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Improvement molluscicidal activity of *Anagalis arvensis* extracted by copper oxide nanoparticles against *Biomphalaria alexandrina* snails

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ABSTRACT

Nanotechnology has received more attention in the field of control of intermediate hosts. In the present study, copper nanoparticles were fabricated and characterized using scanning electron microscopy (SEM), transmission electron microscope (TEM), x-ray fluorescence (XRF) and fourier transform infrared (FTIR). Improvement of the molluscicidal activity of *Anagalis arvensis* ethanolic extract was made using CuO NPs by forming plant nanocomposite (ACuO NC). The results showed that ACuO NC has high molluscicidal activity against *B. alexandrina* snails; recording LC_{50} and LC_{90} were 1.76 and 3.16 ppm, respectively. Also, biochemical parameters (Total protein, ALT, AST and ALP) revealed highly significant changes in treated snails with sublethal concentrations (LC_{10} and LC_{25}) of ACuO NC. However, histopathological changes occurred in the hermaphrodite glands of snails exposed to these concentrations of nanocomposite were detected, where, the ova and sperms degenerated and there were losses of connective tissues between acini. ACuO NC was more safety when tested against the biological indicator *Daphnia magna*. These observations prove that ACuO NC has potent molluscicidal activity against the intermediate hosts of *Schistosoma mansoni* with more safety for aquatic environment.

INTRODUCTION

Schistosomiasis is a widespread neglected tropical parasitic disease transmitted by snails (WHO, 2017). Freshwater snails of *Biomphalaria* genus are the intermediate hosts of *Schistosoma mansoni* in Egypt (Ibrahim and Abdalla, 2017). It has been long documented that the most important trematode disease of man is schistosomasis, also called Bilharziasis after the German physician Theodor Bilharz who discovered the causative organisms of the disease in Cairo more than 140 years ago (Bilharz, 1853).

Human infection with *Schistosoma mansoni* is closely related to the existence of its intermediate host of the genus *Biomphalaria alexandrina* snails. The distribution of these snails extended from the Nile Delta and present throughout the country along the tributaries of the Nile (Abou-El-Naga *et al.*, 2011).

There are several strategies have been used to control snail populations through breaking the life cycle (El-Ghany and El-Ghany, 2017). Manufactured molluscicides is an imperative part in the incorporated schistosomiasis control programs (Abdel-

Ghaffar *et al*., 2016), but due to they have high cost and being poisonous to creatures of land and water (WHO, 2014), have stimulated interest to find suitable plant molluscicides (Elsareh *et al*., 2016).

The molluscicidal activity of some plants extracts not only control the vector snail but they would also seem to be less expensive, readily available, and rapidly biodegradable and have low toxicity to non-target organisms (Adewumi *et al.*, 2013).

Nanotechnology is the science of manipulating matter at a very small, molecular scale where dimensions and tolerances of less than 100 nanometres. It has recently been regarded as a promising field of high technologies capable of covering many vitally important spheres of human activities. The development of nanotechnology is ahead of the assessment of their impact on the environment, plants, animals and humans. Moreover, the currently available data are variegated and contradictory (Andrievsky *et al.,* 2005; Oberdorster *et al.*, 2005; Oberdorster *et al,* 2007; Wang *et al.,* 2017).

Nanomaterials have unique properties compared with their larger counterparts. Due to small size and hence higher specific surface area of the nanoparticles, these can easily bind with and transport toxic pollutants. The synthesis of nanoparticles takes place for many applications in various fields of science, technology, medicine, drug delivery, health impacts, personal care applications (Shi *et al.*, 2002; Sun *et al.,* 2018).

The effect of engineered nanoparticles on aquatic organisms and the issues of penetration and accumulation of them in the body of aquatic organisms and their toxic effect, biotransformation, and migration along food webs are considered. It is demonstrated that the behaviour of nanomaterials in the environment and their effect on living organisms have been studied insufficiently and require close attention because their release into the environment will increase in the very near future (Krysanov *et al.*, 2010).

The potential impact of NPs on aquatic ecosystems has attracted special attention due to their unique physicochemical properties (Wiench *et al*., 2009; Ali *et al*. 2012; Sales, 2013). Ecotoxicological studies on NPs are more limited, with only a few reports on the acute toxic effects of NPs on aquatic organisms (Park and Choi, 2010; Ma *et al*. 2013). Freshwater snails are an ecologically important species because they serve as sensitive biomarkers of aquatic ecosystem pollution (Jagtap *et al*., 2011).

There are many nanoparticles show significant effect as molluscicides such as zinc oxide and copper oxide nanoparticles. The potential impacts of ZnO NPs on aquatic ecosystems have attracted special attention due to their unique properties. It was designed to evaluate the effects of ZnO NPs on freshwater snail *B. alexandrina*. ZnO NPs showed molluscicidal activity on *B. alexandrina* snails, it elicited a significant decrease in the different behaviors of treated snails. These changes in behavior would potentially impact the snail's ability to survive in the field (Habib *et al.*, 2016).

