Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Metabolic and behavioral effects of PHMB on *Biomphalaria glabrata*: a strategy for schistosomiasis control

Efeitos metabólicos e comportamentais do PHMB sobre *Biomphalaria glabrata*: uma estratégia para o controle da esquistossomose

M. E. A. Almeida[a], V. C. S. Amaral[b], T. L. Costa[c], K. B. Machado[d], J. C. B. Bezerra[c], M. C. Vinaud[c] and L. Damacena-Silva[a]*

[a]Universidade Estadual de Goiás – UEG, Laboratório de Pesquisa da Interação Parasito-Hospedeiro, Anápolis, GO, Brasil
[b]Universidade Estadual de Goiás – UEG, Laboratório de Farmacologia e Toxicologia de Produtos Naturais e Sintéticos, Anápolis, GO, Brasil
[c]Universidade Federal de Goiás – UFG, Instituto de Patologia Tropical e Saúde Pública, Goiânia, GO, Brasil
[d]Universidade Estadual de Goiás – UEG, Instituto Acadêmico de Ciências da Saúde e Biológicas, Laboratório de Biogeografia e Ecologia Aquática, Anápolis, GO, Brasil

Abstract

The snail *Biomphalaria glabrata* is an important transmitter of *Schistosoma mansoni*, the parasite that causes schistosomiasis. One of the strategies for controlling the disease involves interrupting the transmission cycle of the parasite by managing the host snail population. In a previous study, the sanitizer polyhexamethylene biguanide hydrochloride (PHMB) caused mortality in *B. glabrata* starting at 1.6 mg L^{-1}. The present study evaluated the activity of PHMB at concentrations of 0.4, 0.6, and 0.8 mg L^{-1} through behavioral changes and biochemical biomarkers. The snails were evaluated every 24 hours for behavioral changes such as lethargy, mucus secretion, shell confinement, exposure of the cephalopodal mass, and lack of movement. After 96 hours, hemolymph was collected via cardiac puncture for analysis of glucose, total protein, urea, uric acid, creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes, and organic acids involved in the glycolytic pathway, tricarboxylic acid cycle, and lipid and protein metabolism. The exposure of the cephalopodal mass with lack of movement showed the highest rates of behavioral changes, ranging from 72% to 95%. Additionally, exposure to PHMB resulted in the use of fatty acids and proteins as energy substrates. Our results suggest a continuation of studies on energy metabolism pathways as a promising target for controlling snail vectors of parasites that cause neglected diseases such as schistosomiasis.

Keywords: behavioral changes, energetic metabolism, schistosomiasis control, sanitizer, snail control.

Resumo

O caramujo *Biomphalaria glabrata* é um importante transmissor de *Schistosoma mansoni*, o parasito causador da esquistossomose. Uma das estratégias para o controle da doença envolve a interrupção do ciclo de transmissão do parasito por meio do controle da população de caramujos hospedeiros. Em um estudo anterior, o sanitizante cloridrato de poliexametileno biguanida (PHMB) causou mortalidade em *B. glabrata* a partir de 1,6 mg L^{-1}. O presente estudo avaliou a atividade do PHMB nas concentrações de 0,4, 0,6 e 0,8 mg L^{-1} por meio de alterações comportamentais e biomarcadores bioquímicos. Os caramujos foram avaliados a cada 24 horas para alterações comportamentais, como letargia, secreção de muco, reclusão na concha, exposição da massa cefalopediosa e ausência de movimento. Após 96 horas, a hemolinfa foi coletada por punção cardíaca para análise de glicose, proteínas totais, ureia, ácido úrico, creatinina, as enzimas aspartato aminotransferase (AST) e alanina aminotransferase (ALT), além de ácidos orgânicos da via glicolítica, do ciclo do ácido tricarboxílico e do metabolismo de lipídios e proteínas. A exposição da massa cefalopediosa com ausência de movimento apresentou as taxas mais altas das alterações comportamentais, variando de 72% a 95%. Além disso, a exposição ao PHMB resultou no uso de ácidos graxos e proteínas como substratos energéticos. Nossos resultados sugerem a continuidade dos estudos sobre as vias de metabolismo energético como um alvo promissor para o controle de caramujos hospedeiros de parasitos causadores de doenças negligenciadas, como a esquistossomose.

Palavras-chave: alterações comportamentais, metabolismo energético, controle da esquistossomose, sanitizante, controle do caramujos.

*e-mail: luciana.silva@ueg.br
Received: December 17, 2024 – Accepted: January 31, 2025

1. Introduction

The freshwater snail *Biomphalaria glabrata* is an important intermediate host in the life cycle for the human parasite *Schistosoma mansoni*, the causative agent of schistosomiasis. About 239 million people in 78 countries worldwide are infected with the disease. Brazil represents the most impacted nation in the Americas, with an estimated 1.5 million cases, primarily in the northeastern states (Brasil, 2014; WHO, 2017).

The control of intermediate host snails represents a strategy for the control of schistosomiasis, with the objective of interrupting the transmission chain of *S. mansoni* (WHO, 2023). The molluscicide Niclosamide is widely utilized in schistosomiasis control initiatives. However, its low selectivity, potential for environmental contamination, and the development of resistance among *Biomphalaria glabrata* snails raise significant concerns regarding its impact on non-target species (Costa et al., 2015; Rangel et al., 2022).

In the search for new molluscicidal compounds, exposure of *B. glabrata* to the disinfectant polyhexamethylene hydrochloride biguanide (PHMB) resulted in lethargy, followed by high mortality rates starting at 2.0 mg L^{-1}. The LC_{50} was 1.49 mg L^{-1} (87.5 ± 12.5%) and 2.6 mg L^{-1} (100 ± 0%) with 96 h of exposure (Melo et al., 2019).

Experimental research has shown that the ecotoxicity of this compound to different aquatic organisms varies by species, depending on concentration and exposure time. In *Danio rerio* embryos, for example, delays in hatching, developmental anomalies, and reduced heart rate were observed at concentrations of 1, 2, 3, and 4 µM, 120 hours after fertilization (Oh et al., 2024). On the other hand, in adult fish liver cell lines, PHMB exhibited low toxicity, with an EC_{50} of 11.02 µg mL^{-1} after 168 hours of exposure (Christen et al., 2017). In other aquatic species, such as *Oncorhynchus mykiss*, the LC_{50} in 96 hours was 0.026 mg L^{-1}, while for the microcrustacean *Daphnia magna*, the LC_{50} in 48 hours was recorded at 0.09 mg L^{-1} (ECHA, 2015).

The responses of *B. glabrata* to PHMB may result from the interaction of this sanitizer with the phospholipids of the plasma membrane, leading to the activation of chloride (Cl^-) and potassium (K^+) ion channels, similarly to what has been described for bacteria, protozoa, and fungi (Niro et al., 2022). Behavioral changes and mortality rates in snails are biomarkers of toxic stressors in water and potential indicators of molluscicides (Bernot et al., 2005; Lürling, 2012; Pieri and Jurberg, 1981). Thus, lethargic behavior is indicative of hypoxia and anoxia (de Zwaan and Putzer, 1985; Fraga, 2002; Lee et al., 2023) and can be enhanced by the concentration of substrates and metabolites involved in the snail's energy metabolism (Patience et al., 1983). *B. glabrata* under hypoxic conditions showed a partial inversion of the tricarboxylic acid (TCA) cycle and end products of anaerobic metabolism (Patience et al., 1983; Bezerra et al., 1999).

