

Impact of the photosensitizers hematoporphyrin coated gold nanoparticles on *biomphalaria alexandrina* snails

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ABSTRACT

The present study was done using two concentrations of the photosensitizer Hematoporphyrin coated Gold Nanoparticles (HpdGNP₃) (5×10^{-6} , 5×10^{-5} mole/dicimeter⁻³), to evaluate their efficacy on survival rate, egg-laying capacity of *Biomphalaria alexandrina* snails and on histological deteriorations in their hermaphrodite gland. *B. alexandrina* snails were incubated for 12 hours at each tested concentration in the dark, thereafter they were exposed to direct sunlight (336.2 W/m^2) for either 2 or 4 hours followed by 24 hours of recovery. Control snails were treated with these concentrations without exposure to light irradiance. Another experiment was carried out simultaneously and the snails were left for 4 weeks of recovery to evaluate their egg-laying capacity (Mx). The results indicate that $5 \times 10^{-5} \text{ Mdm}^{-3}$ (HpdGNPs) with 4 hours of exposure sunlight suppressed the survival rate of *B. alexandrina* snails by 50%. Meanwhile, control snails incubated with $5 \times 10^{-5} \text{ Mdm}^{-3}$ HpdGNPs were not affected and still alive (100%). For snail's fecundity (Mx), treated snails laid low number of eggs throughout the recovery period (4 weeks), in comparison with that of control ones. The highest value of Mx for snails treated with $5 \times 10^{-5} \text{ Mdm}^{-3}$ Hpd coated GNPs was recorded at the 3rd week of recovery period, being 6.7 eggs/snail, compared to 37.6 eggs/control snail. This has a negative reflect on the reproductive rate (R_o) of treated snails as it was reduced under these conditions by 76.6% and 86.1%, respectively. Histological tests revealed injuries in spermatocytes, oocytes, several degenerations of *B. alexandrina* hermaphrodite gland then evacuations in many gonad's cells which severely suppressed their capacity for egg-laying. It is concluded from the present

work that exposing *B. alexandrina* snails to sublethal concentrations of the photosensitizer Hpd coated GNPs (12 hours incubation, 4 hours exposure to 336.2 W/m^2) significantly reduced their reproductive capacity that may have a negative reflect on schistosomiasis transmission.

Keywords: *Biomphalaria Alexandrina* Snails; Hematoporphyrin Coated Gold Nanoparticles; Snail's Egg-Laying Capacity; Histology

1. INTRODUCTION

Schistosomiasis is an important public health problem in Egypt and several developing countries (Pasvol and Hoffman, 2001). Control of this parasitic disease could be through treatment of infected persons, health education, sanitation and snail control. Thus, snail control strategies are considered a priority for preventing or minimizing schistosomiasis transmission (Lardans and Dissous, 1998). These strategies have been based upon elimination of the snail intermediate hosts chemically, biologically or environmentally. However, further techniques are required in this field. So, photosensitizing compounds could be evaluating for controlling noxious water parasites, since sunlight activated dyes characterized by a high efficiency, lack of mutagenic activity and negligible toxicological risk to humans and other mammalian species (Ben Amor *et al*, 1998).

Hematoporphyrin derivatives have been used as photosensitizing agent in the presence of sunlight as well as artificial light (Spikes, 1984). Later, Abdel-Kader *et al* (2001) observed that incubation of *Lymnaea natalensis* snails with 10^{-3} Mdm^{-3} Hematoporphyrin (Hpd) for 12 hrs, followed by 30 min of exposure to sunlight (300 W/m^2) killed these snails after 24 hrs of recovery. El-Tarky (2005) noticed that mortality rate of adult *B. alexandrina* snails exposed for 4 hrs to 438.8 w/m^2 of sunlight after 12 hours of their incubation with 10^{-5}

Hpd, was 52%. The author added that the effect of Hpd against snails increased in the alkaline media (pH = 8), while it decreased at the acidic ones (pH = 6). It was stated that porphyrins and their derivatives exhibited a potent phytotoxic effect against gram-positive bacteria (Bertoloni *et al.*, 1993). In 2006, El-Sayed and El-Sherbini recorded that LC₅₀ of Hematoporphyrin (Hpd) against adult *B. alexandrina* snails was 5×10^{-5} M/dm³ after 12 hours of incubation and 2 hours of irradiation at ~ 336 W/m² in sunlight and at 2×10^{-5} Mdm⁻³ Hpd, they did not lay eggs. The authors added that infection rates of these snails with *S. mansoni* were significantly suppressed by their exposure to 2×10^{-5} Mdm⁻³ Hematoporphyrin (Hpd) either pre- or postmiracidial exposure, in addition to the significant reduction in cercarial production/snails treated with 4×10^{-5} Mdm⁻³ Hpd. Moreover, histological examinations showed a severe damage in the hermaphrodite gland of snails exposed to the sublethal concentrations of Hpd.