Fahmy *et al.* (2014) studied the highlights potential of ecological implications of ZnO NPs release in aquatic environments .Moreover, copper oxide nanoparticles have been used as molluscicides which have significant effect on the snail's ability to survive in the field (Handy *et al.*, 2008; Nowack, 2009).

In the present study, testing the efficacy of synthesized CuO NPs in improvement the molluscicidal activity of ethanolic extract of *Anagallis arvensis* plant extraction was carried out.

MATERIALS AND METHODS

Material and methods

Preparation of ethanolic *A. arvensis* extract

Anagalis arvensis was collected from Orman garden, Giza governorate during full growing spring season according to (WHO, 1965). The ethanol extract was prepared by soaking 250 g of dry plant powder in 1000 ml of the ethanol in a conical flask. The mouth of the flask was covered with aluminum foil and incubated in an oven for 24 hours. After incubation time, the extract was obtained by a filtered solution using whatman filter paper (No.1). The solvent was evaporated using a water bath temperature at 50°C and kept in an oven at 50°C to dry completely. Finally, the residues were collected and the extract was stored in dry clean dark glass beaker till use (Bakry *et al*., 2002).

Preparation of copper oxide NPs

The copper oxide nanoparticles were prepared by precipitation method using copper sulphate as starting material. Sodium hydroxide solution (2M) was added to copper sulphate solution (1M) drop by drop touching the walls of the vessel under constant stirring (160 rpm) on a magnetic stirrer, (JSHS-180, Korea) for 2 hours. The solution was allowed to settle overnight, and then filtrated carefully through whatman filter paper (No. 1). The precipitate was washed several times using distilled water. CuO NPs were dried in an oven at 50°C overnight (Manyasree *et al*., 2017) (Fig. 1).

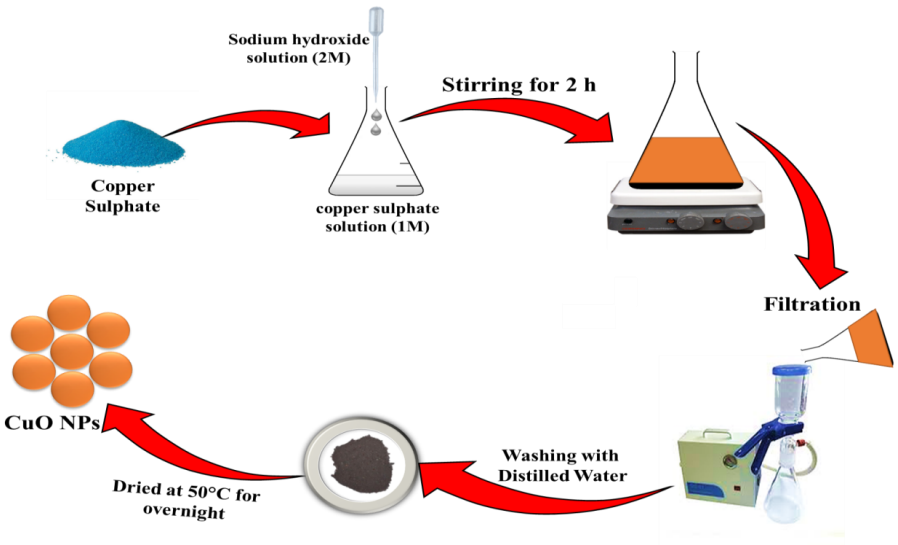

Fig. 1: Schematic diagram of copper oxide nanoparticles synthesis.

Characterization of CuO nanoparticles and plant nanocomposite

Prepared nanomaterials were characterized with the help of multiple techniques. Morphology of the nanoparticles was studied using scanning electron microscopy (SEM) analysis. Samples were placed on SEM grids coated with gold palladium on a pumped-rotary sputter coater Q150R ES (Quorum Technologies Ltd., USA). SEM was carried out on a JEOL JSM-7600F electron microscope (JEOL Ltd., Tokyo, Japan) at a voltage of 2.0 kV. This method is considered to examine each particle, including the aggregate particles (Lee *et al*., 1996).

The particles size of the resulting nanoparticles was analyzed using a transmission electron microscope (TEM) (JEOL Ltd, USA) connected to a high

resolution imaging system. Samples for TEM studies were prepared by placing drops of nanoparticles solutions on carbon-coated TEM copper grids.

X-ray fluorescence (XRF) that was performed to detect the main chemical elemental analysis of the minerals that are present in synthesized CuO nanoparticles. XRF measurements were carried out using the JSX-3222 element analyzer.

Fourier transform infrared (FTIR) spectroscopy of nanoparticles and nanocomposites were analyzed using Nicolet iZ10, thermos-Scientific, Waltham via the KBr pressed disc method, in the spectral region of 4000 to 400 cm^{-1} wave numbers.

