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Assessment of molluscicidal and larvicidal activities of CuO nanoparticles on *Biomphalaria alexandrina* snails

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Abstract

Background: Schistosomiasis is a major, but generally overlooked, tropical disease carried by snails of the genus *Biomphalaria*, which have a large distribution in Egypt. Control of the intermediate host snail is critical in limiting schistosomiasis spread. On the topic of snails' management, nanotechnology has gained more interest.

Results: Copper oxide nanoparticles, characterised by transmission electron microscopy and X-ray diffraction, showed a single crystal structure with an average crystallite size around 40 nm by X-ray diffraction and typical transmission electron microscopy (TEM) image. Also, the UV–VIS spectrophotometer displayed a sharp absorption band of CuO NPs. Molluscicidal activity of copper oxide nanoparticles against *B. alexandrina* snails was observed. Following exposure to CuO NPs (LC_{50} and LC_{90} was 40 and 64.3 mg/l, respectively), there was a reduction in the growth and reproductive rates of treated *B. alexandrina* at the sub-lethal concentrations, as well as, a drop in egg viability. Moreover, CuO NPs exhibited a toxic effect on miracidiae and cercariae of *S. mansoni*. Scanning electron microscopy (SEM) investigations of the head-foot and mantle of control and treated snails to the sub-lethal concentrations of CuO NPs (LC_{10} 15.6 mg\l–LC_{25} 27.18 mg\l) indicated morphological alterations in the ultrastructure.

Conclusions: CuO NPs caused a significant effect against the intermediate hosts of *S. mansoni* and provide a considerable scope in exploiting local indigenous resources as snail molluscicidal agents.

Keywords: *Biomphalaria alexandrina*, *Schistosoma mansoni*, CuO NPs, Molluscicide, Scanning electron microscope

1 Background

Schistosomiasis is the second neglected tropical parasitic infection after malaria [1, 2]. Human infection with *Schistosoma mansoni* is closely related to the existence of its intermediate host, *Biomphalaria alexandrina* [3]. These snails were found across Egypt, particularly in the Nile Delta and along the Nile's tributaries with a high prevalence [4]. Controlling these snails remains one of the most promising strategies for combating schistosomiasis [5–7]. Snail populations have been managed using a number of methods that interrupt their life cycle [8]. Although chemotherapy is one of the most effective techniques for schistosomiasis management, there is still a need for more selective and efficient molluscicides for snail vector control [9, 10]. Currently, nanoparticles are being used in a growing variety of products [11, 12] due to their unique properties compared with their larger counterparts, such as ultra-small size, large surface-area-to-mass ratio, and high reactivity [13] and have recently gained popularity in biomedical sciences as antibacterial [14], antiviral [15], antifungal [16], antiprotozoal [17, 18] and anthelmintic agents [19]. But only few studies have looked into NPs as cercaricides or molluscicides [20, 21]. Therefore, the goal of this study was to assess the effect of CuO NPs on *B. alexandrina* snails and how it is reflected on survival, growth by reproductive rates, egg

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hatchability, larvitoxicity and topographical architecture of these snails.

2 Methods

2.1 Snails

Adult *B. alexandrina* snails (8–10 mm) from Medical Malacology department, Theodor Bilharz Research Institute (TBRI), Giza, Egypt, were obtained. Ten snails were placed in each aquarium filled with one litre of dechlorinated water (pH 7–7.5) and covered with glass plates. Water temperature was adjusted to (25 ± 2 °C) and illumination was provided from 80 watts ceiling-level fluorescent lamps. Dead snails were collected every day and the water was changed twice weekly. Oven dried lettuce leaves, blue green algae (*Nostoc muscorum*) and dried flakes (TetraMin, Hanover, Germany) were used for feeding. Small pieces of polyethylene sheets were put into the aquaria to gather egg masses according to Pellegrino et al. [22] daily, then kept in tiny jars until they hatched according to El-Fiki and Mohamed [23] and Liang et al. [24].

2.2 Characterisation of CuO NPs

Copper oxide nanopowder (CuO NP) < 50 nm particle size was purchased from Sigma–Aldrich, St. Saint Louis, MO 63,103, USA. Structural studies of CuO NPs were done by high-resolution transmission electron microscope (FETEM, JEM-2100F, JEOL Inc., Japan) that used for the purpose of imaging and made by Nanotechnology and Advanced Material Central Lab (NAMCL), Agriculture Research Center (ARC). Two different modes of imaging were employed; the bright field at electron accelerating voltage 200 kV using lanthanum hexaboride (LaB6) electron source gun and the diffraction pattern imaging. The crystalline nature of CuO NPs was determined by observing the X-ray diffraction (XRD) pattern. The average hydrodynamic size and Zeta potential of CuO NPs also were determined by dynamic light scattering (DLS) (Nano-Zeta sizer-ZS, Malvern Instrument, UK). The optical absorption of the CuO NPs suspension was measured using a double beam UV–Vis-NIR spectrophotometer (Varian-Cary 5000) in the wavelength range of 200–800 nm at room temperature.

