J. Animal and Poultry Prod., Mansoura Univ., Vol. 5 (7): 453-458, 2014

EFFECT OF SOME CHELATING AGENTS ON BALANCE AND TISSUE DISTRIBUTION OF ESSENTIAL TRACE ELEMENTS IN TILAPIA FLORIDA

Ohaida, A. M. I.
Zoology Department. Faculty of Science. University of Misurata Libya.

ABSTRACT

Three chelating agents, being EDTA, thiamin and ascorbic acid were tested for balance and tissue distribution of four essential trace elements, mainly, copper. zinc, iron and manganese, in Tilapia Florida. Essential trace element balance was less affected by thiamin administration. Thiamin produced minimum alterations in the tissue levels of essential trace elements. Alterations produced by EDTA were more pronounced than those occurred with ascorbic acid and thiamin. Copper, zinc and iron levels in all tissues were drastically depleted by EDTA. The present results suggested that thiamin had the best efficacy as a metal chelating agent.
Keywards: Tilapia Florida – Chelating agents – Trace elements.

INTRODUCTION

The therapeutic mechanism of chelating agents involves their interaction with toxic metals leading to their rapid excretion from the body. However, because of their affinity for various metal ions, the potential interaction between these chelating agents and endogenous trace metals is of concern (Cantilena and Klaassen, 1982 and Tandon *et al.,* 1984). Thus the toxic manifestations of chelating agents may partly be the result of their interaction with essential metal which may ultimately lead to various characteristic biochemical and pathological alterations (Sigel, 1983). Therefore, it was considered of interest to evaluate the potential of chelating agents to modify the balance and distribution of essential trace elements. The objective of this study was to compare the acute influences of three chelating agents being, EDTA, thiamin and ascorbic acid on the balance and concentrations of four essential trace elements mainly copper, zinc, iron and manganese in different tissues of Tilapia Florida.

MATERIALS AND METHODS

Materials:

Tilapia Florida were collected from the Tawarga pond at Misurata, Libya, weighing 100- 110 g. The fish were immediately transported after catching to special aquaria in the laboratory filled with aerated fresh water. The water temperature was adjusted thermostatically at 22± 0.05˚C. Continuous aeration through aerators was maintained throughout the experimental period. Commercial fish pellets were used as food. Feeding was stopped 24^{hr} before and during the experimental period.

Treatment:

The acclimated fish were divided into four groups consisting of 32 fish in each. The animals of each of the first three groups received a concentration of 3.8, 3.4 or 0.6 ppm of EDTA, thiamin or ascorbic acid (Sigma), respectively. The concentrations of the chelating agents were

selected because none of the fish died over a period of 14 days due to these levels. The fourth group of fish served as a control. Because the fish were not fed during the experimental period, treatments were terminated after 4 days to preclude potential nutritional stresses.

Metal analysis:

Six surviving animals from each group were selected, sacrificed and analyzed for essential trace elements at 24, 48, 72 and 96^{hr} after treatment. Samples of blood, liver, kidneys and muscles were obtained from each fish. Blood was collected in heparinized vials by heart puncture, and centrifuged at 2500 rpm for 5 min. Serum protein (0.3 ml) was precipitated with 1.5 ml of 6% trichloroacetic acid and metals were determined in the resulting supernatant. The other collected tissues (liver, kidneys and muscles) 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. Metal levels in all types of tissues were determined by atomic absorption analyses using a shimadzw A.A. 630-11 atomic absorption spectrophotometer (Langmyher and Amodt, 1976).

Statistical analysis:

Values are presented as means ± SD of six individual observations. The means of the test and control groups were compared using student's t-test. The results were significant when set at $P<0.05$ (Joan, 1987).

RESULTS

The effects of acute administration of three chelating agents, EDTA, thiamin and ascorbic acid, on the tissue levels of four endogenous essential trace elements, copper, zinc, iron and manganese at various time intervals are shown in Tables 1-4.

Table 1: Comparative influences of EDTA, thiamin and ascorbic acid on blood levels of essential trace elements at 24, 48, 72 and 96^{hr} after treatment .