Fabrication of plant extract nanocomposite

Following the method of (Rostami-Vartooni *et al.*, 2015), 0.2 g of the CuO nanoparticles was added to 35 ml ethanol under condition of heating 40˚C±1 with stirring (200 rpm) for 15 min, then 25 ml Glutaraldehyde solution (25%) was added as cross a linkage agent with vigorous stirring for 6 hours (Hotplate and magnetic stirrer, JSHS-180, Korea). After that, the precipitate was filtered and washed three times with ethanol. The precipitate was put in clean flask, then 100 ml ethanol was added with stirring (200 rpm), after that the ethanolic *A. arvensis* extract was added dropwise, at 40˚C±1. The formed nanocomposite (NC) was filtrated and washed with ethanol three times to remove any unlinked compounds. The nanocomposite dried in an oven at ◦0°C overnight and stored until testing its molluscicidal activity (Fig. 2).

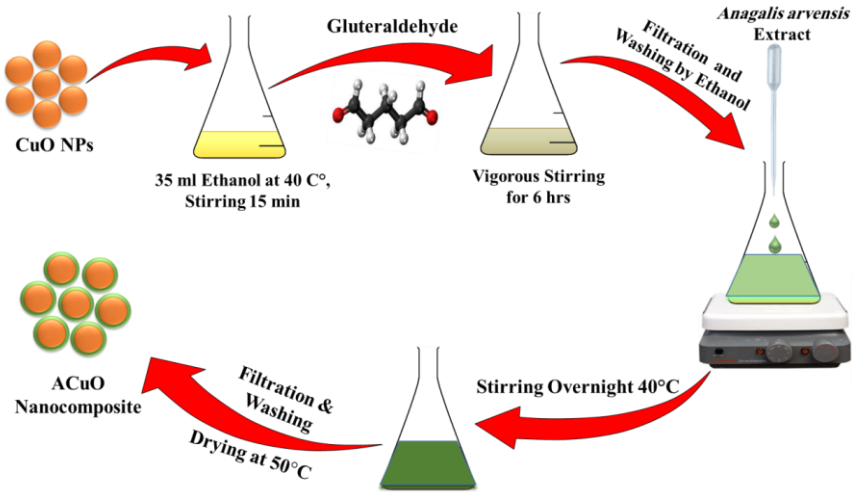

Fig. 2: Schematic diagram of plant–nanocomposite synthesis.

Detection of molluscicidal activity

The snails used in the present work were *B. alexandrina*, the snail intermediate host of *Schistosoma mansoni*, they were collected from canals in Giza governorate, Egypt during the spring season, 2017. Snails kept for four weeks under laboratory conditions before used to accommodate to these conditions. To estimate the lethal doses, series of concentrations expressed in terms of ppm were prepared for *A. arvensis* ethanolic extract, CuO nanoparticle and nanocomposite on the basis of weight/volume using dechlorinated tap water (Borai *et al.*, 2005). Three replicates of one hundred snails were exposed for target concentrations for 24 hours each and then recovered for another same time. Control snails were maintained under the same experimental conditions in dechlorinated water. Snail mortality was confirmed by immersion tested snails in sodium hydroxide solution (20%) (Nolan *et al.*, 1953). The result was calculated as the average of these replicates. The test was carried out at

room temperature using water with pH 7.0 and TDS 235 ppm. The molluscicidal agent tested has been expressed in terms of lethal concentrations values via statistical analysis.

Effect of some environmental factors on molluscicidal activity of ACuO NC

It was necessary to determine the molluscicidal activity of ACuO NC against snails at a different temperature, pH and TDS. The temperature (15, 25 and 30 °C) were adjusted using a water bath (Lemma, 1970). pH concentrations (5, 7 and 9) were prepared using 0.1 M sodium hydroxide solution or 0.1 M hydrochloric acid solution (Salah El-Din, 1999). Different TDS (300, 600, 900 and 1200 ppm) were prepared using sodium chloride solution, pH and TDS values were adjusted using pH and TDS Bench meter (Romania) (Wetzel *et al*; 2001).

Effect of ACuO NC on biochemical parameters of *B. alexandrina*

Biomphalaria alexandrina snails were exposed to LC_{10} and LC_{25} of ACuO NC for two weeks (Bakry, 2009). Three replicates of one hundred snails were exposed for each concentration, hemolymph was collected from these treated groups (each replicate in Eppendorff vial) and another group was maintained in dechlorinated water as control (Sminia, 1972). Hemolymph of the snails were collected by using the techniques described by Nduka and Harrison (1980). The hemolymph was obtained via a small hole made in the shell directly above the heart and capillary tube was inserted then it was drawn into tube by capillary suction. The collected hemolymph was kept in ice for biochemical parameters analysis.

Biochemical parameters were determined spectrophotometrically using kits purchased from BioMerieux and Boehringer 3.1 Mennheim companies. Total protein was estimated according to the method of Bradford (1976). Transaminase enzymes (ALT and AST) were examined according to the method of Reitman and Frankel (1957). Alkaline phosphatase (ALP) was estimated according to the method of Babson (1965).

