



Biosynthesis of silver nanoparticle (AgNPs) using *Lactobacillus* and their effects on oxidative stress biomarkers in rats



Abbas Sabbar Dakhil

Faculty of Medicine, University of Al-Qadisiyah, 58001, Iraq

ARTICLE INFO

Article history:

Received 8 April 2017

Accepted 15 May 2017

Available online 18 May 2017

Keywords:

Silver nanoparticles

Oxidative stress

Scanning electron microscope (SEM)

ABSTRACT

Biosynthesis method of nanoparticles acquires very important area due to their economic and ecofriendly benefits. The present study was aimed to the biosynthesis of silver nanoparticles (AgNPs) using *Lactobacillus* mixture and evaluating their antioxidant activity.

The characterization and biosynthesis AgNPs was achieved, using Ultra Violet (UV)–Visible spectrophotometry. Scanning electron microscope (SEM) was used to detect the size, shape and distribution of AgNPs. The occurrence of elemental silver was analyzed by Energy Dispersive-X-ray Spectroscopy (EDS) analysis. To evaluate the antioxidant activity of AgNPs and *Lactobacillus* in vivo, forty healthy adult rats were used and divided into eight equal groups, first group served as control, second group received LAB mix1 (1 ml/kg) and three groups were administrated with three concentration of AgNPs (5, 50 and 500 mg/kg AgNPs) respectively and other three groups were administrated with the same concentration of AgNPs along with LAB mix1 for two weeks.

The results revealed significant increased ($p < 0.05$) in total antioxidant capacity (TAC) of (LAB mix1 1 ml/kg, 5 mg/kg AgNPs, 5 mg/kg AgNPs + LAB mix1, 50 mg/kg AgNPs + LAB mix1 and 500 mg/kg AgNPs + LAB mix1) and significantly decreased ($p < 0.05$) in treatments of (50 and 500 mg/kg AgNPs).

The current study demonstrated that *Lactobacillus* afforded beneficial role by increasing the antioxidant activity with AgNPs and as ameliorative function for the effect of high dose of AgNPs.

© 2017 The Author. Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Nanotechnology is a modern field of science deals with synthesis and application of nanoparticles (NPs), it have a size of 1–100 nm. NPs have been studied extensively because of their unique physicochemical characteristics including antibacterial properties, catalytic activity, optical properties, electronic properties, and magnetic properties (Murugan and Shanmugasundaram, 2014)

Silver nanoparticles (AgNPs) have received significant attention because of their antimicrobial activity and prevention the biofilm formation, as well as their unique physical, chemical, biological properties, and their applicability in electronics, optics and medicine (Ansari et al., 2014). There are several number of physical,

chemical, biological, and hybrid methods available for synthesizing different types of nanoparticles, physical and chemical methods which are more expensive, energy consuming and potentially toxic to the environment (Liu et al., 2011). Development of reliable, non-toxic, and eco-friendly methods for synthesis of nanoparticles are the most important to expand their biomedical applications. One of the options to achieve this goal is to use microorganisms to synthesize nanoparticles (Gade et al., 2008). The extracellular synthesis of silver nanoparticles using *Lactobacillus* species appears to be low cost effective and ecofriendly (Chaudhari et al., 2012) and to develop new effective antimicrobial agents that overcome the multiple antibiotics resistance of microorganisms (Franci et al., 2015). Nanoparticles exhibit new or improved properties based on specific characteristics such as size, distribution and morphology. New applications of nanoparticles and nanomaterial are increasing rapidly. Nanotechnology can be termed as the synthesis, design, manipulation of structure of particles with dimension smaller than 100 nm (Ahmad et al., 2003).

Reactive oxygen species (ROS) are produced by living organisms as a result of normal cellular metabolism and environmental factors. The human body is equipped with a variety of antioxidants that serve to counterbalance the effect of oxidants (Birben et al.,

E-mail addresses: abbas.sabbar@qu.edu.iq, abbassd992@gmail.com

Peer review under responsibility of King Saud University.



<http://dx.doi.org/10.1016/j.jksus.2017.05.013>

1018–3647/© 2017 The Author. Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

2012). Biosynthesizing AgNPs exhibited antioxidant and anti-inflammatory (El-Rafie and hamed, 2014).

Lactobacillus have probiotic functions, due to their antimicrobial and antioxidative properties, adjusting the balance of intestinal flora, reducing blood cholesterol, inhibiting and reducing the risk of tumors and cancer, stimulating the immune system, stimulation of vitamin C production and enhancement of digestion (Songisepp et al., 2004) (Fig. 1).

Therefore the present study has been designed to biosynthesis of silver nanoparticles using *Lactobacillus* mixtures and study antioxidant activity of nanoparticles and *Lactobacillus* mixture *in vitro* and *in vivo* in rats.

