BIOCHEMICAL CHANGES IN THE LIVER OF
TILAPIA NILOTICA EXPOSED TO LEAD

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ABSTRACT

Fry of *T. nilotica* were exposed to 2 mg/l and 4 mg/l of lead for 90 days after which the livers of the fish were dissected out for biochemical tests.

The concentration of reduced glutathione (GSH) in the fish liver was decreased as the concentration of lead increased. The GSH concentrations of replicates I and II in the control group were 25.85 ± 0.05 μmole/ml and 24.41 ± 0.89 μmole/ml respectively. In the 2 mg/l group , the GSH concentrations were 21.73 ± 0.97 and 16.86 ± 1.46 μmole/ml for the two replicates. In the 4 mg/l group, the GSH concentrations were 14.18 ± 1.13 and 16.77 ± 1.81 μmole/ml.

The activity of glucose-6-phosphate dehydrogenase (G-6-PD) in the fish liver was lowered as the concentration of lead increased. For replicate I, G-6-PD activity of the control group was 1.18 ± 0.06 units/mg. For replicate II, G-6-PD activity of the control group was 1.15 ± 0.07 units/mg. At 2 mg/l lead exposure, G-6-PD activities were 0.84 ± 0.05 and 0.62 ± 0.03 units/mg. At 4 mg/l exposure, G-6-PD activities were 0.51 ± 0.04 and 0.56 ± 0.04 units/mg for replicates I and II respectively.

Glucose-6-phosphatase (G-6-Pase) activity in the fish liver was reduced as the concentration of lead increased. G-6-Pase activities in the control group were 2.55 ± 0.13 and 2.52 ± 0.11 μmole/mg for replicates I and II respectively. At 2 mg/l exposure, replicates I and II had G-6-Pase activities of 2.21 ± 0.21 and 2.16 ± 0.16 μmole/mg respectively. At 4 mg/l exposure, G-6-Pase activities were 1.83 ± 0.19 and 1.78 ± 0.17 μmole/mg.

INTRODUCTION

Lead is a non-essential, bioaccumulative and ubiquitous toxic metal. It enters the aquatic environment through precipitation, lead smelters, soil erosion and industrial effluents[1,2,3].

Lead is known to complex with enzymic and structural proteins, nucleic acids, amino acids and peptides, particularly those with sulfhydryl (SH) groups such as cysteine and glutathione[4]. Lead binds with SH groups, thus inhibits the activity of enzymes that are dependent on the presence of these SH groups for their activity[5]. One enzyme found to be extremely sensitive to lead is δ-aminolevulinic acid dehydratase (ALAD) which is involved in heme synthesis[6,7,8]. Decrease in ALAD activity was observed in birds poisoned with lead[9,10,11]. There is scant information regarding effects of lead on other proteins. This study reports on the effects of lead on the concentration of reduced glutathione (GSH) and the activities of glucose-6-phosphate dehydrogenase (G-6-PD) and glucose-6-phosphatase (G-6-Pase) in the liver of *Tilapia nilotica*.

MATERIALS AND METHODS
Lead exposure

Fry of *T. nilotica* obtained from breeders reared at the University of the Philippines College of Fisheries were acclimated in the laboratory for 3—4 days prior to initial exposure to lead.

Forty fish were placed in a 20-liter aquarium with continuously aerated tap water. The water of the treated groups was spiked with 2 and 4 mg/l of lead nitrate respectively. The same number of fish was used for the control group. Test water was changed every 48 h. Commercial fish feed, Pisces, was given to the fish twice a day.

The study was conducted for 90 days employing a static method of heavy metal exposure. The experiments were done in duplicate.

Biochemical tests

After 90-day exposure, the fish were sacrificed and their livers dissected out for biochemical tests.

For the reduced glutathione (GSH) and glucose-6-phosphate dehydrogenase (G-6-PD) assays, pooled liver was homogenized in 4 volumes of ice-cold 0.1 M phosphate buffer, pH 7.4, using Heidolph mechanical homogenizer.

For determination of reduced glutathione, based on the method of Jollow *et al.*[12], an aliquot was deproteinized by adding an equal volume of 4% sulfosalicylic acid and centrifuged at 3,000 rpm for 5 min in ice. A hundred μl (0.1 ml) of supernatant 970 μl 0.1 M phosphate buffer, pH 7.4 and 30 μl cold Ellman's reagent were mixed in a cuvette and read at 412 nm. Ellman's reagent served as blank. Reduced glutathione concentration in the sample was determined from the reduced glutathione standard curve. All values were reported in μmole/ml.

