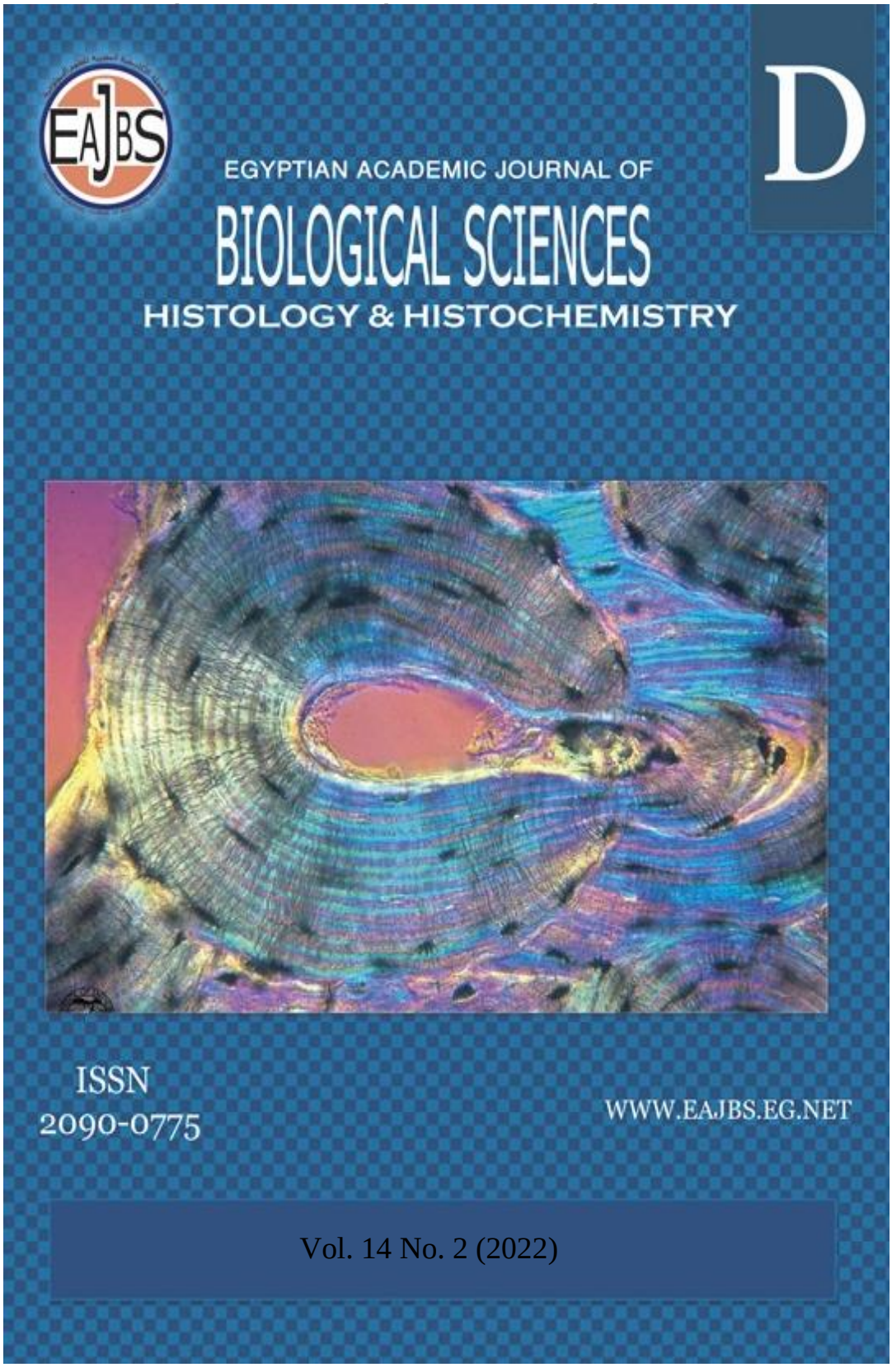
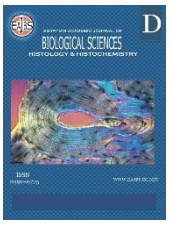


Citation: Egypt. *Acad. J. Biolog. Sci. (D-Histology and histochemistry) Vol.14(2) pp215-219(2022)*
DOI: **10.21608/EAJBSD.2022.277987**

