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Effects of green-synthesized silver nanoparticles from *Azadirachta indica* on growth performance and liver function parameters in male albino rats

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Abstract. *Arowora KA, Ugwuoke KC, Abah MA, Ugwoke BC. 2023. Effects of green-synthesized silver nanoparticles from* Azadirachta indica *on growth performance and liver function parameters in male albino rats. Cell Biol Dev 7: 28-34.* The effects of green-synthesized silver nanoparticles (AgNPs) from *Azadirachta indica* A.Juss. aqueous leaf extract on growth performance and liver function parameters in male albino rats were evaluated. Synthesis of AgNPs from $AgNO_3$ and plant extract was achieved using standard methods, while the characteristic peak of the synthesized AgNPs was determined using a UV-VIS spectrophotometer at a resolution of 1 nM. The crystal structure of AgNPs was determined using the technique of Fourier Transform Infrared Spectroscopy (FTIR). Growth performance study was carried out using the AOAC method. A total of 16 male albino rats weighing between 120 and 180 g were randomly allotted into four experimental groups having four rats each. 100, 250, and 500 mg/kg AgNPs were administered daily to Groups 2, 3, and 4 respectively for 14 days, while Group 1 served as the control. At the end of the administration, rats were sacrificed under anaesthetization, and serum samples were collected for analysis. The result revealed that the absorption characteristic peak of the ultraviolet-visible spectrum of the silver nanoparticles synthesized was 450 nM. It was also observed that the growth performance of the experimental animals gave no significant difference in average weight gain, feed intake, and feed efficiency ratio. The feed conversion ratio of Group 4 (6.94) is significantly higher than all the groups. However, Groups 2 (4.56) and 3 (4.74) were found to be non-significant (P>0.05), and significantly higher than the Control Group (3.75). Synthesized silver nanoparticles from *A. indica* affected the level of some selected biochemical parameters such as ALT, ALP, TP AST, and Albumin in a non-significant way. This study found no evidence of hazardous effects from silver nanoparticles, which could be attributed to the minimal dosage of AgNPs used or the source of the nanoparticles.

Keywords: *Azadirachta indica*, growth performance, liver function tests, silver nanoparticles

INTRODUCTION

Silver nanoparticles (AgNPs) are extremely small silver atoms with sizes ranging from 1 nM to 100 nM (Graf et al. 2003). Owing to their antibacterial qualities, silver NPs have been used extensively in the food and medical industries (Pulit et al. 2015). Sahayaraj and Rajesh (2011) reported that several public places in China utilize AgNPs as an antimicrobial agents, and it has been used greatly to lessen the infections of implanting surgical catheters in the course of surgery. It is no longer news that many researchers have put forward many biological activities; these AgNPs possess, ranging from anti-angiogenic, anti-fungal, anti-permeability, and to anti-inflammatory activities (Gurunathan et al.2009; Kalishwaralal et al. 2009; Sheikpranbabu et al. 2009).

In recent years, the biosynthesis of AgNPs has been at the forefront of contemporary nanotechnology (Gou et al. 2015; Parang and Moghadamnia 2018), and it has been identified that several biological agents, including microbes and plant extracts, have the capability of reducing silver ions to AgNP (Kahrilas et al. 2014; Dewi et al. 2023). According to several investigations, oxidative stress that causes lipid peroxidation and even apoptosis or necrosis which emanates from an imbalance between the generation of Reactive Oxygen Species (ROS) and antioxidant defenses, is the mechanism that significantly contributes to the cytotoxic effects of nanosilver (Kim and Ryu 2013; Völker et al. 2013). Reactive oxygen species production and alterations in enzyme activity linked to antioxidant defense systems were revealed to be induced by AgNPs (Dziendzikowska et al. 2012).

The likelihood of these nanoparticles becoming harmful rises with their widespread use. According to several scientific and medical studies, nanoparticles can enter the body through the skin, lungs, and gastrointestinal tract and cause negative health effects (Wijnhoven et al. 2009). Several consumers' items often contain silver nanoparticles because of their antibacterial and anti-fungal characteristics. These Ag NP-containing items include fabric (Kulthong et al. 2010), aerosolized such as nasal sprays or air sanitizers (Chao et al. 2011), wound dressings solvent (Silver et al. 2006), kinds of toothpaste, and colloidal silver suspensions (Nowack et al. 2011), has direct or indirect exposure to humans.