Considering that in gastropods glucose molecules are used as the main energy substrate and that this carbohydrate is mobilized during anoxia independently or together with the use of hemolymph glucose (Hochachka et al., 1983), the strategy of metabolic depression is considered as a physiological adaptation mechanism that redirects its metabolic flow in an anaerobic direction, using amino acids and fatty acids as substrates in gluconeogenesis and, to a lesser extent, the use of ketone bodies (Bezerra et al., 1999; Meyer et al., 1986; Bezerra and Becker, 1993; Silva et al., 2017).

The presence of substances that alter the host's metabolic energy metabolism may have an unfavorable impact on the establishment of the parasite or may promote significant alterations to certain substrates that maintain the redox balance. Therefore, the aim of this study was to quantify the behavioral changes, as well as to verify the use of alternative metabolic pathways by *B. glabrata* through the detection of glycolysis metabolites, the TCA cycle, acids related to fatty acid catabolism, ketone bodies and protein metabolism. The hypothesis that PHMB promotes behavioral and metabolic changes in *B. glabrata* was tested.

2. Materials and Methods

2.1. Preparation of experimental PHMB solutions

The commercial formulation of Polyhexamethylene biguanide hydrochloride (PHMB), (Vantocil IB®) (CAS n 32289-58-0; 20% w/w) was supplied by Lonza Microbial Control (São Paulo, Brazil). The PHMB stock solution (100 mg L^{-1}) was prepared using dechlorinated water. The test solutions at concentrations of 0.4, 0.6 and 0.8 mg L^{-1} were prepared from the stock solution. The choice of PHMB concentrations (0.4, 0.6 and 0.8 mg L^{-1}) was based on toxicity data previously established by Melo et al. (2019), with the purpose to evaluate the effects of the sanitizer at levels below the LC_{50} for embryos (0.98 mg L^{-1}) and newly hatched and adult snails (1.43 and 1.49 mg L^{-1}, respectively).

2.2. Snails

The snail *B. glabrata*, sexually mature (confirmed by oviposition), with shell diameter 11 ± 1 mm; total weight: 0.23 ± 0.06 g were kept at the Laboratory for Research on Parasite-Host Interaction (LAPIPH) of the Research and Postgraduate Center - State University of Goiás (UEG), according to OECD guideline n 243 (OECD, 2016). The snails were kept in aquaria (20 x 13 x 13 cm), containing 3 L of dechlorinated water under a 12:12 h light/dark photoperiod, temperature (25 ± 1 °C) and pH (7.0 ± 1). They were fed ad libitum with lettuce leaves (*Lactuca sativa*) three times per week.

2.3. Experimental design

A total of 80 snails were used in the experiment, distributed into four experimental groups with 20 individuals each. These groups were exposed to different concentrations of PHMB (0.4, 0.6, and 0.8 mg L^{-1}). The snails in the control group were kept in dechlorinated water. The experiment was conducted in quadruplicate, with 5 snails per replicate. Glass aquariums were used for the experiment (180 mL), under a 12:12 h light/dark photoperiod and a temperature of 25 ± 1 °C. Snails were fed 1.0 g of fresh lettuce leaves (*Lactuca sativa*) every other day.

The snails were observed daily for behavioral changes such as position of the snails in the aquarium (bottom or surface), exposure of the cephalopodal mass, absence of movements (lethargy), reclusion in the shell and secretion of mucus. The observation time was 1 minute per day for each individual (Caixeta et al., 2021).

For the biochemical analyses, the snails were randomly removed from the experimental aquariums and transferred to a 100 mL beaker containing ice cubes to be anesthetized by hypothermia. Subsequently, their shells were dried with absorbent paper for hemolymph extraction through cardiac puncture. The hemolymph was obtained using a 1 mL syringe with a 0.7x25 needle (type 22G 1"). The samples were transferred to 1 mL microtubes and kept at 0 °C to preserve their stability, according to the protocol of Guder et al. (2001) and Cuhadar et al. (2013). Subsequently, the hemolymph samples were frozen in liquid nitrogen and stored in a freezer at -80 °C. Due to the low volume of hemolymph obtained from each snail, each sample was composed of hemolymph from 5 snails, in quadruplicates, with the aim of increasing statistical precision and controlling biological variability, making the data more robust and representative.

Hemolymph samples were thawed at room temperature and 100 µL were used to obtain concentrations of glucose, total proteins, urea, uric acid, creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes using Abbott reagents and read on an Alinity spectrophotometer (Lima et al., 2022).

The organic acids present in the hemolymph were extracted using an ion exchange extraction column (Bond Elut® Agilent®) as described by Bezerra et al. (1999). The columns were activated with the aid of a Manifold box under vacuum through successive washes with 1 mL of 0.5M HCl, 1 mL of methanol, and 2 mL of HPLC-grade water. Loading was accomplished with 200 µL of hemolymph and 2 mL of water. After disconnecting from the column was removed from the Manifold box under the vacuum pump, 250 µL of 0.5 M sulfuric acid was applied to facilitate the elution of organic acids retained on the matrix of Bond-Elut®. The eluate underwent centrifugation at 1200 rpm for 5 minutes, and the resulting supernatant from the test tube was stored at -20 °C until subjected to analysis via high-performance liquid chromatography.

2.4. Statistical analysis

Statistical analysis was performed using the R program (R Core Team, 2024). The one-way ANOVA one-way test followed by pairwise comparison post-test were used to evaluate possible differences in the concentrations of organic acids and biochemical data in the different concentrations of exposure to PHMB. Differences were considered statistically significant when $p < 0.05$. Descriptive statistics were used to determine the mean and standard deviation and to assess the differences between the groups analyzed. The one-way ANOVA was performed using the RVAideMemoire (Hervé, 2023) package and p value estimated by 999 permutations (Mangiafico, 2024). The pairwise comparison post-test was executed using the rcompanion package.

3. Results and Discussion

3.1. Behavioral changes of Biomphalaria glabrata

Exposure to PHMB caused the snails to project the cephalopodal mass, followed by a lack of movement (Figure 1A). The occurrence of this behavior varied, with rates of 80-90% for the concentration of 0.4 mg L^{-1}, 72-95% for 0.6 mg L^{-1} and 90-94% for 0.8 mg L^{-1}. Additionally, the behavior of reclusion into the shell (Figure 1B) was observed in 10-20% of snails at 0.4 mg L^{-1}, 5-28% at 0.6 mg L^{-1}, and 6 -10% at 0.8 mg L^{-1}.

The behavior of concentrating on the bottom of the aquaria (Figure 1C) without moving when exposed to PHMB at a concentration of 0.8 mg L^{-1} varied between 69 and 95% of the snails. While 10 - 90% and 10 - 78% of the snails exposed to 0.4 and 0.6 mg L^{-1}, respectively, remained on the surface of the aquaria throughout the exposure time (Figure 1D). Mucus secretion was observed in 90% of the snails exposed to 0.6 and 0.8 mg L^{-1} PHMB.

The prolonged presence of snails at the bottom of experimental aquariums, with their visceral mass protruding outside the shell and lacking mobility, can be attributed to the mechanism of action of PHMB, as previously described in the context of bacteria and fungi. In this process, the polymer binds to the phospholipids of the cytoplasmic membrane, forming a polyhexanide-phospholipid complex. This results in the activation of Cl^- and K^+ ion channels, as well as the dysfunction of membrane receptors and enzymes (Niro et al., 2022). Consequently, PHMB promotes the inactivation of neurons that stimulate muscle contractions, which are responsible for the snail's movements. Additionally, the lethargic condition prevented the snails from feeding, which resulted in high mortality rates (Silverthorn, 2010; Pavlova, 2010)

Snails exposed to 0.4 mg L^{-1} fed normally, similar to the control group, for a total of 2 g of lettuce in 96 h. At 0.6 mg L^{-1}, the snails ate approximately 0.25 g of lettuce every 24 h, for a total of 0.5 g in 96 h. At 0.8 mg L^{-1} PHMB, the snails did not feed during the entire exposure period.

