Original Article

Evaluation of the immunological effect of beta alanyl-l-histidine against Schistosoma mansoni antigens in rabbits

Sanaa Ahmed Ali

Department of Therapeutic Chemistry, National Research Centre, Dokki, Giza, Egypt

Abstract

Introduction: The influence of vaccination on healthy (non-infected) rabbits treated with *Schistosoma mansoni* egg antigens, cercariae, and worms as prophylactic agents against infection, and the benefit of beta alanyl-l-histidine treatment against *Schistosoma mansoni* infection were investigated.

Methodology: This study involved individual injection of three *Schistosoma mansoni* antigens: soluble egg antigen (SEA), cercarial antigen preparation (CAP) and soluble worm antigen preparation (SWAP), in three rabbit groups, respectively. Three other groups each received the same specific antigen in conjunction with the administration of (beta alanyl-l-histidine) L-carnosine. Hepatic total protein, glycogen and glycogen phosphorylase, total serum protein and one diminution electrophoresis of protein fractions (180 KDa; 116 KDa; 97, 4 KDa, serum albumin 66 KDa; 48. 5 KDa; 29 KDa; 18.400 KDa; 14.200 KDa; 6.5 KDa) were measured in all the rabbit groups.

Results: Elevation in most parameters was observed in the immunized groups. Carnosine treatment of rabbit groups immunized with SEA, CAP and SWAP in comparison to the non-carnosine-treated immunized groups resulted in amelioration of serum protein fractions in all SEA and SWAP-immunized animals, and reduction in glycogen phosphorylase b in SWAP animals alone. In addition, changes in glycogen content were observed in the CAP-immunized group.

Conclusion: L-carnosine has a beneficial effect in the amelioration of most biochemical parameters as a result of *S. mansoni* antigen immunization.

Key words: antigens; protein; glycogen content; glycogen phosphorylase b

J Infect Dev Ctries 2012; 6(2):166-175.

(Received 02 September 2010 – Accepted 06 December 2010)

Introduction

Vaccination, ultimately, is anticipated to be the most effective form of schistosomiasis treatment and control [1]. If proved to be effective and inexpensive enough for worldwide distribution, it would eliminate the need for snail and reservoir host control. To date, a vaccine based on irradiated cercariae offers almost complete protection in experimental animals [2]. The first generation vaccines were directed against infection and/or worm fecundity but currently there is a natural balance, tempering anti-schistosomal responses by stimuli down-regulating the granulomatous reaction against eggs in the tissue [3]. *Fasciola* spp. can be used as a source of antigen because the fasciolosis worm has common or cross-reacting antigens with schistosomes, is associated with high eosinophil levels, and is capable of inducing specific immunological defenses against schistosomiasis [4].

It has been shown that *Fasciola* and *Schistosoma* worm antigens mixed with or without saponin as well as saponin alone are successful in protecting mice against *S. mansoni* infection. The combinations are more efficacious than saponin and *Fasciola* antigens alone [5]. This protection is achieved by a reduction in the total number of male and female worms, as well as in the levels of toxins elaborated by them. The role of these antigens in eliminating the products of oxidative stress and assistance in immune-mediated destruction of eggs that ameliorate the histopathological picture of liver cells and preserve its function has been demonstrated [5]. For example, in liver sections, egg granuloma size was reduced in animals vaccinated with *Fasciola* or *Schistosoma* eggs as well as *Fasciola* or *Schistosoma* eggs with saponin antigens [6].

Mice immunized with soluble worm antigen preparation (SWAP) and interleukin-12 show a highly statistically significant increase in resistance when compared with those immunized with SWAP only [7]. Laboratory investigations of mechanisms of mammals' immunological responses have long been under intensive scrutiny. Leukocytes react directly with

soluble egg antigen (SEA) or SWAP thus losing their property of adherence to glass. SEA induces apoptosis only in T cells from patients [8]. SEA and SWAP, through distinct intracellular signaling pathways, induce peripheral blood mononuclear cell proliferation [9]. The products secreted by schistosome eggs captured in the hepatic granulomata may promote angiogenesis within its granuloma by upregulating endothelial cell vascular endothelial growth factor [10]. L-carnosine (beta alanyl-L-histidine) is present at surprisingly high levels (up to 20 mM) in muscle and nervous tissues in many animals. L-carnosine inhibits lipid peroxidation and oxidative modification of proteins in muscle tissue since it acts as a reactive oxygen species (ROS) scavenger [11]. The recorded minimum effective concentrations of L-carnosine for protection of lipid and protein from oxidation were 2.5 and 1 mM, respectively [12]. It was proved to react with protein carbonyls, thereby generating "carnosinylated" polypeptides and thus protecting the molecules against modification. Conversely, glycation alters protein structure and decreases biological activity. Glycated proteins that have accumulated in affected tissue are reliable markers of disease. The L-carnosine dipeptide does not only prevent glycation but also plays a role in the disposal of glycated protein [13]. The present work investigated the effect of L-carnosine administration in SEA, cercerial antigen preparation (CAP) or SWAP-immunized rabbits. The investigated parameters included hepatic total proteins, glycogen content, and 97.4 KDa phosphorylase b. The serum total and certain fractional serum proteins were also tested.

Methodology

Chemicals

All fine chemicals and the L-carnosine used were products of the Sigma Chemical Company, St. Louis, USA.

Animals

Twenty-one male New Zealand white rabbits weighing approximately 2 kg were obtained in-house from the Research Institute of Ophthalmology, Cairo. They were routinely inspected for ecto-and endo-parasites. Anesthetic procedures complied with the legal ethical guidelines approved by the Ethical Committee of the Federal Legislation and National Institutes of Health Guidelines in the United States, and were approved by the ethical committee of the National Research Centre in Egypt. An overdose of ether was given gradually to the rabbits to ensure that the animals did not suffer at the final stage of the experiment.

