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INFLUENCE OF CHLORELLA VULGARIS ON COPPER METAL CAUSED PHOSHOGLUCOMUTASE VARIATION IN DIFFERENT BRAIN REGIONS OF TELEOSTS DUE TO DETOXIFICATION

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ABSTRACT

The autotroph *Chlorella vulgaris* reduced the metal (sub-lethal concentration copper) induced enzyme variations *PGM* in followed by cerebrum, diencephalon, medulla oblongata and cerebellum in *Labeo rohita*, *Clarias batrachus* and *Channa punctatus*. The variations recorded in the above said enzyme subjected to sub-lethal copper concentration in presence of microbe recorded less fall in various brain regions than the one exposed directly to sub- lethal concentration of copper concentration in presence of microbe recorded less fall in various brain regions than the one exposed directly to sub-lethal concentrations of cooper educates that the autotroph *Chlorella vulgaris* has the detoxification ability.

INTRODUCTION

Heavy metal exposure is ascribed to the formation of free radicals and reactive oxygen species in various animal species including fish species and the formation of the above said perhaps the root cause of a no. of acute and chronic disorders and subsequent dysfunction of cell /organ and to induce pathological conditions (Barron 2003). Every animal including fish has the ability and capacity to reduce the free radicals created damage through enzymatic and non- enzymatic events in a routine way. If the normal level of antioxidant defense mechanism is not potential enough to contain and remove free radicals, then the application of exogenous antioxidants through innovative approach are required to protect the cell from oxidative stress.

Contaminants caused damage to all organizational aspects of animal i.e. morphology, anatomy, development, growth, reproduction, neuro-endocrine mechanisms, embryology, physiology, biochemistry and genetics (Akiyama *et al*. 2001). If proper and appropriate innovative detoxification devises are not devised/applied to protect environment the time is not very for when contamination prove destroys to life including Homo sapiens globally.

In the present investigation the investigators made an attempt to investigate the detoxification of sub-

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lethal and lethal concentrations of copper on brain region (Cerebrum, diencephalon, cerebellum & medulla oblongata) biochemical compartmentation (Phoshoglucomutase (PGM)induced variations in Labeo rohita(Ham.),Claries batrachuse (Linn.) and Channa punctatus (Bloch.)).These inland fish sp. Which are economically, culturally, nutritionally important once from atropical habitat on comparative basis by the application of the autotrophs i.e. Chlorella vulgaris under acute studies.

MATERIAL AND METHODS

Alive, healthy, mature, disease-free and active *Labeo rohita* (Ham.), *Clarias batrachus* (Linn.) and *Channa punctatus*(Bloch.) 120-130 g of 18-20 cm. (standard length) were obtained from few selected local ponds to avoid ecological variation and acclimatized in the laboratory condition for a period of seven days and were subjected for various exposures and investigations.

Determination of safety, Sub-lethal and lethal concentration

Safety, sub-lethal concentrations of copper was determined on *Labeo rohita, Clarias batrachus* and *Channa punctatus* by the Probit Analysis Method (Finney, 1971). Higher concentration of copper was used and slowly reduced the amount of concentration to know the Lc 50/100 value for 96-hour exposure.

Acute studies

The *Labeo rohita, Clarias batrachus* and *Channa punctatus* (120-130 g) of 18-20 cm (standard length) were taken separately and kept in twenty groups and each group consist of forty eight fish species. No food was given to the above fish species during this period (08, 16 and 24hrs). The first set of *Labeo rohita , Clarias batrachus* and *Channa punctatus* were exposed to sub-lethal and lethal concentration of zinc and the detail were described somewhere else (Shaffi and Kakaria, 2006).

Preparation of tissue extract

The termination of the experiment,preparation of tissue extract and enzyme assays were described elsewhere (Colowick and Kaplon, 1975; Shaffi and Habbibulla, 1977).

Statistical analysis

The experiments with acute and chronic studies were repeated at least seven times separately to subject the data for analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Exposure to sub - lethal and lethal concentrations of copper caused marked changes in *PGM* in cerebrum, diencephalons, cerebellum and medulla oblongata of *Labeo rohita* (sub-lethal concentration of Cu- 0.10 mg/L), *Clarias batrachus* (sub-lethal concentration of Cu- 0.50 mg/L), *Channa punctatus* (sub-lethal concentration of Cu- 0.80mg/L) under acute studies. Safety level concentrations of copper metals were determined for Labeo rohita (Cu-0.06mg/L.), *Clarias batrachus* (Cu- 0.22 mg/L) and *Channa punctatus* (Cu- 0.30 mg/L). The influence of Chlorella vulgaris on sub-lethal and lethal concentrations of copper was investigated and the details are in Table 1.