Histological effect of ACuO NC on hermaphrodite glands of *B. alexandrina*

Snails exposed to LC_{10} and LC_{25} of ACuO NC for two weeks (Bakry, 2009). Three replicates of one hundred snails were prepared for each concentration. Changes in histology of hermaphrodite gland of treated snails compared with control snails were done according to Mohamed and Saad (1990).

Effect of nanocomposites on biological indicator *Daphnia magna*

The effect of ACuO NC on *Daphnia magna* was accomplished by collecting water from natural snail's habitat, transferred to the laboratory and examined microscopically. *Daphnia magna* was cultured in dechlorinated water in plastic containers and feed daily on yeast. Healthy ones were exposed to LC_{10}, LC_{25}, LC_{50} and LC_{90} of ACuO NC. Three replicates of one hundred Daphnia were used for experiment and control (Mostafa, 2006). After exposure time (½, 1, 3, 6. 12, 24, 48, 72 and 96 hours), the mortality rates were recorded.

Statistical analysis

Data were analyzed using statistical package for the social sciences (SPSS, 18). Differences between the untreated and treated samples were tested for significance using a T-test (P value < 0.05 was considered statistically significant).

RESULTS AND DISCUSSION

Characterization nanoparticles

The scanning electron microscope image provides spherical nanoparticles morphology (Fig. 3A). However, TEM image showed that the CuO NPs have average

size 29.0 nm (Fig. 3B). Srivastava *et al*. (2013) have synthesized CuO NPs with an average particle size16 nm that observed from TEM image. Moreover, XRF recorded that 98.5 % of the elemental composition of CuO NPs powder is pure Cu (Fig. 4).

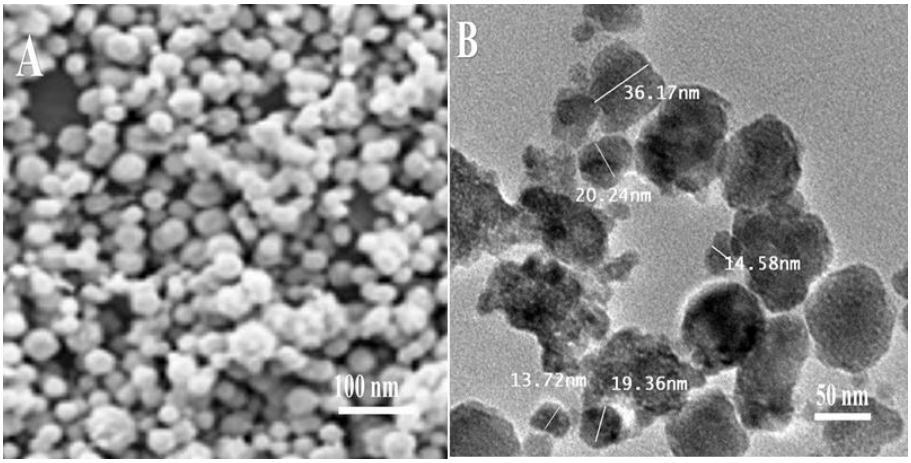

Fig. 3: A) SEM and B) TEM for copper oxide nanoparticles.

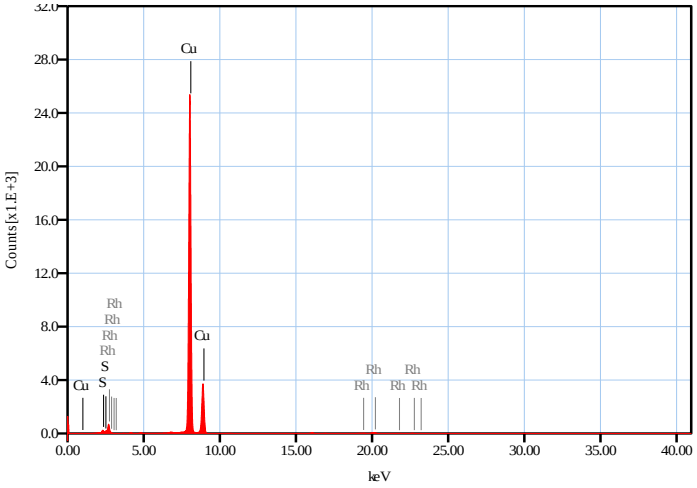

Fig. 4: XRF for copper oxide nanoparticles

The FTIR spectra of ACuO NC composites compared with pure CuO NPs as shown in Figure (5). Data showed wide and stronger absorption bands at 3854 cm^{-1}, which indicated that the hydroxyl (–OH) functional group is present on the surface of NPs and NC. Moreover, the strong link of CuO NPs to amide groups of *A. arvensis* was present. However, the absorption peak at 2926 cm^{-1} was attributed to asymmetric stretching of CH_3 and CH_2 of the plant. While the peaks at 1624–1099 cm^{-1} referred bending vibration of ($–NH_2$) group and (C–O) stretching group. New broad absorption band in the range of 820–494 cm^{-1} was appeared in the FTIR spectra of ACuO NC due to the formation of metal oxygen (Cu–O) bond. Also, the peaks of $–NH_2$, 3'-OH, 5'-OH and C=O groups were found due to hydrogen bonds between plants and NPs as recorded by Li *et al.* (2010).

