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Impact of pesticide Rogor toxicity on serum phosphomonoesterase levels of freshwater catfish *Clarias batrachus*

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Abstract: The exposure of pesticide Rogor had significantly altered serum alkaline and acid phosphatase activities in freshwater catfish, *Clarias batrachus* at different concentrations and duration under static conditions. However, the intensity of activity of both the enzymes were interestingly different. The fish elicited marked behavioral changes with increasing concentrations and exposure duration. The rising trend in enzyme levels which mediate membrane transport mechanism and biosynthesis of macromolecules, have been correlated with the induction and leakage of respective enzymes from liver following membrane damage and necrosis due to pesticide toxicity.

Keywords: Catfish, Rogor pesticide, Serum phosphatase, Toxicity

INTRODUCTION

Varied pollutants from industries and agricultural processes have contributed to contamination of freshwater systems, making adverse impact on aquatic biota. Among freshwater inhabitants fishes are frequently prone and economically the most important that suffer from exposure to different toxicants directly or indirectly in various ways, leading to major decline in their population and human health (Wang, 2002, Kalita *et al.*, 2003; Deutremepuits *et al.*, 2004; Shukla *et al.*, 2007; Agtas *et al.*, 2007; Yoon *et al.*, 2008; Despande *et al.*, 2011). The degree of toxicity due to pollutants could be predicted by applying short as well as long term tests, using fish as a bioindicator (Mullick and Konar, 1995; Athikesawan *et al.*, 2006). Alterations observed in physiological and biochemical parameters of toxicant treated fishes are sensitive index of the changes due to pesticide toxicity and can constitute important diagnostic tool in water quality assessment in the field of environmental toxicity.

Phosphatases are an important and critical enzymes in the biological system responsible for detoxification processes, metabolism and biosynthesis of energetic macromolecules for different functions. It's clinical value have been correlated with anaemia, malnutrition, hyperglycemia, skeletal and liver disorders (Bell *et al.*, 1980). Blood being an integrated and enevitable part of every tissue, piscine haematology becomes useful in assessing the health and general condition of the animal subjected to changing ecophysiological situations (Kori Siakpera and Ubogu, 2008). The present paper embodies observations on toxic effects of pesticide Rogor on serum alkaline and acid phosphatase level of freshwater catfish *Clarias batrachus*, an active and hardy fish of economic importance due to its food and medicinal value.

MATERIALS AND METHODS

Live *Clarias batrachus* were collected from local resources with the help of fishermen, transported to laboratory and treated with $KMnO_4$ (2mg/l). Apparently healthy looking fishes of weight range 180-200 grams were selected, transferred to glass aquaria and acclimatized for seven days under standard laboratory conditions as described earlier (Chandra, 2008). They were fed properly and then starved for 24 hours before the experiment. Fish from the same group kept under normal conditions were used as control. Static bioassay tests were performed as given by Doudoroff *et al.* (1951) and APHA (2005). Four different concentrations of pesticide Rogor ($C_5H_{12}O_3NPS_2$ formulation liquid 30EC) were selected for the experiment on the basis of 80-100% survival of fishes (Table 1). Fish were exposed to different concentrations of pesticide from 24 to 96 hours depending upon their survival at the particular concentration. The control and pesticide exposed fishes from each concentration were taken out every 24 hours interval, were washed with distilled water, blotted dry and blood was collected in a clean dry tube, after severing off its caudal peduncle. Blood was allowed to clot at room temperature for 15 minutes, then centrifuged and clear serum decanted in a vial, kept in refrigerator until analyzed. Fish were carefully examined for any visible disease or parasitic infection. Biochemical estimation of serum alkaline and acid phosphatase were performed following method of King and Wootton (1959), using Bausch and

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Lomb spectronic-20 spectrophotometer at 620µm. The physico-chemical characteristics of dechlorinated tap water during the experiment were temperature 19-20°C; pH 7.6-7.8; dissolved oxygen 6.0-7.5mg/l; total alkalinity 110-118 mg/l and total hardness 120-125 mg /l.

RESULTS

A comparative data of serum alkaline phosphatase and acid phosphatase levels in control and pesticide exposed fish upto 96 hours at different concentrations have been presented in Table 2. Significant increase in activity of both phosphomonoesterases were observed due to rogor toxicity in *C. batrachus* at different concentrations and exposure periods. At 7.50 mg /l pesticide exposure, serum alkaline phosphatase levels increased by 38.80%, 36.05%, 16.29% and 9.96% after 24, 48, 72 and 96 hours respectively. At 8.25 mg/l concentration the enzyme level rose by 39.55%, 29.38% and 12.09% after 24, 48 and 72 hours respectively above control. At 8.75 mg /l pesticide exposure the level elevated by 44.55% and 23.58% after 24 and 48 hours versus control and fish did not survive beyond that. At 9.50 mg /l rogor exposure serum alkaline phosphatase level increased 31.80% above control after 24 hours and the dose proved lethal. The enzyme level revealed maximal escalation during initial 24 hours exposure at all concentrations and then gradually decreased but remained above control level. The difference between initial and terminal increase in enzyme level at 7.50 mg/l, 8.25 mg/l and 8.75 mg/l pesticide exposure were gradual denoting 36.24%, 31.23% and 27.44% respectively.

