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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 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 (R_0) and total levels of progesterone, testosterone and estradiol were evaluated. Besides, we determined the production of cercariae of S. *mansoni*. The concentrations LC_{10} and LC_{25} of Cu-chl and Mg-chl significantly reduced the fecundity and R_0 of the treated snails which could be partially due to the decrease in the level of steroid sex hormones. The reduction rates of R_0 for the snails treated with LC_{10} 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 LC_{10} 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 (R_0) 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-HOMMOSSANY & EL-SHERBINI (2011) found that exposure of *B. alexandrina* to sub-lethal concentrations of hematoporphyrin-coated gold nanoparticles (Hpd

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GNPs) for 12 hours followed by four hours of light exposure (336.2 W/m^2) 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 (Wholleb et al. 2009). Photosensitisers negatively affect different microorganisms, such as drug-resistant bacteria, yeasts, viruses and parasites. Furthermore, the requirement for both photosensitiser 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 cholorophyllin 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 LC_{10} and LC_{25} 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 LC_{10} and LC_{25} 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/m^2) (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-

pernatants were subject to ELISA runs to estimate the total levels of testosterone (DRG Instruments Gmbh, Germany Cat. EIA-1559), progesterone (DRG Instruments Gmbh, Germany Cat. EIA-1561) and estradiol (Diagnostics Biochem Canada Inc. "DBC"; Cat. CAN-E-430). The absorbance of the calibrators, controls and the test samples was measured using ELISA reader, stat fax, device, USA.

INFECTION WITH *S. MANSONI*

Each of the 13 groups included 30 snails: six for each of Cu-chl and Mg-chl and one as the light control. Snails of each group were individually exposed overnight to miracidia of *S. mansoni* (5–8 miracidia/snail 2 mL dechlorinated water). They were then transferred to clean dechlorinated water in plastic aquaria (10 snails/L). The snail groups three and 21 days after miracidial exposure were incubated with LC_{10} and LC_{25} of each of Cu-chl and Mg-chl, followed by exposure to sunlight, as mentioned above. Then, they were transferred to clean dechlorinated water for prepatency. Light and dark control groups were run parallel to the tested groups. During the prepatency 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.

STATISTICAL ANALYSIS

The data were statistically analysed for the significance of differences between the treated and control groups by means of Student t test and the values were expressed as mean ± S.D. The statistical programme for Windows "Graph Pad Prism soft-ware" was used along with the statistical package for social sciences (SPSS version 17.0) to calculate the data significance using one-way ANOVA and t-test (MILLER & MILLER 2010).

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_{25} 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_{25} 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 and six weeks of recovery. Thus, after six weeks of recovery the Mx values for the snails at LC_{10} of Cu-chl and Mg-chl were 4.2 and 1.0 eggs/snail/week, respectively, compared to 15.6 eggs/snail/week for the light control group. Moreover, during the recovery period the snails surviving at LC_{25} and LC_{10} in each tested compound ceased egg laying for one or two weeks. Similarly, the dark control group LC_{25} Mg-chl ceased egg laying for four weeks during the recovery period and laid few eggs; their Mx values were very small compared to the light control group: 0.71 and 0.95 eggs/snail/week during the 3rd and 4th weeks of recovery.

The reproductive rate (R_0) was considerably decreased, reflecting the reduction in both Lx and Mx of the treated snails compared to the values for the light control group (Fig. 1). The reduction rates in

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_{25})			Cu-chl LC_{25}			Cu-chl LC_{10}			Mg-chl dark control (LC_{25})			Mg-chl LC_{25}			Mg-chl LC_{10}		
	Lx	Mx	LxMx	Lx	Mx	LxMx	Lx	Mx	LxMx	Lx	Mx	LxMx	Lx	Mx	LxMx	Lx	Mx	LxMx	Lx	Mx	LxMx
1	1.0	3.08	3.08	0.87	2.36	2.05	0.70	2.70	1.89	0.90	1.03	0.92	0.86	0.0	0.0	0.8	0.4	0.32	0.9	0.0	0.0
2	0.8	1.16	0.92	0.83	0.00	0.00	0.66	0.00	0.00	0.90	0.00	0.00	0.86	0.0	0.0	0.8	0.5	0.4	0.9	0.0	0.0
3	0.8	0.45	0.36	0.66	0.82	0.54	0.63	0.0	0.00	0.73	0.18	0.13	0.7	0.71	0.49	0.66	1.3	0.85	0.66	1.0	0.66
4	0.73	2.54	1.85	0.56	1.58	0.88	0.60	2.25	1.35	0.73	0.55	0.40	0.7	0.95	0.66	0.66	1.05	0.69	0.5	0.73	0.36
5	0.73	0.00	0.00	0.53	0.00	0.00	0.53	1.0	0.53	0.66	0.90	0.59	0.7	0.0	0.0	0.66	0.0	0.0	0.5	0.6	0.3
6	0.73	15.6	11.39	0.53	0.38	0.20	0.40	7.44	2.97	0.66	4.20	2.77	0.63	0.0	0.0	0.63	0.93	0.56	0.5	1.0	0.5
R_0			17.6			3.67			6.74			4.81			1.15			2.82			1.82
Reduction (%) of R_0 ± 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_0 – net reproductive rate (sum of Lx and Mx).

