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Effect of copper sulphate on protein content in mature female *Hydrophilous olivaceous*

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Abstract : Proteins are complex organic macromolecules that contain carbon, hydrogen, oxygen, nitrogen and usually sulphur and are composed of one or more chains of amino acids. These are fundamental components of all living cells and include many substances, such as enzymes, hormones and antibodies that are necessary for proper functioning of an organism. Various aspects of protein metabolism in insect development, such as the pattern of free amino acid pool, the intermediary pathway of individual amino acids and their derivatives, qualitative and quantitative changes in haemolymph and whole body protein synthesis and activity of specific enzymes have been studied by many insect's biochemists.[1-7] It is well known fact that the copper sulphate is an important hazardous water pollutant. The effect of which can be felt at all bio-chemical parameters of an aquatic life. The bio-assay experiment in the present investigation, it was realized that Copper Sulphate badly affected the protein metabolism at different levels in mature females of *H. olivaceous*. Copper sulphate act as major toxicants which affect all body actions directly or indirectly of all aquatic organisms. Almost all heavy metals are toxic at higher concentration and some are severely poisonous even at low concentration. In present investigation on mature female *Hydrophilous olivaceous*, it was observed that copper sulphate had an initial protein lowering effect and latter elevating trend on the haemolymph, fatbody and ovary of female insect. Overall initial decline of the protein content in haemolymph, fatbody and ovary were observed in insect exposed to sub-lethal concentration of copper sulphate.

Key words: Copper sulphate, protein, *Hydrophilous olivaceous*, haemolymph, fatbody, ovary.

INTRODUCTION

Proteins are complex organic compounds that contain carbon, hydrogen, oxygen, nitrogen and usually sulphur and are composed of one or more chains of amino acids. They include many substances, such as enzymes, hormones and antibodies that are necessary for proper functioning of an organism.

Aquatic environment is said to be the ultimate sink for the toxic compounds. Heavy metals from motor vehicles via urban storm, water runoff reaches to water reservoirs.

A number of works on the effect of pesticides and other chemicals indicate that although in low concentration, they may not lead to the death of aquatic organisms but can cause alterations in their metabolic processes and chemical constituents. Roan and Hopkins 1961 & O'Brien 1967 gave review of insecticides action on metabolism.

Effects of heavy metals on protein and free amino acids of certain aquatic insects on proteins and free amino acids were also studied by Chaudhary (1996)[3].

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Copper is a highly toxic metal posing great threat to aquatic organisms. The impact of copper and its salt can be felt at all trophic levels. The toxicity of copper and its compound to different aquatic organisms have been determined. It has been reported that copper sulphate was highly toxic and that there was tissue accumulation in the stone loach *Noemachelius barbatulus* in hard water, Solbe and Cooper, 1976. The acute lethality of copper over wide range of combinations of hardness and pH to rainbow trout has been determined,Howrath and Spraque, 1978. Thus it is expected that the copper sulphate causes alterations in quantitative and qualitative levels of proteins involved in metabolism. Keeping this in view, in present investigation mature *Hydrophilous olivaceous* and *Laccotrephes maculatus* both female and male were exposed to sub-lethal doses of Copper Sulphate and quantitative estimation of proteins were done at 24 hrs, 48 hrs, 72 hrs and 168 hrs of treatment in each case separately.

The present investigation deals with the biochemical studies of sub-lethal doses of copper sulphate on the insect *H. olivaceous* and *L. maculatus* and the main emphasis has been given on the quantitative change of carbohydrates, proteins, alkaline phosphatase and lactate dehydrogenase content in the haemolymph, fatbody, ovary and testes of the mature female and male insects.

MATERIALS AND METHODS

Quantitative Estimation of Protein

Quantitative estimation of haemolymph proteins, fatbody proteins, testes proteins of treated and control insects in mature stages were done by Lowry's method.

Principle of Lowry's Method

Proteins react with Folinciocalteaue reagent to give a coloured complex. The colour so formed is due to the reaction of Alkaline Copper with the proteins and then reduction of the Phosphomolybdate by Tryosine and Tryptophans.

Reagents

(a) Alkaline Sodium Carbonate Solution (2% of Na_2CO_3 in N / 10 NaOH).

(b) Copper Sulphate Sodium potassium tartarate (5% $CuSO_4$ in 1% Na, K tartarate) was freshly prepared by mixing stock solution.

(c) Alkaline Solution- It was prepared on day or working by mixing 50 ml of Alkaline Sodium Carbonate solution and 1 ml of Copper Sulphate Sodium Potassium tartarate solution.

(d) Folinciocalteau Reagent - The commercial reagent was diluted the equal volume of water on the day of use. This is a solution of Sodium Tongstate and Sodium Molybdate in Phosphoric Acid and HCL.

(e) Standard Protein Solution - Albumin solution 0.2 mg / ml [Bovine serum albumin Loba (Ltd.)]