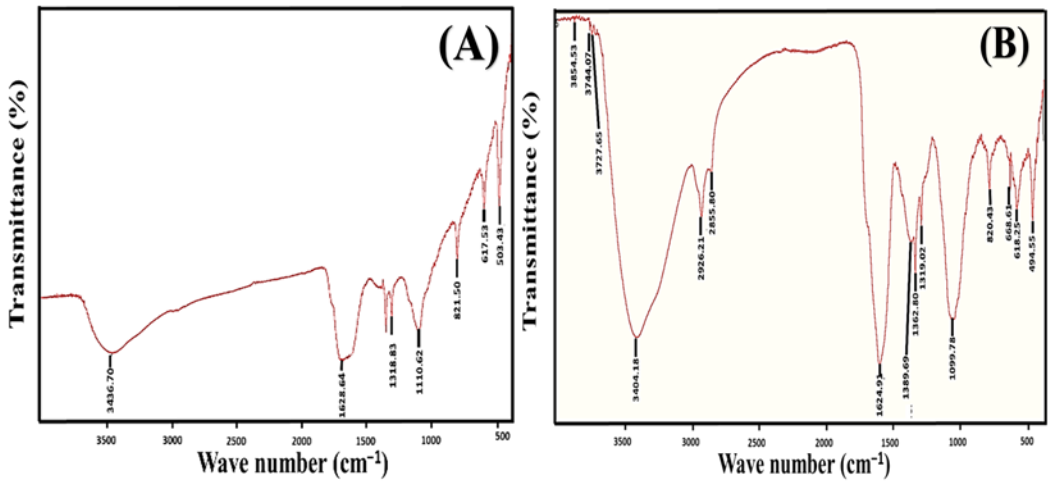

Figure 5: Fourier transform infrared spectroscopy of A) Copper oxide nanoparticles and B) *A. arvensis* – copper oxide nanocomposite.

Molluscicidal activity of ACuO NC

Data from (Table 1) showed that different lethal concentrations of ACuO NC against *B. alexandrina* snails. The results revealed that the molluscicidal activity increased by increasing the concentration. Where, the lethal concentrations LC_{50} and LC_{90} of ACuO NC were 1.76 and 3.16 ppm, respectively (Fig. 7). However, Ibrahim and Ghoname (2018) showed that low molluscicidal activity of the aqueous leaves extracts of *A. arvensis* against *B. alexandrina* snails with LC_{50} and LC_{90} reached to 37.9 and 48.3 ppm, respectively. Also, the present data showed that survival rates of adult *B. alexandrina* snails were markedly reduced which may arise from metabolic disorders as a result of saponine compounds present in the plant extracts or the type of solvent used. In most recent studies, the toxicity of CuO NPs increased when the rate of Cu^{++} ions increased and bioavailability increased in solution (Aruoja *et al.*, 2009; Mortimer *et al.*, 2010).

Table 1: Molluscicidal activity of *A. arvensis* - nanocomposite against *B. alexandrina.*

Tested plant NC	**Lethal concentrations (ppm)**				
	LC_5	**LC_{10}**	**LC_{25}**	**LC_{50}**	**LC_{90}**
***A. arvensis* – CuO NC**	**0.18**	**0.35**	**1.01**	**1.76**	**3.16**

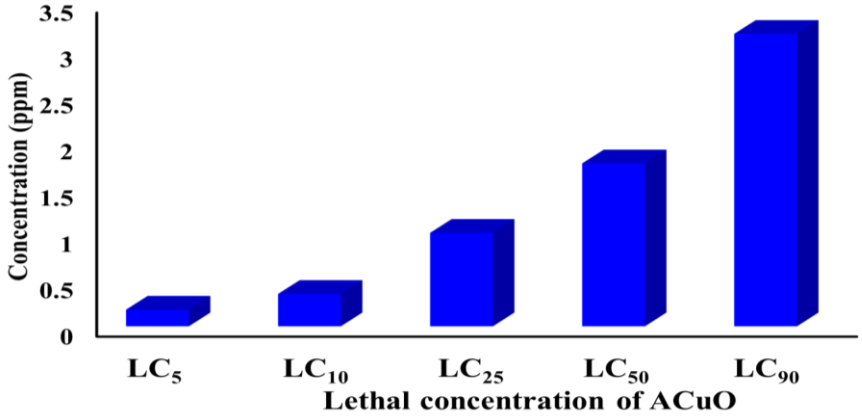

Fig. 7: Lethal concentrations of ACuO NC against *Biomphalaria alexandrina* snails.