LC_{10} – 5×10^{-6} mol/L, LC_{25} – 10^{-5} mol/L.

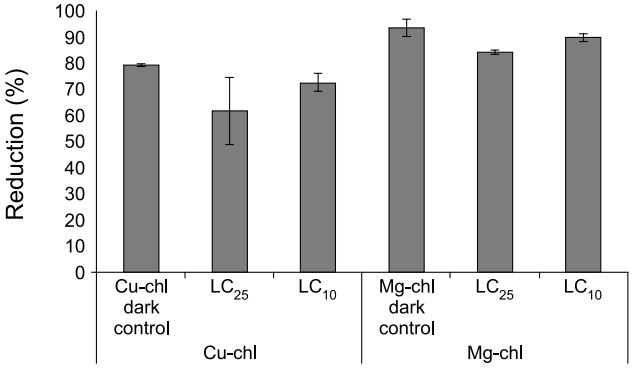

Fig. 1. Reduction (mean % ± S.D.) of reproductive rate (R_0) of *B. alexandrina* incubated in the dark with copper cholorophyllin (Cu-chl) and magnesium chlorophyllin (Mg-chl) then exposed to light followed by six weeks of recovery

this parameter in the snails incubated with LC_{10} Cu-chl and Mg-chl were 72.7% and 89.7%, respectively.

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_{25} 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} and LC_{10} Cu-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)	
	Cu-chl	Mg-chl	Cu-chl	Mg-chl	Cu-chl	Mg-chl
Light control	6.3±0.26	6.3±0.26	12.6±0.31	12.6±0.3	212.6±7.61	212.6±7.61
Cu-chl LC_{25} 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_{25}) 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^{-5} mol/L.

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 21days 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 (LC_{25})	LC_{10}	LC_{25}	Dark control (LC_{25})	LC_{10}	LC_{25}	Dark control (LC_{25})	LC_{10}	LC_{25}	Dark control (LC_{25})	LC_{10}	LC_{25}	
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	21.0± 8.08	7±0 ***	0	38.5± 10.5
Total Cercariae/ snail	0	0	0	0	3,126± 0 ***	0	117±0	3,145.2± 103.4 ***	0±0	3,656.7± 1,889.3	107± 0 ***	0	9,778.0± 562.0

LC_{10} – 5×10^{-6} mol/L, LC_{25} – 10^{-5} mol/L.

