Effect of ziram on pyruvate and lactate levels in fresh water teleost, *Labeo rohita* (Hamilton)

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SUMMARY

Freshwater fish, *Labeo rohita* were exposed to technical grade dithiocarbamate fungicide, ziram for 96 hours and the LC_{50} was determined as 0.66 mg/l. One-tenth of LC_{50} (0.066mg/l) was selected as sublethal dose for studies on pyruvate and lactate levels for a period of 1,7,15 and 30 days. The depletion in pyruvate and lactate levels and an increase in muscle lactate were observed significantly.

Key words : Ziram, Pyruvate, Lactate, Sub-lethal studies, *Labeo rohita*.

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Pesticides are chemical substances which are used to kill or to control pests. Although pesticides produce many results in the control of pests, their harmful effects results on non-target animals, because pesticides leave residues in the soil and water even after several days of their application. These possess a constant threat to the non-target organisms, especially to the fish (Pandey, 2000). Dithiocarbamate fungicides form a large group of chemicals that have numerous uses in agriculture and medicine. It may be applied to the foliage of plants, as seed treatment and also used as a bird and rodent repellent. Ziram (zinc dimethyldithiocarbamate) belongs to a class of fungicide used worldwide in agriculture. It is generally used because of low cost, good efficacy and broad spectrum of antifungal activity. The fungicides have caused extensive damage to various tissues of fish as reported by many workers (Rani *et al.*, 1990; Tiwari and Mishra, 1996; Nivedhitha *et al.*, 1998; Banurekha and Dawood 1999; Thangavel *et al.*, 2004; Sindhe *et al.*, 2006).

Lethal effects are rare in nature because the organisms are exposed to low concentrations, which are normally sublethal (Dehn and Schriff, 1986). Hence, the objective of this study was to investigate sublethal effects of ziram on pyruvate and lactate levels of freshwater fish, *Labeo rohita* which are one of the commercially important edible fish.

MATERIALS AND METHODS

The fish, *Labeo rohita* were procured from the Department of Fisheries, Anantapur, Andhra Pradesh. They were acclimated to laboratory conditions for fifteen days prior to the experiment. During acclimatization, fish were fed daily with rice bran and oil cake in the ratio of 2:1. *Labeo rohita* weighing 10±2 gm were selected from the stock. Technical grade ziram (90%) was obtained from Rallis India Ltd., Bangalore. The toxicity tests were conducted as per the recommendations of APHA *et al.* (1998). Fish were exposed to different concentrations of ziram and mortality rate was recorded. The data were subjected to Probit analysis (Finney, 1971) and Dragstedt-Beherens equation (Carpenter, 1975) to determine LC_{50} values. In the present study, $1/10^{th}$ of 96 hours LC_{50} value of ziram (0.066 mg/l) was selected as sublethal concentration for chronic studies (1, 7, 15 and 30 days) to observe the pyruvate and lactate levels in various tissues of fish, *Labeo rohita*. Pyruvate was estimated by the method of Friedman and Haugen (1942) and lactate was estimated by the method of Barker and Summerson (1941). The values were expressed as mg/gm wet weight of tissue.

RESULTS AND DISCUSSION

Pyruvate and lactate levels were observed in gill, liver and muscle tissues of *L. rohita*. In the present study, relative to controls, the level of pyruvate progressively decreased (Table 1) and the level of lactate decreased in gill and liver, where as the lactate levels increased in muscle tissue (Table 2).

Evaluation of carbohydrate metabolism in

Table 1 : Pyruvate level (mg/ gm wet wt) in the tissues of fish, *Labeo rohita* on exposure to sublethal concentrations of ziram

			Exposure period in days			
Sr. No.	Tissue	Control	24 hrs	7th day	15th day	30th day
1.	Gill	0.224	0.295	0.327	0.265	0.203
	SD±	0.0030	0.0040	0.0032	0.0030	0.0031
	% change		+33.48	+46.60	+27.60	-8.14
2.	liver	0.295	0.375	0.433	0.354	0.244
	SD±	0.0032	0.0040	0.0030	0.0032	0.0035
	% change		+26.17	+45.30	+19.79	-16.77
3.	Muscle	0.265	0.355	0.313	0.286	0.205
	SD±	0.0025	0.0040	0.0035	0.0037	0.0035
	% change		+35.36	+20.53	+7.22	-23.19

Means are ± SD (n=6) for a tissue in a column followed by the same letter are not significantly different (P<0.05) from each other according to DMR test. The values below the mean are per cent change over control.

