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RESEARCH ARTICLE

ANTIOXIDANT ROLE OF CAPSICUM ANNUM ON ZINC INDUCED ALTERATION ON GLYCOGEN CONTENT IN FRESHWATER MUSSELS, LAMELLIDENS CONSOBRINUS

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ABSTRACT

The present study describes the antioxidant role of zinc sulphate induce toxicity in an experimental model the freshwater mussel, *Lamellidens consobrinus*. The effect of mussels was studied under five groups, Group A was maintained as control, Group B mussels were exposed to chronic LC_{50/10} doses and zinc sulphate (0.21 ppm) for 21 days, while group C mussels were exposed to respective chronics concentrations of heavy metals with 5 ml /lit and extract from *Capsicum annum*. The glycogen content from all groups were estimated after 7,14 and 21 days. Mussels from group B were divided into two groups after 21 days exposure to heavy metal into D and E group. Mussels and D groups were allowed to cure naturally. While those with *Capsicum annum* L. (5ml/liter) and their glycogen content were studied after 7,14 and 21 days. Remarkable decrease in the glycogen content was observed in zinc exposed mussels. The group exposed to heavy metal along with extract from *Capsicum annum* L. showed more glycogen content than those exposed to heavy metals. Pre exposed mussels to heavy metals showed fast recovery and higher glycogen content than those which were allowed to cure naturally. The probable antioxidant role of extract from *Capsicum annum* L. is discussed in the present paper.

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INTRODUCTION

The aquatic environment has always been subjected to different types of pollutants of industrial, domestic and agricultural wastes. (Farkas *et al*, 2000) freshwater bivalves provides significant role in providing source of food for human being and other aquatic birds from all over the world. (Malathi and Thippeswamy, 2013). The aquatic ecosystem are very sensitive to heavy metal pollution and this is a primary problem due to their persistence as they are not removed by biodegradation. Heavy metals are the major contaminants a aquatic environment and a toxic towards aquatic organisms (Witeska *et al*, 1995). The most important heavy metals in water pollution are zinc, copper, lead, cadmium, mercury, nickel, chloride and chromium (Abel, 1989) (Javed and Usmani, 2012) reported that the water of sewage fed aquaculture pond of Panethi, Aligarh, which is used as source of commercial fish food contains the heavy metal zinc. Many water bodies have been polluted by effluents released by industries, factories, power stations, domestic waste, etc. which disturbing the quality of water and also degrade the protein source in the form of aquatic food (Baki *et al*, 2011. Taweel *et al*, 2012; Fatima and Usmani, 2013), Zinc accumulation was beyond the permissible limits (100ppm) set by FAO/WHO (1989) in muscles of *M. armatus*. Although Zn is an essential element as it is carefully

regarded on a potential hazard that can endanger both animal and human health.

Agency for toxic substances and Disease Registry (ATSDR, 2005), suggest that ingesting high levels of zinc for several months may cause anaemia, damage to pancreas and decreased level of high density lipoprotein (HDL) cholesterol. The *Capsicum annum* have high concentration of antioxidants such as polyphenols Carotenoids, ascorbic acid and flavinoids which fight against hazardous oxidative damage to the cell. (Odukoya *et al*, 2007 a Karandenz *et al* 2005). Banerjee *et al*, 2005 reported that the phenolics of green peeper has higher DPPH radical scavenging activity i.e. it traps the free radicals and protect the cell from oxidative damage. So the purpose of this study is to find out the antioxidant role of *Capsicum annum* on Zn induced alterations on glycogen content in fresh water mussel *Lamellidens consobrinus* as *Lamellidens* is used as important food source in different parts of the world.

MATERIAL AND METHODS

Preparation of aqueous extract of *Capsicum annum*

The plant *Capsicum annum* Linn (1 kg) was collected from a local market in Bhusawal (M.S.), India. The dried green chillis

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were ground to a fine powder and were extracted with boiling water (5 L) for 30 min by Soxhlet technique. The filtrate was evaporated at < 70°C in a vacuum dryer to give a final yield of 128.69 g was stored at 4°C. It was dissolved in distilled water whenever needed for experiments.

Treatment with heavy metal salt

The fresh water mussels *Lamellidens consobrinus* were collected from Hartala lake near Muktainagar Dist. Jalgaon (M.S.). Bivalve of similar size were collected and washed in order to remove the algal biomass and other waste. They are acclimatized for 4-5 days at laboratory conditions. Medium sized, healthy and active bivalve were used for experiment. They are divided into three groups such as group A, B and C, the group A of acclimatized mussels are kept as control set. The group B of acclimatized mussel was exposed to chronic concentration (LC₅₀ value of 96 hrs/10) of heavy metal salt ZnSO₄ (0.21ppm) as chronic doses upto 21 day. While group C of acclimatized bivalve was exposed to chronic concentrations (LC₅₀ value of 96hrs/10) with 5ml/lit extract from *Capsicum annum* upto 21 days. After exposure to heavy metal for mussel from group B were divided into two subgroups, such as D and

E groups. The mussels of group D were allowed to self cure naturally in normal water and mussels of group E were exposed to 5ml/lit extract from *Capsicum annum* upto 21 days. During experimentation, mussels were fed on fresh water algae. In each group 10 animals were selected. After every 7th, 14th, 21st day of interval, 10 animals from each set were taken out, dissected and tissues such as digestive glands, gills were separated and whole body mass of remaining animals was taken.