Experimental design and immunizations

The rabbits were divided into seven groups of three rabbits each. Group 1 (G1) was designated the healthy control group. The six treated groups were divided into two large collections each comprised of three groups according to treatment. Batch A was comprised of groups 2, 3 and 4, each of which was injected with a specific antigen (egg, cercariae or worm antigens of *S. mansoni*) as follows: Group 2, SEA-I; Group 3, CAP-I, Group 4: SWAP-I. Batch B was comprised of groups 5, 6 and 7, each of which was injected with L-carnosine in addition to a specific antigen as follows: Group 5, SEA-I-C; Group 6, CAP-I-C; Group 7, SWAP-I-C. The six groups of rabbits (groups 2-7) were subcutaneously injected with 0.5 mg of the specified antigen formulated with an equal volume of Freund's complete adjuvant. A second booster dose of 1 mg in Freund's incomplete adjuvant (v/v) was injected on day 21. A third booster dose of 1 mg in Freund's incomplete adjuvant (v/v) was injected on day 26. Sera were collected two days after the last immunization in groups 2 to 4. Groups 5 to 7 were injected with an intramuscular dose of L-carnosine at 100 mg/day for 15 days [14]. Blood was collected from control and injected rabbits after decapitation. Samples were allowed to clot for two hours at room temperature and overnight at 4°C. After centrifugation at 100 x *g* for 15 minutes, sera were separated and stored at -20°C until used.

Preparation of antigen

All antigens used for this research (cercariae, worm and egg antigens of *S. mansoni*) were obtained from the Theodore Bilharz Research Institute, Giza, Egypt. SEA, CAP and SWAP antigens of *S. mansoni* were prepared as previously described [15].

Preparation of liver homogenate

Liver tissue prepared for estimation of total protein content was homogenised, in a normal physiological saline solution at a ratio of 1:10 w/v. The homogenate was centrifuged for 5 minutes at 300 x *g* at 4°C and the supernatant was used. One gram of liver tissue was boiled in 5 ml 30% KOH for estimation of glycogen. Liver tissue for estimation of glycogen phosphorylase was homogenised in 1:2 w/v of 100 mM maleate-NaOH buffer (pH 6.6) containing 20 mM NaF, 1 mM EDTA, 0.5 mg/ml bovine serum albumin and 10 mM

Table 1. Effect of L-carnosine on total protein concentration, glycogen content and glycogen phosphorylase activity in SEA, CAP and SWAP groups

Parameters	Control	Immunized groups			Treatment groups		
		SEA-I	CAP-I	SWAP-I	SEA-I-C	CAP-I-C	SWAP-I-C
	G_1	G_2	G_3	G_4	G_5	G_6	G_7
Total serum protein	94.5 ± 3.41 (6)*	97.5 ± 1.29 (5,6)*	92.5 ± 1.91 -	95.5 ± 2.52 (6)*	87.5 ± 5.97 (2)*	84.5 ± 8.69 (1,2,4,7)*	93.5 ± 2.52 (6)*
Total hepatic protein	36.25 ± 2.22 (5,6)*	35.5 ± 3.87 (4, 5,6)*	32.7 ± 2.2 (4,5,6)*	42.5 ± 1.91 (2,3,6)*	44.75 ± 4.11 (1,2,3,6)*	57.0 ± 5.29 (1,2,3,4,5,7)*	38.75 ± 3.4 (6)*
Glycogen	19.67 ± 2.12 (3,4,5,7)*	19.49 ± 0.75 (3,4,5,7)*	16.29 ± 0.47 (1,2,4,5)*	11.08 ± 0.81 (1,2,3,6,7)*	13.06 ± 1.52 (1,2,3,6)*	18.31 ± 1.87 (4,5,7)*	15.14 ± 1.26 (1,2,4,6)*
Glycogen-phosphorylase b	1.30 ± 0.12 (5, 6)*	1.41 ± 0.21 (4,5,6)*	1.55 ± 0.12 (4,5,6)*	1.07 ± 0.046 (2,3,6,7)*	1.03 ± 0.095 (1,2,3,6,7)*	0.71 ± 0.064 (1,2,3,4,5,6)*	1.43 ± 0.18 (4,5,6)*

-Data are expressed as means ± SD of rabbits in each group as follows: I = immunized by antigen; I-C = immunized and L-carnosine- treated
-Analysis of data was performed by one-way analysis of variance (ANOVA) accompanied by *post hoc* least significant difference (LSD; SPSS computer programme).
-P is level of significance, where $P < 0.05$ is significant
-ANOVA $P < 0.0001$ for all parameters except total protein where $P < 0.006$
*Number of groups which have a significant correlation between each other

DL-dithiothreitol. The supernatant obtained after centrifugation at 3,000 x *g* at 4 °C used.

Biochemical determinations

The following biochemical parameters were measured: (a) total protein content [16]; (b) glycogen (assayed according to the method of Nicholas *et al.*) [17]; (c) glycogen phosphorylase [18]; and (d) serum protein fractions [19], using 10-20% gradient polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. The gel was stained with Coomassie Brilliant Blue R250 (CBB R250) followed by silver staining, which is more sensitive than CBB R250 alone. To enhance the detection sensitivities for other proteins, the gel was dried according to the method of Jaung *et al*. [20]. The following individual protein fractions were used as standards: alpha-2-macroglobulin, 180 kDa; galactosidase, 116 kDa; phosphorylase b, 97.4 kDa; serum albumin, 66 kDa; fumarase, 48.5 kDa, carbonic anhydrase, 29 kDa; beta-lactoglobulin, 18.4 kDa; alpha-lactalbumin, 14,200 kDa and aprotinin, 6.5 kDa [21]. The electrophoretic results were recorded as percentages of the concentration of total protein, expressed as mg protein/ml. Electrophoretic separation bands were measured using a Helena Scanner (Junior 24, Helena, Beaumont, TX, USA) at 600 nm.

Statistical analysis

Data are expressed as means ± SD, and were analyzed using analysis of variance (ANOVA) (SPSS, Chicago, IL, USA).