Egypt. Acad. J. Biolog. Sci., 14(2): 215-219 (2022)

Egyptian Academic Journal of Biological Sciences
D. Histology & Histochemistry
ISSN 2090 – 0775
http://eajbsd.journals.ekb.eg



Effect of Silver Nanoparticles on Histological Parameters on Mulberry Silkworms, *Bombyx mori* L. (Lepidoptera: Bombycidae)

Hamed M. Mady[2], Ghazy, M.U. [2], Omer.R.E.M. [1], El-khayat E. F. [1] and El-Shewy A.M. [1]

1-Faculty of Agriculture, Moshtohor, Benha university, Egypt.
2-Plant Protection Research Institute, Agricultural Research Center, Egypt.
E.Mail :Drhamed042@gmail.com

ARTICLE INFO
Article History
Received:20/9/2022
Accepted:26/11/2022
Available:2/12/2022

***Keywords*:**
Mulberry leaf, silkworm, nanoparticles, *Bombyx mori*, histological.

ABSTRACT

The silkworm, *Bombyx mori* being a monophasic insect, derives all nutrients required for its development from mulberry leaves itself. Silkworm feeding is one of the most important major areas of research in sericulture. The distinctive characteristics of nanomaterials have the ability to enhance and development of various sectors including agriculture, industry, cosmetics and medicine. Silver nanoparticles (AgNPs) it is one of the most common nanoparticles that are known as a noble metal, a widely used antibacterial material, and non-antibiotic, and have demonstrated toxic and other potentially adverse effects on mammals. However, the deleterious effects of Silver nanoparticles on insects are still unknown. Here, we researched the effects of silver nanoparticles on the model invertebrate organism Silkworm, *Bombyx mori* L.

The aim of this study was to evaluate the effects of ingesting Silver nanoparticles through food to assess their toxicity effects on the silkworms, After treatment with various concentrations (5,10,50 and 100 ppm), of silkworm and midgut histopathological analysis. Our research showed abnormalities histopathological of midgut-damaged epithelium cells detached from the broken basement membrane, some cells become thicken and large size than control in some areas and the space between the peritrophic membrane layers and the epithelial layer, emptied them cytoplasmic contents.

INTRODUCTION

Silkworm, *Bombyx mori* L. a group of insects, is called the "Queen of textiles" for producing economically significant silk fibre (Soumya *et al*. 2017). Silkworms are an important economic insect; they play a significant part in rural life in many countries. Silkworms produce many silk products of high-quality fibers and silk fabrics of attractive colors. There are many variations for silk production lines and strains. Several factors affect the production of cocoons and raw silk, such as the potential genetic diversity of commercial cultivars, climate, mulberry leaf quality, breeding and management plans, silk reeling techniques, and the quality of silkworm eggs (Bizhannia and Seidavi, 2008). In insects, the midgut is the primary location for food digestion and absorption of digested food it is well-known as acts as a barrier to the different parasitic organisms due to the chemical composition of gut contents, availability of some non-specific surface inhibitors also the creation of a protective peritrophic membrane by the gut epithelium working to protect the food (Tinsley, 1975).

Nanotechnology is a general term for a branch of applied science and technology. Its unifying theme is the control of matter on the molecular and atomic scale (Anima Nanda and Saravanan, 2009). The diameters of nanomaterials range from (1 to 100 nm) and they have unique physical and chemical characteristics. Silver nanoparticles (AgNPs) It is one of the most modern and commercialized nanomaterials, and they have effective antibacterial activity. They are widely used in various fields, such as engineering, chemistry, food packaging, medical devices, communications, and cosmetics (suh *et al*., 2009, seal and kayn, 2014). However, some research has implied that they may be risky. (sekhon 2014, and stensberg *et al*.,2017) Ag nanoparticles (AgNPs) are playing an active role in the field of nanotechnology, water treatment, and sunscreen lotions and nanomedicine.

Silver nanoparticles are a brand name for pure de-ionized water with suspended superfine silver. (AgNPs) size range from (5 to 50nm). Metallic Ag nanoparticles are the primary form of silver. The remaining silver is in ionic form. due to the small particle size, the total silver surface area exposed in the solution is maximized, this results in the biggest impact per unit of silver.

