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Alterations in the Haemolymph Biochemical Parameters of Silkworm *Bombyx mori L*. Treated with Selenium

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Abstract: The effect of selenium on silkworm *Bombyx mori.L* larvae at lethal and sub lethal dose was studied at 3, 4, 5 and 6 day of V instar. The treatment with selenium resulted in significant alterations in various biochemical parameters of haemolymph such as proteins, total carbohydrate contents in larvae treated with selenium compared to untreated controls. The increase in haemolymph carbohydrates and decrease in protein content was observed at lethal dose (32.39 µ gm/ k.g body wt.) of selenium. Silkworms exposed to sub lethal dose (6.47 µ gm/ k.g body wt.) of selenium showed an insignificant increase in haemolymph carbohydrates and significant increase in protein content. Selenium at lethal dose impaired the normal biochemical parameters whereas sub lethal dose exhibited beneficial effects on haemolymph biochemical parameters.

Key words: Silkworm · Haemolymph · Total proteins · Total carbohydrates · Selenium

INTRODUCTION

Selenium is an essential trace element in small quantities [1, 2]. Selenium is well known for its beneficial effects. Selenium is a cofactor in many enzymes protecting the cell against free radicals and maintains high tissue anti oxidant levels [3]. Further it has the potential capacity to counteract viral, tumor properties and inflammatory activities. It promotes cell growth [4], stability of cellular membranes [5] affecting DNA replication [6] and cellular immunity [7]. Hence selenium is an essential element in amounts varying from trace to parts per million doses for men and animals. Therefore the study reports the possible effect of selenium supplementation on silkworm hemolymph biochemical parameters.

It is known that stress induce many biochemical changes in the hemolymph of insects [8, 9]. Since hemolymph is reservoir of various biochemical components, the alterations in the bio chemical composition of hemolymph of selenium treated silkworms is affected. In the present study an attempt has been made to elucidate the effect of selenium on alteration in hemolymph composition of the treated insect silkworm which has been under the patronage of man for its economic value.

MATERIALS AND METHODS

The rearing of Silkworm *Bombyx mori.L* with a parentage of PM X NB4D2 was maintained on the leaves of Mulberry Morus alba, V1 variety at 23± 1°C and 70±5 % Relative humidity (R.H). V instar larvae were selected for the present study. A group of 100 larvae of V instar were fed on selenium treated leaves for 4 days, in order to analyze the effect of lethal (32.39 µ gm/ k.g body wt.) and sub lethal dose (6.47 µ gm/ k.g body wt.) of selenium toxicity.Untreated controls received equivalent amounts of distilled water and were treated in a similar way. After scheduled exposure the silkworms were sacrificed. Haemolymph samples were collected from silkworm body at 15± 1°C to carry out further work. Haemolymph was obtained by cutting proleg at its base and collected through micro pipette in small ice cooled test tubes rinsed with phenyl thiourea solution (1%W/V). About 50 to 60 larvae were sacrificed for each experiment. The haemolymph was centrifuged at 3000 rpm for 10 minutes. The plasma so obtained was used for biochemical estimations. Total protein content was estimated by the method of [10]. Total carbohydrate content was determined using the method of Roe [11]. Duncan's multiple range tests [12] was applied to check the difference between treated groups and controls and the significance was calculated at 5% level (P).

RESULTS AND DISCUSSIONS

As seen in Table 1, total carbohydrate content in haemolymph was found significantly in larvae treated with lethal dose of selenium. This increase at 3 day of exposure was greater than the untreated controls and progressed

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Table 1: Biochemical changes in the haemolymph of selenium treated silkworm *Bombyx mori L.* larvae

Stage of larvae	Day exposed	Dose	Protein (mg/ml)	Carbohydrates (mg/100 ml)
V Instar	3 day	Control	186.35[a]	28.9a
		Lethal	220.37[c] (+18.25)	32.01c (+10.76)
		S. Lethal	210.32[b] (+12.8)	29.9b (+3.77)
	4day	Control	192.16[b]	30.1 a
		Lethal	170.52[a] (-11.2)	32.20 b (+6.97)
		S. Lethal	221.36[c] (+18.2)	30.30 a (+0.6)
	5 day	Control	210.57 [b]	30.86 a
		Lethal	152.38[a] (-27.6)	33.33 b (+8.0)
		S. Lethal	229.38[c] (+8.9)	31.08 a (+0.71)
	6 day	Control	229.80 [b]	32.50 a
		Lethal	123.13[a] (-46.42)	35.24 b (+8.4)
		S. Lethal	241.69[c] (+5.1)	32.95 a (+1.3)

* Each value is a mean of eight estimations

** Percentage decrease/increase over control is given in parenthesis

Means within column followed by the same letter are not significantly different (P>0.05) from each other according to Duncan's multiple range test

subsequently at 4, 5, 6 days in the haemolymph of silkworms on exposure to lethal dose. The results are in corroboration with the earlier findings [13] who reported that homeostatic status of carbohydrates in haemolymph is maintained by substances secreted by the neurosecretary cells of the brain which can be compared to the pituitary glands of vertebrates. Hence, selenium might have caused an indiscriminate release of neurohormones which ultimately led to the metabolic balance at lethal dose of selenium. The increase in carbohydrates content in haemolymph of animals treated with selenium leading to disruption of carbohydrate metabolism was also reported [14]. This indicates the persisted effect of selenium on the carbohydrate metabolism under prolonged period of exposure to lethal dose. Shifts in carbohydrate metabolism are natural under stress which may lead to impairment of homeostasis. Silkworms treated with the sub lethal dose of selenium showed insignificant increase in total carbohydrate in haemolymph. This insignificant increase in haemolymph carbohydrate suggests normalcy as like controls. It appears that the overall system is thus regulated both allosterically by substrate and products and by post translational modification of enzymes in response to selenium.

A significant increase in protein content was observed (Table 1) in larvae exposed to lethal dose of selenium at 3 day of exposure. But these protein levels were declined significantly on further exposure periods to lethal dose of selenium. The decrease in total protein content in haemolymph suggests the suppression of protein synthesis or utilization of protein for energy processes. Suppression of protein synthesis is observed in the silkworm exposed to lethal dose indicates the breakdown of proteins due to active stress. Proteolysis is dominated over its synthesis [15]. Silkworms showed hyper protenemia exposed to sub lethal dose of selenium on all exposure periods. This increase in protein content in haemolymph can be attributed to stimulated synthesis of protein producing factors. Selenium treatment at sub lethal dose might have enhanced protein synthetic ability. Sub lethal dose of selenium in trace amounts is stimulatory and do not exert toxic effect on silkworm protein metabolism.

Our results also coincide with the earlier findings which exhibited that selenium in sub lethal treated silkworms activated the protein synthetic machinery and physiological activities in silkworm resulting in the increased silk production in silkworm, *Bombyx mori. L* and exhibited that lower dose is beneficial to the silkworm growth and cocoon production [16]. On the whole it can be concluded that selenium is a trace mineral that is essential for good growth of insects but required only in small doses as supplement.

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