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Altitude Specific Pro-Oxidant Activities in Diapausing Pupa of Tasar Silk Moth, Antheraea Mylitta

KEYWORDS: A. mylitta, altitude, antioxidants.

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ABSTRACT *Quantitative analyses of proteins, ascorbic acid, reduced glutathione and level of lipid peroxidation in the haemolymph (HL) and fat body (FB) tissues of the diapausing pupa of Daba ecorace of tasar silk moth, Antheraea mylitta were carried out to find out if any altitude specific variation exists among them. Analyses revealed that, the pro-oxidant activity was comparatively high concomitant with better antioxidant protection in the fat body of diapausing pupa of tasar silk moth, A. mylitta. It was further observed that between the two groups of pupa collected from low and high altitude, the latter one experience more pro-oxidant assault with simultaneous induction of antioxidants.*

INTRODUCTION

To maintain physiological function at high altitude, under reduced environmental oxygen availability, the capacity to transport O_2 must increase (Carvalho and Goncalves, 2011). Environmental cues influence not only the span of life cycle but also the voltinism (number of life cycles per year). Voltinism of a species depends upon its geographic distribution, temperature, day length (duration of light), moisture, nutrition, seasonal changes and altitude (Jolly *et al.* 1968; Sharan *et al.* 1994). Voltinism of *Antheraea mylitta* increased with decreasing in altitude (Saxena *et al.* 1997). Oxidative stress is the result of an imbalance between pro-oxidant species and the levels of the defences resulting from the generation of reactive oxygen species (ROS) or pro-oxidants (Santoro and Thiele, 1997) like superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) etc. Reactive oxygen species is related to ageing and life span (Orr and Sohal, 1994) and plays a significant role in the innate immune response of insects (Hao *et al.* 2003). Ascorbic acid is redox catalyst which can reduce and neutralise ROS such as hydrogen peroxide (Padayatty *et al.* 2003). Glutathione (GSH) is one of the most important cellular antioxidants (Meister and Anderson, 1983). Oxidative stress in the cells is a result of increase exposure to oxidants or from decreased protection against oxidants, or even from both the events occurring simultaneously (Cadenas, 1989). Acute or chronic oxidative stress may result in uncontrolled lipid peroxidation (LPX) and protein oxidation (Levine *et al.* 1981), which leads to cell death and impair cell function.

The tropical tasar silk moth, *Antheraea mylitta* is a phytophagous insect and has the second largest capacity for silk production among all the silk spinning insects (Akai, 2000). Different ecoraces of *A. mylitta* are reared in low and high altitudes of the forest. Information about the pro-oxidant and antioxidant defences in diapausing pupa of Daba ecorace of *A. mylitta* Drury is scanty. So, emphasis has been given on the analysis of quantities of proteins, ascorbic acid (ASA), reduced glutathione (GSH) and level of lipid peroxidation (LPX) (nmol MDA formed per mg protein) in the haemolymph (HL) and fat body (FB) tissues of the diapausing pupa of tasar silk moth, *A. mylitta* to find out if any altitude specific variation exist among them.

MATERIALS AND METHODS

About the animal: The live diapausing pupa of Daba ecorace of tasar silk moth, *Antheraea mylitta* were collected from the primary host plant like Asan (*Teminalia tomentosa*) from low altitude (51-300m ASL) and high altitude (600-900m ASL) (Nayak *et al.* 1999) of Similipal Biosphere Reserve, Mayurbhanj, Odisha.

Tissue preparation: Haemolymph (HL) was collected in 3% phenylthiourea coated with prechilled appendorf tube to inhibit denaturing or blackening of the haemolymph (Mishra *et al.* 2009) by cutting the body segment of pupa and centrifuged at 7,000 x g for 10 minutes at 4°C to settle the haemocytes. Pupae were dissected to collect the fat body (FB). After washing in ice-cold physiological saline (0.67% NaCl) the tissues were retained in ice separately. The tissues were weighed in monopan digital balance and homogenised (10%) in 50 mM phosphate buffer pH 7.4 with 1mM EDTA using hand homogeniser under ice (Patra *et al.* 2011). Homogenates were centrifuged at 10,000 x g for 20 minutes at 4°C. The supernatant was collected for further chemical analyses.

