

Metabolic changes in *Biomphalaria glabrata* infected with *Schistosoma mansoni* exposed to latex of *Euphorbia milii* solution versus times of preparation

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ABSTRACT

In order to establish how long after the first application of the latex of *Euphorbia milii* it remains with activity on the metabolic pathways of molluscs host, this study aimed to screen the effects of latex exposure from 24 hours until 35 days after exposure to the latex solution to check how long the solution exhibits harmful effects on the carbohydrate metabolism and on the nitrogen excretion products of molluscs. Snails exposed to the solution presented a reduction of the glycogen levels in the tissues analyzed and changes in the ways of excreting nitrogen and the concentration of total proteins in the hemolymph, an effect that was strongest in the groups exposed up to 14 days after preparation of the solution. In both cases, the glycogen concentrations and the nitrogen excretions products in the treated groups only returned near the levels in the control groups from the 21 days onwards, but there were still statistically significant differences. These results contribute to the optimization of the use of this product as a molluscicide of natural origin and the development of protocols for control of schistosomiasis.

Keywords: *Biomphalaria glabrata*; *Schistosoma mansoni*; *Euphorbia milii*; Metabolism.

INTRODUCTION

Studies on vegetable materials with molluscicidal activity have been carried out since 1930s aiming to control *Schistosoma mansoni* intermediate hosts. The molluscicidal activities of *Euphorbia milii* were firstly studied by Vasconcellos and Schall (1986) who showed lethal action on *Biomphalaria glabrata* under laboratory conditions. The snail *B. glabrata* is the main intermediate host of *S. mansoni* and is closely associated with the occurrence of mansonic schistosomiasis in Brazil. The use of molluscicides is a complementary action of the program for global control of schistosomiasis (WHO

1993; 2006). In Brazil, the latex of *E. milii* is one of the most promising natural molluscicides (Mello-Silva, et al., 2006a; 2007; Vasconcellos and Amorin, 2003). This latex has been investigated in a large number of studies around the world, researches about the molluscicidal miliiamines (Zani, et al., 1993) and the influence of environmental factors on the molluscicidal activity and comparative toxicity of the latex and synthetic molluscicides to *B. glabrata* embryos (Oliveira-Filho, et al., 1999; 2010), the absence of tumor promoting activity (Delgado, et al., 2003), biochemical and physiological features (Yadav, et al., 2006; 2008). One of the advantages of this product is its biodegradability, but it only remains active in aqueous solution for up to 30 days because of its photosensitivity (Schall, et al., 1992).

Alterations on snail's energetic metabolism are a natural condition of the relationship with trematode larvae which depletes the nutrients in the hemolymph required by the host to develop. Changes in the total protein levels of snails are closely linked to the exhaustion of carbohydrate stores. The most common stress factor is infection by trematode larvae. According to Becker (1980), they drain energy reserves from the snail host to develop, generating alterations in the snail's levels of proteins and free amino acids. Consequently, the process of spoliation of energy sources, with extensive degradation of amino acids and proteins to maintain the basic metabolic activities, leads to an increase in nitrogen excretion products-urea, uric acid and ammonium.

Studies on the performance of the *E. milii* latex solution on the energetic metabolism of *B. glabrata* infected or not by *S. mansoni* have been made by Mello-Silva, et al., (2010; 2011). In order to establish how long after the first application of the latex it remains with activity on the metabolic pathways of molluscs host, this study aimed to screen the effects of latex exposure from 24 hours until 35 days after exposure to the latex solution to check how long the solution exhibits harmful effects on the carbohydrate metabolism and on the nitrogen excretion products of molluscs. This type of work on this plant is reported first time here.

MATERIAL AND METHODS

Plant: The crude latex of *E. milii* was collected in the august of 2010 on the *campus* of the Instituto Oswaldo Cruz in Rio de Janeiro, Brazil, the collection was carried out using the latex of a cross-section on the same day that the tests were conducted, about 10cm below the apical meristem of each branch; the crude latex was collected in a glass container to prevent coagulation and transported to laboratory, according to the method described by Vasconcellos and Amorim (2003a).

