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Biosynthesized Silver Nanoparticles Demonstrate Hepatoprotective Biochemical Safety in Female Wistar Rats

Suhad Khalid Sgheer [1] , Ali Tariq Abdul Hassan [2]

[1] Directorate of Education of Kerbala, Ministry of Education, Kerbala, Iraq
[2] Directorate of Education of Kerbala, Ministry of Education, Kerbala, Iraq

Email: [1] suhad.khalid@ec.edu.iq , [2] alial-aboodi@alameed.edu.iq

Abstract. **General Background:** Silver nanoparticles (AgNPs) are widely applied in biomedical and technological fields, yet conventional chemical and physical synthesis methods raise safety and environmental concerns. **Specific Background:** Biosynthesis using probiotic bacteria such as *Lactobacillus rhamnosus* has emerged as a green alternative with potential biocompatibility advantages. **Knowledge Gap:** Limited in vivo evidence exists regarding the biochemical and histological safety of biosynthesized AgNPs, particularly their hepatic effects. **Aims:** This study aimed to evaluate the biochemical and histological effects of AgNPs biosynthesized by *L. rhamnosus* in female Wistar albino rats. **Results:** Biosynthesized AgNPs significantly reduced liver enzymes (ALT, AST, and ALP), significantly increased vitamin B12 levels, showed no significant change in body weight, and preserved normal liver tissue architecture. **Novelty:** The study demonstrates the hepatic safety profile of *L. rhamnosus*–mediated AgNPs in vivo compared with risks reported for conventionally synthesized AgNPs. **Implications:** These findings support probiotic-mediated biosynthesis as a safer and environmentally friendly approach for developing silver nanoparticles for biomedical applications.

Keywords: Biosynthesized Silver Nanoparticles, Lactobacillus rhamnosus, Green Nanoparticle Synthesis, Liver Enzyme Biomarkers, Wistar Albino Rats

Highlights:

1. Significant reductions in AST, ALT, and ALP without alterations in body mass.
2. Serum vitamin B12 concentrations increased markedly following 30-day administration.
3. Liver histology preserved normal architecture, indicating absence of hepatocellular damage.

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Introduction

Nanotechnology is the discipline focused on the production of nanoparticles (NPs). The utilization of silver nanoparticles (AgNPs) is predominantly in the production of medicinal, domestic and industrial items [1].

In this decade, researchers have shown great interest in nanomaterials due to their diverse biological, physical and chemical properties, that have diverse applications in cosmetics, agriculture, industry, healthcare, packaging and medicine [2]. Extensive research has focused on the synthesis of various metal and metal oxide nanoparticles, including zinc oxide NPs, silver NPs and gold NPs, using conventional and unconventional methods [3]. The production and use of silver and silver nanoparticle-based materials has become a prominent field within nanomaterials due to their distinct morphology, biophysical properties and stability. It has been widely used in the biomedical field as an antiangiogenic, antibacterial, anti-inflammatory, antifungal, antiviral, and anticancer drug [4].

Nanotechnology is a very promising technology in the 21st century; Nanomedicine represents an innovative approach to the application of nanotechnological systems for the detection and treatment of diseases [5]. Nanomaterials have particles smaller than 100 nm in at least one dimension [6]. Solutions derived from nanotechnology, especially NPs, have gained prominence in medical research due to their significant efficiency and safety advantages compared to traditional therapeutic and diagnostic methods [7]. The properties of NPs vary according to their surface and size functionality. Due to their small size, NPs have a large surface-to-volume ratio. Due to its higher surface/volume ratio, it shows greater reactivity and therefore greater efficiency compared to bulkier materials [8]. NPs provide a bunch of therapeutic benefits in medicine. NPs synthesized by green technologies show significant cytotoxic and anticancer properties against cancer cells, making them interesting candidates for cancer therapy [9][10][11].