The liver, being one of the most crucial organs, is the main target for any routes of exposure involving bloodstream translocation, and prior studies have demonstrated a significant accumulation of NPs inside the liver following injection (Hirn et al. 2011), retention of particles in the liver after ingestion (Schleh et al. 2012), and liver affected following inhalation. To ensure customers' safety, it is necessary to screen for AgNPs' potential toxicity. Therefore, this work is aimed at determining the effect of synthesized silver nanoparticles from a plant origin (*Azadirachta indica* A.Juss.) on the performance and liver function tests in male albino rats.

An expanding field of study is the green production of nanoparticles, which is a well-known substitute for conventional techniques. This method utilizes plant extracts for the biosynthesis of nanoparticles. The advantages of green syntheses over chemical and physical approaches include environmental friendliness, cost-effectiveness, and ease of scaling up for large-scale nanoparticle production. In addition, there is no need to utilize harmful compounds or high temperatures, pressures, or energies (David et al. 2014). Taking into account the enormous potentiality of plants as a source, this work synthesized AgNPs using *A. indica* aqueous leaf extract.

The *A. indica*, usually called neem, is a species from a family called Meliaceae, which was used to bioconvert silver ions into nanoparticles. This plant is widely distributed in India, and from ancient times, all parts of the tree have been employed as natural remedies for a variety of human maladies, including viral infections. The plant leaves contain β-sitosterol (a flavonoid that is polyphenolic), and quercetin that possess anti-fungal and antibacterial activities (Srivastava et al. 2020). According to reports, neem leaves and their constituents have immuno-modulatory, anti-inflammatory, anti-hyperglycemic, ulcer-healing, antioxidant, anticarcinogenic, anti-mutagenic, antimalarial, antibacterial, anti-fungal, and antiviral properties (Girish and Shankara 2008).

MATERIALS AND METHODS

Chemicals and plant material collection

All the reagents purchased were of analytical grade and used without any further purification. Silver nitrate ($AgNO_3$) was purchased from Merck with a ≥99.5% purity. Neem leaves (*A. indica*) were collected from Wukari environs into a polyethylene zipper bag and transported to the Biochemistry Research Laboratory, Federal University Wukari, Taraba State, Nigeria.

Preparation of plant sample

The extraction of the plant sample was done using the method described by David et al. (2014). The *A. indica* leaf extract was prepared with distilled water. It was thoroughly washed with tap water and then with distilled water and cut into small pieces. The chopped leaves were then ground using mortar and pestle and 10g was weighed and heated in 100 mL of distilled water for 30 minutes at 60°C. The leaf broth was then cooled and filtered using Whatman No. 1 paper.

Synthesis of silver nanoparticles and its characterization

The biosynthesis of Silver Nanoparticles was done using plant materials as described by Sahayaraj and Rajesh (2011). A stock solution of 1 mM silver nitrate ($AgNO_3$) was prepared with distilled water.Ten (10) mL of *A. indica* leaf extract was added to 200 mL of 1 mM $AgNO_3$ solution placed on a hot plate with mild stirring. After heating the solution at 60°C for 30 minutes, there was a change in colour from dark green to yellow which indicates the formation of silver nanoparticles. The characteristic peaks were determined using a UV-VIS spectrophotometer at a resolution of 1 nM, by periodically scanning the optical absorbance between a range of 300-600 nM to investigate the reduction rate of silver ions by the leaf extract. The crystals of green synthesized silver nanoparticles produced was subjected to FTIR spectroscopy for its characterization.

Experimental animals

Male rats of Wistar strain weighing between 120 g and 180 g were obtained from National Veterinary Research Institute Vom, Plateau state, Nigeria. Animals were housed in a hygienic environment and allowed to acclimatize for a week before the commencement of treatments. Animals were fed with commercial rat chows and given potable water *ad libitum.*

Animal groupings and treatments

Animals were randomly assigned into four experimental Groups 1-4 of four rats per group. Group 1 served as control and received potable water daily. Groups 2, 3, and 4 were daily administered with 100, 250, and 500 mg/kg AgNPs respectively for 14 days. All treatments were orally administered with the aid of a cannula.

