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Behavioral, Biochemical, and Histological Evaluation of Artificial Light on Infected Freshwater Snails *Bimophalaria alexandrina* by *Schistosoma mansoni*

Ahmed A. A. Hussein[1*], Marwa I. Saad El-Din[2], Nahla S. El-Shenawy[2], Sara S. M. Sayed[1]

[1]Division of Environmental Research and Medical Malacology, Theodor Bilharz Research Institute (TBRI), 30 Imbaba, 12411 Giza, Egypt.

[2]Zoology Department, Faculty of Science, Suez Canal University, Ismailia 41522, Egypt.

*Correspondence: ahmed.abdelazeez@science.suez.edu.eg

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ABSTRACT

Artificial light near rivers and lakes can have a substantial impact on aquatic species, especially freshwater snails, in terms of behavioral and physiological parameters. The extent to which this alternation may occur is unknown, particularly if this snail serves as an intermediate host for the parasite *Schistosoma mansoni*. As a result, the goal of this study is to see how light from high-pressure sodium (HPS) and light-emitting diode (LED) lights interact with *Schistosoma mansoni* infection in *Bimophalaria alexandrina* snails. The survival rate of infected snails and the mean number of cercariae/snails exposed to HPS was significantly higher than those exposed to LED. The investigation revealed that infected snails were more active and traveled longer distances at a higher speed post the 4th week of the exposure to LED light. Results of biochemical parameters showed a highly significant elevation in malondialdehyde (MDA) activity post-exposure to LED, while a highly significant increase in total antioxidant capacity (TAC) was recorded after exposure to HSP. The histopathological examination of infected snails exposed to LED showed highly affected digestive tissue, degenerated sporocysts, and incomplete mature cercariae, while the infected snails exposed to HPS showed well-developed sporocysts and mature cercariae with differentiated heads and tails. Snails exposed to LED showed a highly significant decrease in serotonin levels compared to those exposed to HPS. Melatonin levels recorded significant differences between the exposed groups to LED and HPS. The current study recommends using artificial LED lighting around freshwater canals instead of HPS lighting, which could limit the transmission of schistosomes.

INTRODUCTION

Schistosomiasis, commonly known as bilharzia, is an infection resulting from a parasitic worm that dwells in fresh water in subtropical and tropical areas. This parasitic worm (genus: *schistosome*) with its different life stages, including cercariae, miracidia, eggs, and adult worms, infects humans, some animals, as well as a specific intermediate host snail **(McManus *et al.*, 2018).** *Schistosoma* spp. infects a minimum of 230 million

individuals worldwide, according to conservative assessments **(Vos *et al.*, 2012; Hazell *et al.*, 2021)**. Many studies have been conducted in an attempt to control snail populations and find a safe solution for schistosomiasis transmission **(Soliman *et al.*, 2017; Abu Almaaty *et al.*, 2021)**. Snail control is a necessary component of a transmission-blocking approach for schistosomiasis elimination, and it can be handled through chemical, environmental, or biological means. It is well established that synthetic and chemical molluscicides are environmentally hazardous **(Singh *et al.*, 1996; Ibrahim *et al.*, 2021)**.

Recently, chronobiologists have noticed that snails have a behavioral reaction to light **(Hori *et al.*, 2014; Hussein *et al.*, 2020b)**. In addition to behavior, some physiological processes and molecular parameters were manipulated with exposure to different intensities and wavelengths **(Bedrosian *et al.*, 2011; Ter Maat *et al.*, 2012; Hussein *et al.*, 2020a)**. Based on his finding that light in the visible spectrum could act as a tolerant sign that mediates egg-laying behavior, **Kumar *et al.* (2017)** concluded that using a specific light band could be a safe protocol for controlling vector snails. This means a probability of controlling snail populations. The latter inspired the evaluation of the effect of exposure to different artificial light spectrums on the snails infected with *Schistosoma* spp.

Besides the previous inspired idea, there is a clear governmental approach to replacing the high-pressure sodium (HPS) lamps used in street lighting with LED lamps to save electrical energy. It is still not known whether this change in the artificial night lighting affects the snail-schistosome interaction or not. Therefore, the purpose of the present investigation is to explore the relationship between the type of light and the infection of *Bimophalaria alexandrina* by *Schistosoma mansoni*. We started by assessing the effects of artificial light emitted from LED and HPS lamps on some biological, behavioral, and biochemical parameters of infected snails.