2.3 Molluscicidal activity of CuO nanoparticles

A stock solution of 1000 mg/l was prepared and serial concentrations of CuO NPs (70, 60, 50, 40, 30, 20, and 10 mg/l) in glass beakers filled with 100 ml water were produced. For each concentration, three replicates of 10 adult snails were used. Each exposure lasted 24 h; at temperature 25 ± 2 °C and pH 7.4. Control snails were kept in dechlorinated water under the same experimental conditions. The snails were taken from each experimental test at the end of exposure period and washed thoroughly with dechlorinated water. Dead snails were documented as the average of the three replicates. The toxicity of CuO NPs has been expressed as LC_{50} and LC_{90} via probit analysis according to the procedure of Finney [25] using statistical program SPSS. The LC_0 was estimated at 1/10 of the LC_{50} value according to El -Gindy et al. [26].

2.3.1 *Effect of CuO NPs on survival and growth rates of juvenile snails*

Four groups of juvenile *B. alexandrina* snails (2–3 mm) from the laboratory breeding colony each of 30 snails were used. A set of these groups was exposed to sub-lethal concentrations (LC_0, LC_{10} and LC_{25}) of Cu NPs for 24 h/week followed by 6 days of recovery in clean dechlorinated water. This technique was repeated for four successive weeks. Another set of snails was maintained in clean dechlorinated tap water as control. Shell diameter was measured weekly under a dissecting microscope by a caliper according to Chernin, Michelson [27]. Dead snails were distinguished by immersion in a small amount of 15–20% sodium hydroxide solution, if bubbles and blood come out of snail, it is recorded as alive and if not, it is recorded as dead, then removed daily, and the survival rate was calculated according to Frank [28] by the following equation:

$$\text{Survival rate } (L_x) = \frac{\text{Number of survived snails}}{\text{Total number of exposed snails}} \times 100$$

2.3.2 *Effect of CuO NPs on egg laying capacity (M_x) and net reproductive rate (R_o) of adult snails*

Adult *B. alexandrina* snails were exposed for 24 h/week for four successive weeks to the tested concentrations of CuO NPs (LC_0, LC_{10} and LC_{25}). For each concentration, three replicates of ten adult snails (8–10 mm diameter) were used. Under the same experimental conditions, 30 snails were used as control group and kept in dechlorinated tap water. For egg deposition, polyethylene sheets were placed in the aquaria of treated and untreated snails, and egg masses were collected and counted weekly. The egg laying capacity is expressed in the form (M_x) and is calculated by dividing the total number of laid eggs in any given week by the total number of living snails at the start of the week as stated by El-Gindy and Radhawy [29] by the following equation.

(x)=Time of exposure in weeks

(L_x) is the survived snails at any given week as a fraction of the correct one (1.0=100%),

Fecundity (M_x) =the mean number of eggs/snail/week,

$$\text{The net reproductive rate } R_o = \Sigma L_x M_x$$

2.3.3 Larvicidal (miracidicidal and cercaricidal) activity

Five ml of water containing about 100 freshly hatched *S. mansoni* miracidia or cercariae were mixed with five ml of double concentration of the tested ones (LC_0 4; LC_{10} 15.6; LC_{25} 27.18; LC_{50} 40 and LC_{90} 64.3 mg/l) of CuO NPs from each. As a control, 10 ml dechlorinated tap water with 100 newly hatched miracidia was used or cercariae according to Ritchie et al. [30]. The mortality rates of stationary one were reported at the end of the experiment since they were presumed to be dead as stated by WHO [31].

2.4 Scanning electron microscopy studies

Ten *B. alexandrina* snails were exposed for 24 h to each sublethal concentration of CuO NPs (LC_{10} 15.6 mg/l and LC_{25} 27.18 mg/l), then rinsed in dechlorinated water for 24 h for recovery. Ten snails were dipped in dechlorinated water as a control. Using a stereomicroscope, the soft parts were detached and washed twice in phosphate buffer saline (PBS) before fixed for 24 h in 2.5% glutaraldehyde and 0.2 Molar cacodylate buffer (pH 7.2). The specimens were rinsed in PBS, cold distilled water followed by dehydration with an ascending series of ethanol (70–100%). The dehydrated specimens were immersed in acetone and isoamyl acetate, and dried using a transitional medium of liquid carbon dioxide. Finally, the samples were coated with gold using an ion-sputter coater apparatus and photographed by a scanning electron microscope (JSM-5200 LA, JOEL Company, USA).

2.5 Statistical analysis

The analyses included the calculation of the mean value, standard deviation, standard error and a "*t*" value at level $p \leq 0.05$ according to Zar [32]. The median lethal concentration (LC_{50}) value was determined by applying regression equation analysis to the probit transformed data of mortality as mentioned by Finney [25] using SPSS v. 17.0 for Windows (SPSS Inc. 2008).

3 Results

3.1 Properties of CuO NPs

Transmission electron microscopy shows the typical (TEM) image of CuO NPs, exhibits that the majority of the particles were polygonal in shape with smooth surfaces, and their average crystallite size was found to be around 40 nm (Fig. 1A). The structure of the CuO NPs was characterised by X-ray diffraction (Fig. 1B) confirmed the single crystal structure. No characteristic peaks of any impurities were detected, suggesting that high-quality CuO NPs were synthesized. The average hydrodynamic diameter and Zeta potential of CuO NPs were 503.6 nm and 23.6 mV, respectively (Fig. 1C, 1D). The UV–VIS spectrophotometer showed a sharp absorption band (Fig. 1E, F).

3.2 Molluscicidal activity of CuO NPs

The present results showed that CuO NPs have a molluscicidal activity against *B. alexandrina* snails after 24 h exposure at LC_{50} 40 mg/l (Table 1, Fig. 2).

