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ANTIOXIDANT STUDIES ON SONATA (FUNGICIDE) INDUCED STRESS IN CHANA PUNCTATUS AND MACROBRACHIUM ROSENBERGII

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ABSTRACT

Acute toxicity tests (48hr, 72hr, 96 hr LC50& Lethal concentration) of Sonata were conducted with two species (fish and prawn) viz. *Chana punctatus* and *Macrobrachium rosenbergii*. At the end of each trail, the animals were dissected and their organs viz. Liver, Gill and Brain were isolated for determination of antioxidant activity. Sonata was significantly more toxic in prawn than fish with 96hr LC 50 values of 12.094ppm and 15.399ppm respectively. Activity of SOD, Catalase, GSH and LPO increased with increasing sonata concentrations in the test mediums. The fish and prawn were kept in control conditions(without sonata stress) showed maximum activity of catalase, SOD,GSH and LPO. Among fish and prawn organs Liver appeared as a target organ that accumulates significantly higher contents of sonata followed by that of gill and brain.

KEYWORDS: Acute toxicity tests *Chana punctatus* and *Macrobrachium rosenbergii*.

INTRODUCTION

Pesticides cover a wide range of compounds used in pest control; including insecticides, fungicides, herbicides, rodenticides and molluscicides.[1,2] Pesticides and fungicides exert their toxic action on arthropods, mussels, fishes, frogs, turtles, water birds and other wild life too. Protection of wild life and water quality is possible when pesticide used selectively, and in combination with other pest control measures in a safe manner. The pollution of surface waters and contamination of aquatic life can be avoided. Excessive use leads to bioaccumulation in farm workers, fruits, vegetables, nuts and food crops, consumers, and it also causes biomagnification at various trophic levels of the food chain. Application of these compounds is most effective and accepted mean for protection of crops.[3] They accumulate

in liver, kidney, salivary glands and fat. Fish are useful bioindicators and integrators of contaminants. Pesticides generally affect the biological active molecules like carbohydrates, proteins, lipids and enzymes. Depletion of oxygen content occurs in medium when pesticides, chemicals, sewage and other effluents contaminating organic matter are discharged into water bodies.

One of the important manifestations of toxic action of chemicals is overstimulation or depression of respiratory activity. Respiration also plays an important role in study in aquatic toxicology. Aquatic orgnisms like prawns, fish, bivalves, crabs respire through gills and it causes reduction in oxygen consumption and leads to physiological imbalance in the organism.[4]

The extensive advantages of the utilization of pesticides are incompletely balanced by generous condition cost. An ecologically pervasive problem is widespread environmental contamination by pesticides, including the chemical compounds residues in aquatic life.[5] The contamination of water sources by pesticides may affect non target aquatic organisms including fish.[6,7,8]

Fish-toxin relationship has impressive significance in the investigation of fish populace and vitality designs in fish stocks of a particular area.[9] The field of fishes and pesticide contamination plainly shows that impacts of individual pesticides on various physiological and biochemical parts of fishes have been broadly examined by an expansive number of specialists.[10,11,12] Through the examinations involving the impact of individual pesticides on fishes have produced valuable information, the problem of pesticidal pollution becomes magnified when the runoff waters from cultivable land drain cause a wide spectrum of different pesticides accumulate into a particular fresh water body such as ponds, lakes or rivers.

Since the physiological changes that occur when organisms are exposed to sublethal levels of pressure could include rate of feeding as well as respiration and excretion, the net outcome could be a change in energy available for growth and reproduction. Since, pollutants uptake from water the most important route is gill the primary target and may be one of the first organs to exhibit symptoms of sublethal toxicity. Besides, there are biochemical parts like starches, for example, proteins and lipids that can likewise go about as vitality sources.[13]

MATERIALS AND METHODS

Animal collection: The fish and prawn specimens samples of the two varities namely *Channa punctatus* and *Macrobrachium rosenbergii* were collected from the freshwater lake located in waddepelly cheru, Warangal district. Specimens were brought to the laboratory immediately and analysed for various biological and nutritive value studies. Fish measuring 14-15cms in length and weighing 250-300gms and prawn measuring 14-18 cms in length and weighing 25-30gms specimens were brought to the laboratory immediately and analysed for various biological and nutritive value studies.