The preparation of the aqueous solution and determination of the lethal (LC₉₀) and sublethal concentration (LC₅₀): Using the freshly latex was prepared the aqueous solution at a concentration of 100mg/l, and from this, solutions were prepared at different concentrations (0.7, 0.8, 1.0, 1.2µl) to use in bioassays. For all dilutions were used volumetric flasks of 1000ml and the volume of each solution was divided into 500ml beakers. Were placed in each receptacle 10 snails of *B. glabrata* (BH strain), measuring 8-12mm in shell diameter, for each concentration being exposed to this solution for 24hours. Two beakers received 500ml of distilled water, where were placed 10 molluscs in each beaker, without latex and used as controls. During this period all molluscs were fasted. After the period of exposure to latex, the snails were removed from the flasks and washed with distilled water to remove residues of the solution and the number of deaths was noted. The snails that escaped the solutions at each concentration were separated in glass beakers containing 50ml of distilled water.

Molluscs which have remained in solution during exposure were placed in vessels containing 500ml of distilled water for 24 hours. During the recovery period, the snails were fed with pieces of fresh leaves of lettuce (*Lactuca sativa L.*). For determination of LC₅₀ was used group of snails exposed to solution concentration of 0.7µl showed that 50% mortality within 24 hours of the experiment. This methodology followed the methods developed by Vasconcellos and Amorim (2003a) and is in line with the recommendations of the WHO (1983) and Mott (1987).

The infection and maintenance of the host snails: Specimens of *B. glabrata* (strain coming from Belo Horizonte-BH strain), maintained at the Laboratório de Esquistossomose Experimental, Instituto Oswaldo Cruz, FIOCRUZ/ RJ, Brazil, were used in this experiment. The snails were placed in polyethylene tanks of 30L capacity. The average water temperature was around 28.5±1°C and relative humidity ranging between 70 to 78% from beginning to end of the experiment. Three times a week the aquariums were cleaned and the snails fed *ad libitum* with fresh leaves of lettuce.

To maintain the *S. mansoni* cycle the Swiss Webster albino mice were used as definitive host, in the Laboratório de Esquistossomose Experimental - FIOCRUZ/ RJ. This procedure followed the same as described by Mello-Silva (1996). The specimens of *B. glabrata* (BH) were individually exposed to 6-8 miracidia of *S. mansoni* BH strain, by snail. The both methods were carried out according to the methods described by Souza and Lima (1990) and Mello-Silva (2007), respectively.

Experiment with Sub lethal Dose: The crude latex collected was used to prepare the stock solution, which was stored in a 1L glass balloon flask at a temperature of 5°C and relative humidity of 50%. Then the LC₅₀ extract was prepared at a concentration of 0.7mg/L. The same day, the *B. glabrata* specimens (BH strain) were infected with 4 to 6 *S. mansoni* miracidia obtained from eggs isolated from Swiss Webster albino mice maintained in the Laboratório de Esquistossomose Experimental – FIOCRUZ/RJ, according to the technique described by Souza and Lima (1990), allowing the infection time to accompany the period since preparation of the solution. All the snails used had shell diameters between 8 and 10 mm.

The *B. glabrata* specimens were organized into four groups: 1- Uninfected and not exposed to the latex (negative control - CTL-), 2- Infected and not exposed to the latex (positive control - CTL+), 3- Uninfected and exposed to the latex (UE); and 4- Infected and exposed to the latex (IE). Each group was formed of 240 snails divided into four replications. The snails were placed in 500-ml glass beakers in groups of 60 each and subjected to the sublethal latex dose (exposed) or distilled water (unexposed). At each weekly interval (1, 7, 14, 21, 28 and 35 days), a group of 240 uninfected snails and another group with 240 snails infected with *S. mansoni* were exposed to the latex solution or distilled water for 24 hours, after which the snails were washed in distilled water and dried. Then the specimens were dissected to collect the cephalopodal mass and digestive gland. During collection, the material was kept in an ice bath, after which it was frozen at -10°C. The treatment beakers were sealed with perforated polyethylene lids to keep the snails from escaping the solution and the snails were not fed during the treatment period.

