FECUNDITY, SEX HORMONES AND RELEASE OF CERCARIAE OF SCHISTOSOMA MANSONI IN BIOMPHALARIA ALEXANDRINA (EHRENBERG, 1831) TREATED WITH COPPER AND MAGNESIUM CHLOROPHYLLIN

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ABSTRACT: Schistosomiasis is a major public health problem in many developing countries. Control of the snail intermediate hosts of Schistosoma is a promising method for eliminating this disease. Copper (Cu-chl) and magnesium chlorophyllin (Mg-chl) are potent photosensitisers used in diverse biological applications. Their effects on the fecundity of Biomphalaria alexandrina (Ehrenberg): its reproductive rate (R₀) and total levels of progesterone, testosterone and estradiol were evaluated. Besides, we determined the production of cercariae of S. mansoni. The concentrations LC₁₀ and LC₂₅ of Cu-chl and Mg-chl significantly reduced the fecundity and R₀ of the treated snails which could be partially due to the decrease in the level of steroid sex hormones. The reduction rates of R₀ for the snails treated with LC₁₀ Cu-chl and Mg-chl were 72.7% and 89.7%, respectively. Moreover, Cu-chl and Mg-chl significantly suppressed the cercarial production which dropped from 9,778 cercariae per snail in the light control group to 3,126 and 107 cercariae per snail in the groups treated with LC₁₀ Cu-chl and Mg-chl 21 days after exposure to miracidia, respectively. Since Cu-chl and Mg-chl negatively interfere with the parasite transmission, they should be considered in schistosomiasis control programmes.

KEY WORDS: schistosomiasis, fresh water snails, photosensitisers, fecundity, reproductive rate

INTRODUCTION

Schistosomiasis is one of the major communicable diseases with socio-economic and health importance in the developing world (Steinmann et al. 2006). Control of the snail intermediate hosts of Schistosoma using molluscicides is still one of the promising methods for the disease control (WHO 2009). Molluscicides of natural origin have several advantages over synthetic compounds (Perrett & Whitfield 1996). Research on the impact of photosensitisers as agents against the snail intermediate hosts of schistosomiasis is limited. The photosensitiser hematoporphyrin causes high mortality of B. alexandrina and their eggs after 12 and 21 hours of exposure to this compound, respectively, followed by four hours of exposure to light with irradiance of 450 and 350 W/m² (El-Sayed & El-Sherbini 2006). The reproductive rate (R₀) of B. alexandrina is greatly suppressed by carbamide perhydrate due to its negative effect on the snails’ glycolytic and glycogenic activities as well as the transaminase and alkaline phosphatase enzymes (Gawish et al. 2009). El-Hommosany & El-Sherbini (2011) found that exposure of B. alexandrina to sub-lethal concentrations of hematoporphyrin-coated gold nanoparticles (Hpd
GPNPs) for 12 hours followed by four hours of light exposure (336.2 W/m²) significantly reduced their reproductive capacity which may suppress schistosomiasis transmission.

Chlorophyllin is a semi-synthetic derivative of chlorophyll, a porphyrin compound commonly used as a food dye. The pigment has significant anti-mutagenic and antioxidant effects, and regulates detoxification (Ferruzzi & Blakeslee 2007, Vesenick et al. 2012). Sodium magnesium chlorophyllin shows viricidal activity in patients with Herpes simplex and H. zoster (Mekler et al. 1969). Besides, photosensitisers with photodynamic reactions show great potential harmful effects in pest control (Erzinger et al. 2011). Water-soluble chlorophyllin kills mosquito larvae within few hours of exposure to solar radiation (Whollebe et al. 2009). Photosensitisers negatively affect different microorganisms, such as drug-resistant bacteria, yeasts, viruses and parasites. Furthermore, the requirement for both photosensitisers and light to cause an effect is an advantage of photosensitisation reaction over conventional chemical reactions (Spikes 1989). Photosensitisers and light are used to kill malignant tumours and to destroy other unwanted tissues in the process referred to as photodynamic therapy, PDT (Redmond 2008). Thus, photosensitisation may help in developing novel and environmentally safe effective means of controlling snail-borne diseases. To this purpose, molluscicidal properties of sodium copper chlorophyllin and sodium magnesium chlorophyllin were evaluated against biological and biochemical parameters of B. alexandrina, the intermediate host of S. mansoni.

