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Studies On The Impact Of Heavy Metal Chromium On Protein Metabolism *In Sphaerodema Rusticum* (Heteroptera: Belastomatidae)

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	Abstract
	The effects of median lethal concentration of heavy metal chromium (14ppm for 48h) exhibited a significant decline in the contents of protein and amino acid in the haemolymph, fat body, testis, seminal vesicle. The toxicity impact of chromium on <i>S.rusticum</i> was appropriate to be comparatively higher than any other insects, since it was its own tolerance limit beyond 48h (14 ppm) concentration in the aquatic environment. This suggests that the non-target organisms like <i>S.rusticum</i> could be used as an effective indicator to assess the extent of chromium pollution in the aquatic environment.
CC License CC-BY-NC-SA 4.0	Keywords: Sphaerodema rusticum, Haemolymph, Fat body, Testis, and Seminal vesicle.

INTRODUCTION

Proteins are complex organic nitrogenous compounds. The building blocks of protein and amino acid. Most of them can synthesized inside the body of animals are called non-essential amino acids. Some of the amino acids, which cannot be synthesized by the body of animal, are called essential amino acids and must be supplemented only through, diet (Candy and Kilby, 1975). Protein synthesis by the fat body consists of range of protein (Riddiford and Dhadialla, 1990). The heamolymph mainly serves to transport diacylglycerols from the fat body to the site of utilization of flight muscles (Chino *et al.*, 1977). Downer (1982) has reported that the proteins synthesized from amino acid excretion in insects is a difficult area in which to work because of the widespread potential presence of amino acid in food stuffs and their ready biochemical inter conversion once inside the insects body (Bursell, 1967).

The impacts of toxicant on protein and amino acids metabolism have been reported in insects for *Odontopus varicornies* (Jayakumar,1988). *Aspongopus janus* (Thiruvasagam,1994); *Catacanthus incanatus* treated with leaf extract of *Pongamia globra* (Ramanathan, 1955); *Laccotrephos ruber* treated with mercuric

(Palanisamy,1997); Sphaerodema rusticum (Navamani, 2019); Odontopus varicornies (Babitha, 2017) to be important, since the food value of insect directly depends on their quantity. However, species-specific sequence databases are still very rarely used in conjunction with protein and proteomics analysis, although this is quite feasible in laboratory cultured insects (Bayram et al., 2017; Bayram et al, 2019). It is evident from the above literature cited that the effects of information regarding the effect of chromium on haemolymph, fat body, testis and seminal vesicle are scanty. Hence, an attempt has been made to find out the effect of heavy metal chromium on biochemical changes with the special reference to protein and amino acid contents in the haemolymph, fat body, testis and seminal vesicle of hemipteran insect adult male Sphaerodema rusticum.

MATERIAL AND METHODS

Adult insects were collected from reading troughs and vivisected in insect Ringer solution (Emphrussi and Beadle, 1936). The following biochemical estimations were made in the tissues of haemolymph, fat body, testis, seminal vesicle.

The protein content in the haemolymph, fat body, testis, and seminal vesicle were determined by adopting the procedure of Lowry *et al.*, (1954). The amino acid content in the heamolymph, fat body, testis and seminal vesicle were determined by adopting the procedure of Moore and Stein (1954).

RESULT AND DISCUSSION

The protein content in haemolymph, fat body, testis and seminal vesicle were found to be decreased in heavy metal chromium treated insects than the control insects about 93.18 to $81.63\mu g/100\mu l$, 16.78 to $13.7\mu g/mg$, 57.30 to $43.20\mu g/mg$ and 20.28 to $19.05\mu g/mg$. respectively (Table 1). The mean protein content of the haemolymph, fat body, testis, and seminal vesicle in control and treated insects were compared for significance of difference. For this purpose, the t-value was calculated and it was given in the table 1. It is clear from the Table 1 that, t-values 246.23, 105.25, 4.193 and 2.896 were significant at 0.01 level. Therefore, it may be concluded that the protein content of haemolymph, fat body, testis, seminal vesicle were significantly different in control and treated insects.

Amino acid content of haemolymph, fat body, testis, and seminal vesicle were increased in the treated haemolymph, fat body, testis, and seminal vesicle than the control insects of about to 57.17 to $62.91\mu g/100\mu l$: 5.42 to $8.14\mu g/mg$: 15.78 to 17.35 $\mu g/mg$ and 2.90 to $4.138\mu g/mg$, respectively (Table 2). It was clear from the table 2 that t-value of haemolymph (246.23), fat body (105.25), testis (4.193) and seminal vesicle (2.896) at 0.01 level. Therefore, it may be concluded that the amino acid content of fat body, haemolymph, testis, seminal vesicle were differ significantly in control and treated insects.

Thus, it was evident from the table-1 that the quantity of protein of all the tissues haemolymph, fat body, testis, and seminal vesicle were decreased significantly than the control insects. But, the quantity of the amino acid of all the tissues has increased significantly during treatment with heavy metal chromium. Jayakumar (1988) has reported that the reduction of protein content in the fat body for *Odontopus varicornis* when exposed to dimetholate increased protein content has been reported for fat body of *Catacanthus* incarnates after 24 hours of exposures to median lethal and sublethal concentrations, of dimethoate (Vijayaprabha, 1990). A similar increase in the quantity of protein has been reported for thr haemolymph of monocrotopus treated *Odontopus varicornis* (Ravichandran, 1994). Prakash *et al.*, (1990) have reported that in fat body and haemolymph *of poecilocerus pictus* treated with endosulfan and those effects have been possibly due to enhanced proteolytic activity. Shanmugavelu (1993) has reported that decline in protein content in the fat body of *Mylabris pustalata* when treated with heavy metal, might be due to an active energy of protein released from the fat body into haemolymph.

