus eugeniae* by measuring different biochemical parameters like Protein, Lipid, Reduced Glutathione, Lipid Peroxidation, Superoxide dismutase and Catalase at different time intervals.

MATERIALS AND METHODS

Eudrilus eugeniae (Earthworm) were purchased from Soil Chemist Office, Takatpur, Baripada, Mayurbhanj. A culture of *Eudrilus eugeniae* was maintained in the laboratory. They were reared in buckets with perforated base, lined with gravel, coconut husk, farmyard manure on which worms are released. Bucket was covered with nylon net and gunny cloth and kept moist by sprinkling water. The cultured earthworms were divided into four groups as C_{E0}, T_{E24}, T_{E48} and T_{E72}. Each group placed in culture tray containing 1 kg of soil. To each tray (T_{E24}, T_{E48} and T_{E72}) 4mg of Ferric Chloride were added and mixed properly as experimental group.. The earthworms were sacrificed after exposure to ferric chloride for different time intervals, 24h (T_{E24}), 48h (T_{E48}) and 72h (T_{c72}).

5-6 number of earthworms were picked up from the designated tray as time intervals and their pooled weight was measured in digital monopan balance (Shimadzu; ELB 300). A 20% homogenate was prepared in ice-cold 50mM phosphate buffer (pH 7.4) using pre-chilled porcelain mortar and pestle by up and down strokes at 4°C. The homogenate was centrifuged at 4500 rpm (1000 Xg) for 10 minutes at 4°C in Cooling Centrifuge (Remi). The supernatant was taken for biochemical assay.

Protein estimation

Protein estimation of the samples was made according to the method of Lowry *et al.,* (1951). Protein content was expressed as mg/g wet weight of the tissue and aqueous BSA (Bovine Serum Albumin) was taken as standard protein.

Lipid

Lipid was estimated by Vanilline-phosphoric acid reagent according to Folch method (1957). The lipid content was expressed in mg/g tissue.

Superoxide Dismutase

Superoxide dismutase (SOD; EC 1.15.1.1) activity was determined according to the method of Das *et al.,* (2000). SOD activity was expressed as units/mg protein

Estimation of Catalase

Catalase (CAT; EC 1.11.1.6) activity was estimated according to Beers and Sizer (1952). The activity of catalase was expressed as nkat/mg protein.

Reduced Glutathione

Reduced glutathione (GSH of the sample were estimated by Ellman (1959) method. The data are expressed as mg /gm tissue.

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). The data are expressed as nmoles of TBARS/ mg protein.

Statistical methods

One-way ANOVA and Post Hoc analysis was carried out to find out the level of significance between *Eudrilus eugeniae* exposed over a period of 24 h, 48 h, 72 h of Ferric chloride in comparison to the 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

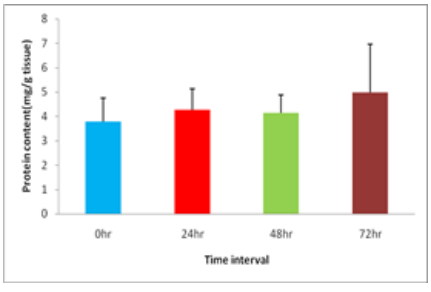

Fig 1. Comparison of protein content (mg/g tissue)of the *Eudrilus* exposed to f erric chloride (5 mg/ml) at different time intervals.

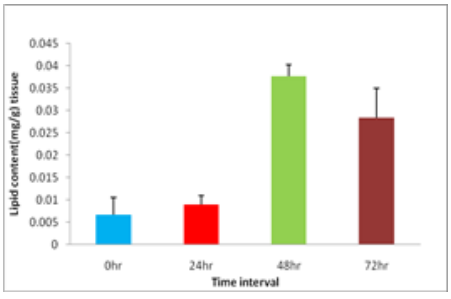

Fig2. Comparison of lipid content (mg/g tissue) of the *Eudrilus* exposed to f erric chloride (5 mg/ml) at different time intervals.

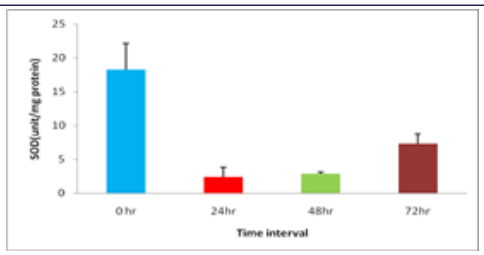

Fig 3. Comparison of SOD activity (Unit/mg protein)of the *Eudrilus* exposed to f erric chloride (5 mg/ml) at different time intervals.