3.2.1 Survival rate of B. alexandrina juveniles

The survival rate of *B. alexandrina* juvenile snails exposed to LC_0 (4 mg/l) of CuO NPs for 24 h/ week decreased gradually during the 1st period of the experiment (Table 2, Fig. 3A). Increasing the concentration to LC_{10} (15.6 mg/l) and LC_{25} (27.18 mg/l) caused a sharp decrease in the survival rate of the treated snails, at the 4th week it was 35 and 5%, respectively, compared to 95% of the control one. At the 6th week, the survival rate was 25 and 5% for the groups exposed to LC_0 and LC_{10}, respectively, while no snails survived at the LC_{25} concentration at this week, compared to 90% for the control group.

3.2.2 Growth rate of B. alexandrina juveniles

The data presented in Table 2 and Fig. 3B showed that there was a highly significant reduction in the growth rates of the snail groups exposed to LC_0 for 24 h/ week for 4 successive weeks of exposure (55%) compared to the control group (47.7%). The same trend was recorded for the treated snail group throughout the 2nd four weeks of the experiment (recovery period), as the growth rate was decreased by 87% compared with the control group. Also, the growth rate of snail group exposed to LC_{10} under these conditions was significantly decreased after the 4 weeks of exposure compared by the control group (86%). Thereafter, the growth rate of the treated snails was less than that of control snails after 2 weeks of the recovery period (at the 6th week), being 96% compared to control snails at this time. For LC_{25}, the growth rate of this snail group was significantly lower than that of control group up to the 4th week of experiment (79.1%), as they died at 5th week.

3.2.3 Survival rate of adult B. alexandrina

The survival rate of adult *B. alexandrina* snails exposed to LC_0 (4 mg/l) CuO NPs was slightly affected, being 0.8 at the 4th week of exposure. Thereafter, through the recovery period of four weeks, the snails survived till the end of the experiment, as their L_x values were 0.25, compared to 0.80 for the control group. Also, exposure of snails to LC_{10} (15.6 mg/l) considerably reduced their survival rate (L_x) to be 0.38 at the 4th week of exposure compared to 0.91 for the control group. This group died

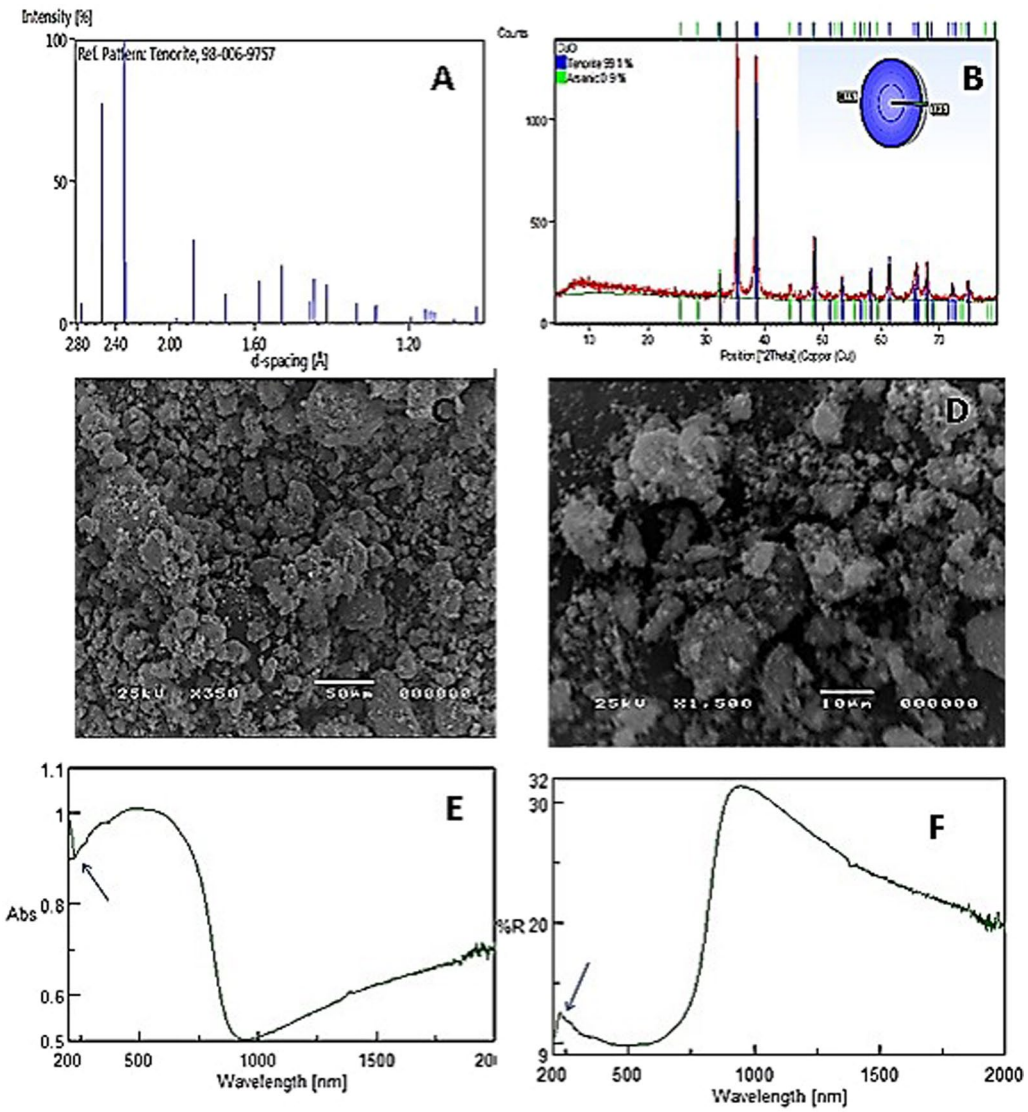

Fig. 1 Characterisation of CuO NPs: **A**, **B** XRD pattern, **C**, **D** TEM image (X 200,000), **E**, **F** UV–visible spectrum of CuO NPs and the narrow peak indicate the small size of the particles. Analysis was performed from the stock solution

