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Seasonal Variation of Protein Content, Lipid Peroxidation and Reduced Glutathione in Eisenia Fetida (Savigny, 1826)

KEYWORDS: Eisenia fetida, season,Protein, Reduced glutathione, Lipid peroxidation

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ABSTRACT *Protein content, lipid peroxidation (LPX) and reduced glutathione levels (GSH) were measured in three different seasons (summer, monsoon and winter) in Eisenia fetida. It was found that protein content, LPX and GSH levels were varies in different season. Protein, LPX and GSH levels were highest in winter, summer and monsoon respectively.*

INTRODUCTION

Formation of reactive oxygen species (ROS) is a normal metabolic process, but can become a problem under stress conditions when ROS production is increased and oxidative stress is generated (Asada, 1999). As a consequence of the instability of these ROSs and their potential to damage cells and tissues, there are both enzymes and small molecular weight molecules with antioxidant capabilities that can protect against the adverse effects of ROS reactions. The extent to which oxyradical generation produces biological damage is dependent on the effectiveness of these antioxidant defences (Machlin and Bendich, 1987). Stenersen *et al*. (1979), Stenersen and Øien (1981) reported the existence of the reduced glutathione (GSH), glutathione-S-transferase (GST) system in earthworms. Saint-Denis *et al.,* (1998) studied the activities of enzymes (catalase, glutathione peroxidase, glutathione-S-transferase and glutathione reductase) involved in antioxidant defence systems in *Eisenia fetida andrei,* which are mainly localized in the cytosolic fractions. The metabolic activities of invertebrates are influenced to a large extent by changes in both biotic and abiotic factors, which depend on season. Changes in environmental factors as a consequence of change in seasons may therefore influence normal metabolic activities of organisms and the induction of oxidative stress as a consequence of increased generation of ROS (Verlecar *et al.*, 2008). In earthworms, very few studies exist concerning antioxidant defense system. Whilst the ability of *Eisenia fetida* to accumulate metals or organic contaminants has been highlighted, very little is known of the endogenous metabolism of this organism.

In order to provide basic data on the antioxidant defenses in *Eisenia fetida* and to underpin the existence of potential biomarkers with respect to seasonal variation the current investigation has been undertaken.

MATERIALS AND METHODS

Earthworm (Eisenia fetida)

Eisenia fetida were purchased from soil conservation office, Baripada, Mayurbhanj. Ten plastic trays were taken measuring 30cmX25cmX6.5cm, each filled with 1Kg of soil rich in cattle manure. Twenty numbers of *Eisenia fetida* were taken in each tray, covered with nylon net, gunny cloth and kept moist by sprinkling of water. *Eisenia fetida* were introduced in the soil, acclimatized for seven days before experimentation.

Preparation of supernatant

Pooled weight of 4-5 *Eisenia fetida*, with well developed clitellum, having average weight 0.0964g was taken. A 10% homogenate was prepared in ice-cold 50mM phosphate buffer (pH7.4). The tissues were ground in a porcelain mortar and pestle at 4°C. The homogenate was centrifuged at 4500 rpm (1000 Xg) for 10 min at 4°C. This supernatant was used for assay of protein, GSH and LPX.

Protein estimation

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

Lipid peroxidation (LPX) Assay

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

Estimation of Glutathione reductase (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 mg/g tissue.

Statistics

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

RESULTS AND DISCUSSION

Protein content, lipid peroxidation (LPX) and reduced glutathione levels (GSH) were measured in three different seasons (summer, monsoon and winter) in *Eisenia fetida*. It was found that protein content was 26.044±2.936 mg/g tissue in summer, 20.78±0.7228 mg/g tissue in monsoon and the highest, which is 32.784±1.0295 mg/g tissue in winter. LPX was the lowest in winter, 0.907±0.206 nmol TBARS/mg protein, highest in summer 1.528±0.293 nmol TBARS/mg protein, and 1.329±0.334 nmol TBARS/mg protein in monsoon. LPX levels were lowest in winter 0.0359±0.00976 mg/g tissue, highest in monsoon 0.0613±0.0058 mg/g tissue and 0.04316±0.0114 mg/g tissue in summer. Thus, protein, LPX and GSH levels were highest in winter, summer and monsoon respectively (Table 1; Figures 1,2,3)

One way analysis of variance (ANOVA) of LPX and GSH were significantly different (p< 0.05) (Tables 2, 3, 4).

Anitha and Jayaraaj (2012), found that in the earthworm powder of *Eudrillus euginae*, protein content was 5.021±0.015 mg/g dried earthworm powder and GSH level was

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7.11±0.020 µmol of

As said earlier, living organisms possess several antioxidative species and mechanisms protecting them against the harmful action of ROS. Hence it can be concluded that least ROS is generated during the winter season in *Eisenia fetida* which leads to the lowest levels of GSH and LPX in the animals as compared to the levels in summer and monsoon.