NPs typically produced during physical and chemical processes, produce dangerous by-products that pose an environmental risk. Also, due to health considerations, these particles are not suitable for medical applications, especially in clinical areas [12]. Bionanotechnology serves as a cost-effective alternative to the chemical and physical techniques used in the production of NPs. The biological synthesis of NPs involves the chemical reduction of bulk materials, that does not require additional energy and avoids the use of organic solvents and toxic chemicals that contribute to the toxicity of NPs [13]. The diverse natural resources required for the green synthesis of NPs include bacteria, plants, yeasts, algae, fungi, viruses, or their by-products, including lipids and proteins, facilitated by a variety of biotechnological methods. Consequently, nanobiotechnology is a possible alternative method for the creation of biocompatible, stable NPs. Numerous scientists utilize cyanobacteria for the synthesis of NPs (Pd, Au, Pt and Ag) either intracellularly [14].

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Research indicates that AgNPs can elicit significant cellular damage, pro-inflammatory responses and oxidative stress in human pulmonary epithelial cells [15]. Some research indicate that AgNPs can induce in vitro liver damage in rats. Nevertheless, there is scant evidence regarding their harmful side effects [16]. Nano-materials cause cellular toxicity by generating reactive oxygen species (ROS) and releasing cytokines [17]. In biological systems, ROS are generated and eliminated by both exogenous and endogenous antioxidants. Elevated generation of ROS can produce oxidative damage to DNA and trigger apoptotic cell death [18]. Apoptosis is triggered by a cascade of sequential events involving caspases, a group of proteins belonging to the protease family [19]

Numerous researches have examined the ability of probiotics to influence the intestinal microbial environment and inhibit pathogenic gut bacteria [20]. Probiotics promote the growth of beneficial microorganisms in the gut and may exert an immunomodulatory effect through the bioactive substances synthesized by these beneficial organisms [21]. Plus, a bunch of studies have evaluated the therapeutic efficacy of lactic acid bacteria in liver fibrosis and cirrhosis, revealing that certain probiotic strains inhibit the progression of liver fibrosis [22].

Methodology

The method of this study had an experimental in vivo design intended to assess the biochemical and histological effects of biosynthesized silver nanoparticles from Lactobacillus rhamnosus. Abstract Silver nanoparticles were biologically synthesized from silver nitrate as a precursor using an activated culture of a Lactobacillus rhamnosus, previously identified by the Vitek 2 compact system. Bacterial biomass was collected and suspended in deionized water and the suspension was progressively added to aqueous solutions of silver nitrate to form the nanoparticles at a relatively low temperature (25°C) and stirring until a characteristic brown color indicated nanoparticle formation. Wistar albino rats were used as an experimental model, as they are physiologically adapted for toxicological and biochemical studies, and female Wistar albino rats were selected for work. Under a standardized housing environment, animals were acclimatized and randomly assigned to 2 groups as control and treatment groups. The experimental group was continuously treated with biosynthesized silver nanoparticles for 30 days, while controls were kept free of exposure. After overnight fasting, blood samples were taken at the end of the experimental period, under anaesthesia, and serum was separated for the biochemical investigation of liver enzymes (ALT, AST and ALP) and of vitamin B12. To evaluate structural integrity and possible tissue changes, liver tissues from the experimental groups were excised, fixed in buffered formalin and prepared for histopathological examination. The statistical analyses of all biochemical and histological data were performed using SAS software for Windows (SAS Institute Inc; 2003, version 9.1), following the least significant difference test at significance level of $p \leq 0.05$ between groups. This combination of techniques allowed the systemic and hepatic safety profile of silver nanoparticles biosynthesized by the Saccharomyces cerevisiae cell-free extract to be evaluated crisply in an integrated manner.

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Results and Discussion

Recent experimental results have confirmed the efficient biosynthesis of silver nanoparticles by Lactobacillus rhamnosus that shows high efficiency in converting silver nitrate into silver nanoparticles with an average size of like 50.47 nm. These results are consistent with Naseer who efficiently produced 30–100 nm silver nanoparticles using Lactobacillus bulgaricus [23].

Koul They emphasize that unlike traditional physical or chemical synthesis methods microbial biosynthesis of NPs represents a faster safer and more environmentally friendly alternative. Various microorganisms such as bacteria fungi yeasts microalgae and viruses have been studied for nanoparticle synthesis [24].

Recent focus has been directed on specific bacterial and microalgal species for their capacity to synthesis metals, metal oxides, and other nanostructured materials through intracellular and extracellular processes, yielding nanomaterials with distinctive and advantageous properties.

