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Individual and combined effect of mercuric chloride, magnesium sulphate and selenium on testis of *Heteropneustes fossilis*

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ABSTRACT

The present study deals with the individual and combined effects of mercuric chloride, magnesium sulphate and selenium on testis of *Heteropneustes fossilis*. Individually all these three chemicals decreased the Na +/K+-ATPase activity in the experimental fishes upto 57.35 %, 34.22 % and 38.40 % respectively. However, in the combined effect the Na +/K+-ATPase activity decreased up to 47.28 %. These findings suggest that loss of Na +/K+-ATPase is due to mercuric chloride which could be recovered up to 10.07% by supplementation of magnesium sulphate and selenium in *H. fossilis* testis .

KEY WORDS: MERCURIC CHLORIDE, MAGNESIUM SULPHATE SELENIUM TOXICITY, NA +/K+-ATPASE ACTIVITY HETEROPNEUSTES FOSSILIS TESTIS

INTRODUCTION

Na+/K+-ATPase is an important energizer for ion transport in epithelial tissue (Tipsmark and Madsen, 2003). This enzyme is also important in determining the milieu of cerbro-microvascular and Neurons (Caspers *et al.*, 1993). Maintenance of cation gradient by Na+/K+-ATPase and Ca+ ATPase has fundamental importance in the control of hydration volume, Nutrient uptake and fluidity of cells. It is also essential for contractibility and excitability properties of muscles and nervous tissues (Mohandas and Shohet, 1978). Mercuric chloride is one of the most toxic forms of mercury and is primarily nephro-toxic (Moraes-Silva, 2014). It is well known as hematotoxic (Durak et al., 2010), hapatotoxic (Joshi et al., 2014; Othman et al., 2014), neurotoxic (Moraes-Silva et al., 2014) and genotoxic (Rozaqai et al., 2005) and exert negative effect on the reproductive system in male

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rat (Kalander et al., 2013). Selenium has ability to reduce the toxicity of several xenobiotics including heavy metals (Agha et al., 2014). Considerable data available on fish Na+/K+-ATPase activity induced by Mercury chloride, Selenium and Magnesium sulphate individually and collectively on testis is very meagre, present study was attempted in *Heteropneustes fossilis*.

MATERIAL AND METHODS

Heteropneustes fossilis (Weight 50-60 gm.) were used as experimental animals which were obtained live from Nolakkha fish market, Indore (M.P.). Following chemicals were used and their doses: Mercuric chloride-1.0 ppm (E-Merck India Ltd., Mumbai) of molecular weight 275.52 Dalton. Selenium -0.9 ppm (Loba Chem India Ltd.) of molecular weight 246.47 Dalton. Magnesium sulphate-0.3 ppm (Loba Chem India Ltd).Ten experimental fishes were placed in separate glass aquarium having 10,000 cc of tap water free from chlorine.Experimental fishes were divided into following groups: Group I (Control group): Contained only chlorine free tap water. Group II (Experimental group 1): Contained 1.0 ppm 10,000 cc aqueous mercuric chloride solution. Group III (Experimental group 2): Contained 0.3 ppm 10,000 cc aqueous solution of Magnesium sulphate. Group IV (Experimental group 3): Contained 0.9 ppm 10,000 cc aqueous selenium solution. Group V (Experimental group 4): Contained 1.0 ppm aqueous mercuric chloride solution +.3 ppm aqueous Magnesium sulphate solution +0.9 ppm aqueous selenium solution inTotal volume of 10000 cc distilled water. Two fishes were removed from each group after 0 hrs, 24 hrs, 48 hrs, 72 hrs and 96 hrs. Na+/K+-ATPase activity of test organ Testis was determined by the method given by Tipsmark and Madsan (2003).