Biochemical Analyses:

Glycogen concentration: The concentration of glycogen was measured in the cephalopodal mass and digestive gland tissues of the infected or uninfected *B. glabrata* snails, exposed or not to the sublethal concentration of the aqueous extract of the *E. milii* latex, according to Pinheiro and Gomes (1994), by the reaction of 3,5 DNS (Sumner 1924). The concentrations of total proteins, uric acid and urea were

determined (expressed as mg/dl) in the hemolymph of *B. glabrata*, infected and not infected, exposed or not to the sublethal dose of the aqueous extract of the latex of *E. milii*.

Glucose: The glucose sample undergoes the action of glucose oxidase in the presence of oxygen to produce hydrogen peroxide, in the presence of this phenol and 4-aminoantipirina suffers the action of peroxidase producing a pinkish-reddish compound with maximum absorbance at 505nm. At this study, three tests were prepared, the first (with the solution) containing only 1.0ml of the enzyme reagent (aqueous buffer pH 7.40, 4-aminoantipirina 0.8mmol/L, phenol 11mmol/L, Glucose oxidase \geq 1000 U/L and p-methyl hydroxybenzoate 6.5mmol/L). The second, test sample, containing 0.01ml of the sample plus 1.0ml of the enzyme reagent. Finally, the pattern solution, containing 0.01ml of the solution pattern (5.56mmol/L glucose in benzoic acid solution 0.25%) plus 1.0ml of the enzyme reagent.

The solutions were homogenized and placed in a water bath at 37°C for 15 minutes. Then we had determined the absorbance of the pattern and of the samples at 505nm, hitting the zero with the white solution.

Total proteins: The serum proteins formed through its peptide linkages colored complexes with cupric ions in alkaline media in the Biuret reagent. The resulting complexes have absorption maximum at 545nm and the intensity of the color formed is easily automatable adapting itself to all available automatic analyzers.

For analyze our samples we had prepared biuret reagent usage (containing sodium hydroxide in 1680mmol/L, treated dual potassium sodium 320mmol/L, copper sulfate 188mmol/L and potassium iodide 300mmol/L). It was transferred to a 500ml volumetric flask and completed with distilled water. The solution was stored in plastic flask sheltered from light.

Then the solutions were prepared for absorbance reading. For the different dosages we had established three tests: the white solution, the sample solution and the standard solution. The white solution containing 0.05ml of distilled water and 2.5ml of Biuret reagent usage. The sample solution containing 0.05ml sample and 2.5ml of Biuret reagent use, and finally the standard solution containing 0.05ml of standard solution (aqueous solution of albumin bovine 4.0g/dl, containing sodium azide 8.5mmol/L) and 2.5ml of Biuret reagent usage. The solutions were homogenized and rested for 15 minutes at room temperature. Then we had determined the absorbance of the samples and the standard at 545nm and hitting the zero with the white solution.

Urea: Urea is hydrolyzed by the enzyme urease producing carbon dioxide and ammonium ions. These in the presence of salicylate, hypochlorite and nitroprusside (Berthelot reaction modified) produce indophenol blue dye which has an absorption maximum around 600nm.