The fish were acclimatized to the laboratory conditions in large plastic tanks with unchlorinated ground water for two weeks at a room temperature of 28±2^{o}C. During the period of acclimatization, the fish were fed with groundnut oil cake and rice bran. Feeding was stopped one day prior to the experimentation. All the precautions laid by committee on toxicity tests to aquatic organisms,[14] were followed.

Fungicide selected for the study: Procurement of technical grade SONATA fungicides technical grade 00.0% purity was supplied by Hyderabad chemical supplies limited Hyderabad.

Sonata acute toxicity assays: The lethal concentrations ensure death even before noticing the behavioural abnormalities.[15] reported that sublethal exposures to longer periods may be dangerous to the organisms. Even when the animal is exposed to low concentrations continuously, many behavioural abnormalities and physiological alterations would be observed. In the present study 96 hr LC50 value was selected as sublethal concentration to study the behavioural alterations and physioloical alterations (As per the recommendations of committee on toxicity studies.[16,14]

Enzyme assays: The fish and prawn used in the acute (48-hr,72-hr,96-hr LC50) and lethal test trails were weighed and removed from the media. All the fish were dissected and organs viz. liver, brain and gills were removed. These organs were kept at -80°C for the further enzyme assays and biochemical analyses.

Preparation of extract: To remove RBCs the dissected organs of each fish and prawn were washed with phosphate buffer (pH 6.5), Organs were weighed and homogenate was prepared in phosphate buffer (0.2M, pH 6.5) with a ratio of 1: 4, respectively. These tissues

homogenates were then centrifuged at 10,000 rpm for 15 minutes at 4◦C. The clear supernatant was preserved and used for further enzyme analysis.

***1. Superoxide dismutase assay*:** The activity of superoxidedismutase was determined by measuring its ability to inhibit the O2- dependent reaction or to inhibit the photo-reductionof nitro-blue tetrazolium (NBT) by superoxide.[16]

***2. Catalase assay*:** The crude enzyme was subjected to enzyme assay and the activities of catalase were measured by following the method of Beer and Sizer (1952).[17] Catalase activity was concluded by measuring its ability to decrease the H2O2 concentration per minute at 240 nm.

3. Glutathione Reductase activity: The activity of Glutathione reductase was determined by measuring its ability to maintains adequate levels of reduced cellular GSH by Carlberg and Mannervik.[17]

4. Lipid peroxidation activity: The activity of Lipid peroxidation was determined by measuring of MDA has been used as an indicator of lipid peroxidation by Utley *et al.*[18]

Statistical analysis: The significance of sample mean between control and Sonata treated fish and prawn was tested using Student's "*t*" test.

RESULTS

The effect of sublethal concentration (15.399 ppm & 12.094 ppm) of sonata on metabolic enzyme activities in gill, liver and brain of *C. punctatus* and *Macrobrachium rosenbergii* is represented in Table 1 and 2. The antioxidant enzyme activities viz., SOD, CAT, GSH and LPO in fish *Chana punctatus* and in prawn *Macrobrachium rosenbergii* exposed to sublethal concentration of Sonata for 48hrs, 72hrs and 96hrs showed significant alterations when compared to control groups. A significant ($p < 0.05$) decrease in SOD activity was observed in gill, liver and brain of *Chana punctatus* and *Macrobrachium rosenbergii* throughout the study period when compared to the control group. The catalase activity in gill, liver and brain of sonata treated fish and prawn showed a significant ($p < 0.05$) decrease throughout the study period. The GSH activity in gill, liver and brain of sonata treated fish and prawn showed a significant ($p < 0.05$) decrease throughout the study period. The LPO activity in gill and brain showed a significant increase ($p < 0.05$) throughout the study period.

