Research Paper | E-ISSN No : 2455-295X | Volume : 2 | Issue : 5 | May 2016

COMBINED IMPACT OF CHLORELLA VULGARIS AND SPIRULINA PLATENSIS ON COPPER METAL CAUSED VARIATIONS OF PHOSPHOGLUCOMUTASE ENZYME

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ABSTRACT

The variations in the above said ***phosphoglucomutase*** enzyme subjected to sub-lethal copper concentration in presence of *Chlorella vulgaris* and *Spirulina platensis* less fall in various brain regions than the one exposed directly to sub- lethal concentration of copper .The combined influence of *Chlorella vulgaris* and *Spirulina platensis* on sub-lethal concentration of copper metal caused enzymatic variations *phosphoglucomutase* in different brain regions [cerebrum, diencephalons, cerebellum & medulla oblongata] of fresh water teleosts i.e. *Labeo rohita* (Ham.), *Clarias batrachus* (Linn.) and *Channa punctatus* (Bloch.) on a comparative basis from a tropical environment under short term exposure. Aquatic autotrophs can be used to remove heavy metals from aquatic system over a wide range of pH. Such events might have taken place even in the present investigation and the less fall in *phosphoglucomutase* in different brain regions of *Labeo rohita, Clarias batrachus* & *Channa punctatus* might be ascribed to a less degree in microbe presence than direct exposure to heavy metals. The autotrophs *Chlorella vulgaris* and *Spirulina platensis* have the detoxification ability and the present change of enzyme levels in different brain regions of three fish species.

KEY WORDS: Chlorella vulgaris, Spirulina platensis, Copper, Phosphoglucomutase, Teleosts.

Introduction:
Heavy metals are regarded as one of the most serious pollutants are due to their environmental persistence and tendency to concentrate in aquatic organisms. Heavy metals are dangerous because they tend to bioaccumulation *[Bevoets et al., 2001 & Chattopadhyay et al.,2002].*

Heavy metal induce oxidative damage in different organs by increasing per-oxidation of membrane chemistry and altering the antioxidant system of the cells/tissues *[Lengke & Southam, 2005; Gupta & Flora, 2005,2006 & Kumar et al. ,2007]*. Interaction of metal ions with the cell organelles cause injury to cellular components. Heavy metal intoxication further depletes glutathione & protein bound sulfa-hydryl groups resulting into the production of reactive oxygen species like hydrogen peroxides, superoxide ions & hydroxyl radicals. These reactive oxygen species induce elevated visceral per-oxidation *[Bano et al., 2007 & Kaur & Bansal, 2008].*

In the present investigation the author made an attempt to investigate the combined influence of *Chlorella vulgaris* and *Spirulina platensis* on sub-lethal & lethal concentration of copper metal caused enzymatic variations [*phosphoglucomutase* in different brain regions [cerebrum, diencephalons, cerebellum & medulla oblongata] of fresh water teleosts i.e. *Labeo rohita* (Ham.), *Clarias batrachus* (Linn.) and *Channa punctatus* (Bloch.) on a comparative basis from a tropical environment under short term exposure (acute studies).

Material & methods :
Alive, healthy, mature, disease-free & active *Labeo rohita* (Ham.), *Clarias batrachus* (Linn.) and *Channa punctatus* (Bloch.) 120-130 gm. 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 gm) 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 & 24hrs). The first set of *Labeo rohita* , *Clarias batrachus* and *Channa punctatus* were exposed to sub-lethal concentration of copper and the detail were described somewhere else (Shaffi & Kakaria2006).

Preparation of tissue extract : The termination of the experiment,preparation of tissue extract and enzyme assays were described elsewhere [Colowick & Kaplon,1975;Shaffi & Habbibulla,1977].

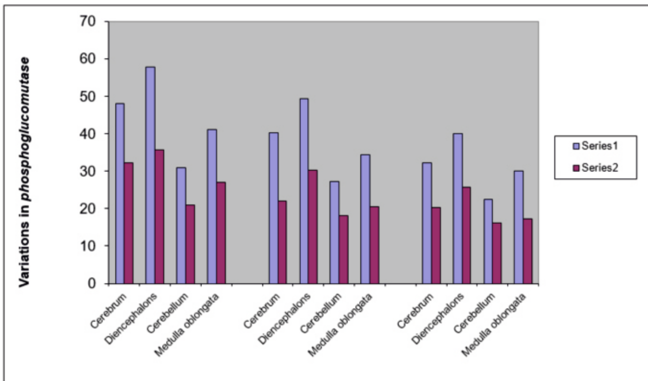
Statistical analysis: The experiments with acute and chronic studies were repeated at least seven times separately to subject the data for analysis of variance.