Results and discussion

In the present study, the CAP-I and SWAP-I groups of animals in batch A showed decreased hepatic glycogen levels (Table 1 and Figure 1). Glycogen phosphorylase b was decreased in the SWAP-I group only. The decreased phosphorylase b activity in the SWAP-1 group may reflect inhibition of enzyme activity due to low concentrations of glycogen [22]. Although phosphorylase b is the inactive form of the enzyme, it represents the source of the active form, phosphorylase a [23]. The disturbance of either glycogen or 97.4 kDa phosphorylase b reflects disturbed metabolic machineries of hepatic energy reactions. The decreases in two energy factors magnify the naturally-targeted local suppressive effect of the experimental (unnatural) high dose of the antigens. These changes could denote the need for these two parasite developmental stages, cercaria and adult worm, to weaken the host liver tissue by disturbing the hepatic energy supply to complete the parasite biological goal (*i.e.*, complete their life cycle). Hence the antigens of both stages have this inherited host energy suppressive ability. Meanwhile, the SEA-I group did not show any deviated value of the three hepatic parameters.

Figure 1. Percent change of serum, hepatic protein concentration, glycogen and glycogen phosphorylase from rabbits immunized with SEA-I, CAP-I, or SWAP-I, or immunized with and treated with L-carnosine, SEA-I-C, CAP-I-C, or SWAP-I-C. Groups are: nothing (G1, control), SEA-I (G2), CAP-I (G3), SWAP-I (G4), SEA-I-C (G5), CAP-I-C (G6), SWAP-I-C (G7).

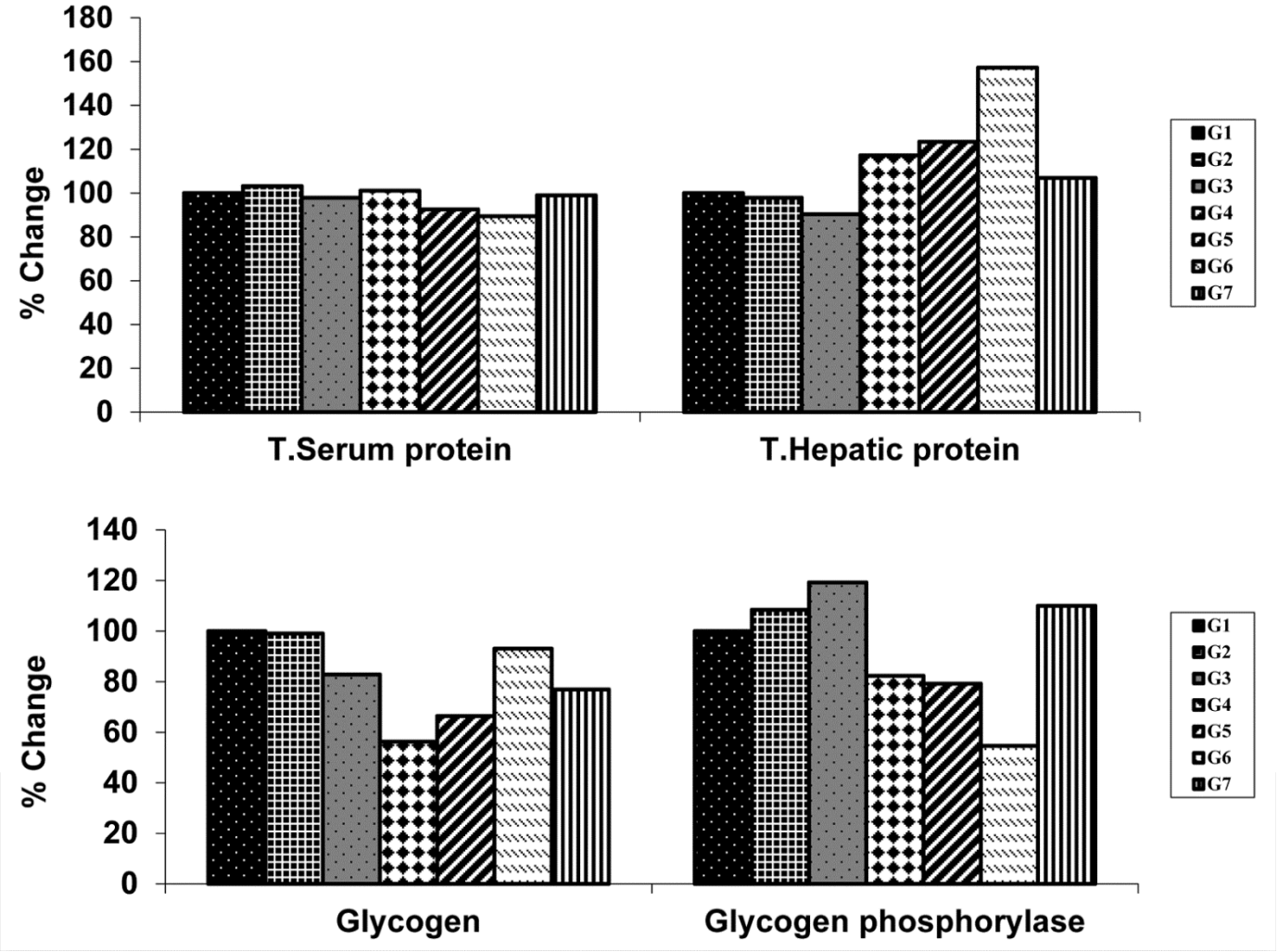

Figure 2. Percent change of different serum protein fractions (180KDa- 6.5 KDa) in rabbits immunized with SEA-I, CAP-I, or SWAP-I, or immunized with and treated with L-carnosine, SEA-I-C, CAP-I-C, or SWAP-I-C. Groups are: nothing (G1, control), SEA-I (G2), CAP-I (G3), SWAP-I (G4), SEA-I-C (G5), CAP-I-C (G6), SWAP-I-C (G7)

