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Toxicological effects of a carbaryl and 2,4-d sodium salt on blood biochemistry of a freshwater catfish, H*eteropneustes fossilis* (bloch)

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Abstract

The aim of the present work was to evaluate the comparative effects of a carbaryl (Carbamate) insecticide and 2,4-D sodium salt (Herbicide) on carbohydrate metabolites and blood chloride levels of a freshwater catfish, H. fossilis, at different concentrations and time intervals. The catfish shows significance differences in their biochemical indices at acute and subacute concentration for acute (96h) and short (10-20 days) term intervals but no remarkable changes were observed in sublethal concentrations at long (30-60 days) term to both the toxicants. It is also observed that carbaryl is more toxic to fish than 2,4-D sodium salt.

Keywords-Carbamate (Carbaryl), herbicide (2, 4-D sodium salt), biochemical parameters, heteropneustes fossilis

Introduction

The aquatic ecosystem is cover the greater part of natural environment which is facing the threat of shrinking genetic base and biodiversity due to indiscriminate use of pesticides (Rahman *et al*., 2002). It also induced alternation in the biochemistry and physiology of aquatic animals (Satyaparameshwar *et al*., 2006; Tilak *et al*., 2007; Srivastava *et al*., 2008; Butchiram *et al.,* 2009). They also accumulate in the body resulted in alternation in various biochemical constituents of body. Now a days highly toxic pesticides are replaced by less toxic pesticides like carbamates and herbicides. The carbaryl (1-naphthyl 1-N-methyl carbamate; sevin) is a moderate carbamate which is less toxic insecticide but at high concentration of carbaryl have caused adverse effect on birds, fish, tadpoles and other animals. The main effect of carbaryl includes reduced production of eggs, ability to run, deformed legs, reduced swimming speed and increased mortality. The other less toxic toxicants which used for uncontrolled growth of various aquatic plants, which causing many problem including blocked navigation ways, obstructed water flow, lowered real estate values, reduced fishing success and impaired recreational use and also provides an ideal breeding ground for mosquitoes. 2,4-D sodium salt is the one example of herbicide whose toxic effects are limited because of rapid degradation. The present work is to study the effects of carbamate (carbaryl) and herbicide (2,4-D sodium salt) on carbohydrate metabolites and blood chloride levels in a freshwater catfish, *Heteropneustes fossilis* (Bloch.), at different doses and time intervals.

Materials and Methods

Live and adult specimen of freshwater catfish, *Heteropneustes fossilis* (Weight 26.30±1.50 gm, length 14.20± 1.20 cm) were procured from the local market and brought to the laboratory in 15 litre plastic

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bucket. They were acclimatized to the laboratory condition for 10 days in dechlorinated water. The water used during experiments was analysed as per the standard method (APHA.1998). The methods (Litchfield and Wilcoxon, 1949) were used to calculate 24, 48, 72 and 96h LC_{50} values and 95% confidence limits. The presumably harmless (safe) concentrations of both the toxicants were also estimated by the formula of Hart *et al*., 1945. The stock solution of both the toxicants were prepared in distil water and the toxicants used were of technical grade (95 to 98% pure) and purchased from Sigma Chemical Company, Mumbai.The fish were exposed to acute ($1/5^{th}$ of 96h LC_{50} value), subacute ($1/10^{th}$ of 96h LC_{50} value) and sublethal ($1/15^{th}$ of 96h LC_{50} value) concentrations for the acute (96h), subacute (10-20 days) and sublethal (30-60 days) term. A parallel group of control fish were also maintained in the toxicant free tapwater and used for the analysis of selected variables for comparision. The physico-chemical characteristics of tapwater were (pH 7.6; dissolve oxygen 7.40 mgL^{-1}; total hardness 250.50 mgL^{-1} as $CaCO_3$ and BOD 15.20 mgL^{-1}).

At autopsy, the fish were anasthetized with MS 222. Blood from the fish were collected from the served caudal peduncle into citrated tuberculin syringes for determination of blood glucose (Oser, 1965) and chloride (Schales and Schales, 1941). Liver and muscle glycogen contents were measured by the method of Van der Vies (1954). The statistical significance between the treated and control groups were calculated by the t-test (Sokal and Rohef, 1981).

Results

The effect of carbaryl and 2,4-D sodium salt on carbohydrate metabolism and blood chloride content in *Heteropneustes fossilis* were observed at acute (Carbaryl 3.28 mgL^{-1} and 2,4-D sodium salt 300 mgL^{-1}) concentrations for four days, subacute (Carbaryl 1.64 mgL^{-1} and 2,4-D sodium salt 150 mgL^{-1}) and sublethal (Carbaryl 1.09 mgL^{-1} and 2,4-D sodium salt 100 mgL^{-1}) concentrations for both short (10-20 days) and long (30-60 days) term (Rai and Srivastava, 2011). The hepatic glycogen content in control fish ranged from 9.25 to 12.10 mgL^{-1} per 100 mg of wet wt. of tissue during the entire experiments. Acute exposure (4 days) at acute dose of both the toxicants resulted in significant decreased levels of hepatic glycogen of the fish.