RESULTS AND DISCUSSION

It was observed that, 96 hrs exposure of 0.1 ppm mercuric chloride reduced the Na+/K+-ATPase activity in the testis of *H.fossilis* up to 57.35 per cent. The decrease in activity at 24, 48, 72 1nd 96 hrs respectively was 47.25, 50.96, 54.25 and 57.35 per cent respectively (Table 1and 2). 96 hrs exposure of 0.9 ppm selenium also reduced the Na+/K+-ATPase activity in the testis of exposed fishes up to 34.22 per cent, the decrease in activity at 24, 48, 72 1nd 96 hrs respectively was 19.66, 23.71, 29.54 and 34.22 per cent respectively (Table 3 and 4). Similarly, 96 hrs exposure of 0.3 ppm of magnesium sulphate reduced the Na+/K+-ATPase activity in testis up to 38.4o per cent. The decrease in activity at 24, 48, 72 1nd 96 hrs was 26.18, 33.27, 33.71 and 38.40 per cent respectively (Table 5 and 6). Combination of mercuric chloride, magnesium sulphate and selenium reduced the Na+/K+-ATPase activity up to 47.28 per cent in 96 hrs. The reduction in enzymatic activity in combined exposure after 24, 48, 72 and 96 hrs were 38.42, 43.66, 47.23 and 47.28 per cent respectively (Table 7 and 8).

It is evident that mercury released in the environment affects the reproductive system of several animals. Mercuric salts elicited direct toxic action on steroid pro-

Table 1: Mercuric chloride (1.0 ppm.) induced changes in the Na+/K+-ATPase activity in testis of *H. fossilis* (Short duration exposure)

S. No.	Exposure Duration (in hours)	Na+/ K+ ATPase activity in ng pi liberated /mg protein			
		Control Value	Experimental Value	Difference	Per cent alter
1.	24	57.10	30.12	26.98	-47.25
2.	48	57.10	28.00	29.90	-50.96
3.	72	57.10	26.12	30.98	-54.25
4.	96	57.10	24.35	32.75	-57.35

Table 2: Mercuric chloride (1.0 ppm.) induced changes in the Na+/K+-ATPase activity in testis of *H. fossilis* (Long duration exposure)

S. No.	Exposure Duration (in days)	Na+/ K+ ATPase activity in ng pi liberated /mg protein			
		Control Value	Experimental Value	Difference	Per cent alter
1.	15	57.10	23.00	34.10	-59.71
2.	30	57.10	22.12	34.98	-61.26
3.	45	57.10	18.25	38.85	-60.03

Table 3: Selenium (0.9 ppm.) induced changes in the Na+/K+-ATPase activity in testis of *H. fossilis* (Short duration exposure).

S. No.	Exposure Duration (in hours)	Na+/ K+ ATPase activity in ng pi liberated /mg protein			
		Control Value	Experimental Value	Difference	Per cent alter
1.	24	57.10	45.87	11.23	-19.66
2.	48	57.10	43.56	13.54	-23.71
3.	72	57.10	40.23	16.87	-29.54
4.	96	57.10	37.56	19.54	-34.22

Table 4: Selenium (0.9 ppm.) induced changes in the Na+/K+-ATPase activity in testis of *H. fossilis* (Long duration exposure)

S. No.	Exposure Duration (in days)	Na+/ K+ ATPase activity in ng pi liberated /mg protein			
		Control Value	Experimental Value	Difference	Per cent alter
1.	15	57.10	33.13	23.97	-41.97
2.	30	57.10	32.21	24.89	-43.59
3.	45	57.10	30.34	26.76	-46.86

Table 5: Magnesium sulphate (0.3 ppm.) induced changes in the Na+/ K+ ATPaseactivity in the testis of *H. fossilis* (Short duration exposure).

S. No.	Exposure Duration (in hours)	Na+/ K+ ATPase activity in ng pi liberated /mg protein			
		Control Value	Experimental Value	Difference	Per cent alter
1.	24	57.10	42.15	14.95	-26.18
2.	48	57.10	38.10	19.00	-33.27
3.	72	57.10	37.85	19.25	-33.71
4.	96	57.10	35.17	21.93	-38.40

duction in the testis (Ng and Liu, 1990). Ramalingam *et al*. (2001 and 2002) have reported adverse effects of mercuric chloride on testis and spermatozoa of experimental animals. Nagar and Bhattacharya (2001) also observed impaired testicular function after an exposure of Swiss albino rats (30+/2g) to mercuric chloride. In the present study effect of mercuric chloride (1.0 ppm) on Na+/K+-ATPase activity of testis of *H. fossilis* was investigated and after 96 hrs it was found reduced upto 57.35 per cent. This showed that mercuric chloride produces toxic effect to testis of *H. fossilis* and inhibited the Na+/K+ATPase enzymatic activity. The effect was exposure dependent. Ramalingam and Vimaladevi (2004) also observed significant decrease in the same mem-

Table 6: Magnesium sulphate (0.3 ppm.) induced changes in the Na+/ K+ ATPaseactivity in testis of *H. fossilis* (Long duration exposure).