**MATERIAL AND METHODS**

**SNAILS**

Laboratory-bred B. alexandrina (Ehrenberg, 1831) (7–9 mm) were obtained from S. mansoni susceptible strain maintained at the Medical Malacology Department, Theodor Bilharz Research Institute (TBRI), Imbaba, Giza, Egypt. They were kept in dechlorinated water (24±1°C) and fed oven-dried lettuce leaves daily.

**MIRACIDIA**

Miracidia of Schistosoma mansoni were obtained from the Schistosomiasis Biological Supply Center (SBSC), TBRI.

**COPPER CHLOROPHYLLIN AND MAGNESIUM CHLOROPHYLLIN**

Sodium copper chlorophyllin (Cu-chl) and sodium magnesium chlorophyllin (Mg-chl) were obtained from the Innovative Research and Development Incorporation (InRaD), Egypt.

The photosensitisers Cu-chl and Mg-chl proved to have considerable toxic effects against B. alexandrina at LC₁₀ and LC₂₅ of 5×10⁻⁶ and 10⁻⁵ mol/L, respectively (Ragheb et al. 2013). Accordingly, a stock solution of 10⁻³ mol/L from each of Cu-chl and Mg-chl was prepared, then the experimental dilutions were made for testing the snails’ fecundity, their steroid sex hormones and their infection with S. mansoni.

**EFFECT ON FECUNDITY**

Three replicates of B. alexandrina (7–9 mm), each of 10 snails/L, were incubated in the dark with LC₁₀ and LC₂₅ of Cu-chl and Mg-chl once only at the beginning of the experiment. The incubation periods were 12 h for Cu-chl and 3 h for Mg-chl (optimum periods for snails to survive incubation) followed by exposure to sunlight for 9 h (average irradiance 391.27 W/m²) (Ragheb et al. 2013). Then, the snails were transferred to clean dechlorinated water for recovery and observation during six consecutive weeks under laboratory conditions (ceiling light, 25±1°C). The dark and light control groups were run parallel to the test groups. The light control snails were kept in clean dechlorinated water without any treatment and exposed to sunlight followed by six weeks of recovery. The dark control snails were incubated with Cu-chl and Mg-chl and without light exposure were transferred to clean dechlorinated water for recovery. The survivorship of snails (Lx) and the number of laid eggs (Mx) were recorded weekly; the reproductive rate (Ro) was calculated at the end of the experiment. Throughout the experimental period, the snails were fed oven-dried lettuce leaves and the aquaria were provided with pieces of foam sheets for egg deposition; the water was changed weekly.

**EFFECT ON STEROID SEX HORMONES**

B. alexandrina (three replicates, 10 snails/L each) were incubated in the dark with Cu-chl and Mg-chl. Then, they were exposed to sunlight and recovered for six weeks as mentioned above. The soft tissues of the surviving snails in the treated and control (light and dark) groups were removed from the shells and homogenised (1 g/2 mL dechlorinated water) using UP 200H ultrasonic processor, and the suspensions were centrifuged at 4,000 rpm for 45 min at 25±1°C. The pellets were discarded while the aliquots of su-
RESULTS

FECUNDITY

The incubation of *B. alexandrina* with Cu-chl and Mg-chl followed by exposure to sunlight and left for six weeks to recover reduced their survivorship (Lx) compared to the light control snails (Table 1). At LC<sub>25</sub>, Cu-chl and Mg-chl the Lx values after six weeks of recovery were 0.40 and 0.63, respectively, compared to 0.73 for the light control group. The results were similar for the dark control groups; their Lx values at LC<sub>25</sub> Cu-chl and Mg-chl were 0.52 and 0.63, respectively. Also, the fecundity (Mx) was generally decreased after incubation with Cu-chl and Mg-chl followed by exposure to sunlight, as mentioned above. Then, they were transferred to clean dechlorinated water for prepaternity. Light and dark control groups were run parallel to the tested groups. During the prepaternity period the snails were fed oven-dried lettuce leaves, dead snails were daily removed and water was changed weekly. On day 28th after miracidial exposure, the surviving snails were individually examined for cercarial shedding and the number of cercariae/snail/week was recorded till the snails’ death or cessation of shedding. The snails’ infection rate and the number of cercariae per snail were calculated.