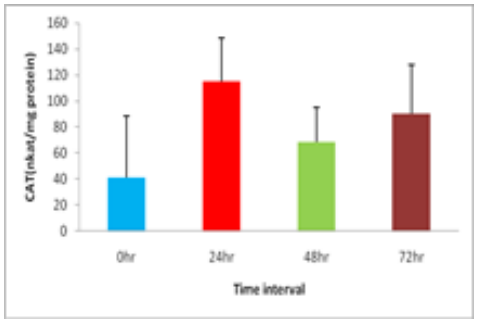

Fig 4. Comparison of CAT activity (Unit/mg protein)of the *Eudrilus* exposed to f erric chloride (5 mg/ml) at different time intervals.

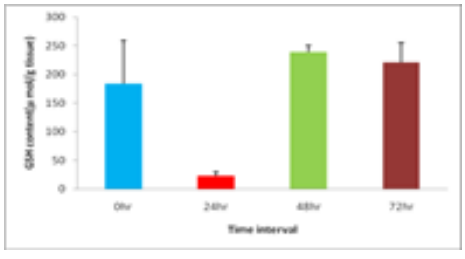

Fig 3. Comparison of GSH content (µ mol/g tissue) of the *Eudrilus* exposed to f erric chloride (5 mg/ml) at different time intervals

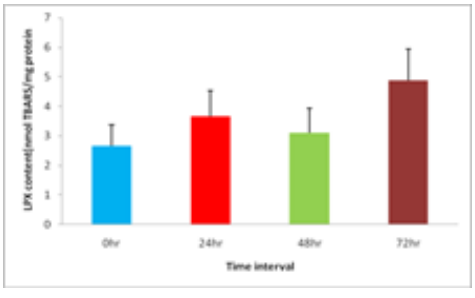

Fig 3. Comparison of LPX level (n mol TBARS/mg protein) of the *Eudrilus* exposed to f erric chloride (5 mg/ml) at different time intervals

Protein content

Protein content (mg/g tissue) in *Eudrilus eugeniae* treated with Ferric chloride (5mg/ml) were 3.778 ±0.978 mg/g tissue, 4.268 ± 0.857 mg/g tissue, 4.151 ± 0.723 mg/g tissue, 4.993 ±1.956 mg/g tissue at different time intervals such as 0 hour, 24 hours, 48 hours and 72 hours respectively. The protein content (mg/g tissue) increased at 24 hours and then slightly increased at 72 hour in comparison to the control (fig.1)

One way ANOVA was performed in order to analyse the effect of ferric chloride (5mg/ml) on the protein content at different time intervals in *Eudrilus eugeniae*. One way ANOVA revealed that the protein content at different time intervals in *Eudrilus eugeniae* is significant [F (3, 11) = 0.514, P=0.684]. Post Hoc analysis revealed that the protein content at different time intervals when treated with ferric chloride in *Eudrilus eugeniae* was not significant at any hour with respect to control.

Lipid content

Lipid content (mg/g tissue) in *Eudrilus eugeniae* treated with ferric chloride(5 mg/ml) were 0.007 ± 0.004 mg/g tissue, 0.009 ± 0.002 mg/g tissue, 0.038 ± 0.003 mg/g tissue, 0.028 ±0.007 mg/g tissue after 0 hour, 24 hours, 48 hours and 72 hours respectively. The lipid content (mg/g tissue) increased at 24 hours, 48 hours and 72 hours. It was highest at 48 hours and lowest at 24 hours in comparison to *Eudrilus eugeniae* exposed to ferric chloride at different time intervals (Fig. 2).

One way ANOVA was performed in order to analyse the effect of ferric chloride (5 mg/ml) on the lipid content at different time intervals in *Eudrilus eugeniae*. One way ANOVA revealed that the lipid content at different time intervals in *Eudrilus eugeniae* is significant [F (3, 11) = 39.399, P=0.000]. Post Hoc analysis revealed that the lipid content at different time intervals when treated with ferric chloride in *Eudrilus eugeniae* was significant at48 hours and 72 hours (P<0.05; LSD) with respect to control.

Superoxide dismutase (SOD) activity

Superoxide dismutase activity (Unit/mg protein) in *Eudrilus eugeniae* treated with ferric chloride(5 mg/ml) were 18.247 ± 3.947 Unit/mg protein at 0 hour (control), 2.412 ± 1.391 Unit/mg protein after 24 hours, 2.864 ± 0.252 Unit/mg protein after 48 hours, 7.332 ± 1.432 Unit/mg protein after 72 hours . The SOD level (Unit/mg protein) of *Eudrilus eugeniae* exposed to ferric chloride was highest at 0 hour (control) and then gradually decreased at 24 hour and 48 hours. The SOD level at 72 hours again increases then 48 hours (Fig. 3).

One way ANOVA revealed that the SOD activity (Unit/mg protein) in *Eudrilus eugeniae* exposed to ferric chloride (5 mg/ml) at different time intervals is significant [F (3, 11) = 33.155, P=0.000]. Post Hoc analysis revealed that the SOD activity (Unit/mg protein) at different time intervals when treated with ferric chloride in *Eudrilus eugeniae* was significant at 24 hours, 48 hours and 72 hours (P<0.05; LSD) with respect to control.