Technique

(a) Technique of Haemolymph Protein: Insects head were pricked with needle and were kept inverted in centrifuge tubes, one insect in each centrifuge tube and were centrifuged at 1500 rpm to collect haemolymph. Then 1 ml of haemolymph was taken in separate centrifuge tube and added 5 ml 10 % TCA. It was centrifuged at 3000 rpm for supernatant, from supernatant test for carbohydrate was carried out.

(b) Techniques of Fatbody and ovary Protein :Insects were dissected, fatbody and ovaries or testes were taken out and crushed in 2 ml of distilled water with the help of mortar and pestle separately. The crushed material was collected in a centrifuge tube and 5 ml of 10 % TCA was added to obtain the precipitate of protein. It was centrifuged at 3000 rpm. The supernatant was kept separately for performing quantitative assay of proteins.

Precipitated protein was dissolved in 5 ml N / 10 NaOH. The protein samples obtained by the above procedure were then subjected to quantitative assay colorimetrically by employing Lowry's method.

Procedure

5 ml of alkaline solution was added to 0.1 ml of test solution. It was mixed thoroughly and .5 ml of diluted folinciocalteau reagent was added to it. The solution was kept at room temperature for 20 minutes, N / 10 NaOH were used as a blank and the absorption was read at 660 nm in colorimeter.

Calculations

The protein content was calculated as follows:

$$\frac{\text{OD of Unknown Solution}}{\text{OD of Known Solution}} \times \text{Concentration of Standard Solution} \times \text{Dilution of Unknown Solution}$$

Where,
OD = Optical Density.

RESULTS AND DISCUSSION

Observation of Protein content in maure female H. olivaceous. The total protein content in the haemolymph, of mature female *H. olivaceous* were 35 ± 7 SD mg / 100 ml and 20 ± 3 SD mg / 100 ml respectively in control insect.

Protein Content in Mature Female *H. olivaceous*

Haemolymph Protein Content

The total protein content in the haemolymph of mature female *H. olivaceous* were 35 ± 7 SD mg / 100 ml in control insect.

On treatment with copper sulphate the haemolymph protein content of mature female declined significantly in comparison to the control insects as 20 ± 4 ($P < 0.01$) SD mg / ml at 24 hrs, 15 ± 3 ($P < 0.001$) SD mg / ml at 48 hrs, 23 ± 4.5 ($P < 0.01$) SD mg / ml at 72 hrs of treatment. A slight but significant ($P > 0.05$) rise of 30 ± 6 SD mg / ml was observed at 168 hrs of treatment, (Table 1, Graph 1).

Fatbody Protein Content

The mature female *H. olivaceous* had a fatbody protein content of 200 ± 9 SD mg / gram in control insects.

On treatment with copper sulphate the fatbody protein content in mature female significantly declined up to 48 hrs of treatment. These were 150 ± 8 ($P < 0.001$) SD mg / gram at 24 hrs and 132 ± 7 ($P < 0.001$) SD mg / gram at 48 hrs of treatment. A highly significant and unusual rise of 140 ± 7.5 ($P < 0.001$) SD mg / gram in protein content of fatbody was noted at 72 hrs of treatment. At 168 hrs of treatment the fatbody protein content showed rising trend as 160 ± 8 ($P < 0.001$) SD mg / gram but still remained significantly lower than that of control insect, (Table 1, Graph 1).

Ovarian Protein Content

The ovary of mature female *H. olivaceous* had protein content of 185 ± 7 SD mg /gram of ovary in control insects.

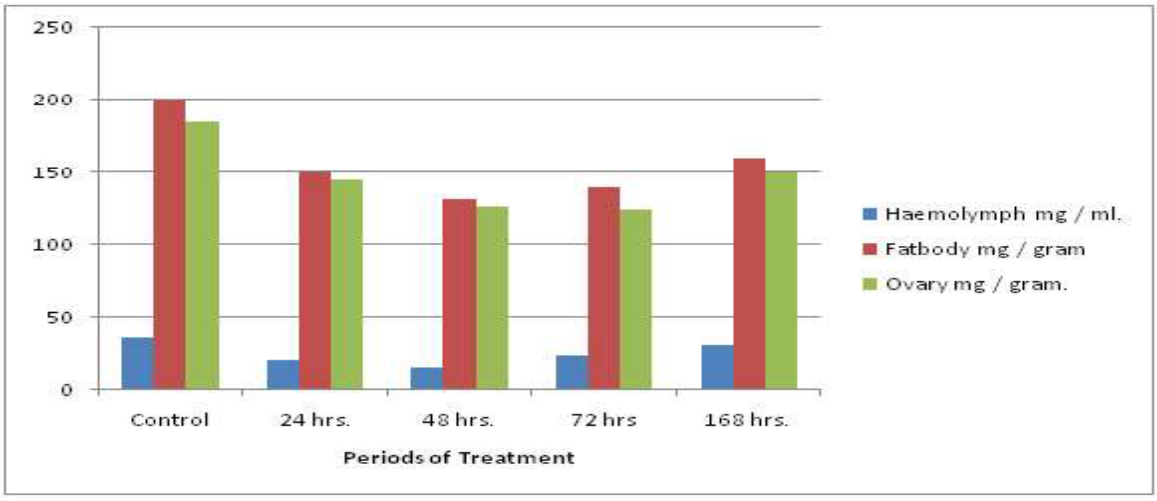
In mature female, similar decline in the ovarian protein content was noted up to 72 hrs of treatment like fatbody, as 145 ± 6 SD mg / gram at 24 hrs, 125 ± 5 SD mg / gram at 48 hrs, 123 ± 4.5 SD mg / gram at 72 hrs of treatment respectively. A significant rise of 150 ± 7 SD mg / gram was noted at the 168 hrs of treatment. All the differences were highly significant ($P < 0.001$) as compared with that of control insects, (Table 1, Graph 1).