Recently, Ragheb (2009) stated that incubation of *B. truncatus* snails for 24 hrs at 10⁻⁴ M/L gold nanoparticles (GNPs) in dark followed by 2 hours of exposure to 650 W/m² irradiance (Solar Simulator) resulted in 100% death of snails. The author added that decreasing GNPs concentration to 10⁻⁵ M/L significantly reduced the snail's reproductive rate (R₀) by 48.3% compared to that of control, and this was partially due to the recorded damage in hermaphrodite gland cells of treated snails. Therefore, the present study aims to evaluate the efficacy of Hematoporphyrin coated Gold Nanoparticles on survival rate, egg-laying capacity of *B. alexandrina* snails and on their hermaphrodite gland cells histologically.

2. MATERIALS AND METHODS

2.1. Snails

Biomphalaria alexandrina snails (6 - 9 mm) were laboratory produced in Medical Malacology Department at Theodor Bilharz Research Institute (TBRI), Giza, Egypt.

2.2. Hematoporphyrin Coated Gold Nanoparticles

A stock Mole/decimeter 3 Hpd concentration Mole/decimeter 3 was prepared by dissolving 672 mg of Hpd in 100 ml of glycerol by stirring overnight and kept in dark. In 250 ml conical flask 5 ml of the stock solution was added to 100 ml of glycerol and boiled with stirring. To the solution 0.01699 g HAuCl₄ was added and the mixture continue boiling until the color of the mixture changes from red to wine red. Heating was removed and

stirring continued till cooling to room temperature. The final concentration is 5×10^{-4} Mdm⁻³ (Hpd + Au).

2.3. Molluscicidal Activity of Hematoporphyrin Coated Gold Nanoparticles

Two concentrations of Hematoporphyrin coated Gold Nanoparticles (5×10^{-6} , 5×10^{-5} Mdm⁻³) were prepared according to El-Tarky (2005) and Ragheb (2009) results, as 10 - 4 Mol/dm⁻³ from each of Hpd and GNPs was lethal to *B. alexandrina* and *B. truncatus* snails, respectively. Therefore, *B. alexandrina* (6 - 8 mm) were incubated for 12 hours at each tested concentration in the dark, thereafter they were exposed to light irradiance (336.2 W/m²) for either 2 or 4 hours. Three replicates for each light exposure period, each of 10 snails/500 ml, were prepared. After that, snails were washed thoroughly with dechlorinated water and maintained in clean water for 24 hours of recovery then mortality rates were recorded. Control snails were treated simultaneously as the tested ones, without exposure to light irradiance. Death of snails was determined by changes in appearance of the shell and internal body (Nolan *et al.*, 1953).

2.4. Effect of Hematoporphyrin Coated Gold Nanoparticles on Snail's Egg-Laying Capacity

B. alexandrina snails (7 - 9 mm) were incubated once for 12 hours in the dark with sublethal concentrations (5×10^{-5} and 5×10^{-6} Mdm³) of Hematoporphyrin coated gold nanoparticles, after that they were exposed to direct sunlight of 336.2 W/m² for 4 hours. Then, they were transferred to clean dechlorinated water and maintained under laboratory conditions (25°C) for 4 weeks. Two replicates, each of 15 snails/L dechlorinated water, were used for each concentration and control group. Each aquarium was provided with polyethylene sheets for oviposition. The snails were daily fed oven dried lettuce leaves, dead ones were removed and the survival rate were recorded weekly. The survivorship (Lx) and number of eggs/snails (Mx) were recorded weekly (Southwood, 1978).

2.5. Histology

Histological preparations were done for snails incubated for 12 hours to the two concentrations, 5×10^{-6} and 5×10^{-5} Mdm⁻³, followed by 2 and 4 hours exposure to direct sunlight (336.2 W/m²). Control snails were treated with these concentrations without exposure to light irradiance. The hermaphrodite gland of treated and control snail groups was carefully incised using fine

scissors and dropped into a fixative Bouin's solution, then the sections (5 - 8 μm) were stained with delafield's haematoxylin and eosin according to Mohamed and Saad (1990).