Table 1. Survivorship and fecundity of *B. alexandrina* incubated in the dark with copper chlorophyllin (Cu-chl, 12 h) and magnesium chlorophyllin (Mg-chl, 3 h) then exposed to light (9 h)

| Recovery period (week) | Light control | Cu-chl dark control (LC<sub>10</sub>) | Cu-chl LC<sub>25</sub> | Mg-chl LC<sub>25</sub> | Mg-chl dark control (LC<sub>10</sub>) | Mg-chl LC<sub>25</sub> |
|------------------------|---------------|--------------------------------------|-----------------------|---------------------|--------------------------------------|-----------------------|
| 1                      | Lx 3.08       | Lx 3.08                              | Lx 3.08               | Lx 3.08             | Lx 3.08                              | Lx 3.08               |
|                        | Mx 3.08       | Mx 3.08                              | Mx 3.08               | Mx 3.08             | Mx 3.08                              | Mx 3.08               |
|                        | LxMx 3.08     | LxMx 3.08                            | LxMx 3.08             | LxMx 3.08           | LxMx 3.08                            | LxMx 3.08             |
| 2                      | 0.8 0.8       | 0.8 0.8                              | 0.8 0.8               | 0.8 0.8             | 0.8 0.8                              | 0.8 0.8               |
|                        | 0.8 0.8       | 0.8 0.8                              | 0.8 0.8               | 0.8 0.8             | 0.8 0.8                              | 0.8 0.8               |
|                        | 0.8 0.8       | 0.8 0.8                              | 0.8 0.8               | 0.8 0.8             | 0.8 0.8                              | 0.8 0.8               |
| 3                      | 0.8 0.4       | 0.8 0.4                              | 0.8 0.4               | 0.8 0.4             | 0.8 0.4                              | 0.8 0.4               |
|                        | 0.4 0.4       | 0.4 0.4                              | 0.4 0.4               | 0.4 0.4             | 0.4 0.4                              | 0.4 0.4               |
|                        | 0.4 0.4       | 0.4 0.4                              | 0.4 0.4               | 0.4 0.4             | 0.4 0.4                              | 0.4 0.4               |
| 4                      | 0.73 0.53     | 0.73 0.53                            | 0.73 0.53             | 0.73 0.53           | 0.73 0.53                            | 0.73 0.53             |
|                        | 0.53 0.53     | 0.53 0.53                            | 0.53 0.53             | 0.53 0.53           | 0.53 0.53                            | 0.53 0.53             |
|                        | 0.53 0.53     | 0.53 0.53                            | 0.53 0.53             | 0.53 0.53           | 0.53 0.53                            | 0.53 0.53             |
| 5                      | 0.73 0.53     | 0.73 0.53                            | 0.73 0.53             | 0.73 0.53           | 0.73 0.53                            | 0.73 0.53             |
|                        | 0.53 0.53     | 0.53 0.53                            | 0.53 0.53             | 0.53 0.53           | 0.53 0.53                            | 0.53 0.53             |
|                        | 0.53 0.53     | 0.53 0.53                            | 0.53 0.53             | 0.53 0.53           | 0.53 0.53                            | 0.53 0.53             |
| 6                      | 0.73 0.53     | 0.73 0.53                            | 0.73 0.53             | 0.73 0.53           | 0.73 0.53                            | 0.73 0.53             |
|                        | 0.53 0.53     | 0.53 0.53                            | 0.53 0.53             | 0.53 0.53           | 0.53 0.53                            | 0.53 0.53             |
|                        | 0.53 0.53     | 0.53 0.53                            | 0.53 0.53             | 0.53 0.53           | 0.53 0.53                            | 0.53 0.53             |
| R<sub>0</sub>          | 1.67          | 1.67                                 | 1.67                  | 1.67                | 1.67                                 | 1.67                  |
| Reduction (%) of R<sub>0</sub> ± S.D. | 79.15 ± 0.57 | 61.70 ± 12.81 | 72.67 ± 3.45 | 93.47 ± 3.35 | 83.98 ± 0.82 | 89.66 ± 1.34 |