2. Materials and methods

2.1. Sources of *Lactobacillus*

The types of *Lactobacillus* mixture were obtained from pharmacy and there are three types identified (*L. Acidophilus* + *L. plantarum*), (*L. acidophilus* + *L. bifidus*) and (*L. delbrueckii* + *L. fermentum*), were used as a source of *Lactobacillus* and give symbols LAB mix1, LAB mix2 and LAB mix3, respectively. Identification of *lactobacillus* was depending on morphological and biochemical tests by the method used by Holt et al. (1994).

2.2. Biosynthesis of silver nanoparticles

2.2.1. Culture of *Lactobacillus* mixture

Each *Lactobacillus* mixtures were inoculated separately in prepared and autoclaved De Man, Rogosa and Sharpe broth (MRS broth, Sigma Aldrich, USA) and then cell free supernatant was prepared according to Chaudhari et al. (2012).

2.2.2. Biosynthesis of silver nanoparticles using cell free supernatant

Silver nitrate (AgNO_3) was used as precursor for biosynthesis of silver nanoparticles by *Lactobacillus*. AgNO_3 was added with a concentration of (1, 2, 3, 4 and 5 mM) to cell free supernatant of each *Lactobacillus* mixtures (LAB mix1, LAB mix2 and LAB mix3) which distributed in sterilized tubes and mixed well in ratio 1:1. The procedure was done according to Maria et al. (2015).

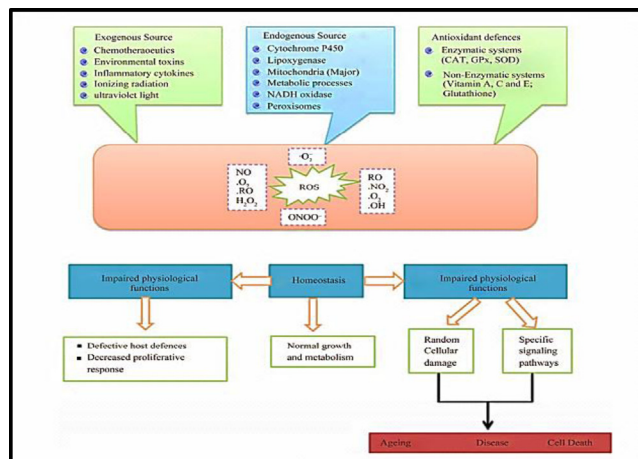


Fig. 1. The sources and cellular responses to reactive oxygen (Rahman et al., 2012).

2.3. Characterization of silver nanoparticles

2.3.1. Ultraviolet (UV)–Visible spectrophotometer analysis

Silver nanoparticles were analyzed using UV–Vis spectrophotometer (Shimadzu, 1600, Japan). Color change of the reaction mixtures was monitored by measuring UV–visible spectrum of the reaction mixture after periodically diluting the reaction mixture with deionized distill water and subsequently measured by UV–Vis spectrophotometer (Caroling et al., 2013).

2.3.2. Analysis by Scanning electron microscope (SEM)

Scanning electron microscope (SEM) was used for characterization the morphological and size of nanoparticles. Preparation of slides by adding small drops of suspension of biosynthesis nanoparticles on slides, and left to dry and then analyzed by SEM (FEI, Netherland). The microscope operated at an accelerated voltage at 5–10 kV at different magnification, low vacuum, a spot size 4 and working distances 5–10 mm (Caroling et al., 2013).

2.3.3. Energy Dispersive-X-ray Spectroscopy (EDS) analysis

Elemental analysis of single particle was carried out using EDS (Braker, Netherland) attached with SEM in electron microscope unit, EDS performed for point analysis with accelerating voltage 10KV and spot size 5, working distances 10 mm, this analysis was used to detect presence of elements nanoparticles (Sarvamangala et al., 2013).

2.4. *In vitro* antioxidant activity of nanoparticles and *Lactobacillus* mixture

DPPH (1, 1-diphenyl-2-picrylhydrazyl) (Sigma–Aldrich, USA) free radical scavenging assay was used to evaluate the ability of the AgNPs and *Lactobacillus* mixture to annihilate the DPPH free radical. The method described by Harbone and Baxter (1995) was used.

2.5. *In vivo* antioxidant activity of AgNPs and *Lactobacillus* mixture

2.5.1. Preparation of AgNPs concentrations for animals treatment

To evaluate the antioxidant activity *in vivo* by AgNPs, three concentrations of AgNPs were used (5, 50 and 500 mg/kg) of body weight and the LD50 for silver nanoparticles was dependent according to Shayesteh et al. (2014).

2.5.2. Preparation of *Lactobacillus* suspension for animals treatment

LAB mix1 was activated at MRS broth (Sigma Aldrich, USA), after incubation, the optical density (O.D) of culture suspension of *Lactobacillus* mixture was measured by spectrophotometer (CECIL, England) at 600 nm and diluted by normal saline until it reached the optical density that is equivalent to 1×10^9 CFU/ml. 1 ml/kg of LAB mix1 culture suspension (1×10^9 CFU/ml) was administered for each dose (Brown, 2010).