Liver function tests

At the end of two weeks of administration, rats were sacrificed under anaesthetization in slight chloroform. Blood samples were obtained by cardiac puncture into plain bottles. The blood samples were allowed to coagulate and then centrifuged for 10 min at 3000 rpm. Serum was obtained by aspiration of the clear yellowish liquid. Estimation of liver function tests, such as AST (Aspartate Transaminase), ALT (Alanine Transaminase), Alkaline phosphatase (ALP), Total Protein (TP), and Albumin (ALB) were carried out using serum with the aid of biochemical kits.

Growth performance study

The growth performance was studied using the method of AOAC (2000), Feed Intake is the sum of the quantity of feed consumed by the rats. The average weekly weight gain, feed efficiency ratio, and feed conversion ratio were calculated using the following formulas:

$$Feed\ Efficiency\ Ratio\ (FER) = \frac{Body\ weight\ gain}{feed\ intake} \quad (1)$$

$$Feed\ Conversion\ Ratio\ (FCR) = \frac{feed\ intake}{Body\ weight\ gain} \quad (2)$$

Weight gained by the rats is an increase in body weight. In each group,

$$Body\ Weight\ Gain\ (BWG) = Average\ final\ live\ weight - initial\ live\ weight \quad (3)$$

Statistical analysis

All the analyses were carried out in duplicates in completely randomized design. The data were subjected to analysis of variance using Statistical Package for Social Science (SPSS) software. Means that were significantly different were separated by the Least Significant Difference (LSD) test. Significance was accepted at P<0.05.

RESULTS AND DISCUSSION

Synthesis of AgNPs from $AgNO_3$ and *Azadirachta indica* leaf extract

The biosynthesis of Silver Nanoparticles was done using plant materials as described by Sahayaraj and Rajesh (2011). After heating the solution at 60°C for 30 minutes, there was a change in colour from dark green to yellow which indicates the formation of silver nanoparticles (Figure 1).

Spectrophotometric evaluation of $AgNO_3$ reduction

In this study, silver nanoparticles were synthesized from plant material. The absorption characteristic peak of the ultraviolet-visible spectrum of the silver nanoparticles synthesized wasobserved at 445 nM (Figure 2).

FTIR spectroscopy characterization of green synthesized silver nanoparticles

The crystals of green synthesized silver nanoparticles produced was subjected to FTIR spectroscopy for its characterization. The changes observed in the reaction converting $AgNO_3$ to AgNPs using FTIR analysis is represented below (Figure 3).