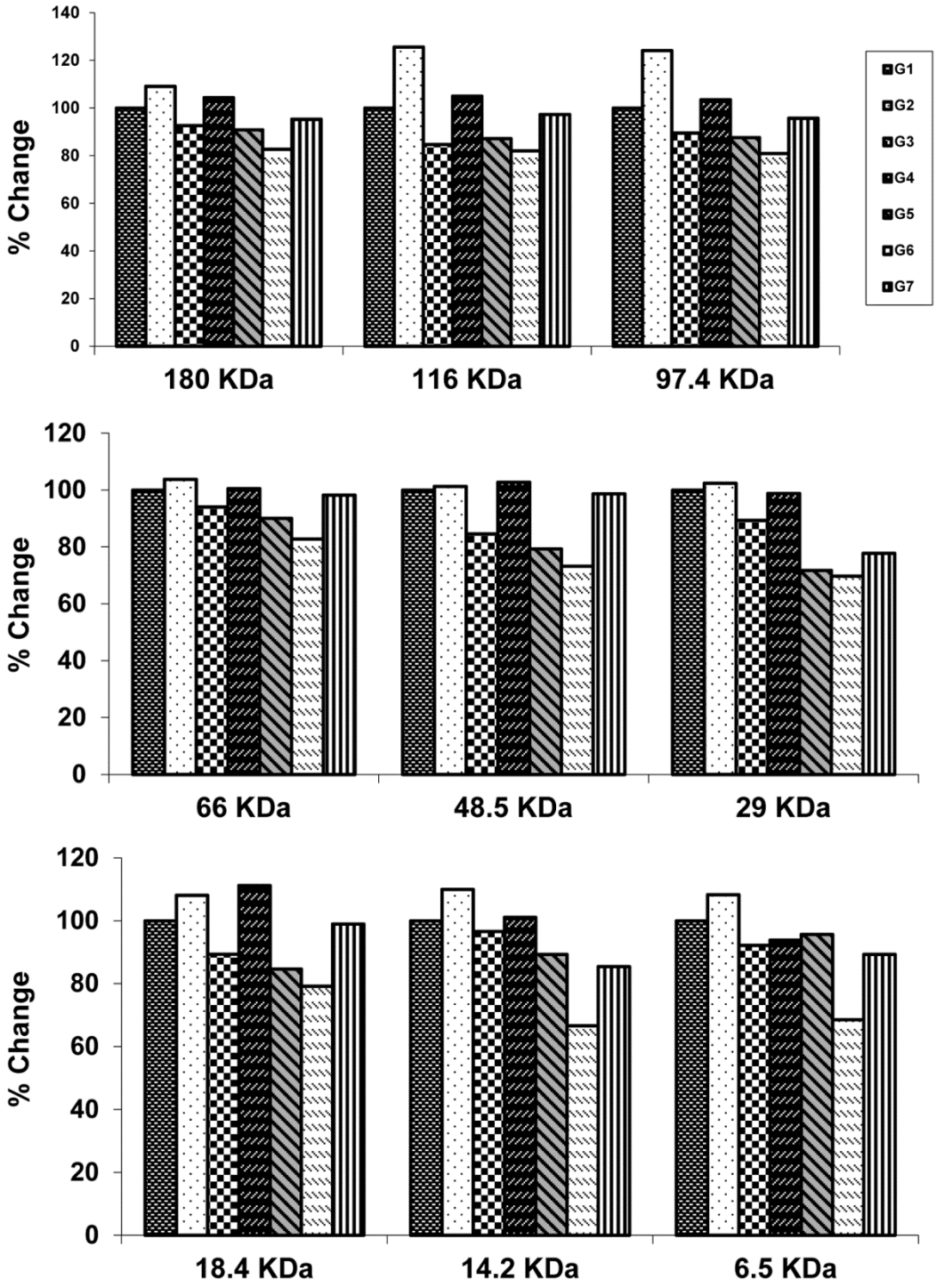

Figure 3. Electrophoretic profile of serum protein fractions (180KDa- 6.5 KDa) from rabbits treated with the following various immunization regimes: nothing (G1, control), SEA-I (G2), CAP-I (G3), SWAP-I (G4), SEA-I-C (G5), CAP-I-C (G6), SWAP-I-C (G7)