To this procedure was prepared the using buffer, wherein the buffer stock (containing sodium salicylate 300mmol/L and methyl p-hydroxybenzoate 6.5mmol/L, sodium nitroprusside 17.5mmol/L EDTA and 25mmol/L at pH 7 in solution 5x concentrated) was transferred to a 500ml volumetric flask and completed with distilled water. The solution was stored in amber bottle. Then the Enzyme Reagent of Use was prepared adding 5.0ml of the enzyme reagent (solution buffered at pH 7, 20x concentrate containing urease $>$ 160KU/L, methyl p-hydroxybenzoate 3.2mmol/L EDTA and 5mmol/L) to 100ml of buffer usage, homogenized and then stored in amber bottle. Finally the Use of Color Reagent (25ml of 20x concentrated solution containing sodium hypochlorite 220mmol/L and sodium hydroxide 3500mmol/L)

where the color reagent stock was transferred to a 500ml volumetric flask supplemented with distilled water and stored in plastic bottle.

For dosing the urea contents we had prepared the white solution (1.0ml enzyme reagent usage), the test solution (0.01ml samples of more than 1.0ml enzyme reagent usage) and the standard solution (0.01ml standard and 1.0ml enzyme reagent usage). These solutions were homogenized and placed in a water bath at 37°C for 5 minutes. Then each solution received 1.0ml of Use of Color Reagent were again homogenized, placed in a water bath at 37°C for 5 minutes. Then we had determined the absorbance of the tests solutions and the standard solution at 600nm hitting the zero with white solution.

Uric Acid: Uric acid undergoes the action of uricase in the presence of oxygen, producing allantoin and hydrogen peroxide, this reagent in the presence of a phenolic (TOOS) and 4-aminoantipyrine suffers the action of peroxidase producing a compound with violet absorption maximum at 545nm.

For measurements of uric acid we had prepared three tests: white solution containing 1.0ml of enzyme reagent (aqueous solution containing pH 7.20 buffer, uricase \geq 120 U/L, peroxidase \geq 1000 U/L, 4 - aminoantipyrine 1.0 mmol/L, TOOS 0.60 mmol/L and sodium azide 0.1g dl), the test solution containing 0.02ml of sample and 1.0ml of enzyme reagent; and the standard solution containing 0.02ml of standard (uric acid 0.476mmol/L aqueous solution and sodium azide 0.1g/dl) and 1.0ml enzyme reagent. The solutions were homogenized and placed in a water bath at 37°C for 10 minutes. Then the absorbance of the test and standard solutions were determined at 545nm, hitting the zero with the white.

Statistical Analyses: The results obtained were expressed as mean \pm standard deviation and subjected to one-way analysis of variance (ANOVA), the Tukey-Kramer test ($\alpha = 5\%$) and polynomial regression (Graphpad Prism v.500).

RESULTS

Glycogen concentrations on the cephalopodal mass and on the digestive gland of the snails: On the first day of exposure to latex there was no difference between the concentrations measured in the infected snails and the CTL+ snails, but in relation to the CTL- snails there was an increase of 35%. From the 7 days to 21 days there were reductions of 75%, 80% and 49%, respectively, in the glycogen levels of the exposed snails in comparison with the CTL+ group. In this same period, there were respective reductions of 90%, 76% and 52% in comparison with the CTL- group. From the 28 days onward, the IE group remained below those observed in the two control groups, but with a trend for normalization of the readings (Table 1).

In the UE group, from the first day to the 14 days, the glycogen concentration in the cephalopodal mass declined 20%, 82.8% and 76% respectively, from the readings in the CTL- group (Table 1).

The concentration of glycogen in the digestive gland showed a similar pattern for the infected snails in relation to the CTL+ and CTL- groups. On the first day there was a 35% increase in the glycogen concentration in relation to the CTL- group, and from the 7 days to the 21 days the respective declines were 90%, 80% and 47%. In this same period, the concentrations in relation to the CTL+ group were 84%, 79% and 42% lower, respectively. For the 28 days and 35 days, the concentrations in the treated infected group remained below those in the two control groups, although they were nearer to normal levels (Table 1).

The UE showed an increase in the glycogen concentration in the digestive gland in relation to the CTL- group of 57% on the first day, but on the 7 days and 14 days this level decreased by 75% and 88%, respectively. On the 21 days and afterward, the difference between the groups became smaller, with the concentrations tending to normalize. Afterwards, there was a tendency for the concentrations to normalize from the 28 days to the 35 days (Table 1).