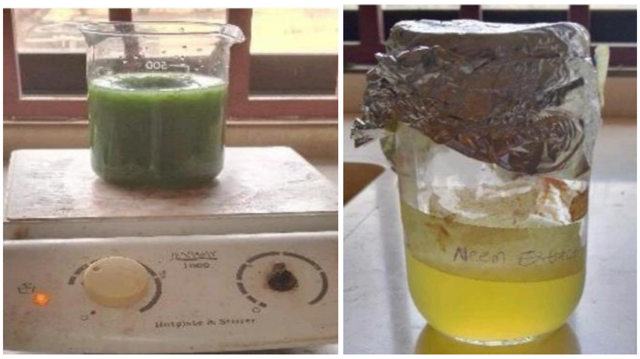

Figure 1. Synthesis of silver nanoparticles

Figure 2. Characteristic peak of silver nanoparticles absorption

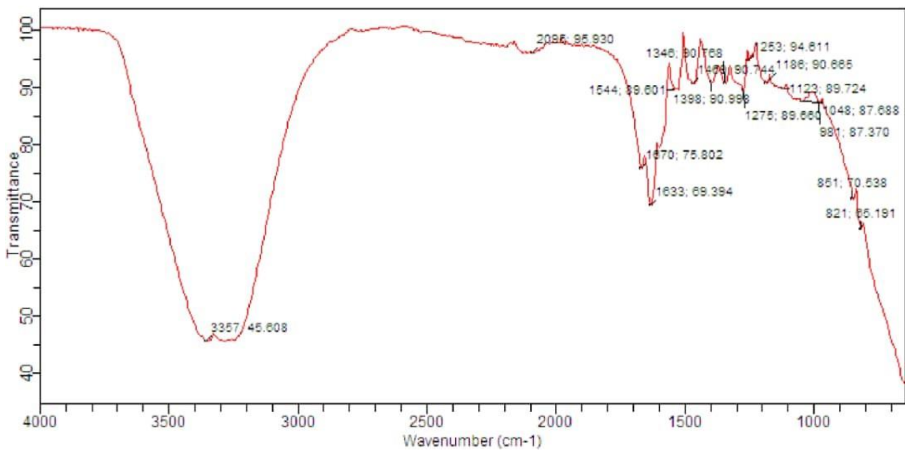

Figure 3. FTIR spectrum of synthesized AgNPs

Effects of experimental diets on serum biochemical parameters of albino rats at the end of 14 days feeding experiment

The result of the serum biochemical parameters (Figure 5) indicated that there were no significant differences (P>0.05) among the groups in the parameters estimated. Also, the effects of experimental diets on the performance of the experimental animals revealed that Group 1 had the highest feed efficiency ratio (0.26), while the lowest feed efficiency ratio of 0.14 was observed for Group 4. There was no significant difference (P>0.05) between Group 2 and Group 3 feed conversion ratio or feed efficiency, however, Groups 1 and 2 were significantly higher when compared with Group 4. However, there was no significant difference (P>0.05) in feed efficiency between Group 2 and 3.

Effects of experimental diet on performance of albino rats at the end of 14 days feeding experiment

The effects of the experimental diet on the growth Performance of the animals at the end of 14 days are shown in Table 1 The results revealed that AgNPs administration had no significant effect on average weekly weight gain, feed intake, and feed efficiency ratio. The average weekly feed intake and weight gain values of Groups 3 and 4 are higher than the Control Group. The result also revealed that the feed efficiency ratio decreased progressively with a significant difference from the Control Group to Group 4, while the feed conversion ratio increased across the group. There is no significant difference in the feed conversion ratio between Groups 2 and 3, however, these groups are significantly higher than the Control Group and at the same time significantly lower than Group 4 (Figure 4).

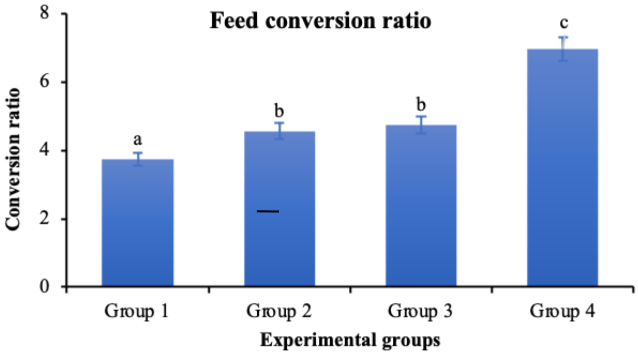

Figure 4. Feed conversion ratio of albino rats at the end of 14 days feeding experiment. Group values with different superscripts are significantly different (P<0.05) using descriptive analysis