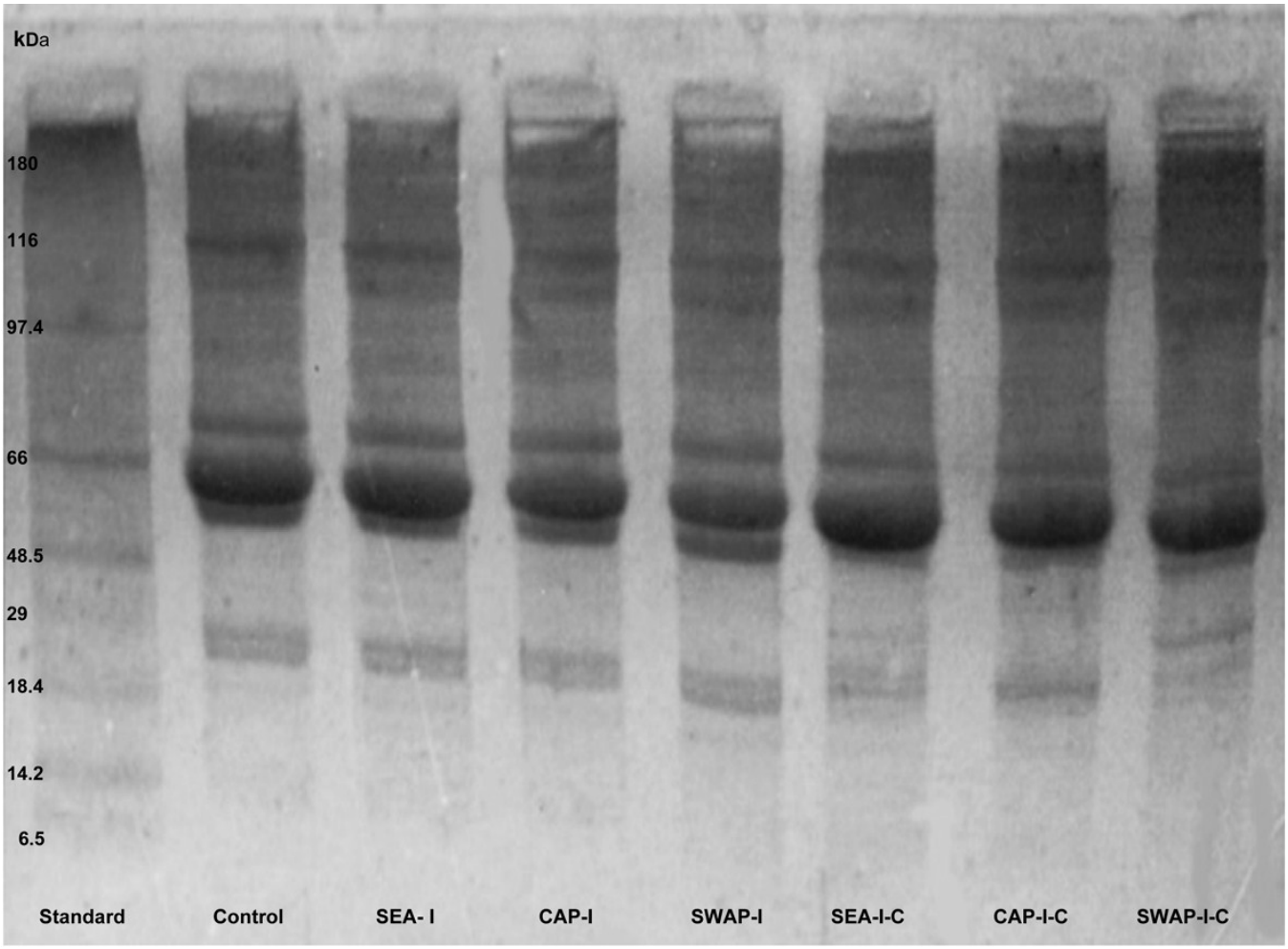

Table 2. Effect of L-carnosine on serum protein fractions in SEA, CAP and SWAP groups

Parameters	Control	Immunized groups			Treatment groups		
		SEA-I	CAP-I	SWAP-I	SEA-I-C	CAP-I-C	SWAP-I-C
	G_1	G_2	G_3	G_4	G_5	G_6	G_7
180 KDa	0.342±0.005 (2,3,5,6)*	0.373±0.0062 (1,3,5,6,7)*	0.317±0.017 (1,2,4,6)*	0.357±0.0047 (3,5,6,7)*	0.311±0.0116 (1,2,4,6)*	0.283±0.0114 (1,2,3,4,5,7)*	0.326±0.0139 (2,4,6)*
116 KDa	0.485±0.021 (2)*	0.609±0.057 (1,3,5,6,7)*	0.411±0.062 (2)*	0.509±0.041 -	0.423±0.043 (2)*	0.398±0.062 (2)*	0.472±0.077 (7)*
97.4 KDa	0.373±0.032 (2)*	0.463±0.058 (2,3,5,6,7)*	0.334±0.0044 (2)*	0.386±0.067 -	0.327±0.029 (2)*	0.302±0.0095 (2)*	0.357±0.059 (2)*
66 KDa	13.13±0.068 (2,3,5,6)*	13.63±0.052 (1,2,3,4,5,6,7)*	12.36±0.122 (1,2,4,5,6,7)*	13.2±0.103 (2,3,5,6,7)*	11.83±0.173 (1,2,3,4,6,7)*	10.87±0.110 (1,2,3,4,5,7)*	12.9±0.154 (2,3,4,5,6)*
48.5 KDa	0.983±0.033 (3,5,6)*	0.996±0.042 (3,5,6)*	0.832±0.046 (1,2,4,6,7)*	1.01±0.047 (3,5,6)*	0.78±0.043 (1,2,4,7)*	0.72±0.076 (1,2,3,4,7)*	0.97±0.67 (3,5,6)*
29 KDa	0.698±0.015 (5,6,7)*	0.715±0.115 (5,6,7)*	0.624±0.056 (6)*	0.69±0.053 (5,6,7)*	0.501±0.044 (1,2,4)*	0.487±0.064 (1,2,3,4)*	0.543±0.055 (1,2,4)*
18.4 KDa	0.490±0.049 -	0.53±0.007 (5,6)*	0.438±0.054 -	0.545±0.050 (5,6)*	0.415±0.051 (2,3)*	0.388±0.048 (2,4)*	0.485±0.048 -
14.2 KDa	0.618±0.041 (6)*	0.680±0.032 (5,6,7)*	0.597±0.048 (6,7)*	0.625±0.066 (6)*	0.552±0.046 (2,6)*	0.412±0.058 (1,2,3,4,5,7)*	0.528±0.086 (6,2)*
6.5 KDa	0.554±0.037 (6)*	0.600±0.051 (6,7)*	0.511±0.040 (6)*	0.52±0.036 (6)*	0.53±0.059 (6)*	0.380±0.053 (1,2,3,4,5,7)*	0.496±0.057 (2,6)*

-Data are expressed as means ± SD of rabbits in each group
-Serum protein fractions are expressed in mg protein/ml
-I = immunized by parasite antigen; I-C = immunized and L-carnosine-treated
-P is level of significance, where $P < 0.05$ is significant
-Analysis of data was performed by one way analysis of variance (ANOVA) accompanied by *post hoc* least significant difference (LSD; SPSS computer programme)
-ANOVA significant $P < 0.0001$ in all parameters and 14.8 KDa $P < 0.005$
*Number of groups which have a significant correlation between each other

The passive effect of egg antigen reflects the difference of biological activities among the three parasite stages, cercaria and worm on one side and egg on the other. This might be explained by the eggs being metabolically quiet, while the cercaria and the adult worm actively secrete their hepatic residue [24].