Behavioral changes in aquatic snails in the face of stressors are considered to be responses to toxicity in water and may also be indicative of agents such as molluscicides (Pieri and Jurberg, 1981). The secretion of mucus by *B. glabrata* was interpreted by Melo et al. (2019) as an attempt to block the action of PHMB. On the other hand, the lethargy associated with fasting makes the animals weaker, more susceptible to predation and death (Bernot et al., 2005).

3.2. Biochemical data

An increase in glucose was observed at an exposure of 0.6 mg L^{-1} compared to the control and 0.8 mg L^{-1} ($p = 0.002$) (Figure 2A). However, exposure to PHMB did not alter the concentrations of the organic acids involved in the glycolytic pathway, such as PEP (Figure 2B), pyruvate (Figure 2C), and lactate (Figure 2D).

Pyruvate is the end product of the glycolytic process and is used as a substrate for the formation of acetyl-CoA, which enters the ATC cycle or forms acetate (Figure 3A) or lactate. The process of oxidative decarboxylation of

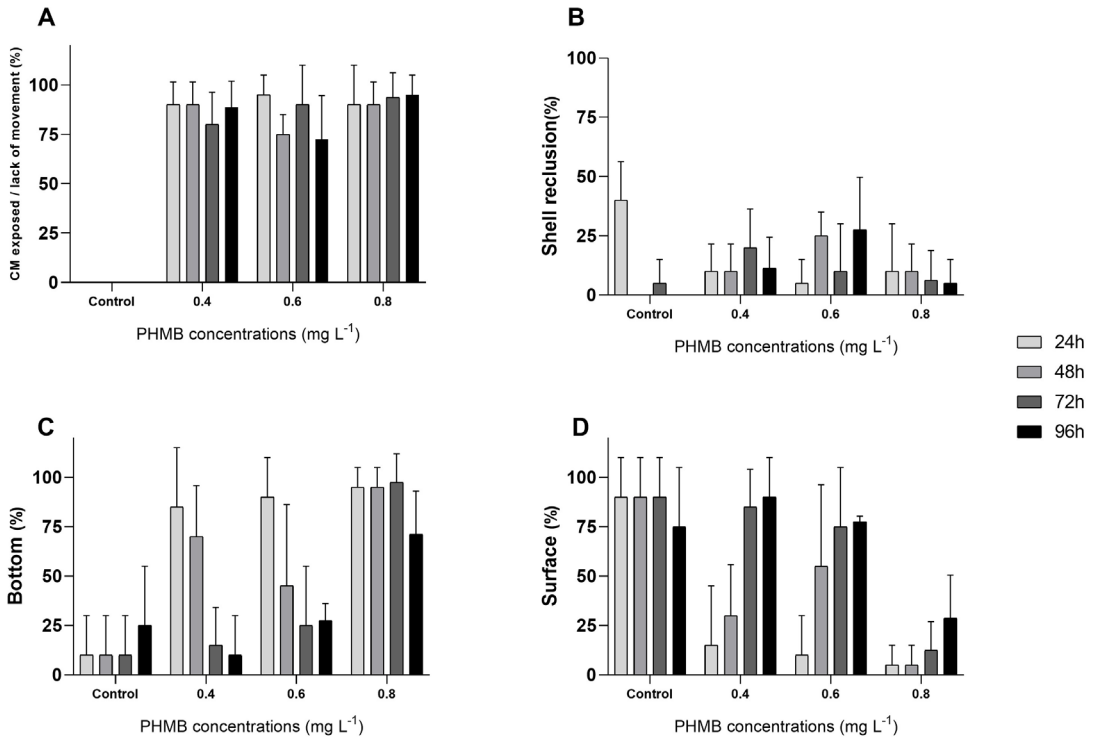

Figure 1. Mean behavioral changes observed in *Biomphalaria glabrata* exposed to PHMB. (A) Exposure of the cephalopodal mass and lack of movement; (B) Shell reclusion; (C) Snails at the bottom of the aquarium during the exposure time; (D) Snails at the surface of the aquarium during the exposure time.

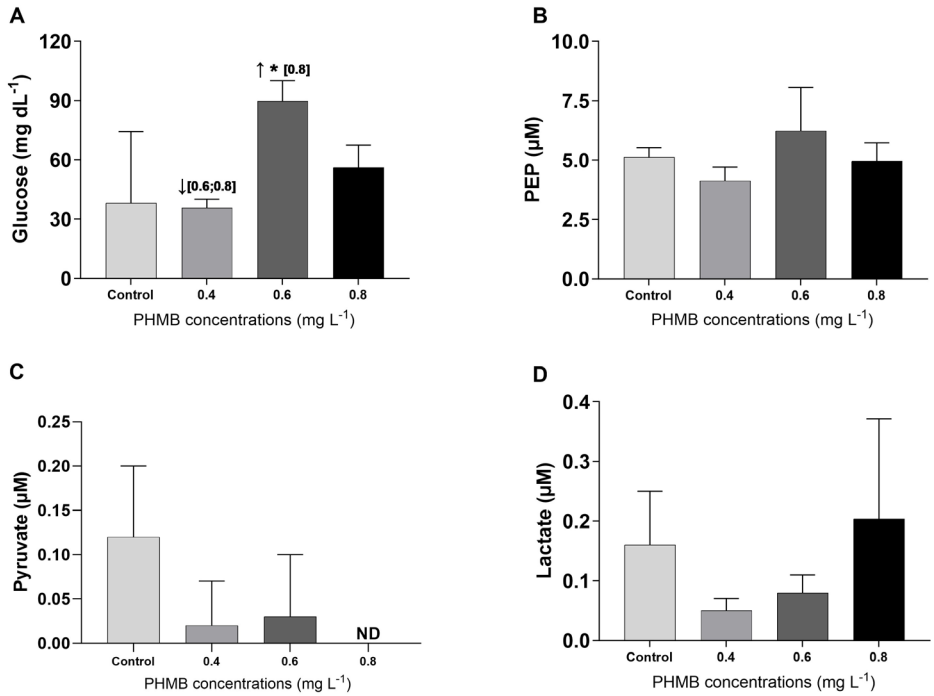

Figure 2. Mean concentrations of glucose and organic acids of the glycolytic pathway detected in the hemolymph of *Biomphalaria glabrata* snails exposed to 0.4, 0.6 and 0.8 mg L^{-1} of PHMB for 96 h. (A) Glucose; (B) PEP; (C) Pyruvate; (D) Lactate. [↑], [↓] increase and decrease; (*) difference compared to control; [0.4]/[0.6]/[0.8] differences between concentrations. Differences were considered statistically significant when $p < 0.05$. ND = Not detected.