MATERIALS AND METHODS

Experimental Design

Two sets of fourty *B. alexandrina* snails have shell diameters ranged from 4 to 6 mm, were acclimatized in plastic aquaria (16 × 23 × 9 cm) for one week under a photo-periodicity of 12 h light/12 h dark, provided by dechlorinated aerated tap water (10 snails/L), covered with glass plates, the water temperature of 30 ± 2°C was adjusted, and dried lettuce leaves were used for feeding.

The first set was maintained with 12 h of light produced from a E3 HPS lamp (produce monochromatic yellow light at 560 to 590 nm, light World and 12 h of dark, while the other set was maintained with 12 h of light produced from a custom-made specialized broad-spectrum LED-light strip system (white cool dimmable SMD5050-300 LED strip, produce strong irradiance peaks around 450 nm, LED Lights World) and 12 h of dark. Dead snails were removed daily from the two different light treatments. After one-week acclimation, all snails from the two different light treatment were individually

exposed to 6-8 miracidia of *S. mansoni* for 24 h **(Anderson *et al.*, 1982; Hussein *et al.*, 2016).**

Examination of snails for cercarial shedding

After 10 days of infection, all live snails from each group were examined for a cercarial shedding test. Positive snails transferred to clean aquaria and maintained under the two tsted light types. Two drops of iodine solution were added, counted the number of cercariae under a stereomicroscope, and attributed their number to each snail. This was repeated each week for five weeks until the death of the last snail.

The survival rate and the snail's infection rate were calculated according to **Yousif *et al.* (1998)** and **Mansour *et al.*** (**2021)** as following:

$$\text{The survival rate (for the infected snails)} = \frac{\text{The number of snails at the first shedding}}{\text{The total number of exposed snails (at the beginning of the experiment)}}$$

$$\text{The snail's infection rate (at the end of the experiment)} = \frac{\text{The number of shedding snails}}{\text{The number of surviving exposed snails (at the first shedding)}}$$

Locomotion activity

Every week post-infection, 12 snails from each tested light type were moved to another setup to evaluate their locomotion activity. In this test, snails were placed individually in rounded containers (10 cm in diameter and 10 cm high), then filled with water with the same characteristics mentioned before. Thereafter, each snail was allowed to acclimate for 10 min. before recording videos for the set of snails (n=12) using a digital camera (Sony, 20MPxl). The camera was fixed above the twelve containers that contain the individual snails by a holder. After recording a thirty-minute video for each tested light type, the snails were transferred back to the original light treatment in their previous setup.

The recorded videos were analyzed using Image J software **(Habib *et al.*, 2016).** Briefly, the recorded video is opened in Image J and converted to grayscale. After that, crop the exact area of all containers together to avoid unnecessary noise. Next, adjust the threshold after selecting the MaxEntropy and light background setting to the level at which only snails without their background are colored red. At this point, the video is ready for analysis by the wrMTrck plugin. This plugin allows analyzing many tracking parameters, but only the distance traveled by each snail during the recorded 30 min., as well as the average speed of snails during this distance.

Determination of Malondialdehyde (MDA) concentration

Malondialdehyde in the whole homogenate was determined as thiobarbituric acid reactive substances (TBARS) according to the method of **Ohkawa *et al.* (1979)**. Briefly, an aliquot of 0.1 ml of the tissue homogenate was add to a tube containing an equal volume of SDS solution. This was followed by adding 0.75 ml of acetic acid, 0.75 ml of TBA, and 0.3 ml of distilled water, The contents were mixed with a vortex. The tubes were placed in a boiling water bath for 1.0 h and then cooled to room temperature. An aliquot of 0.5 ml of distilled water was added to each tube, followed by the addition of 2.5 ml of n-butanol. The contents of the tubes were vigorously mixed with a vortex then rotated in a centrifuge at 2500 × *g* for 10 minutes. The absorbance of the organic layer was read at 532 nm in a spectronic 21 spectrophotometer against a blank prepared and treated exactly like the sample, but containing phosphate buffer solution instead of the sample. The concentration (nmol/ml) of MDA in the sample was obtained from a standard curve made by preparing serial dilutions of tetramethoxypropane (TMP), 1, 2, 4, 6, 8, and 12 nmol/ml in ethanol and treating them like the sample.

Total antioxidant capacity (TAC) determination

The total antioxidant capacity was determined using an assay kit (Colorimetric) (Abcam, ab65329, Cambridge, UK).The assay was performed according to the manufacturer's protocol. Where in the Cu^{2+} ion is converted to Cu^{+} by both small molecules and proteins. The protein mask prevents Cu^{2+} reduction by protein, enabling the analysis of only the small molecule antioxidants. The reduced Cu^{+} ion is chelated with a colorimetric probe, giving a broad absorbance peak around OD 570 nm, proportional to the total antioxidant capacity.

