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NANOPARTICLE-MEDIATED PATHOLOGICAL ALTERATIONS IN CULEX PIPIENS LARVAE: A HISTOLOGICAL AND BIOCHEMICAL EXAMINATION FOR EFFECTIVE LARVICIDAL STRATEGIES

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ABSTRACT

There is significant vector-borne disease spread worldwide by Culex pipiens. Insect resistance and environmental pollution have been caused by the extensive use of synthetic insecticides. Applications of nanoparticles in insect management are a necessary alternative control strategy. A study was carried out to assess the effects of copper oxide nanoparticles (CuONPs) on the histological and cellular damage in larval Culex pipiens tissues. A regression log concentration-response line was established after 24 hours of treatment to determine the lethal concentrations LC10, LC25, LC50 and LC90. Each concentration was replicated four times with 25 third-instar larvae participating per replicate. As copper oxide nanoparticles (CuONPs) concentrations increased for LC10, LC25, LC50, and LC90, mortality rates increased significantly. These percentages 0.040, 0.099, 0.268, and 1.767 mg/ml were the concentrations, respectively. As a result of this study, levels of cellular damage enzymes There are two major sources of nitric oxide and lipid peroxidase increased significantly, whereas levels of total protein decreased significantly. Among larvae treated with increasing concentrations of lethal CuONPs, a significant increase in albumin concentrations was observed in tissue homogenates. Furthermore, histological studies indicated that the exposed larvae exhibited severe deterioration of their tissue architecture, which increased with an increase in CuONP concentration. Accordingly, CuONPs cause cellular damage to Culex pipiens larvae, deteriorating their histological structure, making them promising and effective larvicidals.

Key words: Vector-Borne Disease, Culex pipiens, Cellular Damage, Larvicidal Effects.

INTRODUCTION

Rift Valley fever, filariasis, and West Nile virus are some of the diseases carried by the culex pipiens family of Diptera. Current mosquito-borne disease control strategies include vector control. However, chemical a method of control that is ineffective is control the target species since it is not selective, leaves residues in the environment, and results in the development of strain resistance. Furthermore, non-target animals may suffer harmful effects, the environment may be negatively affected, and human health may be affected.

Insecticides that are eco-friendly and effective have gained more and more attention throughout the world. Copper oxide nanoparticles (CuONPs) have gained considerable attention over the past several years due to their highly diverse applications in biological research, pharmaceuticals, chemicals, industrial science, and medicine. Oxide copper nanoparticles bind four oxygen molecules with a copper ion at their center. Oxide copper nanoparticles are made of copper and oxygen. Copper-oxide nanostructures are effective in preventing water pollution because they act as organic dye-degradation materials. A catalytic reaction is also known to be carried out by them. A crucial role is played by CuONPs in the prevention of fungi, bacteria, and microbial infections.

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As well as it's a combo of Bacillus subtilis, Staphylococcus aureus, and E. coli, CuONPs inhibit bacterial growth in a variety of ways. Furthermore, CuONPs are considered biocidal and used widely in biomedicine. It has been demonstrated that CuONPs have a high degree of efficacy in biomedical concerns, however, their toxicity is their major drawback. Numerous factors, including Reactive oxygen species are produced more frequently, influence the toxicity of CuONPs in mammalian cells and invertebrates. Oxidative stress produced by these nanoparticles leads to DNA and mitochondrial damage in pulmonary epithelium cells, promoting toxicity. Detection of diseases and viruses that infect humans would be the most promising biomedical applications of CuONPs. For the detection of flu viruses, a smart method has been developed in the study described. Since CuONPs have antimicrobial properties capable of controlling almost all bacteria types, they are currently used as antimicrobial agents in hospitals. In addition to their antifungal properties, CuONPs have a wide range of applications. The fact that they do not irritate or sensitize the skin is of great interest. As long as they are used externally and in low quantities, they are generally safe for humans. As a result, the present study attempts Culex pipiens larvae, in relation to their histological and cellular damage, were investigated and elucidated for their toxic effects by copper oxide nanoparticles.