Table 1 Molluscicidal activity of CuO NPs against adult *B. alexandrina* snails after 24 h exposure

Nanomaterial	LC_0	LC_{10}	LC_{25}	LC_{50}	LC_{90}	Slope
CuO NPs	4	15.6	27.18	40.0	64.3	1.2

at 7th week of recovery. Rising the concentration to LC_{25} (27.18 mg/l), a quick and severe death of treated snails through the first 4 weeks of the experiment as their L_x was 0.25, then these survived snails could not tolerate treatment as they died by the 5th week of the experiment (Table 3, Fig. 4A).

3.2.4 Reproductive rate (R_O) of B. alexandrina

Copper oxide NPs revealed that the reproductive rate of treated snails exposed to the tested concentrations was extremely highly suppressed ($p < 0.001$) in comparison to the control group. At LC_0, the reproductive rate (R_o) was 4.507 with 88.8% reduction than control group (40.266).

Table 2 Survival rate (%) and mean shell diameter (mm) of juvenile *B. alexandrina* snails exposed to the sub-lethal concentrations of CuO NPs for 24 h/ week for 4 successive weeks followed by 4 weeks of recovery

Weeks	Control		LC_0 (4 mg\l)		LC_{10} (15.6 mg\l)		LC_{25} (27.18 mg\l)	
	% Survival	Shell diameter ± SD	% Survival	Shell diameter ± SD	% Survival	Shell diameter ± SD	% Survival	Shell diameter ± SD
0	100	2.41 ± 0.37	100	2.41 ± 0.37	100	2.41 ± 0.37	100	2.41 ± 0.37
1	99	2.7 ± 0.21	90	2.6 ± 0.19	82	2.4 ± 0.1	75	2.6 ± 0.2
2	98	2.85 ± 0.13	87	2.8 ± 0.21	70	2.5 ± 0.2	50	2.7 ± 0.2
3	96	3.4 ± 0.12	80	2.9 ± 0.13	45	2.7 ± 0.21	25	2.8 ± 0.17
4	95	3.9 ± 0.12	70	3.1 ± 0.11	35	2.8 ± 0.23	5	2.9 ± 0.1
5	94	4.3 ± 0.3	50	3.5 ± 0.13	18	3.2 ± 0.12		
6	90	4.9 ± 0.35	25	3.8 ± 0.1	5	3.3 ± 0.13		
7	85	5.9 ± 0.15	15	4.1 ± 0.15				
8	85	6.5 ± 0.12	5	4.3 ± 0.12				
Mean	4.3 ± 1.38		*3.38 ± 0.14		*2.8 ± 0.98		*2.7 ± 0.67	
Growth reduction % at 1st 4 weeks	47.7%		55%		86%		79.1%	
Growth reduction% at 2nd 4 weeks	52.29%		87%		96%			

*Significant compared to control at $p < 0.05$

Table 3 The survival rate (L_x) and fecundity (M_x) of adult *B. alexandrina* snails exposed for 24 h/ week to the sub-lethal concentrations of CuO NPs for 4 weeks followed by 4 weeks of recovery

Weeks	Control			LC_0 (4 mg\l)			LC_{10} (2.417 mg\l)			LC_{25} (27.18 mg\l)		
	L_x	M_x	$L_x M_x$	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	3.96	3.96	1.00	3.96	3.96	1.00	3.96	3.96	1.00	3.96	3.96
1	0.99	1.13	1.11	0.96	0.75	0.72	0.88	0.15	0.132	0.81	0.11	0.089
2	0.98	2.70	2.64	0.90	0.41	0.369	0.76	0.42	0.319	0.73	0.1	0.073
3	0.95	4.55	4.32	0.85	0.28	0.238	0.55	0.21	0.115	0.36	0.1	0.036
4	0.91	9.5	8.64	0.80	0.6	0.48	0.38	0.21	0.079	0.25	0	0
5	0.90	4.3	3.87	0.70	2.8	1.96	0.25	0	0			
6	0.85	5.6	4.76	0.5	0.33	0.165	0.07	0	0			
7	0.85	8.7	7.39	0.3	0.60	0.18						
8	0.80	9.4	7.52	0.25	0.46	0.115						
$R_o = \Sigma L_x M_x$	40.266			4.507***			0.645***			0.198***		
Reduction %				88.8			98.3			99.5		

***Highly significant from control at $p < 0.001$, compared to control

Also, the R_o values of snails treated with LC_{10} and LC_{25} were 0.645 and 0.198, respectively, compared to 40.266 for control one (Table 3, Fig. 4B).