This research aims to synthesize AgNPs using a safe and inexpensive biochemical method, employing Lactobacillus rhamnosus bacteria, instead of harmful chemical or physical methods. The results will be verified by evaluating their effects on body weight, liver enzymes and vitamin B12 in rats.

Materials and Methods

Biological synthesis of silver NPs using L. rhamnosus

This study aimed at the possibility of using the bacterium L. rhamnosus as a biological agent to reduce silver nitrate to silver nanoparticles according to the following steps.

The bacteria L. rhamnosus

The bacteria, L. rhamnosus, were obtained for molecular identification by the Vitek 2 compact method at Al-Ameen Center for Research and Advanced Biotechnology in Al-Ataba Al-Alawiya - Najaf.

Development and activation of L. rhamnosus

On solid MRS agar medium, which was created by dissolving 70 g of powder in 1 L of distilled water and then bringing the pH level to 7, bacteria were grown and activated as per the manufacturer's instructions, then sterilizing in an autoclave at a temperature of 121 °C and under a pressure of 1 atmosphere for 15 mins, then pouring into Petri dishes, and then Bacterial culture was reported by Harley and Prescott [25].

Preparation of L. rhamnosus bacteria solution

The bacteria solution was prepared according to the method mentioned by Saifuddin with some modifications, as 10 ml of deionized water was added to each Petri dish, and the bacteria were harvested by scraping the top layer and using a sterile razor, and then the solution containing deionized water was collected with the bacteria in Opaque cans until use in the manufacture of nano.

Prepare a silver nitrate solution

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Labanni followed the method of preparing a silver nitrate solution with a concentration of 1 mM (0.169 mg) and 2 mM (0.340 mg) of silver nitrate in 1 liter of deionized water under dark conditions, covering the glass beaker with commercial aluminum (silophone) and keeping the solution. In a dark way until use [26].

*Synthesis of silver nanoparticles mediated by the bacterium L. rhamnosus

The method of Saifuddin was followed with some modifications for the manufacture of silver nanoparticles by bacteria, as the manufacture was done by taking 10 ml of the bacteria solution and adding it in the form of drops to 90 ml of the silver nitrate solution with a silver nitrate solution placed on a stirrer Magnatic for 30 minutes at a temperature of 35 °C, after which the color change is observed, which is a first sign of the formation of AgNPs [27].

The bacteria proved effective in reducing silver nitrate and converting it into AgNPs, as the solution gradually changed color to brown within 120 hours, which is evidence of particle formation.

Experimental Animals

Twelve female Wistar albino rats, weighing 250–300 g and aged 14 weeks, were used in this study. It was divided into two groups. The animals were obtained from the Animal House, College of Pharmacy, University of Karbala. They were housed in plastic cages with metallic lids and wood shavings as bedding, which was replaced periodically. Standard pellet diet and water were provided ad libitum. Animals were maintained under controlled environmental conditions of proper ventilation, a temperature of (25 ± 2) °C, and a 12 h light/12 h dark cycle. An acclimatization period of two weeks was allowed before the commencement of the experiment. The second group was then inoculated with silver nanoparticles produced by L. rhamnosus bacteria for 30 days.

Blood Collection and Serum Preparation

After a 12-hour fasting period the animals were anesthetized by inhalation of chloroform with a cotton pad soaked in anesthetic and placed in a sealed container. After confirmation of anesthesia approximately 5 ml of blood was taken directly from the heart by cardiac puncture.

Blood samples were transferred into gel tubes designed for biochemical assays to obtain serum for the analysis of liver enzymes (Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT)) and vitamin B12. Serum was isolated using centrifugation at 3000 revolutions per minute for 15 minutes. The supernatant was carefully transferred into clean, labeled Eppendorf tubes and stored at –20 °C until further biochemical analysis [28].

Tissue Preparation

Histopathological analysis

After blood collection, the animals were dissected to obtain the required tissues. The liver was excised, gently rinsed with saline solution to remove blood residues, dried using filter paper, and cut into small longitudinal and transverse pieces. Tissue samples were

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placed in clean, tightly sealed containers filled with 10% formalin solution and stored until histopathological examination.

Statistical Analysis

The data were processed with SAS software, and the findings were analyzed using the LSD at a probability level of 0.05.

