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METALS EFFECT ON FISH TISSUES
I. EFFECTS OF CHRONIC MERCURY AND SELENIUM TREATMENT ON YOUNG TILAPIA TISSUE ENZYMES AND LIPID PEROXIDATION

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Abstract

Chronic treatment was conducted with different concentrations of mercury chloride ($HgCl_2$) and sodium selenite (Na_2SeO_3) alone and an in combination. Effect of chronic treatment was studied on some metallo-enzyme and lipid peroxidation of liver and muscle tissues.

Both metals showed a strong concentration-dependent toxic effect on enzyme activities.

Key words: Mercury chloride and sodium selenite intoxication, tilapia hybrid, enzymes activity changes, lipid peroxidation.

Introduction

Numerous studies deal with the characteristic effect of Hg^{2+} and Se on vertebrates. In earlier works where the effect of $CuSO_4$ respectively, were studied in fishes it was demonstrated that the compounds are affected by the formation of "oxidative stress" in fish tissues. Upon treatment with $ZnSO_4$ oxidative stress similar but slighter is induced (RADY et al., 1988).

It was demonstrated that Hg^{2+} salts induce oxidative damage (state of oxidative stress) first of all in the kidney tissues of rat by dismutating superoxide (O_2^-) anion which resulted in H_2O_2 formation.

Thereby accumulation of H_2O_2 is the cause of oxidative damage. Oxidative

stress presumably decreases the amount of reduced glutathion (GSH) in the tissues, while increases lipid perocidation (LP) (LUND et al., 1991, MILLER et al., 1991). It is known that Se protects the organisms from Hg^{2+} intoxication and is a good anti-oxidant. Therefore the aims of present examination are the study of different concenterations of Se and Hg^{2+} salts alone, and in combination, as supplementation of the artificial diet on the metabolic enzymes and lipid peroxidation on tilapia fingerlings.

Materials and Methods

Experiments with fishes

Tilapia *(Oreochromis niloticus)* fingerlings weighing about 2.3—2.9 g/fish in average, were obtained from Research STation Faculty of Agriculture, Alexandria University, Egypt. They were kept in a glass jar (105 1 capacity) at temperature 28 °C and fed for seven days on a basic diet as adaptation period and the healthy fish were selected for the experiments.

They were then randomly distributed into twenty glass jars filled with tap water which was stored for two days before use. Water was changed every day. Water temperature was thermostatically controlled at 28±1 °C. Diets were prepared by mixing thoroughly the dry ingredients at first and followed wiht oil.

The composition of the basic diet is given in table 1

Table 1. Ingredient on basis dry matter (% d. m.)

Ingredient	% d. m.
Fish meal	20.00
Soybean meal	35.00
Yellow corn	40.00
Bone meal	2.00
Fish oil	2.00
Trace elements	0.70
Vitamin premix	0.30

Before the beginning of the mixing process, stock solution of mercury chloride and sodium selenite were prepared by dissolving 2.187 g of mercury chloride ($HgCl_2$) (solution A) and 1.35599 gm of sodium selenite (Na_2SeO_3) (solution B) in one litre of distilled water. The formulated basic diet consisted of fish meal, yellow corn and bone meal, fish oil, vitamins and minerals and was used as a control diet without the addition of solutions A or B. The other diets tested were prepared by adding 0.25, 0.5 and 1.0 ml of solution A, and 0.5, 1.0 and 1.5 ml of solution B per kg of basic diet, respectively. Mixture of mercury chloride (solution A) and sodium selenite (solution B) were prepared by adding 0.25, 0.5 and 1.0 ml of solution A with 1.5, 1.0 and 0.5 ml of solution B, respectively.

Feeding strategy:

Fish were fed on the experimental diets (two jars for each diet) for 112 days. The feeding rate was as follows: 10% of the total biomass of fish daily for 14 days and 4% of the total biomass of fish for 70 days.

The daily feed ration was divided into two equal portions and give at 9.00 a.m. and 1.00 p.m. The glass jars were cleaned daily to prevent the accumulation of faeces to reduce algae growth.