Chelating agent	Time interval (hr)	Element level (µg/ml)			
		Copper	Zinc	Iron	Manganese
Control		0.18±0.04	0.91±0.15	2.83±0.27	0.06±0.03
EDTA	24	0.16±0.05	0.84±0.27	2.41±0.98	0.10±0.05
	48	0.13±0.09	0.72±0.29	2.53±0.89	0.08±0.02
	72	0.11±0.04*	0.57±0.11*	2.44±0.22*	0.07±0.05
	96	0.11±0.03*	0.59±0.21*	1.72±0.38*	0.05±0.04
Thiamin	24	0.17±0.12	0.89±0.61	2.79±1.11	0.07±0.03
	48	0.16±0.06	0.91±0.58	2.74±1.02	0.06±0.02
	72	0.17±0.10	0.91±0.41	2.80±1.14	0.06±0.01
	96	0.17±0.04	0.87±0.52	2.77±1.06	0.04±0.03
Ascorbic acid	24	0.17±0.08	0.86±0.39	2.59±1.39	0.09±0.06
	48	0.15±0.13	0.89±0.60	2.61±0.86	0.09±0.05
	72	0.14±0.05	0.87±0.11	2.53±0.27	0.06±0.03
	96	0.16±0.15	0.71±0.99	2.03±0.33*	0.08±0.05

*** P<0.05, relative to control**

Among all chelating agents, EDTA produced comparatively more drastic effects on the tissue levels of endogenous essential trace elements. This was indicated by significant depletions in the blood, liver and muscle

levels of copper, zinc and iron (72 and 96 ^{hr}) and renal levels of copper, zinc (24, 48 and 72 ^{hr}) and iron (48, 72 and 96 ^{hr}). The fish treated with thiamin had lesser alterations in the levels of the essential trace elements than those treated with either EDTA or ascorbic acid. Thiamin caused significant depletion of hepatic manganese (48 and 72 ^{hr}) and renal levels of copper, zinc, and iron (48 ^{hr}), but in all cases with the exception of hepatic manganese values returned to normal control levels after 72 ^{hr} of treatment. Ascorbic acid statistically decreased the levels of renal zinc (24 and 72^{hr}), copper (24 and 48^{hr}) and iron (48 and 72^{hr}), blood iron (96^{hr}), hepatic iron, muscle zinc (72^{hr}), muscle iron (96^{hr}) and raised the levels of copper in liver (24 and 72^{hr}) and muscle copper (96^{hr}).

Table 2: Comparative influences of EDTA, thiamin and ascorbic acid on the liver levels of essential trace elements at 24, 48, 72 and 96^{hr} after treatment .

Chelating agent	Time interval (hr)	Element level (ug/g dry weight)			
		Copper	Zinc	Iron	Manganese
Control		11.89±1.06	55.21±4.23	143.16±6.01	2.31±0.09
EDTA	24	11.28±0.98	51.16±3.06	132.73±10.21	2.18±0.18
	48	11.19±2.43	46.81±7.91	133.98±7.46	2.20±0.18
	72	9.21±0.80*	38.37±4.82*	96.09±10.10*	2.25±1.01
	96	7.35±1.14*	33.21±2.21*	92.17±3.93*	2.23±0.30
Thiamin	24	12.25±2.11	54,23±2.72	134.51±9.18	2.24±1.61
	48	12.01±0.89	52.09±1.31	137.32±5.06	1.93±0.27
	72	11.83±1.70	53.16±4.21	138.76±6.53	1.93±0.30
	96	11.30±1.95	49.76±3.81	141.93±0.98	2.05±0.94
Ascorbic acid	24	13.37±0.86*	52.16±7.28	133.41±8.01	2.24±0.87
	48	12.95±1.08	50.35±3.74	134.09±9.63	2.15±1.11
	72	13.33±0.95*	51.61±1.02	129.17±2.73*	2.13±1.09
	96	12.47±0.90	49.91±3.34	133.35±9.91	2.16±0.61

*** P<0.05, relative to control**

Table 3: Comparative influences of EDTA, thiamin and ascorbic acid on the kidney levels of essential trace elements at 24, 48, 72 and 96^{hr} after treatment .

Chelating agent	Time interval (hr)	Element level (ug/g dry weight)			
		Copper	Zinc	Iron	Manganese
Control		5.81±1.10	39.26±0.92	105.93±0.86	1.38±0.27
EDTA	24	3.50±0.84*	30.75±1.01*	104.65±1.33	1.34±0.17
	48	3.16±0.94*	32.43±0.55*	98.35±1.09*	1.31±0.25
	72	2.92±0.19*	29.60±0.63*	97.49±2.18*	1.30±0.09
	96	4.37±1.16	37.19±1.91	100.22±2.89*	1.28±0.32
Thiamin	24	4.69±0.80	38.09±1.99	105.13±2.11	1.36±0.34
	48	4.13±0.64*	36.94±1.11*	99.81±1.14*	1.35±0.61
	72	4.97±1.59	37.28±1.96	103.93±2.23	1.33±0.11
	96	5.16±1.60	38.99±2.12	105.70±2.12	1.37±0.29
Ascorbic acid	24	3.48±0.90*	35.86±0.71*	104.39±1.37	1.32±0.21
	48	3.55±0.67*	37.78±1.78	100.91±0.80*	1.33±0.44
	72	4.38±1.09	35.19±2.05*	100.85±1.46*	1.35±0.73
	96	4.39±1.03	37.98±1.09	103.98±2.11	1.30±0.34

***P<0.05, relative to control**

Table 4: Comparative influences of EDTA, thiamin and ascorbic acid on the muscle levels of essential trace elements at 24, 48, 72 and 96^{hr} after treatment .