The Results and discussion

We observed in the results of our 30-day experiment shows a significant decrease in ALP, ALT and AST in AgNPs -treated group compared to the control group (G1) (Table. 1).

Our findings indicate that the biosynthesis of AgNPs using Lactobacillus rhamnosus bacteria did not cause the problems associated with conventional nano-extraction. Liver enzyme (AST, ALT and ALP) levels decreased significantly, there was non-significant difference in body weight in the experimental rats and vitamin B12 levels increased significantly.

Table (1) Effect of Biosynthesized Silver Nanoparticle Solution in Female Rats on ALT, ALP , AST , B12 and Bady wight

S.E ± Means					
groups	**Bady wight**	**ALP**	**ALT**	**AST**	**B12**
control	279.83 ± 5.40 A	289.00 ± 3.81 A	39.50 ± 1.05 A	170.33 ± 0.71 A	1107.17 ± 15.43 B
AgNPs with *Lactobacillus rhamnosus* bacteria	286.00 ± 4.38 A	274.50 ± 2.43 B	37.83 ± 0.75 B	131.33 ± 5.04 B	1229.83 ± 51.70 A
L.S.D 0.05	**15.506**	**10.617**	**2.5233**	**12.765**	**120.23**

* P value Significant ≤ 0.05

Histological examinations of the treated group revealed normal liver tissue architecture. The central vein appeared normal, and the hepatic cords, liver cells, and their nuclei were well-organized, with the presence of normal sinusoid, showed Figure (2). Compared to G1, there is non-significant difference, showed Figure (1).

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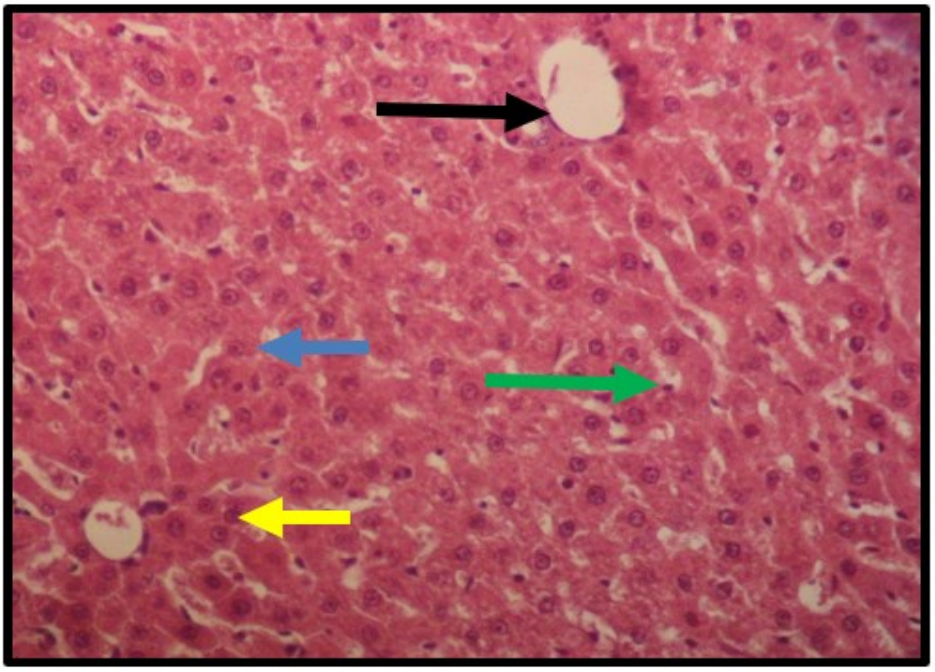

Figure (1): A cross-section of the rat liver tissue of the control group it is noted that there is a normal central vein ( ) and the organization of the hepatic cords ( ) and the liver cells and their nuclei ( ) with the presence of normal sinusoids ( ) (E & H 200X).