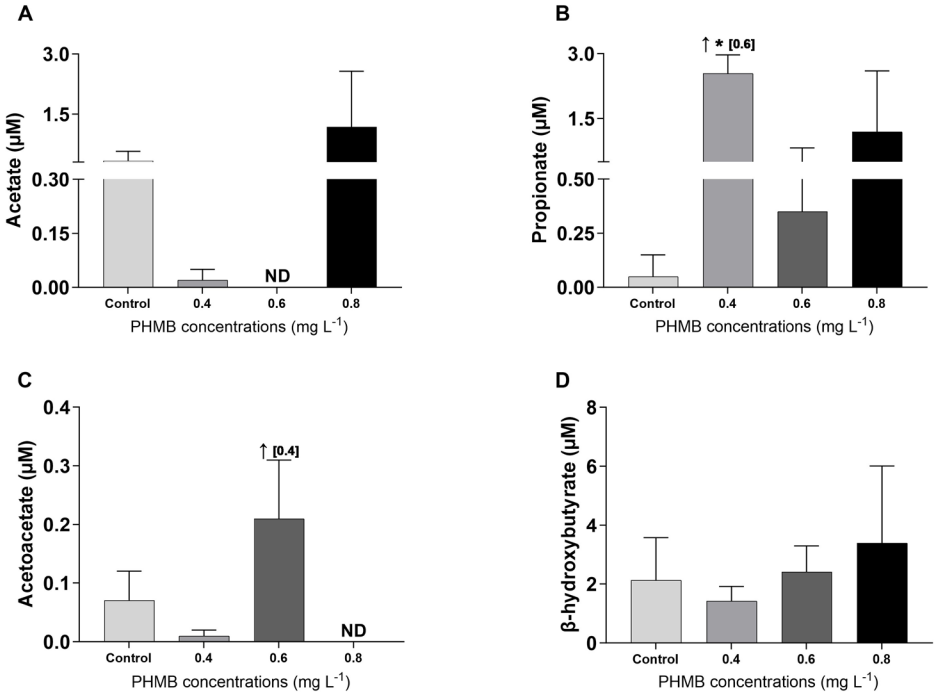

Figure 3. Mean concentrations of organic acids from fatty acid oxidation detected in the hemolymph of *Biomphalaria glabrata* snails exposed to 0.4, 0.6 and 0.8 mg L^{-1} of PHMB for 96 h. (A) Acetate; (B) Propionate; (C) Acetoacetate; (D) β-hydroxybutyrate. [↑], [↓] increase and decrease; (*) difference compared to control; [0.4]/[0.6]/[0.8] differences between concentrations. Differences were considered statistically significant when $p < 0.05$. ND =Not detected.

pyruvate to acetyl-CoA in *B. glabrata* can produce free acetate and ATP through acetyl phosphate. This process can lead to partial anaerobic metabolism, as observed in other invertebrates (Wieser, 1980; Wolmarans, 1987). The production of acetate likely occurred due to a decrease in oxygen consumption by the snail, as well as an imbalance in the redox system (Bezerra et al., 1999). Furthermore, the reduction in citrate ($p = 0.01$) in the hemolymph observed on exposure to 0.6 mg L^{-1} compared to the control and 0.8 mg L^{-1} suggests a decrease in normoxic activity in the Krebs cycle, influenced by PHMB.

Propionate (Figure 3B) increased ($p < 0.05$) at the lowest concentration of PHMB tested. This organic acid, as well as lactate and succinate are described as the end products of anaerobic metabolism (Wieser, 1980; Wolmarans, 1987). In addition to succinate, fumarate and malate were detected in the hemolymph of *B. glabrata* exposed to PHMB, with malate increasing at 0.8 mg L^{-1} compared to the 0.4 and 0.6 ($p < 0,05$). The presence of these acids in the hemolymph of *B. glabrata* under hypoxic conditions indicated a partial inversion of the ATC cycle and the end products of anaerobic metabolism (Patience et al., 1983; Bezerra et al., 1999).

The increase in the concentration of acetoacetate (Figure 3C) when exposed to 0.6 mg L^{-1} of PHMB can be explained by the fasting state of the snails exposed to this concentration of PHMB. Acetoacetate may have originated from succinate produced in the TCA cycle (Meyer et al., 1986) or from the digestive gland, an organ analogous to the liver in vertebrates (Zarai et al., 2011; Faro et al., 2013). Free acetoacetate is reduced to D-β-hydroxybutyrate through a reversible reaction catalyzed by D-β-hydroxybutyrate dehydrogenase, a mitochondrial enzyme. Acetoacetate and β-hydroxybutyrate (Figure 3D) are used as energy substrates for other tissues through ketolysis (Meyer et al., 1986).

Furthermore, although the oxidation of fatty acids is the main source of ketone bodies, they are also produced by the catabolism of certain amino acids, such as: leucine, isoleucine, lysine, tryptophan, phenylalanine, and tyrosine. These amino acids are called ketogenic because their carbon chains are catabolized to acetyl-CoA or acetoacetyl-CoA, substrates for the synthesis of ketone bodies (Motta, 2005).

The increase in glucose from the intermediate concentration (0.6 mg L^{-1}) and the reduction of total proteins (Figure 4A) in the hemolymph of *B. glabrata* exposed to 0.8 mg L^{-1} compared to the control ($p = 0.04$) indicates that glucose resulted from gluconeogenesis due to the degradation of lipids, as previously described, and proteins, resulting in intermediate substrates from oxidative pathways (Becker, 1980; Pinheiro et al., 2009; Tunholi et al., 2013). The use of alternative pathways for energy production maintains the homeostatic balance that is destabilized under adverse ecophysiological conditions (Becker, 1980; Pinheiro et al., 2009; Mello-Silva et al., 2010; Tunholi et al., 2013; Silva et al., 2019).

Although exposure to PHMB did not cause changes in the concentrations of the enzymes alanine aminotransferase