S. No.	Exposure Duration (in days)	Na+/ K+ ATPase activity in ng pi liberated /mg protein			
		Control Value	Experimental Value	Difference	Per cent alter
1.	15	57.10	34.12	22.98	-40.24
2.	30	57.10	30.00	27.10	-47.46
3.	45	57.10	29.12	27.98	-49.00

Table 7: Mercuric chloride (1.0 ppm), Selenium (0.9 ppm) and Magnesium sulphate (0.3 ppm.) induced changes in the Na+/ K+ ATPaseactivityof testis of *H. fossilis* (Short duration exposure).

S. No.	Exposure Duration (in hours)	Na+/ K+ ATPase activity in ng pi liberated /mg protein			
		Control Value	Experimental Value	Difference	Per cent alter
1.	24	57.10	35.16	21.94	-38.42
2.	48	57.10	32.17	24.93	-43.66
3.	72	57.10	30.13	26.97	-47.23
4.	96	57.10	10.10	27.00	-47.28

Table 8: Mercuric chloride (1.0 ppm), Selenium (0.9 ppm) and Magnesium sulphate (0.3 ppm.) induced changes in the Na+/ K+ AT Paseactivity of testis of *H. fossilis* (Long duration exposure).

S. No.	Exposure Duration (in days)	Na+/ K+ ATPase activity in ng pi liberated /mg protein			
		Control Value	Experimental Value	Difference	Per cent alter
1.	15	57.10	36.72	20.38	-35.69
2.	30	57.10	38.11	18.99	-33.25
3.	45	57.10	40.33	16.77	-29.36

brane bound enzyme of rat testis when it was treated with low and high dose of mercuric chloride. Mercury generally inhibits the function of ion dependent ATPase leading to disturbances in the ion homeostasis. Disturbances in the ion homeostasis results in impaired signal transduction, altered cellular metabolism, changes in the cell membrane permeability, integrity and disturbances of vital functions (Ramalingam and Vimaladevi, 2003). An inhibition of Na+/K+-ATPase has been shown to be linked with intracellular accumulation of sodium ,which reverse the direction of the sodium-calcium exchange and exacerbates the intracellular calcium ion accumulation (Goddard and Robinson 1976; Akerman and Nicolls, 1982; DiP0l0 and Beauge, 1983) which could further increase lipid peroxidation,membrane derangement and excitotoxicity/apoptosis (Farber,1981 and Choi,1993)In the present study it was inferred that the inhibition of ATPase in the testis of *H. fossilis* was due to mercuric chloride treatment which altered biochemical functioning of the testis.

Selenium is an essential trace element, but when its presence is higher than the normal level in water it causes adverse health effects, (Beyers and Sodergren, 2002). In the present study exposure of 0.9 ppm aqueous solution of selenium to *H. fossilis* caused depletion in Na+/ K+-ATPase activity in testis. Adverse effects of selenium to reproductive system of fishes were also observed by Choudhary *et al.* (1983), Hilton (1986), Lemley (1993), Kaur and Bansal (2004 and 2005), Demerdash (2004) and Pyle *et al.* (2005). In the present study magnesium sulphate (0.3 ppm) exposure of 96 hrs inhibited Na+/K+-ATPase activity in testis up to 38.40 percent. According to Hoffmann *et al.* (1994) magnesium plays important role in preventing hypoxia. Hang (1984) also suggested that magnesium may play direct role in intracellular potassium homeostasis.

In the present investigation interactions of mercuric chloride, selenium and magnesium sulphate were also studied in order to examine the combined effect of these metals on Na+/K+-ATPase activity of testis. Results showed reduction of Na+/K+-ATPase up to 47.28 percent, which is less in comparison to the individual exposure of mercuric chloride which was observed up to 60 percent. There was a recovery of Na+/K+-ATPase activity up to 11 percent. The present data are in agreement with the statement of Halmy *et al. (*1987) that uptake of one metal decreases in the presence of the others, and thus supports Demerdash (2004) statement that selenium could be able to antagonize the toxic effect of mercury. On the basis of present study it is concluded that loss of Na+/K+-ATPase due to mercuric chloride could be prevented up to reasonable level by supplementation of selenium and magnesium sulphate in fish.

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