

A COMPARATIVE STUDY OF REDUCED GLUTATHIONE AND LIPID PEROXIDATION IN PRE-CLITELLATED AND CLITELLATED *Eisenia fetida* (Savigny, 1826)

Sudipta Chakrabarti¹, Puspanjali Parida¹ and Kajari Das²

¹P.G. Dept. of Zoology, North Orissa University, Baripada-757003, Odisha, India

²P.G. Dept. of Zoology/Biotechnology, CBSH, OUAT, Bhubaneswar-751003, India

E-mail: p_parida2000@yahoo.co.in

Abstract: Lipid peroxidation and reduced glutathione levels were measured in two different stages of life cycle (pre-clitellated and clitellated) in *Eisenia fetida*. It was found that level of lipid peroxidation (LPX) was higher in pre-clitellated *E. fetida* while reduced glutathione (GSH) level was higher in clitellated *E. fetida*.

Keywords: *Eisenia fetida*, reduced glutathione, lipid peroxidation.

Introduction

Depletion of antioxidant defences and/or rises in reactive oxygen species (ROS) production can tip the ROS antioxidant balance and cause oxidative stress (Sies, 1991), which may result in tissue injury. Oxidative stress can produce major interrelated derangements of cell metabolism including DNA-strand breakage (Halliwell and Aruoma, 1991).

Glutathione is a ubiquitous tripeptide that is regarded as one of the most important non-protein thiols in biological systems (Kosower and Kosower, 1978). GSH functions as an important overall modulator of cellular homeostasis, and serves numerous essential functions including detoxification of metals and oxy-radicals (Miester and Anderson, 1983; Christie and Costa, 1984).

Lipid peroxidation has been reported as a major contributor to the loss of cell function under oxidative stress situations. For example, peroxidation attack on microsomal membranes can lead to Calcium release and uncontrolled activation of Calcium dependent proteases and lipases (Orrenius *et al.*, 1990, Geeraerts *et al.*, 1991), where as attack on mitochondrial membranes can alter permeabilities and induce a disruption of cellular energetic (Bindoli, 1988).

In the present study, level of reduced glutathione and lipid peroxidation in two stages of life cycle of *Eisenia fetida*, namely clitellated and pre-clitellated stages were measured.

Materials and Methods

Earthworm (*Eisenia fetida*)

Eisenia fetida were purchased from soil conservation office, Baripada, Mayurbhanj. Ten plastic trays were taken measuring 30cm X 25cm X 6.5cm, each filled with 1 Kg of soil rich in cattle manure. Twenty numbers of *Eisenia fetida* were taken in each tray, covered with nylon net and gunny cloth and kept moist by sprinkling of water. *Eisenia fetida* were introduced in the soil and kept for seven days for acclimatization prior to experimentation.

Preparation of supernatant

Clitellated *Eisenia fetida*, 4-5 in number, were picked up from a tray and their pooled weight was taken. Similarly, pre-clitellated *Eisenia fetida*, 9-10 in number were picked up from a tray and their pooled weight was taken. A 10% homogenate was prepared in ice-cold 50mM phosphate buffer (pH 7.4) of the two stages, separately. The tissues were ground in a porcelain mortar and pestle at 4°C. The homogenates were centrifuged at 4500 rpm (1000 Xg) for 10 min at 4°C. The supernatants of clitellated and non-clitellated earthworms were used for assay of protein, GSH and LPX.

Protein estimation

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

Estimation of Reduced Glutathione (GSH)

GSH content of the tissue samples was determined according to the method of Ellman (1959) with slight modifications. Absorbance at 412 nm was recorded against a blank containing only DTNB. GSH content of the tissue samples was expressed as $\mu\text{mol/g}$ tissue.

Lipid peroxidation (LPX) Assay

Lipid peroxidation level was estimated as thiobarbituric acid reacting substance (TBA-RS) by thiobarbituric acid (TBA) according to the method of Ohkawa *et al.*, (1979). The absorbance of the supernatant was measured at 532 nm against an appropriate blank and lipid peroxidation was expressed as nmol TBA-RS/mg of protein.

Statistics

One way analysis of variance (ANOVA) was employed and P values < 0.05 considered as significant.

Results and Discussion

Table I. Comparison of GSH level and of LPX level in *Eisenia fetida* pre-clitellated stage and *Eisenia fetida* in clitellated stage. Data are expressed in mean \pm SD

Earthworms (n = 8)	GSH level ($\mu\text{mol /g tissue}$)	LPX level (nmol TBA-RS/mg protein)
Pre-clitellated	0.059 \pm 0.005	1.29 \pm 0.009
Clitellated	0.067 \pm 0.008	1.123 \pm 0.006

Level of GSH in *Eisenia fetida* in pre-clitellated stage ranged from 0.0521 to 0.0711 $\mu\text{mol /g}$ tissue. Level of GSH in *Eisenia fetida* in clitellated stage ranged from 0.0432 to 0.0827 $\mu\text{mol /g}$ tissue. Level of LPX in *Eisenia fetida* in pre-clitellated stage ranged from 1.231 to 1.331 nmol TBA-RS/mg protein. Level of LPX in *Eisenia fetida* in clitellated stage ranged from 1.118 to 1.311 nmol TBA-RS/mg protein. The difference between GSH level in pre-clitellated *Eisenia fetida* and clitellated *Eisenia fetida* was statistically not significant. The difference between LPX level in pre-clitellated *Eisenia fetida* and clitellated *Eisenia fetida* was statistically not significant.

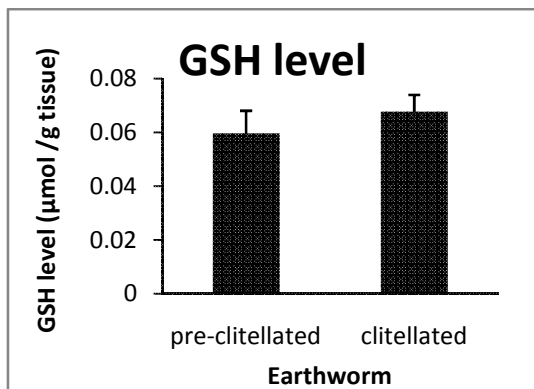


Fig. 1: Comparison of GSH level ($\mu\text{mol /g tissue}$) in pre-clitellated and clitellated *Eisenia fetida*

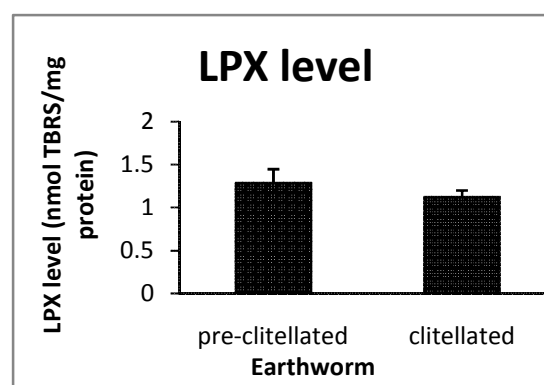


Fig. 2. Comparison of LPX level (nmol TBARS/ mg protein) in pre-clitellated and clitellated *Eisenia fetida*

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