The total protein content in the snails showed a tendency to increase due to exposure to the latex, in the first two observation periods (one and seven days), when the total protein levels in the unexposed snails were higher than in the exposed specimens (Table 2). On the 14th day, the levels for the two groups did not differ as significantly as in the previous periods.

Glucose and Total protein circulating in the hemolymph: On the first day the UE had a reduction in glucose concentration (61%), whereas the IE group, and the CTL + showed an increase of 57%, when compared to CTL -. At 7th day the IE group had elevated concentration of free glucose in the hemolymph. The UE group presented on the 14th day a high concentration of free glucose in the hemolymph. From the 21st to the 28th day the values of glucose concentrations of UE and IE groups were higher than their respective control groups. With values of 103% and 119%, respectively, compared between the groups at 21 days and 109% and 125% after 28 days. On day 35 a decrease was observed in these concentrations, the UE showed 98% of the glucose concentration of the CTL- and the IE showed 39% of the same concentration of the CTL+ (Table 2).

The alterations in the concentration of total proteins on the influence of the latex are reflected in the uncompensated regulation of the urea and uric acid levels excreted by the infected *B. glabrata*. On the first day, exposure to the latex induced a decline in the urea concentration (-79.0%) in the infected snails, unlike in the uninfected ones (no difference) in comparison with the CTL- group (Table 3).

In the relation between the total protein concentrations and the different latex preparation times, in the period between 1 and 14 days there was an increase in the total protein concentrations in the hemolymph of the snails exposed to the latex, mainly in the infected specimens. From the 21st day onward the protein concentration was lower in the IE. The activity of the latex on this parameter receded as of the 21st day since solution preparation, but this decline was only significantly different in comparison with the CTL- group.

Nitrogen excretion products concentration: The levels of urea in the hemolymph of the snails exposed to the latex were reduced by up to 82.35% in relation to the unexposed snails. For the infected and exposed snails, this level was affected starting in the second week, when the urea concentration started to increase, reaching a concentration 3,150 times that of the infected snails not exposed after 7 days.

The concentration of uric acid in the uninfected and unexposed snails was always higher than in the uninfected exposed ones, but on the 28th and 35th days of observation, the uric acid concentration was higher.

DISCUSSION

Carbohydrates are the primary and immediate source of energy (Lehninger, 1978). Under stress conditions, carbohydrate reserves are depleted to meet increased an energy demand (Arasta, et al., 1996). Depletion of glycogen may be due direct utilization for energy generation, a demand caused by latex and/or infection-induced hypoxia.

The development of trematode larvae within *S. mansoni* causes intense drainage of nutrients from the host, mainly of glucose. Although the glucose concentration in the hemolymph of snails is very precisely regulated (Thompson, et al., 1986), the continuing reduction of the level of this carbohydrate in the hemolymph leads to mobilization of the snail's energy reserves, causing changes its concentration (Becker, 1983; Pinheiro and Amato, 1994). In response to this ongoing drainage, the glucose levels in the hemolymph decline and the metabolic routes that catalyze the degradation of glycogen molecules accelerate (Tielens, et al., 1992).

The results obtained in the present study indicate there was an increase in energy demand, with a consequent acceleration of the catabolism of carbohydrates, triggered by the infection and exposure to the latex, which in turn induced an increase in the degradation of glycogen molecules in the reserve tissues studied, the cephalopedal mass and digestive gland. The decline in the glycogen levels in both types of tissue was evident starting on the 7 days of exposure to the latex, coinciding with the first week of infection, at the moment when the miracidia become primary sporocysts and begin their development. This effect continues when they develop into secondary sporocysts and attach themselves to the digestive gland (Coelho, et al., 2008). According to Bezerra, et al., (1999), the larvae located in the inter-follicular tissues of the digestive gland are bathed by the hemolymph, from where they obtain the glucose necessary for energy metabolism (oxidative or aerobic), causing the host to mobilize its carbohydrate deposits to maintain its normal glycemia.