Table 1: SOD, Catalase, GSH, MDA activity in the Gill, liver and Brains of C. punctatus (n=5), exposed to the sublethal concentration of Sonata for 48,72,96hrs. The values given are mean ± S.E.M. *= p<0.05.

	Chana punctatus									
		Brain			Liver			Gill		
	Incubation	Control	Experiment		Control	Experiment		Control	Experiment	
			10 PPM	20PPM		10 PPM	20PPM		10 PPM	20PPM
SOD (IU/Mg/protein)	48hrs	581±1.78	483±1.42	352±1.43	332±0.62	261±0.90	122±1.21	63±1.56	50±1.31	44±0.79
	72hrs	662±0.71	544±0.81	486±1.22	447±1.75	397±1.30	269±0.83	70±0.78	58±0.52	39±1.73
	96hrs	735±0.96	653±1.01	535±0.99	523±0.05	423±0.09	323±0.99	82±1.00	50±0.45	39±0.56
Catalase (µmol/mg/protein)	48hrs	52±0.90	45±1.32	32±1.34	42±1.09	38±0.57	33±1.19	23±1.55	11±0.65	7±1.19
	72hrs	63±1.15	49±1.19	38±1.42	55±0.75	49±0.62	33±0.83	41±1.39	32±0.71	20±1.48
	96hrs	81±0.85	86±0.99	94±0.96	68±0.96	81±1.05	91±0.65	66±0.89	69±0.84	84±0.54
GSH (mU/mg/protein)	48hrs	702±0.95	582±1.39	422±1.10	441±1.23	389±0.79	312±1.14	115±1.29	87±0.87	62±1.45
	72hrs	783±0.82	676±1.42	579±1.23	512±1.35	443±0.90	333±1.36	154±1.48	139±1.21	129±1.69
	96hrs	815±0.58	915±1.56	996±0.98	598±0.98	625±1.91	721±0.58	172±0.68	175±0.95	197±0.87
MDA (nmol/mg/protein)	48hrs	6.68±0.94	7.36±1.16	8.29±1.22	4.66±0.84	5.88±1.61	5.99±0.88	0.54±0.89	0.83±1.10	1.7±0.78
	72hrs	8.03±0.99	9.25±1.05	12.1±1.28	6.11±1.09	7.63±1.52	8.72±0.92	1.39±0.92	1.91±1.33	2.9±1.13
	96hrs	12.58±0.58	13.49±0.96	18.7±1.45	11.78±1.58	9.58±1.68	11.05±1.02	1.76±0.68	2.54±0.84	5.1±0.85

Table 2: SOD, Catalase, GSH, MDA activity in the Gill, liver and Brains of M.rosenbergii (n=5), exposed to the sublethal concentration of Sonata for 48,72,96hrs. The values given are mean ± S.E.M. *= p<0.05.