Histopathological examination

After four weeks of cercarial shedding, about 3 to 5 infected snails were randomly selected from each LED and HPS exposure, then carefully dissected and pulled the soft bodies of snails, fixed in Bouin's solution for 24 h, placed in gradually ascending concentrations of ethanol, cleared with xylol, embedded in paraffin, and finally sectioned at 6 μm, and stained with hematoxylin and eosin stain.The slides were examined under a light microscope (Olympus System Microscope BX2 Series), then photographed **(Borges *et al.*, 1998).**

Serotonin Concentration

Serotonin, or 5-hydroxytryptamine (5-HT), is a monoamine neurotransmitter. Biochemically derived from tryptophan, serotonin is primarily found in the gastrointestinal tract (GI tract), blood platelets, and the central nervous system (CNS) of animals, including humans. BioVision's Serotonin ELISA kit (USA) was used for the quantitative measurement of serotonin in the whole tissue homogenate according to the manufacturer's protocol. The density of color is proportional to the amount of serotonin captured from the samples.

Melatonin Concentration

Melatonin is a hormone that is produced by the pineal gland in humans and animals. The melatonin ELISA kit from BioVision (USA) was used as a competitive ELISA assay for quantitative measurement of melatonin in whole tissue homogenate. The density of color is proportional to the amount of melatonin captured from the samples.

Statistical analysis

The data was grouped according to the different light treatments. The data of infection and survival rates were analyzed using chi-square test. Results of cercarial shedding, malondialdehyde (MDA), total antioxidant capacity (TAC), serotonin, and melatonin were presented as mean ± SEM and analyzed using a T-test to compare the significant differences between the two exposed groups at $P \leq 0.05$. All statistical analyses were accomplished by GraphPad Prism 8.

RESULTS

Survival, infection rates, and cercarial shedding

Data demonstrated that exposure to two types of light influenced the survival rate of infected snails. Specifically, the survival rate of infected snails exposed to HPS was significantly higher than that of those exposed to LED (Chi-square test, $\chi^2 = 4.39$, $df = 1$, at $P < 0.05$) (Fig. 1a**)**. Furthermore, the infection rate showed no significant difference between exposure to LED and HPS (Chi-square test, $\chi^2 = 0.13$, $df = 1$, at $P > 0.05$) (Fig. 1b). However, the infected snails that were exposed to HPS showed a significantly higher mean number of cercariae/snail than those exposed to LED at (t-test, $t_{(6)} = 2.858$, at $P < 0.05$) as shown in Fig. (1c).

Locomotion activity

The distance traveled by snails was checked weekly four times after exposure to the two light conditions. The results reveal no statistical difference in the distance traveled by snails (n = 12) after one, two, and three weeks (t-test, $t_{(22)} = 0.7778$, $P = 0.45$; $t_{(22)} = 0.6336$, $P = 0.53$; and $t_{(22)} = 0.07633$, $P = 0.94$, respectively) with a mean traveled distance within the range of 1-1.5 m during the thirty min. period of observation. However, after four weeks, snails exposed to LED light conditions traveled longer by about 33% than snails exposed to HPS light conditions(t-test, $t_{(22)} = 14.60$, $P < 0.0001$) (Fig. 2).