The results found in this study are corroborated by those of Mello-Silva, et al., (2010; 2011), studying the influence of exposure to the latex of *E. splendens* on the carbohydrate stocks at different sites and the glucose in the hemolymph of *B. glabrata* infected with *S. mansoni*. In those two studies, the authors found that the levels of glycogen, in the same two tissue types, of the infected snails also declined in relation to the control group and the association of infection with exposure to the latex caused a reduction in the concentration of the energy reserves in the cephalopedal mass and digestive gland. According to Tiwari, et al., 2005 exposure to sublethal doses of 40 and 60% of 24- and 96- h LC₅₀ of the aqueous extract of *Euphorbia tirucalli* latex significantly altered the levels of glycogen, pyruvate, and lactate and the activity of dehydrogenase (LDH), succinic dehydrogenase (SDH) and cytochrome oxidase in the nervous, hepatopancreatic and ovotesti tissues of the snail *Lymnaea acuminata*.

The findings of the present study suggest that the alterations caused by exposure to the LC₅₀ of the aqueous solution made from the *E.* latex affect both infected and uninfected snails. However, the glycogen concentration in the sites analyzed remained depressed for a longer period in the infected than in the uninfected snails.

All the biochemical changes observed in the snails in this study indicate that the actions triggered by the host are responses to maintain homeostasis, both in function of the infection and the exposure to the latex.

The effects of latex of *E. milii* on the carbohydrate metabolism of snails *B. glabrata* was shown by Mello-Silva, et al., (2010), but the results here presented confirmed the effects of this solution on the carbohydrate metabolism of the snails and we also can state that the latex solution of *E. milii* did not lose its moluscicidal activity until de 28th day after preparation. The LC₅₀ did not killed the snails, but it is possible to observe the toxic effects of it on the energetic metabolism of the snails, being these effects more severe in the infected snails.

Following the metabolic changes in the snails exposed to *E. milii* latex solution, it was possible observe in the initial periods of infection an increase of the total

proteins content. Tunholi, et al., (2011) and Pinheiro, et al., (2009) observed in different species of snails infected by the larval trematode *Echinostoma paraensei* an immediate reduction of the total protein content in the hemolymph of the host. In the present study, the elevation of the total protein content was higher than the values observed in control group until 7 days of infection. Probably this is not related to the latex solution action, it seems to be more a response to the infection, once the penetrating miracidia and the migrating sporocysts cause tissue disruption and cellular lysis, with consequent releasing of intracellular proteins in the hemolymph.

Another scenario is viewed in the snails 14 days post infection and exposed to latex prepared 14 days, where the total protein content is reduced, being this reduction significantly different from the control snails in the last period analyzed. This observation evidenced that the snails exposed to the stress of infection and the toxic action of latex solution, had their carbohydrates metabolism changed, clearly presenting a homeostasis break, reducing the glycogen stores in the tissues and compromising the glycemia of the snail. In this situation, the snails make use of non glycidic substrates to obtain alternatively the energy needed to their survival and to ensure the larval development of the developing trematodes. So, they metabolize amino acids, using the carbon skeleton of these molecules in the gluconeogenesis pathway, ranging the maintenance of the glicemia and more substrates to energetic metabolism, and reducing the proteins and free amino acids content in the hemolymph. This kind of changing in the proteic content in infected snails was observed by some authors (Mello-Silva, et al., 2006; Pinheiro, et al., 2009; Tunholi, et al., 2011), reinforcing our results.