METHODS AND MATERIALS

It is believed that Culex pipiens larvae exist as follows

A dipping method was used to obtain mosquito larvae from stagnant water around the greater Cairo area. Larvae collected at the Research Institute of Medical Entomology's Mosquito Research Department were identified as Culex pipiens. A lab totally isolated from any source of insecticide exposure maintained Culex pipiens colonies at 29 degrees Celsius, 80 percent relative humidity and 12 hours of darkness and light. Once the larvae have grown into pupae, they are placed on enamel plates, fed yeast granules and rusk powder. After emergence, mosquitoes were allowed to feed on cotton pads impregnated with 10% sucrose solution inside wooden cages with plastic bowls filled with water and cotton pads contained in wooden cages. Pigeon's blood meals were used to provide mosquitoes with the protein they needed to produce eggs. We selected specimens to run the bioassay experiments after hatching eggs and breeding for many generations.

The Sol-Gel method is used to prepare copper oxide nanoparticles:

Deionized water was used to dissolve copper nitrate powder. Nitrate solution of copper was mixed with A drop-by-drop approach to acetic acid and heated to 100°C for one hour. An additional hour of reaction at 100°C was conducted after adding the reaction mixture should be diluted with sodium hydroxide solution. By adjusting the lab oven to 500°C, we obtained a black precipitate that the filter paper used was Whatman. Transmission electron microscopy was used to characterize copper oxide nanoparticles powder.

Testing Copper Oxide Nanoparticles for Detection of SLDs The third instar larvae of Culex pipiens are susceptible:

By using dechlorinated water as diluent, copper oxide nanoparticles were prepared at varying concentrations (0.03, 0.07, 0.1, 0.2, 0.5, and 0.7 mg/ml). Contains each concentration of test solution in a 500ml beaker contained 25 larvae in the third instar. Each concentration was replicated four times. Only dechlorinated water was used for control experiments. After 24 hours of treatment, larval mortality was assessed. Prodding the larva with a fine dowel would indicate that it was dead. A mortality rate of 24 hours after exposure was used to determine lethal concentrations. Lethal concentrations were detected and slope values were obtained via probit analysis. Based on Abbott's formula, control mortality was corrected.

A method for preparing larval homogenates is as follows:

Using the regression log concentrator response lines, larvae were administered sublethal concentrations of copper oxide nanoparticles after 24 hours of incubation. An ultrasonic processor UP 200H was used for homogenizing the non-mortal larvae, and 1 gram of tissue was added to a 5 ml solution of phosphate buffer (PH 7.4) for further processing. After centrifuging at 4000 rpm at room temperature for 45 minutes, the suspension was obtained. In order to determine cellular damage marker enzyme activities, pellets of supernatants were discarded and aliquots of supernatants were used.

Using copper oxide nanoparticles to treat Culex pipiens larvae show reduced levels of total protein and albumin

We analyzed Total protein and albumin levels were determined in step I using larval tissue homogenates by spectrophotometry to assess cellular functions. DiaSys diagnostic systems, Gmbh, Germany, provided instructions for detecting total protein levels. According to Diamond diagnostics' instruction manual, albumin levels were determined in tissue homogenates.

An analysis of Culex pipiens larval enzymes responsible for cellular damage in the presence of copper oxide nanoparticles

A spectrophotometric analysis of Step I: preparation of larval tissue homogenates was conducted to evaluate cellular damage by determining nitric oxide and lipid peroxidase levels. Following the instructions of the BIODIAGNOSTICS kit Egypt, colorimetric measurements

were performed to determine lipid peroxidation levels. Following the instructions included in the A colorimetric method was used to measure nitric oxide in tissue homogenates as per the instructions provided with the BIODIAGNOSTICS kit Egypt.

Studies on histology:

A method based on Picric acid and formalin were used in Bouin's fixative oxide nanoparticles was used to fix Culex pipiens larvae of the third instar. After embedding, sectioning (5-8 m), and staining The fixings were embedded and stained with Delafield's hematoxylin and eosin.

Analyses based on statistics:

Analysis of the data was conducted using SPSS version 25. A mean x standard deviation was used to summarize data in this study. The difference between groups was assessed through analyses of variance (ANOVA) with more than two parameters. When the difference between groups reached p 0.05, it was considered significant.