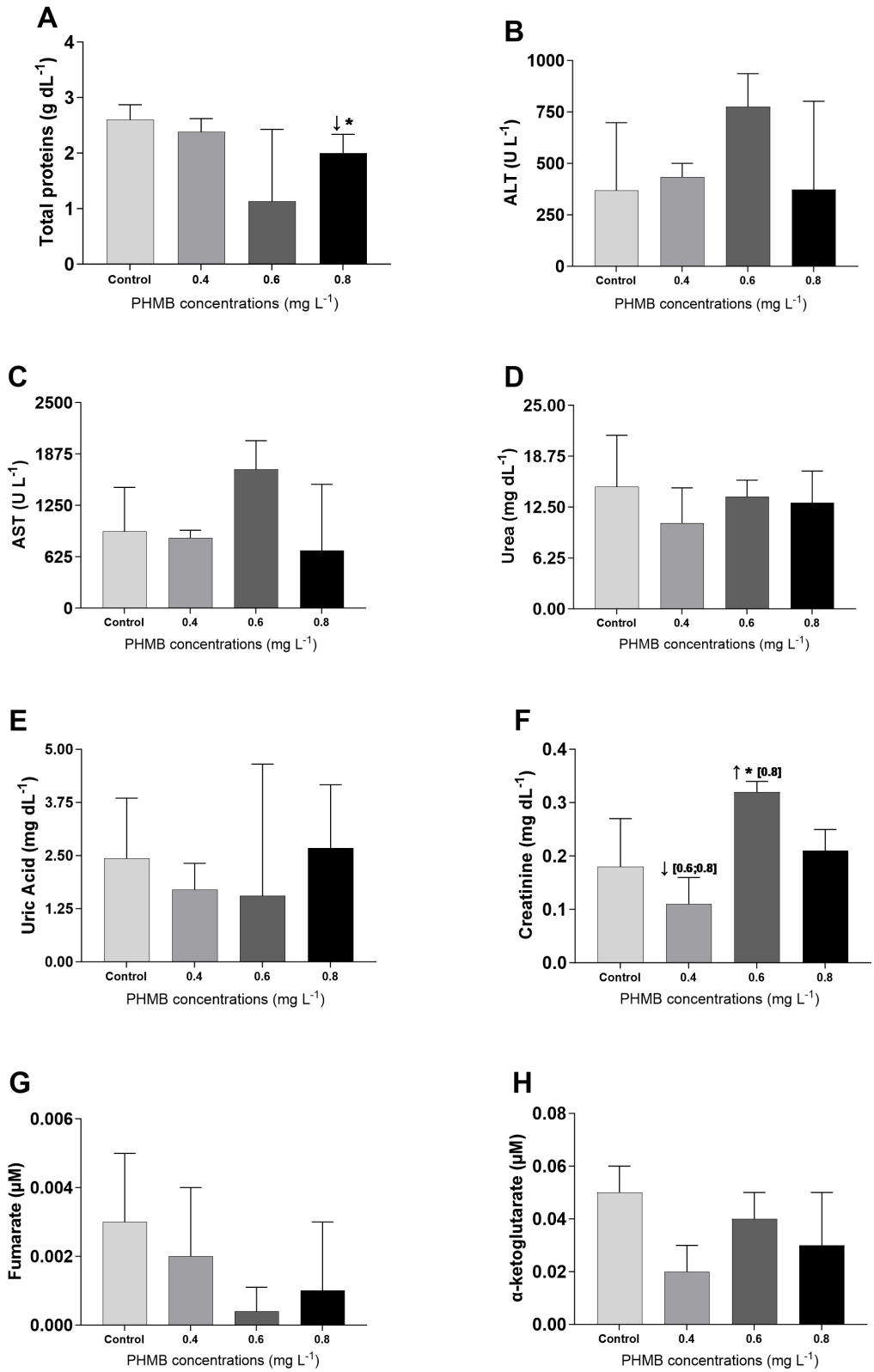

Figure 4. Mean concentrations of total proteins, aminotransferases and metabolites related to protein catabolism detected in the hemolymph of *Biomphalaria glabrata* snails exposed to 0.4, 0.6 and 0.8 mg L^{-1} of PHMB for 96 h. (A) Total proteins; (B) ALT; (C) AST; (D) Urea; (E) Uric acid; (F) Creatinine; (G) Fumarate; (H) α-ketoglutarate. [↑], [↓] increase and decrease; (*) difference compared to control; [0.4]/[0.6]/[0.8] differences between concentrations. Differences were considered statistically significant when $p < 0.05$.

(ALT) (Figure 4B) and aspartate aminotransferase (AST) (Figure 4C). The increase in ALT and AST concentrations in the hemolymph of gastropods has been interpreted as an activation of gluconeogenesis (Christie and Michelson, 1975; Pinheiro et al., 2001; Tunholi-Alves et al., 2013), where a greater consumption of carbon structures from amino acids, a reduction in total protein concentrations, and an accumulation of nitrogenous excretion products were observed in snails infected with trematodes (Pinheiro et al., 2001). Additionally, ALT is considered a biomarker of tissue injury, located in both the cytosol and mitochondria of cells in vertebrates and invertebrates (Christie and Michelson, 1975; Pinheiro et al., 2001; Tunholi-Alves et al., 2013). Increased ALT in the hemolymph indicates cellular damage induced by inflammatory and/or necrotic processes in the tissues (Pinheiro et al., 2001).

As a result of the amino acid deamination process, nitrogenous degradation products such as urea (Figure 4D) and uric acid are formed (Figure 4E) (Becker, 1983; Pinheiro, 1996; Tunholi et al., 2011). In this study, PHMB did not alter the concentrations of urea and uric acid in the hemolymph of *B. glabrata*. Uric acid is a less toxic metabolite for aquatic snails and may act as a non-enzymatic antioxidant (Becker, 1983).

A creatinine (Figure 4F) as the end product of the urea cycle (Silva et al., 2019; De Cian et al., 2000), was suggested on exposure to 0.6 mg L^{-1} compared to the control and 0.8 mg L^{-1} ($p < 0.05$). Fumarate (Figure 4G) is also a metabolite in the urea cycle and can be converted into cytosolic malate. Malate, when in the mitochondria, is utilized in the TCA (tricarboxylic acid) cycle (Becker, 1980).

The results of this study indicate that *B. glabrata* exposed to PHMB resorts to metabolic pathways associated with fatty acid oxidation and protein degradation as alternative energy sources (Figure 5). This change in energy metabolism suggests a state of physiological

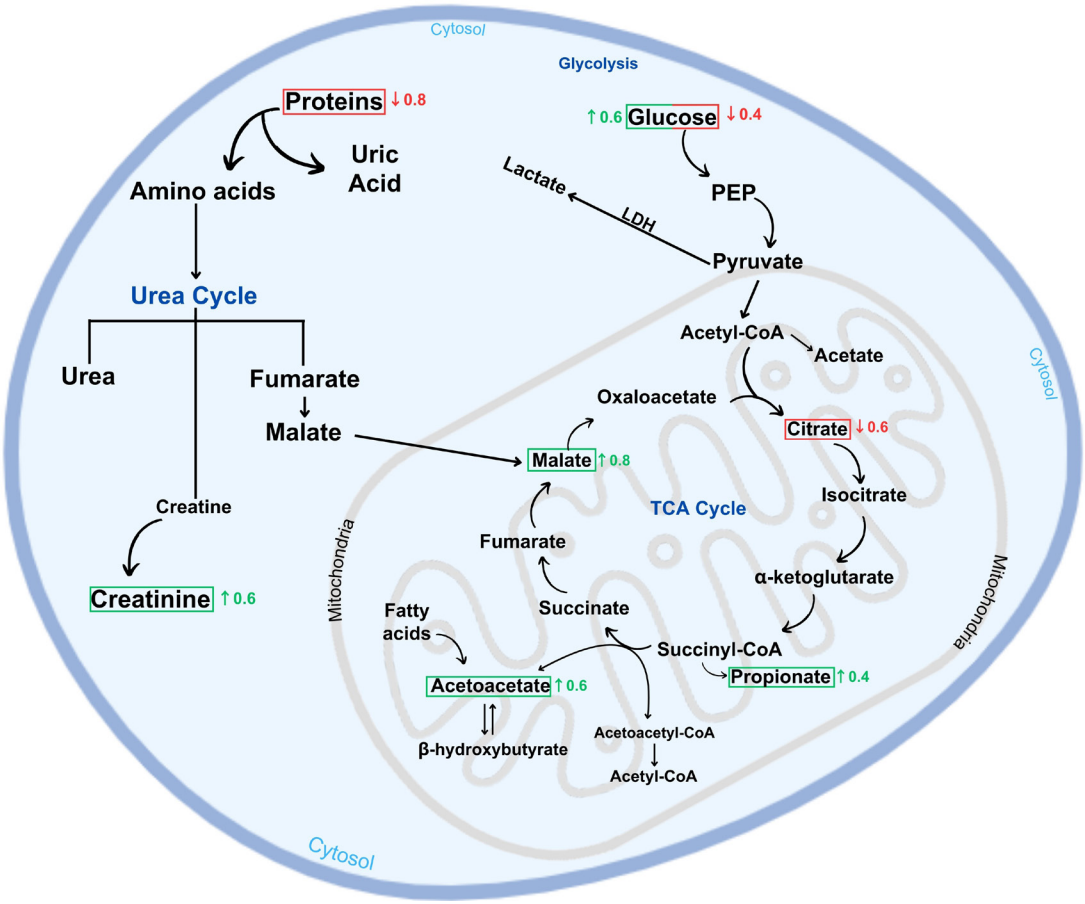

Figure 5. Diagram illustrating the main changes detected in the energy metabolism of *Biomphalaria glabrata* exposed to concentrations 0.4, 0.6 and 0.8 mg L^{-1} of PHMB, indicating the main significant differences ($p<0.05$). The increase in hemolymphatic glucose indicates the demand used in the production of the organic acids involved in the glycolytic pathway. The decrease in citrate suggests a decrease in the activity of the tricarboxylic acid (TCA) cycle. In anaerobic metabolism, propionate appears as the end product of the TCA cycle. An increase in malate indicates a partial inversion of the TCA cycle or a product of protein metabolism. Succinate has been converted to acetoacetate, which also comes from fatty acid oxidation or the reversible reaction with β-hydroxybutyrate. The reduction in total protein in the hemolymph suggests its use as an energy substrate in gluconeogenesis. An increase in creatinine indicates activation of the urea cycle. Both urea and uric acid are nitrogenous excretion products of snails. [↑] indicates an increase in concentration, [↓] a decrease in concentration.