Catalase (CAT) activity

Catalase activity (nkat/mg protein) in *Eudrilus eugeniae* treated with ferric chloride(5 mg/ml) were 41.316 ± 47.230 nkat/mg protein, 115.285 ± 33.499 nkat/mg protein, 68.19 ± 26.629 nkat/mg protein, 90.183 ± 37.797 nkat/mg protein, after 0 hour, 24 hours, 48 hours and 72 hours respectively. The CAT level (nkat/mg protein) was highest at 24 hours and only slightly increased at 48 hours and 72 hours with respect to control (Fig. 4).

One way ANOVA revealed that the CAT activity (nkat/mg protein) at different time intervals in *Eudrilus eugeniae* exposed to ferric chloride (5 mg/ml) is significant [F (3, 11) =2.170, P=0.170]. Post Hoc analysis revealed that the CAT activity (nkat/mg protein) at different time intervals in *Eudrilus eugeniae* exposed to ferric chloride was only significant at24 hours (P<0.05; LSD) with respect to control.

Reduced Glutathione (GSH) Content

The GSH content in *Eudrilus eugeniae* treated with ferric chloride(5 mg/ml) were 183.493 ± 75.572 mg/g tissue, 23.25 ± 6.495mg/g tissue,238.727 ± 11.809 mg/g tissue, 220.723 ± 34.231 mg/g tissue tissue after 0 hour, 24 hours, 48 hours and 72 hours respectively. The GSH content (mg/g tissue) decreased at 24 hours and increased at 48 hours and 72 hours. It was highest at 48 hours in comparison to *Eudrilus eugeniae* exposed to ferric chloride at different time intervals (Fig. 5).

One way ANOVA was performed in order to analyse the effect of ferric chloride (5 mg/ml) on the GSH content at different time intervals in *Eudrilus eugeniae*. One way ANOVA revealed that the GSH content at different time intervals in *Eudrilus eugeniae* is significant [F (3, 11) = 16.444, P=0.001]. Post Hoc analysis revealed that the GSH content at different time intervals when treated with ferric chloride in *Eudrilus eugeniae* was only significant at 24 hours (P<0.05; LSD) with respect to control .

Lipid Peroxidation (LPX)

The LPX content in *Eudrilus eugeniae* treated with ferric chloride(5 mg/ml) were 2.654 ± 0.725TBA-RS/mg, 3.655 ± 0.878 TBARS/mg ,3.109 ± 0.822TBA-RS/mg, 4.881 ± 1.052TBA-RS/mg after 0 hour, 24 hours, 48 hours and 72 hours respectively. The LPX (TBARS/mg) content gradually increased at 24 hours, 48 hours and 72 hours. It was highest in 72 hours in comparison to *Eudrilus eugeniae* exposed to ferric chloride at different time intervals (Fig. 6).

One way ANOVA was performed in order to analyse the effect of ferric

chloride (5 mg/ml) on the LPX content at different time intervals in *Eudrilus eugeniae*. One way ANOVA revealed that the LPX content at different time intervals in *Eudrilus eugeniae* is significant [F (3, 11) = 3.604, P=0.065]. Post Hoc analysis revealed that the LPX content at different time intervals when treated with ferric chloride in *Eudrilus eugeniae* was only significant at72 hours (P<0.05; LSD) with respect to control.

REFERENCES

1. Beers, R. F., Jr. and Sizer, I. W., (1952) A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem., 195: 133-140.
2. Bouche´, M.B (1972). Lombriciens de France, Ecologie et Syste´matique, INRA, Paris. Bouche´, M., 1984. Ecotoxicologie des lombriciens. 2. Surveillance de la contamination des milieux. Oecologia Applicata 5, 291–301.
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. Das, K., Samanta, L. and Chainy, G. B. N., (2000) A modified spectrophotometric assay of superoxide dismutase using nitrite formation by superoxide radicals. Ind. J. Biochem. Biophys., 37: 201-204.
5. Ellman, G.L. (1959). Tissue sulphydryl groups. Arch. Biochem. Biophy. pp 82:70-77
6. Folch J, Lees, M and Sloane Stanley, GH (1957). A simple method for isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-509.
7. Kennette, D., Hendershot, W., Tomlin, A., Sauve´, S., (2002). Uptake of trace metals by the earthworm Lumbricus terrestris L. in urban contaminated soils. Applied Soil Ecology 19, 191–198.
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. Ohkawa H., Ohishi, N. and Yagi, K. (1979). Assay of Lipid Peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. Physiol.118C (1):33-37.
11. Wang S, Shi X (2001). Molecular mechanisms of metal toxicity and carcinogenesis. Mol Cell Biochem; 222:3–9.