Fish sampling:

At the end of the experiment five fish from each jar were taken, and their muscle and livers were removed and kept under cold condition for biochemical analysis.

Selenium in fish organs were determined spectrophotometrically by 3,3-diaminobenzidine described by MARCZENKO (1976). Mercury in fish organs was determined by flameless atomic absorption spectrophotometer (perkin Elmer 4305) by the method of CHAPMAN et al. (1961).

Biochemical-measurements:

Alkaline and acid phosphatase (AlPh-ase; EC 3.1.3.1), (AcPh-ase; EC 3.1.3.2) activities were measured according to the method of BERGMEYER (1974) phenol released by enzymatic hydrolysis from phenyl phosphate under defined conditions of time, temperature and pH was measured colorimetrically at 400 nm after using 1N NaOH to stop reaction.

Lactic dehydrogenase activity (LDH; EC 1.1.1.27) was determined colorimetrically using the method reported by ANON (1971) the method depends on reduction of pyruvate by incubation with enzyme in the presence of reduced nicotinamide adenine dinucleotide (NADH). The reaction was stopped by adding dinitrophenyl hydrazine solution, which reacts with the remaining pyruvate forming hydrazone. The colour produced was measured at 510 nm.

Glutathione transferase avtivity (GSH-S-Tr-ase; EC 2.51.18). Enzyme activity is expressed as μmoles of 4-chloro-1,3-dinitrobenzene (CDNB) conjugated/minute/mg protein according to the method of VESSEY et al., (1984), in the presence of reduced glutathione. Glutathione peroxidase activity (GP-ase: EC 1.11.1.9) was measured spectrophotometrically using cumene hydroperoxide and GSH solutions as substrates (CHIU et al., 1976; SEDLAK et al., 1968).

Lipid peroxidation (LP) was measured by the thiobarbituric acid spectral method of PLACER et al. (1966).

Total protein was measured by the Folin-reagent according to the method of LOWRY et al., (1951).

Statistical analysis: The data obtained in the present study were statistically analyzed according to the method of SNEDECOR et al., (1967).

Result

In Fig. 1 it can be seen that AlPh-ase activity of liver homogenate is increasing steadily after selenite treatments, only the highest selenite concentraton (1.5 mg) decrease significantly the enzyme activity. The mercury salt treatment after the acitivation of enzyme, in highest concentration (1.0 mg) totally inhibited the enzyme activity. The two metals together only in the concentrations of 1.5 mg and 0.25 mg respectively inhibited significantly the AlPh-ase activity.

The AcPh-ase activity changes only after the treatment of 1.0 mg of Na-selenite, it activates the enzyme and after the highest mercury treatment (1.0 mg) the activity decrease considerably.

The mild treatment of selenite (1.0 mg) and mercury (0.5 mg) respectively decrease the LDH activity on a concentration dependent manner.

The further effect of metals on the liver enzymes activities and LP summarised in Fig. 2. The selenite treatment activate the GSH-S-Tr-ase as well as GP-ase at lower concentration (0.5 mg) but inhibits them in the highest one similar to mercury. The highest selenite and mercury treatment significantly