The aim of the study is mainly concerned with the treatment of silver nanoparticles at different concentrations on the histological midgut of Silkworms, *Bombyx mori* L.

MATERIALS AND METHODS

Insect Rearing:

Mulberry of *Morus alba* var Kokuso-27used for feeding silkworm larvae. Chopped leaves were offered to the young silkworm. Silkworm larvae were reared under normal conditions the average temperature was 22.54±1.606 and the relative humidity was 60.27±6.35 Silkworm eggs were acquired from the sericulture Research department, Agricultural Research Center – Giza Egypt.

Sources of Silver Nanoparticles (AgNPs):

The nanoparticles (AgNPs) were obtained from the City of Scientific Research and Technological Application (SRTA-CITY), Borg El-Arab city.

Bombyx mori 5th instar larvae were fed on the following mulberry leaves. The larvae were divided into two groups (control and treated). control group larvae feed with normal mulberry leaves. The treated group was treated with four concentrations of 100, 50,10 and 5 ppm of silver nanoparticles. After soaking in each concentrate for 30 minutes, fresh mulberry leaves were air-dried for 10 minutes. Leaves treated with different concentrations were used to feed the 5th instar larvae of the silkworm, *Bombyx mori*.

Histological Studies:

Nanosilver was applied on the fifth instar larvae of *B. Mori* using the leaf dipping technique method at concentrations of 100,50,10 and 5 ppm.

of silver nanoparticles. The larvae were taken 24 hours post-treatment and put it in Bouin's solution The larvae were rinsed in a series of ethanol solutions and the yellow was removed from Bowen's solution, which was used as a fixative. The larvae then went through several steps with a series of alcohol treatments for about 2 hours at room temperature starting with (80, 90, and 96)% and ending with ethyl alcohol 100%. The larvae are left after drying for 24 hours at 50 °C in a mixture of amyl acetate solution and soft paraffin wax. A mixture of hard paraffin wax was added to the larvae.

The larvae were dipped in the wax mixture and then serial sections were made at 6 μm by microtome and mounted on clean slides using Mayer's albumin. Sections are mounted on glass slides and stained with haematoxylene (Humason, 1963). and prepared for examination and photomicroscopy.

RESULTS AND DISCUSSION

Histological Studies:

1-The transverse section in the normal midgut of 5th instar larvae of *B.mori*:

A cross-section in the midgut of normal larvae of silkworm, *Bombyx mori* showed epithelium of midgut rounded by the basement membrane conspicuous nuclei almost central in position (Fig.1). The microvilli of columnar cells that extend from their free ends are what give the epithelium its striated appearance (brush border).

There are two separate layers of muscle fibres in the gut wall, called longitudinal muscles. and circular muscle fibers inside, connective tissue has filled the spaces between the various layer of the gut wall layer.

2. After treatment with a concentration of 100ppm nanosilver

The present histological study on the impact of Silver nanoparticles (AgNPs) on the mid-gut of treated *Bombyx mori* larvae revealed certain changes shown within Fig. (2) that appeared epithelium cells separated from basement membrane in many areas and thicknesses. The space between epithelial cells and the peritrophic membrane is filled with cytoplasmic and broken.

3. After treatment with a concentration of 50ppm of nanosilver

Figure (3) showed that certain cells become thickened and deformed, their cytoplasmic contents filled in the space between epithelial and peritrophic membrane layers. Additionally, the epithelium cells are destroyed, broken and separated completely from the ruptured basement membrane.

4. After treatment with a concentration of 10ppm of nanosilver:

Figure (4) some abnormalities were found in tshe internal components of the midgut compared with the normal larvae. The epithelial cells sometimes become elongated in size and appeared distorted or damaged and occasionally the epithelium cells detached from the basement membrane. Additionally, compared to the control, silver nanoparticles caused the peritrophic membrane to disintegrate.

5. After treatment with a concentration of 5ppm of nanosilver:

Figure (5) appeared cells become thick and deformed in many areas. The space between epithelial and peritrophic membrane layers is filled with cytoplasmic. Also, destroyed epithelium cells detached from the broken basement membrane.

The midgut represents the primarily targeted organs and the main site of digestion and absorption of materials in silkworm *Bombyx mori* larvae (Franzetti *et al*., 2015). The release of enzymes from the midgut has been shown in several studies to have a protective effect against foreign and toxic compounds (Zhang *et al*., 2014; Li *et al*., 2017; Wang *et al*., 2015).