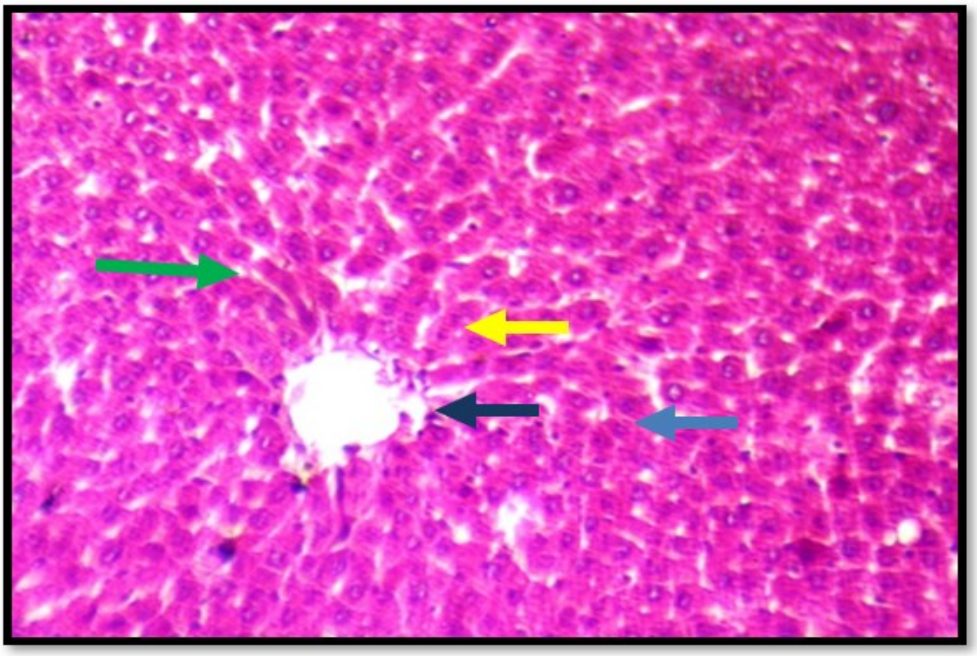

Figure (2): A cross-section of the rat liver tissue of the treated group. It is noted that there is a normal central vein ( ) and the organization of the hepatic cords ( ) and the liver cells and their nuclei ( ) with the presence of normal sinusoids ( ) (E & H 200X)

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In vitro studies demonstrate the cytotoxicity of AgNPs and indicate their ability to induce oxidative stress in human liver cells. augment Deoxyribonucleic Acid damage by the generation of ROS [29].

Blanco demonstrated that the oral treatment of AgNPs increases Oxidative stress Disorder in the livers [30]. These nanoparticles enhanced catalase and superoxide dismutase activity in the livers. Moreover, AgNPs at a concentration of 200 mg/kg increase the generation of ROS, resulting in cellular damage [31]. Furthermore, Ali examined the possible effects of AgNPs on Deoxyribonucleic Acid damage and apoptotic cell death in mice under in vivo settings. AgNPs have been demonstrated to significantly elevate symptoms of liver injury, including increased levels of ALP, ALT, and AST enzymes. These particles induced hepatocyte apoptosis and DNA fragmentation in lymphocytes [32][33].

Furthermore, Teodoro demonstrated that prolonged exposure to minimal dosages of AgNPs can lead to alterations in liver mitochondrial activity in rats [34]. Susan also observed that the varying impacts of NPs, based on their diameter and dispersion in bodily tissues, were directly correlated. AgNPs free radicals target hepatocytes, releasing stored ATP into the bloodstream. In reaction to this exogenous stimulus, mice exhibit an increase in white blood cell count to engulf the AgNPs [35].

Guo discovered that intravenous administration of AgNPs leads to kidney and liver toxicity by reducing endothelial junctions associated with intracellular reactive oxygen species.

Furthermore, Teodoro showed that long-term exposure to low-dose AgNPs can lead to changes in liver mitochondrial activity in mice. Susan also noted that the differential effects of nanoparticles are directly related to their diameter and distribution in body tissues. AgNPs free radicals target liver cells and release stored ATP into the bloodstream. In response to this external stimulus, the number of white blood cells needed to ingest AgNPs increased in the mice [36].

According to a recent study, NPs can enter cells and disrupt their structure and biological functions by generating ROS or increasing intracellular oxidative stress.