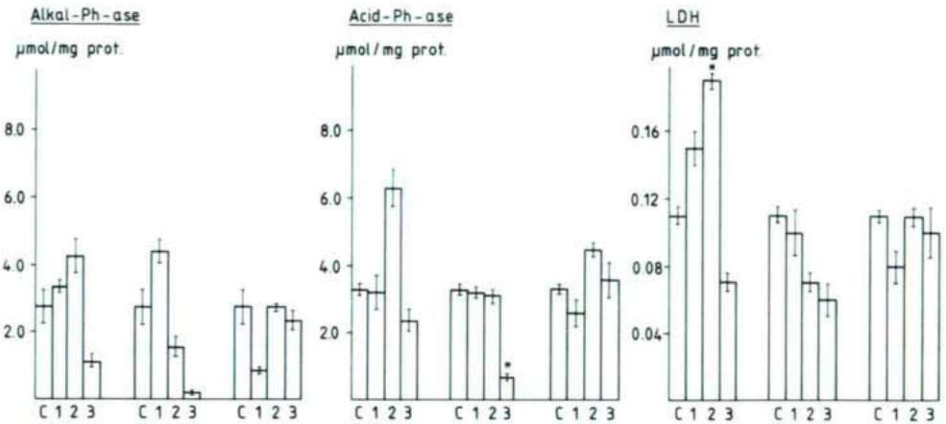

Fig. 1. First series of column shows the changes enzyme activities in liver homogenates if 0.5, 1.0 and 1.5 mg/kg sodium selenite is mixed in the feed (columns No. 1, 2, 3). The second revies of column demonstrates the same with 0.25, 0.50 and 1.0 mg/kg $HgCl_2$. The first colounna of the third series shows the simultaneous effect of 1.5 mg/kg sodium selenite and 0.25 $HgCl_2$; while the second and third coloumnes indicate the simultaneous effect of 1.0 mg: 0.5 mg and 0.5 mg: 1.0 mg selenite and $HgCl_2$, respectively.

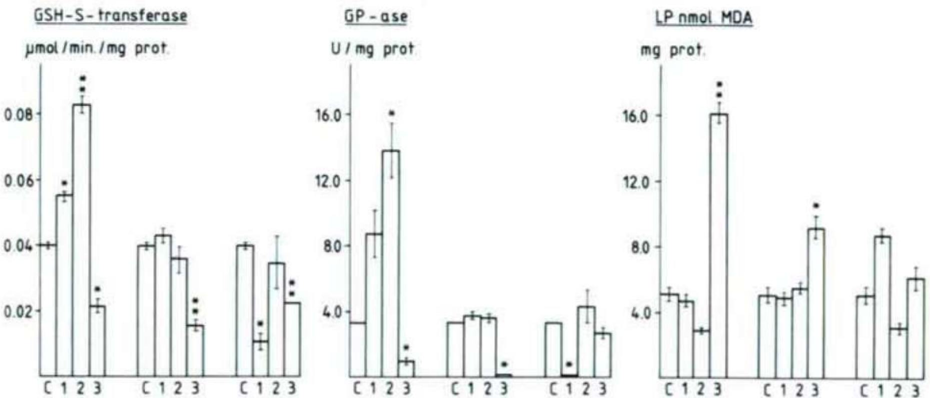

Fig. 2. Effects of metals as listed in Fig. 1 on liver enzyme activities and LP are shown.

increased the LP. Changes in the AlPh-ase, AcPh-ase and LDH activity in muscle are shown (Fig. 3) as wel as the GSH-S-Tr-ase and GP-ase activity and LP (Fig. 4).

The treatments did not influence very much the LDH activity, but the lower concentration of Na- selenite activates the AlPh-ase and AcPh-ase, but the highest selenite and mercury concentrations significantly inhibited the enzymes. Selenite and mercury in their lower concentration significantly activate the GSH dependent enzymes, but in highest concentrations significantly inactivate them (see Fig. 4.). The highest selenite and mercury treatments activated the muscle

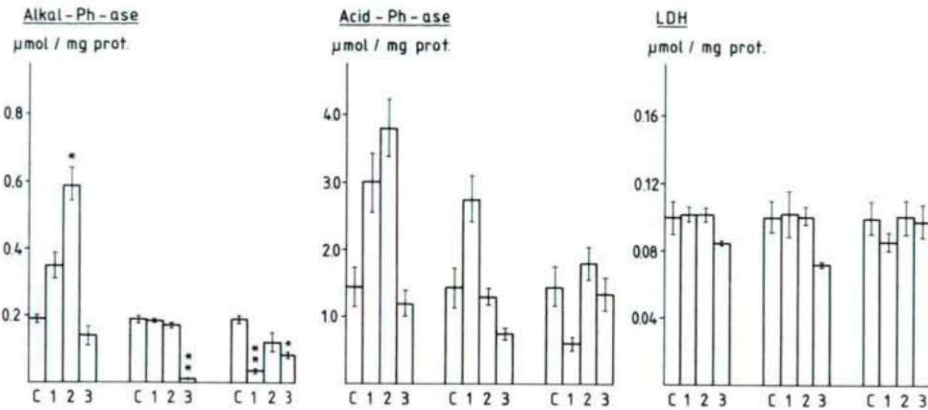

Fig. 3. Response of muscle tissue enzymes upon treatment with metals as indicated in details in Fig. 1.