Table No.-1 : Combined influence of *Chlorella vulgaris* & *Spirulina platensis* on copper metal (sub-lethal) caused toxicity in three fresh water teleosts Phosphoglucomutase Chronic studies

REGIONS OF THE BRAIN	CONTROL	DURATION OF SUB-LETHAL CONCENTRATION EXPOSURE			% OF FALL/ RISE	DURATION OF SUB-LETHAL CONCENTRATION EXPOSURE WITH Chlorella vulgaris & Spirulina platensis			% OF FALL/ RISE
		15 DAYS	30 DAYS	45 DAYS		15 DAYS	30 DAYS	45 DAYS	
(a) Labeo rohita (HAM)									
Cerebrum	0.421 .158	0.276 c .036	0.248 c .028	0.219 c .026	47.98	0.348 c .032	0.309 c .038	0.285 c .042	32.30
Diencephalon	0.242 .039	0.259 .042	0.162 c .019	0.126 c .024	57.85	0.248 .042	0.210 .024	0.192 .028	35.78
Cerebellum	0.229 .028	0.208 .022	0.169 .026	0.158 c .032	31.00	0.204 .028	0.198 .021	0.181 .016	20.96
Medulla Oblongata	0.336 .032	0.246 c .038	0.212 c .019	0.198 c .024	41.07	0.286 .036	0.258 .042	0.245 c .032	27.08
(b) Clarias batrachus (LINN.)									
Cerebrum	0.381 .081	0.268 c .028	0.236 c .020	0.228 c 0.036	40.15	0.314 .029	0.305 .019	0.297 .024	22.04
Diencephalon	0.268 .042	0.252 .039	0.184 .021	0.136 c .016	49.25	0.212 .032	0.196 .021	0.187 .018	30.22

Cerebellum	0.188 .036	0.171 .021	0.149 .031	0.137 .019	27.12	0.170 .026	0.164 .028	0.154 .022	18.08
Medulla Oblongata	0.302 .028	0.236 .023	0.214 c .028	0.198 c .021	34.43	0.264 .038	0.252 .021	0.240 .032	20.52
(c) Channa punctatus (BLOCH)									
Cerebrum	0.301 .019	0.256 .042	0.224 .031	0.204 c .019	32.22	0.264 .042	0.256 .028	0.240 .032	20.26
Diencephalon	0.222 .022	0.198 .026	0.148 .024	0.133 c .022	40.09	0.194 .024	0.174 .019	0.166 .016	25.72
Cerebellum	0.161 .028	0.148 .022	0.134 .014	0.125 .019	22.36	0.140 .019	0.138 .012	0.135 .013	16.14
Medulla Oblongata	0.267 .032	0.247 .042	0.194 .021	0.187 .026	29.96	0.251 .022	0.234 .028	0.221 .021	17.22

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



Different brain region of *(a) L. rohita (b) C. batruchus (c) C. punctatus.* *Series 1- Duration of sub- lethal Cu concentration,* Series -2 Sub-lethal Cu Concentration with *Chlorella vulgaris & Spirulina platensis*

Diagram 1 :- Table No. 1

Results:

The combined impact of *Chlorella vulgaris* and *Spirulina platensis* was investigated on sub - lethal concentrations of copper toxicity on *phosphoglucomutase* in various brain regions of *Labeo rohita*(sub-lethal concentration of,Cu- 0.10 mg/ltr *Clarias batrachus* (sub-lethal concentration of Cu- 0.50 mg/ltr Cu- 0.96 mg/ltr.) and *Channa punctatus* (sub-lethal concentration of Cu- 0.80mg/ltr at 08, 16 & 24 hrs. exposure under acute studies **.** Please see **Table-01** and chart diagrams.

The *phosphoglucomutase* fall in diencephalon under the influence of microbes though highest but less in comparison to sub-lethal copper toxicity induced fall than in cerebrum, medulla oblongata & cerebellum in *Labeo rohita* at 08 hrs of exposure in comparison to 16 & 24 hrs. exposure. In *Clarias batrachus* the fall in *phosphoglucomutase* was optimum in diencephalon at 16 hrs. exposure than at 08 & 24 hrs. exposure followed by cerebrum, medulla oblongata and cerebellum. In *Channa punctatus* the fall in *phosphoglucomutase* was noticed in diencephalons at 16 Hrs. of exposure than at 08 & 24 Hrs. exposure followed by cerebrum, medulla oblongata & cerebellum.