2.6. Experimental animals

Healthy adult female albino rats weighting between 240 and 266 g were obtained from Faculty of Veterinary Medicine/University of Wastte – Iraq. The animals were placed under standard environment condition (temperature 25–28 °C and 12 h. light-dark cycles). Standard pellets feed and water were provided to animals.

2.7. Experimental design

The study protocol was approved by the ethical committee of the Faculty of Medicine – University of Al-Qadisiyah. Forty mature

female rats were distributed randomly into eight groups (5 rats for each group). One group served as control and second group received LAB mix1, three groups treated with AgNPs at concentration of (5, 50 and 500 mg/kg) of body weight and other three groups treated with same concentration of AgNPs along with 1 ml/kg of LAB mix1 (1×10^9 CFU/ml) for each animal using a medical syringe that was inserted orally, the dosing was daily for two weeks.

2.8. Sample collection

At the end of the experimental period, all rats were weighted. They were anesthetized, using a mixture of ketamine 0.2 ml and xylazine 0.1 ml (Denver, Netherlands) and then they were sacrificed (Schiller and McNamara, 1999). The recording of body weight of each animal was done initially and at the end of experiment. The blood sample was obtained from animal through heart puncture by using a disposable medical syringe, then placed in tubes without anticoagulant and centrifuged at 3000 rpm for 10 min. The blood serum was separated, transferred into 1.5 ml micro centrifuge tubes. All samples were kept in a refrigerator at -20°C until the time of analysis.

2.9. Measurement of total antioxidant capacity

Measurement of total antioxidant capacity was performed through the immunosorbent assay (ELISA) (Biotek, USA) using TAC kit which was supplied by the manufacturing company (Elabscience, China) (Apak et al., 2008).

2.10. Statistical analysis

The data were analyzed by using windows software packages Graphpad prism V6. Data are expressed as (mean \pm standard error). T-test was used for the statically comparison between control group and treated groups, P values of less than 0.05 were considered to be statistically significant (Motulsky, 2003).

3. Results

3.1. Morphological and microscopic properties of *Lactobacillus* mixtures

Morphological and microscopic examinations were used to confirm each type of probiotic mixture of *Lactobacillus*. Morphologically they were characterized through observing their small convex, smooth, glistening colonies and without pigments microscopically, after stained with gram stain (Himedia, India) the bacteria appeared gram positive, rod shaped, arranged singly, pairs or short chains (Fig. 2).

3.2. Biosynthesis of AgNPs by supernatant of *Lactobacillus* mixtures

Lactobacilli showed their ability for extracellular biosynthesis of silver nanoparticles using cell free supernatant of each *Lactobacillus* mixture (LAB mix 1, LAB mix2 and LAB mix3) after adding silver nitrate (AgNO_3) in a different concentrations (1, 2, 3, 4 and 5 mM) to cell free supernatant of each *Lactobacillus* mixture (see Section 2.2.2). Changing the color of reaction mixture from yellow to reddish brown after incubation of mixtures for 24 h at 37°C in shaking incubator (150 rpm) (Labtech, USA) which represents as indicator for biosynthesis the AgNPs by *Lactobacillus* mixture.

3.3. Characterization of silver nanoparticles

3.3.1. UV-visible spectrophotometer analysis

The biosynthesis of nanoparticles can be confirmed by visual observation and measuring the surface Plasmon resonance (SPR) band using UV-visible spectroscopy. The absorption spectrum of nanoparticles formed in the reaction mixture has an absorption peak at 410 nm for AgNPs (Fig. 3) this evidence of the presence of surface Plasmon resonance (SPR) of nanoparticles and a single SPR band indicates that nanoparticles have spherical shape.

3.3.2. SEM analysis of nanoparticles

The results showed well-dispersed silver nanoparticles and homogenous with a diameter of (30–100 nm) with variable shapes most of them present in spherical form (Fig. 4). According to the results of characterization of nanoparticles by SEM it was observed difference in the ability of each *Lactobacillus* mixture for biosynthesis of silver. LAB mix1 and LAB mix2 showed their ability for biosynthesis AgNPs, while they were distinctive in their characteristics when formed by LAB mix1 through noticing the typical shape (spherical), size (30–100 nm) and homogenous distribution than their being formed by LAB mix2. LAB mix3 was less in its ability for synthesizing AgNPs. The concentration of AgNO_3 which was added to the supernatant also have effect on characterization of formed nanoparticles, the best concentration in the biosynthesis of AgNPs was 4 mM AgNO_3 in comparison with other concentrations.

3.3.3. EDS analysis of nanoparticles

The presence of elemental silver is indicated the reduction of silver ion in the reaction mixture by *Lactobacillus* mixtures into silver metal. The EDS spectrum was recorded in the spot-profile mode, strong signals from the Ag atom are observed while medium signals from oxygen and weaker signals from other atoms. The weight percentage of elemental constituents for AgNPs that were formed by LAB mix1 was (67.35%) silver and (32.65%) oxygen (Fig. 5). The optical absorption peak of AgNPs was observed at 3 keV which is atypical absorption of metallic AgNPs.