All of our conclusions are consistent with previous research that showed that silver nanoparticles caused serious effects on the midgut tissues after a period of 96 hours of feeding leaves treated with different concentrations(1to100) Mg/L. The midgut of worms treated with nanoparticles (AgNPs) displayed significant histopathological alterations when compared to the control group (Nouara *et al*., 2018).

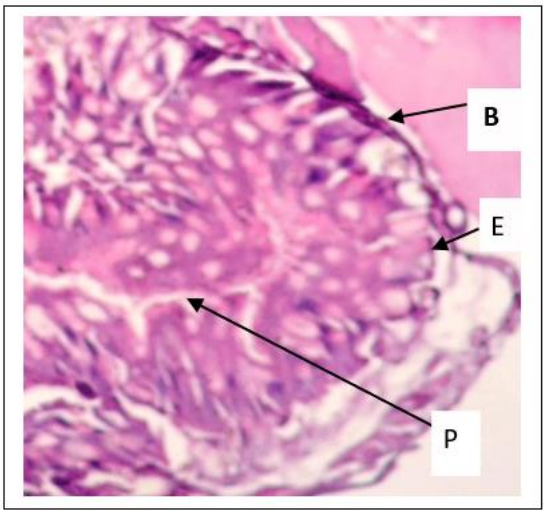


Fig.1: T.S. in the normal midgut of 5th instar larvae of *B.mori*

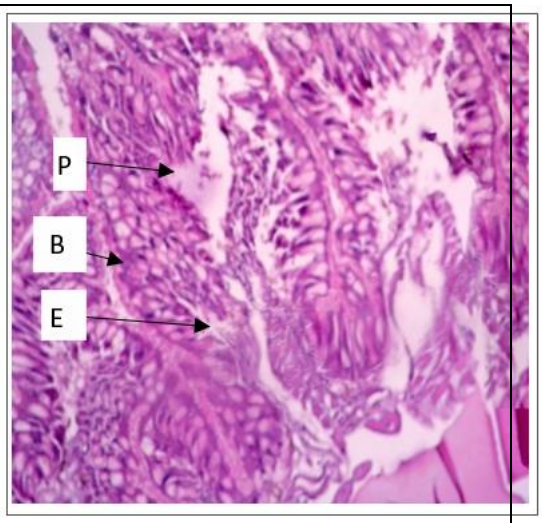


Fig.2: T.S. After treatment with a concentration of 100ppm nanosilver

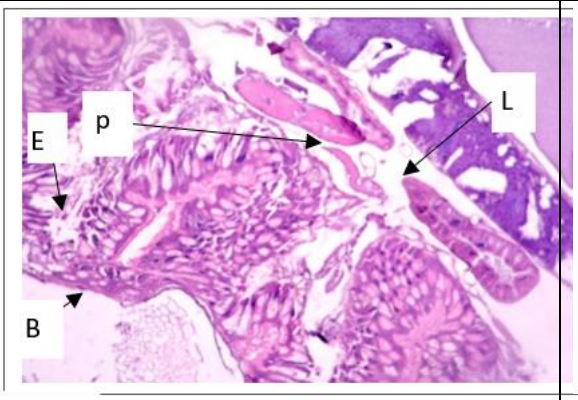


Fig.3: T.S. after treatment with a concentration of 50ppm of nanosilver

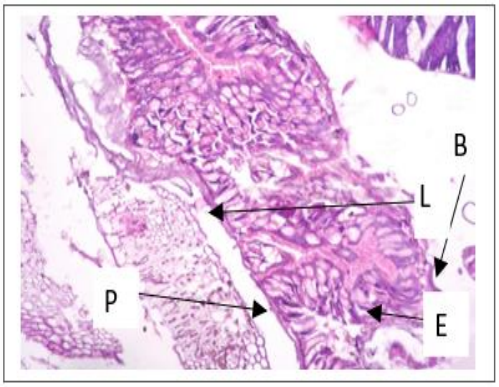


Fig.4: T.S. after treatment with a concentration of 10ppm of nanosilver

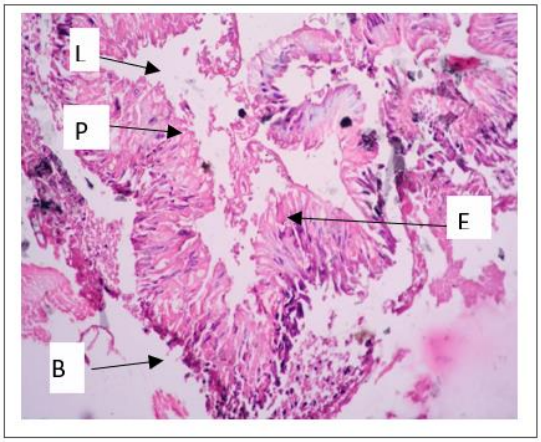


Fig.5: T.S. after treatment with a concentration of 5ppm of nanosilver

B: Basement membrane
E: Epithelial cell
L: Lumen
P: Peritrophic membrane

REFERENCES

Abdelli Nouara[1,2], Peng Lü[2], Liang Chen[2], Yan Pan[2], Yanhua Yang[2] and Keping Chen[1,2](2018): Silver effects on silkworm, Bombyx mori. *Journal of Toxicological Sciences*, 43(12): 697-709.

Anima Nanda. and Saravanan, M. (2009): Biosynthesis of silver nanoparticles from Staphylococcus aureus and its antimicrobial activity against MRSA and MRSE. *Nanomedicine*, 5(4):452-6. doi: 10.1016/j.nano.2009. 01. 012. Epub 2009 Feb 13.

Bizhannia AR.and Seidavi AR. (2008): Principles and Techniques of Silkworm Breeding (Ed., Lea H Z,). Haghshenass Press; 150pp.

Franzetti, E., Romanelli, D., Caccia, S., Cappellozza, S., Congiu, T., Rajagopalan, M., Grimaldi, A., de Eguileor, M., Casartelli, M. and Tettamanti, G. (2015): The midgut of the silkmoth *Bombyx mori* is able to recycle molecules derived from degeneration of the larval midgut epithelium. *Cell Tissue Research*, 361, 509-528.