The carbohydrate and proteins alterations will be reflected on the nitrogenous products of degradation and excretion in the snails. Firstly, the infection establishment and the intoxication by the *E. milii* latex solution causes a metabolic disorder in the host, but from the 7 days of infection and exposition to latex solution prepared 7 days onward the urea and uric acid contents are increased. Pinheiro, et al., (2009) also observed this response in *Lymnaea columella* infected with *E. paraensei*. These authors showed that the stressed snails use the amino acids to obtain energy, but to obtain the carbon skeleton of these molecules the amino acids must be deaminated, rising the nitrogen, as NH_3^+ , in the snail organism. This excess of nitrogenous products must be detoxified. The freshwater snails excrete mainly uric acid, but in stress conditions the increased level of this nitrogenous product must lead to intoxication of the animal. So, the snails accelerate the metabolic activity of urea cycle, increasing the urea formation and excretion, in a less toxic way. This metabolic resource is doubly useful, once the increasing of urea cycle activity also provides larger amounts of arginine, essential amino acids to larval development of trematodes (Kurelec, 1975). The results found in this study are corroborated by those of studying of the toxic effect of the aqueous and serially purified latex extracts of *E. pulcherima* and *E. hirta* (Singh, et al., 2004) and the aqueous stem bark and leaf extracts of plant *E. hirta* (Singh, et al., 2005) on the *L. acuminata*. The sulethal doses (40 and 80% of LC_{50}) of the both experiments also significantly alter the levels of total protein, total free amino acid, nucleic acid (DNA and RNA) and the activity of enzyme protease and acid and alkaline phosphatase in nervous tissues of the snails in time and dose dependent manner.

According to the biochemical tools used in the present study, it is possible to observe that the period of greatest activity of this solution on the carbohydrate metabolism of *B. glabrata* was from the first to the 14 days after solution preparation,

with pronounced changes in the carbohydrates and nitrogenous products metabolism of the snails, being stronger on the infected than the uninfected snails. Therefore, these results contribute to the optimization of the use of this product as a molluscicide of natural origin and the development of protocols for control of schistosomiasis. Suggesting a pattern of field use, and may establish ranges of application.

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Table-1: Concentration of glycogen (mg glucose/g of tissue) in the cephalopodal mass and digestive gland of *B. glabrata*, infected or uninfected by *S. mansoni* and exposed to the latex of *E. milii*, at a concentration of 0.7mg/L, in the period from 1 to 35 days.

Groups	Time since preparation of the latex (in days)					
	1	7	14	21	28	35
	X ± SD	X ± SD	X ± SD	X ± SD	X ± SD	X ± SD
Cephalopodal Mass						
CTL -	3.7 ± 0.2a	2.9±0.05a	2.8 ± 0.06a	2.9 ± 0.06a	2.7 ± 0.05a	2.8 ± 0.06a
CTL +	5.5 ± 0.2b	1.9± .04b	2.0 ± 0.07b	2.7 ± 0.04a	2.7 ± 0.1a	2.3 ± 0.03b
UE	3.0 ± 1.0ac	0.5± .04c	0.4 ± 0.1bc	2.4 ± 0.05b	2.4 ± 0.05a	2.8 ± 0.04a
IE	5.0 ± 0.3b	0.3± .06d	0.4±0.06bc	1.4 ±0.04c	2.2 ± 0.2b	2.7 ± 0.07a
Digestive Gland						
CTL -	2.6 ± 0.3a	2.8±0.06a	2.5 ± 0.02a	2.6 ± 0.02a	2.6 ± 0.05a	2.6 ± 0.05a
CTL +	5.5 ± 0.3b	1.8±0.04b	2.3 ± 0.04b	2.4 ± 0.1a	2.3 ± 0.1b	2.1 ± 0b
UE	3.9 ± 0a	0.7±0.04c	0.3±0.01c	2.3 ± 0.5a	2.3 ± 0.06b	2.6 ± 0.07a
IE	4.1 ± 1.4b	0.3±0.03d	0.5 ±0.1c	1.4 ± 0.1b	2.1 ±0.09b	2.6 ± 0.09a

- X± SD = mean ± standard deviation.
- Different letters mean significant differences in comparison to control group, a= $P<0.05$, b= $P<0.01$, c= $P<0.001$.
- CTL-=uninfected and unexposed to latex; CTL+=uninfected and exposed to latex; UE=uninfected and exposed to latex; IE=infected and exposed to latex.