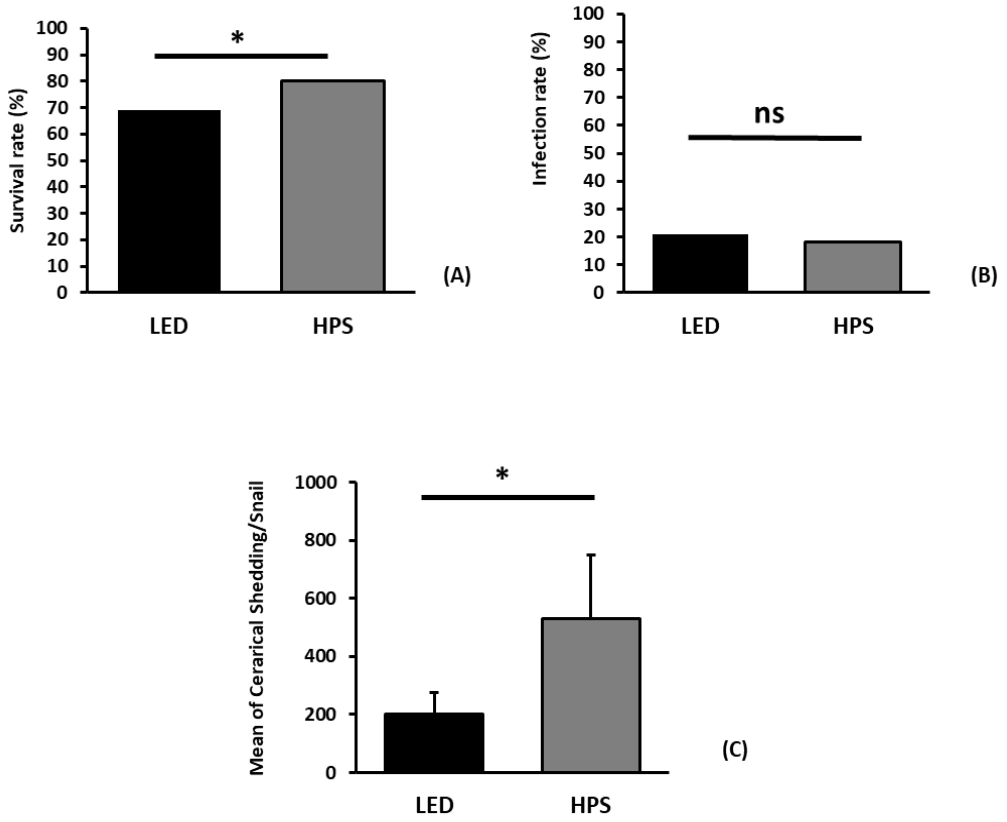

Fig. 1 Survival rate (a), mean number of cercariae/snail (b), and infection rate (c) of infected *Biomphalaria alexandrina* snails exposed to LED and HPS. The asterisk symbol (*) refers to significant differences between two groups at *P<0.05*, while "ns" refer to insignificant results between treatment groups at *P >0.05.*

Correspondingly, the data on speed matches the data of the traveled distance meaning that when snails travel a longer distance in one group than another group in the same period, this indicates that this group was traveling faster. The data demonstrated that snails traveled by almost the same speed (n=12) after one, two, and three weeks of exposure to the two tested light types with average speed sranging from 0.7: 1.0 mm/S. However, after four weeks of exposure to the same light conditions, a significant difference arises. Snails exposed to HPS light were slower than those exposed to LED light (t-test, $t_{(22)} = 11.72$, $P< 0.0001$) (Fig. 3).

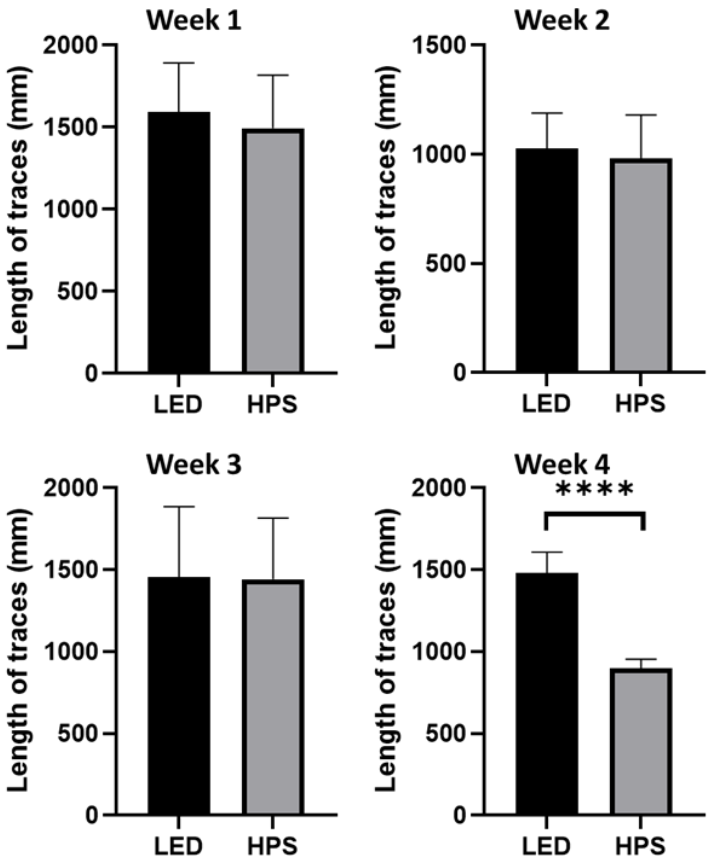

Fig. 2 The traceroute of the snails during 30 min, after the 1st, 2nd, 3rd, and 4th week of exposure to the light treatments (n = 12). The data have plotted the mean with vertical whiskers indicating SE. The asterisk symbol (****) refers to significant differences between two groups at *P<0.0001*.

