RESEARCH ARTICLE

Green Synthesis of Silver Nanoparticles *via* Black and Green Tea and Study its Toxicity on few Vital Organs of Female Mice

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ABSTRACT

This study included synthesizing silver nanoparticles (AgNPs) in a green method using AgNO3 solution with glucose exposed to microwave radiation. The prepared NPs were also characterized using ultraviolet and visible (UV-vis) spectroscopy and scanning electron microscopy (SEM). The UV/vis spectroscopy confirmed the production of AgNPs, while SEM analysis showed that the typical spherical AgNPs were 30 nm and 50 nm in size for the NPs prepared using black tea (B) and green tea (G) as reducing agent, respectively.

The changes in some of the biochemical parameters related to the liver and kidneys have been analyzed to evaluate the probable toxic effects of AgNPs. 40 adult male mice were included in this study.

To assess the probable health effects of the prepared AgNPs, an experiment has been designed that includes 40 adult mice, and it was randomly divided into three groups as follows: Group I (C): The control group consisting of 10 animals was injected intraperitoneally with PBS for 15 days. Second (G): included 10 animals who were injected with 0.1 mL of (2) mg/kg (G) intraperitoneally for 15 days. Group B included 10 animals injected with 0.1 mL of (2 mg/kg) (B) solution intraperitoneally for 15 days. Changes in some biochemical parameters related to the liver and kidneys were analyzed to assess potential toxic effects on the function of these vital organs.

The serum levels of alanine aminotransferase (ALT)(IU/L), alkaline phosphatase (ALP) (IU/L), and cholesterol (mg/dL) as parameters of liver function were analyzed, and the serum levels of uric acid, creatinine, and urea (mg/dL) were analyzed as parameters of kidney function. The results revealed that no significant changes occurred in organ weight in groups treated with AgNPs compared with the control. However, the results showed a significant increase in ALT enzyme level in the third group compared with its level in the control group. Meanwhile, there were no significant differences in ALT level between the second group and the control. The level of the anaplastic lymphoma kinase (ALK) enzyme was significantly increased in both groups (2 and 3) in comparison with its level in the control group. No significant differences were found in cholesterol levels between the groups. Also, there were no significant differences found in uric acid levels between the groups. At the same time, creatinine level increased significantly in group 2 (where the NPs prepared using Black tea as a reducing agent) compared to its level in the control group. Urea level increased significantly in both groups (2 and 3) in comparison with its level in the control group. We conclude that the submission to AgNPs causes toxicity in the liver and kidneys.

Keywords: Silver, Nanoparticles, Black tea, Green tea, Synthesis, Characterization, Toxicity.

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INTRODUCTION

Nanoparticles (NPs) are widely used in various aspects of modern life due to their unique properties such as their small size and high surface area, making them very irritable and harmless and can cause injury to human and animal cells by the mechanism of oxidative stress.[1]

Many publications demonstrated that NPs have relatively more toxicity than particles of large sizes because NPs are highly reactive and cause oxidative stress in humans and animals.[2]

The science of the synthesis of NPs has augmented wide interest in current times, and the latest developments have included the production of nanoparticles using environmentally friendly methods called green synthesis. Also, physical and chemical methods can be used to manufacture NPs. These require the usage of strong and weak chemical-reducing agents

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and protecting agents such as sodium borohydride, sodium citrate, and alcohol. Often, these agents are toxic and flammable and cannot be easily removed due to ecological problems with low production rates.[3,4]

The most popular methods used to synthesize AgNPs include chemical reduction by various organic and inorganic reducing agents. Most traditional methods of producing NPs require expensive chemicals and have dangerous effects. Therefore, researchers have used green methods to produce nanoparticles, as they are economical, clean, and environmentally friendly. These methods included using plant extracts as reducing agents in the green synthesis of AgNPs. On the other hand, biological methods can reduce the toxic effects of NPs in the human body.[5]

Silver NPs are one of the most attractive considerable kinds of metal NPs because of their marvelous physical (optical, electrical, and magnetic) properties, which can be incorporated into antimicrobial uses, bio-sensing materials, composite fibers, cryogenic high-efficiency conductive resources, beautifying products, and electronic parts.[6]

The current study evaluated the toxicity level of AgNPs prepared using black and green tea extracts.