Table-2: Concentration of glucose and total protein (mg/dl) in the hemolymph of *Biomphalaria glabrata*, infected or uninfected by *Schistosoma mansoni* and exposed to the latex of *Euphorbia milii*, at a concentration of 0.7mg/L, in the period from 1 to 35 days.

Groups	Time since preparation of the latex (in days)					
	1	7	14	21	28	35
	X ± SD	X ± SD	X ± SD	X ± SD	X ± SD	X ± SD
Glucose						
CTL -	2.6 ± 0.3a	2.8±0.06a	2.5 ± 0.02a	2.6 ± 0.02a	2.6 ± 0.05a	2.6 ± 0.05a
CTL +	5.5 ± 0.3c	1.8±0.04b	2.3 ± 0.04b	2.4 ± 0.1a	2.3 ± 0.1b	2.1 ± 0b
UE	3.9 ± 0b	0.7±0.04c	0.3±0.01c	2.3 ± 0.5a	2.3 ± 0.06b	2.6 ± 0.07a
IE	4.1 ± 1.4c	0.3±0.03d	0.5 ±0.1c	1.4 ± 0.1b	2.1 ±0.09b	2.6 ± 0.09a
Total protein						
CTL -	14 ± 01a	14±004a	13 ± 02a	13 ± 01a	12 ± 02a	14 ± 002a
CTL +	14 ± 01a	12± 01b	17 ± 003b	10±006ab	10±003ab	11 ± 002b
UE	29 ± 04b	17±003c	16 ± 02ab	10 ± 004b	07 ± 003b	08 ± 008c
IE	24 ± 01b	33±002d	14±004ab	09± 009b	09 ± 009b	09 ± 002d

- Footnotes are same as given in table-1.

Table-3: Concentration of urea and uric acid (mg/dl) in the hemolymph of *Biomphalaria glabrata*, infected or not infected by *Schistosoma mansoni* and exposed to the latex of *Euphorbia milii*, at a concentration of 0.7mg/L, in the period from 1 to 35 days.

Groups	Time since preparation of the latex (in days)					
	1	7	14	21	28	35
	X ± SD	X ± SD	X ± SD	X ± SD	X ± SD	X ± SD
Urea						
CTL -	14,7±0,7a	1.2 ± 0a	15.2 ± 2.5a	15.0± 2.3a	16.4 ± 0.1a	17.0± 0.06a
CTL +	4,5 ± 0,2b	0.4 ± 0a	5.6 ± 0.2b	7.0 ± 0.3b	6.0 ± 0.2b	11.2 ± 0.1b
UE	14,0 ± 1,4a	0.4 ± 0a	26.0 ± 1.4c	7.0 ± 0.1b	4.4 ± 0.3c	3.0 ± 0.4c
IE	3,4 ± 1,3b	13.0±0.6b	8.0 ± 0.3b	9.0 ± 0.2b	19.0 ± 0.4d	6.0 ± 0.4d
Uric acid						
CTL -	1.3± 0.4 a	1.6± .02a	1.2 ±0.03a	1.5± 0.06a	1.1 ± 0.2a	7.2± 0.06a
CTL +	1.7 ± 0.2a	1.7 ± 0.1a	0.8 ± 0b	1.0 ± 0.2b	0.4 ± 0b	0.7 ± 0b
UE	0.5 ± 0.1b	1.0± 0.2b	0.2 ± 0c	0.3 ± 0.02c	1.5 ± 0c	1.6 ± 0.02b
IE	0.4± .01b	8.7±0.07c	7.3 ± 0.2d	8.1 ± 0.2d	10.0 ±0.1d	12.4 ± 0.8c

- Footnotes are same as given in table-1.