Results of serum protein fractions from the three groups in batch A (carnosine non-treated) revealed a relatively smaller number of affected parameters in comparison to the batch B groups (carnosine-treated). (Table 2, Figures 2 and 3). The ten deviated parameters from batch A were distributed between the SEA-I (four increased parameters) and CAP-I groups (four decreased parameters) while SWAP-I animals showed two decreased parameters. The four increased protein fractions of the SEA-I group were 180 kDa, 116 kDa, 97.4 kDa and serum albumin 66 kDa. Serum albumin is, among other proteins, involved in the inflammatory acute phase reaction response. Acute phase response is a constellation of host reactions indicating acute or chronic diseases [25]. In a study involving the administration of a huge number of *Schistosoma* eggs (20,000) into the portal veins of mice, high levels of 180 kDa alpha-2-macroglobulin were correlated with significantly greater mean size of the granuloma at four weeks post-injection in relation to cercariae infestation control [26]. Another study recorded the expression of 180 kDa and its mRNA by granuloma cells but not the surrounding liver parenchymal cells or serum [27]. The present study's increase of the serum 180 kDa fraction is not wholly contradictory as the SEA was systematically injected. Furthermore, an increase in the 180 kDa fraction has previously indicated a decreased immunity to the galactosidase enzyme, which has hydrolytic abilities, among others, to disturb specific cell surface receptors [28]. The increase in the 116 kDa galactosidase might, in an ordinary infestation, help to facilitate the exit of

the numerous eggs by disturbing the surface of hepatic cells to weaken the host tissue's exit passageway. These three deviations might facilitate the native biological goal of the whole egg to exit out of the liver, where it was laid, into the environment. Natively, the intact egg, in a natural infestation has two main weapons to help its exit: the injurious spine and SEA. Both together could elicit mechanical and metabolic disturbances that could weaken the tissue by mild, chronic, local inflammation to facilitate egg exit.

The decrease of the 180 kDa fraction points to a high native defense of the animal host against the cercaria. De Meirleir [29] stated that a deficiency of the 180 kDa (alpha-2-macroglobulin) fraction also showed an increased host defense against invading organisms. As either direction in deviation from normal is considered negative for the host, the decrease of the 48.5 KDa fraction meant a reduction of a powerful host energy agent by CAP, the representative of the cercaria. The suppressed 48.5 kDa (fumarase) fraction reflected the huge furnace machine of the cell, the citric acid cycle. The increased hepatic weakening ability of the cercaria antigen compared to that of the worm is attributable to the *Schistosoma* life cycle.

In the batch B animals, L-carnosine caused modifications of the results of tested parameters in comparison to the corresponding values from batch A animals. The L-carnosine increased liver glycogen content in both the CAP-I-C and SWAP-I-C groups (Figure 1 and Table 1). Previous records indicating the ability of L-carnosine to save glycogen metabolic machineries under stressful conditions support these results. For example, L-carnosine caused recovery of glycogen levels experimentally depleted with noradrenaline in rats [30]. In addition, L-carnosine was also stated to restore phosphorylase b activity during muscle contraction accompanying moderate pH changes [31]. L-carnosine caused greater glycogen recovery in the CAP-I-C than in the SWAP-I-C group, indicating that it nullified the strong action of the short-lived cercaria. L-carnosine has been suggested to have an activating effect on glycogen and glycogen phosphorylase, which catalyses the central reaction in glycolysis [32]. Glycogen recovery was proportional to decreased phosphorylase activity in the CAP-I-C group. This moderate difference in the increase of glycogen content between both groups, CAP-I-C and SWAP-I-C, by L-carnosine cannot account for the huge difference in the change of the enzyme activity in both conditions. However, the fact that metabolic integrations allow multiple different factors to interact is a plausible explanation. The results of the three groups, SEA-I-C and CAP-I-C and SWAP-I-C (Table 2, Figures 2 and 3), collectively showed twelve significant deviated parameters from the controls. However, these animals presented more deviations (30 parameters) relative to the corresponding parameters from the animals in group A. The changes of all protein parameters all showed significant reduction, thus, in both comparisons, the deviations were greater in the SEA-I-C and CAP-I-C groups than in SWAP-I-C. The SEA-I-C group showed twelve out of the 30 group B parameters, presenting further decreases. In the CAP-C group, seven parameters were reduced, while SWAP-I-C animals presented only one lowered level (the 29 kDa fraction). The small number of deviated parameters in SWAP-C animals might reflect an inherited ability of the animal's body to combat the adult worm. The decrease of almost all parameters in SEA-I-C and CAP-I-C animals, as opposed to the small number in SWAP-I-C animals, can be attributed to the biological interrelations between both parasite and host tissues. Also, the high (experimental) dosages of antigen may not have matched the corresponding amounts in natural infestation. The natural short time-span of both developmental stages (*i.e.*, eggs and cercariae which are transient in the liver) in host tissues support this explanation. After the eggs are laid, the majority are expelled outside the liver and intestine. On the host side, serum albumin has, evolutionarily, long been exposed to worm antigen. However, L-carnosine, a natural host component, might have not ever experienced such a high dosage of egg or cercerial antigens during evolution. The presence of L-carnosine, being unable to normalize the decreased protein parameters, resulted in further numerous decreases [32]. Dixit *et al*. [33] proved that vitamin C, in large doses, caused inhibition of key enzymes in carbohydrate metabolism. The foregoing immunological results support the relative actions of the three antigens. However, the authors stated that the histopathological picture of SWAP-C-treated animals showed a greater immunological defence in lymph nodes' cellular reaction, presented as sinus histiocytosis [34]. Nassr *et al*. recorded that anti-SWAP IgG1 and IgG4 were increased in schistosomiasis [35]. However, to potentiate the SEA vaccine action against schistosomiasis, combined administration of SEA in conjunction with 28 kDa glutathione-S-transferase antigens in infected animals was suggested [36]. Nevertheless, the above-mentioned conservation of hepatic glycogen and phosphorylase by carnosine combating the high