Table 2 : Lactate levels (gm/wet wt) in the tissues of fish, *Labeo rohita* on exposure to sublethal concentrations of ziram

			Exposure period in days			
Sr. No.	Tissue	Control	24 hrs	7th day	15th day	30th day
1	Gill	3.735	4.564	4.843	4.961	3.143
	SD±	0.0040	0.0041	0.0030	0.0030	0.0035
	% change		+22.20	+31.33	+34.74	-15.84
2	liver	12.065	14.515	15.424	16.573	10.684
	SD±	0.0020	0.0026	0.0045	0.0020	0.0036
	% change		+20.29	+27.83	+37.35	-11.45
3	Muscle	8.684	10.182	10.984	11.465	12.312
	SD±	0.0015	0.0020	0.0035	0.0036	0.0020
	% change		+17.24	+26.47	+32.01	+41.77

Means are ± SD(n=6) for a tissue in a column followed by the same letter are not significantly different (P<0.05) from each other according to DMR test. The values below the mean are per cent change over control.

fish could prove useful and rapid for assessing acute toxicity of the toxicants. Pyruvate and lactate levels form useful biochemical indices of oxygen debt (Huckabee, 1958) and mitochondrial redox state (Krebs *et al.*, 1969). The concentration of pyruvate and lactate in the tissues can be used to assess the stress condition (Ramalingam, 1988). Pyruvate represents an important junction point in carbohydrate catabolism and in the cell under aerobic conditions pyruvate is the end point of glycolysis and pushed into the citric acid cycle, where as under anaerobic conditions, pyruvate is reduced to lactate.

In the present study, there was an initial gradual increase in pyruvate level (Table 1) which may be due to the conversion of amino acids to pyruvate to initiate gluconeogenesis (Exton, 1972) and the elevation in pyruvate levels may be due to recycling of pyruvate occurring during starvation (Friedmen *et al.*, 1971). Further, decrease in pyruvate levels may be due to rapid mobilization into Krebs cycle for the production of more ATP to meet the energy demand evoked by the toxic stress and also may be due to rearrangement of mitochondrial integrity and less availability of oxygen. Similar findings were observed by some workers when the fish were exposed to various pesticides (Suresh *et al.*, 1993; Padmavathi and Premkumari, 2006). Impaired pyruvate oxidation seems to be a probable physiological adaptation of fish to mitigate starvation stress.

Lactate is a relatively stable and saturated ketoacid, which is the end-product of glycolysis under anaerobic conditions. Lactate acts as an index to the prevalence of anerobiosis and tissue susceptibility or resistance to hypoxic condition under physiological stress and accumulation of lactate is considered as an index of physiological stress (Theya, 1971).

In the present study, the initial increase in the lactate level (Table 2) suggests that the oxygen supply was not met with demands, thus creating oxygen debt. An elevation in lactate level in all three tissues at 15 days indicates that the fish was in stress condition and is an indicative of a shift from aerobic to anaerobic metabolic pathway. Further, hyperlactimecia in muscle tissue may be due to utilization of hepatic glycogen seems to be common phenomenon in fish (Black *et al*., 1962). The initial elevated levels of lactate observed in the present investigation could be due to release of lactate into the blood following cellular damage and the action of catecholamines (Anitha Kumari and Sreeram Kumar, 2006). Further, decrease in lactate levels in gill and liver may be due to detoxification mechanism adapted by the fish during stress conditions. In muscle, the gradual increase in lactate levels may be the fish relied more on the energetically less efficient anaerobic glycolysis than on oxidative metabolism (Sreenivasa Reddy *et al*., 2006).

The inter convertibility of pyruvate and lactate has a great advantage in the operation of carbohydrate metabolism and thus facilitates the synthesis of energy molecules and prevents the cells from accumulating the end products.

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REFERENCES

Anitha Kumari, S. and Sree Ramkumar, N. (2006). Impact of pollution- stress on blood glucose, lactate and pyruvate levels in certain fish collected from Hussainsagar lake, Hyderabad, Andhra Pradesh. *J. Aqua. Biol*., **22** (1): 179-183.

APHA AWWA WEF. (1998). Standard methods for the examination of water and wastewater. 20th Ed. Washington, D.C., American Public Health Association, American Water Works Association and Waters Environment Federation.

Banurekha, P. and Shaik Dawood, A. (1999). Effect of fungicide, zoom on the freshwater teleost, *Sarotherodon mossambicus*. *J. Ecotoxicol. Environ. Monit*., **9** (2): 103-108.

Barker, S.B. and Summerson, W.H. (1941). The calorimetric determination of lactic acid biological materials. *J. Biol. Chem*., **139** : 535-554.