2.6. Statistical Analysis

Survival rates of treated snails were analyzed by Chi-square values of contingency tables (Southwood, 1978).

3. RESULTS

From **Table 1**, it is clear that 5×10^{-5} Mdm^{-3} (HpdGNPs) with 4 hours of exposure to 336.2 W/m^2 sunlight suppressed the survival rate of *B. alexandrina* snails by 50%. Meanwhile, control snails incubated with 5×10^{-5} Mdm^{-3} HpdGNPs were not affected and still alive (100%). It is also, seen that snail's survival rate was concentration and light period dependent. So, increasing the concentration from 5×10^{-6} to 5×10^{-5} Mdm^{-3} reduced the survival rate from 100% to 62.7%, respectively. Similar conclusion was observed by elongation of the light exposure period from 2 to 4 hours, as the rates of snails incubated with 5×10^{-5} Mdm^{-3} decreased from 62.7% to 50%, respectively.

The data in **Table 2** and (**Figure 1** and **2**) represent the effect of 5×10^{-6} and 5×10^{-5} Mdm^{-3} Hpd coated GNPs on snail's fecundity (Mx) and their survivorship (Lx) post 4 weeks of recovery followed their exposure once to this compound. It was observed that survivorship (Lx) of treated snails was significantly decreased during the 1st, 2nd and 3rd weeks of recovery in comparison with that of control group ($P < 0.01$). The Lx values of snails exposed to 5×10^{-6} and 5×10^{-5} Mdm^{-3} Hpd coated GNPs at the 1st week were 0.75 and 0.46, respectively compared to 1.00 for control ones. It was also, noticed that survived snails at 5×10^{-5} Mdm^{-3} Hpd coated GNPs tried to somewhat overcome the harmful effects of the tested compound through the 2nd and 3rd weeks of recovery as their Lx was stable, being 0.46, but they suffered from another significant reduction in this parameter at the 4th week (Lx = 0.23) compared to 0.6 for control ones ($P < 0.05$).

Table 1. Survival rate (%) of *Biomphalaria alexandrina* snails exposed to Hematoporphyrin coated gold nanoparticles (12 hours incubation, and 4 hours exposure to 336.2 W/m^2 , 24 hours recovery).

Concentrationnm (Mdm^{-3})	Survival rate (%) after	Light exposur for
5×10^{-6}	100	4 hours
5×10^{-5}	62.7	50.0
Control*	100	100

*Incubated with 5×10^{-5} Mdm^{-3} without light exposure.

For snail's fecundity (Mx), treated snails laid low number of eggs throughout the recovery period (4 weeks), in comparison with that of control ones. The highest value of Mx for snails treated with 5×10^{-5} Mdm^{-3} Hpd coated GNPs was recorded at the 3rd week of recovery period, being 6.7 eggs/snail, compared to 37.6 eggs/control snail. This has a negative reflect on the reproductive rate (R_o) of treated snails as it was 20.3 and 11.8 for groups exposed to 5×10^{-6} and 5×10^{-5} Mdm^{-3} of this compound, respectively, compared to 84.8 for control group. The reduction rates of this parameter in this case were 76.1% and 86.1%, respectively.

Histological effects on the hermaphrodite gland of *B. alexandrina* snails revealed that the hermaphrodite gland of control snails (**Plate 1**) consists of a number of closely connected tubules where each tubule is lined with distinct germinal epithelium gives rise to all the stages of oogenesis and spermatogenesis. Oogonia are found in groups which are arranged along the periphery of the tubules. Oocytes are usually aggregated in the lumen of the tubules. The mature ova deeply stained with H-E than oocytes due to the accumulation of yolk material in their cytoplasm. On the other hand, spermatogonia become arranged in small groups along the side walls of the tubules. As a result of their division, spermatocytes are produced. Spermatids are developed from the secondary spermatocytes and are found in small groups around them. Spermatozoa (sperms) are found in large groups free in the lumen; each consists of an oval head and thread like long tail (Mohamed and Saad, 1990).

For snails treated with Hpd coated GNPs (5×10^{-6} and 5×10^{-5} Mdm^{-3}), **Plates 2** and **3** indicated that the cells of their hermaphrodite gland suffered from injuries in spermatocytes, oocytes, besides several degenerations then evacuations were seen in many gonad's cells. The acini lost their normal shape and an almost damage to their connective tissues was noticed which severely suppressed their capacity for egg-laying.