MATERIALS AND METHODS

Synthesis AgNPs by Black and Green Tea Potion

To prepare 50 mg L^{-1} of black tea, put 1.5 g of black tea in 250 mL of boiled distilled water, 60 mL of the solution was withdrawn by a pipette and diluted to 500 mL. Adjust the pH of the solution to 10 by adding a small amount of potassium carbonate. The same method used to prepare green tea by dissolving 1.3 g in 200 mL instead of 1.5 g and then placing it in the microwave for 60 seconds to speed up the reaction. An individual $AgNO_3$ stock solution was also prepared by dissolving 54 mg in 200 mL of distilled water.

Characterization of Nanoparticles

The UV-visible absorption spectra were measured at 200–1200 nm with a spectrophotometer that used an easy method to estimate the diameter and shape of nanoparticles. The UV-visible absorption spectra revealed an observable shift in the LSPR bands of AgNPs. Scan electron microscope (SEM) was used to determine the size and shape of nanoparticles found about 30 and 50 nm for using black and green tea, respectively.[7]

Experiment Design

Forty adult mice aged 6 to 8 weeks were conditioned; Weighing between 25–30 g, they were placed in the animal house of the Biotechnology Research Center, Al-Nahrain University in separate cages at room temperature (25°C) and provided with an adequate diet in addition to water. The standard ethical procedures of the ethics committee of the College of Science and Research, University of Baghdad, in the care and use of laboratory animals are followed.

The animals were randomly divided into three groups as follows:

*First group (C): (The Control group) includes 10 animals that were injected intraperitoneally with PBS for 15 days.
*Second group (G): include 15 animals were injected with 0.1 ml of (2 mg/kg) (G) solution intraperitoneally for 15 days.
*Third group (B): include 15 animals were injected with 0.1 ml of (2 mg/kg) (B) solution intraperitoneally for 15 days.
The solution concentration was determined according to the work of Sardari *et al*., 2012).[8]

Serum

Serum samples were obtained after collecting blood from the animals using a heart puncture and left to coagulate for at least 20 minutes before being subjected to centrifugation. The serum samples were then stored at -20°C.

Determination of Liver Function Tests and Liver Function Tests

The serum levels of ALT (IU/L), ALK, and Cholesterol (mg/dL) were analyzed by kits (Cloud-clone corp. / USA). The biochemical tests were quantified using the manufacturer's protocol.

Determination of Kidney Function Tests

The serum levels of uric acid, creatinine, and urea (mg/dL) were analyzed by kits (Cloud-clone corp./ USA). The biochemical tests were quantified using the manufacturer's protocol.

Statistical Analysis

Version 21 of the IBM SPSS computer program was used to achieve statistical treatments. The statistical differences between groups were analyzed using the means of the ANOVA table. The data are stated as (mean ± SE). A *P-value* of 0.05 was reflected as statistically significant.

Results and Discussion

AgNPs synthesized by green tea reduced $AgNO_3$ by the activity of their content of catechins and flavanols, and we can expect that all these substances are working as reducing and capping agents for NP synthesis based on black and green tea. The mechanisms of the same molecules that provide antioxidant stuff can stimulate the reduction of Ag^+ ions to Ag atoms.[9] Green and black tea have been used to prepare AgNPs as reducing agents, the conception of H is the main mechanism since polyphenol has OH groups.[10] The extraction procedure from dried black and green tea yielded a greenish liquid which has been observed spectroscopically.

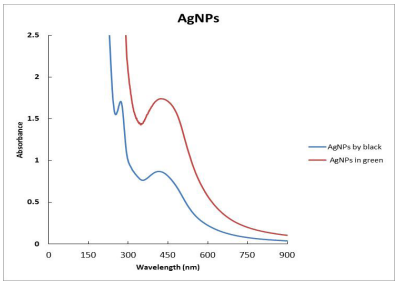

Figure 1: UV–vis absorbance for AgNPs synthesized by black and green tea

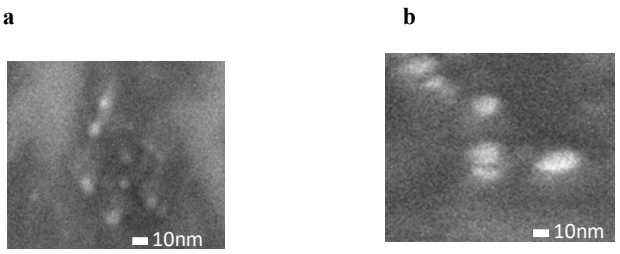

Figure 2: SEM image of the a) AgNPs synthesized by green tea b) AgNPs synthesized by black tea