stress, resulting in a depression of metabolism and directly impacting the snail's ability to respond to external stimuli. As a consequence, the animal becomes more vulnerable to predators and less able to react to environmental changes, compromising its survival (Lutz and Nilsson, 1997). The decline in the population viability of *B. glabrata* enhances the effectiveness of environmental control, aiding in the disruption of the life cycle of *S. mansoni* and contributing to the long-term reduction of schistosomiasis transmission. Furthermore, studies utilizing *in silico* technology have demonstrated that PHMB exhibits high stability in water and low chemical reactivity, suggesting it is an environmentally safe and non-toxic compound (Celik and Tanis, 2022). Despite the apparent environmental safety of PHMB, its ecotoxicological impacts vary depending on concentration, exposure time, and organism sensitivity. In *Danio rerio*, concentrations of 1-4 µM caused delayed hatching, anomalies, and reduced heart rate after 120 hours (Oh et al., 2024). In liver cells of adult fish, toxicity was low (EC_{50} = 11.02 µg mL^{-1} at 168 h) (Christen et al., 2017). For *Oncorhynchus mykiss*, the 96 h LC_{50} was 0.026 mg L^{-1} and for *Daphnia magna*, it was 0.09 mg L^{-1} at 48 h (ECHA, 2015).

4. Conclusion

The use of PHMB shows strong potential in controlling *S. mansoni* transmission by targeting its intermediate host, *B. glabrata*. PHMB induces behavioral responses in snails, such as exposing the cephalopod mass with no movement, and biochemical changes indicative of metabolic stress, utilizing alternative energy sources. This includes partial anaerobic metabolism, with fatty acids and proteins as substrates, and metabolites like propionate and malate. These changes, especially the behavioral and metabolic stress, highlight PHMB's potential to reduce snail host viability and disrupt the lifecycle of schistosomiasis-causing parasites.

From a cost-benefit perspective, PHMB (aqueous solution) offers advantages over niclosamide, including greater stability, lower toxicity, and reduced environmental impact. Unlike niclosamide, which has low biocidal selectivity and poses a risk of environmental contamination and resistance, PHMB is less persistent, minimizing long-term environmental effects and harming non-target organisms less.

In this context, as future prospects, we suggest the incorporation of new approaches in metabolic studies of *B. glabrata*, including experimental investigations on the effects of sublethal concentrations of PHMB through complementary biomarkers. Among these approaches, we highlight histopathological analysis of the gonads and digestive gland, studies of reproductive biology, and histochemistry for the evaluation of antioxidant enzyme activity, such as catalase or superoxide dismutase. Furthermore, we recommend conducting more in-depth studies on the half-life of PHMB in the aquatic environment, with the aim of validating its effectiveness as a sustainable tool for controlling schistosomiasis.

Acknowledgements

This work was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, demanda social, No. 001/2022) and the Fundação de Amparo à Pesquisa do Estado de Goiás (FAPEG, No. 03/2022 - Programa de Auxílio à Pesquisa Científica e Tecnológica - No. 202310267000204), Conselho Nacional de Desenvolvimento Científico e Tecnológico. The authors would also like to thank Lonza Microbial Control for the donation of the PHMB.

References

BECKER, W., 1980. Metabolic interrelationship of parasitic trematodes and molluscs, especially *Schistosoma mansoni* in *Biomphalaria glabrata*. *Zeitschrift für Parasitenkunde*, vol. 63, no. 2, pp. 101-111. http://doi.org/10.1007/BF00927526. PMid:7456639.

BECKER, W., 1983. Purine metabolism in *Biomphalaria glabrata* under starvation and infection with *Schistosoma mansoni*. *Comparative Biochemistry and Physiology. B, Comparative Biochemistry*, vol. 76, no. 2, pp. 215-219. http://doi.org/10.1016/0305-0491(83)90061-5. PMid:6641159.

BERNOT, R.J., KENNEDY, E.E. and LAMBERTI, G.A., 2005. Effects of ionic liquids on the survival, movement, and feeding behavior of the freshwater snail, *Physa acuta*. *Environmental Toxicology and Chemistry*, vol. 24, no. 7, pp. 1759-1765. http://doi.org/10.1897/04-614R.1. PMid:16050594.

BEZERRA, J.C.B., KEMPER, A. and BECKER, W., 1999. Profile of organic acid concentrations in the digestive gland and hemolymph of *Biomphalaria glabrata* under estivation. *Memórias do Instituto Oswaldo Cruz*, vol. 94, no. 6, pp. 779-784. http://doi.org/10.1590/S0074-02761999000600012. PMid:10585654.

BEZERRA, J.C.B. and BECKER, W., 1993. Konzentration organischer Säuren in der Hämolymphe von *Biomphalaria glabrata* unter verschiedenen physiologischen Zuständen. *Verhandlungen der Deutschen Zoologischen Gesellschaft*, vol. 86, pp. 81.

BRASIL. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância das Doenças Transmissíveis, 2014. *Vigilância da esquistossomose mansoni: diretrizes técnicas.* 4ª ed. Brasília: Ministério da Saúde.

CAIXETA, M.B., ARAÚJO, P.S., RODRIGUES, C.C., GONÇALVES, B.B., ARAÚJO, O.A., BEVILAQUA, G.B., MALAFAIA, G., SILVA, L.D. and ROCHA, T.L., 2021. Risk assessment of iron oxide nanoparticles in an aquatic ecosystem: A case study on *Biomphalaria glabrata*. *Journal of Hazardous Materials*, vol. 401, pp. 123398. http://doi.org/10.1016/j.jhazmat.2020.123398. PMid:32763694.

CELIK, S. and TANIS, E., 2022. Toxic potential of Poly-hexamethylene biguanide hydrochloride (PHMB): a DFT, AIM and NCI analysis study with solvent effects. *Computational & Theoretical Chemistry*, vol. 1212, pp. 113709. http://doi.org/10.1016/j.comptc.2022.113709.

CHRISTEN, V., FALTERMANN, S., BRUN, N.R., KUNZ, P.Y. and FENT, K., 2017. Cytotoxicity and molecular effects of biocidal disinfectants (quaternary ammonia, glutaraldehyde, poly(hexamethylene biguanide) hydrochloride PHMB) and their mixtures in vitro and in zebrafish eleuthero-embryos. *The Science of the Total Environment*, vol. 586, pp. 1204-1218. http://doi.org/10.1016/j.scitotenv.2017.02.114. PMid:28236482.

CHRISTIE, J.D. and MICHELSON, E.H., 1975. Transaminase levels in the digestive gland-gonad of *Schistosoma mansoni*-infected *Biomphalaria glabrata*. *Comparative Biochemistry and Physiology.*

B, Comparative Biochemistry, vol. 50, no. 2B, pp. 233-236. http://doi.org/10.1016/0305-0491(75)90268-0. PMid:1109822.