Oxidation evaluation

Results of oxidation parameters are summarized in Fig. (4). Datashowed a highly significant increase (t-test, $t_{(4)}$ = 7.827, P = 0.0014) in malondialdehyde (MDA) (Fig. 4a) activity post-exposure to LED. Meanwhile, results showed a highly significant elevation (t-test, $t_{(4)}$ = 7.180, P = 0.0020) in total antioxidant capacity (TAC) (Fig. 4b) after exposure to HSP.

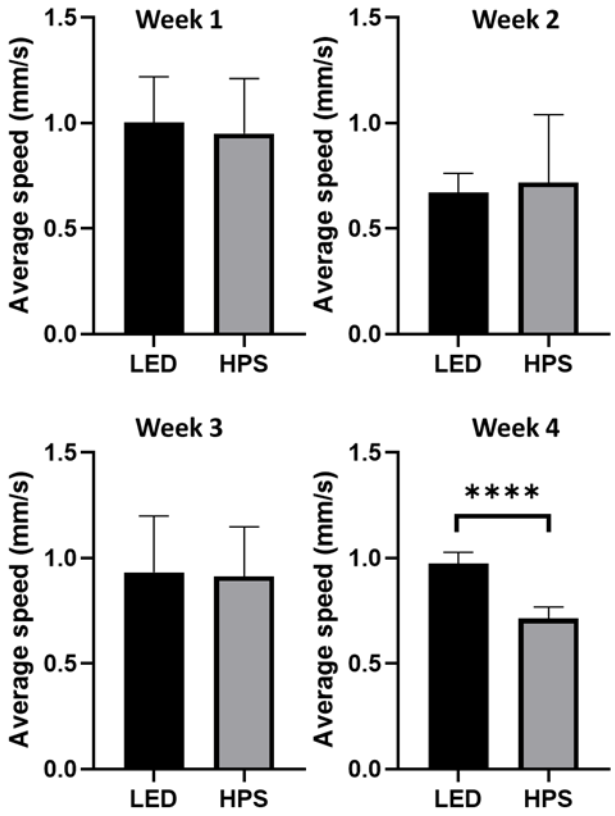

Fig. 3 The mean values of speed traveled by snails during 30 min period after the 1st, 2nd, 3rd, and 4th week of exposure to the light treatments (n = 12). The data have plotted the mean with vertical whiskers indicating SE. The asterisk symbol (****) refers to significant differences between two groups at *P<0.0001*.

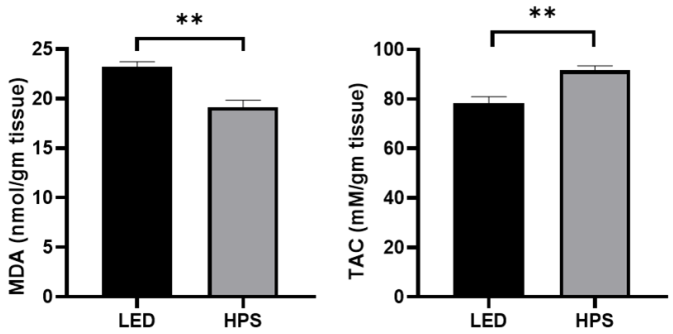

Fig. 4 Comparative effect of LED and HPS on malondialdehyde (MDA) (a), total antioxidant capacity (TAC) (b) in infected *B. alexandrina* snails. The asterisk symbol (**) refers to significant differences between two groups at *P<0.01*.

Histopathological examination

The histopathological examination of the digestive gland tissue of infected *B. alexandrina* snails exposed to LED showed damaged digestive cells with dense secretory cells. Sporocysts were observed in digestive gland tissue with degenerated germinal cells. Numerous hemocytes around some immature cercariae try to retard their development. Furthermore, deformed immature cercariae were detected and incomplete mature cercariae were observed, although the formation of their heads and tails (Fig. 5a- 5d).

