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An Investigation of Vitamin Levels of the Carp (*Cyprinus carpio* L.) Muscle Tissue Subjected to Thiazole Copper (II) Complex

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Abstract: In this study, four groups of carp, each containing 12 fish (*Cyprinus carpio* L.) were chosen. The first group of fish was fed with Cu(II) added pellet fish food, the second group, was fed with thiazole ligand added pellet fish food and the third group was fed with thiazole Cu(II) complex added pellet fish food. The forth group was kept as a control group and was fed only with pellet fish food. Retinol, α-tocopherol and ascorbic acid levels in fish's muscle tissues were measured using HPLC. Antioxidant vitamin levels of the fish which fed with Cu(II) thiazole complex, were significantly lower than those fed without Cu(II) thiazole complex ($p < 0.005$). It was found that the decrease in vitamin levels correlated with the Cu(II) thiazole complex concentration.

Key words: Thiazole, thiazole Cu(II) complex, retinol, α–Tocopherol, ascorbic acid, HPLC

Introduction

Thiazoles and their derivatives are known to posses antitubercular (Ashtekar *et al.*, 1987) antibacterial, antiviral, cytotoxic (Agarwal, 1997; Gu *et al.*, 1999), antimicrobial (Fahmy, 1997), antitumor, antineoplastic (El-Subbagh and Al-Obaid, 1996), immunomodulatory (Schorlemmer *et al.*, 1989), antifungal (Mishra and Singh, 1991), hypotensive (Nakahara *et al.*, 1985), anti-inflammatory (Labanauskas, *et al.*, 2000) and analgesic activities (Body *et al.*, 1999).

Thiazole and their derivatives have also biological significance, for example vitamin-B1 and coenzyme cocarboxylase contains thiazole ring (Breyer, 1963). It is known that 2-aminothiazole is a biologically active compound with broad range of activity and is also an intermediate in the synthesis of antibiotics and dyes. The penicillin molecule too contains a thiazolidine ring (Tortora *et al.*, 1995).

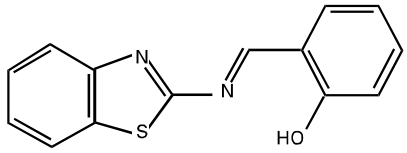
Determination of antiarthritic properties of a novel thiazole derivative (Nishikaku *et al.*, 1994) and several thiazolyl derivatives are reported to act as lipoxygenase inhibitors affecting inflammation and psoriasis (Hadjipavlou-Litina and Geronikaki, 1997). Bonomo *et al.* (1995) reported that Cu(II) complexes cause an oxidative stress in erythrocytes. Antibiotics increase free radicals in cell (Bulkley, 1983). Superoxide radical's scavering from organism by enzymatic dismutation while antioxidant vitamin (A, E and C vitamin) scavering oxygen radicals from organism (Badwey and Karnowsky, 1980).

Because Cu(II) thiazole complex contains biologically active thiazole group (Tortora *et al.*, 1995), it affects the antioxidant level in organisms. Therefore, the effect of Cu(II) thiazole complex on the level of retinol, α–tocopherol, ascorbic acid on Carp tissue was thought to have important result. Thiazoles are important constituents of many biologically active compounds, especially drugs and dyes. These compounds find way to ground water, this in turn may affect living organisms in water. Therefore, it was thought that antioxidant level of the Carp tissue may be affected from thiazole containing compound in water originated from environmental pollution.

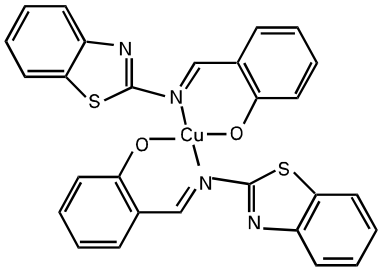
To our knowledge, the effect of Cu(II) on antioxidant levels has not been reported in the literature so far. Therefore, in this work, we have investigated the effects of Cu(II) thiazol on the level of antioxidant vitamins in the tissue of carp.

Materials and Methods

Fish used in this work were caught from Keban Dam Lake in Elazığ, situated south east of Turkey, by fishing cast-net in July 2000. The fish ages were determined from scale (Robert *et al.*, 1990) and they were found to be one years of age. The length and weight of the fish were 11± 2 cm and 40± 7 g, respectively. Four groups were formed with 12 fish in each. The first group of fish was fed with 450 μg ligand /g food (Fig. 1a) and second group, with 500 μg thiazole Cu(II) complex/g food (Fig. 1b) and third group was fed with pellet fish food containing 88.6μg $CuCl_2 . 2H_2O$/g food. The forth group was kept as control and only fed with pellet fish food.



(a)



(b)

Fig. 1: a) 2-hydroxybenzildin-2-aminobenzothiazol (LH) ligand
b) 2-hydroxybenzildin-2-aminobenzothiazol Cu(II) complex

The concentration Cu(II) and Cu(II) complex were chosen below the toxic level which is over 0.3 μg/g (Howarth and Sprague, 1978). It was thought that a fish would consume 1% of its weight (g/day) fish food and food additives. So the

amount of food was adjusted accordingly. To prepare the fish food, the additives were dissolved in ethanol mixed with corn oil and then added to commercially supplied fish food. The pellets were made from this mixture. The fish food for the control group was made in the same way except that this time no additives were introduced. The fish food used in the experiments was supplied from Pınar ™. Feeding continued for 21 days and then fish were killed. Retinol, α–tocopherol, ascorbic acid levels were measured in their muscle.