COSTA, A.V., ALMEIDA, B.R., GONÇALVES, L.V., CRICO, K.B., IGNACCHITI, M.D.C., PEREIRA JUNIOR, O.S., PINHEIRO, P.F. and QUEIROZ, V.T., 2015. Efeito moluscicida do óleo essencial de *Cymbopogon winterianus* Jowitt (Poaceae) sobre *Lymnaea columela* (Say, 1817) e *Biomphalaria* tenagophila (D'Orbigny, 1835). *Revista Brasileira de Plantas Medicinais*, vol. 17, no. 4, pp. 707-712. http://doi.org/10.1590/1983-084X/14_017.

CUHADAR, S., KOSEOGLU, M., ATAY, A. and DIRICAN, A., 2013. The effect of storage time and freeze-thaw cycles on the stability of serum samples. *Biochemia Medica*, vol. 23, no. 1, pp. 70-77. http://doi.org/10.11613/BM.2013.009. PMid:23457767.

DE CIAN, M., REGNAULT, M. and LALLIER, F.H., 2000. Nitrogen metabolites and related enzymatic activities in the body fluids and tissues of the hydrothermal vent tubeworm *Riftia pachyptila. The Journal of Experimental Biology*, vol. 203, no. Pt 19, pp. 2907-2920. http://doi.org/10.1242/jeb.203.19.2907. PMid:10976028.

DE ZWAAN, A. and PUTZER, V., 1985. Metabolic adaptations of intertidal invertebrates to environmental hypoxia (a comparison of environmental anoxia to exercise anoxia). *Symposia of the Society for Experimental Biology*, vol. 39, pp. 33-62. PMid:3914721.

EUROPEAN CHEMICALS AGENCY – ECHA, 2015. *Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products. Polyhexamethylene biguanide (Mn = 1600; PDI =1.8) (PHMB).* Paris.

FARO, M.J., PERAZZINI, M., CORREA, L.R., MELLO-SILVA, C.C., PINHEIRO, J., MOTA, E.M., SOUZA, S., ANDRADE, Z. and MALDONADO JÚNIOR, A., 2013. Biological, biochemical and histopathological features related to parasitic castration of *Biomphalaria glabrata* infected by *Schistosoma mansoni*. *Experimental Parasitology*, vol. 134, no. 2, pp. 228-234. http://doi.org/10.1016/j.exppara.2013.03.020. PMid:23541880.

FRAGA, L.S., 2002. *Efeito da anóxia sobre o metabolismo de carboidratos no sistema nervoso central do caracol Megalobulimus oblongos (Gastropoda: Pulmonata).* Porto Alegre: Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, 110 p. Dissertação de Mestrado em Ciências Biológicas (Fisiologia).

GUDER, W.G., FONSECA-WOLLHEIM, F., HEIL, W., TOPFER, G., WISSER, H. and ZAWTA, B., 2001. The quality of diagnostic samples. *GIT Verlag*, vol. 30, pp. 50-54.

HERVE, M., 2023 [viewed 12 September 2024]. *RVAideMemoire: testing and plotting procedures for biostatistics. R package version 0.9-83-7* [software]. Vienna: R Foundation for Statistical Computing. Available from: https://CRAN.R-project.org/package=RVAideMemoire&gt

HOCHACHKA, P.W., FIELDS, J.H.A. and MOMMSEN, T.P., 1983. Metabolic and enzyme regulation during rest-to-work transition: a mammal versus mollusc comparison. In: P.W. HOCHACHKA, ed. *The Mollusca metabolic biochemistry and molecular biomechanics.* New York: Academic Press, pp. 55-89. http://doi.org/10.1016/B978-0-12-751401-7.50009-0.

LEE, Y., BYEON, E., KIM, D.H., MASZCZYK, P., WANG, M., WU, R.S.S., JEUNG, H.D., HWANG, U.K. and LEE, J.S., 2023. Hypoxia in aquatic invertebrates: occurrence and phenotypic and molecular responses. *Aquatic Toxicology*, vol. 263, pp. 106685. http://doi.org/10.1016/j.aquatox.2023.106685. PMid:37690363.

LIMA, N.F., ANDRADE PICANÇO, G., COSTA, T.L. and VINAUD, M.C., 2022. *In vitro* metabolic stress induced by nitazoxanide and flubendazole combination in *Taenia crassiceps* cysticerci. *Experimental Parasitology*, vol. 238, pp. 108265. http://doi.org/10.1016/j.exppara.2022.108265. PMid:35525309.

LÜRLING, M., 2012. Infodisruption: pollutants interfering with the natural chemical information conveyance in aquatic systems. In: C. BRONMARK and L.A. HANSSON, eds. *Chemical ecology in aquatic systems*. Oxford: Oxford University Press, pp. 250-271. http://doi.org/10.1093/acprof:osobl/9780199583096.003.0018.

LUTZ, P.L. and NILSSON, G.E., 1997. Contrasting strategies for anoxic brain survival–glycolysis up or down. *The Journal of Experimental Biology*, vol. 200, no. Pt 2, pp. 411-419. http://doi.org/10.1242/jeb.200.2.411. PMid:9050250.

MANGIAFICO, S.S., 2024 [viewed 12 September 2024]. *rcompanion: Functions to Support Extension Education Program Evaluation: version 2.4.36* [software]. Vienna: R Foundation for Statistical Computing. Available from: https://CRAN.R-project.org/package=rcompanion

MELLO-SILVA, C.C., VILAR, M.M., VASCONCELLOS, M.C., PINHEIRO, J. and RODRIGUES, M.L.A., 2010. Carbohydrate metabolism alterations in *Biomphalaria glabrata* infected with *Schistosoma mansoni* and exposed to *Euphorbia splendens* var. *hislopii* latex. *Memórias do Instituto Oswaldo Cruz*, vol. 105, no. 4, pp. 492-495. http://doi.org/10.1590/S0074-02762010000400024. PMid:20721497.

MELO, A.O., SANTOS, D.B., SILVA, L.D., ROCHA, T.L. and BEZERRA, J.C.B., 2019. Molluscicidal activity of polyhexamethylene biguanide hydrochloride on the early-life stages and adults of the *Biomphalaria glabrata* (Say, 1818). *Chemosphere*, vol. 216, pp. 365-371. http://doi.org/10.1016/j.chemosphere.2018.10.035. PMid:30384305.

MEYER, R., BECKER, W. and KLIMKEWITZ, M., 1986. Investigations on the ketone body metabolism in *Biomphalaria glabrata*: influence of starvation and of infection with *Schistosoma mansoni. Journal of Comparative Physiology. B, Biochemical, Systemic, and Environmental Physiology*, vol. 156, no. 4, pp. 563-571. http://doi.org/10.1007/BF00691043.

MOTTA, V.T., 2005. *Bioquímica básica*. 2ª ed. São Paulo: Laboratório Autolab Ltda.

NIRO, A., PIGNATELLI, F., FALLICO, M., SBORGIA, A., PASSIDOMO, F., GIGLIOLA, S., NACUCCHI, A., SBORGIA, G., BOSCIA, G., ALESSIO, G., BOSCIA, F., ADDABBO, G., REIBALDI, M. and AVITABILE, T., 2022. Polyhexamethylene biguanide hydrochloride (PHMB)-properties and application of an antiseptic agent: a narrative review. *European Journal of Ophthalmology*, vol. 33, no. 2, pp. 655-666. http://doi.org/10.1177/11206721221124684. PMid:36083163.

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT – OECD, 2016. OECD test nº 243: *Lymnaea stagnalis* reproduction test. In: ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT – OECD, ed. *OECD guidelines for the testing of chemicals, section 2.* Paris: OECD Publishing. http://doi.org/10.1787/9789264264335-en.