Chelating Agent	Time interval (hr)	Element level (ug/g dry weight)			
		Copper	Zinc	Iron	Manganese
Control		7.16±0.88	34.13±1.33	31.08±2.95	0.31±0.54
EDTA	24	6.52±0.62	36.18±2.04	28.78±3.15	0.25±0.73
	48	6.70±0.67	31.93±2.10	30.06±1.36	0.25±0.11
	72	5.39±0.13*	24.38±2.23*	19.28±3.19*	0.30±0.01
	96	4.17±0.80*	24.01±3.25*	23.51±3.41*	0.27±0.21
Thiamin	24	7.11±0.92	34.01±2.39	30.12±3.25	0.31±0.03
	48	6.99±0.34	34.12±3.12	31.19±4.79	0.31±0.13
	72	7.15±1.14	32.45±1.94	30.06±1.03	0.30±0.15
	96	7.10±1.55	33.07±3.41	27.98±1.56	0.27±0.11
Ascorbic acid	24	6.89±0.41	33.41±1.13	29.11±1.36	0.27±0.13
	48	6.91±1.28	33.25±2.83	29.63±1.06	0.26±0.05
	72	8.67±3.11	28.71±1.12*	29.81±3.17	0.29±0.16
	96	10.13±1.02*	32.93±1.15	25.29±1.85*	0.28±0.13

*** P<0.05, relative to control**

DISCUSSION

Comparison of the data of the present study reveals that trace element balance was greatly affected by EDTA followed by ascorbic acid, while it is less affected by thiamin. These chelating agents either individually or in combination have been proven to be effective in promoting the excretion of toxic metal ions from the body organs (Chisolm, 1978; Bratton *et al .,*1981; Tandon and Flora, 1989; Ghazaly, 1991and Misra *et al.,* 2008). The efficacy of the chelating agents to remove metals from the biological system depends on their ability to form stable complexes with the toxic metal ions and to enhance their excretion from the body without affecting the levels of essential trace elements (Misra *et al.,* 2008). The high affinity of EDTA for copper, zinc and iron may be due to the high stability constants of copper, zinc and iron-EDTA complexes in solution. This could explain the cause for the depletion of tissue copper, zinc and iron levels after EDTA administration. These data are inconsistent with those of Misra *et al*. (2008) who reported that EDTA has no effect on the trace metal content in rats.

The effect of EDTA was more pronounced than that of thiamin or ascorbic acid in depleting tissue levels of essential trace elements. Among the chelating agents evaluated in the present study, thiamin exhibited least potential to interact with endogenous trace elements as evidenced by nonaltered levels of the trace elements in the tissues. The low affinity of thiamin for the metals investigated herein may be due to the low stability constant of metal-thiamin complex in solution. The changes observed in the tissue metal concentrations of Tilapia Florida after acute administration of the chelating agents points to the best efficacy of thiamin. Dhawan *et al.* (2010) observed that thiamin did not cause any major reduction in body metal content, but a combination of thiamin and ascorbic acid improved the ability of animals to excrete metals, thereby reducing the body metal content. Similarly, Bratton *et al.* (1981) found that thiamin had no effect on the trace

metal content of plasma in cattle. In contrast, Flora *et al.* (1986); Tandon *et al.* (1984) and Tandon *et al.* (1987) observed that thiamin is effective in reducing the metal content. Analogues effects of ascorbic acid were reported by Calabrese and Kamp (1985). The present results seem to support the hypothesis that chelating agents may alter trace metal metabolism and are in general, consistent with those noted previously for other species (Torronen and marselos, 1978; Plana-Bohne, 1979; Milne and Omaye, 1980; Kostniak and Clarkson, 1981; Solecki *et al.,* 1984 and Tandon *et al.,* 1984).

REFERENCES

Bratton, G. R.; Zmudzki, J.; Bell, M. C. and Warnock, L. G. (1981): Thiamin (vitmminB$_1$) effects on lead intoxication and deposition of lead in tissues. Theraptutic potential. Toxicol. Appl. Pharmacol., 59: 164-172.

Calabesese, E. D and Kamp, J. (1985): The effects of ascorbie acid supplementa lion on copper-induced oxidative changes in human erythrocytes. J. Environ. Sci. Health, 20 (2):239-250.