Effect of some environmental factors on molluscicidal activity of ACuO NC

Data from Figure (8) revealed that the molluscicidal activity of ACuO NC was increased as the temperature was increased. LC_{50} of ACuO NC was 5.0, 1.76 and 1.0 ppm at 15, 25 and 30˚C, respectively. The snail species were highly sensitive to temperature elevation in which may causes thermal stress on snail and reduces the dissolved oxygen content of the water body (Hofkin *et al*., 1991). Also, El-Emam and Madsen, (1982) observed that the survival of *B. alexandrina* was reduced at temperatures above 33 ˚C and below 10 ˚C.

The data indicated that the molluscicidal activity of ACuO NC increased at acidic pH. LC_{50} of ACuO NC were 0.9, 1.79 and 1.79 ppm, respectively at pH 4, 7 and 9. (Fig. 8). pH 7 showed the most promising results of the nanocomposite. It was found that nanoparticles were more soluble at pH 5 than at pH 7 and 9, suggesting higher toxicity of the nanoparticles at lower pH due to the increased release of M^{2+} ions which indicated that M^{2+} ions are key agents of toxicity (Bondarenko *et al*., 2012). The acidic pH may increase the ability of nanoparticles to penetrate cells and it may prove that toxicity depends on pH due to the increased solubility of nanoparticles in acidic pH. The increased release of M^{2+} in acidic pH may increase the production of reactive oxygen species and affect the mortality of snails (Kaweeteerawat *et al*., 2015).

The molluscicidal activity of ACuO NC decreased as TDS increased, where LC_{50} of ACuO NC was 1.76, 3.07, 7.8 and 20.9 ppm at TDS 300, 600, 900 and 1200 ppm, respectively (Fig. 8). However, Saad *et al.* (2012) indicated that *B. alexandrina* snails were found at wide TDS range of (601-1000 ppm). While, Goncalves *et al*. (2017) indicated that Cu^{2+} dissolution with inverse salinity dependence, the toxic effects observed for CuO NPs might be due to intrinsic toxicity mechanisms related to the nanoform and their aggregation plays a key role in their toxicity.

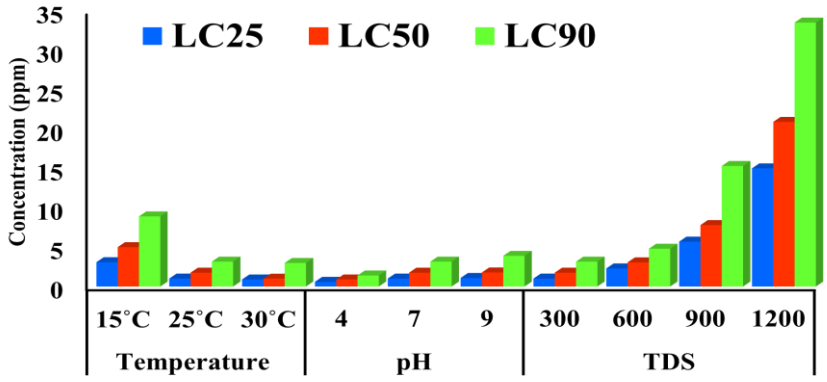

Fig. 8: Effect of temperature, pH and TDS on molluscicidal activity of ACuO NC against *B. alexandrina* snails.

Effect of ACuO NC on biochemical parameters of *B. alexandrina*

Table (3) showed that the total protein contents of the treated snails were affected with LC_{10} and LC_{25} of ACuO NC. The results revealed a significant reduction in total protein content compared with the control group ($p<0.05$). It noticed that the reduction in total protein was increased by raising the concentration of nanocomposite (Fig. 9). This decrease in total protein content might due to the physiological adaptation of the snails to compensate the toxic stress which led to the stimulation of protein catabolism (Hasheesh *et al*., 2011) and determines how various toxic agents affect protein synthesis. This result agreed with result recorded by

Fahmy *et al*. (2014), who showed that the total protein contents in the hemolymph of *B. alexandrina* snails were reduced after their exposure to sublethal concentrations of zinc oxide nanoparticles.

Also, data from (Table 3) revealed that the activities of transaminase enzymes (ALT and AST) and ALP with highly significant elevation in comparison with those of control (p<0.01 and p<0.001) (Fig. 9). These results were in agreement with the past investigation of Abdel Kader *et al*. (2004) who reported severe damages in vital activities of *B. alexandrina* snails when exposed to low doses of synthetic and natural molluscicides.

Table 3: Effect of ACuO NC on some biochemical parameters against *B. alexandrina* snails.

Lethal concentrations	Total protein (g/l)	ALT (U/l)	AST (U/l)	ALP (U/L)
Control	6.00 ± 0.5	77.2 ± 6.3	87 ± 2.8	82.5 ± 5.5
LC_{10}	5.35 ± 0.4	112± 4.٥**	99 ± 3.6**	103 ± 3.9**
LC_{25}	4.57 ± 0.39*	150.7 ± 8.٥***	120.4 ± 3***	127 ± 2.8***

Data is expressed as Mean ± SD. *Significant compared to control at p< 0.05; **highly significant compared to control at p< 0.01; ***Very highly significant compared to control at p<0.001.