4. DISCUSSION

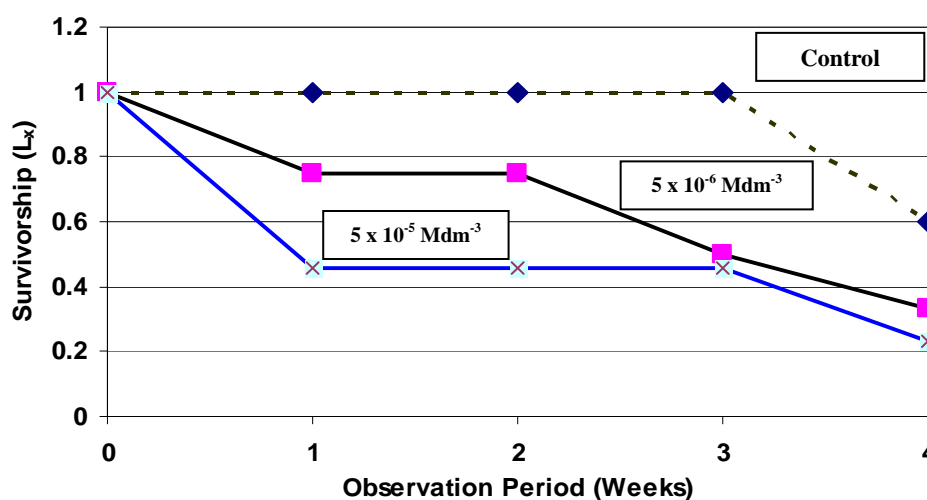
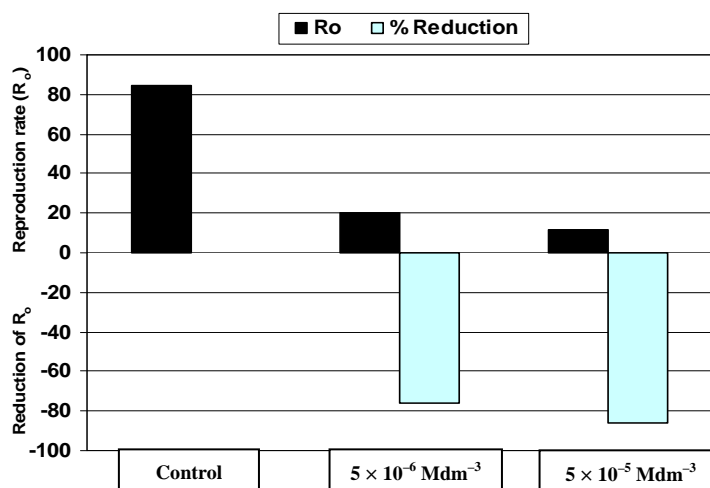
The present lethal effect of the photosensitizer Hematoporphyrin coated gold Nanoparticles (Hpd coated GNPs) against *B. alexandrina* snails was in parallel with that of Hpd on *L. natalensis* (Abd El-Kader *et al.*, 2001) and *B. alexandrina* snails (El-Tarky, 2005 and El-Sayed and El-Sherbini, 2006). As well, the harmful effects of GNPs against *B. truncatus* snails (Ragheb, 2009) support the present study.

Concerning snail's fecundity (Mx) and reproductive rate (R_o), the present results showed that exposure of *B. alexandrina* snails to Hpd coated GNPs markedly reduced these parameters. This could be attributed to the

Table 2. Survivorship (L_x) and fecundity (M_x) of *Biomphalaria alexandrina* snails exposed to Hematoporphyrin coated gold nanoparticles (12 hours incubation, 4 hours exposure to 336.2 W/m² sunlight).

Observation Period (week)	Control			$5 \times 10^{-6} \text{ Mdm}^{-3}$			$5 \times 10^{-5} \text{ Mdm}^{-3}$		
	L_x	M_x	$L_x M_x$	L_x	M_x	$L_x M_x$	L_x	M_x	$L_x M_x$
0	1.00	6.7	6.7	1.00	6.7	6.7	1.00	6.7	6.7
1	1.00	14.0	14.0	0.75***	3.7	2.8	0.46***	2.2	1.0
2	1.00	5.6	5.6	0.75***	1.2	0.9	0.46***	0.8	0.4
3	1.00	37.6	37.6	0.50***	8.2	4.1	0.46***	6.7	3.1
4	0.60	34.9	20.9	0.33n.s.	17.7	5.8	0.23*	2.6	0.6
$R_0 =$			84.8			20.3			11.8
Reduction(%)					76.1			86.1	

L_x = Survivorship, M_x = Mean number of eggs/snail/week; R_0 = net reproductive rate, Sum $L_x M_x$; *** = Highly Significant, $P < 0.01$; * = Significant, $P < 0.05$; n.s. = Not significant, $P > 0.05$.