The assay of G-6-PD was based on the method of Livingstone[13]. A mixture composed of 2 mM G-6-P, 0.5 mM NADP, 10 mM $MgCl_2.H_2O$ and 60 mM Tris. HCl, pH 7.4 was prepared to a volume of 2.7 ml. 0.3 ml of the liver homogenate was added to the mixture and read at one-minute interval for 5 min at 340 nm. Mixture without G-6-P was used as blank. One unit of activity was defined as the amount of the enzyme that reduced 1 μmole of NADP per minute. The specific activity of G-6-PD was expressed as units/mg protein[14].

Protein content of the liver was determined using the Lowry method[5] and read at 540 nm. 22% bovine albumin was used as standard.

Glucose-6-phosphatase (G-6-Pase) activity was assayed based on the method of Dyce and Bessman[16]. Pooled liver was homogenized in 4 volumes of ice-cold distilled water using Heidolph mechanical homogenizer. Liver homogenate was incubated in 0.1 M glucose-6-phosphate (substrate) and 0.1 M Tris-citrate buffer, pH 6.5 for 30 min. 1 ml 10% trichloroacetic acid was added to stop the reaction after 30 min incubation. The mixture was cooled in ice for 5 min. 0.36 N H_2SO_4 was added to 0.2 ml of the sample to make a total volume of 2 ml. 0.5 ml ascorbic acid, 0.5 ml ammonium molybdate and 1 ml of stabilizing reagent were added to the mixture one at a time by mixing between addition. The stabilizing reagent contained 3% each of sodium citrate, sodium arsenite and glacial acetic acid. The optical density of the sample was read at 660 nm, 15 min after the addition of the stabilizing reagent. 0.36 N H_2SO_4 was used as blank. Phosphate concentration in the sample was determined from the phosphate standard curve. Enzyme activity was expressed in terms of μmoles of inorganic phosphate released in 30 min of enzyme-substrate incubation per milligram protein.

All biochemical tests were assayed using the UV-VIS Perkin-Elmer 55B spectrophotometer.

Statistical analyses

Data gathered from the biochemical tests were computed for their arithmetic means and corresponding standard deviations.

Single factor ANOVA and student t-test were used to find out if there were significant differences between treated and control groups at 5% level[17].

RESULTS

Reduced glutathione (GSH)

The mean GSH values decreased as the concentration of lead increased (Table 1). In the two sets of experiments (Replicates I and II), GSH values of the 2 mg/l group are reduced by as much as 15.9% and 30.9% respectively compared to the control. Between the control and 4 mg/l groups, the reduction is 45.2% and 31.3% in the two sets of experiments. There is significant difference between mean values of control and the treated groups. In the first replicate, there is significant difference between 2 and 4 mg/l groups. However, in the second replicate, there is no significant difference between the treated groups, even though the GSH value of the 2 mg/l group is slightly higher than that of the 4 mg/l group.

TABLE 1

REDUCED GLUTATHIONE (GSH) CONCENTRATION IN THE LIVER OF
T. NILOTICA AFTER EXPOSURE TO LEAD FOR 90 DAYS.
FOR EACH VALUE, N = 15.

Exposure concentration (mg/l)	GSH concentration (μmole/ml) mean $\pm$ std. dev.	
	I	II
0 (Control)	25.85 ± 0.50	24.41 ± 0.89
2	21.73 ± 0.97^{a}	16.86 ± 1.46^{a}
4	$14.18 \pm 1.13^{a,b}$	16.77 ± 1.81^{a}

[a]statistically significant differences between treated and control groups per replicate at P<0.05.

[b]statistically significant differences between treated groups per replicate at P<0.05.

Glucose-6-phosphate dehydrogenase (G-6-PD)

The activity of G-6-PD was lower in the treated groups compared to that of the control (Table 2). The activity of G-6-PD at 2 mg/l is decreased by as much as 28.8% and 46.1% in the two sets of experiments. At 4 mg/l, the reduction is 56.8% and 51.3% compared to the control. There is significant difference between mean values of control and treated groups. Likewise, there is also significant difference between treated groups in both sets.

TABLE 2

GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G-6-PD) ACTIVITY IN THE LIVER OF *T. NILOTICA* AFTER EXPOSURE TO LEAD FOR 90 DAYS. FOR EACH VALUE, N = 15.