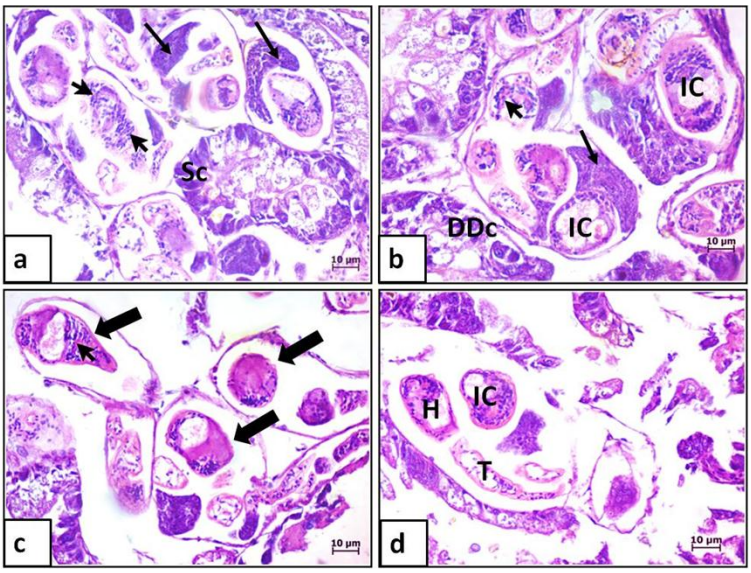

Fig. 5 Photomicrographs of the digestive tissue of infected *B. alexandrina* snails exposed to LED; (**a**) showing degenerated germinal cells (thin arrows), aggregation of hemocytes (head arrows) around immature cercaria and dense secretory cells (Sc), (**b**) showing damaged digestive cells (DDC), immature cercariae (IC), (**c**) showing deformed immature cercariae (thick arrows), (**d**) showing immature cercaria (IC) and cercaria during its differentiation into the head (H) and tail (T) affected by hemocytes – tissue reaction.

On the other hand, *B. alexandrina* snails exposed to HPS showed moderately affected digestive cells and secretory cells. Single and multiple sporocysts were observed with well-developed germinal cells surrounded by tegument penetrating the cell. Many developmental stages of cercariae were detected, although few hemocytes aggregated on their surface. In addition to mature cercariae with differentiated heads and tails, others were observed (Fig. 6a-6d).

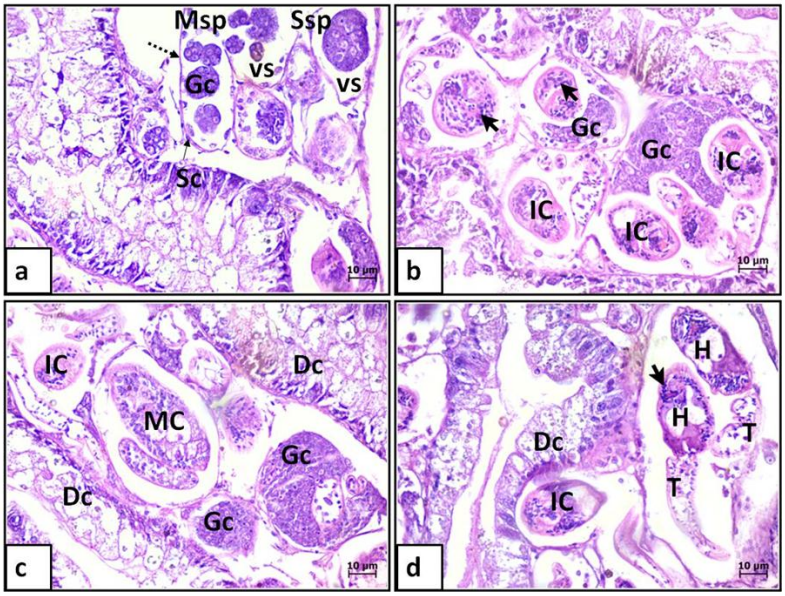

Fig. 6 Photomicrographs of the digestive tissue of infected *B. alexandrina* snails exposed to HPS; (**a**) showingsingle sporocyst (Ssp), multiple sporocysts (Msp), tegument (dotted arrow), penetrating gland (short arrow), vacant space (vs) and secretory cells (Sc), (**b**) showing germinal cells (Gc), hemocytes (head arrows) aggregated on the surface of immature cercariae (IC), (**c**) showing formed mature cercaria (MC) and digestive cells (Dc), (**d**) showing some differentiated cercariae with head (H) and tail (T).

Serotonin and melatonin levels

Snails exposed to LED showed a highly significant decrease (t-test, $t_{(4)} = 7.186$, $P = 0.0020$) in serotonin levels than those exposed to HPS (Fig. 7a). Also, melatonin levels recorded significant differences (t-test, $t_{(4)} = 4.421$, $P = 0.0115$) between the exposed groups to LED and HPS (Fig. 7b).