Lx – survivorship, Mx – mean number of eggs/snail/week (1.41 eggs at the start of the experiment), R<sub>0</sub> – net reproductive rate (sum of Lx and Mx).

LC<sub>10</sub> – 5×10<sup>-6</sup> mol/L, LC<sub>25</sub> – 10<sup>-3</sup> mol/L.
Steroid Sex Hormones

The incubation of *B. alexandrina* with Cu-chl and Mg-chl followed by exposure to light disturbed the activity of their steroid sex hormones (Table 2). The levels of progesterone in the tissue homogenate of snails incubated with Cu-chl and Mg-chl were lower than those of the light control group. The progesterone concentrations in the group incubated with LC 

10 Cu-chl and Mg-chl followed by six weeks of recovery after light exposure were 1.0 and 1.5 nmol/L, respectively, compared to 6.3 nmol/L for the light control group (p<0.001).

The testosterone levels in the tissue homogenate of snails incubated with LC 

25 Cu-chl and Mg-chl and left to recover for six weeks after light exposure were significantly lower than those of the light control group: 9.7, 8.6 and 12.6 nmol/L, respectively (p<0.05 and 0.01). Such concentrations of Mg-chl raised the levels of this hormone in the treated snails but without significant differences from those of the light control group. Similarly, LC 

25 Cu-chl and Mg-chl raised the levels of this hormone in the dark control groups.

The levels of estradiol were significantly decreased in the tissue homogenate of the groups incubated with the tested concentrations of Cu-chl and Mg-chl compared to that of the light control group. The recorded levels of this hormone in the groups incubated with LC 

25 Cu-chl and Mg-chl, and then recovered for six weeks, were 102.4 and 92.1 pg/mL, respectively, compared to 212.6 pg/mL for the light control group (p<0.001). However, the only significantly higher level of this hormone (275.0 pg/mL) was observed in the snails incubated with LC 

10 Cu-chl and left to recover for six weeks (p<0.001).

Infection with *S. Mansoni*

Survivorship on the 1st day of shedding

The incubation of *B. alexandrina* with Cu-chl and Mg-chl three and 21 days after their exposure to miracidia of *S. mansoni* reduced their survivorship at the 1st shedding compared to the light control group (Table 3). The survivorship of the snails incubated with LC 

10 Cu-chl and Mg-chl 21 days following miracidial exposure was 10% for each tested compound compared to 40% for the light control group. On the other hand, under these conditions, no snail could survive at LC 

25 of any of the tested compounds (0%). The dark control groups incubated with LC 

25 Mg-chl three and 21 days after miracidial exposure exhibited small survivorship at the 1st shedding: 10% and 30%, respectively, compared to 40% for the light control snails.

Prepatent period and duration of cercarial shedding

The incubation of *B. alexandrina* with Cu-chl and Mg-chl three and 21 days after miracidial exposure shortened these periods compared to the light control snails (Table 3). The prepatent period of the snails in-

Table 2. Effect of copper chlorophyllin (Cu-chl) and magnesium chlorophyllin (Mg-chl) on the sex hormones of *B. alexandrina* (Mean ± S.D. * – P< 0.05, ** – P< 0.01, *** – P< 0.001)

| Treatment | Progesterone (nmol/L) | Testosterone (nmol/L) | Estradiol (pg/mL) |
|-----------|-----------------------|-----------------------|-------------------|
| Light control | 6.3±0.26 | 6.3±0.26 | 212.6±7.61 | 212.6±7.61 |
| Cu-chl LC 

10 without recovery | 3.7±0.29*** | 4.7±2.31 | 10.7±1.43 | 18.8±2.95 | 89.9±5.22*** | 187±9.93*** |
| Dark control (LC 

