

## Inorganic Elements Alteration in *Biomphalaria alexandrina* Snails Naturally Parasitized with *Echinostoma liei* or *Schistosoma mansoni*

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### ABSTRACT

Calcium, potassium, magnesium and sodium are inorganic ions which play important role in the life of gastropod animal *Biomphalaria alexandrina*, the intermediate host of *Schistosoma mansoni* and *Echinostoma liei* in Egypt. The present study identified the effect of the previous two parasites on the concentration of inorganic ions mentioned before with special concern to hypercalcification hypothesis of the shell of infected snails. Flame atomic absorption spectrometry was used to study the concentration of such ions in the soft parts and shells of *B. alexandrina*, naturally parasitized with *S. mansoni* or *E. liei*, collected from Kafer Alsheikh and Menofia Provinces, Egypt. Alterations in the concentration of such elements were observed due to the infection with *S. mansoni* or *E. liei* within shells and soft parts. The hypercalcification hypothesis was not upheld in all of the snail-larval digenean system studied here and there was no fixed pattern for inorganic ions alterations in snails infected with digenean trematode and such pattern was variable according to trematode parasite infecting the snails and the habitat of the snails.

**Key words:** Flame atomic absorption spectrometry, *Schistosoma*, *Echinostoma*, *Biomphalaria alexandrina*, hypercalcification, inorganic elements, kafer alsheikh province, menofia province, Egypt

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### INTRODUCTION

Inorganic ions play an important role in the life of mollusks. Calcium and sodium play an essential role in the nerve-muscle transmission; Ca (HCO<sub>3</sub>)<sub>2</sub> functions as a buffer in the haemolymph<sup>1</sup>. The phagocytic activity of hemocytes and lectins cooperating in defense reactions depends on the presence of calcium ions in hemolymph<sup>2</sup>. Mishkin and Jokinen<sup>3</sup> reported that environmental calcium has a positive effects on the fecundity and cercarial production of *B. glabrata* infected with *S. mansoni*. Moreover, large amount of calcium are used in the reproduction of the snails<sup>4</sup>. Magnesium plays a fundamental role in the regulation of many cellular functions such as protein synthesis and enzyme activation. Amongst the enzymes in which Mg<sup>2+</sup> acts as an essential co-factor are those concerned with glycolysis, respiration and membrane transport processes, e.g. Na<sup>+</sup>

and Ca<sup>2+</sup> pumps<sup>5</sup>. Na<sup>+</sup>/K<sup>+</sup>-ATPase has a critical vital function in the maintenance of plasma membrane potential difference in all animal cells, pumping Na<sup>+</sup> and K<sup>+</sup> against their concentration gradients to maintain high sodium levels outside cells and high potassium inside. The pump consumes a great deal of energy; for example, in resting endotherms it is responsible for 5-40% of total ATP consumption, depending on cell type<sup>6</sup>.

The disturbance occurred in the metallic ion concentrations in the snails infected with trematode a larva was considered as one of the causes of alterations occurred in the biological activities and the increase in the mortality of the infected snails<sup>7,8,9,10</sup>.

Alteration in the calcium content was in the focus of interest of many authors due to its importance in the life of mollusks and the hypothesis of hypercalcification; that is, the increase in the calcium content of the shell of their snail hosts due to larval trematodes induce was discussed by several investigators<sup>9,10,11,12,13,14,15,16</sup>.

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The present study aimed to study the changes in the concentration calcium, potassium, magnesium and sodium in soft parts and shells of *Biomphalaria alexandrina* naturally infected with *Schistosoma mansoni* or *Echinostoma liei* and to examine the hypothesis of hypercalcification in shells of infected snails.

## MATERIALS AND METHODS

*Biomphalaria alexandrina* snails were collected from Nile River at Kafer Alsheikh and Menofia Provinces, Egypt and transferred to Medical Malacology laboratory at Theodor Bilharz Research Institute (TBRI), Egypt. Snails were examined immediately for trematode infection by exposing them to artificial light to induce cercarial shedding. Shedding snails were isolated and kept at -20°C until used in analysis. The remaining snails were crushed and examined under binocular microscope; snails free from any trematode larval stages were isolated and kept at -20°C until used for comparison with infected snails from the same field. Non-infected, lab bred *B. alexandrina* snails were obtained from Medical Malacology laboratory at TBRI and used for comparison with non-infected, field collected snails. The diameter of the shells used in the present study was ranged from 5-8 mm.