	Macrobrachium rosenbergii									
		Brain			Liver			Gill		
	Incubation	Control	Experiment		Control	Experiment		Control	Experiment	
			10 PPM	20PPM		10 PPM	20PPM		10 PPM	20PPM
	48hrs	21.4±0.21	14.6±0.65	12.4±0.54	18±0.45	13±0.48	8±1.01	23.2±0.42	11.5±0.21	5.6±0.11
SOD (IU/Mg/protein)	72hrs	26±0.18	15±0.84	9.7±0.98	12±0.58	8±0.78	4±1.24	26.9±0.68	16.8±0.56	7.8±0.48
	96hrs	31±0.47	25±0.65	19.2±0.67	25±0.96	20±0.62	14±0.95	28.5±0.54	21.5±0.64	16.9±0.52
	48hrs	24.8±0.21	21.5±0.84	17.4±0.47	22.4±0.58	12.4±0.34	7.9±0.48	29.5±0.62	17.5±0.15	9.5±0.48
Catalase (µmol/mg/protein)	72hrs	29.5±0.19	19.9±0.65	13.8±0.58	27.9±0.69	17..7±0.68	14.5±0.78	33.4±0.84	26.8±0.39	13.5±0.68
	96hrs	37.6±0.35	25.6±0.48	11.1±0.24	32.4±0.47	23.9±0.75	12.1±0.96	38.7±0.56	24.5±0.96	11.8±0.56
	48hrs	1.65±0.15	1.47±0.58	1.34±0.47	1.54±0.64	1.37±0.58	1.24±0.47	3.25±0.13	2.68±0.68	1.85±0.74
GSH (mU/mg/protein)	72hrs	1.99±0.28	1.89±0.91	1.57±0.82	1.97±0.38	1.89±0.91	1.57±0.82	6.89±0.35	2.65±0.89	4.47±0.89
	96hrs	2.32±0.48	1.92±0.99	1.38±0.96	2.35±0.79	1.92±0.99	1.28±0.96	9.9±0.59	9.6±0.97	7.5±0.95
	48hrs	0.89±0.18	1.14±0.28	1.28±0.24	0.46±0.34	0.89±0.87	1.01±0.98	0.86±0.21	1.02±0.35	1.24±0.85
MDA (nmol/mg/protein)	72hrs	1.25±0.34	1.59±0.67	1.86±0.54	0.98±0.56	1.48±0.95	1.68±1.05	1.12±0.56	1.35±0.47	1.58±0.99
	96hrs	2.65±0.84	2.84±0.59	2.99±0.68	1.23±0.24	1.96±0.65	2.05±1.24	1.24±0.69	1.48±0.69	1.96±1.02

DISCUSSION

The toxicity of many contaminants in aquatic organisms is mediated through oxidative damage when reactive oxygen species (ROS) are formed. Under normal conditions, ROS are removed from the cell by the action of antioxidant defence systems. If the production of ROS is in excess, the balance between the formation and removal of ROS will be destroyed and it will produce the oxidative stress.[19] Lipid peroxidation (LPO) has been reported as a major contributor to the loss of the cell function under oxidative stress conditions and it is usually indicated by TBARS in fish.[20] To minimise the potential toxic effects of ROS, fish have evolved an enzymatic antioxidant defence system composed of SOD, CAT, GPx, GR and other molecules to inhibit the formation of oxygen radicals.[19] SOD is a primary oxygen radical scavenger of tissues converting the superoxide anion radical to H2O and H2O2.[21] CAT and GPx act cooperatively as scavengers of hydrogen peroxide (both enzymes) and other hydroperoxides (GPx).[22] GR plays an important role in cellular antioxidant protection and adjustment processes of metabolic pathways.[23]

In the present investigation tissue specific responses in the activities of antioxidant enzymes such as SOD, catalase, GSH and LPO were observed during acute and sublethal sonata exposure which may indicate the different rates of free radical generation and different antioxidant potentials of these tissues and also the varied concentration of Sonata in these tissues as reported by Monteiro[24] The present study also demonstrated that sonata has a high oxidative-stress-inducing potential in *Chana punctatus* and *Macrobrachium rosenbergi*i and gill is the most sensitive organ in both acute as well as sub lethal concentration.

CONCLUSION

The results of the present investigation indicate that acute and sublethal exposure of sonata induces significant changes in the enzymatic profiles in *C. Punctatus and Macrobrachium rosenbergii*. The presence of such level of sonata in the natural environment is dangerous to the ecosystem and will definitely affect the survival of fish. Gills, due to their large surface area and permeability, are the primary sites for absorption. The experimental data obtained with *C. Punctatus and Macrobrachium rosenbergii* can be considered as a useful reference for comparisons with biomarker responses of organisms living in polluted environments. These parameters can be used as biomarkers in assessing the pesticide toxicity in aquatic ecosystem.

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