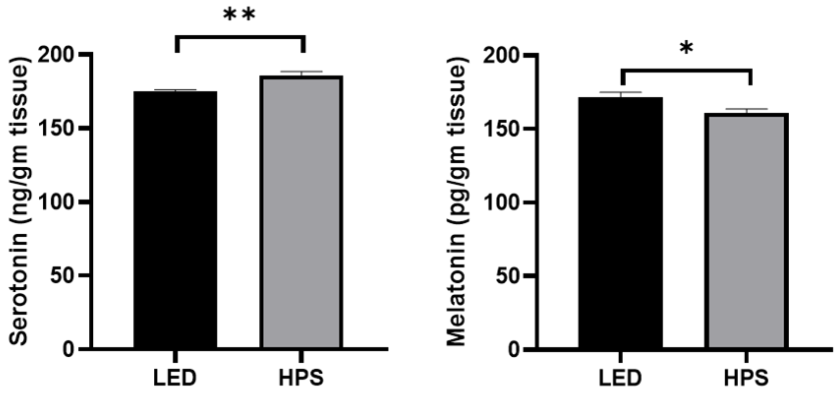

Fig. 7 Effect of LED and HPS on serotonin (a) and melatonin (b) levels in infected *B. alexandrina* snails. The asterisk symbol (*) refers to significant differences between two groups at $P<0.05$, while (**) refers to significant differences between two groups at $P<0.01$.

DISCUSSION

Variations in photoperiods and thermoperiods are among the abiotic factors that mediate many physiological and behavioral processes in aquatic molluscs **(Hussein *et al.*, 2020b)**. Also, this may influence the infectivity and periodic emergence of cercariae in infected snails with schistosomes **(Théron, 2015)**. In reviewing the literature, no data was found on the association between light type and snail infection. Therefore, this study set out with the aim of assessing the relationship between exposure to different types of artificial light and schistosome infection in *Biomophalaria alxendrina.*

The current study found that snails exposed to LED survived less than those exposed to HPS, while the number of shedding cercariae/snails in HPS light treatment was significantly higher than in LED light treatment. **Morley *et al.*(2010)** suggested that the cercariae liberation from snails is activated by light, where a larval positive phototropism occurs when the tissue of the snail emerges to light, this time is governed by the circadian light-dark cycle along with temperature differences. Also, these results might be due to the light intensity and wavelengths, which in turn may affect the physiology, behavior, and reproduction of the organism **(Baz *et al.*, 2022)**.

Moreover, **Achiorno and Martorelli (2016)** reported that temperature, light, and water conditions are the most important drivers of cercarial emergence and have a role in the acceleration of cercariae maturation and their liberation.

Locomotor activity is one of the behaviors that may impact the survival of snails and other organisms because snails move to provide proper foraging and avoid environmental stress and predators **(Luarte *et al.*, 2016; Hussein *et al.*, 2020b)**. This behavior is known to be impacted by the change in the chemical condition of the environment **(Habib *et al.*, 2016).** Although the relationship between locomotion activity and exposure of infected snails to different light types is not investigated before,the results of the current study provide novel data of this exposure impacts on the locomotion behavior by the semi-automated method using the free available image-j software. The data revealed that infected snails were more active and traveled longer distances at a higher speed after four weeks of exposure to LED light. One explanation could be that snails under LED treatment exhibit more stress. The latter forced snails to move faster to find shelter to avoid such stress.

The underlying mechanism of the relationship that resulted in making snails more active is unknown. However, the neuromodulator role of monoamines has been well confirmed in the regulation of snail locomotion **(Pavlova, 2019)**. It is assumed that light information influences ciliary activity directly, either by directly influencing 5-HT receptors or by hitting the serotonergic regulatory pathway(s) via an afferent-efferent system that includes receptor cells on the foot's surface and central (pedal) serotonergic efferent elements **(Vehovszky *et al.*, 2019)**. More investigations are needed to establish the stress level that LED light offers to the aquatic environment, including snails.

Malondialdehyde (MDA) is a product of lipid peroxidation **(Cui *et al.*, 2018)**. The present work showed that LED lighting caused an increase in MDA levels in infected snails. This increase might be due to enhanced oxygen-free radical production caused by LED lighting exposure. This result agrees with **Gawel *et al.* (2004)** who declared that the elevation of free radicals causes an increase in MDA level and **(Khoubnasabjafari *et al.*, 2015)** who stated that MDA content is considered a marker of oxidative stress.