RESULTS

By estimating LC10, LC25, LC50 and LC90 levels and As shown in the figure below, log-concentration probit lines slope upward after 24 hours, copperoxide nanoparticles (CuONPs) were assessed to determine Culex pipiens larvae are killed by CuONPs due to their larvicidal properties. There was a very high effectiveness of CuONPs against mosquito larvae as demonstrated by the results. A level of 0.040, 0.099, 0.268, and 1.767 mg/ml was determined for the LC10, LC25, and LC50. As steepness increases, slope increases in reverse relation to LC90 / LC50. Copper oxide nanoparticles showed a slope of 0.96 on the efficacy regression line, and a ratio of 6.59 on the Lc90/Lc50.

Table 1: Larval responses to copper oxide nanoparticles in Culex pipiens.

CuONPS Concentration (mg/ml)	No. of larvae Tested	Died	Alive	Mortality%
0.03	100	12	90	12
0.07	100	16	86	16
0.1	100	25	77	25
0.2	100	31	71	31
0.5	100	61	41	61
0.7	100	91	11	91
0.0	100	0.0	101	0.0

Table 2: Copper oxide nanoparticles are effective against Culex pipiens larvae of the third instar at different lethal concentrations

Lc values(mg/ml)				$LC_{90}LC_{50}$	Slope $\pm$ SE
LC_{10} (lower-upper)	LC_{25} (lower-upper)	LC_{50} (lower-upper)	LC_{90} (lower-upper)		
0.040 (0.026 -0.062)	0.099 (0.064 -0.153)	0.268 (0.174 -0.413)	1.767 (1.147 -2.722)	6.60	1.660 $\pm$ 0.096

Among Protein content of Culex pipiens larvae is depicted in Table (3) levels decreased significantly, and albumin levels increased significantly as well as enzymes of cellular damage.

Table 3: CuONPs were administered at different lethal concentrations to Culex pipiens mosquito larvae to measure Nitric oxide levels, albumin levels, and total protein levels

Copper oxide nanoparticles concentrations (mg/ml)	Total protein (g/dL) ± SE	Albumin (g/dL) ± SE	Lipid peroxidation level (nmol/g. tissue) ± SE	Nitric oxide (umol/L) ± SE
LC10 (0.040)	0.0816 ±0.00004**	0.046±0.0004**	24.82±0.004**	16.33±0.004**
LC 25 (0.099)	0.06±0.0040**	0.051±0.0004**	31.41±0.004**	16.83±0.004**
LC50 (0.268)	0.04±0.0040**	0.058±0.0004**	33.33±0.004**	17.82±0.004**
LC90 (1.767)	0.02±0.0040**	0.066±0.0004**	75.44±0.004**	23.26±0.004**
0.00	0.142±0.0004**	0.033±0.0004**	11.32±0.004**	14.85±0.004**

A highly significant value = P 0.001 is represented as mean + SE.

The histological effects of CuONP treatment on Culex pipiens larvae are illustrated as their midgut tissues deteriorated at a rapid rate, which indicated that as CuONP concentrations increased, so did such damages.