3.4. In vitro antioxidant activity of nanoparticles and *Lactobacillus* mixture

The results revealed ability of nanoparticles and LAB mix1 to scavenging DPPH free radicals that indicated by observing the color change from the original purple color of DPPH into yellow color. The inhibition proportion of DPPH scavenging free radicals by *Lactobacillus* mixture growth was (17%) and their supernatant was (10.3%). DPPH reducing activity of nanoparticles increased with increasing their concentration that indicated by the elevated percentage of inhibition of DPPH which increased with increase concentration of AgNPs. It was (92.4% in 1.5 mg/ml) while the inhibition percentage was 65.7% at 0.5 mg/ml (Fig. 6).

3.5. Effect of AgNPs and LAB mix1 on serum level of TAC activity

The results in Fig. 7 showed that serum total antioxidant capacity (TAC) was significant increased ($p < 0.05$) in LAB mix1 of 1 ml/kg and 5 mg/kg AgNPs treated groups respectively in comparison with control group. While observed significant decreased ($p < 0.05$) of TAC with increase of AgNPs concentrations in (50 and 500 mg/kg) treated groups respectively in comparison with control and 5 mg/kg AgNPs treated groups. Treatment of rats with (1 ml/kg) of LAB mix1 along with 5, 50 and 500 mg/kg AgNPs concentration respectively showed significant increased ($p < 0.05$) of TAC activity in 50 and 500 mg/kg AgNPs + LAB mix1 in comparison with AgNPs groups.

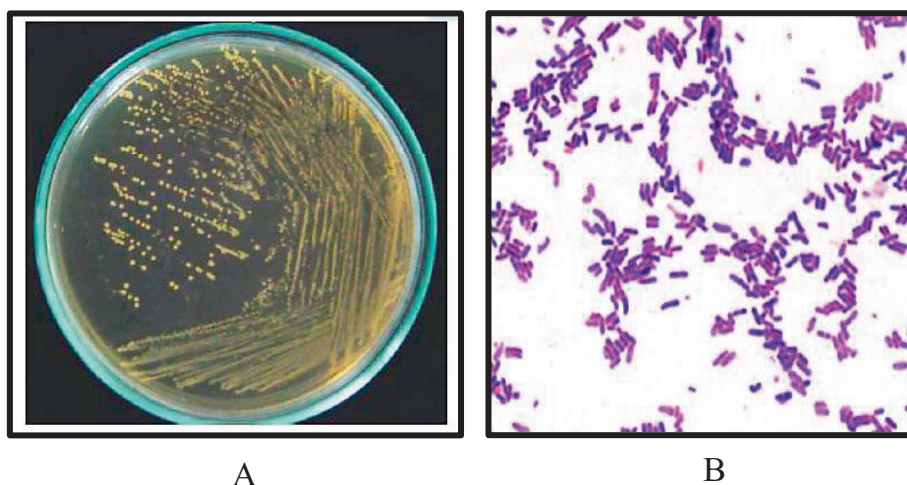


Fig. 2. Morphological and microscopic properties of *Lactobacillus* mixtures. (A) *Lactobacillus* on MRS agar medium, 37 °C, 48 h, and anaerobic condition. (B) *Lactobacillus* stained with gram stain under light microscope (X1000).

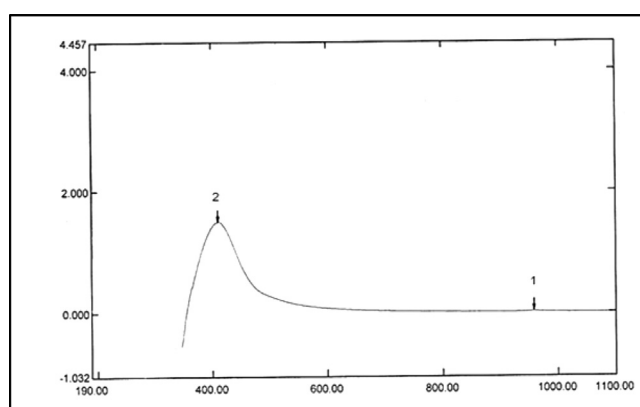


Fig. 3. UV-visible absorption spectrum of AgNPs synthesized by LAB mix 1. The absorption spectrum of AgNPs exhibited a strong broad peak at 410 nm.