Soft parts were separated from shells and both were rinsed, at least, three times with deionized water. Excess water was removed from the specimens by using filter papers. Three pools of soft parts and shells from: snails shedding *S. mansoni* cercariae, snails shedding *E. liei* cercariae, non-infected, field collected snails and non-infected, lab bred snails were prepared for analysis. Wet-weighted samples were digested in 10 mL of concentrated nitric acid by boiling to dryness. The residue from each digested sample was diluted to 25 mL with deionized water in a volumetric flask.

Elemental analysis by flame atomic absorption spectrometry using Perkin-Elmer model 3100 AAS was performed to determine the concentration of heavy metals Al, Cd, Cu, Fe, Mn, Pb and Zn in the soft parts and shells of the snails. The flame wavelength and sample aspiration rate were optimized according to the manufacturer's recommendations and four aqueous standards having analytic concentrations within the linear response range of the instrument and containing the same concentration of nitric acid as the samples were used for calibration. Each sample, standard and blank, was analyzed using three 10 sec integrations. The reagent blank was prepared and its value was subtracted

to give the final concentration. The final element concentration (C) was calculated according to the following equation:

$$C = \frac{F \times V}{wt \times 1000}$$

where, F is the standard factor calculated from the standard curve, V is the volume of sample and wt is the wet weight of sample. Data of potassium, magnesium and sodium are expressed in micrograms of element per gram of wet tissue and data of calcium are expressed in percentage of weight of wet tissue. Results were subjected to one-way ANOVA test followed by post hoc Duncan test using SPSS program version 8 to determine the significant of data.

## RESULTS

In comparison with lab-bred snails, the Ca content in non-infected snails collected from Kafer Alsheikh province was insignificantly higher; in contrast it was significantly lower in snails collected from Menofia province. Generally, Ca concentration was significantly higher in shell than soft parts regardless the status of snails. In comparison with non-infected, field collected snails, the infection with *E. liei* leads to increase of Ca content in the shells of snails collected from Kafer Alsheikh and Menofia. However, the infection with *S. mansoni* leads to insignificant decrease of the same element in the shell if compared with non-infected, field collected snails (Table 1).

The concentrations of K and Mg were significantly higher in soft parts than shells in all tested snails. However, the total concentration of the two elements was significantly higher in non-infected, lab-bred than in non-infected, field-collected ones. Na concentration was significantly higher in the shell than soft part in all snails examined. In non-infected snails, the total concentration of Na was higher in field collected snails than in lab-bred snails (Table 2).

In comparison with non-infected, field collected snails, infection with *E. liei* leads to significantly increase in the concentrations of K, Mg and Na elements in snails collected from Kafer Alsheikh and Menofia provinces. On other hand, in snails infected with *S. mansoni*, K<sup>+</sup> and Na<sup>+</sup> concentrations were significantly lower when compared with non-infected, field collected snails and Mg concentration was significantly higher when compared with the same snails.

Table 1: Alteration in the concentration of Ca ion (%) in non-infected and trematode-infected *Biomphalaria alexandrina* collected from Kafer Alsheikh and Menofia provinces (Egypt) compared with Lab bred non-infected snails

province	Lab bred non-infected snails			Non-infected, field collected snails			<i>Echinostoma liei</i> infected snails			<i>S. mansoni</i> infected snails		
	Total	Soft parts	Shell	Total	Soft parts	Shell	Total	Soft parts	Shell	Total	Soft parts	Shell
Kafer Alsheikh	13.00±0.57 <sup>b</sup>	1.21±0.12 <sup>a</sup>	11.79±0.69 <sup>b</sup>	13.55±0.71 <sup>b</sup>	0.31±0.02 <sup>b</sup>	13.24±0.71 <sup>b</sup>	16.31±0.35 <sup>a</sup>	1.46±0.15 <sup>a</sup>	14.85±0.50 <sup>a</sup>	12.69±0.41 <sup>b</sup>	0.45±0.10 <sup>b</sup>	12.24±0.43 <sup>b</sup>
Menofia	13.00±0.57 <sup>b</sup>	1.21±0.12 <sup>b</sup>	11.79±0.69 <sup>b</sup>	11.1±0.22 <sup>c</sup>	3.11±0.10 <sup>a</sup>	7.99±0.29 <sup>c</sup>	23.44±0.46 <sup>c</sup>	1.32±0.18 <sup>b</sup>	22.12±0.64 <sup>a</sup>	-	-	-