The exposure to sub-lethal concentrations of copper in presence of *Chlorella vulgaris* led to highest fall in diencephalons *PGM* in comparison to cerebrum, medulla oblongata and cerebellum in *Labeo rohita* ,in *Clarias batrachus* and in *Channa punctatus* (Table 1). The impact of *Chlorella vulgaris* on sub-lethal copper treated remained same but to a less extent than, in *Labeo rohit*a. The maximum fall in *PGM* was in diencephalons followed by cerebrum, medulla oblongata and cerebellum at 08 hrs. exposure than at 16 and 24 hrs. exposure in *Clarias batrachus*.The fall in *PGM* in *Channa punctatus* was optimum at 16 hrs. in diencephalon, in comparison to , medulla oblongata, and cerebellum than at 08 hrs and 24 hrs in exposure to sub-lethal concentrations (Table 1). The exposure to sub-lethal concentrations of copper in presence of *Chlorella vulgaris* led to highest fall in diencephalons *PGM* in comparison to cerebrum, medulla oblongata and cerebellum in *Labeo rohita*, in *Clarias batrachus* and in *Channa punctatus* (Table 1). The impact of *Chlorella vulgaris* on sub-lethal copper treated remained same but to a less extent than, in Labeo rohita. The exposure to sub-lethal concentrations of copper in presence of *Chlorella vulgaris* led to highest fall in diencephalons *PGM* in comparison to cerebrum, medulla oblongata and cerebellum in *Labeo rohita*, in *Clarias batrachus* and in *Channa punctatus* (Table 1).

The impact of *Chlorella vulgaris* on sub-lethal copper treated remained same but to a less extent than, in *Labeo rohita*. The maximum fall in *PGM* was in diencephalons followed by cerebrum, medulla oblongata and cerebellum at 08 hrs. exposure than at 16 and 24 hrs. exposure in *Clarias batrachus*.The fall in

Table 1. Influence of Chlorella vulgaris on copper (sub-lethal) caused phosphoglucomutase variations in different brain regions of three fresh water fish species

Regions of the Brain	Control	Duration of sub-lethal concentration exposure			%of Fall /Rise	Duration of sub-lethal concentration exposure with *Chlorella vulgaris*			%of Fall /Rise
		08 Hrs.	16 Hrs.	24 Hrs.		08 Hrs.	16 Hrs.	24 Hrs.	
(A) *Labeo rohita* (Ham.)									
Cerebrum	0.430±.060	0.342 c±.038	0.162 a +.044	0.112 c ±.024	74.00	0.305+.032	0.196 a+.024	0.133 c +.020	69.06
Diencephalons	0.306 ±.046	0.146 b±.030	0.092 a +.026	0.061 c ±.012	80.00	0.176 +.024	0.126 b+.019	0.073 a +.014	76.14
Cerebellum	0.235 ±.028	0.186 c±.022	0.142 a +.052	0.098 b ±.021	58.00	0.265 +.019	0.164 c +.024	0.115 +.012	51.06
Medulla oblongata	0.345 ±.066	0.262 a±.049	0.158 b +.032	0.119 ±.025	66.00	0.282 +.034	0.194 a +.018	0.144 c +.036	58.26
(B) *Clarias batrachus*(Linn.)									
Cerebrum	0.390 +.054	0.301 c+.038	0.184 a +.029	0.120 b +.016	69.23	0.325 +.022	0.245 b +.025	0.111 c +.019	63.07
Diencephalons	0.274 +.042	0.245 +.029	0.132 b +.032	0.066 a +.015	75.91	0.206 +.012	0.146 a +.036	0.079 b +.012	71.16
Cerebellum	0.194 +.046	0.162 +.036	0.126 c +.028	0.091 b +.017	53.09	0.175 +.018	0.137 b +.020	0.102 c +.021	47.42
Medulla oblongata	0.309 +.036	0.236 +.038	0.165 a +.020	0.121 b +.015	60.84	0.245a+.024	0.182 c +.036	0.145 +.029	53.07
(C) *Channa punctatus* (Bloch)									
Cerebrum	0.315 +.038	0.209 b +.032	0.156 a +.024	0.115 c +.061	64.12	0.282 +.062	0.174 b +.029	0.132 c +.016	58.09
Diencephalons	0.234 +.028	0.156 a +.041	0.115 b +.032	0.070 b +.024	70.08	0.184 +.036	0.124 a +.032	0.079 b +.013	66.23
Cerebellum	0.167 +.030	0.132 +.028	0.105 +.015	0.085 b +.013	49.10	0.124a+.024	0.102 +.014	0.093 c +.017	44.31
Medulla oblongata	0.274 +.032	0.198 +.022	0.146 a +.017	0.120 b +.024	56.20	0.195b+.034	0.159 c +.041	0.143 +.028	47.81

Values are mean ± SDM of seven replicates . The data was subjected to test of ANOVA . The super scripts (a, b & c) indicates that P >0.01, P>0.02, & P>0.05 respectively.

PGM in *Channa punctatus* was optimum at 16 hrs. in diencephalon, in comparison to, medulla oblongata, and cerebellum than at 08 hrs. and at 24 hrs.in exposure to sub-lethal concentrations (Table 1).

