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EFFICACY OF ASCORBIC ACID (VITAMIN C) ON EXPERIMENTAL COPPER INTOXICATION IN TILAPIA ZILLII

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ABSTRACT

The influences of two different concentrations of Ascorbic acid on the experimental Copper intoxication in Tilapia zillii was investigated. Ascorbic acid has been shown to have protective and therapeutic effects against Copper intoxication. The efficacy of Ascorbic acid was more clearly indicated in Tilapia by a reduction of mortality, absence of poisoning signs, lowered metal content of tissues and preventing the inhibition of blood GOT and LDH activities. Treatment with a concentration of 0.432 ppm Ascorbic acid was most effective than the other treatment (0.216 ppm Ascorbic acid) which appeared to have less preventive effect on Copper intoxication. In blood, liver, kidney, gills, muscle and brain, Ascorbic acid interacted with Copper in some way prevent tissue deposition. Enhanced transport of Copper to kidney was most pronounced at different concentrations of Ascorbic acid as shown by the metal level in each tissue. The present results demonstrate that Ascorbic acid has been found to be an effective antidote of Copper intoxication.

INTRODUCTION

The existence of small amounts of many of the relatively toxic heavy metal cations into aquatic environment causes multiple changes in the internal dynamics of aquatic organisms, even at sublethal levels (Christensen et al., 1972). Copper is a surprisingly widespread pollutant of water (Sprague, 1968). Trace concentrations of this metal (one-tenth to one-twentieth of the accepted standards for drinking water) can be lethal for fish in regions where surface water is very soft (Sprague, 1968). Metal intoxication and the consequent fish mortalities can be probable. Chelation therapy is

the most successful modality for the management of heavy metal poisoning (Kostyniak and Clarkson, 1981; Williams and Halstead, 1983). Ascorbic acid, Thiamin, Folic acid and Pyridoxine have been proven to be effective in reducing the toxic manifestations of Lead (Flora and Tandon, 1986; Flora **et al.**, 1986; Tandon et al., 1987; Ghazaly, 1991). Sprague (1968) utilized the Tri-Sodium salt of Nitrilotriacetic acid (NTA) in treatment of Copper poisoning in the brook trout (Salvelinus fontinalis). D-Penicillamine and Tetramines were also used by Borthwick et al. (1980) to control Copper accumulation in rats.

The objective of this study is to investigate whether Ascorbic acid (vitamin C) could prevent experimental Copper intoxication in Tilapia zillii. This metal was selected not only because very little studies on heavy metal-chelation-therapy in fish were carried out but also because it is found in aquatic environments as a natural and industrial contaminant. The fish species was chosen on the basis of its relative abundance, and on frequent use in laboratory studies in Egypt.

MATERIALS AND METHODS

Test fish and acclimation:

Adult healthy specimens of the freshwater fish Tilapia zillii (weight 100 ± 3g) were collected from El-Nasr channel, 70 Km west of Alexandria, and were acclimated to laboratory conditions for two weeks. Well-aerated, dechlorinated and filtered tap water (hardness 81 ppm as $CaCO_3$; pH 7.1 ± 0.1; temperature 22 ± 1°C) was used throughout the study. Aeration was by diffuser stones connected to a compressed air main. Commercial fish pellets were used as food.

Exposure and treatment studies:

Initially the 96 hr TL_m was determined to establish the test concentration of Copper for the ensuing sublethal exposure. All bioassay procedures were conducted according to the methods of the American Public Health Association (1965). All tests were carried out in 50l glass tanks. Copper was added as Copper Sulphate ($CuSO_4.7H_2O$) and concentrations are given as ppm of Cu^{++}. Concentrations of the chelating agent Ascorbic acid were also measured as ppm. The previously acclimated fish were randomly divided into four groups: Group I, control; Group II, Copper exposed; Group III and IV, Copper plus Ascorbic acid-treated. Each group consisted of 40 animals (10 animals/tank). Copper Sulphate was provided to the fish of Groups

II, III and IV in a concentration of 0.144 ppm; equivalent to 0.6 of the 96-hr TL_m value, determined by the present static bioassays. The fish of Groups III and IV received in addition to Copper, concentrations of 0.216 and 0.432 ppm Ascorbic acid, respectively. Concentrations of Ascorbic acid, were selected on the basis of mortality. Water was renewed every four days. Sublethal exposure and treatment studies continued for 28 days. Changes in behaviour and individual deaths of the control, intoxicated and treated fish, if any, were recorded throughout the experimental period. Each tank was examined at 24 hr intervals. Animals were considered to be dead when they were immobile, showed no respiratory activity, and failed to respond to probing of the caudal peduncle.