Value are expressed as Mean ± S.E., Values are not sharing superscripts letters differ significantly at p < 0.05

Table 2: Alteration in the concentration of K, Mg and Na ions in non-infected and trematode-infected *Biomphalaria alexandrina* collected from Kafer Alsheikh and Menofia provinces (Egypt) compared with Lab bred and field collected, non-infected snails

Province	Lab bred non-infected snails			Non-infected, field collected snails			<i>Echinostoma liei</i> infected snails			<i>S. mansoni</i> infected snails		
	Total	Soft parts	Shell	Total	Soft parts	Shell	Total	Soft parts	Shell	Total	Soft parts	Shell
Kafer Alsheikh	72.81 ± 0.68 <sup>b</sup>	553.65 ± 2.35 <sup>f</sup>	626.46 ± 1.66 <sup>f</sup>	67.16 ± 1.82 <sup>e</sup>	129.22 ± 0.64 <sup>d</sup>	196.38 ± 1.12 <sup>e</sup>	127.2 ± 1.84 <sup>d</sup>	264.5 ± 2.14 <sup>b</sup>	391.7 ± 0.29 <sup>b</sup>	67.79 ± 1.61 <sup>e</sup>	107.52 ± 1.32 <sup>d</sup>	175.31 ± 0.28 <sup>f</sup>
Mg	24.63 ± 0.41 <sup>c</sup>	267.99 ± 1.72 <sup>e</sup>	292.62 ± 1.31 <sup>e</sup>	29.4 ± 0.81 <sup>b</sup>	196.26 ± 4.20 <sup>d</sup>	225.66 ± 3.40 <sup>e</sup>	68.11 ± 1.79 <sup>c</sup>	231.04 ± 3.44 <sup>b</sup>	299.15 ± 1.64 <sup>d</sup>	25.25 ± 0.72 <sup>e</sup>	218.68 ± 1.62 <sup>e</sup>	248.93 ± 0.91 <sup>f</sup>
Na	424.03 ± 2.32 <sup>e</sup>	265.82 ± 2.99 <sup>f</sup>	689.85 ± 1.6 <sup>e</sup>	580.33 ± 3.07 <sup>b</sup>	239.59 ± 1.96 <sup>d</sup>	819.92 ± 5.04 <sup>b</sup>	655.79 ± 2.43 <sup>a</sup>	608.31 ± 4.79 <sup>a</sup>	1264.1 ± 2.36 <sup>a</sup>	422.41 ± 4.85 <sup>e</sup>	276.08 ± 1.39 <sup>b</sup>	689.55 ± 3.45 <sup>e</sup>
Menofia	72.81 ± 0.68 <sup>b</sup>	553.65 ± 2.35 <sup>f</sup>	626.46 ± 1.66 <sup>f</sup>	90.48 ± 2.00 <sup>c</sup>	117.38 ± 1.21 <sup>c</sup>	207.86 ± 0.79 <sup>f</sup>	77.58 ± 0.62 <sup>b</sup>	150.97 ± 1.74 <sup>c</sup>	228.58 ± 1.13 <sup>c</sup>	-	-	-
Mg	24.63 ± 0.41 <sup>b</sup>	267.99 ± 1.72 <sup>a</sup>	929.62 ± 1.31 <sup>a</sup>	34.6 ± 0.81 <sup>a</sup>	127.18 ± 2.31 <sup>c</sup>	161.78 ± 1.50 <sup>f</sup>	22.48 ± 1.43 <sup>b</sup>	148.33 ± 2.92 <sup>b</sup>	170.81 ± 1.49 <sup>b</sup>	-	-	-
Na	424.03 ± 2.32 <sup>e</sup>	265.82 ± 2.99 <sup>f</sup>	689.85 ± 0.66 <sup>e</sup>	493.44 ± 5.45 <sup>b</sup>	296.33 ± 2.49 <sup>b</sup>	789.99 ± 2.95 <sup>b</sup>	667.94 ± 3.49 <sup>e</sup>	311.37 ± 0.94 <sup>a</sup>	979.31 ± 2.55 <sup>a</sup>	-	-	-

Value are expressed as Mean ± S.E.; Values are not sharing superscripts letters (a, b, c, d) differ significantly at p < 0.05

## DISCUSSION

In the present investigation, the calcium content of non-infected snails collected from Kafer Alsheikh province was significantly higher than that in lab-bred, non-infected snails and the contrast is true for snails collected from Menofia province. Such changes may be due to the calcium content in the water from which snails collected, such suggestion was supported by Zblkowska<sup>14</sup> who found no differences in the calcium carbonate concentrations in *Lymnaea stagnalis* naturally infected with digenean larvae except in a case where the infected snails came from a lack with a low calcium concentration; such author suggested that the calcium content of water was responsible for calcium carbonate concentration in the snails rather than the presence of digenean larvae within it. Moreover, Young and Harris<sup>17</sup> reported that reduction of calcium concentration in water can reduce the occurrence of the snails in aquatic system.