4. Discussion

It has been recently established that microorganisms have been explored as potential bio-factories for synthesis of metallic NPs (Mukherjee et al., 2002). In the present study an attempt was made to employ the potentials of three *Lactobacillus* mixtures to synthesize AgNPs. The appearance of a brown color in solution is a clear indication of the formation of silver nanoparticles in the reaction mixture due to reduction of Ag^+ ions to Ag metal by the reducing agents in the cultural supernatants (Sreedevi et al., 2015). The exact reaction mechanism leading to the formation of silver nanoparticles by all organisms is yet to be elucidated. Extracts from microorganisms may act both as reducing and capping agents in AgNPs synthesis (Kalimuthu et al., 2008). The increase in intensity of the surface Plasmon resonance (SPR) peak could be due to increasing number of nanoparticles formed as a result of reduction of silver ions present in the aqueous solution. The surface Plasmon band in the silver nanoparticles solution remains close to 410 nm throughout the reaction period, indicating that the particles are dispersed in the aqueous solution with no evidence for aggregation (Ahmad et al., 2003). Experimental results showed well-dispersed nanoparticles with diameter of (30–100 nm) with variable shapes most of them present in spherical form, these results agreed with study achieved by Aldujaili et al. (2015). EDS analysis detect the presence of elemental silver, the optical absorption peak was

observed at 3 keV which is a typical absorption of metallic AgNPs, these results are similar with study of Bhakya et al. (2015).

DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging activity of nanoparticles increased with increasing their concentration that indicated by the elevated percentage of inhibition of DPPH which increased with increase concentration of AgNPs that exhibited more inhibition (92.4% in 1.5 mg/ml) due to more an electron donated and accepts by DPPH (Xing et al., 2015). The mechanisms of antioxidants are not only by scavenging free radicals, but also by inhibiting production of free radicals (Ranjbar et al., 2014). The inhibition proportion by bacterial suspension of LAB mix1 was (17%) and cell free supernatant was (10.3%). The different mechanisms involved in the radical-antioxidant reactions may explain the different in scavenging potentials of the compounds (Niki, 2010; Xing et al., 2015).

The elevated and decreased of total antioxidant capacity (TAC) indicates that AgNPs with 5 mg/kg are more effective antioxidant than 50 mg/kg and 500 mg/kg which can induce the oxidative damage through a reactive oxygen species (ROS-mediated process), these results related with other study obtained by Schluesener and Schluesener (2013), they showed that AgNPs decrease the oxidative stress due to decreased superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities and increase the TAC level in low dose of AgNPs, but the TAC level was decreased when SOD and GPx increased in high dose of AgNPs. These results also agreement with other study that indicated AgNPs with a concentration of (5 mg/kg) decrease the oxidative stress by observed increase TAC activity and a decreased catalase, but in 250 mg/kg and 500 mg/kg decrease TAC and increase catalase activity (Shayesteh et al., 2014). Ag-NPs concentrations that were used play very significant role to evaluate the antioxidants status, this implies that dose in addition to the size and chemical composition of nanoparticles are properties of great importance when appraising nontoxicity (Fabrega et al., 2009; El Badawy et al., 2010). However, it remains to be investigated whether Ag NPs induce free radicals directly or indirectly through depletion of antioxidant defense mechanisms depending dose by interactions with antioxidant systems (Schluesener and Schluesener, 2013). TAC was significantly increased ($p < 0.05$) after treatment of rats with suspension of LAB mix1 beside concentrations of AgNPs (5, 50 and 500 mg/kg AgNPs + LAB mix1) due to the antioxidant activity of *Lactobacillus* by using their antioxidant enzymes that play a critical role in defense against reactive oxygen species (ROS) such as superoxide dismutase (SOD) eliminates direct toxic-

