ABSTRACT

In the era of intensive scientific development and the possibility of using modern technologies, increased interest in the antibacterial properties of nano-sized silver can be observed. With its specific properties, nano-sized silver is widely used in a number of everyday products. The widespread presence of products containing silver nanoparticles increases concerns about their use. Today, nanoparticles are known to have a harmful effect on organisms and to contribute to serious damage to cellular structures. Disturbances in homeostasis leading to impairment of physiological processes may have consequences not only for a specific species exposed to a specific agent, but also for many other species interlinked through trophic interactions.

Spiders are considered important regulators of the size of many insect populations in meadow, forest and agricultural ecosystems. At the same time, they are among the invertebrates with the highest recorded concentrations of metals. To date, analyses of metal content in their tissues have referred to the molecular forms of metal compounds only. There is no data on the accumulation and cellular effects of metal nanoparticles (including nanosilver) in these predatory invertebrates. This project is primarily intended to verify whether and to what extent silver with the chemical formula AgNO3 and in two different nano-sized forms (PVP-AgNPs; nanosilver in groundwork polyvinylpyrrolidone and AgNPs; non-ionic colloidal silver) administered to *Steatoda grossa* (Theridiidae) spiders in a simplified food chain model (medium supplemented with various forms of silver → *Drosophila hydei* flies → spider) can cause cytotoxic effects in selected organs and tissues (the ampullate silk glands, midgut glands, haemolymph) and modify certain physicochemical properties of the hunting web. The research on the aspect of short- and long-term in'toxication will also make it possible to assess the extent of degenerative and metabolic changes in the organ.

The tested parameters as well as the methods and techniques of their analysis are summarized in the table below:

Parameter	Method / technique
Silver concentration in the bodies of spiders and their victims	Atomic absorption spectrometry (AAS)
Oxidative stress parameters	Spectrophotometry, Flow cytometric analysis
Quantitative assessment of apoptotic cells, necrotic cells and autophagic cells	Flow cytometric analysis
Quantitative assessment of cells with depolarized mitochondria	Flow cytometric analysis
ADP/ATP ratio and ATP concentration	Luminometric methods

Quantification of metallothioneins (MTs)	Enzyme-linked immunosorbent assay (ELISA)
Assessment of tissue ultrastructure	Transmission electron microscopy (TEM)
Quantitative and qualitative assessment of amino acid composition in spider silk	Thin layer chromatography (TLC) with densitometry
Spider web architecture assessment	Scanning electron microscopy (SEM)
Analysis of the elemental composition of spider webs	Energy Dispersive X-Ray Analysis (EDX)
Assessment of the calorific value of spider webs	Oxygen micro-bomb calorimetry MBC-3

Exposure of *S. grossa* females to food supplemented with selected forms of silver resulted in the bioaccumulation of this metal in their bodies, as the concentration of the metal increased along with the extension of the intoxication time. Contamination of prey with silver nanoparticles resulted in a lower concentration of this element in the spiders' bodies after long-term exposure than when $AgNO_3$ was used. Moreover, dietary supplementation with silver compounds resulted in the activation of antioxidant reactions, intensified production of metallothionein-like proteins and the occurrence of apoptotic, necrotic and autophagic changes in the analysed organs. These changes were concurrent to a decrease in the level of mitochondrial potential and ATP concentration, and an increase of the ADP/ATP ratio. However, the results indicated a stronger pro-oxidant effect of $AgNO_3$ impurities than of the silver nanoparticle forms used. The greatest changes in the levels of the above parameters were recorded in the cells of the midgut gland, which may confirm its significant role as a filter of xenobiotics and a place of detoxification processes to which compounds entering the body through the digestive system are subjected. A reduction in the diameter of silk fibres and the calorific value of the spider webs in conditions of exposure to silver compounds was also confirmed. However, the extent of these changes in the analysed groups was comparable regardless of the form of silver used. This may indicate that energy is allocated to energy-intensive detoxification reactions to counteract the toxicity of dietary silver.

Keywords: *Steatoda grossa*, spiders, nanosilver, antioxidant parameters, apoptosis, necrosis, autophagy, haemolymph, midgut gland, ampullate silk glands, spider silk