Humason, G. L. (1963): Animal tissue techniques. W. H. Freeman and Company.

Li, F., Xu, K., Ni, M., Wang, B., Gu, Z., Shen, W. and Li, B. (2017): Effect of oxidative phosphorylation signaling pathway on silkworm midgut following exposure to phoxim. *Environmental. Toxicology*, 32, 167-175.

Seal S.and Karn B. (2014): Safety aspects of nanotechnology based activity. Saf Sci 63:217–225 3. Sekhon BS Nanotechnology in agri-food production: an overview. *Nanotechnology, Science and Applications*,8:31–53.

Sekhon, B.S. (2014): Nanotechnology in agri-food production: an overview. *Nanotechnology, Science and Applications*, 8:31–53.

Soumya M, Harinatha Reddy A, Nageswari G, Venkatappa B. (2017): Silkworm (*Bombyx mori*) and its constituents: a fascinating insect in science and research. *Journal of Entomology and Zoology Studies*,5(5):1701–1705.

Stensberg MC, Wei Q, McLamore ES, Porterfield DM, Wei A, Sepúlveda MS. (2017): Toxicological studies on silver nanoparticles: challenges and opportunities in assessment, monitoring and imaging. *Nanomedicine*, 6:879–988.

Suh WH, Suslick KS, Stucky GD, Suh YH. (2009): Nanotechnology nanotoxicology, and neuroscience. *Progress in Neurobiology*,87:133–170.

Tinsley, T. W. (1975): Factors affecting virus infection of insect gut tissue. In "Invertebrate immunity" (K. Maramorosch and R. E. Shope, eds.), pp. 55-63. Academic press, New York.

Wang, L., Su, M., Zhao, X., Hong, J., Yu, X., Xu, B., Sheng, L., Liu, D., Shen, W., Li, B. and Hong, F. (2015): Nanoparticulate TiO2 protection of midgut damage in the silkworm (*Bombyx mori*) following phoxim exposure.*Archives of Environmental Contamination Toxicology*, 68, 534-542.

Zhang, Y., Dong, Z., Wang, D., Wu, Y., Song, Q., Gu, P., Zhao, P. and Xia, Q. (2014): Proteomics of larval hemolymph in Bombyx mori reveals various nutrient-storage and immunity-related proteins. *Amino Acids*, 46, 1021-1031.