Biochemical estimation: The amount of proteins was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard. Ascorbic acid (ASA) content was measured according to the method of Jagota and Dani (1982). The reduced glutathione (GSH) content in the tissue samples was determined according to the method of Ellman (1959). The sample after centrifugation was used for the estimation of

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lipid peroxidation (LPX) by monitoring the formation of malondialdehyde (MDA) by the method of Ohkawa *et al*. (1979). The amount of MDA formed was calculated from the extinction coefficient of 1.56 x 105 M-1 cm-1 (Wills, 1969).

Statistics: To know the difference between means of two independent samples Fisher's t-test and for dependent samples Paired t-test were employed (Chainy *et al*. 2008).

RESULTS AND DISCUSSION

To compare the same tissue of the pupa of different altitudes, Paired t-test was employed. The concentration of protein, ascorbic acid ASA and GSH of the haemolymph (HL) and fat body (FB) tissues was found higher in the pupa collected from low altitude than that of the high altitude. The concentration of protein in the HL and FB tissues was significantly higher ($P< 0.001$) in the pupa collected from low altitude than that of the high altitude (Fig. 1). The concentration of ascorbic acid in HL tissue was found significantly higher ($P< 0.01$) in the pupa collected from low altitude than that of the high altitude (Fig. 2). A similar trend was found in the case of FB tissue where the concentration of ASA was found higher in the pupa collected from low altitude than that of the high altitude which is statistically not significant (Fig. 2). The concentration of reduced glutathione GSH in the HL tissue was found significantly higher ($P< 0.01$) in the pupa collected from low altitude as compared to the high altitude (Fig. 3). However, the concentration of GSH of FB tissue was found significantly higher ($P< 0.001$) in the pupa collected from low altitude than that of the high altitude (Fig. 3). The level of LPX in HL tissue was found significantly higher ($P< 0.001$) in the pupa collected from high altitude than that of the low altitude (Fig. 4). Whereas, the LPX level in the FB tissue was found more in the pupa of high altitude than that of the low altitude which is statistically not significant (Fig. 4).

To compare the different tissue of pupa collected from low and high altitude Fisher's t-test was employed. The analyses revealed that, the concentration of protein, ASA, GSH including the level of LPX in the FB tissue was significantly higher ($P < 0.001$) than that of the HL tissue (Figs.1, 2, 3 and 4).

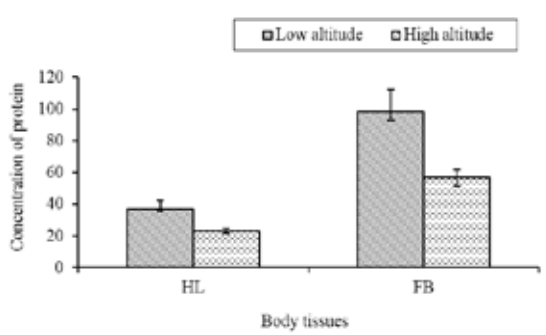

Fig. 1. Concentration of protein in mg/ml in the haemolymph (HL) and mg/g in fat body (FB) tissues of diapausing pupa of Daba ecorace of tasar silk moth, *A. mylitta* **collected from low and high altitude. Data are mean ± SD (n= 10 each).**

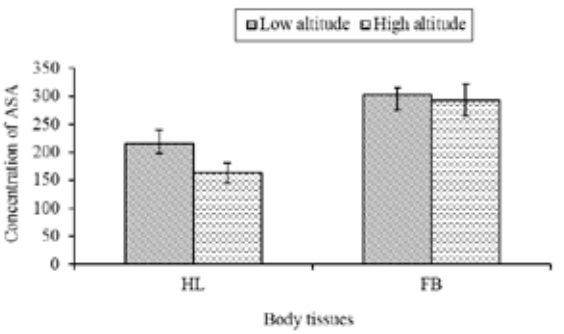

Fig. 2. Concentration of ascorbic acid (ASA) in µg/ml in the haemolymph (HL) and µg/g in fat body (FB) tissues of diapausing pupa of Daba ecorace of tasar silk moth, *A. mylitta* **collected from low and high altitude. Data are mean ± SD (n= 10 each).**