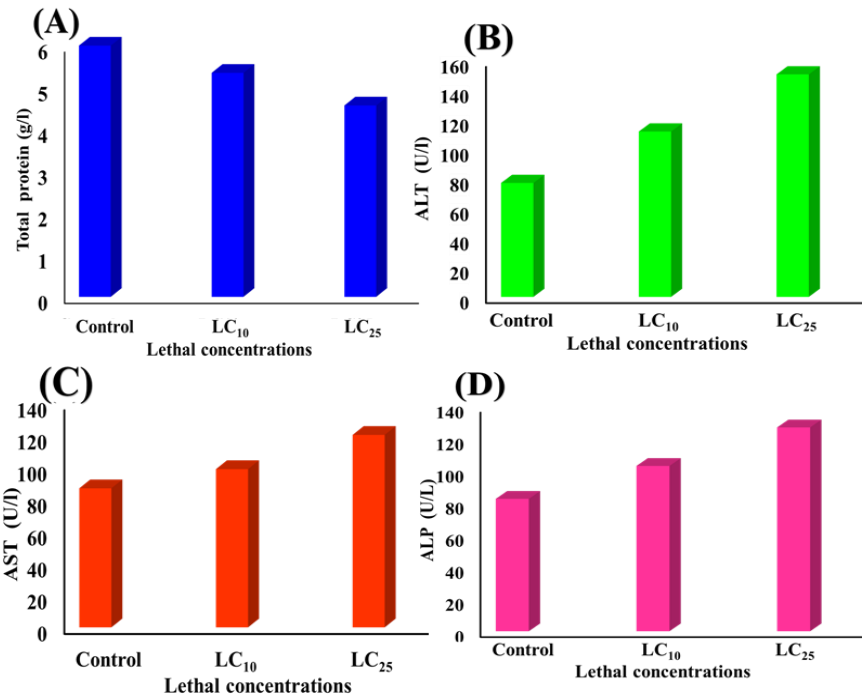

Fig. 9 : Effect of ACuO NC on (A) Total protein, (B) ALT, (C) AST and (D) ALP of treated *B. alexandrina* snails compared with control group.

Effect of ACuO NC on hermaphrodite glands of *B. alexandrina*

The hermaphrodite gland of *B. alexandrina* snails was detected by light microscope, where the normal hermaphrodite gland (control) showed spermatogenesis and oogenesis stages (Fig. 10, A). Snails exposed to LC_{10} of ACuO NC showed moderate changes in the hermaphrodite gland structure. Most of the primary oocytes degenerated and became crumpled. Sperms degenerated and scattered in acinus (Fig. 10, B). While, snails exposed to LC_{25} of ACuO NC suffered from great damage in gonadal cells represented by degenerated mature ova and sperms (Fig. 10, C). These results were in agreement with El-Khayat *et al*. (2018),

who indicated that the hermaphrodite gland of *B. alexandrina* infected treated with the plant extract of *A. arvnsis* showed induced degeneration and disappearance in the gonadal cells with severe deformation, destruction in the reproductive units and severe suppression when evacuations occur in many gonads cells.

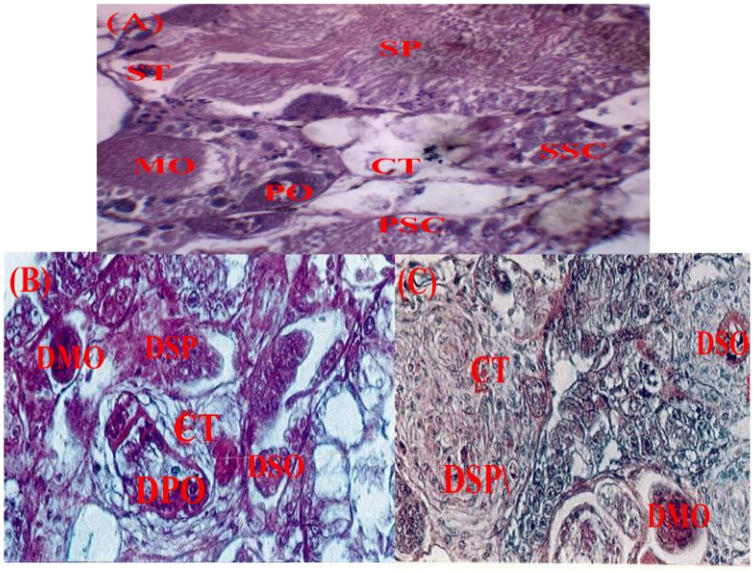

Fig. 10: Light micrograph showing sections in hermaphrodite glands of snails exposed to *A. arvensis* – CuO nanocomposite (×400): (A) normal *B. alexandrina* snails showing connective tissue (CT), mature ova (MO), secondary oocyte (SO), primary spermatocytes (PSC), secondary spermatocytes (SSC), sperms (SP) and spermatids (ST). (B) Snails exposed to LC_{10} of the composite, degenerated mature ova (DMO), degenerated primary oocyte (DPO), degenerated secondry oocyte (DSO) and degenerated sperms (DSP). (C) Snails exposed to LC_{25} of the composite.