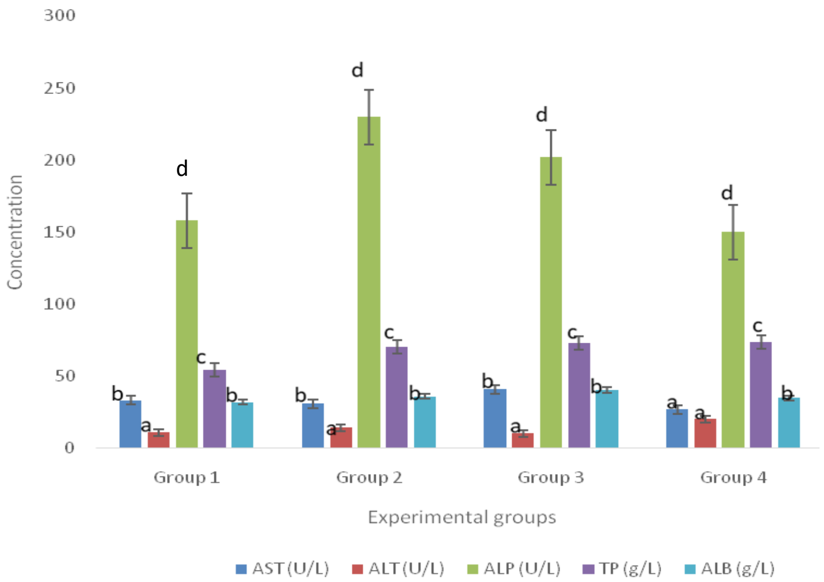

Figure 5. Effects of experimental diets on serum biochemical parameters of albino rats at the end of 14 days feeding experiment. Note: Values are presented as means ± SD for duplicate determinations, and values with different superscripts within the same column have no significant difference (P>0.05). Group 1 served as control and received potable water daily. Groups 2, 3, and 4 were daily administered with 100, 250, and 500 mg/kg AgNPs respectively for 12 days. Aspartate Aminotransferase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), Total Protein (TP), and Albumin (ALB)

Table 1. Effects of experimental diet on performance of albino rats at the end of 14 days feedingexperiment

Treatments	Group 1	Group 2	Group 3	Group 4
Average weekly Weight gain (g)	144.50±04	86.50±10	117.50±09	88.50±12
Average weekly Feed intake (g)	550.00±13	397.00±11	557.50±08	614.00±07
Feed efficiency Ratio	0.26±09^{a}	0.22±12^{b}	0.21±14^{b}	0.14±15^{c}

Discussion

An expanding field of study is the green production of nanoparticles, which is a well-known substitute for conventional techniques. This method utilizes plant extracts for the biosynthesis of nanoparticles. The advantages of green syntheses over chemical and physical approaches include environmental friendliness, cost-effectiveness, and ease of scaling up for large-scale nanoparticle production. In addition, there is no need to utilize harmful compounds or high temperatures, pressures, or energies (David et al. 2014). Taking into account the enormous potentiality of plants as a source, this work synthesized AgNPs using *A. indica* aqueous leaf extract.

In this study, silver nanoparticles were synthesized from plant material. The absorption characteristic peak of the ultraviolet-visible spectrum of the silver nanoparticles synthesized was observed at 445 nM (Figure 2). FTIR spectroscopy was used to identify the functional groups and biomolecules available in the crystal structure of silver nanoparticles (Figure 2). These biomolecues were identified based on the wavelength of their absorption and transmittance. The peak at this wavelength (3357 nM) was due to the absorption by hydroxyl group that could be found in flavonoids andpolyphenolics. This band appeared for AgNPS at transmittance of 45.608, the shift occurred is indicative of the chelative between silver nanoparticles via OH group, which could be alcohols i.e. aromatic alcohol. Unsaturated hydrocarbons could be present, since the stretches at 2095 nM could be due to the alkyne (c≡c) bond absorption, while 1670 and 1663 nM could be due to alkene (c═c) and alkanes within the crystal structure of Ag-NPs.

To study the effects of silver nanoparticles on the liver function enzymes and growth performance, the nanoparticles synthesized were administered to the experimental rats at different concentrations for 14 days. The result of the serum biochemical parameters (Figure 5) indicated that there were no significant differences (P>0.05) among the groups in the parameters estimated. Also, the effects of experimental diets on the performance of the experimental animals revealed that Group 1 had the highest feed efficiency ratio (0.26), while the lowest feed efficiency ratio of 0.14 was observed for Group 4. There was no significant difference (P>0.05) between Group 2 and Group 3 feed conversion ratio or feed efficiency, however, Groups 1 and 2 were significantly higher when compared with Group4. However, there was no significant difference (P>0.05) in feed efficiency between Groups 2 and 3.

The activity of AST and ALT enzymes is the first stage in examining liver damage, normally, an increase in AST and ALT indicates a liver problem (Gavanji et al. 2014). The most sensitive and the most practical recognizing enzymes in the liver are aminotransferases. The enzymes normally exist within the liver cells. When the liver gets damaged, the cells flow the enzymes to the blood and the increase in the level of the enzymes indicates liver damage (Reitman and Frankel 1957). ALP is a hydrolytic enzyme whose activity is observed in alkaline pH and different forms of these enzymes exist in the blood. The level of ALP serum in blood increases in pathologic conditions as well as in bone and liver damage (Soochan et al. 2012).