In the present study, it has observed that the increased amount of amino acid content in all the target tissues may be due to heavy metal chromium intoxication. Thiruvasagam (1994) has also reported the similar result in the tissues of *Aspongapus janus*, when treated with nimbicidin. Shrivastava (1998) and Anita *et al.*, (1999) have also reported the reduction of protein content after an initial increased in the certain tissues of Heteropheustes fossils and in to the kidney of *Catla catla* exposed to endosulfan, Pyrethoid and fenvalerate, respectively. Seminal fluid protein classes are generaly conserved across a wide taxanomic range (Mueller et al., 2004;

Braswell et al., 2006; Collins et al., 2006; Davies and Chapman, 2006) but several proteins themselves evolve more rapidly, on average, than other proteins (Clark et al.,1995; Civetta and Clark,2000; Andres et al., 2006; Panhuis and Swanson,2006). Most of these proteins fall into similar biochemical protein classes as male-derived reproductive proteins in other insect and salivary gland and digestive tract proteins in blood-feeding arthropods (Laura K. Sirot et al., 2008).

Proteins are the most important organic constituents of animal tissues including insects and play an important role in energy production Hakan Bozdogan et al., (2016). In the development process, various agents such as protein in required for the synthesis of ATP Taskin and Aksoylar, (2011). Ravichandran *et al.*, (1994) studied the protein level of freshwater fish *Oreochromis missambicus* exposed to sub lethal concentration of phenol and reported an increase in amino acid content and protein content was decreased in the kidney. Boran (1995) also investigated the effect of organ in certain tissues of *Heteropheustes fossilis* found reduction in protein content. Thus, the biochemical variations in the quantity of protein and amino acid in the haemolymph, fat body, testis and seminal vesicle of the adult *S.rusticum* seems to be due be to treatment with heavy metal, chromium.

Table 1: Protein content haemolymph, fat body, testis and seminal vesicle of control and Chromium treated adult male insect *S. rusticum* (Quality expressed μ g/mg wt in case of tissues and μ g/100 μ l in case haemolymph)

Tissues	Control	Treated	Percent change	t-value
Chart Area			over control	
Haemolymph	93.18 ± 2.15	81.63 ± 3.20	-12.31	246.23*
Fat body	16.78 ± 1.58	13.7 ± 2.11	-24.50	105.25*
Testis	57.30 ± 3.14	43.20 ± 1.85	-11. 50	4.193*
Seminal vesicle	20.28 ± 3.43	19.05 ± 3.32	-13.45	2.896*

+ Data represent value of six observations. There is significant difference between the normal and treated tissues and haemolymph protein content ** denotes significant at 0.01 level

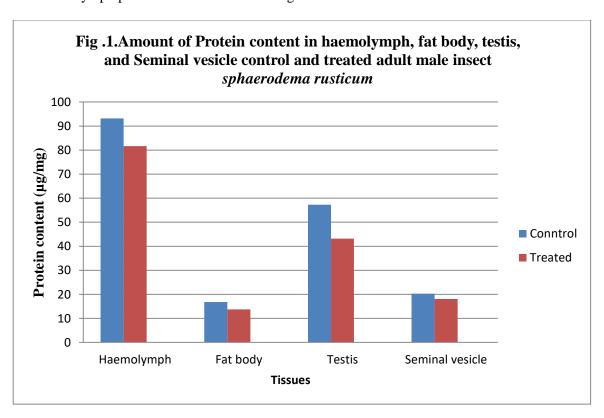
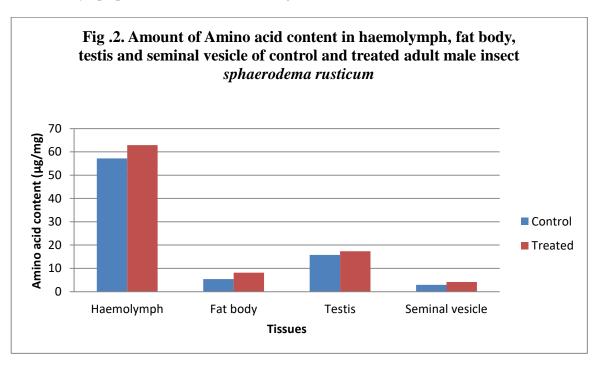


Table 2: Amino acid content haemolymph, fat body, testis and seminal vesicle of control and Chromium treated adult male insect *S. rusticum* (Quality expressed μ g/mg wt in case of tissues and μ g/100 μ l in case haemolymph)

Tissues	Con Chart Area	Treated	Percent change	t-value
			over control	
Haemolymph	57.17 ± 0.08	62.91 ± 670.07	-10.97	20.85*
Fat body	5.42 ± 1.04	8.14 ± 2.05	-32.18	34.60*
Testis	15.78 ± 2.04	17.35 ± 30.05	-5.72	31.52*
Seminal vesicle	2.90 ± 7.06	4.138 ± 90.05	-42.36	14.21*

⁺ Data represent value of six observations. There is significant difference between the normal and treated tissues and haemolymph protein content ** denotes significant at 0.01 level



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