3.2.5 Larvicidal activity of CuO NPs

a- Mortality rate of miracidiae: CuO NPs exhibited a larvicidal activity, where, after 20 min of exposure of miracidiae to CuO NPs LC_{10}, moderate mortality rates of *S. mansoni* miracidia were observed (40%), while the miracidial mortality rate for LC_{25} was 75%, compared to 5% for the control group. Furthermore, prolonging miracidial exposure to LC_{10} and LC_{25} concentrations resulted in 100% mortality after 60 and 50 min, respectively, compared to 20 and 45% for the control group. Increasing the concentration to LC_{50} and LC_{90} induced severe and rapid mortality of treated miracidia during short exposure times, with a 100% death rate after

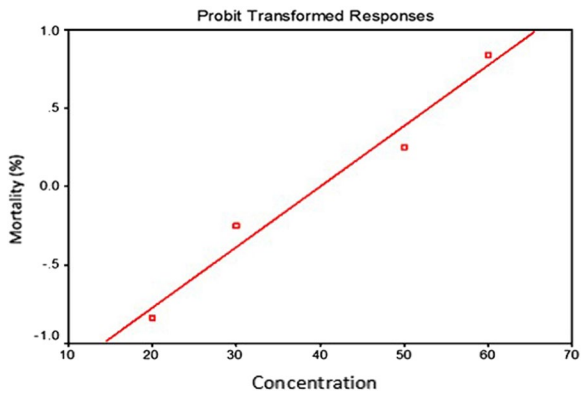

Fig. 2 Molluscicidal activity of CuO NPs (mg/l) against adult *B. alexandrina* snails after 24 h of exposure under laboratory conditions

10 min at the LC_{90} concentration and 15 min at the LC_{50} concentration (Table 4, Fig. 5A).

b- Mortality rate of cercariae: The mortality rate of cercariae increased with increasing the concentration of CuO NPs and the time of exposure. After 30 min of exposure to LC_{90}, and 50 min for LC_{25} and LC_{50}, 100% of cercariae die, while after exposure to LC_{10} for 50 min, the death rate of cercariae was 45% compared to 10% of control group and 100% death was after 90 min of exposure compared to 75% of control group (Table 5, Fig. 5B).

3.3 Microscopic examination

Scanning Electron Microscopy (SEM) studies of the head-foot region of control *B. alexandrina* snails showed normal manner with a smooth tegmental surface and conspicuous microvilli. The tentacles have a flat surface with fine cilia, and the mantle has a smooth tegmental surface (Fig. 6A, B). After exposure of *B. alexandrina* snails to LC_{10} 15.6 mg\l, the tentacles have rough folds with erosion at their apex. The tegmental surface of the mantle became turgid, rough, blebbing, peeling, and tortuosity (Fig. 6C, D). At LC_{25} (27.18 mg\l) mantle is ruptured, nipples appeared, and tegmental surface showed erosion and tortuosity. Also, tentacles are ruptured with rough folds and becoming more tortuose (Fig. 6E, F).

4 Discussion

Metal oxide nanoparticles, such as copper oxide nanoparticles (CuO NPs), have gained great interest among these nanomaterials due to their antibacterial, anticancer, antiprotozoal anthelmintic agents and antioxidant efficiency [33, 34]. The current study found that CuO NPs exhibit molluscicidal activity on adult *Biomphalaria alexandrina* snails with LC_{50} (40 mg/l). The calculation of LC_{50} value is critical because it aids in determining the safe amount or tolerance threshold of any contaminant [35]. These findings are consistent with those of Ganesan et al. [36], who discovered that CuO NPs was toxic to the freshwater crustacean *Daphnia magna* with LC_{50} values ranging from 0.06 to 9.80 mg/l. Also, Abd El-Atti et al. [37] validated the toxicity of CuO NPs on the crayfish *Procambarus clarkii*, finding that mortality rates were 0%, 6.7%, and 36.7% after exposure to 25, 125, and 250 mg/l of CuO NPs, respectively. Svobodová et al. [38] attributed these mortalities to the direct harmful effects of these nanoparticles on gill epithelium, which resulted in hypoxia and osmoregulatory stressors. The present study stated that exposing *B. alexandrina* juvenile snails to CuO NPs at concentrations of LC_0, LC_{10} and LC_{25} is dramatically reduced their survival and growth rates

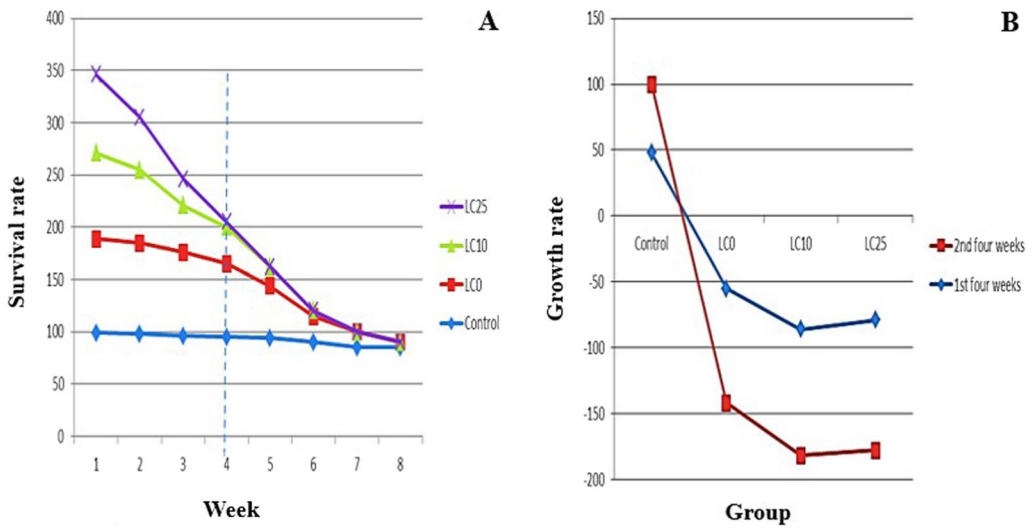

Fig. 3 Histogram showing; **A** The reduction percent in the survival rate; **B** The percent reduction in the growth rate of juvenile *B. alexandrina* snails exposed to the sub-lethal concentrations of CuO NPs for 24 h/ week for 4 weeks followed by 4 weeks of recovery