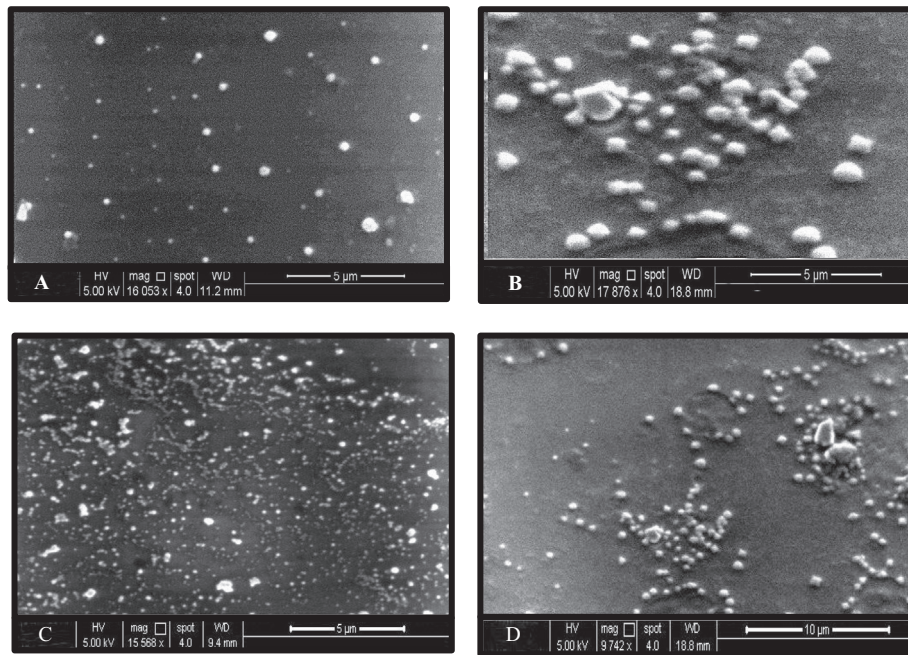


Fig. 4. SEM of silver nanoparticles synthesized by LAB mix1. The shape of AgNPs was spherical and homogenous, size between (30–100 nm), magnification (16053 \times , 17876 \times , 15568 \times , 9742 \times), the voltage (5 kV) and spot size 4 for A, B, C and D respectively.

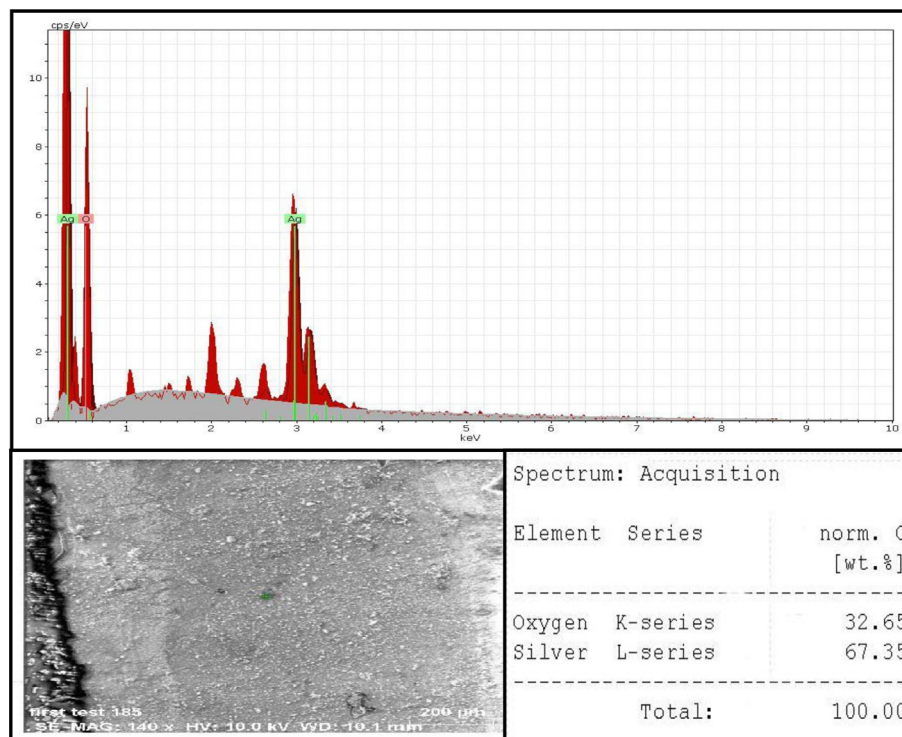


Fig. 5. EDS analysis of AgNPs synthesized by LAB mix1 illustrated strong signals from the Ag, medium signal from O₂, the optical absorption peak of Ag was observed at 3 keV, the weight percentage of silver (67.35%) and oxygen (32.65%).

ity of superoxide anions and glutathione peroxidase (GPx) scavenges H₂O₂ and hydroxyl radicals or non-enzymatically by metal ion chelating (Persichetti et al., 2014). *Lactobacillus* have antioxidant effect similar to the effect of other antioxidants such as plant extracts or vitamins to present synergistic effect through increasing antioxidant activity and decrease the oxidative stress caused by high dose of nanoparticles (Diab et al., 2015).

5. Conclusions

Three test *Lactobacillus* mixtures were capable of synthesizing silver nanoparticles by reaction silver nitrate (AgNO₃) for AgNPs supernatant. Comparatively, AgNPs synthesis was better in term of quality; minimum size and less polydispersity, with LAB mix1 (*L. Acidophilus* + *L. plantarum*). It has been also concluded that *Lac-*

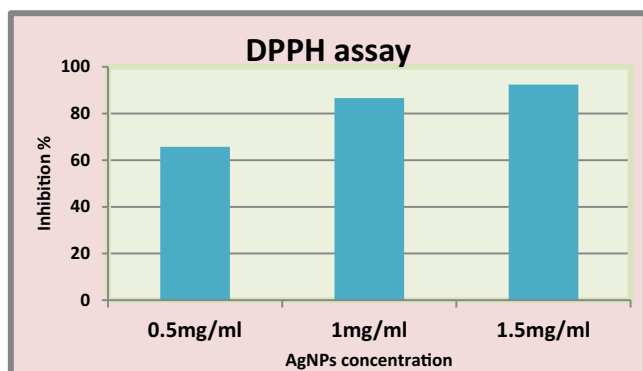


Fig. 6. DPPH free radical scavenging ability of silver nanoparticles.