DISCUSSION

Anopheles stephensi and Aedes aegypti are three mosquito species are some of the vector diseases studied in recent studies that demonstrate the Copper oxide nanoparticles have larvicidal properties. The CuONPs cellular toxicity is influenced by a number of factors, which influences their usefulness as pesticides. Small particles are more toxic than larger ones because of their surface charge and positive charges, which enhance interaction between CuONPs and cells. pH and temperature of the solution affect the dissolution of CuONPs, as well as the concentration. To prepare CuONPs with dimensions ranging from 10 to 40 nm and ensure proper CuONP size synthesis, the sol-gel method was used in this study. In animal cells, CuONPs may elucidate their molecular toxicity mechanisms by promoting There are three types of DNA damage: mitochondrial, oxidative, and peroxidative. Further, CuONPs have been found to damage cell membranes. At low concentrations, CuONPs can induce cell death due to cytotoxicity. There was a significant increase in An increase in CuONP concentrations will result in a lower mortality rate, a finding that was also described in this study, not only due to the nano-size and structural shape of the particles but also as a result of their concentration. The concentrations of these CuONPs increase in a dose-dependent manner, resulting in an increase in mortality rates due to damaged cell membranes. CuONPs concentrations increased significantly in this study, resulting in significant reductions in total protein levels in Culex pipiens larval tissue homogenates. The surface of CuONPs may be adsorbing cellular metabolites or proteins or CuONPs may interact with biomolecules in the biological media, such as proteins, nucleic acids, phospholipids, nucleic acids and glucolipids. One possible explanation for the decrease in protein levels observed in this study might be this interaction or adsorption. Further, CuONP surfaces may alter the structures and functions of adsorbed proteins, affecting their bioactivity. In fact, CuONPs altered protein conformation and structure, affecting protein-protein interactions downstream as well as DNA transcription as well as cell signaling, which in turn resulted in the loss of enzyme activity, resulting in loss of bio vital functions, and eventually death of the organism, which may also contribute to mosquito mortality. This study showed significant elevations in albumin levels in Culex pipiens larval tissue homogenates. The level of albumin can indicate the viability and functionality of cells and is known to be an important cellular marker. Albumin supports osmotic pressure regulation, antioxidant defenses, and binding and transporting various endogenous and exogenous compounds. Due to its ability to bind tightly to copper and weakly to iron, it exhibits antioxidant properties. Furthermore, it provides thiol groups in pathological conditions and scavenges free radicals. An increase in albumin levels may therefore be an excellent biomarker of increased oxidative protein damage. In the case of cellular stress caused by CuONPs, such as dehydration or oxidative stress, the levels of albumin are likely to increase. Among the most accepted mechanisms for CuONP toxicity are lipid peroxidation and oxidative stress. The surface properties of metal nanoparticles like CuONPs or the elaborated metal ions are likely to be responsible for oxidative stress in these nanoparticles. Using a lipid peroxidase enzyme assay and nitric oxide measurements, we assessed the ability of CuONPs to induce oxidative stress. Lipid peroxidase was elevated in this study, indicating oxidative stress and associated cell damage. A trend towards increased levels of lipid peroxidation was observed upon administration of green silver nanoparticles to Culex pipiens larvae and zinc oxide nanoparticles to Musca domestica larvae. Copper nanoparticles were shown to increase the levels of lipid peroxidase in spleens and livers of rats exposed to CuONPs in accordance with the notion that they were dealing with biological entities. The levels of lipid peroxidase were reported to increase dose-dependently. In addition, CuONP treatment of kidneys of albino rats had demonstrated an increase in lipid peroxidation levels. An increase in nitric oxide levels always correlates with an increase in lipid peroxidase levels. Additionally, copper oxide toxicity is believed to be mediated by lipid peroxidase, nitric oxide levels, and oxidative stress. Moreover, lipid peroxidation and nitric oxide levels were also increased in this study, resulting in oxidative stress and cellular damage. There is agreement between these results.

As a result of CuONP treatment, ROS generation and oxidative stress are enhanced, inflammation is induced, cellular dysfunction is induced, and apoptosis occurs in vivo. Histopathological changes also follow. Studies on Culex pipiens larvae in their third instar revealed brush protrusions encircling midgut epithelial cells indicated severe damage to the midgut. As compared to the Brush in good condition and tight borders in the control sample, the brush border became thin in the midgut epithelial cells in the present study. According to this finding, silver nanoparticles have the ability to damage In comparison to control larvae that weren't treated, the epithelial cells and brush border cells were significantly smaller.

CONCLUSION:

Copper oxide nanoparticles were shown to play a critical role in larvicidal efficacy The third instar larvae of Culex pipiens are resistant in this study. As a result of altering the configurational structure of such nanoparticles,

protein function mechanisms can be altered, tissues become dehydrated, oxidative stress is induced, resulting in cellular damage that eventually leads to the organism's death due to deterioration of the histological architecture. As a result, copper oxide nanoparticles could be used to reduce the prevalence of larvae in larvicidal programs.

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