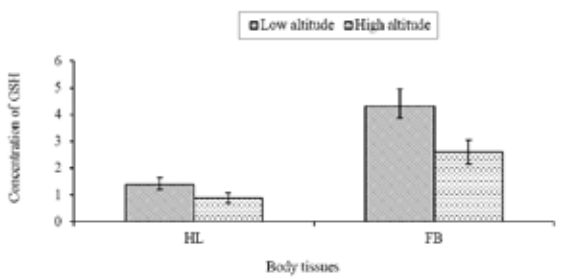

Fig. 3. Concentration of reduced glutathione (GSH) in µmol/ml in the haemolymph (HL) and µmol/g in fat body (FB) tissues of diapausing pupa of Daba ecorace of tasar silk moth, *A. mylitta* **collected from low and high altitude. Data are mean ± SD (n= 10 each).**

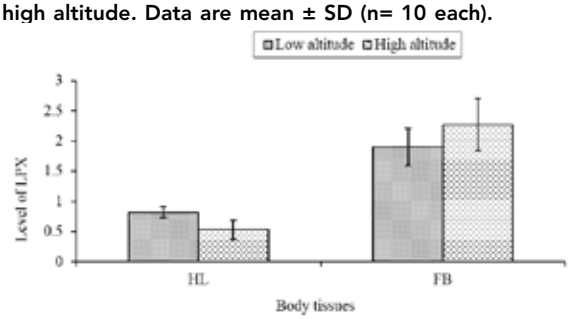

Fig. 4. Level of lipid peroxidation (LPX) (nmol MDA, i.e., malondialdehyde formed per mg protein) in the HL and FB tissues of diapausing pupa of Daba ecorace of tasar silk moth, *A. mylitta* **collected from low and high altitude. Data are mean ± SD (n= 10 each).**

Dean (1991) had opined that decrease in protein content of tissues in different pathological conditions is due to oxidative damage. The low protein concentration in the pupa collected from high altitude is an indication of oxidative damage in the HL and FB tissues as reported by Dean (1991). The higher concentration of ascorbic acid in the pupa collected from high altitude indicates its effective role in protection against pro-oxidant phenolics. Ascorbic acid can also act as pro-oxidant (Summers and Felton, 1994). The higher concentration of GSH in the tissue of pupa collected from low altitude indicates the activity of cellular antioxidant to protect the cell from oxidative damage as reported by Meister and Anderson (1983). Low level of LPX in the pupa collected from lower altitude indicates a protective mechanism to limit the tissue oxidation

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during metamorphosis of the post-diapausing pupa into winged adult. The higher level of LPX in the pupa collected from high altitude can be linked to increase in O_2 consumption as reported by Rath *et al*. (2005) for *A. mylitta*.

High protein content in FB tissue indicates that the animal stores its antioxidant components in the respective tissue to maintain its antioxidant defence system. The higher ascorbic acid content observed in the FB than HL might be an adaptive antioxidant response against elevated oxidative assault. The higher concentration of GSH in FB tissue corroborates with the findings of Meister and Anderson (1983) that this tissue maintains the cellular antioxidants like GSH. The high level of LPX in FB tissue indicates that this tissue challenged higher rate of tissue oxidation than HL.

From the present findings it is concluded that the oxidative stress was comparatively high concomitant with better antioxidant protection in the fat body of diapausing pupa of tasar silk moth, *A. mylitta*. It was further observed that, between the two groups of pupa collected from low and high altitude of Similipal Biosphere Reserve, Mayurbhanj, Odisha, the latter one experience more oxidative assault with simultaneous induction of non-enzymatic antioxidants (GSH and ascorbic acid) probably as an adaptive cellular response due to higher rate of oxygen consumption.