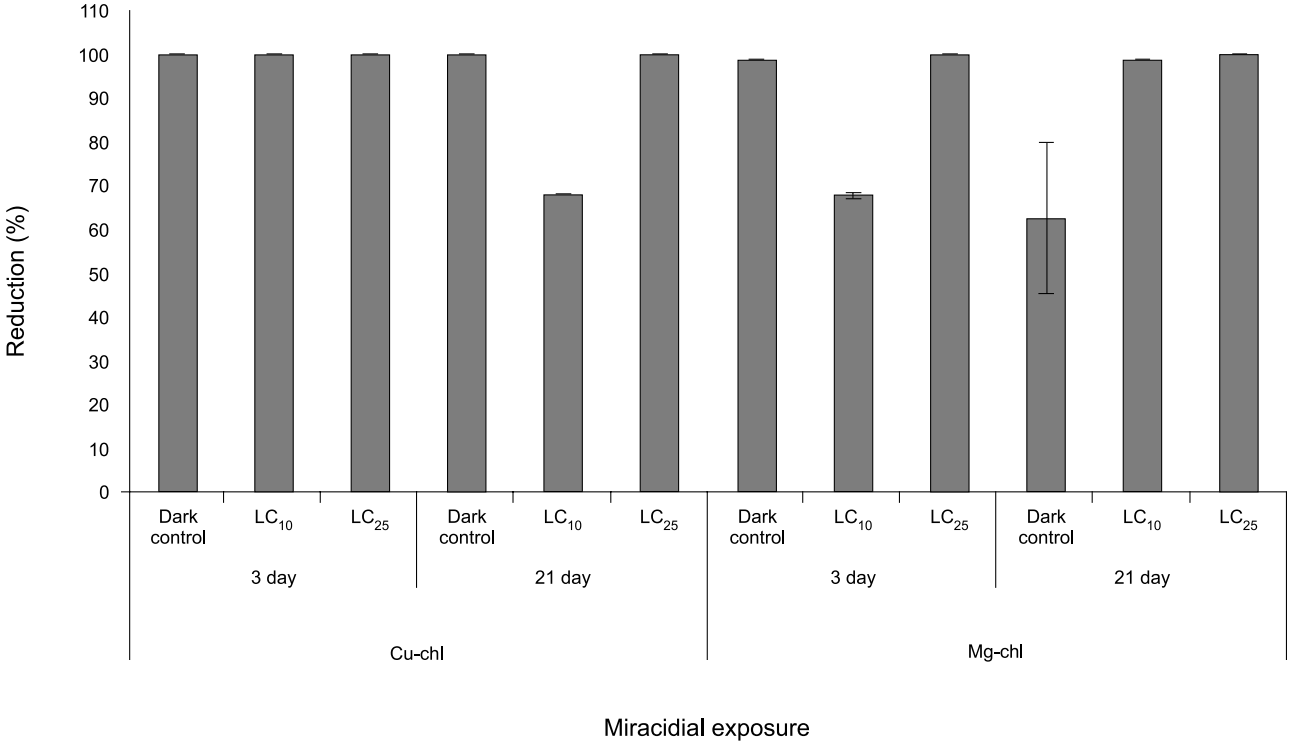

Fig. 2. Effect of copper cholorophyllin (Cu-chl) and magnesium chlorophyllin (Mg-chl) on cercarial production of *B. alexandrina* exposed to *S. mansoni* (mean values ± S.D. are given)

cubated with LC_{10} 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 LC_{10} 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 LC_{25} Cu-chl and Mg-chl.

DISCUSSION

Although chemotherapy is one of the most valuable methods of schistosomiasis control, there is still a need for more selective and efficient molluscicides 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_0) 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_0 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. citrinus* (BAKRY et al. 2013, EL-EMAM et al. 2017). The reduction in Mx and R_0 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. citrinus* 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, Atriplex 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 (LC_{25}& LC_{10}) 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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REFERENCES

BAKRY F. A. 2009. Use of some plant extracts to control *Biomphalaria alexandrina* snails with emphasis on some biological effects. Pesticide Biochem. Physiol. 95: 159–165. https://doi.org/10.1016/j.pestbp.2009.08.007

BAKRY F. A., ABD EL-ATTI M. S., ISMAIL S. M. 2013. Effect of *Zingiber officinale* (ginger) on electrophoresis analysis and biochemical aspects of *Biomphalaria alexandrina* snails infected with *Schistosoma mansoni*. J. Sci. Eng. Res. 4: 1147–1154.

EL-EMAM M. A., OSMAN G. Y., ABDEL-HAMID H., MOHAMED A. H., ALI R. E. M. 2017. Determination of egg laying capacity, sex hormones and mortality of *Biomphalaria alexandrina* snails exposed to methanol extract from curcumin and the plants *Callistemon citrinus* and *Zingiber officinale*. J. Biosci. Appl. Res. 3: 97–109.

EL-HOMMOSSANY K., EL-SHERBINI S. A. 2011. Impact of the photosensitizer hematoporphyrin coated gold nanoparticles on *Biomphalaria alexandrina* snails. Open J. Anim. Sci. 1: 54–60. https://doi.org/10.4236/ojas.2011.12007

EL-SAYED K., EL-SHERBINI S. 2006. Impact of hematoporphyrin and different laser sources on *Biomphalaria alexandrina* snails and their infection with *Schistosoma mansoni*. J. Biol. Chem. Environ. Sci. 1: 320–340.

ERZINGER G. S., WOHLLEBE S., VOLLRATH F., SOUZA S. C., RICHTER P., LEBERT M., HÄDER D. P. 2001. Optimizing conditions for the use of chlorophyll derivatives for photodynamic control of parasites in aquatic ecosystems. Parasitol. Res.: 109: 781–786. https://doi.org/10.1007/s00436-011-2322-7

FERRUZZI M. G., BLAKESLEE J. 2007. Digestion, absorption and cancer preventative activity of dietary chlorophyll derivatives. Nutr. Res. 27: 1–12. https://doi.org/10.1016/j.nutres.2006.12.003

GAWISH F. A. 2008. Activity of the plant *Syzygium jambos* against *Biomphalaria alexandrina* snails' reproduction and infection with *Schistosoma mansoni*. New Egypt. J. Med. 39: 103–110.

GAWISH F. A., EL-SHERBINI, S. A., ALY H. F. 2009. Effect of photosensitization process of carbamide perhydrate on *Biomphalaria alexandrina* snails and their infection with *Schistosoma mansoni*. J. Appl. Sci. Res. 5: 46–56.