Table No-1 Blood chloride values and carbohydrates metabolits for the catfish, Heteropneustes fossilis following exposure to acute concentrations of carbaryl (3.28 mgL^{-1}) and 2, 4-D sodium salt (300 mgL^{-1}) for 96 h

Parameters	Control	Experimental	
		Carbaryl	2,4-D Na salt
Blood chloride(mM/ l)	65.25±2.10	85.50±2.65***	76.45±1.95**
Liver glycogen (mg/ 100mg wet wt.)	10.25±1.05	8.85±0.75**	9.87±0.85*
Muscle glycogen (mg/ 100mg wet wt.)	1.05±0.02	0.80±0.04**	0.94±0.02*
Blood glucose (mg/ 100 ml)	40.25±1.35	50.85±1.25**	45.15±1.65*

Note: Values are mean ± SE (N=6). Significant differences are indicated by asterisks.

Exposure of the fish to subacute and sublethal concentrations of both toxicants also resulted reduced hepatic glycoge at all time interval except at subacute and sublethal level during long (30-60 days) term to 2,4-D sodium salt. (Table 1-3). The mean muscle glycogen levels of control fish was ranged between 0.80 to 1.05 mgL^{-1} per 100 mg wet wt. of tissue. Acute exposure (4 days) at acute dose of both the toxicants resulted in significant decreased levels of muscle glycogen concentration of the fish. Exposure of the fish to subacute and sublethal concentrations of both toxicants also exhibited reduced muscle glycogen at all time interval except at subacute and sublethal levels during long (30-60 days) term to 2,4-D sodium salt exposure. (Table 1-3).

The blood glucose and chloride levels in the control fish varied from 38.32 to 45.72 mg/ 100 ml and to 60.25 to 65.45 mM/l respectively. Acute exposure to both toxicants caused significantly increased levels of both blood parameters in the fish. They showed hyperglycaemia and hyperchloraemia during both short and long term exposures to each of subacute and sublethal concentrations of both the toxicants except in 2,4-D sodium salt for long (30-60 days) term. (Table 1-3)

Discussion

Glycogen is the from in which carbohydrate is stored in animals mainly in the liver and muscle. It may provide a reserve for acute demand occurring as a result of transient stress (Love, 1980). A stress situation, such as that caused by acute hypoxia, strong muscular exercise (Nakano and Tomlinson, 1968), or pesticides (Singh and Srivastava, 1982; Srivastava and Srivastava, 1988; Ferrando and Andreu-Moliner, 1991; Srivastava and Srivastava, 1995), has been reported to decrease the hepatic and muscle glycogen contents in fish. Stress evokes circulating levels of both catecholamines (Nakano and Tomlinson, 1968) and glucocorticoids in fish. Catecholamines deplete the liver and muscle store glycosen of the fish. Thus the marked glycogenolysis in the liver and muscle after acute as well as short term exposure to different concentrations of both the toxicants in this study was most likely due to the stress-induced increase in circulating catecholamines. Studies on the brown bullhead *Ictalurus nebulosus* indicated that both epinephrine and glucagon bring about a decline in hepatic glycogen concentrations and an increase in the specific activity of hepatic total glycogen phosphorylase assayed with AMP (Umminger and Benziger, 1975). This provides substantial support regarding glycogenolysis in this study with the activation of the adrenal medullary hormones which are important mediators of stress-induced glycogenolytic response. Alteration of the blood sugar level is the primary metabolite sign in vertebrates subjected to a stressful situation. Metal toxicity (Larsson and Haux, 1982), exposure to pulp effluent (Mc Leay and Brown, 1979) and pesticides (Srivastava and Mishra, 1982; Gluth and Hanke, 1984; Mishra and Srivastava, 1984; Srivastava Anil and Srivastava Ashok, 1988; Ferrando and Andreu- Moliner, 1991; Srivastava Arun and Srivastava Anil, 1995) produce hyperglycaemia in fish. The occurrence of both the toxicants induced hyperglycaemia in the catfish in this study may have been due to the mobilization of both hepatic and muscle glycogen stores perhaps brought about by the individual or combined effects of enzymatic, hormonal and respiratory disturbances. Bhatia *et al*., 1973 reported an increase in blood glucose concentration due to the gluconeogenic effect of the steroid hormone. A similar impairment in calcium permeability at the level of the cell membrane of the pancreas may inhibit insulin release (Yau and Mennear, 1977) resulting in hyperglycaemia. The inorganic electrolytes sodium and chloride have often been used as an index of osmoregulation and they have been reported to react quite similarly in freshwater teleosts under situations of stress (Umminger and Gist, 1973; Das and Das, 1976). The catfish in this study elicited hyperchloremia following acute, short and long periods of exposure to both the toxicants. A review of literature shows that organochlorine pesticides evoke hyperchloremia in fish (Grant and Mehrle, 1973; Haux and Larsson, 1979; Srivastava and Mishra, 1982; Mishra and Srivastava, 1984). The toxicity of DDT and PCBs in fish is due to the disruption of osmoregulation (Kinter *et al*., 1972).