Table 1: Protein content in haemolymph, fatbody and ovary of mature female *H. olivaceous* during control and after different periods of treatment with Copper sulphate

Periods of Treatment	Haemolymph mg/ml	Fatbody mg/gram	Ovary mg/gram
Control	35 ± 7	200 ± 9	185 ± 7
24 hrs	20 ± 4**	150 ± 8***	145 ± 6***
48 hrs	15 ± 3***	132 ± 7***	125 ± 5***
72 hrs	23 ± 4.5**	140 ± 7.5***	123 ± 4.5***
168 hrs	30 ± 6	160 ± 8***	150 ± 7***

Mean ± SD
SD = Standard Deviation
* = $P < 0.05$ (Significant)
** = $P < 0.01$ (Significant)
*** = $P < 0.001$ (Significant)

Graph – 1



On observing effect of copper sulphate on protein content of mature female *H. olivaceous* it was found that the total protein content in the haemolymph, fatbody and ovary of mature female *H. olivaceous* were 35 ± 7 SD mg/100 ml, 200 ± 9 SD mg/gram and 185 ± 7 SD mg/gram respectively in control insect.

In mature *H. olivaceous* copper sulphate had a protein lowering effect in haemolymph up to 48 hrs but in fatbody there was protein lowering effect up to 72 hrs of treatment. In ovary, the lowering tendency was maintained up to 72 hrs of treatment. A gradual rise in protein content in female haemolymph was observed from 72 hrs in all the tissues.

The overall drop in protein content in all cases female *H.olivaceous* in early stages might be due to the interference of this chemical in protein synthesizing machinery or due to rapid catabolic breakdown of protein into glucose to produce energy during chemical stress. The lesser food intake and rapid utilization of protein under stress may also be a factor leading to the loss of protein in haemolymph, fatbody, ovary.

But after 72 hrs of treatment gradual rise in protein content of haemolymph, fatbody, ovary was observed. It showed active entry of protein from ovary and fatbody into haemolymph. It suggests stability in the protein synthesis of the insects after facing stress of sub-lethal dose of Copper Sulphate.

After treatment with Copper Sulphate a sharp decline in protein content at 24 hrs was noted in all the experimental tissues in female. Then a gradual rise was noted up to 7 days of the treatment except fatbody where declining trend was maintained up to 72 hrs

The overall drop in protein content in early stages of treatment confirmed the inhibitory effects of copper sulphate in protein synthesis machinery. The rise in protein content after 72 hrs of treatment confirmed the metabolic stability of the insects due to detoxification. A similar result was observed by Islam and Roy (1983)[5] in Chrysocoris stolli exposed to various concentrations of Cadmium Chloride.

Copper Sulphate thus affected the protein synthesizing machinery of the mature female and male *H. olivaceous* and *L. maculatus*. Fatbody being the main site of protein synthesis had been affected in the initial stages of the treatment. This accordingly showed effects in the decline in haemolymph, testis and ovarian protein contents. Increase in protein content in later stages suggests some stability in protein synthesizing of insects after facing initial hazards.

The study of physiological changes due to the responses of individual insects or other aquatic animals in the presence of various types of pollutants is of immense help to aquatic toxicologists. It is obvious that the changes in an aquatic organism that occurs as a result of pollution also have real practical use. An organism makes a marvelous integration of the effect of the any type of changes in chemical and physical factors in aquatic environment. The usual approach of performing chemical analysis and physical measurements of natural water may or may not detect changes that are harmful to the aquatic organisms. In real sense aquatic organisms themselves are the best judge of that different physiological parameters are generally used as tools for the study of aquatic pollution by chemical and other means. The physiological parameters are Hematology, measurement of haematochrit, hemoglobin concentration, erythrocytes and leucocytes count being the main aspects; and blood chemistry, which ranges from the determination of blood clotting time to the estimation of hormones and tissues chemistry.

Effects of heavy metal on the blood proteins of fishes have been reported by various workers like Baskaran (1991)[8], Choudhary (1998)[9], Winteringham (1959)[10] Ehteshamuddin (1985)[11].

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