Table 1: Comparison of Protein (mg/g tissue), GSH (mg/g tissue) and LPX (n mol TBARS/mg protein) in Eisenia fetida in summer, monsoon and winter. Data are expressed in mean ± SD

	Summer	Monsoon	Winter
Protein	26.044 ± 2.936	20.78 ± 0.7228	32.784 ± 1.0295
GSH	0.04316 ± 0.0114	0.0613± 0.0058	0.0359 ± 0.00976
LPX	1.528± 0.293	1.329± 0.334	0.907± 0.206

Table 2: Summary of calculations for one way analysis of variance (f-Test) of data of Protein conc. (mg/g tissue) in Eisenia fetida in summer, monsoon and winter

Source of variation	Degree of freedom	Sum of squares	Mean of squares	F ratio
		1	3	
Between seasons	3-1=2	* BSS= 579.28	* BMS=289.64	F_C=BMS/ WMS=20.28
Within seasons	7-2=5	*[2]WSS= 71.4	*[4]WMS=14.2	$F_{0.05}$= 2.069
Total	7			

Table 3: Summary of calculations for one way analysis of variance (f-Test) of data of GSH level (mg/g tissue) in Eisenia fetida in summer, monsoon and winter

Source of variation	Degree of freedom	Sum of squares	Mean of squares	F ratio
Between seasons	3-1=2	BSS= 0.0018	BMS=0.0009	F_C=BMS/ WMS=0.0009/0.0001 =9
Within seasons	5-2=3	WSS= 0.0003	WMS=0.0001	$F_{0.05}$= 9.55
Total	5			

Table 4: Summary of calculations for one way analysis of variance (f-Test) of data of LPX conc. (nmol TBARS/mg protein) in Eisenia fetida in summer, monsoon, winter.

Source of variation	Degree of freedom	Sum of squares	Mean of squares	F ratio
Between seasons	3-1=2	BSS= 1.2	BMS=0.6	F_C=BMS/ WMS=0.6/0.4 =1.5
Within seasons	5-2=3	WSS= 1.2	WMS=0.4	$F_{0.05}$= 9.55
Total	5			

*[1]BSS: Between sum of squares; *[2]BMS: Betweeen mean of squares; *[3] WSS: Within sum of squares; *[4] WMS : Within mean of squares

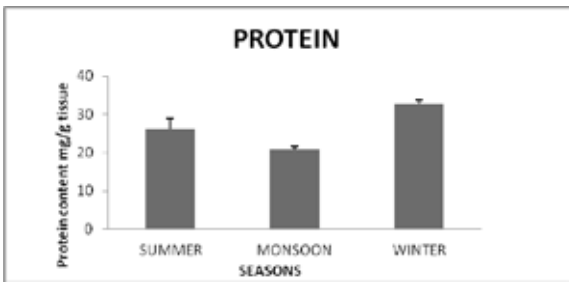

Figure 1: Comparison of protein content (mg/g tissue) in different seasons

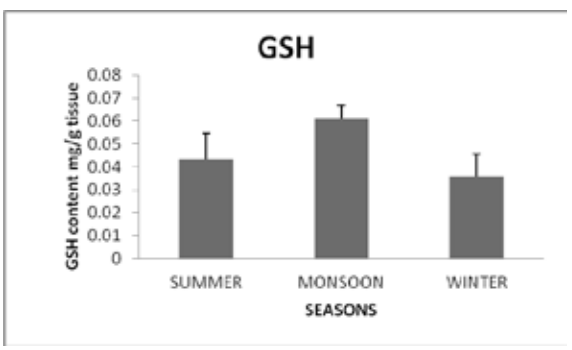

Figure 2: Comparison of GSH (mg/g tissue) content in different seasons

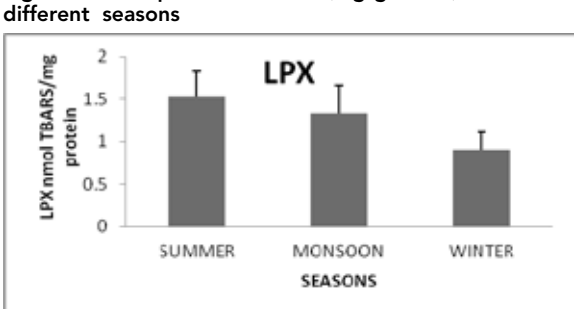

Figure 3: Comparison of LPX (TBARS/mg protein) content in different seasons

REFERENCE

1. Anitha, J. & Jayaraaj, A. I. 2012. Nutritional and antioxidant R. P. (Ed.), Free Radicals | in Biology,5: Academic press, New York 223-254. | 2. Asada, K. 1999. The water-water cycle in chloroplasts: Scavenging of active oxygens and dissipation of excess photons. Annu Rev Plant Physiol Plant Mol Biol, 50 :601–639. | 3. Ellman, G. D. 1959. Tissue sulphydryl groups. Arch Biochem Biophys, p.82-70. | 4. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. J Biol Chem, 193: 265-75. | 5. Ohkawa, H., Ohishi, N. & Yagi, K. 1979. Assay for Lipid Peroxides in animal tissues by | thiobarbituric acid reaction. Anal Biochem, 95: 351-358. | 6. Machlin L. J, Bendich A. (1987) Free radical tissue damage: protective role of antioxidant nutrients. Faseb J, 1:441–445. | 7. Saint-Denis, M., Labrot, F., Narbonne, J. F. & Ribera, D., 1998. Glutathione, glutathione related enzymes, | and catalase activities in the earthworm Eisenia fetida andrei. Archives of Environmental Contamination and Toxicology, 35, 602-614. | 9. Stenersen J. & Øien N. (1981) Glutathione-S-transferase in earthworms Lumbricidae). Substrate specificity, tissue and species distribution and molecular weight. Comp Biochem Phys,69C :243–252 | 8. Stenersen J., Guthenberg C. & Mannervik B. (1979) Glutathione-Stransferases in earthworms (Lumbricidae). Biochem J, 181 :47–50 | 9. Verlecar, X. N., Jena K. B. & Chainy G. B. N. 2008. Seasonal variation of oxidative | biomarkers in gills and digestive gland of green-lipped mussel Perna viridis from Arabian Sea. Estuarine, Coastal and Shelf Science, Vol. 76(4) : 745-752p.