The presence of AgNPs was confirmed by UV spectra. The sample absorbance reached a peak in the range of 409 nm and 419 nm for black and green tea, respectively. The reduction of silver ions in AgNPs was determined by the change of color of the silver nitrate solution to brown within 1 minute in the microwave while the color of the control $AgNO_3$ solution (without leaf extract) did not change.

During samples preparation of AgNPs, adding the plant extract accompanied by a change in visual color indicates the start of the reduction reaction. At first, the color of the reaction was yellowish after the reaction started color became dark. The color change is strongly indicated the formation of AgNPs. In Figure 1, the spectra show a pronounced peak around 400nm, as expected.

Figures 2 a and b presented that AgNPs were about 30 and 50 nm in size for the black and green tea synthesized AgNPs, respectively.

The results revealed that non-significant changes occur in organ weight in both groups treated with AgNPs compared with the control (Table 1). Our findings are in contrast with the results of previous studies. The non-significant change in organ weight between the groups can be explained by the short time of exposure to AgNPs.

The results of Almansour *et al*., 2015[11] study highlighted the importance of the shape and size of the nanoparticles in the intensity of the effect and found that the smaller particles have a greater effect than the larger ones, when exposing healthy adult male mice (BAL/C) to 10, 20, 40, 60 and 100 nm of AgNPs for 35 days caused an insignificant decrease in average weight, a significant decrease in food consumption, an increase in water intake, unilateral blindness, tanning and cholestasis with a decrease in the relative weight of the liver, kidney, and spleen concerning the body. It was also found that

Table 1: Effect of AgNPs (prepared by black and green tea as a reducing agent) in organ weight (g) of the White mice

Groups	*Mean ± SE*		
	Liver	*Spleen*	*Kidney*
G1/Control	1.29 ± 0.03 a	0.216 ± 0.01 a	0.136 ± 0.02 a
G2/B	1.30 ± 0.05 a	0.233 ± 0.03 a	0.133 ± 0.003 a
G3/G	0.796 ± 0.30 a	0.240 ± 0.06 a	0.136 ± 0.003 a
LSD value	0.615 NS	0.154 NS	0.036 NS

NS: Non-significant.

Table 2: Effect of AgNPs (prepared by black and green tea as a reducing agent) in liver functions of the white mice

Groups	*Mean ± SE*		
	ALT (IU/L)	*ALK (IU/L)*	*Cholesterol (mg/dL)*
G1/Control	15.33 ± 1.76 b	112.67 ± 3.48 c	132.33 ± 6.11 a
G2/B	17.50 ± 0.99 b	193.67 ± 4.62 a	128.33 ± 2.96 a
G3/G	36.50 ± 2.32 a	147.25 ± 4.58 b	134.00 ± 3.02 a
LSD value	5.300 *	15.491 *	12.184 NS

*($p<0.05$), NS: Non-significant.

mice exposed to 10 and 20 nm were more susceptible than mice that received larger nanoparticles.

Organ weight is one of the most sensitive indicators of toxic agents that reflect an impact on the metabolism due to effects on the health and immunological status of the body.[12]

In another study in which mice were administrated by oral repeatedly with doses of 30, 300, and 1000 mg/kg of AgNPs (60 nm) in carboxylmethylcellulose for 28 days, it was found that the nanoparticles did not produce any obvious changes in body weight. As for tissue accumulation, it depended on the size of the dose, and the reactions related to genotoxicity did not appear in all groups.[13]

The results in Table 2 showed a significant increase in ALT enzyme level in G3 (where the NPs prepared using green tea as a reducing agent) in comparison with its level in the control group, while there are no significant differences in ALT level between G2 and control. The enzyme ALK level significantly increased in both groups (2 and 3) compared to its level in the control group. No significant differences were found in cholesterol levels between the groups (Table 2).