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REFERENCE Akai, H. (2000). Cocoon filament characters and post-cocoon technology. International Journal of Wild silkmoth and Silk, 5: 255-259. Cadenas, E. (1989). Biochemistry of Oxygen toxicity: Annu. Rev. Biochem., 58: 79-110. Carvalho, O. and Gonçalves, C. 2011. Comparative Physiology of the Respiratory System in the Animal Kingdom. The Open Biology Journal, 4: 35-46. Chainy, G. B. N., Mishra, R. and Mohanty, P. K. (2008). Basic Biostatistics. Kalyani Publishers, New Delhi, pp. 331. Dean, R. T. (1991). Protein damage and repair: An overview. In: Oxidative Damage and Repair: Chemical, Biological and Medical Aspects, Davies K. J. A. (Ed.), Pergamon Press, New York, USA. pp. 341-347. Ellman, G. L. (1959). Tissue sulfhydril groups. Arch. Biochem. Biophys. 82: 72-77. Hao, Z., Kausumba, I. and Aksoy, S. (2003). Proventricules (cardia) plays a crucial role in immunity in tsetse fly (Diptera : Glossinidiae). Insect Biochemistry and Molecular Biology, 33: 1155-1164. Jagota, S. K. and H. M. Dani, (1982). A new colorimetric technique for the estimation of vitamin-C using folin phenol reagent. Clinical Biochemistry, 127: 178-182. Jolly, M. S., Chaturvedi, S. N. and Prasad, S. (1968). A survey of tasar crop in India. Indian Journal of Sericulture, 9: 23-25. Levine, R. L., Oliver, C. N., Fulks, R. M. and Stadtman, E. R. (1981). Turnover of bacterial glutamine syntheses: Oxidative inactivation proceeds proteolysis: Proceeding National Academy of Science, USA, 78: 2120- 2124. Lowry, O. H., Resebrough, N. J., Farr, A. L. and Randall, R. J. (1951). Protein Measurement with the Folin Phenol Reagent. Journal of Biological Chemistry, 19: 265-275. Meister, A. and Anderson, M. E. (1983). Glutathione. Annual Review of Biochemistry, 52: 711-760. Mishra, P. K., Kumar, D., Jaiswal, L., Kumar, A., Singh, B. M. K., Sharan, S. K., Pandey, J. P. and Prasad, B. C. (2009). Role of Haemolymph Biochemical Constituents and Brain Proteins in Maintenance of Pupal Diapause in Daba Bivoltine Ecorace of Tropical Tasar Silkworm Antheraea Mylitta Drury. Journal of Ecophysiology and Occupational Health, 9: 243-252. Nayak, B. K; Patro, K. B. G. and Dash M. C. (1999). Colour diversity among cocoons of tropical tasar ecoraces of A. paphia and A. mylitta (Lepidoptera:Saturniidae) at three altitude zones in Orissa. Sericologia, 39(3): 371-382. Ohkawa, H., Ohishi, N. and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry, 95: 351-358. Orr, W. C. and Sohal, R. S. (1994). Extension of life-span by over expression of superoxide dismutase and catalase in Drosophila melanogaster. Science, 263: 610-612. Padayatty, S., Katz, A., Wang, Y., Eck, P., Kwon, O., Lee, J., Chen, S., Corpe, C., Dutta, A., Dutta, S. and Levine, M. (2003). Vitamin C as an antioxidant: evaluation of its role in disease prevention. J. Am. Coll. Nutr., 22 (1): 18-35. Rath, S. S., Negi, B. B. S., Singh, B. M. K., and Thangavelu, K. (2005). Rate of oxygen uptake in Antheraea mylitta in various stages of development and during diapause. International Journal of Industrial Entomology, 10(1): 45-49. Patra, G. C., Mohanty, N. and Dey, D. G. (2011). Oxidative stress in the diapausing pupae of tasar silkworm, Antheraea mylitta Drury exposed to high temperature. Journal of Advanced Zoology, 32 (2): 111-115. Santoro, N. and Thiele, D. J. (1997). Oxidative stress responses in the yeast: Yeast Stress Responses. Springer, 171-211. Saxena, S. K., Yadav, G. S., Somasundaram, P., Harlapar, V. K. and Srinivasu, R. (1997). Impact of abiotic factors and microclimate on cocoon and seed productivity of Antheraea mylitta. Indian Silk, Oct. pp 27. Sharan, S. K., Singh, M. K., Ojha, N. N. G. and Sinha, S. S. (1994). Regulation of voltinism in Antheraea mylitta by manipulation of rearing period larval stages. Int. J. Wild Silkmoths & Silk, 1: 191-194. Summers, C. B. and Felton, G. W. 1994. Prooxidant effects of phenolic acids on the generalist herbivores Helicoverpa zea (Lepidoptera: Noctuidae): Potential mode of action for phenolic compounds in plant anti-herbivore chemistry. Insect Biochemistry and Molecular Biology, 24: 943-953. Wills, E. D. (1969). Lipid Peroxide Formation in Microsomes: General Considerations. Biochemistry Journal, 113: 315 - 324.