10) after 6 weeks of recovery | 2.5±0.58* | 4.2±2.6 | 16.0±2.41 | 13.6±3.06 | 89.6±4.70*** | 177.3±5.83*** |
| Cu-chl LC 

25 after 6 weeks of recovery | 1.0±0.12*** | 1.5±0.11*** | 9.7±3.34* | 16.2±4.27 | 102.4±7.81*** | 92.1±4.44*** |
| Cu-chl LC 

10 after 6 weeks of recovery | 1.1±0.05*** | 4.4±0.35 | 8.6±1.79** | 12.7±1.93 | 275.0±9.88*** | 70.1±6.27 |

LC 

10 – 5×10 

6 mol/L, LC 

25 – 10 

6 mol/L.
Egg laying capacity of *B. alexandrina* incubated with Cu-and Mg-chlorophyllin

Incubated with LC10 Cu-chl and Mg-chl after 21 days of miracidial exposure was 21 days for each compared to 24.5 days for the light control group. The duration of cercarial shedding for the treated groups was 21 and seven days, respectively, compared to 38.5 days for the light control group (p<0.05 & 0.001).

Cercarial production

The cercarial production in the snails incubated with Cu-chl and Mg-chl after three and 21 days of miracidial exposure (Table 3 and Fig. 2) was smaller: for LC10 Cu-chl and Mg-chl after 21 days of miracidial exposure it was 3,126 and 107 cercariae/snail, respectively, compared to 9,778 cercariae/snail in the light control group (p<0.001). Similar results were obtained for the dark control snails incubated with LC25 Cu-chl and Mg-chl.

Table 3. Infection rate (%) and cercarial production (mean ± S.D) in infected *B. alexandrina* incubated with copper chlorophyllin (Cu-chl) and magnesium chlorophyllin (Mg-chl) three and 21 days after their exposure to *S. mansoni* miracidia (*– p<0.05, *** – p<0.001)

| Parameters of treated snails | Cu-chl | Mg-chl | Light control |
|-----------------------------|--------|--------|---------------|
|                            | 3 days post miracidial exposure | 21 days post miracidial exposure | 3 days post miracidial exposure | 21 days post miracidial exposure |
| Dark control (LC10) | LC10 | LC25 | LC10 | LC25 | LC10 | LC25 | LC10 | LC25 |
| Survivorship (%) at 1st shedding | 10 | 0 | 0 | 10 | 10 | 0 | 10 | 50 | 0 | 30 | 10 | 0 | 40 |
| Infection (%) | 0 | 0 | 0 | 0 | 100 | 0 | 100 | 100 | 0 | 100 | 100 | 0 | 50 |
| Prepatent period day | 0 | 0 | 0 | 0 | 21±0 | 0 | 21±0 | 24.5±3.5 | 0±0 | 21±0 | 21±0 | 0 | 24.5±3.5 |
| Shedding period day | 0 | 0 | 0 | 0 | 21±0 * | 0 | 7±0 | 21.0±0 * | 0±0 | 7±0 | 8.08 | 0 | 38.5±10.5 |
| Total Cercariae/ snail | 0 | 0 | 0 | 0 | 3,126±0 *** | 0 | 117±0 | 3,145.2±0 *** | 0±0 | 3,656.7±0 107±0 *** | 1,889.3 | 0 *** | 9,778.0±562.0 |

LC10 = 5×10−6 mol/L, LC25 = 10−5 mol/L.

Fig. 2. Effect of copper chlorophyllin (Cu-chl) and magnesium chlorophyllin (Mg-chl) on cercarial production of *B. alexandrina* exposed to *S. mansoni* (mean values ± S.D. are given)
DISCUSSION

Although chemotherapy is one of the most valuable methods of schistosomiasis control, there is still a need for more selective and efficient molluscidic for controlling the snail intermediate hosts of this parasite (WHO 2017). In this context, the photosensitisers copper chlorophyllin and magnesium chlorophyllin were evaluated against biological and biochemical parameters of B. alexandrina and their infection with S. mansoni.