The TAC is a straight forward technique for determining the amount of free radicals in biological samples **(Ghiselli *et al.*, 2000)**. The current results showed an increase in the TAC content post-exposure to HPS lighting, this might be due to inducing antioxidant defenses. Conversely, the decreased level of TAC detected after exposure to LED lighting might be due to the defense diminished and the oxidative stress has occurred **(Kaloyianni *et al.*, 2009)**.

The present findings of histopathological examinations confirmed the biochemical results, where the exposure to LED showed highly affected digestive tissue, degenerated sporocysts, and incomplete mature cercariae, these might be attributed to extensive immune defense stimulated by LED lighting, which is represented as hemocytes aggregation around immature cercariae. **Hussein *et al.* (2016)** claimed that the infected snails exposed to inorganic fertilizers showed tissue reaction by hemocytes dispersed around sporocystsand damaged their teguments.

These findings support the hypothesis proposed by **Le Clec'h *et al.* (2019)** that *S. mansoni* cercariae cause generally harm to snail health. It might happen during larval stages production that consumes snail tissue, by the cercarial shedding that causes damage to snail tissue, or because of transformation of the snail's energetic resources to defend against oxidative stress. **(Bayne *et al.*, 2001; Moné *et al.*, 2011)**.

On the contrary, the infected snails exposed to HPS lighting showed well-developed sporocysts and mature cercariae with differentiated heads and tails, and thiswas confirmed by the observed high number of shedding cercariae/snail exposed to HPS. In parallel, the parasites with high cercarial liberation displayed low virulence toward the snail host **(Davies *et al.*, 2001)**. However, these results might be attributed to the effect of HPS lighting on suppressing the immune response in snails' tissue, resulting in the tolerance for parasites to grow and complete their life cycle without retarding them.

Prior studies have noted the importance of biogenic amines, specifically serotonin, in the maturation of miracidia to sporocysts in infected snails with *S.mansoni* **(Manger *et al.*, 1996)**. Miracidia use serotonin secreted by host tissue to transform sporocysts. This consumption of serotonin leads to a reduction in its level in the host tissue **(Delgado *et al.*, 2012; Vallejo *et al.*, 2014)**. However, HPS light could stimulate infected snails to secrete more serotonin, which aids in significantly more cercariae production. This means the formation of more sporocysts. This may explain the significant rise in serotonin levels in the infected snails that exposed to HPS. On the other hand, infected snails exposed to LED light had insufficient serotonin secretion, and so more uptake by miracidia had led

to the depletion of serotonin levels in the surrounding tissue. One more explanation could be that LED light causes an increase in apoptosis to the sporocyst-surrounding tissue including nerve endings, which are responsible for the production and depletion of serotonin **(Habib *et al.*, 2020)**. Accordingly, this apoptosis might be less likely to increase serotonin levels.

The endocrine melatonin is secreted in the dark part of the day regardless of the animal's diurnal or nocturnal status **(Pévet, 2003)**. In the literature, there is a strong relationship between melatonin and immune enhancement, free radicals, and preventing the oxidation of hormones and enzymes **(Wu and Swaab, 2005; Shin *et al.*, 2011)**. This matches with the significant increase in melatonin levels in snails exposed to LED light, since LED light treatment enhanced immunity by increasing MDA levels and decreasing TAC. This also corresponds with the histological examination of the digestive tissue sections of snails exposed to LED light treatment, which showed more injury than those exposed to HPS. One explanation for melatonin levels rising in LED light treatment could be that LED lighting stimulates an increase in the activity of the arylalkylamine N-acetyltransferase (AANAT) enzyme. This enzyme is the precursor of melatonin and has a fundamental role in melatonin synthesis **(Iuvone *et al.*, 2005; Klein, 2007)**. This result is also in parallel to a previous work that declared that melatonin levels increase with exposure to LED (red spectrum) in yellow tail clownfish, *Amphiprion clarkia* **(Shin *et al.*, 2011).**

CONCLUSION

Infected *B. alexandrina* exposed to LED lighting suffered from a lower survival rate, dramatically damaged digestive tissue, and oxidative stress. Besides, degenerated sporocysts and incomplete mature cercariae were observed along with low cercarial shedding. Furthermore, LED alters the serotonin and melatonin levels in the snails' tissue, which in turn may involve multiple physiological processes. The current findings suggest for the first time that LED lighting may act as a limiting factor for the transmission of schistosomes in general by decreasing the number of cercarial shedding and lowering the risk percentage of transferring the infection from the intermediate host to the definitive host.

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