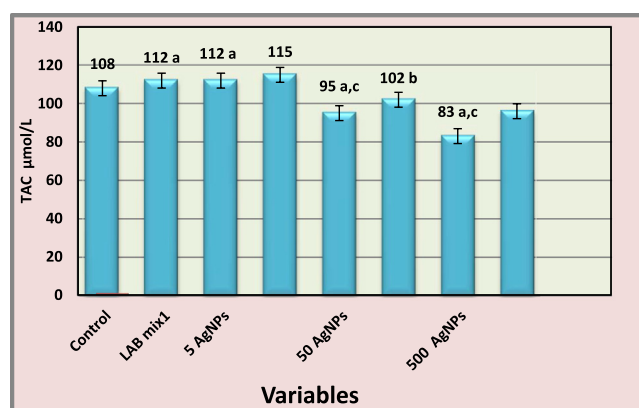


Fig. 7. Effect of AgNPs and LAB mix1 on serum level TAC- a: represents significantly different from control at ($p < 0.05$) b: represents significantly different from AgNPs groups at ($p < 0.05$) c: represents significantly different from 5 mg/kg AgNPs group at ($p < 0.05$).

tobacillus afforded beneficial role by increasing the antioxidant activity with AgNPs and as an ameliorative function for the effect of high dose of AgNPs.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgements

The author wishes to thank the technical staff of Faculty of pharmacy and Science/University of Kufa/Iraq, for numerous assistances during this research, they contributed equally to the work, they also supported by characterization of nanoparticles.

References

Ahmad, A., Mukherjee, P., Senapati, S., Mandal, D., Khan, M.I., Kumar, et al., 2003. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. *Colloids Surf. B: Biointerfaces* 28, 313–318.

Aldujaili, N.H., Abdullah, N.Y., Khaqani, R.L., Al-tfaly, S.A., Al-Shammari, A.H., 2015. Biosynthesis and antibacterial activity of Titanium nanoparticles using *Lactobacillus*. *Inter J. Recent Sci. Res.* 6, 7741–7751.

Ansari, M.A., Khan, H.M., Khan, A., Cameotra, S.S., Pal, R., 2014. Antibiofilm efficacy of silver nanoparticles against biofilm of extended spectrum B-lactamase isolates of *Escherichia coli* and *Klebsiella pneumoniae*. *Appl. Nanosci.* 4, 859–868.

Apak, R., Guclu, K., Ozyurek, M., Celik, S.E., 2008. Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay. *Microchim. Acta* 160, 413–419.

Bhakya, S., Muthukrishnan, S., Sukumaran, M., Muthukumar, M., 2015. Biogenic synthesis of silver nanoparticles and their antioxidant and antibacterial activity. *Appl. Nanosci.* 6, 755–766.

Birben, E., Sahiner, U.M., Sackesen, C., Erzurum, S., Kalayci, O., 2012. Oxidative stress and antioxidant defense. *WAOJ* 5, 9–19.

Brown, T.A., 2010. Gene cloning and DNA analysis: an introduction. Wiley-Blackwell Publishing, UK, pp. 123–127.

Caroling, G., Tiwari, S.K., Ranjitham, A.M., Suja, R., 2013. Biosynthesis of silver nanoparticles using aqueous broccoli extract-characterization and study of antimicrobial cytotoxic effects. *Asian J. Pharm. Clin. Res.* 6, 165–172.

Chaudhari, P.R., Masurka, S.A., Shidore, V.B., Kamble, S.P., 2012. Antimicrobial activity of extracellularly synthesized silver nanoparticles using *Lactobacillus* species obtained from vizylac capsule. *J. Appl. Pharmaceut. Sci.* 02, 25–29.

Diab, A.A., Zahra, M.H., AL-dohim, S.I., Hassan, N.J., 2015. The impact of Moringa Oleifera extract and vitamin E against zinc oxide nanoparticles induced hepatotoxicity in male albino rats. *J. Am. Sci.* 11, 33–41.

El Badawy, A.M., Silva, R.G., Morris, B., Scheckel, K.G., Suidan, M.T., Tolaymat, T.M., 2010. Surface charge-dependent toxicity of silver nanoparticles. *Environ. Sci. Technol.* 45 (1), 283–287.

El-Rafie, H.M., Hamed, M.A., 2014. Antioxidant and anti-inflammatory activities of silver nanoparticles biosynthesized from aqueous leaves extracts of four Terminalia species. *Adv. Nat. Sci. Nanosci. Nanotechnol.* 5, 35–37.

Fabrega, J., Fawcett, S.R., Renshaw, J.C., Lead, J.R., 2009. Silver nanoparticle impact on bacterial growth: effect of pH, concentration, and organic matter. *Environ. Sci. Technol.* 43 (19), 7285–7290.