Conclusion

There is evidence also that DDT can affect striodogenesis and steroid storage which is involved in the maintenance of ionic regulation. The hyperchloremia observed in catfish during acute, short and long term exposures to both the toxicants might be due to the cortisol interference. The catecholamines, especially epinephrine, secreted during stress, may also be the cause of induced hyperchloremia. Exogenous epinephrine has been shown in induce hyperchloremia in the eel. Carbonic anhydrase also plays a vital role in electrolyte regulation in fish, inhibition of carbonic anhydrase is

Table No-2.Blood chloride values and carbohydrate metabolites for the catfish, *Heteropneustes fossilis* following exposure to subacute concentrations of carbaryl (1.64mgL^{-1}) and 2,4-D sodium salt (150mgL^{-1}) for short (10-20 days) and long (30-60 days) terms

Parameters	Short Term						Long Term					
	10 days			20 days			30 days			60 days		
	Control	Experimental		Control	Experimental		Control	Experimental		Control	Experimental	
		Carbaryl	2,4-D Sodium salt		Carbaryl	2,4-D Sodium salt		Carbaryl	2,4-D Sodium salt		Carbaryl	2,4-D Sodium salt
Blood chloride (mM/ l)	60.25±1.50	80.75±2.15**	70.35±2.22**	62.35±1.80	80.32±1.25**	72.25±1.70**	64.35±2.10	75.25±1.75*	70.75±2.15*	65.45±1.60	77.23±1.55*	68.33±1.28
Liver glycogen (mg/100mg wet wt.)	9.25±1.05	8.33±1.05*	9.15±1.80*	10.15±1.55	8.35±1.70*	9.18±1.15*	11.25±2.10	9.99±1.88*	10.85±1.10*	12.10±1.85	10.98±1.38*	11.77±1.83
Muscle glycogen (mg/100mg wet wt.)	0.80±0.03	0.74±0.02*	0.78±0.02*	0.99±0.02	0.90±0.04*	0.96±0.02*	1.04±0.03	0.98±0.03	1.01±0.02	1.08±0.02	1.02±0.02	1.06±0.02
Blood glucose (mg/100ml)	38.32±1.28	44.25±1.32**	40.15±1.22*	40.28±1.38	45.72±1.83*	43.25±1.72*	42.33±1.57	45.28±1.92*	43.13±1.78*	44.25±1.35	48.25±1.82*	45.72±1.75

Note: Values are mean ± SE (n=6). Significant differences are indicated by asterisks. (* 1 % and ** 5% level of significance)

Table No-3 . Blood chloride values and carbohydrate metabolites for the catfish, *Heteropneustes fossilis* following exposure to sublethal concentrations of carbaryl (1.09 mgL^{-1}) and 2,4-D sodium salt (100 mgL^{-1}) for short (10-20 days) and long (30-60 days) terms

Parameters	Short Term						Long Term					
	10 days			20 days			30 days			60 days		
	Control	Experimental		Control	Experimental		Control	Experimental		Control	Experimental	
		Carbaryl	2,4-D Sodium salt		Carbaryl	2,4-D Sodium salt		Carbaryl	2,4-D Sodium salt		Carbaryl	2,4-D Sodium salt
Blood chloride (mM/ l)	61.38±1.38	70.27±2.18**	65.28±1.98*	61.57±1.48	70.28±1.37**	66.01±1.29*	62.01±1.78	68.36±1.89*	63.28±1.32	62.33±2.10	65.77±1.39*	63.33±1.18
Liver glycogen (mg/100mg wet wt.)	9.88±1.07	9.50±1.28*	9.84±1.76	9.90±1.73	9.85±1.79	9.88±1.79	9.95±1.89	9.88±1.64	9.95±1.76	10.01±1.75	9.96±1.86	9.98±1.69
Muscle glycogen (mg/100mg wet wt.)	0.88±0.02	0.86±0.01	0.87±0.01	0.92±0.02	0.89±0.01	0.90±0.02	0.98±0.01	0.96±0.02	0.97±0.01	0.99±0.02	0.97±0.01	0.98±0.02
Blood glucose (mg/100ml)	39.78±1.38	43.38±1.48*	40.01±1.78	40.88±1.78	44.28±1.19*	42.73±1.93	43.38±1.89	45.27±1.78*	43.91±1.93	45.72±2.10	46.38±1.38	46.01±1.38

Note: Values are mean ± SE (n=6). Significant differences are indicated by asterisks (* 1 % and ** 5% level of significance)

followed by a decreased in Cl^- uptake. Thus hyperchloremia in the present study might have occurred due to decreased activity of carbonic anhydrase following exposure of both the toxicants.

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