In the present work, calcium content was significantly higher in the shells than in the soft parts of the snails, regardless infected or non-infected. This observation was correlated with that of White *et al.*<sup>15</sup> who mentioned that under conditions of variable Ca concentrations in the water and trematode parasitism, pulmonate snails are able to maintain a high concentration of CaCO<sub>3</sub> in their shells.

Hypocalcification observed in the shells of *S. mansoni*-infected *B. alexandrina* snails in the present study was correlated with the observation of White *et al.*<sup>15</sup> on their study on the effect of *S. mansoni* on *Heliosoma trivolvis*, *B. glabrata* and *Physa* sp. and with the observation of Mostafa<sup>16</sup> on his study on *B. alexandrina* and *Bulinus truncatus* snails shedding *S. mansoni* and *S. haematobium* cercariae respectively<sup>17</sup>. In addition, Mostafa<sup>9</sup> observed a hypocalcification in *L. natalensis* infected with *Fasciola gigantica*. The cercariae of *S. mansoni* sequester large amounts of calcium in their pre-acetabular glands and such sequestration probably occurs at expense of calcium in the shell and haemolymph of the snail<sup>18</sup>. Therefore, we can suggest that hypocalcification observed in the shells of *S. mansoni*-infected *B. alexandrina* snails in the present study may be due to the cercariae within that snails utilized large amount of calcium which may be compensated by calcium from the shell and in contrast the hypercalcification noted in the shells of *E. liei*-infected *B. alexandrina* snails may be due to the *E. liei* cercariae within that snails utilized small amount of calcium. Davies and Erasmus<sup>19</sup> reported that *B. glabrata* containing the larval stages of *S. mansoni* at 40 days post infection show disintegration of the calcareous corpuscles in Type-A calcium cells and erosion of the inner surface of the shell.

The hypercalcification observed in the shells of snails infected with *E. liei* was in agreement with several authors. Mazuran *et al.*<sup>4</sup> reported that large amounts of calcium were used in reproduction of snails; the inhibition of reproduction caused by the parasites could affect calcium distribution, leading to its deposition in the shells and mantle<sup>12</sup>.

Thus we can report that the hypercalcification hypothesis was not upheld in all of the snail-larval digenean system studied here. The present findings have not validated the generalization of hypercalcification hypothesis of snail shells due to infection by parasites.

In the present investigation, infection of *B. alexandrina* snails with *E. liei* lead to increase in the concentration of K, Mg and Na in the soft parts of snails collected from both Kafer Alsheikh and Menofia provinces; this was in agreement with Ong *et al.*<sup>20</sup> in their study of the effects of *S. mansoni* infection on *B. glabrata* snails. However, the concentration of Mg and Na in the soft parts of *B. alexandrina* infected with *S. mansoni* was increased agreement with Ong *et al.*<sup>20</sup> but the concentration of K was decreased in snails collected from Kafer Alsheikh. In addition Layman *et al.*<sup>21</sup> reported that Na was present in the digestive gland-gonad complex at higher concentration in infected snails relative to the uninfected snails. However, these results are in contrast to the findings of Evans *et al.*<sup>22</sup> and Bergey *et al.*<sup>23</sup> in which parasitism lowered the amounts of certain elements in infected hosts. On other hand, the alteration pattern of the same element within the same snails from the same habitat may differ according to the type of the digenean infection. This was correlated with the observation of Hassan<sup>24</sup> in here study on the effects of digenean infection on the metallic ions of *Lanistes carinatus* snails collected from River Nile at Sohage province; she found that the K ion was increased in digestive gland of snails infected with xiphidiocercariae and was decreased in digestive gland of snails infected with gymnocephalus cercariae. Thus we can concluded that there was no fixed pattern of inorganic ions alterations in snails infected with digenean trematode.

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