Franci, G., Falanga, A., Galdiero, S., Palomb, Rai, M., Morell, G., et al., 2015. Silver nanoparticles as potential antibacterial agents. *Molecules* 20, 8856–8874.

Gade, A.K., Bonde, P., Ingle, A.P., Marcato, P.D., Durã, N., 2008. Exploitation of *Aspergillus niger* for synthesis of silver nanoparticles. *J. Biobased Mater. Bioenergy* 2, 243–247.

Harbone, J.B., Baxter, H., 1995. *Phytochemical Dictionary, A Handbook of Bioactive Compounds from Plants*, second ed. Taylor and Francis, London. 143–55.

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T., Williams, S.T., 1994. *Bergey's manual of determinative bacteriology*. Williams & Wilkins, USA, pp. 532–551.

Kalimuthu, K., Babu, R.S., Venkataraman, D., Mohd, B., Gurunathan, S., 2008. Biosynthesis of silver nanocrystals by *Bacillus licheniformis*. *Colloids Surf. B Biointerfaces* 65, 150–153.

Liu, J., Qiao, S.Z., Hu, Q.H., Lu, G.Q., 2011. Magnetic nanocomposites with mesoporous structures: synthesis and applications. *Small* 7, 425–443.

Motulsky, H.J., 2003. *Fitting Model to Biological Data using Linear and Nonlinear Regression. A Practical Guide to Curve Fitting*. San Diego, CA, Graph Pad Software Inc.

Mukherjee, P., Senapati, S., Mandal, D., Ahmad, A., Khan, M.I., Kumar, R., Sastry, M., 2002. Extracellular synthesis of gold nanoparticles by the fungus *Fusarium oxysporum*. *Chem. Bio. Chem.* 3, 461–463.

Murugan, A., Shanmugasundaram, K.K., 2014. Biosynthesis and characterization of silver nanoparticles using the aqueous of vitex Negundo Linn. *World J. Pharm. Pharmaceut. Sci.* 3, 1385–1393.

Niki, E., 2010. Assessment of antioxidant capacity in vitro and in vivo. *Free Radic. Biol. Med.* 49, 503–515.

Persichetti, E., De Michele, A., Codini, M., Traina, G., 2014. Antioxidative capacity of *Lactobacillus fermentum* LF31 evaluated in vitro by oxygen radical absorbance capacity assay. *Nutrition.* 30, 936–938.

Rahman, T., Hosen, Ismail, Islam, M.M.T., Shekhar, H.U., 2012. Oxidative stress and human health. *Adv. Biosci. Biotechnol.* 3, 997–19.

Ranjbar, A., Ataie, Z., Khajavi, F., Ghasemi, H., 2014. Effects of silver nanoparticle (Ag NP) on oxidative stress biomarkers in rat. *Nanomed. J.* 1, 205–211.

Sarvamangala, D., Kondala, K., Shivakumar, N., Sharatchandrababu, M., Manga, S., 2013. Synthesis characterization and antimicrobial studies of AgNP's using probiotics. *Inter Res. J. Pharm.* 4, 240–243.

Schiller, N.K., McNamara, D.B., 1999. Balloon catheter vascular injury of the alloxan-induced diabetic rabbit: the role of insulin-like growth factor-1. *Mol. Cell. Biochem.* 202, 159–167.

Schluesener, J.K., Schluesener, H.J., 2013. Nanosilver: application and novel aspects of toxicology. *Arch. Toxicol.* 87, 569–576.

Shayesteh, T.H., Khajavi, F., Ghasemi, H., Zijoud, S.M.H., Ranjbar, A., 2014. Effects of silver nanoparticle (Ag NP) on oxidative stress, liver function in rat: hepatotoxic or hepatoprotective? *Issues Biol. Sci. Pharm. Res.* 2, 40–44.

Songisepp, E., Kullisaar, T., Hutt, P., Elias, P., Brilen, T., Zilmer, M., 2004. A new probiotic cheese with antioxidative and antimicrobial activity. *J. Dairy Sci.* 87, 2017–2023.

Sreedevi, T.P., Thilagam, M., Tamil Selvi, A., Chandrasekaran, B., 2015. Synthesis, characterization and antibacterial studies of silver nanoparticles using *Lactobacillus Plantarum*. *World J. Pharm. Res.* 4, 1757–1773.

Sumi Maria, B., Devadiga, A., Shetty Kodialbail, V., Saidutta, M.B., 2015. Synthesis of silver extracts nanoparticles using medicinal Zizyphus xylopyrus bark extract. *Appl. Nanosci.* 5, 755–762.

Xing, J., Wang, G., Zhang, Q., Liu, X., Gu, Z., Zhang, H., Chen, Y.Q., Chen, W., 2015. Determining antioxidant activities of *Lactobacilli* cell-free supernatants by cellular antioxidant assay: a comparison with traditional methods. *PLoS ONE* 10, 3.