antigen doses could be ascribed to its well-documented antioxidant action [11]. This apparently contradicts its decreasing effect on the tested serum parameters, which could be ascribed to a pro-oxidant effect. Pro-oxidant effects were previously recorded for other antioxidant agents as well as L-carnosine when given in high dosages, for example vitamin C [37]. The peculiarity in the present results was the record of the presence of both antioxidant action (favorable increase in glycogen content) as well as moderate pro-oxidant effect (the decreases in serum protein concentrations). However, this difference could be explained by the previously reported selectivity of L-carnosine in regulating animal body biochemical machineries to pathological challenge [38]. The mechanisms by which L-carnosine could cause selective changes are multiple. L-carnosine intracellular defense mechanisms against ROS were stated to protect phospholipid organelles that could help the biochemical energy machinery of host cells [11]. L-carnosine is a potent and selective scavenger of alpha, beta-unsaturated aldehydes, typical by-products of membrane lipid peroxidation (considered second messengers of the oxidative stress response), and inhibits aldehyde-induced protein-protein and DNA-protein cross-linking in neurodegenerative disorders such as Alzheimer's disease [39]. L-carnosine involvement in the activation of natural systems of host immune resistance has been reported. It induced liberation of the immune modulator intermediates, cytokines and interleukins. It could also bind to immune system cell receptors that stimulated the liberation of hepatocyte growth factor and cytokines, causing liver recovery [40]. L-carnosine can be useful as a prophylactic treatment to protect the liver against hypoxia-reoxygenation damage [41]. It also promoted liver regeneration in partially hepatectomized mice, which was shown in highly significant increases in weight, RNA content, and many hepatic proteins. The modulation of host immune response against SEA by L-carnosine could be comparable to the immune modulation of *S. mansoni* egg recorded by immunomodulators [42,43].

In conclusion, immunological studies should investigate further the effect of smaller dosages of L-carnosine combined treatment. The selectivity of L-carnosine proved favorable for hepatic glycogen content. The various decreased protein parameters should be tested with smaller L-carnosine dosages. It is expected that smaller dosages may be able to normalize most tested proteins.

Acknowledgement

The author expresses her sincere gratitude to Professor Kawser Soliman, Department of Biochemistry, Faculty of Medicine, Cairo University, Egypt, for providing facilities during this research.

References

1. Kojima S (2004) Overview: from the horse experimentation by Prof. Akira Fujinami to paramyosin. Parasitol Int 53: 151-162.
2. Doenhoff MJ, Modha J, Lambertucci JR (1988) Anti-schistosome chemotherapy enhanced by antibodies specific for a parasite esterase. Immunol 65: 507-510.
3. Capron AG, Riveau JM, Grzych D, Boulanger M, Capro, Pierce R (1995) Development of a vaccine strategy against human and bovine schistosomiasia. Background and update. Mem Inst Oswaldo Cruz 90: 235-240.
4. Hillyer GV (1979) *Schistosoma mansoni*: Reduced worm burdens in mice immunized with isolated Fasciola hepatica antigen Exp. Parasitol.48: 287-295.
5. Maghraby AS, Hamed MA, Ali SA (2010) Bioimmunological response to *Schistosoma mansoni* and Fasciola gigantica worm homogenates either mixed with or without saponin. J Infect Dev Ctries 4: 334-344.
6. Maghraby AS, Hamed MA, Ali HF, Ali SA (2010) Antischistosomal activity of Fasciola gigantica eggs influenced by Egyptian saponin extracted from Atriplex nummularia. J Am Sci 6: 368-381.
7. Abdel-Aaty HE, Ramadan NI, Mahmoud MS, Abdel-Aziz SS, Khalil HM, El- Badawy NM, Khalil HH (1999) Role of recombinant interleukin-12 as an adjuvant on vaccine-induced immunity in murine *Schistosoma mansoni* infection. J Egypt Soc Parasitol 29: 1-11.
8. Carneiro-Santos P, Martins-Filho O, Alves-Oliveira LF, Silveira AM, Coura-Filho P, Viana IR, Wilson RA, Corea-Oliveria R (2000) Apoptosis: a mechanism of immunoregulation during human Schistosomiasis mansoni. Parasite Immunol 22: 267-277.
9. Hilpela P, Oberbanscheidt P, Hahne P, Hund M, Kalhammer G, Small JV, Bahler M (2003) SWAP-70 identifies at transitional subset of actin filaments in motile cells. Mol Biol Cell 14: 3242-53.
10. Loeffler DA, Lundy SK, Singh KP, Gerard HC, Hudson AP, Boros DL (2002) Soluble egg antigens from *Schistosoma mansoni* induce angiogenesis-related processes by up-regulating vascular endothelial growth factor in human endothelial cells. J Infect Dis 185: 1650-1656.
11. Soliman KM, Hamed, AM, Ali SA (2006) Hepatoprotective Effect of Carnosine on Liver Biochemical Parameters in Chronic Ethanol Intoxicated Rat. J Med Sci 6: 528-536.
12. Nagasawa T, Yonekura T, Nishizawa N, Kitts D D (2001) *In vitro* and *in vivo* inhibition of muscle lipid and protein oxidation by carnosine. Mol Cell Biochem 225: 29-34.
13. Yeargans GS and Seidler NW (2003) Carnosine promotes the heat denaturation of glycated protein. Biochem Biophys Res Commun 300: 75-80.
14. Soliman K and Abo-El_Doubal S (2003) Effect of carnosine administration on the immune response in rabbit to *Schistosoma mansoni* antigens. J Egypt Soc Prasit 33: 663-678.
15. Da-Silva LC and Ferri RC (1968) *Schistosoma mansoni* homogenate for active immunization of mice. Amer J Trop Med Hyg 17: 367-371.