Exposure concentration (mg/l)	G-6-PD (units/mg) mean ± std. dev.	
	I	II
0 (Control)	1.18 ± 0.06	1.15 ± 0.07
2	0.84 ± 0.05[a]	0.62 ± 0.03[d]
4	0.51 ± 0.04[a,b]	0.56 ± 0.04[a,b]

[a]statistically significant differences between treated and control groups per replicate at $P<0.05$.

[b]statistically significant differences between treated groups per replicate at $P<0.05$.

Glucose-6-phosphatase (G-6-Pase)

There is lowered activity of G-6-Pase as the exposure concentration of lead increased (Table 3). The reduction in the two replicates at 2 mg/l is 13.3% and 14.3% compared to the control. At 4 mg/l , the reduction is 28.2% and 27.4%. There is significant difference between G-6-Pase activities of the treated and control groups. Between treated groups, there is significant difference in the two replicates.

TABLE 3

CLUCOSE-6-PHOSPHATASE (G-6-PASE) ACTIVITY IN THE LIVER OF *T. NILOTICA* AFTER EXPOSURE TO LEAD FOR 90 DAYS. FOR EACH VALUE, N = 15.

Exposure Concentration (mg/l)	G-6-Pase (umole/mg) mean ± std. dev.	
	I	II
0 (Control)	2.55 ± 0.13	2.52 ± 0.11
2	2.21 ± 0.21[a]	2.16 ± 0.16[a]
4	1.83 ± 0.19[a,b]	1.78 ± 0.17[a,b]

[a]statistically significant differences between treated and control groups per replicate at $P < 0.05$.

[b]statistically significant differences between treated groups per replicate at $P < 0.05$.

DISCUSSION

Reduced glutathione (GSH), a tripeptide of glycine, glutamic acid and cysteine is a primary low molecular weight cellular thiol and a major reserve of cellular cysteine. It participates in diverse biological processes such as detoxification of xenobiotics, removal of hydroperoxides and protection of membranes against lipid peroxidation-induced damage[18,19,20]. It is released from cells during oxidant stress[21]. Buthionine sulfoximine caused depletion of GSH in mice which brought about mitochondrial damage[22]. Liver of rat exposed to polyacetal, a polymer of formaldehyde exhibited decreased GSH[23]. In yearling channel catfish, liver showed significant depletion of reduced GSH when exposed to naphthoquinones[24]. In the present study, exposure to lead caused a decrease in hepatic GSH concentration. Lead is known to bind with proteins, particularly those with SH groups such as glutathione[4,25,26]. This binding inhibits the activity of enzymes that are dependent on the presence of these sulfhydryl groups for their activity[5]. This could explain the decrease in glucose-6-phosphate dehydrogenase (G-6-PD) activity in the liver of lead-treated *T. nilotica* in the present study. G-6-PD is the first enzyme in the pentose phosphate pathway. This pathway generates NADPH, which is required to maintain the intracellular concentration of reduced glutathione[27,28,29]. G-6-PD is found in almost all animal tissues such as blood cells, liver, kidney, heart and skeletal muscle[30]. It was observed that in G-6-PD deficient cells, disulfide GSSG is not reduced to GSH and the oxidative damage (disulfide formation) in the membrane is not repaired[31].

There is limited information regarding toxicological studies on G-6-PD. Beliles (cited by Dubale and Shah[32]) noted that the harmful effect of cadmium is attributed to its effects on sulfhydryl enzymes especially dehydrogenases. Decreased activity of G-6-PD was observed in CCl_4 treated liver[33].

In hepatic toxicity, the endoplasmic reticulum (ER) is adversely affected and is usually the first site of detectable morphological changes[34]. Studies on the liver of rats and baboons showed that lead caused disruption in the membranes of ER[35,36]. It was observed that destruction of ER caused by administration of toxicants is accompanied by a reduction in the activity of glucose-6-phosphatase (G-6-Pase)[37,38,39]. G-6-Pase is a specific enzyme in the liver and kidney[40] and is found exclusively in the membranes of the ER[34,41]. Since it has been shown that lead caused destruction in the ER membranes, it may be assumed that enzymes associated with ER like G-6-Pase may likewise be affected. Results of the present study showed that the activity of G-6-Pase in the liver of lead-treated *T. nilotica* was significantly decreased. Similar findings were reported in the liver of rats and fish treated with CCl_4 and mercury[42,43,44]. Reduction in G-6-Pase activity is an indication that both glycogenolysis and gluconeogenesis are retarded by toxin administration[43,44].

The present study showed that lead caused a depletion in GSH concentration as well as reduction in the activities of G-6-PD and G-6-Pase.

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