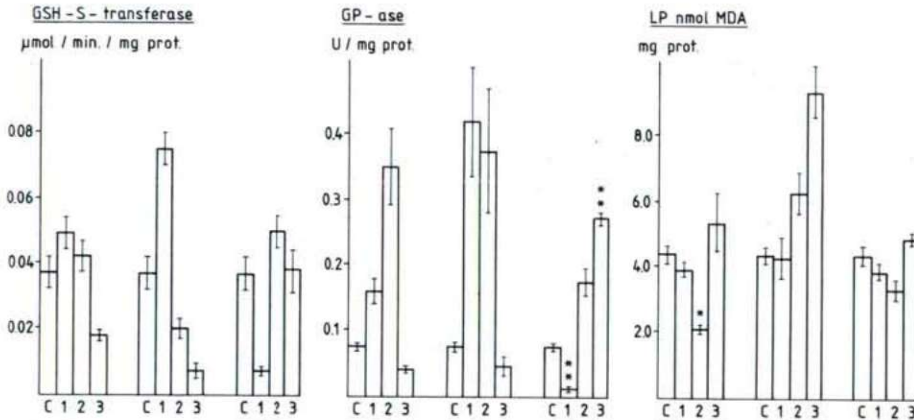

Fig. 4. Effects of metal treatments on muscle tissue enzyme activities and LP vith the same series details as given in Fig. 1.

LP. Table 2. summarises the selenium and mercury measured after the treatments in fish tissues. The Table well demonstrates the concentration dependent increase of metals in the tissues studied upon Se or Hg^{2+} treatments. At simultaneous administration a certain competition could be observed.

Discussion

Se is an enzyme active site specific trace element. Hg^{2+} is a rather toxic pollutant, the amount of which unfortunately is increasing in most of the natural waters. Facts mentioned above made the conducted studies justified. It is also known that both metals, but first of all Se affects the immune system, in such a

Table 2. Concentrations of mercury and selenium of investigated tissues of Tilapia *(Oreochromis niloticus)* fed on different diet contained different levels of mercury and/or selenium.

Treatment	Mercury		Selenium	
	Muscle	Liver	Muscle	Liver
Control	–	–	0.5	1.2
Na_2SO_3 mg/kg feed				
0.5	–	–	0.4	3.8
1.0	–	–	1.5	4.5
1.5	–	–	1.9	5.2
$HgCl_2$ mg/kg feed				
0.25	1.3	1.6	0.5	1.7
0.50	1.3	1.6	0.5	1.7
1.00	1.6	7.9	0.4	1.3
Na_2SO_3:$HgCl_2$ mg/kg feed				
1.5:0.25	0.35	0.23	0.94	5.9
1.0:0.50	0.30	0.14	0.90	2.2
0.5:1.00	0.05	0.30	0.40	3.8

way that Se is an immune activator while Hg^{2+} is immune suppressor element. Immune-toxicity of Hg^{2+} depends upon the Se content of the tissue. Toxic effect of Se manifests, first of all, in its ability to replace S (Sulphur) atom, mostly the active S, e.g. the active S in the Met.

Se can be regarded as the antidote of the toxic effect of Hg (HELLAWELL, 1986 and HEATH, 1987). The dynamic cconcentration dependent of the uptake of the two metals tested with joint treatment in muscle and liver of fish is well illustrated by data in Table 2.

From the studies it appears that Se in the highest concentration used (1.5 mg/kg) is rather toxic for enzymes. It decreases the activities of all other enzymes while increases LP in both liver and muscle homogenates. Cyto-, but first of all nephrotoxic characteristics of Hg are well-known (LUND et al., 1991). Therefore, its tissue enzyme inhibiting and LP enhancing characteristics is not surprising.

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