16. Bradford MM (1976) A rapid and sensitive method for the Quantitation of Microgram Quantities of protein utilizing the principal of protein. Dye binding Anal Biochem 72: 248-254.
17. Nicholas V, Carroll B, Longley W, Joseph HR (1956) the determination of glycogen in liver and muscle by the use of anthrone reagent. J Biol Chem 220: 585-593.
18. Hedrick JL and Fischer EH (1965) On the role of pyridoxal 5 phosphate in phosphorylase, absence of classical vitamin B6 dependent enzymatic activities in muscle glycogen phosphorylase. Biochem 4: 1327-1347.
19. De Moreno MR, Smith JF, Smith RV (1985) Silver staining of proteins of polyacrylamide gels: increased sensitivity through a combined Coomassie Blue- Silver stain procedure. Anal Biochem 151: 466-470.
20. Jaung RH, Chang Y, Sung Di, Yi H, Su JC (1984) Oven-drying method for polyacrylamide gel slab packed in cellophane sandwich. Anal Biochem 141: 348-350.
21. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685.
22. Bray D (1995) Protein molecule as computational element in living cells. Nature 37: 376-407.
23. Buschiazzo A, Ugalde J E, Guerin M E, Shepared W, Ugalde RA ,Alzhari PM (2004) Crystal structure of glycogen synthase: homologus enzyme catalase glycogen synthetase and degradation. EMBO J 23: 3196-3205.
24. Manson-Bahr PEC and Apted FIC (1985) Trematode Infections, Schistosomiasis. In: Mansons`s Tropical Disease, 18th Ed., English Language BookSociety\Balliere Trindall, London, 206-228.
25. Tiemeier H, Hofman A, van Tuijl HR, Kiliaan AJ, Meijer J, Breteler MM (2003) Inflammatory proteins and depression in the Elderly. Epidemiology 14: 103-107.
26. Van Gool J, Van Tiel D, Doenhoff M J, Van Vugt H (1991) Effect of acute phase proteins, especially, alpha 2-macroglobulin, on granuloma formation around *Schistosoma mansoni* eggs in rat. Parasit 102: 49-56.
27. Tegglman AM, Boers M, Moorman AF, De Boer PA, Van-der-Loos CM, Rotman JP, Chamleau RU (1996) Localization of alpha-2-macroglobulin and messenger RNAin rat liver fibrosis: evidence for the synthesis of 2- macroglobulin within *Schistosoma mansoni* egg granuloma. Hepatol 23: 1260-1267.
28. Lehrman H (2001) Oligosaccharide-based information in endoplasmic reticulum and other biological systems. J Biol Chem 276: 8623-8628.
29. De Meirleir L (2002) Defects of pyruvate metabolism and the Krebs cycle. Child Neurol 3: 33-34.
30. Coopersmith R and Leon M (1995) Olfactory bulb glycogen metabolism: noradrenergic modulation in the young rat. Brain Res 674: 230-237.
31. Severin SE, Skolysheva LK, Shur SA, Vulfsan PL (1990) The pH dependent conformational transition in glycogen phosphorylase b. The effect of carnosine and anserine on its activity. Biochem Int 20: 227-238.
32. Begum G, Cunliffe A, Leveritt M (2005) Physiological Role of Carnosine in Contracting Muscle. International J Sport Nutri Exerc Metabol 15: 493-514.
33. Dixit A, Baquer NZ, Roa AR (1992) Inhibition of key enzymes of carbohydrate metabolism in regenerating mouse liver by ascorbic acid. Biochem Int 26: 143-151.
34. Soliman K, Abdel-Aziz M, Nassar, Y, Abdel-Sattar S, El-Ansary A (2002) Effects of carnosine on bilharzial infestation in hamsters: biochemical and histochemical studies. Comp Biochem Physiol 132: 535-542.
35. Nassr AKh, Hassan MM, Abdel Salam FM, Lashin AH, Shahin WA, Amin H (2002) IgG isotypes in schistosomiasis patients before and after praziquantel. J Egypt Soc Parasitol 32: 931-952.
36. Lebens M, Sun JB, Sadeghi H, Backstroom M, Olsson I, Mielcarek N, Li BL,Capron A, Czerkinsky C, Holmgren J (2003) A mucosally administrated recombinant fusion protein vaccine against shcistosomiasis protecting against immunopathology and infection. Vaccine 21: 514-520.
37. Paolini M, Pozzetti L, Pedulli GF, Marchanarchesi F, Cantelli-Forti G (1999) The nature of pro-oxidant property of vitamin C. Life Science 64: 273-278.
38. Soliman KM, Mohamed AM, Metwally NS (2003) Corrective action of carnosine on liver enzymes in ethanol intoxication. Med J Cairo Univer 71: 135-143.
39. Guiotto A, Calderan A, Ruzza P, Borin G (2005) Carnosine and carnosine-related antioxidants: a review Curr Med Chem 12: 2293-2315.
40. Soliman K M, Ali HF (2003) Stimulatory effect on serum proteins in normal and partially hepatectomized mice. Egypt J Physiol 25: 7-28.
41. Amr AF, Mahmoud AE, Hala KM (2007) The hepatoprotective effect of carnosine against ischemia/reperfusion liver injury in rats. Eur J Pharmacol 572: 61-68.
42. Jakubzick C, Wen H, Matsukawa A, Keller M, Kunekel SL, Hogboa N (2004) Role of CCr4 Ligands, CCL17 and CCL22 during *Schistosoma mansoni* egg induced pulmonary granuloma formation. Am J Pathol 165: 1211-1221.
43. Leinonen JS, Saari KA, Seppanen JM, Myllyla HM, Rajaniemi HJ (2004) Immunohistochemical determination of carbonic anhydrase isoenzymVI (CAVI) expreesion in lower airways. J Histochem Cytochem 52: 1107-1112.

Corresponding author

Dr. Sanaa Ahmed Ali
Assistant Professor
Therapeutic Chemistry Department
National Research Center
Tahrir St., Dokki
Cairo, Egypt 12622
Telephone: 00202 33371499; Fax: 00202 33370931
Email: sanaa_ahmedibrahim@yahoo.com

Conflict of interests: No conflict of interests is declared.