Black, E.C., Robertson, A.C., Cheung, L.K. and Chiu, W.G. (1962). Changes in glycogen, pyruvate and lactate in the rainbow trout, Salmo gairdneri during and following muscular activity. *J. Fish. Res. Bd. Can*., **19** : 409-414.

Carpenter, P.L. (1975). *Immunology and Serology*, 3rd Ed. W.B.Saunders Co., U.S.A.

Dehn, D.F. and Schriff, V.R. (1986). Energy metabolism in large mouth bass *Micropterus floridanus salmoides* from stressed and non-stressed environment. Adaptations in the secondary stress response.*Comp. Biochem. Physiol*., **84** : 523 – 528.

Exton, J.H. (1972). Progress in endocrinology and metabolism-gluconeogenesis metabolism, **21** (10): 945-990.

Finney, D.J. (1971). *Probit Analysis, 3rd Ed*. Cambridge University Press, London. 333pp.

Friedman, T.E. and Haugen, G.E. (1942). Pyruvic acid : Collection of blood for the determination of pyruvic acid and lactic acid. *J. Biol. Chem*., **144** : 67-77.

Friedman, B., Edward, H., Goodman, Jr. L, Harry, Saunders, Vincent, Kostos and Sidney, Weinhouse. (1971). Regulation of metabolism in the liver. *Metabolism*, **20** (1) : 53-59.

Huckabee, W.E. (1958). Relationship of pyruvate and lactate during anaerobic metabolism, exercise and formation of O_2 debt. *J. Clin. Invest*., **37** : 255-263.

Krebs, H,A., Freedland, R.A., Hems, R. and Stubbs, M. (1969). Inhibition of hepatic gluconeogenesis by ethanol. *J. Biochem.*, **112** : 117-124.

Nivedhitha, H., Thangavel, P., Dawood, A.K.S. and Ramaswamy, M. (1998). Adaptive changes in the patterns of carbohydrate metabolites in blood, liver, muscle and heart tissues of *Sarotherodon mossambicus* (Peters) exposed to carbamate fungicide ziram. *Pest. Sci*., **52** (2) : 133 – 137.

Pandey, A.C. (2000). Aquatic pollution and fish reproduction. A bibiliographical review. *Indian J. Fish*., **47** (3) : 231-236.

Padmavathi, G. and Premakumari, R. (2006). Pyruvate sparing-Probable strategy to mitigate starvation stress in climbing perch, *Anabas testudineus* (Bloch). *J. Aqua Biol*., **21**(1) : 151-154.

Ramalingam, K. (1988). Toxic effects of DDT, malathion and mercury on the tissue carbohydrate metabolism of *Sarotherodon mossambicus* (Peters.). *Proc. Indian Acad. Sci., (Animal Science*). **97** (5) : 443-448.

Rani, S., Dawood, A.K.S. and Ramaswamy, M. (1990). Toxicity of a carbamate fungicide Cuman L to an edible freshwater fish, *Sarotherodon mossambicus. J. Aquatic Organ*. pp. 28 –36.

Sindhe, S.C.S., Indira Pala and Butchiram, M.S. (2006). Ziram induced histological changes in the liver of freshwater fish, *Labeo rohita* (Hamilton). *J. Aqua Biol*., **21** (1) : 239-242.

Sreenivasa Reddy, A., Venkat Reddy, M and Radhakrishnaiah, K. (2006). Impact of lead on the energetics of common carp *Cyprinus carpio* (Linn). *J. Aqua. Biol*., **21**(2) : 234-238.

Suresh, A., Sivaramakrishna, B. and Radhakrishnaiah, K. (1993). Effects of lethal and sublethal concentrations of cadmium on energetics in the gills of fry and fingerlings of *Cyprinus carpio. Bull. Environ. Contam. Toxicol*., **51**: 920-926.

Thangavel, P., Nivedhitha, H. and Ramaswamy, M. (2004). Comparative study on individual and combined effects of Dimercon and Ziram on carbohydrate metabolism in liver, muscle, heart and blood of a freshwater teleost, *Sarotherodon mossambicus* (Peters.). *Bull. Environ. Contam. Toxicol*., **72** (2) : 365-372.

Theya, R.A. (1971). Effect of halothane, anoxia and haemorrhage upon *Canine* whole body skeletal muscle and splanchnic excess lactate production. *Anaesthesiology*, **35** : 394-400.

Tiwari, C. and Mishra, K.D. (1996). The residual toxicity of fungicide to fish, *Puntius ticto. Polln. Res*., **15** (2): 129-132.