The liver is one of the vital organs most vulnerable to nanoparticles by its function to rid the body of toxins and metabolize drugs. The changes in the level of liver enzymes may indicate an organ's attempt to eliminate the stress imposed on it.[14] In previous reports, it was found that the most common target organs for AgNPs are the liver and lungs in a 90-day inhalation study.[15,16]

A study by Kim *et al*. (2008)[17] aimed to investigate the toxicity and geno-toxicity of AgNPs with an average size of 60 nm and to investigate the accumulation and distribution of these particles in the reproductive tissues of rats. The animals were given 30, 300, or 1000 mg/kg of AgNPs suspended in carboxy-methylcellulose. The results of the experiment showed that there is a significant change in body weight as well as significant dose-dependent changes in the values of alkaline phosphatase and cholesterol in either male or female rats. It will be concluded from the experiment that exposure to levels greater than 300 mg/kg of AgNPs may lead to minor damage to the liver.

Park *et al*. (2010a)[18] found that dosing mice at a dose of 1 mg/kg for 28 days induced biochemical changes characterized by elevated levels of liver enzymes) alkaline phosphatase and aspartate transaminase (in both male and female mice, as well as elevated levels of alanine transaminase after taking a high

Table 3: Effect of AgNPs (prepared by black and green tea as a reducing agent) in kidney functions of the White mice

Groups	*Mean ± SE*		
	Uric acid	*Creatinine*	*Urea (mg/dL)*
G1/Control	3.90 ± 0.11 a	0.276 ± 0.03 b	30.33 ± 2.02 c
G2/B	3.95 ± 0.07 a	0.478 ± 0.04 a	50.67 ± 1.80 a
G3/G	3.75 ± 0.31 a	0.315 ± 0.04 b	39.50 ± 0.64 b
LSD value	0.590 NS	0.144 *	5.614 *

* ($P < 0.05$), NS: Non-significant.

dose but only in Female mice. While Hussain *et al*., 2005[19] showed that AgNPs with a size ranging from 15 to 100 nm at doses ranging from 10 to 50 mg/mL caused an increase in the level of the enzyme lactate dehydrogenase (LDH) as evidence of acute liver toxicity in the immortalized mice (BRL 3A), A dose greater than 5 µg/mL also showed a positive result on the mitochondrial function assessment (MTT- test).

After the intestine absorbs the AgNPs,it binds to the plasma proteins and travel by the blood to numerous tissues and organs of the body such as the liver, kidneys, heart, lymph nodes, brain, lung, stomach and testicles. AgNPs enter the liver through the portal vein and because it acts as a key checkpoint for everything that absorbs and effectively removes compounds from the blood and converts them into chemical forms that can be easily excreted, so it makes sense that the liver is one of the first organs affected.[20,21]

AgNPs cause physiological changes in liver function characterized by elevated serum levels of ALT, AST, and ALP enzymes, which are indicative of liver damage in male and female mice given AgNPs that may be caused by severe disruption of the oxidative system in these cells. The intensity of the physiological changes depends on the size of the particles, the smaller their size, the more side effects they have.[22]

No significant differences were found in Uric acid levels between the groups (Table 3). At the same time, creatinine level increased significantly in group 2 (where the NPs prepared using black tea as a reducing agent) compared to its level in the control group. Urea level increased significantly in both groups (2 and 3) compared to its level in the control group.

AgNPs that are taken orally are accumulated in the membranes of the renal glomeruli.[23] Patho-morphological changes have been observed in the tissues of organs such as the spleen (shrinking the area of the red pulp and white pulp expansion) and the kidneys (necrosis) in the 30 days dosed mice with 1–2 mg/kg of AgNPs (70 nm).[8]

Taking low doses of silver salts for a long time causes chronic symptoms, including fatty relapse in the liver and kidneys, alterations in some blood indicators,[24] and a deterioration in kidney function, expressed through a significant increase in the level of creatinine and N-acetyl-bD glucosaminidase (NAG), which are symptoms common in poisoning associated with some types of heavy metals such as cadmiu.[25]

CONCLUSION

In all the previous results, it is noted that the presence of tea extract with nanoparticles has reduced its harmful effects on liver and kidney functions, and this may be attributed to the presence of chemical compounds that may bind with the nanoparticles and reduce their harm.

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