Bioaccumulation studies:

Six surviving fish from each group were analyzed for Copper at the end of the test (28 days). Samples of blood, liver, kidney, gills, muscle and brain were obtained from each fish. Blood was collected in heparinized vials by heart puncture, and centrifuged at 2500 rpm for 5 min. Plasma samples (0.3 ml) were precipitated with 1.5mL of 6% Trichloroacetic acid and Copper was determined in the resulting supernatant. The other collected tissues (liver, kidney, gills, muscle and brain) were dried at 105°C for 48 hr, and digested until clear in reagent grade Nitric acid. The digests were diluted to 100 ml using bidistilled water. Copper levels in all types of tissues were determined by Atomic Absorption Spectrophotometry.

Biochemical analyses:

Samples of blood were also obtained from surviving fish and serum was separated by centrifugation. Serum GOT activity was assayed according to the method of Reitman and Frankel (1957)) and serum LDH activity was measured by the method of Kornberg (1955).

Data evaluation:

Comparisons between groups were made using Student's t-test at probability level of <0.05 as significant. Data of metal concentrations and biochemical analyses were expressed as mean values $\pm$ standard deviations of 6 animals.

RESULTS

Ascorbic acid caused a marked decrease in Copper poisoning in Tilapia zillii (Table 1). Survival was prolonged to the end of the experimental period (28 days) in both groups of fish receiving Copper plus Ascorbic acid. Forty percent of the experimental fish lived after treatment with 0.216 ppm of Ascorbic acid, and ninety percent for Ascorbic acid concentration of 0.432 pp.

The fish group receiving only Copper; exhibited extreme signs of hyperexitability, darting towards the surface every 4-5 min., body torsion, and difficulty in respiration. Increased mucus secretion from the body was observed. These symptoms were not observed in fish receiving Copper plus 0.432 ppm Ascorbic acid. In fish treated with 0.216 ppm Ascorbic acid, these symptoms were only seen before death.

Copper contents in tissues of fish receiving metal alone and fish receiving metal plus chelating agent are given in Table 2. Both concentrations of Ascorbic acid when given with Copper were effective in decreasing blood, liver, kidney, gills and muscle Copper levels. Brain Copper decreased only by the higher concentration of Ascorbic acid (0.432 ppm).

Although the levels of Copper in tissues from fish treated with the therapeutic levels of Ascorbic acid were significantly higher than controls, the levels were still less than levels of Copper found in tissues from fish not treated with Ascorbic acid.

Estimations of blood GOT and LDH activities, biological indicators of Copper poisoning, are shown in Table 3. In fish receiving only Copper (Group II) blood GOT and LDH activities showed statistically significant decreases. The higher concentration of Ascorbic acid (0.432 ppm) was effective in preventing these changes at 28 days. The lower concentration of Ascorbic (0.216 ppm) succeeded in preventing only the inhibition of LDH activity.

DISCUSSION

Use of Ascorbic acid as a chelating agent for Copper is a new suggestion. It seems practicable because it can lengthen survival of Tilapia zillii. Survival of these fish increased obviously after treatment with Ascorbic acid. The efficacy of this naturally occurring chelating agent was confined mainly to the fish group treated with the higher concentration of Ascorbic acid (0.432 ppm). The present data suggest the

Table (1): Toxicity of Copper for Tilapia zillii with and without Ascorbic after 28 days of treatment.

Treatment	Abnormal (%)	Death (%)
Group I (control)	0	0
Group II (copper)	100	85
Group III (copper + 0.216 ppm Ascorbic acid)	85	60
Group IV (copper + 0.432 ppm Ascorbic acid)	0	10

Table (2): Therapeutic efficacy of Ascorbic acid on tissue Copper concentrations (ug/g) in Copper intoxicated fish at 28 days after treatment.