Our results show a marked reduction in the survivorship (Lx) of B. alexandrina incubated with Cu-chl and Mg-chl, exposed to light and recovering for six weeks in clean dechlorinated water. Similar results were obtained after keeping B. alexandrina with the plants Panicum repens and Solanum nigrum (IBRAHIM et al. 2004), Furcraea selloa marginata (OSMAN et al. 2011), Zingiber officinale and curcumin (EL-EMAM et al. 2017).

The egg laying capacity (Mx) and reproductive rate (R.), of B. alexandrina were markedly reduced after incubation with Cu-chl and Mg-chl followed by six weeks of recovery. Moreover, the surviving snails stopped oviposition for two and five weeks of recovery. This agrees with the results of HASHEESH et al. (2011) on the egg laying capacity (Mx) of B. truncatus maintained with methanol extract of the plant Sesbania sesban. Similarly, Mx and R., of B. alexandrina decreased after treatment with chloroform extract of the plant Haplophyllum tuberculatum (RIZK et al. 2012) and methanol extract of the plants Z. officinale and C. citrinas (BAKRY et al. 2013, EL-EMAM et al. 2017). The reduction in Mx and R., of B. alexandrina following incubation with Cu-chl and Mg-chl may be due to the harmful effect of such compounds on the regulation of the oviposition. This is supported by the observed decrease in the concentrations of progesterone, testosterone and estradiol in the snails’ tissues. The changes in the level of steroid hormones of the treated B. alexandrina are compatible with the results of RIZK et al. (2012) who found that exposing the snails to chloroform extract of H. tuberculatum caused a decrease in the level of their sex hormones. The authors recorded disappearance of testosterone, decrease in epiandrosterone and progesterone, while the level of 11α-hydroxy-progesterone was greatly elevated in the digestive and hermaphrodite glands of B. alexandrina. They suggested that the tested plant might contain compounds which could inhibit the biosynthetic pathways of testosterone in the treated snails and decrease the concentrations of progesterone which is the precursor of the other hormones; the absence of testosterone could explain the decrease in the egg production (Mx). OEHLMANN et al. (1996) recorded an increase in the testosterone concentrations in Mucella labillus after exposure to organotin compounds. Later, EL-EMAM et al. (2017) found that exposure of B. alexandrina to methanol extract of C. citrinas and Z. officinale decreased the concentrations of progesterone and testosterone in the haemolymph, while that of 17β-estradiol was elevated compared to the control group.

Our data on the infection of B. alexandrina with S. mansoni show that although the prepatent period of the parasite within the snail tissues following incubation with Cu-chl and Mg-chl three and 21 days after exposure to S. mansoni miracidia was not significantly different from that of the light control group, the duration of cercarial shedding and cercarial production per infected snail were significantly reduced. Similar results were obtained by GAWISH (2008), BAKRY (2009), MAHMOUD et al. (2011) and RIZK et al. (2012) who found that exposure of B. alexandrina to the plants Syzygium jambos, Euphorbia splendens, Atriplax stylosa, Datura stramonium, S. sesban and H. tuberculatum had an obvious negative effect on the duration of shedding of cercariae of S. mansoni and on the total cercarial production per infected snail. The authors conjectured that the reason could be disturbances in the activities of the snail enzyme system and the total protein concentration in the tissues and haemolymph which suppressed the development of parasite larval stages in the snail tissues. Similarly, exposure of S. mansoni-infected B. alexandrina to the photosensitiser carbamide perhydrate significantly reduced the cercarial production per treated snail compared to the control snails (GAWISH et al. 2009).

It can be concluded that sub-lethal concentrations of copper chlorophyllin (Cu-chl) and magnesium chlorophyllin (Mg-chl) as photosensitising treatment agents (LC50 & LC90) are capable of inducing significant changes in the snails’ egg laying capacity, sex hormones (progesterone, testosterone and estradiol) and susceptibility to infection with miracidia of S. mansoni. These photosensitising agents are inexpensive, environmentally friendly and should be considered in schistosomiasis control programmes.

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