The average amounts of AST, ALT, and ALP were observed to be 33.50±3.54, 11.00±9.90, and 158.00.0±31.11 U/L respectively in the Control Group. The highest amount of AST (41.00±25.46 U/L), ALT (20.50±0.7), and ALP (230.00±0.00) were observed to be non-significant with a silver nanoparticle concentration of 250 mg/kg (Group 3), 500 mg/kg (Group 4), and 100 mg/kg (Group 2) respectively. These findings are similar to research carried out by Gavanji et al. (2014) who reported that there was no meaningful difference in AST and ALP levels in all groups, although, the level of ALT lead to a meaningful effect when compared with control. This result differs from that reported by Parang and Moghadamnia (2018) who observed a significant increase in the mean serum concentrations of AST, ALT, and ALP with an increase in silver nanoparticle concentrations. This could be attributed to the type of synthesis used and dosage of administered AgNPs. Also, the change in the mean of albumin and total protein levels in the experimental groups was observed to be non-significant. However, Parang and Moghadamnia (2018) reported a significant increase in all the treated groups compared to the Control Group.

Al Gurabi and associates in 2015 have looked into the possible impacts of silver nanoparticles on DNA damage and apoptotic cell death in albino mice in vivo (Ali et al. 2012). It has been demonstrated that silver nanoparticles significantly worsen liver injury symptoms, resulting in elevated ALP, ALT, and AST enzyme levels. Silver nanoparticles were employed to treat mice, and it was discovered that the particles caused DNA damage and cell death in lymphocytes and the liver. 7.8 mg/kg of silver nanoparticles significantly damaged DNA and resulted in cell death (Ali et al. 2012).

According to Guo et al. (2015), intravenous administration of silver nanoparticles causes the liver and kidneys to become toxic by weakening endothelial connections that are associated with intracellular ROS (Guo et al. 2015). Additionally, through intravenous exposure, alterations of endothelial cells brought on by silver nanoparticles can influence widespread peripheral inflammation in the liver and kidneys (Guo et al. 2015). Also, systemic exposure to silver nanoparticles was demonstrated to cause liver damage and NLRP3-dependent inflammation in a study by Ramadi et al. (2016). The concentrations of AST, ALT, and LDH were also raised by these nanoparticles. The findings of this study, which indicate that silver nanoparticles increase blood serum levels in a non-significant manner, are in agreement with some researchers' investigations, and also differ slightly from other studies.

The effects of the experimental diet on the growth Performance of the animals at the end of 14 days are shown in Table 1. The results revealed that AgNPs administration had no significant effect on average weekly weight gain, feed intake, and feed efficiency ratio. The average weekly feed intake and weight gain values of Groups 3 and 4 are higher than the Control Group. The result also revealed that the feed efficiency ratio decreased progressively with a significant difference from the Control Group to Group 4, while the feed conversion ratio increased across the group. There is no significant difference in the feed conversion ratio between Groups 2 and 3, however, these groups are significantly higher than the Control Group and at the same time significantly lower than Group 4. These significant differences could be caused by increasing levels of AgNPs suggesting their accumulation at different levels which may induce function alternation and may consequently affectthe metabolic rate of the body.

In conclusion, synthesized silver nanoparticles from *A. indica* altered the level of some selected biochemical parameters such as ALT, ALP, TP AST, and Albumin in a non-significant way. This study found no evidence of hazardous effects from silver nanoparticles, which could be attributed to the minimal dosage of AgNPs used or the source of the nanoparticles. Also, the growth performance of AgNPs administered rats showed a progressive significant increase in their feed conversion ratio, which may be attributed to varying concentration of AgNPs administered to the group.

REFERENCES

Ali D, Alkahtani S, Gurabi M, Alarifi S. 2012. In vivo DNA damaging and apoptotic potential of silver nanoparticles in Swiss albino mice. Onco Targets Ther 8: 295-302. DOI: 10.2147/OTT.S77572.

AOAC. 2000. Official Methods of Analysis 17th Edn. Association of Official Analysis Chemist (AOAC) International, Gaithersburg, M.D.