Table 4 Effect of the sub lethal concentrations of CuO NPs on *Schistosoma mansoni* miracidia

Concentration (mg\l)	% cumulative mortality of miracidia after the following intervals (min)							
	5	**10**	**15**	**20**	**30**	**40**	**50**	**60**
LC_{10} (15.6)	5	20	30	40	75	85	95	100
LC_{25} (27.18)	25	35	60	75	85	100		
LC_{50} (40)	35	75	100					
LC_{90} (64.3)	80	100						
Control	0	0	0	5	12	17	20	45

Table 5 Effect of the sub lethal concentrations of CuO NPs on *Schistosoma mansoni* cercariae

Concentration (mg\l)	% cumulative mortality of cercariae after the following intervals (min)							
	15	**30**	**40**	**50**	**60**	**70**	**80**	**90**
LC_{10} (15.6)	5	15	30	45	65	75	90	100
LC_{25} (27.18)	25	45	65	100				
LC_{50} (40)	30	65	95	100				
LC_{90} (64.3)	65	100						
Control	0	0	5	10	20	45	65	75

compared to the control group, and this reduction was concentration dependent. These results are in accordance with Perreault et al. [39] who displayed that CuO NPs inhibited the development of *Lemna gibba* due to the release of copper ions from the NPs in the media. Also, Wu et al. [40] indicated that CuO NPs could considerably reduce the algal growth rate, *Daphnia magna* survival, and zebra fish hatching and attributed this toxicity to the combined actions of both soluble Cu ions and CuO NPs (Fig. 3).

The present results showed that the survival rate (L_x) of adult *B. alexandrina* snails was markedly reduced post their exposure to sublethal concentrations (LC_0, LC_{10} or LC_{25}) of CuO NPs compared to the control group. Likewise, Shin et al. [41] who stated that copper induces reduction in survival rate of the bivalve *Tegillarca granosa*. Similarly, Croteau et al. [42] demonstrated the toxicity of CuO NPs on *Lymnaea stagnalis* (Fig. 4).

Also, the reproductive rate (R_o) and fecundity (M_x) of adult *B. alexandrina* snails were significantly decreased. This agrees with findings of Ibrahim and Ghoname [43] who attributed this decline to severe histological alterations in the snail's hermaphrodite gland cells and their findings were supported by lower testosterone and estradiol concentrations in the snails' tissues. In a similar line, Pang et al. [44] showed that nano-CuO had a negative impact on the reproduction of the deposit-feeding snail, *Potamopyrgus antipodarum*. Similarly, Azzam et al. [45] revealed that both *B. alexandrina* and *B. truncatus* snails subjected to sub lethal doses of lupine extracts NPs and copper sulphate NPs did not lay any egg masses following treatments. Sakran and Bakry [46] also discovered that persistent exposure to Bayluscide and copper sulphate completely suppressed the fertility of *B. alexandrina* snails. The inhibition of egg laying production after exposure to some metals may result from the tested metals' actions on steroid hormones [47].

According to the current results, CuO NPs have miracidicidal and cercaricidal effects against *S. mansoni* larval stages and these activities were concentration and time dependent. Di Giulio and Hinton [48] stated that the concentration– response correlation may reflect the link between the quantity of chemical pollutant and the degree of organism response. Exposing *S. mansoni* larval stages to LC_{25} of CuO NPs resulted in 100% mortality after 50 min, compared to 20% and 45% for the control group. Increasing the concentration to LC_{50} and LC_{90} induced severe and rapid mortality of treated miracidia during short exposure times, with a 100% death rate after 10 min at the LC_{90} concentration and 15 min at the LC_{50} concentration. While the mortality rate of cercariae increased with increasing the time of exposure, after 30 min of exposure to LC_{90}, and 50 min for LC_{50}, 100% of cercariae die. This is in accordance with Kovrižnych et al. [49] who demonstrated that CuO NPs were acutely lethal to zebra fish embryos at LC_{50} value 960 mg/l and reasoned this toxicity to the released Cu ions from the CuO NPs which accumulated in the zebra fish embryos

