

“EFFECT OF CHELATORS ON CALCIUM AND POTASSIUM CONTENT OF FRESH WATER BIVALVE, *PARREYSIA CORRUGATA*”

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ABSTRSCT:

Chronic exposure of fresh water bivalve, *Parreysia corrugata* to the chelating agents CaNa₂- EDTA and L- cysteine adversely affect the calcium and potassium content of different body parts. *Parreysia corrugata* were exposed to 40 mg/L concentrations of CaNa₂- EDTA and 50 mg/L concentration of L- cysteine for 8 and 15 days. There was decrease in calcium and potassium content of whole tissues and mantle due to Ca Na₂ EDTA and L-cysteine treatment. The depletion in potassium content is less than that of calcium in whole tissue as well as mantle of the *P. corrugata*. The depletion in the calcium and potassium is possibly, due to formation and elimination of their complexes with the chelators Ca Na₂ EDTA and L- cysteine.

INTRODUCTION:

Chelating agents are used to detoxify poisonous metal agents such as, mercury, arsenic, lead, zinc etc. The use of chelating agents in the treatment is approved by the U.S. Food and Drug Administration in 1991. Chelation is also used in the treatment of cardiovascular disorders like arteriosclerosis, atherosclerosis, etc. Although, chelating agents are significantly important in the treatment of heavy metal poisoning, they are life threatening also. According to U.S. CDC report (2006), use of disodium EDTA instead of calcium EDTA has resulted in fatalities due to hypocalcemia. Many investigators studied effect of chelating agents on metabolism and excretion of various minerals in patients (Leo Lutwak, 1964; Herta Spencer et.al., 1952; Premysl Ponka et.al, 1979; Leo S Jensen and Frank R Mraz, 2011). Chelation is the formation or presence of separate coordinate bonds between chelating agents and a single central atom. The chelating agents or chelants are the organic compounds. The chelating agent forms a chelate complex with the substrate. Chelants, according to ASTM-A-380, are "chemicals that form soluble, complex molecules with certain metal ions. The word chelation is derived from Greek chelè, meaning claw; the ligands lie around the central atom like the claws of a lobster.

All chelators seem to remove some vital minerals from the body along with the toxins and these are not so easily replaced. EDTA pulls heavy metals out of the body. On its way out of the body, it removes metals and minerals from the body and excreted through the kidneys, hence there is a

possibility of kidney damage. Minerals like calcium, potassium, sodium, phosphorous are physiologically important minerals in the body. Elevations or depletions of these important minerals can cause problems and/ or even death. Preserving constant potassium level in the blood and cells is essential to body function. Massey and Whiting (1993) reported that, oral doses of caffeine increase the urinary excretion of calcium, magnesium, sodium and chloride for at least 3 hrs. after consumption. Allain P et.al. (1991) studied effect of infusion on the urinary elimination of several elements in healthy subjects. The ratio of the increase of urinary elimination induced by EDTA Ca Na₂ was about 2 for Fe, 5 for Al, Pb & Mn and 15 for Zn. Clarke et. al. (1956) reported that, patients with occlusive peripheral vascular disease said they felt better after treatment with EDTA. Leo Lutwak (1964) reported increased loss of minerals from the body of patient due to infusion of disodium EDTA.

MATERIALS AND METHODS:

The fresh water bivalves, *Parreysia corrugata* were exposed to 40 mg/L concentrations of CaNa₂-EDTA and 50 mg/L concentration of L- cysteine. One group of bivalves was maintained as a control. The exposure of bivalve to each chelator was continued for 8 and 15 days. Bivalves from each experimental group were sacrificed to obtain whole tissue and mantle. These tissues were dried in oven at 80 °C. and blended into dry powder. 1 gram of dry tissue powder was mixed with 10 ml of acid mixture (9:4 mixtures of Nitric acid and Perchloric acid). The content was then heated to dissolve solid particles until the clear and colourless solution was obtained and the volume reduced to 3 - 5 ml. The content was allowed to cool and then diluted to 100 ml with deionized water and kept overnight. The content was filtered and the filtrate was used for estimation of calcium and potassium with atomic absorption spectrophotometry. Amount of calcium and potassium in tissues was calculated and expressed as mg per gram of dry tissue.

OBSERVATIONS AND RESULTS:

Alteration in mineral content of whole tissue, mantle and foot of freshwater bivalve, *Parreysia corrugata* after exposure to chronic doses of chelators, Ca Na₂ EDTA and L- cysteine for 8 and 15 days are summarized in table No.1 and 2.

The calcium content in whole tissue and mantle of bivalves in control group was 24.3155 mg and 25.6143 mg/ g of dry tissue respectively for 8 days and 23.971 mg and 22.321 mg/ g of dry tissue respectively for 15 days. Calcium content in whole tissue and mantle of bivalves exposed to L- cysteine (50 mg/L) was 22.7475 mg and 23.6985 mg/g of dry tissue respectively after 8 days exposure and it was 21.6985 mg and 20.5697 mg/g of dry tissue respectively after 15 days exposure.

In the bivalves exposed to both the chelators viz. Ca Na₂ EDTA (40 mg/L) and L- cysteine (50 mg/L) the calcium content in whole tissue and mantle was lowered than that of bivalves in control group. The potassium content in whole tissue and mantle of bivalves in control group was 10.7465 mg and 8.4129 mg/g of dry tissue respectively for 8 days and 10.2017 mg and 8.2561 mg/g of dry tissue

respectively for 15 days. There was depletion in the potassium content of bivalves treated with Ca Na_2 EDTA (40 mg/L) and L- cysteine (50 mg/L) for the same period of 8 and 15 days. The depletion in potassium content is less than that of calcium in whole tissue as well as mantle of the *P. corrugata*.