Chao JB, Liu JF, Yu SJ, Feng YD, Tan ZQ, Liu R, Yin YG. 2011. Speciation analysis of silver nanoparticles and silver ions in antibacterial products and environmental waters via cloud point extraction-based separation. Anal Chemother (83): 6875-6882. DOI: 10.1021/ac201086a

David L, Moldovan B, Vulcu A, Olenic L, Perde-Schrepler M, Fischer-Fodor E, Florea A, Crisan M, Chiorean I, Clichici S. 2014. Green synthesis, characterization and anti-inflammatory activity of silver nanoparticles using European black elderberry fruits extract. Colloids Surf B: Biointerfaces 122: 767-777. DOI: 10.1016/j.colsurfb.2014.08.018.

Dewi FRP, Lim V, Rosyidah A, Fatimah, Wahyuningsih SPA, Zubaidah U.2023. Characterization of silver nanoparticles (AgNPs) synthesized from *Piper ornatum* leaf extract and its activity against food borne pathogen *Staphylococcus aureus*. Biodiversitas 24: 1742-1748. DOI: 10.13057/biodiv/d240348.

Dziendzikowska K, Gromadzka-Ostrowska J, Lankoff A, Oczkowski M, Krawczynska A, Chwastowska J, Sadowska-Bratek M, Chajduk E, Wojewodzka M, Dusinska M, Kruszewski M. 2012. Time-dependent bio distribution and excretion of silver nanoparticles in male Wistar rats. JAppl Toxicol 32: 920-928. DOI: 10.1002/jat.2758.

Gavanji S, Sayedipour SS, Doostmohammadi M, Larki B. 2014. The effect of different concentrations of silver nanoparticles on enzyme activity and liver tissue of adult male wistar rats in-vivo condition. Intl J Sci Res Knowl 2 (4): 182-188. DOI: 10.12983/ijsrk-2014-p0182-0188.

Girish K, Shankara BS. 2008. Neem-a green treasure. Elec J Biol 4 (3): 102-111.

Gou Y, Zhou R, Ye X, Gao S, Li X. 2015. Highly efficient in vitro biosynthesis of silver nanoparticles using *Lysinibacillus sphaericus* MR-1 and their characterization. Sci Technol Adv Mater 16: 015004. DOI: 10.1088/1468-6996/16/1/015004.

Graf C, Vossen DL, Imhof A, Van-Blaaderen A. 2003. A general method to coat colloidal particles with silica. Langmuir 19 (17): 6693-6700. DOI: 10.1021/la0347859.

Guo H, Zhang J, Boudreau M, Meng J, Yin J-j, Liu J, Xu H. 2015. Intravenous administration of silver nanoparticles causes organ toxicity through intracellular ROS-related loss of inter-endothelial junction. Part Fibre Toxicol 13: 1. DOI: 10.1186/s12989-016-0133-9.

Gurunathan S, Kalishwaralal K, Vaidyanathan R, Venkataraman D, Pandian SRK, Muniyandi J, Hariharan N, Eom SH. 2009. Biosynthesis, purification and characterization of silver nanoparticles using *Escherichia coli*. Colloids Surf B: Biointerfaces 74 (1): 328-335. DOI: 10.1016/j.colsurfb.2009.07.048.

Hirn S, Semmler-Behnke M, Schleh C, Wenk A, Lipka J, Schäffler M, Takenaka S, Möller W, Schmid G, Simon U, Kreyling WG. 2011. Particle size-dependent and surface charge-dependent biodistribution of gold nanoparticles after intravenous administration. Eur J Pharm Biopharm (77): 407-416. DOI: 10.1016/j.ejpb.2010.12.029.

Kahrilas GA, Wally LM, Fredrick SJ, Hiskey M, Prieto AL, Owens JE. 2014. Microwave-assisted green synthesis of silver nanoparticles using orange peel extract. ACS Sustain Chem Eng 2 (3): 367-376. DOI: 10.1021/sc4003664.

Kalishwaralal K, Banumathi E, Pandian SBRK, Deepak V, Muniyandi J, Eom SH. 2009. Silver nanoparticles inhibit VEGF induced cell proliferation and migration in bovine retinal endothelial cells. Colloids Surf B: Biointerfaces 73: 51-7. DOI: 10.1016/j.colsurfb.2009.04.025.