3 was used to detect the safety of new molluscicide ACuO NC, where results showed that LC_{10} of ACuO NC caused very lower mortality for *D. magna* by 4% after 96 hours of exposure. However, the LC_{25} recorded 11 % mortality, after the same time. While LC_{50} represented 20 % mortality after 96 hours of exposure. On the other hand, LC_{90} of ACuO NC caused 100% mortality after 4 hours (Fig. 11). Karlsson *et al*. (2008) explained the toxicity of CuO nanoparticles that may be attributed to the release of Cu^{+2} ions. Also, Abdel Kader *et al*. (2003) showed that *A. arvensis* has the least toxic effect on *Daphnia pulex* than *C. micrantha* and Bayluscide.

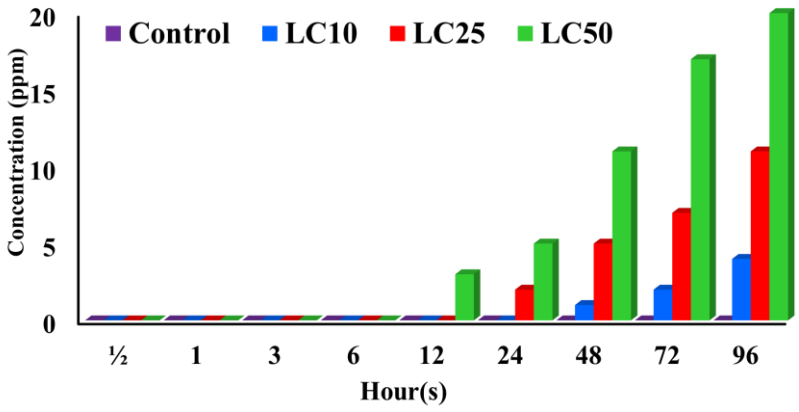

Fig. 11: Effect of Lethal concentrations of ACuO NC on *Daphnia magna.*

CONCLUSION

It can be concluded that the present study succeeded in improving the molluscicidal activity of *A. arvensis* extract fabricated new ACuO agent nanocomposite with promising molluscicidal activity. This new molluscicide has high significant alterations in the biochemical and histopathological aspects in the freshwater snail *B. alexandrina* the intermediate host of *Schitosoma mansoni*. It is advisable to use such nanocomposite in schistosomiasis control planning to limit water contamination as it is inexpensive and environmentally safe, where it showed very high molluscicidal activity with more safety against *Daphnia magna* as an indicator of water contamination.

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ARABIC SUMMARY

تحسين النشاط الإبادى لمستخلص نبات *Anagalis arvensis* باستخدام اكسيد النحاس النانومترى تجاه قواقع *Biomphalaria alexandrina*

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زاد الاهتمام حديثاً بتطبيقات النانوتكنولوجى حيث تم إختبار بعض الجزيئات النانومترية كمبيدات لقواقع العوائل الوسيطة. حيث تم فى هذه الدراسة تحضير اكسيد النحاس النانومترى وتوصيفه بإستخدام المجهر الإلكتروني الماسح (SEM)، المجهر الإلكتروني النافذ (TEM)، الأشعة السينية (XRF) وطيف الأشعة تحت الحمراء (FTIR). يهدف هذا البحث لتحسين النشاط الابادى لمستخلص الايثانول لنبات *الاناجاليس ارفينسيس* باضافة اكسيد النحاس النانومترى لتكوين مركب نباتى نانومترى (ACuO). وقد أظهرت النتائج ان هذا المركب له تاثيرإبادى كبير تجاه قواقع *بيومفلاريا الكسندرينا* حيث كانت قيم التركيزات المميتة (LC_{50}) و(LC_{90}) ١.٧٦ و ٣.١٦ جزء في المليون، على الترتيب. كما تم بيان تأثير التركيزات تحت المميتة للمركب النانومترى على بعض المؤشرات البيوكيميائية مثل البروتين الكلى ونشاط بعض الانزيمات ALT, AST, ALP. وقد اوضحت النتائج تغييرات معنوية كبيرة فى القواقع المختبرة مقارنة بالمجموعة الضابطة. كما أوضحت الدراسة الهستولوجية للقواقع المعالجة بالتركيزات تحت المميتة (LC_{10}) و (LC_{25}) حدوث تغيرات فى الغدد المخنثة للقواقع حيث تحللت البويضات و الحيوانات المنوية و الانسجة الضامة بين الخلايا. كما اكدت النتائج ان هذا المدمج النانومترى اكثر اماناً تجاه *الدافنيا ماجنا*. وتشير هذه النتائج الى إمكانية إستخدام هذا المركب النانومترى (ACuO) فى مكافحة العائل الوسيط لمرض البلهارسيا المعوية لما له من نشاط ابادى قوى وفعال واكثر امناً على البيئة المائية.