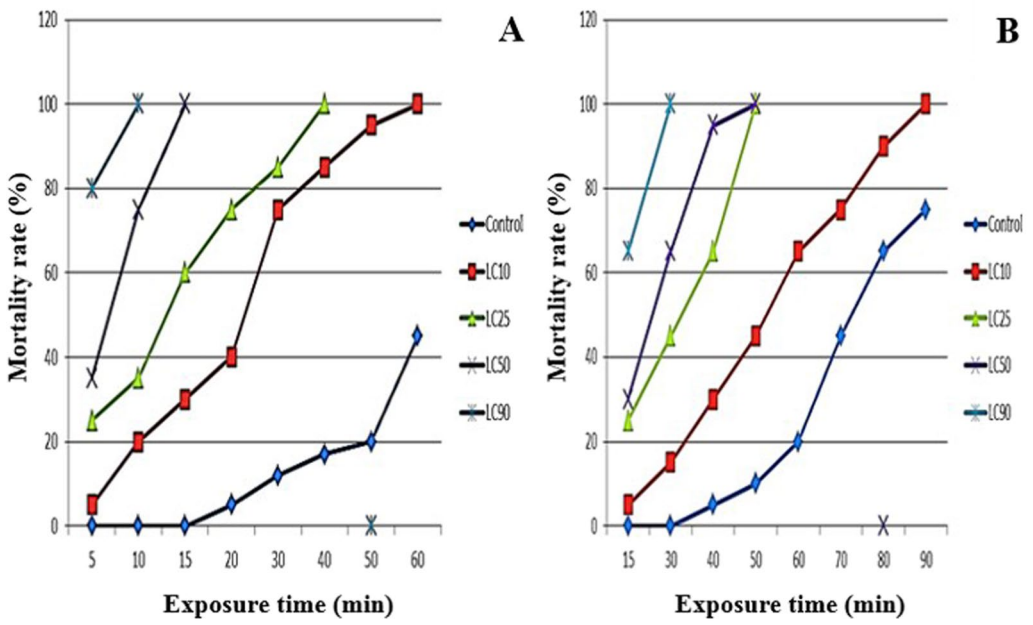

Fig. 5 Histogram showing mortality rate (%) of; **A** *Schistosoma mansoni* miracidia; **B** *Schistosoma mansoni* cercariae post exposure to CuO NPs sub lethal concentrations

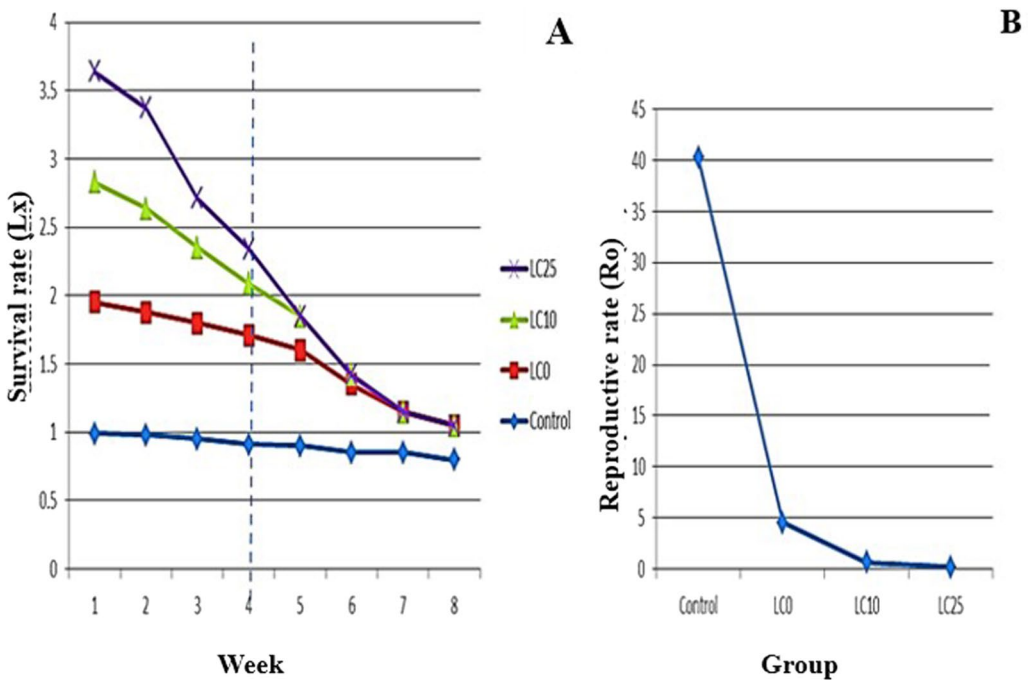

Fig. 4 Histogram showing; **A** The survival rate (L_x). **B** The reproductive rate (R_0) of adult *B. alexandrina* snails exposed to the sub-lethal concentrations of CuO NPs for 24 h/ week for 4 weeks followed by 4 weeks of recovery