Treatment	Blood	Liver	Kidney	Gills	Muscle	Brain
Group I	0.01 ± 0.12	0.31 ± 0.04	0.08 ± 0.12	0.22 ± 0.06	0.02 ± 0.01	0.02 + 0.01
Group II	4.54 ± 0.13^{a}	21.67 ± 3.08^{a}	9.85 + 1.41^{a}	16.10 ± 1.31^{a}	5.62 ± 1.14^{a}	2.93 + 1.17^{a}
Group III	1.09 ± 0.97^{c}	3.25 ± 0.16^{bc}	5.78 + 1.03^{bc}	3.02 + 0.82^{ac}	0.96 ± 0.61^{bc}	2.87 ± 1.08^{b}
Group IV	0.23 ± 0.16^{c}	0.67 ± 0.51^{c}	2.94 ± 0.98^{bc}	1.65 ± 0.35^{bc}	0.13 ± 0.10^{c}	0.61 ± 0.24^{bc}

Group I = (control) Group III = (Copper + 0.216 ppm Ascorbic acid)
Group II = (copper) Group IV = (Copper + 0.432 ppm Ascorbic acid)

a Represents significant difference between control (Group I) and Copper-exposed fish (Group II).
b Represents significant difference between control (Group I) and each of Ascorbic acid-treated fish groups (III and IV).
c Represents significant difference between Copper-exposed (Group II) and each of Ascorbic acid-treated fish groups (III and IV).

Table (3): Therapeutic efficacy of Ascorbic acid after sublethal Copper exposure on serum Glutamate Oxaloacetic transaminase (GOT) and Lactic Dehydrogenase (LDH) activities in Copper intoxicated fish at 28 days after treatment.

Treatment	GOT*	LDH*
Group I (Control)	16.5 ± 1.9	298.7 ± 10.2
Group II (Copper	10.9 ± 2.2[a]	269.1 ± 8.7[a]
Group III (Copper + 0.216 ppm Ascorbic acid)	13.1 ± 0.9[c]	286.8 ± 3.6[c]
Group IV (Copper + 0.432 ppm Ascorbic acid)	15.4 ± 1.4[c]	290.9 ± 2.3[c]

a = significant difference between control (Group I) and Copper-exposed fish (Group II).
b = significant difference between control (Group I) and each of Ascorbic acid treated fish groups (III and IV).
c = significant difference between Copper-exposed (Group II) and each of Ascorbic acid-treated fish groups (III and IV).
* = Values are expressed in n moles pyruvate/min/mL serum.
** = Values are expressed in n moles of NADH oxidized/min/mL serum.

value of Ascorbic acid for the protecting fish against Copper. Ascorbic acid has been shown to have protective effects against lead intoxication and to enhance the efficacy of CaNa EDTA to counteract Lead toxicity (Papaioannou et al., 1978; Tandon et al., 1987). Tandon and Flora (1989) used Ascorbic acid to enhance the role of DMSA (Dimercaptosuccinic acid) in urinary Lead excretion.

The most important finding in this study is the observation that Ascorbic acid markedly decreased the deposition of Copper in the different tissues examined. This finding suggest the beneficial use of Ascorbic acid in preventing tissue accumulation of Copper. It may be due to Ascorbic acid inhibition or interference with Copper absorption, due to complex formation of Copper with Ascorbic acid or one of its metabolites followed by subsequent excretion.

However, the significantly increased concentrations of Copper in tissues from fish of both Groups III and IV when compared with fish of Group I ($p < 0.05$) would suggest that Ascorbic acid does not totally interfere with Copper absorbed from the intestine.

The lower tissue concentrations of Copper obtained after Ascorbic acid supplementation show that either less of Copper was absorbed or the metal was excreted at a higher rate. The only slightly lower levels of Copper in kidney with the marked reduction of Copper levels in other tissues after supplementation of Ascorbic acid might indicate enhanced transport of Copper to the kidney.

It is worthly mentioning that Other chelating agents are also protecting fish against copper toxicity. These are the Di-Sodium salt of Ethylene-diamine- teracetic acid (EDTA) and Trisodium salt of Nitrilotriacetic acid (NTA) (Sprague, 1968). About six times as much EDTA as metal was required, in accord with the higher molecular weight of EDTA. Ascorbic acid is of more practical interest because of its effectiveness, safety and lower cost. Sodium Citrate proved to be only moderately effective, and the Polyphosphate (Calgon) was ineffective against copper toxicity (Sprague, 1968).

Changes of blood GOT and LDH activities are biological indicators of Copper poisoning (Christensen et al., 1972). These changes have been considered as a tool to study the variation in cell viability and changes in membrane permeability (Gentry et al., 1984). It seems likely that the changes in these enzymes are due to cellular

degradation by Copper. Ascorbic acid at concentration of 0.432 ppm has a beneficial effect in preventing the inhibition of both enzymes. The detrimental effects of Copper on GOT activity did not seem to be alleviated by the lower (0.216 ppm) therapeutic concentration of Ascorbic acid. The insignificant change ($p > 0.05$) of GOT activity from fish in Group III compared with fish of Group II may be explained by the low dose of Ascorbic acid used in this study.

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