All tissues were dried at 70-80°C in oven till constant weight were obtained. The dried powders of different tissues of control and experimental animals were used for estimation of glycogen. The amount of glycogen was expressed in mg of glycogen/100mg of dry weight tissue. The estimation of glycogen is done by using standard Anthrone reagent method, given by (Dezwann and Zandee, 1972). The value of estimate was subjected to statistical analysis.

Each observation was confirmed by taking at least three replicates. The percentage of decrease or increase over control was calculated for each value.

Table I Impact of heavy metal ZnSO₄ on glycogen content on *Lamellidens consobrinus* after Chronic exposure

Sr.No	Sets	Types of Tissues	Glycogen Content					
			7 Days	14 Days	21 Days	28 Days	35 Days	42 Days
1.	A (Control)	Gills	3.90	3.77	3.64	-	-	-
			± 0.0082	± 0.0064	± 0.0049	-	-	-
		Digestive Gland	6.11	5.98	5.85	-	-	-
			± 0.0030	± 0.0037	± 0.0022	-	-	-
		Whole Body	6.50	6.37	6.24	-	-	-
			± 0.0022	± 0.0030	± 0.0024	-	-	-
2.	B (Treated with LC _{50/10} of ZnSO ₄ i.e. 0.21ppm)	Gills	3.25	2.99	2.73	-	-	-
			± 0.0057	± 0.0036	± 0.0050	-	-	-
		Digestive Gland	(-16.67%)	(-20.69%)	(-25.00%)	-	-	-
			5.33	5.07	4.81	-	-	-
		Whole Body	± 0.0017	± 0.0022	± 0.0030	-	-	-
			(-12.77%)	(-15.22%)	(-17.78%)	-	-	-
3.	C (Treated with LC _{50/10} of ZnSO ₄ + Extract of <i>C. annum</i> .)	Gills	5.85	5.59	5.33	-	-	-
			± 0.0017	± 0.0010	± 0.0030	-	-	-
		Digestive Gland	(-10.00%)	(-12.24%)	(-14.58%)	-	-	-
			3.51	3.38	3.12	-	-	-
		Whole Body	± 0.005	± 0.007	± 0.010	-	-	-
			(-10.00%)	(-10.34%)	(-14.29%)	-	-	-
4.	D. Normal Cure	Gills	5.59	5.33	5.07	-	-	-
			± 0.0033	± 0.0024	± 0.0036	-	-	-
		Digestive Gland	(-8.51%)	(-10.87%)	(-13.33%)	-	-	-
			6.20	5.98	5.72	-	-	-
		Whole Body	± 0.0020	± 0.0041	± 0.0017	-	-	-
			(-4.67%)	(-6.12%)	(-8.33%)	-	-	-
5.	E (Extract of <i>Capsicum annum</i> 5ml/lit.)	Gills	2.86	2.99	3.25	± 0.0073	± 0.0017	± 0.0010
			-	-	-	(-26.67%)	(-20.69%)	(-10.71%)
		Digestive Gland	4.94	5.07	5.20	± 0.0017	± 0.0022	± 0.0030
			-	-	-	(-19.15%)	(-15.22%)	(-11.11%)
		Whole Body	5.46	5.59	5.85	± 0.0024	± 0.0033	± 0.0030
			-	-	-	(-16.00%)	(-12.24%)	(-6.25%)
		Gills	3.12	3.51	3.77	± 0.0044	± 0.0024	± 0.0030
			-	-	-	(-20.00%)	(-6.90%)	(-3.57%)
		Digestive Gland	5.20	5.46	5.85	± 0.0014	± 0.0033	± 0.0017
			-	-	-	(-14.89%)	(-8.70%)	(-0.00%)
		Whole Body	5.85	6.24	6.50	± 0.0030	± 0.0041	± 0.0030
			-	-	-	(-10.00%)	(-2.04%)	(+4.17%)

RESULT AND DISCUSSION

Impact of $LC_{50/10}$ concentration i.e 0.21 ppm of zinc sulphate were studied along with the extract of *Capsicum annum linn.*, to determine the glycogen in various tissues. The different tissues like Gills, digestive gland and whole body were analyzed to observe the effect after 7,14 and 21 days. Levels of glycogen from control and exposed tissues of mussels are presented in table 1.

The glycogen contents in the gills, digestive gland and whole body of bivalve in presence of $ZnSO_4$ (0.21ppm) decreases with increase in the exposure period. The glycogen content were more in $ZnSO_4$ with extract of *Capsicum annum Linn*, exposed bivalve as compared to those exposed to only $ZnSO_4$ for the corresponding period of exposure. The bivalves pre exposed to $ZnSO_4$ show fast recovery in glycogen content in presence of *Capsicum annum* extract than those allowed to cure naturally.