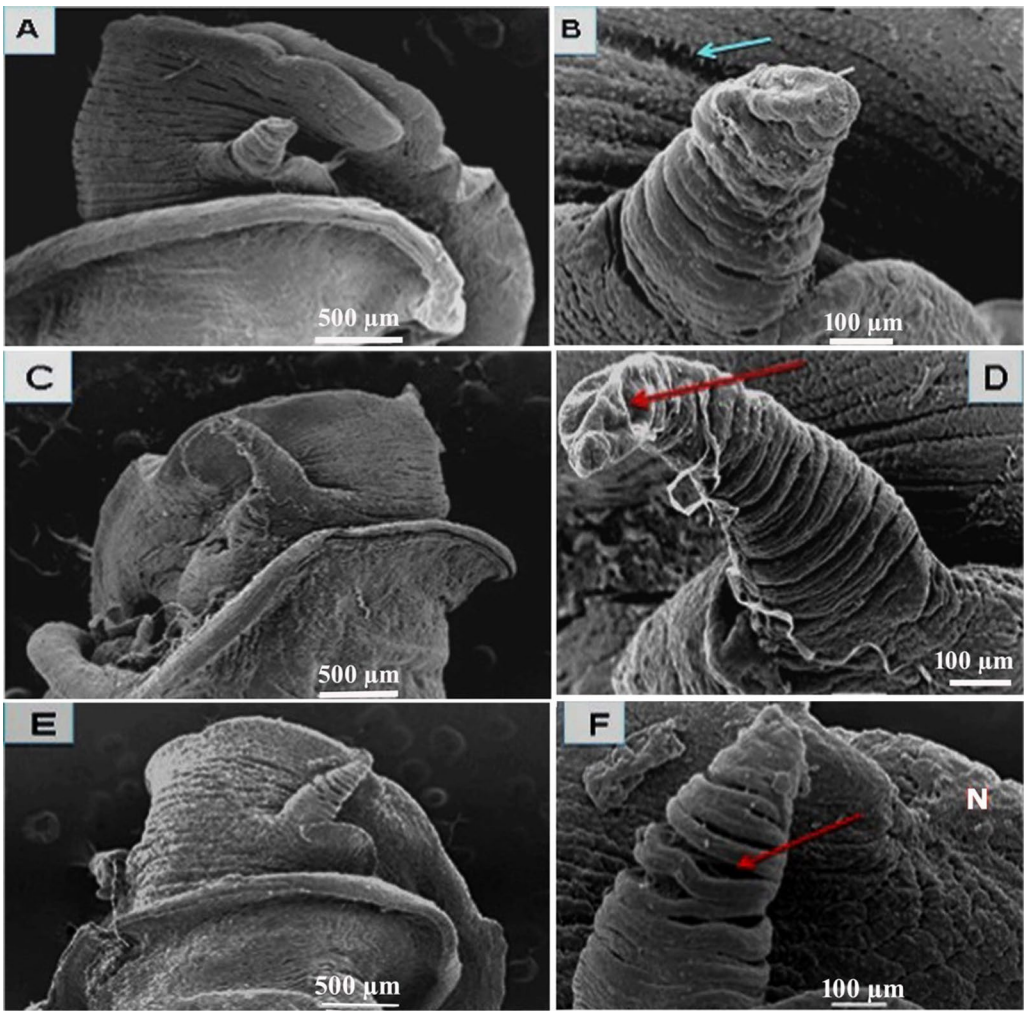

Fig. 6 Scanning electron micrographs of *B. alexandrina* snails (soft parts) showing **A** Control snails displaying normal ultrastructure of head-foot region with smooth tegmental surface of mantle and conspicuous microvilli and the tentacle with fine cilia **B** Higher magnification of the tentacles with a smooth surface and fine spines in the tegmental surface of mantle (the arrow). **C**, **D** Snails exposed to LC_{10} (15.6 mg/l) of CuO NPs showing **C** Mantle became turgidity, rough, blebbing, peeling, and tortuosity. **D** Tentacles was rough with erosion at apex (the arrow). **E**, **F** Snails exposed to LC_{25} (27.18 mg/l) of CuO NPs showing **E** Ruptured and peeling mantle with rough tegmental surface **F** Tentacles became more rough with erosion of its folds, tortuosity (the arrow) and presence of nipples (N)

causing oxidative stress. Also, Sun et al. [50] revealed hepatotoxicity and neurotoxicity in zebra fish eggs and larvae after short-term exposure to CuO NPs at high concentrations. Furthermore, Ebodi and Ahmed [51] showed that cercariae were more resistant to *Randia nilotica* fruit extract as a molluscicidal agent than miracidia. El-Deeb et al. [52] further claimed that the difference in mortality rates between the two larval stages appears to be due to the chemical structure of the tested agents rather than the biological character of these larvae (Fig. 5).

Scanning electron microscopy is an effective method for assessing the effects of environmental stressors on the biological structures of aquatic species [53]. In the current study, the ultrastructure of the head-foot region of *B. alexandrina* snails examined by SEM, displayed normal topography such as a smooth tegmental surface, noticeable microvilli, and tentacles with a flat surface and fine cilia. In contrast, exposing *B. alexandrina* snails to sublethal doses of CuO NPs caused several morphological changes on the snails' outer surface. Tentacles exhibit rough folds with erosion at their apex, and the mantle's tegmental surface has turgidity, roughness, blebbing, peeling, and tortuosity. Furthermore, the mantle is burst and showed nipples emerging on its tegmental surface with erosion, tortuosity, and tentacles have ruptured with rough folds, becoming more tortuose as CuO

NPs concentrations increase. Similarly, Moëzzi et al. [54] revealed ultra-morphological changes in the gills of the swan mussel *Anodonta cygnea* following CuO NPs exposure. Also, Heinlaan et al. [55] observed ultrastructural alterations in the midgut epithelium of *Daphnia magna* after exposure to CuO NPs. Finally, Attia et al. [56], Rasel et al. [57] and Ibrahim et al. [58] concluded that these nanomaterials modifications had an effect on the membrane structures and macromolecules of treated snails, resulting in their mortality (Fig. 6).

5 Conclusions

Copper oxide nanoparticles have the potential to be an effective molluscicide against *B. alexandrina* snails, the intermediate host of *S. mansoni*. As a result, more research is required to determine the best strategy for using such tested agents to reduce schistosomiasis while limiting water pollution and protect non-target species.

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FA, MF and AI have provided guidance during development of idea and MF, HH and AI prepared different figures required, MF and AI wrote and revised the manuscript. All authors read and approved the final manuscript.

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