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

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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.

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, nontoxic, 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.,

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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. Acidophillus* + *L. plantarum*), (*L. acidophillus* + *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, Rogasa 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).

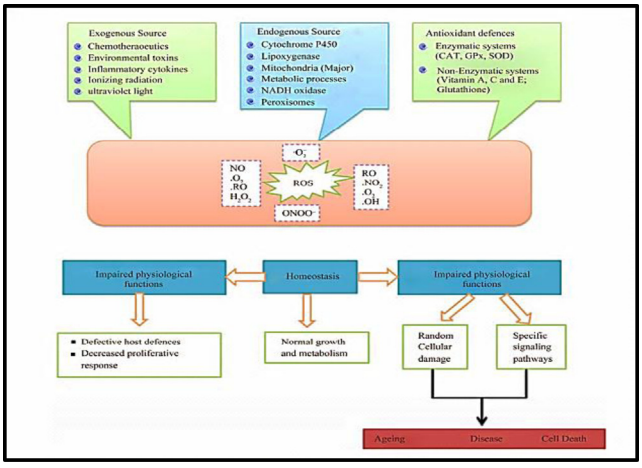

Fig. 1. The sources and cellular responses to reactive oxygen (Rahman et al., 2012).

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 1×109 CFU/ml. 1 ml/kg of LAB mix1 culture suspension (1×109 CFU/ml) was administrated 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