The heavy metal $ZnSO_4$ affect the metabolism of freshwater bivalve *Lamelliden consobrinus*. Alterations in metabolic processes following exposure to heavy metal stress have always been used as indicator of stress. Such stressful condition is variable from heavy metal to heavy metal and animal to animal.

The decrease in glycogen level was reported in fish due to ammonia toxicity (Tilak *et al* , 2002) and pesticide toxicity (Rani *et al*,1989). In the present study,the glycogen from gill and digestive gland dropped significantly under the stress of heavy metals. Shrivastava and Shrivastava (2008) reported that glycogen consistently decreased from 8.18 to 5.3mg g⁻¹ in *Channa punctatus*. When exposed to sublethal concentrations of $ZnSO_4$. The glycogen content was significantly reduced in *Mystus cavasius* to electroplating industrial effluent (Palinsamy *et al*, 2011). This is due to liver has highest metal pollution index, therefore it is assumed that high level of heavy metals in the liver impaired the activity of enzymes which contributes to glycogen synthesis, leading to decrease in glycogen content.

The percentage of decreased glycogen content after chronic treatment at 7,14 and 21 days with $ZnSO_4$ was -10.00%, -12.24%, and -14.58% in Whole body, -16.67%,-20.69% and -25.00% in Gills and -12.77%,-15.22% and -17.78% in Digestive gland. The percentage of glycogen content after treatment of $ZnSO_4$ and extract of *Capsicum annum* shows that the increase in glycogen content after 7,14 and 21 days at the manner as -10.00%,-10.37% and -14.29% in the Gills respectively. -8.51%,-10.87% and -13.33% in the Digestive gland and -6.00%,-6.12%, and -8.33% from the Whole body respectively. The percentage of glycogen content increased slowly in the natural cure i.e. set D in the table, but there is rapid recovery of increasing glycogen content in the set E along with the extract of *Capsicum annum*.

The heavy metals from domestic waste water containing Cu, Fe, Pb, Cd, and Zn directly alter the various parameters in the liver and blood of fish *Clarias gariepinus* (Authman *et al*, 2013).

These alterations are due to dissolved heavy metal must diffuse to reach the blood stream and prolonged exposure to heavy metals can lead to degeneration of the epithelium causing hyperplasia.

Glycogen is one of the cheapest source of energy in the animal food and its plays major role in metabolism. It is the principle and immediate source of energy precursor in animals under the stress conditions. (Patil *et al*, 2012) reported that, maximum level of heavy metal concentration in fish was mostly found in liver which have high metabolic activities and widely recognized as bioindicator of pollution (Sarojini,1990) reported the decline in the glycogen content in *Barytephusa guerini* exposed to zinc sulphate. (Mane and Kulkarni,1999) reported the significant decrease in the glycogen content in the bivalve *Lamellidens marginalis* when the bivalve exposed for the 96 hours with concentration Cadmium 1.00ppm, and 2.114ppm (Kharat *et al*, 2009). The decrease in the level of glycogen in all tested tissues of *Macrobranchium. kistenensis* due to elevated levels of glycolytic enzymes which enhanced glycogenolysis for combating the stress by TBTCL action. A marked decrease in hepatopancreas glycogen is due to extensive utilization of stored glycogen to meet the extra demands of energy necessary during toxic stress. The effect of colour pigments on the glycogen contents in different tissues like muscle, mantle, gills, foot, hepatopancreas in bivalve *Lamellidens marginalis* is studied by (Phadnis *et al*, 2013).

The *Capsicum* has highest concentration of antioxidants such as polyphenols, carotenoids, ascorbic acids, and flavinoids which fight against hazardous oxidative damage to the cell. (Odukoya, 2007; Karandenz *et al*, 2005) Banerjee *et al*, 2005 shows that phenolics of green peepers has higher DPPH radical scavenging activity. Carotenoids which are fat soluble antioxidants found in the peepers have received considerable interest by researchers due to their antioxidant properties (Rao and Rao, 2007).

In the present study, decrease in glycogen content was observed due to high energy demands of the organisms under toxic stress of heavy metal $ZnSO_4$, which leads to hyperglycemia in the set C. The increase of glycogen content observed this is due to probable role of antioxidants present in *Capsicum annum* which may reduces the toxic heavy metal stress, by slight increasing the glycogen content in whole body, Gills and digestive glands. The carotenoids, flavinoids phenolic compound show radical scavenging activity and thereby which are able to serve as donor antioxidant in the free radical mediated oxidation process and is able to reduce the heavy metal $ZnSO_4$. So in conclusion, probable role of the antioxidant increases the glycogen content from the whole body, digestive gland and gill of the bivalve *Lamellidens consobrinus* so in future, there is need to find out the exact role of the antioxidant to prevent the heavy metal stress against the glycogen content.

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