The *phosphoglucomutase* fall in diencephalon under the microbe influenced copper toxicity is lesser than the fall with the direct copper exposure followed by cerebrum, medulla oblongata and cerebellum to a maximum extent in *Labeo rohita* **(Table-01)**. In *Clarias batrachus* **(Table-01)** optimum fall in *phosphoglucomutase* was noticed in diencephalons than cerebrum medulla oblongata, & cerebellum at 16 hrs. Than at 08 & 24 hrs. exposure. In *Channa punctatus* **(Table-01)** the two microbes influenced copper toxicity was more or less same as recorded in *Clarias batrachus*. The lethal level influence of copper in presence of microbes decreased the *phosphoglucomutase* level in diencephalons lesser than copper exposed once in diencephalons followed by cerebrum, medulla Oblongata and cerebellum in *Labeo rohita* in comparison to *Clarias batrachus* & *Channa punctatus* **(Table-01)**.

In all these investigations the fall in the above mentioned enzymes were optimum with copper exposed once respectively both at sub-lethal levels than in presence of microbes.

Discussion and conclusion:

Heavy metal exposure causes enzyme inactivation, reduction in R.B.C., lifespan, fall in hemoglobin surface area, alteration in electron transport, damage to genetic material, immunological variations and change in bio-chemical makeup of different fish species *[Rao et al., 2001; Warner et al., 2003 & Naskar et al., 2004 & 2005].*

The *Chlorella vulgaris* and *Spirulina platensis* influenced the sub-lethal effect of copper caused variations in brain compartmentation (cerebrum, diencephalons, cerebellum & medulla oblongata) of *phosphoglucomutase* in *Labeo rohita*, *Clarias batrachus & Channa punctatus* under acute or short term exposure. The sub-lethal levels of copper inhibited the *phosphoglucomutase* to a highest extent in diencephalon than in cerebrum, medulla oblongata & cerebellum in *Labeo rohita* in comparison to *Clarias batrachus & Channa punctatus* but lesser than the fall of the enzymes in the above said fish species directly exposed to sub-lethal levels of copper directly without any microbe compelled us to develop an insight to understand the positive impact on important bio-chemical parameters like enzymes that are important to promote a variety of anabolic & catabolic processes in an organism effectively reflects that microbes act as antidote effect fall heavy metal toxicity and the less fall of the four enzymes under investigation may be that microbes has a soothing impact and hence the microbes are able to decrease the sub-lethal toxicity of heavy metals.*[Mehta & Gaur, 2001*; *Thomas et al., 2001; Milutinovi et al., 2002* & *Holden & Adams , 2003]*

The following finding may help to understand the microbe-metal interaction and sub sequent detoxification of the metal to a less extent in a better way*[Lu et al. ,2006; Shaffi, 2007]* . The sub-cellular regions of Cyanobacteria and *Anabaena cylindrica* could trap the lead through its phosphate and precipitates in the form of lead phosphate on the cell wall inside the cell *[Bert et. al., 2009]*. Similar kind of mechanism might have taken place in the present findings i.e. less fall of enzymes in which the cellular components of *Chlorella vulgaris* and *Spirulina platensis* might have precipitated the metal into compound with the help of its cellular components and the present findings i.e. less fall of enzymes in presence of a autotrophs than the enzyme fall when directly exposed to copper sub-lethal levels should understand on similar lines.

Such bodies not only function in polyphosphate storage and further functions as a detoxification process such a mechanism is not rule out even in the present investigation and the fall of *phosphoglucomutase* with the metal exposure directly on one side and metal exposure in presence of *Spirulina* in *Labeo rohita*, *Clarias batrachus & Channa punctatus* on both side educates that the presence of the aquatic autotroph significantly checked the fall off the enzymes in different brain regions of the above said fish species is quite innovative and need further investigation on a large scale for the application in the aquatic system and to check the menace of pollution *[Upasani & Balaraman,2003; Sharma & Sharma, 2005; Ansari & Bhandari ,2008 & Bert et. al. 2009]*.

This investigation further helps that aquatic autotrophs can be used to remove heavy metals from aquatic system over a wide range of pH. Such events might have taken place even in the present investigation and the less fall in *phosphoglucomutase* in different brain regions of *Labeo rohita, Clarias batrachus* & *Channa punctatus* might be ascribed to a less degree in microbe presence than direct exposure to heavy metals.

Among the brain regions, diencephalon registered maximum variations in comparison to cerebrum, medulla oblongata & cerebellum in *Labeo rohita* than in *Clarias batrachus & Channa punctatus* .

ACKNOWLEDGEMENTS:

Author thankful to Prof. S.A.Shaffi Ex-Dean Of Regional Education of Institute (NCERT) Bhopal-13and Dr.V.K.Kakaria Asso.Prof. Of Regional Education of Institute (NCERT) Bhopal-13 for supervision of this work and encouragements and also Principal and Staff of Regional Education of Institute (NCERT) Bhopal-13 for providing necessary laboratory facility.

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