Impact of rogor toxicity on serum acid phosphatase level of *C. batrachus* was observed to be directly proportional to exposure period during their absolute percentage survival at different pesticide concentrations. The enzyme level rose 11.89%, 29.56%, 36.55% and 20.11% after 24, 48, 72 and 96 hours of exposure respectively at 7.50 mg/l pesticide concentration. Serum acid phosphatase activity enhanced by 15.24%, 24.98% and 15.24% after 24, 48 and 72 hours respectively at 8.25mg/l concentration. Enzyme level indicated 17.78% and 13.27% gain after 24 and 48 hours duration at 8.75 mg/l pesticide concentration. At 9.50 mg/l rogor exposure acid phosphatase activity was 26.78% higher as compared to control during 24 hours, beyond that fish could not survive. The fish elicited marked behavioural changes during the experiment. They appeared restless, hyperactive showing erratic movements, rapid opercular beats, surfacing, loss of balance, copious mucus secretion, pale colouration followed by calmness an finally death.

DISCUSSION

Biochemical parameters are sensitive indices of structural and functional status of body organs, useful in understanding metabolic alterations and constitute an important diagnostic tool in toxicological studies and investigating diseases (Edsall, 1999; Shaikh and Negi, 2004). Phosphatases being important lysosomal brush border enzymes which catalyzes the splitting of phosphoric acid from certain phosphate esters and generally located on absorptive and secretory surfaces of cells, mediating membrane transport mechanism, are thus early indicators of environmental degradation. Any interference in enzyme activity lead to biochemical impairment, cellular dysfunction, metabolic and physiological activities of the fish. Abnormal behavioral patterns, exhibited by *C. batrachus* during the experiment indicated its efforts to ward off the situation and were identical to that reported in fishes subjected to different pesticides by Tandon and Dubey, 1983; Tripathi and Shukla, 1988; Lata *et al.,* 2001 and Chandra, 2008. Hyperphosphatemia has been noticed in fish after exposure to various toxicants (Gupta *et al.,* 1975; Singh *et al.,* 1996; Yogesh and Saxena, 1999; Singh and Saxena, 2001). Rogor exposure had significantly increased serum alkaline and acid phosphatases in these experiments at all concentrations, the action and the intensity of reaction of both the enzymes were interestingly however, different. Serum alkaline phosphatase level increased sharply during initial periods of exposure, followed by gradual decrease with increasing exposure duration at all concentrations till the fish survived, whereas the acid phosphatase level elevated gradually with increasing exposure period, till the fish was active, then repressed. This clearly elicited different mode of action of alkaline and acid phosphatase to coordinate with impairing cellular, metabolic and physiological activities of fish due to harmful effects following acute and subacute treatment with rogor. Gills being most sensitive to changes in surrounding water become primary target of the contaminants, adversely affecting gas exchange and ionic regulation (Camargo and Martinez, 2007) leading to physiological stress suggesting prevalence of hypoxic environment in the blood of the fish. Biochemical changes have been reported due to shift in respiratory metabolism caused by pesticide in the ambient environment and utilization of organic reserves for energy liberation, needed to compensate the body stress and it was evident in *C. batrachus* following rogor toxicity. Linear

Table 1. Percentage survival of fish during the expriment on exposure to pesticide Rogor.

Rogor conc. (mg/l)	Time of exposure in hours			
	24	48	72	96
Control (0 %)	100%	100%	100%	100%
7.50	100%	100%	100%	60%
8.25	100%	100%	40%	
8.75	100%	100%	20%	
9.50	100%	10%		

Table 2. Serum alkaline and acid phosphatase levels of *C. batrachus* following pesticide Rogor toxicity.

Rogor conc. (mg/l)	Time of exposure in hours			
	24	**48**	**72**	**96**
	Serum alkaline phosphatase (KA units/100ml) Mean±SD			
	Control-5.96±0.42			
7.50	9.74±1.83	9.32±0.41	7.12±0.60	6.60±0.82
8.25	9.86±1.60	8.44±1.01	6.78±0.72	
8.75	10.75±0.74	7.80±0.73		
9.50	8.74±0.78			
	Serum acid phosphatase (KA units/100ml)			
	Control-11.56±1.87			
7.50	13.12±0.92	16.42±2.09	18.22±1.40	14.47±1.26
8.25	13.64±1.24	15.42±2.14	13.64±2.02	
8.75	14.06±2.85	13.33±2.84		
9.50	13.86±2.66			

No. of observations 10 in each experiment

increase in serum phosphatase have been reported in *C. batrachus* following asphyxiation and the balance in acid and alkaline phosphatase level appeared to be nearly adjusted by hormonal machinery which comes to play under such situation of stress (Chandra, 1994; Nath *et al.,* 2000; Verma and Nath, 2004). Similar observations have been reported in different biochemical parameters of fish following exposure to different pesticides by Singh and Srivastava, 1998; Nath, 2003; Padmini *et al.,* 2004; Thosar and Lonker, 2004; Deshmukh and Sonawane, 2007 and Chandra, 2007. Biochemical alterations clearly indicated shifting of metabolic reactions to utilize organic reserve for energy to accommodate, adapt and resist with toxic stress, till it was lethal. Long standing exposure to organosphosphate compounds may produce adaptive mechanism expressed by tendency towards an increase of synthesis of various serum enzymes (Janardan and Sisodia, 1990). Rising trend in serum acid and alkaline phosphatase levels have also been related due to induction and leakage of the respective enzymes following liver necrosis (Foster, 1980; Tomer *et al.,* 1995; Rahman *et al.,* 2000), indicating their role as bioindicator of membrane damage and tissue necrosis, which can be widely interpreted to predict and detect early warning of pesticide toxicity in aquatic environment and as a useful tool to save deterioting fish population and their biodiversity in its habitat.

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