Nanoparticle-induced reduction of aspartate aminotransferase and ALP activity may result from affinity-related disruption of AgNPs to thiol (eSH) groups, that alters the functional state of proteins.9 In a landmark study by Abbas showed that AgNPs inactivate aminotransaminases [37]. Disruption of proteins or enzymes can affect critical metabolic reactions, with serious consequences for cellular integrity. Elevated levels of ALT and AST in the blood can lead to cell damage, especially in the liver [38][39][40].

Lactobacillus rhamnosus previously considered a subspecies of L. casei has now been classified as a separate species [41]. L. rhamnosus is a well-researched probiotic. A recent study showed that L. rhamnosus GG prevents fibrosis in a liver cirrhosis model induced by bile duct ligation.

Treatment with Lactobacillus rhamnosus resulted in significant reductions in liver inflammation, injury and fibrosis, that reduced liver bile acids (BA) in mice. This

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treatment modified the gut microbiota and was associated with increased BA deconjugation and greater BA excretion in feces and urine. a bunch of studies suggest that L. rhamnosus is effective in reducing lannin aminotransferase A and aspartate aminotransferase levels in models of alcoholic liver disease [42].

Treatment with silver nanoparticles induced different changes in the cellular structure of liver and kidney tissues in mice than in the control group. Cell degeneration and inflammation induced by nanoparticles demonstrate their ability to induce biochemical changes that lead to cell damage. Recent studies show that nanoparticles can accumulate in tissues and thus cause cellular damage [43].

Plus silver nanoparticles have been observed to contribute to an increase in reactive oxygen species associated with apoptosis deoxyribonucleic acid damage and necrosis [44]. Previous research has suggested different cytotoxic mechanisms of AgNPs including disruption of the mitochondrial respiratory chain leading to ROS production and inhibition of adenosine triphosphate synthesis that subsequently leads to deoxyribonucleic acid damage [45]. impairment of essential macromolecules containing phosphorus and sulfur such as proteins and Deoxyribonucleic Acid [46], and interaction with thiol-rich enzymes, causing cellular damage or hepatocyte death [47].

Nonetheless, the application of silver nanoparticles with Lactobacillus rhamnosus bacteria resulted in normal liver tissue, devoid of any histological abnormalities, in comparison to the control group. Damage to essential phosphorus and sulfur containing molecules such as proteins and deoxyribonucleic acid and interaction with thiol-rich enzymes causing cellular damage or hepatocyte death.

However the use of Lactobacillus rhamnosus silver nanoparticles resulted in normal liver tissue that was free of histological abnormalities compared to G1.

This contradicts the issues previously noted when silver nanoparticles were utilized without Lactobacillus rhamnosus bacteria.

In conclusion findings indicate that the biosynthesis of AgNPs using Lactobacillus rhamnosus bacteria did not cause the problems associated with conventional nano-extraction. AST, ALT and ALP levels decreased significantly, there was no significant difference in body weight in the experimental rats and vitamin B 12 levels increased significantly.

No hazardous accumulation accumulation of silver was observed in the body tissues of rats treated with the AgNPs solution, although the small amounts used and the 30-day treatment period may explain this. However, a longer duration could lead to a significant accumulation, especially over many years; therefore, we recommend studies consisting of longer-term experiments.

Conclusion

Overall, the results from this study confirm that Lactobacillus rhamnosus biosynthesized silver nanoparticles possess a good biochemical & histological safety profile in female Wistar albino rats. The treatment resulted in significantly decreased liver enzymes (ALT, AST, and ALP), significantly increased vitamin B12 levels, and only a minimal effect on

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body weight, showing no systemic toxicity. Histopathological examination strongly supported the normal liver architecture along with an absence of cellular degeneration and inflammatory changes confirming the biocompatibility of the biosynthesized nanoparticles. Our results demonstrate that the use of probiotics for green synthesis of nanoparticles may serve as a safer alternative to traditional chemical and physical methods of nanoparticle production for biomedical and therapeutic applications. The conclusion can be drawn from the study that the silver nanoparticles derived from L. rhamnosus may abstain the general oxidative and hepatotoxic effects of AgNPs reported for chemically synthesized AgNPs. However, due to the short exposure duration and small dose range, additional studies are needed in which these and other biochemical properties are explored after prolonged administration, potential bioaccumulation, dose–response relationships, and mechanistic pathways, including examination of other organs and functional systems.

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