Kim S, Ryu DY. 2013. Silver nanoparticle-induced oxidative stress, genotoxicity and apoptosis in cultured cells and animal tissues. J Appl Toxicol 33: 78-89. DOI: 10.1002/jat.2792.

Kulthong K, Srisung S, Boonpavanitchakul K, Kangwansupamonkon W, Maniratanachote, R. 2010. Determination of silver nanoparticle release from antibacterial fabrics into artificial sweat. Part Fibre Toxicol Rev (7): 8. DOI: 10.1186/1743-8977-7-8.

Nowack B, Krug HF, Height M. 2011. 120 years of nanosilver history: Implications for policy makers. Environ Sci Technol J 45 (4): 1177-1183. DOI: 10.1021/es103316q.

Parang Z, Moghadamnia D. 2018. Effects of silver nanoparticles on the functional tests of liver and its histological changes in adult male rats. Nanomed Res J 3(3): 146-153. DOI: 10.22034/nmrj.2018.03.005.

Pulit J, Banach M, Kowalski Z. 2015. Nanosilver-making difficult decisions. Ecol Chem Engin 18 (2): 185-196. WOS: 000292468700003.

Ramadi KB, Mohamed YA, Al-Sbiei A, Almarzooqi S, Bashir G, Al Dhanhani A, Sarawathiamma D, Qadri S, Yasin J, Nemmar A, Fernandez-Cabezudo MJ, Haik Y, Al-Ramadi BK. 2016. Acute systemic exposure to silver-based nanoparticles induces hepatotoxicity and NLRP3-dependent inflammation. Nanotoxicology 10 (8): 1061-74. DOI: 10.3109/17435390.2016.1163743.

Reitman S, Frankel SA. 1957. A colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Am J Clin Pathol 28: 59-63. DOI: 10.1093/ajcp/28.1.56.

Sahayaraj K, Rajesh S. 2011. Bionanoparticles synthesis and anti-microbial applications. In: MéndezVilas A (eds). Science Against Microbial Pathogens: Communicating Current Research and Technological Advances Formatex Research Center, Spain.

Schleh C, Semmler-Behnke M, Lipka J, Wenk A, Hirn S, Schäffler M, Schmid G, Simon U, Kreyling WG. 2012. Size and surface charge of gold nanoparticles determine absorption across intestinal barriers and accumulation in secondary target organs after oral administration. Nano-toxicol (6): 36-46. DOI: 10.3109/17435390.2011.552811.

Sheikpranbabu S, Kalishwaralal K, Venkataraman D, Eom SH, Park J, Gurunathan S. 2009. Silver nanoparticles inhibit VEGF-and IL-1b-induced vascular permeability via Src dependent pathway in porcine retinal endothelial cells. J Nanobiotechnol 7: 8. DOI: 10.1186/1477-3155-7-8.

Silver S, Phung LT, Silver G. 2006. Silver as biocides in burn and wound dressings and bacterial resistance to silver compounds. J Indust Microbiol Biotechnol (33): 627-634. DOI: 10.1007/s10295-006-0139-7.

Soochan D, Keough V, Wanless I, Molinari M. 2012. Intra and extra-hepatic cystadenoma of the biliary duct. Review of literature and radiological and pathological characteristics of a very rare case. BMJ Case Rep 20: 1-7. DOI: 10.1136/bcr.01.2012.5497.

Srivastava SK, Agrawal B, Kumar A, Pandey A. 2020. Phytochemicals of *Azadirachta indica* source of active medicinal constituent used for cure of various diseases: A review. J Sci Res 64 (1): 285-290. DOI: 10.37398/JSR.2020.640153.

Völker C, Oetken M, Oehlmann J. 2013. The biological effects and possible modes of action of nanosilver. Rev Environ Contam Toxicol 223: 81-106. DOI: 10.1007/978-1-4614-5577-6_4.

Wijnhoven SWP, Peijnenburg WJG, Herberts CA, Hagens WI, Oomen AG, Heugens EHW, Roszek B, Bisschops J, Gosens I, van de Meent D, Dekkers S, deJong WH, van Zijverden M, Sips AJAM, Geertsma RE. 2009. Nano-silver: A review of available data and knowledge gaps in human and environmental risk assessment. Nanotoxicol 3: 109-38. DOI: 10.1080/17435390902725914.