**Figure 1.** Survivorship (L_x) of *Biomphalaria alexandrina* snails exposed to Hematoporphyrin coated gold nanoparticles (12 hrs incubation and 4 hrs exposure to 336.2 W/m² sunlight).**Figure 2.** Reproduction rate (R_0) of *Biomphalaria alexandrina* exposed to Hematoporphyrin coated gold nanoparticles (12 hrs incubation and 4 hrs exposure to 336.2 W/m² sunlight).

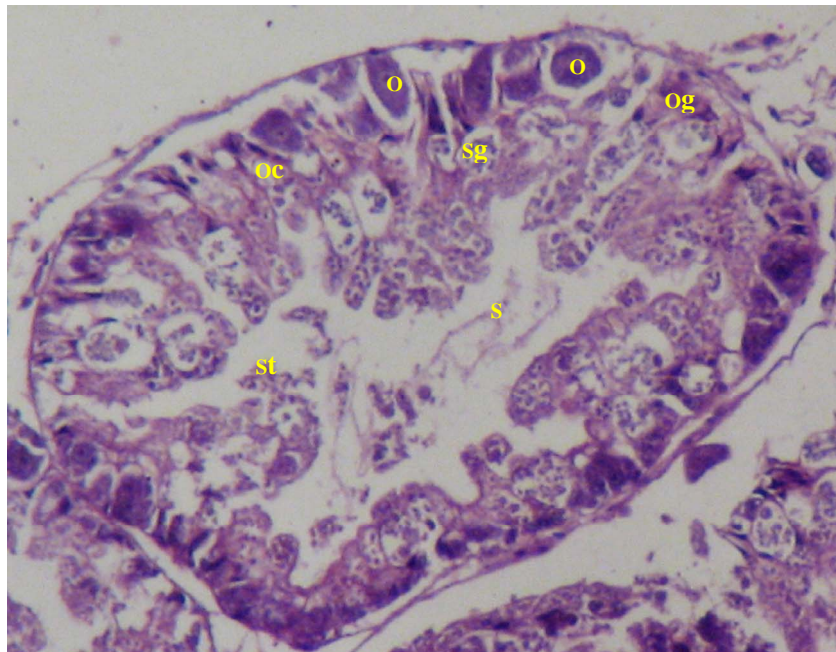


Plate 1. Light photomicrograph transverse section in the hermaphrodite gland of unexposed *Biomphalaria alexandrina* snails (Control), x 500. O=ova; Og = Oogonia; Oc= oocyte; Sg = Spermatogonia; St = Spermatid; S= sperms.