OH, H.N., YOO, D., PARK, S., LEE, S. and KIM, W.K., 2024. Assessment of poly(hexamethylenebicyanoguanide-hexamethylenediamine) hydrochloride-induced developmental neurotoxicity via oxidative stress mechanism: integrative approaches with neuronal cells and zebrafish. *Journal of Hazardous Materials*, vol. 465, pp. 133146. http://doi.org/10.1016/j.jhazmat.2023.133146. PMid:38064952.

PATIENCE, R.L., THOMAS, J.D. and STERRY, P.R., 1983 [viewed 12 September 2024]. Production and release of carboxylic acids during oxic and anoxic metabolism by the pulmonate snail *Biomphalaria glabrata* (Say). *Comparative Biochemistry and Physiology* [online], vol. 76B, pp. 253-262. Available from: https://www.sciencedirect.com/science/article/pii/0305049183900676

PAVLOVA, G.A., 2010. Muscular waves contribute to gliding rate in the freshwater gastropod *Lymnaea stagnalis. Journal of*

Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral Physiology, vol. 196, no. 4, pp. 241-248. http://doi.org/10.1007/s00359-010-0509-5. PMid:20157713.

PIERI, O. and JURBERG, P., 1981. Comportamento de *Biomphalaria glabrata* (Say 1818) como critério de toxicidade em ensaios biológicos com moluscicidas. *Memórias do Instituto Oswaldo Cruz*, vol. 76, no. 2, pp. 147-160. http://doi.org/10.1590/S0074-02761981000200006. PMid:7348773.

PINHEIRO, J., 1996. The influence of starvation on glycogen and galactogen contents in the snail *Bradybaena similaris* (Férussac, 1821) (Mollusca, Gastropoda). *Brazilian Archives of Biology and Technology*, vol. 39, pp. 349-357.

PINHEIRO, J., GOMES, E.M. and CHAGAS, G.M., 2001. Aminostransferases activity in the hemolymph of *Bradybaena similaris* under starvation. *Memórias do Instituto Oswaldo Cruz*, vol. 96, pp. 1161-1164. http://doi.org/10.1590/S0074-02762001000800022. PMid:11784939.

PINHEIRO, J., MALDONADO JÚNIOR, A. and LANFREDI, R.M., 2009. Physiological changes in *Lymnaea columella* (Say, 1817) (Mollusca, Gastropoda) in response to *Echinostoma paraensei* Lie and Basch, 1967 (Trematoda: Echinostomatidae) infection. *Parasitology Research*, vol. 106, no. 1, pp. 55-59. http://doi.org/10.1007/s00436-009-1630-7. PMid:19777261.

R CORE TEAM, 2024 [viewed 12 September 2024]. *R: a language and environment for statistical computing* [software]. Vienna: R Foundation for Statistical Computing. Available from: https://www.R-project.org/&gt

RANGEL, L.D.S., PASSOS DE OLIVEIRA, A., FALCÃO, D.Q., SANTOS, M.G., VON RANKE, N.L., RODRIGUES, C.R., SANTOS, J.A.A., ROCHA, L. and FARIA, R.X., 2022. Nanoemulsion of Sideroxylon obtusifolium as an Alternative to Combat Schistosomiasis. *Frontiers in Plant Science*, vol. 13, pp. 853002. http://doi.org/10.3389/fpls.2022.853002. PMid:35693155.

SILVA, L.D., ALVES, S.M.F., LEICHTWEIS, K.S., SANTOS, D.B., CASTRO, A.M., MELLO-SILVA, C.C., MACHADO, K.B., REZENDE, H.H.A. and BEZERRA, J.C.B., 2019. Cálcio exógeno como biomarcador do uso de via metabólica alternativa por *Biomphalaria spp*. (Mollusca, Planorbidae). *Revista de Biologia Neotropical*, vol. 16, no. 2, pp. 61-69. http://doi.org/10.5216/rbn.v16i2.53324.

SILVA, L.D., AMARAL, V.C.S., VINAUD, M.C., CASTRO, A.M., REZENDE, H.H.A., SANTOS, D.B., MELLO-SILVA, C.C. and BEZERRA, J.C.B., 2017. Changes in energetic metabolism of *Biomphalaria glabrata* (Mollusca, Planorbidae) in response to exogenous calcium. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 77, no. 2, pp. 304-311. http://doi.org/10.1590/1519-6984.13315. PMid:27599101.

SILVERTHORN, D.U., 2010. *Fisiologia humana: uma abordagem integrada*. 5ª ed. Porto Alegre: Artmed.

TUNHOLI, V.M.A., TUNHOLI-ALVES, V.M., LUSTRINO, D., CASTRO, R.N., SANT'ANA, L., GARCIA, J., MALDONADO JÚNIOR, A., SANTOS, M.A., RODRIGUES, M.L.A. and PINHEIRO, J., 2013. Aerobic to anaerobic transition in *Biomphalaria glabrata* infected with different miracidial doses of *Echinostoma paraensei* by High-performance liquid chromatography. *Experimental Parasitology*, vol. 133, pp. 403-410. http://doi.org/10.1016/j.exppara.2013.01.012. PMid:23376444.

TUNHOLI, V.M., LUSTRINO, D., TUNHOLI-ALVES, V.M., MELLO-SILVA, C.C., MALDONADO JÚNIOR, A., PINHEIRO, J. and RODRIGUES, M.L., 2011. Biochemical profile of *Biomphalaria glabrata* (Mollusca: Gastropoda) after infection by *Echinostoma paraensei* (Trematoda: Echinostomatidae). *Parasitology Research*, vol. 109, no. 3, pp. 885-891. http://doi.org/10.1007/s00436-011-2330-7. PMid:21537991.

TUNHOLI-ALVES, V.M., TUNHOLI, V.M.A., GÔLO, P., LIMA, M.G., GARCIA, J., GUEDES, E., MALDONADO JÚNIOR, A. and PINHEIRO, J., 2013. Effects of infection by larvae of *Angiostrongylus cantonensis* (Nematoda, Metastrongylidae) on the lipid metabolism of the experimental intermediate host *Biomphalaria glabrata* (Mollusca: Gastropoda). *Parasitology Research*, vol. 112, no. 5, pp. 2111-2116. http://doi.org/10.1007/s00436-013-3308-4. PMid:23377121.

WIESER, W., 1980. Metabolic end products in three species of marine gastropods. *Journal of the Marine Biological Association of the United Kingdom*, vol. 60, no. 1, pp. 175-180. http://doi.org/10.1017/S0025315400024231.

WOLMARANS, C.T., 1987. *Biomphalaria glabrata*: respiration, calcium and end products of carboydrate metabolism. *Comparative Biochemistry and Physiology. A. Comparative Physiology*, vol. 87, no. 3, pp. 785-790. http://doi.org/10.1016/0300-9629(87)90401-4. PMid:2887362.

WORLD HEALTH ORGANIZATION – WHO, 2017 [viewed 12 September 2024]. *Field use of molluscicides in schistosomiasis control programmes: an operational manual for programme managers* [online]. Available from: https://www.who.int/publications/i/item/9789241511995

WORLD HEALTH ORGANIZATION – WHO, 2023 [viewed 10 September 2024]. *Schistosomiasis* [online]. Available from: https://www.who.int/news-room/fact-sheets/detail/schistosomiasis

ZARAI, Z., BOULAIS, N., MARCORELLES, P., GOBIN, E., BEZZINE, S., MEJDOUB, H. and GARGOURI, Y., 2011. Immunohistochemical localization of hepatopancreatic phospholipase in gastropods mollusc, *Littorina littorea* and *Buccinum undatum* digestive cells. *Lipids in Health and Disease*, vol. 10, no. 1, pp. 219. http://doi.org/10.1186/1476-511X-10-219. PMid:22114916.