Table -1: Effect of Ca Na₂ EDTA and L- cysteine on Calcium content in whole tissue and mantle of fresh water bivalve, *Parreysia corrugata*.

Sr. No.	Tissue	Control		Ca Na ₂ EDTA(40 mg/ L)		L – Cysteine (50 mg/ L)	
		8 days	15 days	8 days	15 days	8 days	15 days
1	Whole tissue	24.3155 ± 0.229	23.971 ± 0.255	22.2842 ± 0.415 (-8.35)**	21.2473 ± 0.305 (- 11.36)*	22.7475 ± 0.531 (-6.44)NS	21.6985 ± 0.255 (- 9.48)NS
2	Mantle	25.6143 ± 1.274	22.321 ± 0.813	23.652 ± 1.046 (- 7.66)NS	20.4195 ± 0.935 (- 8.51)NS	23.6985 ± 0.333 (- 7.47)NS	20.5697 ± 1.106 (- 7.84)NS

1. Values are expressed as mg/g of dry tissue
2. + or – percent variation over control
3. ± indicates S.D. of three observations

Table - 2: Effect of Ca Na₂ EDTA and L- cysteine on Potassium content in whole tissue and mantle of fresh water bivalve, *Parreysia corrugata*.

Sr. No.	Tissue	Control		Ca Na ₂ EDTA(40 mg/ L)		L – Cysteine (50 mg/ L)	
		8 days	15 days	8 days	15 days	8 days	15 days
1	Whole tissue	10.7465 ± 0.408	10.2017 ± 0.578	8.7691 ± 0.772 (-18.40)*	8.7495 ± 0.392 (-14.23)*	9.5895 ± 0.567 (-10.76)*	9.0742 ± 0.756 (-11.05)NS
2	Mantle	8.4129 ± 0.435	8.2561 ± 0.554	7.8573 ± 0.652 (-6.60)NS	7.3165 ± 0.472 (-11.38)NS	7.8572 ± 0.334 (-6.60)NS	7.1869 ± 0.894 (-12.95)NS

1. Values are expressed as mg/g of dry tissue
2. + or – percent variation over control
3. ± indicates S.D. of three observations

DISCUSSION:

The chelating agents like EDTA when administered in the blood stream, chelates metals and minerals from the body on its way out. EDTA also facilitates removal of inappropriate deposition of calcium from tissue. Calcium moves to atherosclerotic plaque in blood vessels, leading to arterial narrowing and blockage. Chelation gently and gradually mobilizes calcium from plaque, restoring elasticity and flow to blood vessels (Ronald L. Hoffman). Hugh D. Riordan et. al., (1990) studied excretion of 11 minerals induced by EDTA chelation therapy in 25 adults. The observations showed, 7-fold increase in mean 24 hrs. excretion for lead and cadmium and nearly 2 – fold increase in aluminum after the first chelation. The calcium excretion was increased by 2 – fold. Thus EDTA infusions increase urinary excretion of all toxic and essential minerals. Juan M. Llobert et. al. (1986) studied comparison of effectiveness of several chelators after single administration on the toxicity, excretion and distribution of cobalt and found the significant increase in urinary excretion of cobalt in male swiss mice after the administration of EDTA, DPTA, L –cysteine, NAC, glutathione and D, L – PEN. EDTA reported to be the most effective agent of those tested in the prevention of acute cobalt intoxication. Allain P et. al. (1991) studied effect of an EDTA infusion on the urinary elimination of several elements like Al, Ba, Cu, Fe, Mn, Sr, Zn, Na, K, Ca, Mg, S and P in healthy subjects and found increased urinary elimination of these elements. Mamduh Sifri et. al. (1978) found greater mortality in chick receiving low Ca diet and feeding on Na₂ EDTA. Probably the excess calcium was complexed with Na₂ EDTA and was excreted rapidly in the complex form (Mamduh Sifri et. al.1978). Funda Kont et. al. (2011) studied effect of chelating agents on the mineral content of root canal dentin and reported significant decrease in Ca level due to paracetic acid, citric acid and EDTA. Leo and Frank (1966) reported significant depression in bone ash and growth rate of chicks fed with basal diet and then supplemented by chelating agents EDTA, hydroxyethylethylenediaminetriacetic acid (HEDTA), nitrilotriacetic acid (NTA), (2-hydroxyethylimino) diacetic acid (HEIDA), 8-hydroxy 5-quinoline-sulfonic acid (HOS) and glutamic acid (GA). None of the chelating agent used in this study improved bone calcification over that obtained with the basal diet.

Present study showed lowering of the mineral content in whole tissue and mantle of freshwater bivalve, *P. corrugata*. There was decrease in calcium and potassium content in both the tissues due to Ca Na₂ EDTA and L-cysteine treatment. The depletion in potassium content is less than that of calcium in whole tissue as well as mantle of the *P. corrugata*. The chelators with their ligands which are organic compounds; form complexes with substrate. The depletion in the calcium and potassium is possibly, due to combinations of chelators with minerals like calcium and potassium and their elimination in the form of complexes from the body tissues along with the

chelators Ca Na₂ EDTA and L- cysteine. EDTA is massively used worldwide with household and industrial applications. It is released in the water bodies through waste waters. EDTA behaves as a persistent substance in the environment (Claudia Oviedo, Jaime Rodriguez, 2003). Persistent existence of such chelating agents in the water bodies is hazardous for aquatic life like molluscs. As these chelants remove vital minerals like Ca, K, etc. from the body which may lead to deficiency of calcium in animals like bivalves resulting in abnormal metabolism and impairment in the development of their shells.

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