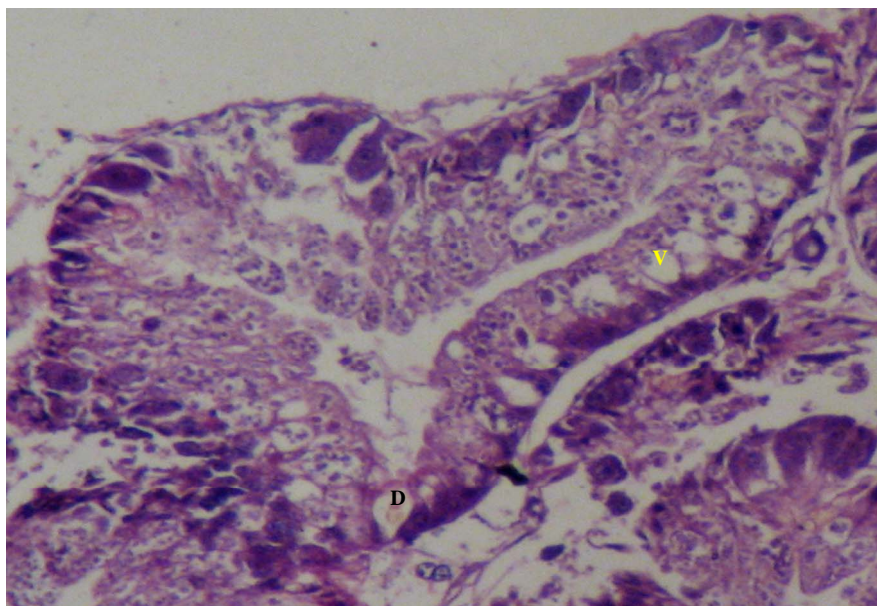


Plate 2. Light photomicrograph transverse section in the Hermaphrodite gland of *B. alexandrina* snails treated with 5×10^{-6} Mdm-3 Hematoporphyrin coated Gold nanoparticles, exposed to direct sunlight for 4hrs, x 500. D = Degenerated cells; V = Vacuoles.

harmful effects of this compound on physiological activities and reproductive system of treated snails, hence considerably reduced their oviposition. This was supported by the present histological results (**Plates 2 and 3**) on the hermaphrodite gland of snails exposed to this compound. These plates revealed marked damages to the gland cells in addition to several evacuations of its tu-

bules from gametogenic stages. This should greatly reduce the offspring of survived snails, hence has a negative reflect on schistosomiasis transmission. These observations are in accordance with the previous ones on Hpd against oviposition of *B. alexandrina* snails (El-Sayed and El-Sherbini, 2006) and on GNPs against reproductive rate of *B. truncatus* snails (Ragheb, 2009).

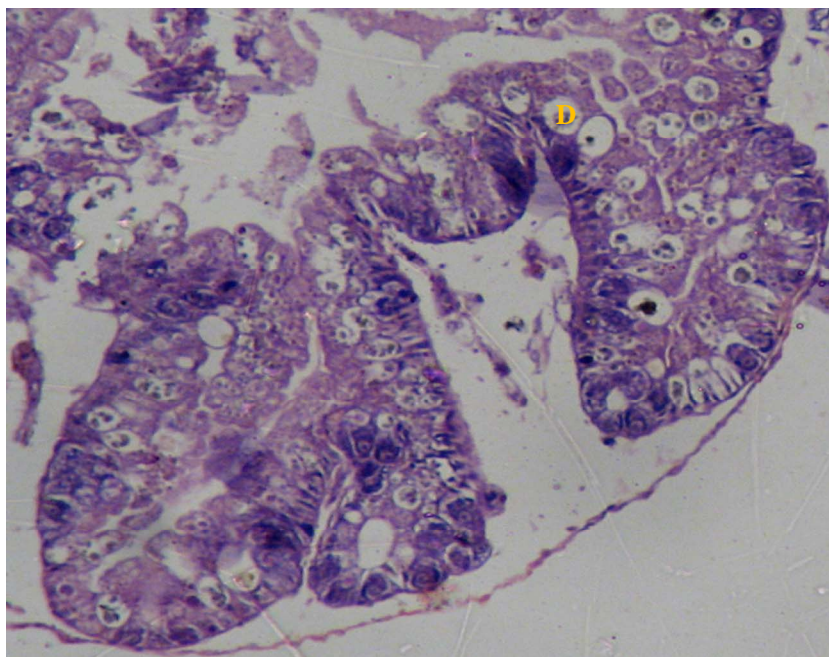


Plate 3. Light photomicrograph transverse section in the hermaphrodite gland of *B. alexandrina* snails treated with 5×10^{-3} Mdm⁻³ Hematoporphyrin coated Gold nanoparticles, exposed to direct sunlight for 4hrs, x 500. D = Degenerated cells.

The harmful effects of Hpds could be partially due to their hydrophobic character, hence localization in and damage the cellular membrane leading to cells death (Ben Amor *et al.*, 1998). As well, these photosensitizers damage the biological targets by photosensitized oxidation which deactivate certain enzymes through destruction of specific amino acids (*e.g.* Methionine and tryptophan), nucleic acid (Primarily of guanine) and by oxidation of unsaturated fatty acids and cholesterol in cell membrane (Abd El-Meguid, 1996). In addition, precipitated GNPs inside snail's tissues when irradiated with some forms of light energy they absorb light and emit localized heat with its lethal or traumas effects (Jana *et al.*, 2007).

It is concluded from the present work that exposing *B. alexandrina* snails to sublethal concentrations of the photosensitizer Hpd coated GNPs (12 hours incubation, 4 hours exposure to 336.2 W/m²) significantly reduced their reproductive capacity that may have a negative reflect on schistosomiasis transmission.

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