Sampling procedure was as follows. The carp (*Cyprinus carpio* L.) muscle was homogenized, 1.0 g of sample was then taken to a polyethylene tube and 2.0 ml ethanol was introduced to the muscle sample to precipitate the proteins. After that, it was separated by centrifuge, followed by the addition of 0.3ml hexane, and mixed well, and then vitamins were extracted. This step was repeated again, and n-hexane phase was added to the first one. Under nitrogen atmosphere, n-hexane was evaporated to dryness. Then 0.2 ml methanol was added to solve the residues followed by injection to HPLC. Mobile phase used in HPLC was methanol, acetonitrile and chloroform (47:42:11 v/v). Flow rate was 1.0 ml/min and analysis wavelengths were 296 nm for α–tocopherol and 326 nm for retinol (Miller *et al.,* 1984).

Ascorbic acid was determined by the following procedure: 1.0g fish muscle sample was taken in 0.5 M perchloric acid (0.5 ml) (Cerhata *et al.,* 1994) and then the volume of sample was brought to 5.0 ml, after that it was centrifuged for ten minutes at 4000 rpm. 20 μl of solution was taken from centrifuge and introduced to HPLC, equipped with C-18 column. Mobile phase was a 0.05 M phosphate buffer (pH= 4), flow rate was 1.0 ml/min; absorption wavelength was 246 nm (Tavazzi *et al.,* 1992).

All the chemical reagents were of analytical grade and obtained from Merck Company (Darmstadt, Germany). Double distilled water was used throughout the work. HPLC was performed with CECIL-1100 (Cambridge, England).

The results are statistically analyzed by calculating the Standard Deviation of the mean (mean ± SD). The results are expressed as mean ± SD and values of $p< 0.005$ were accepted as significant.

Results and Discussion

The recovery rates, found for retinol, α–tocopherol and ascorbic acid were 97.8 %, 99.2 % and 96.7 % respectively. Thiazole and benzothiazole can generate oxidative stress *in vivo* (Kim and Cho, 1996), if constantly being subjected to oxidative stress. Aerobic organisms are protected against oxidative damage by a variety of antioxidant systems under normal conditions. The antioxidant system is divided into two groups as enzymatic and nonenzymatic. Nonenzymatic system constitutes antioxidant vitamins. Oxidative damage, however, may occur when antioxidant potential is decreased and when oxidative stress is increased (Ibrahim *et al.,* 2000). It was observed that, the fish feed with 33 μg Cu(II) (added as $CuCl_2$. H_2O to fish food) showed lower amount of antioxidants α–tocopherol, retinol and ascorbic acid as compared with the control group (Table 1). As can be seen from Table 1, the fish supplemented with 450 μg thiazol ligand/g food showed lower α–tocopherol, retinol and ascorbic acid level in comparison with the control group and Cu(II) group.

Table 1 show that the fish supplemented with 500 μg thiazole Cu(II) complex/g food showed the lowest level of antioxidants, α–tocopherol, retinol and ascorbic acid level in comparison with control and other groups.

Table 1: The levels of retinol, α–tocopherol, ascorbic acid in tissue of carp (*Cyprinus Carpio* L.)

	Groups			
	Control	33µg Cu(II) g^{-1} food	450µg Ligand g^{-1} food	500µg complex g^{-1} food
N	12	12	12	12
Length (cm)	12± 1.8	11± 2.4	11± 2.1	12± 1.5
α-Tocopherol (µg/g)	12± 2.1	10± 2.0	8.0± 1.8	6.3± 2.1
Retinol (µg/g)	1.25± 0.22	0.95± 0.18	0.73± 0.26	0.38± 0.20
Ascorbic acid (µg/g)	65± 7.5	57± 5.9	45± 6.6	34± 7.3

This indicated that, antioxidant vitamin levels of fish feed with supplemented feed result in lower level of vitamins in comparison with the control group ($p< 0.005$).

Cupper produces oxidative stress in erythrocytes, causing the production of free radicals (Bonomo *et al.,* 1995). This explains the lower level of antioxidant vitamins determined in this work. Some metabolic effects caused by antibiotic treatment produce free oxygen radical which in turn has a toxic effect on cell membrane, enzymes, nucleic acid and polysaccharides causing tissue deformations (Bulkley, 1983; Wolff *et al.,* 1986).

Antibiotics increase the formation of free radicals in the body (Wolff *et al.,* 1986). It seems that the thiazol ligand acts the same way as antibiotics. The fish fed with the Cu(II) thiazol complex supplemented food showed considerable decrease in the antioxidant vitamin levels compared with the other groups ($p< 0.005$). This is because the complex has both the Cu(II) and thiazol groups which collaborately decrease the antioxidant vitamin levels.

In conclusion, it can be suggested that, Cu(II), thiazol ligand and Cu(II) thiazol complex produce more free radicals and in turn decrease the level of antioxidant vitamins in the tissue of carp. From these results, we suggest that, antioxidant vitamins such as vitamin A, E, and C should be taken along with the medicine containing thiazole group to compensate the vitamin deficiency caused by the drugs.

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