HASHEESH W. S., MOHAMED R. T., ABD EL-MONEM S. 2011. Biological and physiological parameters of *Bulinus truncatus* snails exposed to methanol extract of the plant *Sesbania sesban*. Adv. Biol. Chem. 1: 65–73. https://doi.org/10.4236/abc.2011.13009

IBRAHIM A. M., EL-EMAM M. A., EL-DAFRAWY S. M., MOSSALEM H. S. 2004. Impact of certain plant species on *Schistosoma mansoni-Biomphalaria alexandrina* system. Proc. 3rd Int. Conf. Biol. Sci. Tanta University 3–4 Apr. 2004. 3: 390–413.

MAHMOUD M. B., IBRAHIM W. L., ABOU-EL-NOUR B. M., EL-EMAM M. A., YOUSSEF A. A. 2011. Biological and biochemical parameters of *Biomphalaria alexandrina* snails exposed to the plants *Datura stramonium* and *Sesbania sesban* as water suspensions of their dry powder. Pest. Bioch. Physiol. 99: 96–104. https://doi.org/10.1016/j.pestbp.2010.11.005

MEKLER L. B., BYCHOVSKY A. F., KRIKUN B. I. 1969. Electron microscope study of the viricidal properties of sodium magnesium chlorophyllin. Nature 22: 574–575. https://doi.org/10.1038/222574b0

MILLER J. N., MILLER J. C. 2010. Statistics and chemometrics for analytical chemistry (6th ed.). Pearson Education Limited, Edinburgh Gate, Harlow, Essex, England.

OEHLMANN J., FIORONI P., STROBEN E., MARKERT B. 1996. Tributiltin (TBT) effects on *Ocinebrina aciculate* (Gastropoda: Muricidae): imposex development, sterilization, sex change and population decline. Sci. Total Environ. 188: 205–223. https://doi.org/10.1016/0048-9697(96)05173-X

OSMAN G. Y., AHMED M. M., ABDEL KADER A., MOHAMED A. A. 2011. Biological studies on *Biomphalaria alexandrina* snails treated with *Furcraea selloa marginata* plant (family: Agavaceae) and *Bacillus thuringiensis kurstaki* (Dipel-2x). J. Appl. Pharm. Sci. 1(10): 47–55.

PERRETT S., WHITFIELD P. J. 1996. Currently available molluscicides. Parasitol. Today 12: 156–159. https://doi.org/10.1016/0169-4758(96)10001-6

RAGHEB M., EL-TAYEB T., AL-EMAM M., AMER M., BASHTAR A. 2013. Copper chlorophyllin and magnesium chlorophyllin as molluscicidal agents against *Biomphalaria alexandrina* snails. Asian Acad. Res. J. Multi-Discipl. 1: 2319–2801.

REDMOND R. W. 2008. Photophysics and photochemistry in photodynamic therapy. In: HAMLIN M. R., MROZ P. (eds). Advances in photodynamic therapy: basic, translational, and clinical. Artech House, Boston and London, pp. 41–58.

RIZK M., METWALLY N., HAMED M., MOHAMED A. 2012. Correlation between steroid sex hormones, egg laying capacity and cercarial shedding in *Biomphalaria alexandrina* snails after treatment with *Haplophyllum tuberculatum*. Exp. Parasitol. 132: 171–179. https://doi.org/10.1016/j.exppara.2012.06.011

SPIKES J. D. 1989. Photosensitization. In: SMITH K. C. (ed.). The science of photobiology. Plenum Press, New York and London, pp.79–110. https://doi.org/10.1007/978-1-4615-8061-4_3

STEINMANN P., KEISER J., BOS R., TANNER M., UTZINGER J. 2006. Schistosomiasis and water resources development, systematic review, meta-analysis and estimates of people at risk. Lancet Infect. Dis. 6: 411–425. https://doi.org/10.1016/S1473-3099(06)70521-7

VESENICK D. C., DE PAULA N. A., NIWA A. M., MANTOVANI M. S. 2012. Evaluation of the effect of chlorophyllin on apoptosis induction, inhibition of cellular proliferation and mRNA expression of CASP8, CASP9, APC and S-catinin. Curr. Res. J. Biol. Sci. 4: 315–322.

WHO (World Health Organization) 2009. The control of schistosomiasis. Tech. Rep. Ser. 922, Geneva.

WHO (World Health Organization) 2017. Schistosomiasis. Fact Sheet No. 115 updated in October 2017. http://www.who.int/mediacentre/factsheets/fs115/en

WHOLLEBE S., RICHTER R., RICHTER P., HÄDER D. P. 2009. Photodynamic control of human pathogenic parasites in aquatic ecosystems using chlorophyllin and pheophorbid as photodynamic substances. Parasitol. Res. 104: 593–600. https://doi.org/10.1007/s00436-008-1235-6

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