Cantilena, L. R. Jr. and Klaassen, C. D. (1982): Effect of chelating agents on the excretion of endogenous metais. Toxicol. Appl. Pharmacol., 63 : 344-350.

Chisolm, J. J. (1978) : The use of chelating agents in the treatment of acute and chronic lead intoxication in childhood. J. Pediat., 73: 1-38.

Dhawan, M.; Kachru, D. N. and Tandon, S. K. (2010): Influence of thiamin and ascorbic acid supplementation of the antidotal efficacy of thiol chelators in experimental lead intoxication. Arch. Toxicol., 62:301-305.

Flora, S. J. S.; Singh, S. and Tandon, S. K. (1986): Chelation in metal intoxication XVIII: Combined effects of thiamin and calcium disodium versenate on lead toxicity. Life Sci., 38: 67-71.

Ghazaly, K. S. (1991) : Influences of thiamin on lead intoxication, lead deposition in tissues and lead hematological responses of *Tilapia zillii.* Comp. Biochem. Physiol., 100C: 417-421.

Joan, F. B. (1987): "Guinness, Gosset, Fisher, and Small Samples". Statistical Science 2 (1): 45–52.

Kostyniak, P. L. and Clarkson, T. W. (1981): Role of chelating agents in metal toxicity. Fund. Appl. Toxicol., 1: 376-380.

Langmyher, F. J. and Amodt, J. (1976): Atomic absorption spectrophotometric determination of some metals. Analytica Chemiea Acta, 78: 483-486.

Milne, D. B. and Omaye, T. (1980) : Effect of vitamin C on copper and iron metabolism in the guinea pig. Int. J. Vitam. Nutr. Res., 50: 301-308.

Misra, M,; Athar, M.; Hasan, S. K. and Srivastave, R. C. (2008): Comparative effects of chelating drugs on trace metal and biochemical alterations in the rat. Bull. Environ. Contam. Toxicol., 41 : 172-184.

Planas-Bohne, F. (1979): Influence of several chelating agents on the exoretion and organ concentration of copper in the rat. Toxicol. Appl. Pharmacol., 50:337-345.

Sigel, H. (1983) : Zinc and its role in biology and nutrition. In: Metal Ions in Biological System, Marcel Dekker, New York, Vol. 15

Solecki, J. J.; Aviv, A. and Bogden, J. D. (1984): Effect of a chelating drug on balance and tissue distribution of four essential metals. Toxicol., 31 : 207-216.

Tandon, S. K. and Flora, S. J. S.(1989) : Therapeutic efficacy of dimercaptosuccinic acid and thiamin / ascorbic acid on lead intoxication in rats. Bull. Environ. Contam. Toxicol., 43 : 705-712.

Tandon, S. K.; Flora, S.J.S. and Singh, S. (1987) : Chelation in metal intoxication XXIV influence of various components of V vitamin B complex on therapeutic efficacy of disodium calcium versenate in lead intoxication. Pharmacol. Toxicol., 60 : 62-67.

Tandon, S. K.; Jain, V. K. and Mathur, A. K. (1984) : Effect of metal chelators on excretion and tissue levels of essential trace elements. Environ. Res., 35 : 237 – 245.

Torronen, R. and Marselos, M. (1978): Changes in the hepatic copper content after treatment with foreign compounds. Fin. Arch. Toxicol., 40: 247-249

تأثير بعض العوامل علي توازن وانتشار العناصر النادرة الفعالة في أنسجة اسماك البلطي (تلابيا فلوريدا)

عبد السلام محمد ابريك اوحيده

قسم علم الحيوان - كلية العلوم- جامعة مصراتة – ليبيا

اختبرت تأثيرات ثلاثة مخلبيات (الإدتا، والثيامين, وحمض الأسكوربيك) على توازن وانتشار أربعة من العناصر النادرة الفعالة (النحاس والزنك والحديد والمنجنيز) في أنسجة أسماك البلطي (تيلابيا فلوريدا). وقد تأثر توازن العناصر النادرة الفعالة بالثيامين تأثيرا أقل من غيره. إذ أحدث هذا العامل القانص أقل تغيرات في معدلات الأنسجة من هذه العناصر. وفي المقابل كانت التغيرات الحادثة من جراء استخدام الإدتا أكثر تاثيرا من تلك الناتجة عن استخدام أي من حمض الأسكوربيك أو الثيامين . اذ سببت الإدتا تناقصا كبيرا في معدلات النحاس الحالية أن الثيامين له فعالية أو كفاءة الادمصاص الأفضل من بين العوامل المخلبية الثلاثة المختبرة.