Chlorella vulgaris is a rich nutritional ingredient and also contain luthein and chlorophyll as antioxidants. Hence the above said species is able to prevent metal caused oxidative stress and cellular damage. Heavy metal including copper depletes glutathione and protein bound sulpha- hydryl groups resulting in enhanced production of reactive oxygen species such as superoxide ions, hydroxyl radicals and hydrogen peroxides. Biological compounds in *Chlorella vulgaris* (Valdia *et al*.2010). Under detoxication studies the author investigated the impact of *Chlorella vulgaris* on sub-lethal copper toxicity on *PGM* in various brain regions of brain i.e. cerebrum, diencephalon, cerebellum and medulla blongata in three important inland teleost viz *Labeo rohita*, *Clarias batrachus* and *Channa punctatus* under short term exposure studies.

The autotroph *Chlorella vulgaris* reduced the metal (sub-lethal concentration copper) induced enzyme variation *PGM* in diencephalon followed by cerebrum, medulla oblongata and cerebellum to a maximum extent in *Labeo rohita*, in comparison to *Clarias batrachus* and *Channa punctatus*. The variations recorded in the above said enzyme subjected to sub-lethal

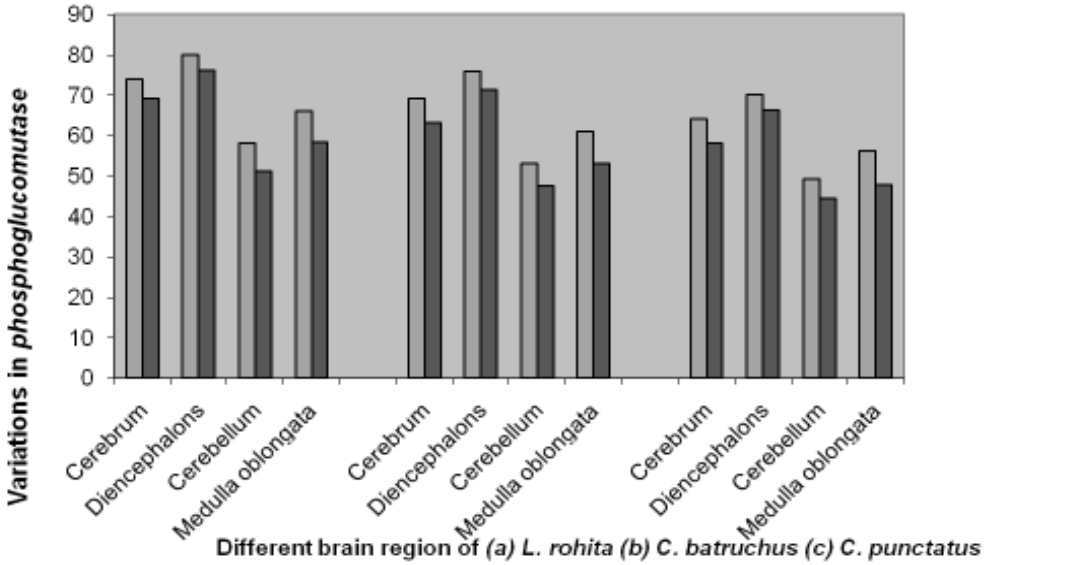

Diagram 1 :- Table No. 1 Series1-Metal treted, Series2-Metal & C. vulgaris treted

copper concentration in presence of microbe recorded less fall in various brain regions than the one exposed directly to sub- lethal concentration of copper concentration in presence of microbe recorded less fall in various brain regions than the one exposed directly to sub-lethal concentrations of cooper educates that the autotroph *Chlorella vulgaris* has the detoxification ability and the present change of enzyme levels in different brain regions of three fish species (Cristina *et al*., 2005; Shaffi *et al*., 2007).

Autotroph bio-mass depletes the copper metal precipitation in fish species as microbial of autotroph bio-mass is capable of absorbing metal ions from aqueous solution. The pattern of absorption of metal ions the autotrophs might have taken place through their general surface and intra-cellularly. The general surface of autotrophs may contain poly- saccharides, proteins and lipids that may act as binding site for heavy metals . The above identified macromolecules on the general surface of autotrophs may provide functional groups and the formal may contain amino, carboxylic, sulpha-hydryl, phosphate and thiol functional groups and they have the ability to bind metals (Page,et.al., 2009; Valdia et al 2010) The metal copper might have accumulated in Chlorella vulgaris and the less impact of copper noticed in the fall of phosphoglucomutase, in various brain regions of Clarias batrachus & Channa punctatus in comparison to directly exposed to copper sub-lethal may be understood on the above described episode. The microbial heavy metal absorption has been a twofold story i.e. a first rapid stage and a second slow stage. In the first rapid stage the heavy metal ions are absorbed at the surface of microorganism and in the next phase (second) the metal ions are transported across the cell membrane in the cytoplasm (Holden & Adams, 2003; Chehregani & Malayou, 2007).

In the present investigation such a mechanism might have taken place and the fall in *PGM* to a highest amount in diencephalon, cerebrum, medulla oblongata and cerebellum in *Labeo rahita* in comparison to *Clarias batrachus* & *Channa punctatus* with sublethal concentrations of copper than copper reflect that microbial bio-mass present in the natural water body may help to contain/control the toxic influence on target and non-target organisms and if the autotrophs are monitored systematically and scientifically this approach certainly help to increase the yield from water bodies and this kind of innovative approach will play a vital role in our national economy and nutritional programme.

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