Green Synthesis, Lead Toxicity and Pharmacokinetic Evaluation of Sumac Silver Nanoparticles *In Vivo*

Ammar H. Salman [1*], Mohammed Mosleh Shwaish [1]

Abstract

Sumac (Rhuscoriaria L., family Anacardiaceae) is rich in bioactive components like tannins and phenolic compounds, exhibiting antioxidant properties. Green synthesis of silver nanoparticles (SAgNP) offers eco-friendly and cost-effective production methods. In this study, the effect of Sumac nano silver particles (SAgNP) on lead pharmacokinetics was assessed. Green synthesis of silver nanoparticles involved adding 25 ml of sumac extract to 200 ml of 1 mM silver nitrate solution under hot stirring. Twenty-one male albino rats were randomly divided into three groups: lead group (60 mg/kg of lead acetate orally), crude group (100 mg/kg of crude sumac extract orally), and nano group (100 mg/kg of SAgNP orally followed by lead acetate). Results demonstrated successful SAgNP formation and significant reduction ($p \leq 0.05$) in blood lead concentration after 6 hours, suggesting SAgNP's potential in lowering lead levels. SAgNP exhibited stronger efficacy than crude extract in reducing blood lead concentrations. Green synthesis of silver nanoparticles offers a safe, cost-effective, and environmentally friendly approach with promising applications in mitigating lead toxicity. Importance of miRNAs in male infertility diagnostics and lays the groundwork for future research in this area. Developing non-invasive diagnostic techniques is crucial for effective management of male infertility.

Keywords: Sumac, Silver nanoparticles, Green synthesis, Pharmacokinetics, Lead toxicity

Significance | Sumac-synthesized silver nanoparticles (SAgNP) showed an eco-friendly approach to reduce blood lead levels, promising safer, cost-effective lead detoxification.

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Introduction

Sumac (Rhus coriaria L., family Anacardiaceae) is widely spread from the Canary Islands and over the Mediterranean area to Iran, Afghanistan, and Southeast Asia, and it is extensively cultivated in southeastern Anatolia of Turkey (Mavlyanov et al., 1997). The fruit of sumac contains many components such as hydrolysable tannins, anthocyanins, phenolic acids, and flavonols, as well as organic acids like citric, tartaric, and malic acids (Kapoor et al., 2018). Additionally, antioxidant materials found in sumac, such as tannins and phenolic compounds, are available in high concentrations. Reports suggest that sumac extract exhibits antioxidant, hypoglycemic, and antibacterial activities (Nasar-Abbas & Halkman, 2004).

Nanotechnology refers to a branch of science in which the activities of different materials occur at the nanoscale level. It is considered a promising science for the 21st century and heralds a new era of development in the human march for prosperity (Haleem et al., 2023). Nanoparticles have a large surface area-to-volume ratio due to their size, allowing them to absorb a large amount of medication in a small size. This enables them to move quickly in the blood circulation and enhance their quality in terms of mechanism, penetration, and activity, making them very useful in

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pharmaceutical applications (Ren et al., 2021). Nanoparticles are classified according to their chemical composition into organic, inorganic, and carbon-based categories (Huang et al., 2018).

There are several routes to prepare nanoparticles, including physical, chemical, and biological methods. Green synthesis routes are used to synthesize metallic nanoparticles like silver nanoparticles (AgNP) because classical chemical processes consume more energy and reagents and may be harmful and toxic compared to biological routes. Biological methods for AgNP synthesis may utilize different plant extracts, as well as chitosan, bacteria, algae, fungi, and other sources (Hasan et al., 2022). Plant-based silver nanoparticles (AgNPs) are of great interest in biomedical applications due to their antioxidant, anticancer, and antibacterial properties, as well as their environmental friendliness and cost-effectiveness (Ahn & Park, 2020). Several reports indicate that plant metabolites such as flavonoids, alkaloids, terpenes, and tannins play a role in the reduction of Ag to AgNP (Ovais et al., 2016). Furthermore, the usage of plant extract in the synthesis of AgNP results in synergistic activity due to combining the activities of both the plant extract and AgNP (Shukla et al., 2018). Subramanian et al. (2013) suggest that the concentration of water-soluble phenolic compounds affects the power reduction of plant extract because these compounds are crucial in the reduction process of Ag. Additionally, other factors influence the size, morphology, and stability of metallic nanoparticles, such as pH, temperature of reaction, type of solvent, method of preparation, nature of reducing agent, and concentration of materials (Kim et al., 2016).

Despite widespread knowledge about lead toxicity, it is still used in various industries such as water pipes, toys, household products, vehicle batteries, and paint (Alonso-Villar et al., 2021). While most cases of lead toxicity occur in dogs and cattle due to their exposure, selective eating habits, and susceptibility, other species may also be affected. Clinical signs of acute lead toxicity are primarily observed in young animals and involve the digestive and nervous systems. Subacute poisoning typically affects older sheep and cattle and is characterized by symptoms such as anorexia, rumen congestion, colic, transient drowsiness, constipation, hypertension, bruxism, hyperesthesia, incoordination, ataxia, salivation, blindness, eyelid and jaw twitching, muscle tremors, and convulsions. Chronic poisoning in cattle may lead to infertility due to embryotoxicity and low semen quality. Symptoms in dogs include gastrointestinal issues, restlessness, hysterical barking, jaw clenching, drooling, blindness, ataxia, muscle spasms, opisthotonos, seizures, and central nervous system depression (MSD MANUAL, 2023).

The aim of the study was to synthesize sumac silver nanoparticles using green synthesis and evaluate the effect of these particles on the pharmacokinetics of lead.

Material and Methods

Preparation of Sumac Extract:

Sumac fruits were obtained from the local market of Fallujah city (Al Anbar province, Iraq) and ground into a fine powder. 200 grams of ground sumac fruit were added to 800 ml of 70% ethanol in a beaker and stirred on a hot plate-magnetic stirrer for 24 hours at 37°C. The mixture was then filtered through multiple layers of gauze due to the thickness of the extract and stored at 4°C until use.

Synthesis of Sumac-silver nanoparticles (SAgNP):

Following the method described by Gur (2022), a 1 mM $AgNO_3$ solution was mixed with 200 ml of sumac extract (total volume of 25 ml). The mixture was stirred at 75°C for 48 hours until the color changed to dark. After cooling to room temperature, the mixture was centrifuged at 6000 rpm for 1 hour, washed with distilled water, and centrifuged again. The supernatant was discarded, and the SAgNP were collected in a petri dish and dried in a dryer at 25°C. The resulting SAgNP were stored at 4°C until use.

Characterization of SAgNP:

To characterize the SAgNP, three tests were conducted at the Biology, Physical, and Chemistry Analysis Center (licensed from Iraqi Chemists Syndicate No.144): UV-vis spectrum, XRD, and SEM.

Experimental Design:

Twenty-one male albino rats were purchased and allowed to adapt for fifteen days. They were then randomly divided into three groups, with seven animals in each group, and treated as follows:

Control group: orally administered lead acetate (60 mg/kg of body weight) only.

Crude group: orally administered Sumac crude extract (100 mg/kg of body weight), followed by oral administration of lead acetate (60 mg/kg of body weight) ten minutes later.

Nano group: orally administered SAgNP (100 mg/kg of body weight), followed by oral administration of lead acetate (60 mg/kg of body weight) ten minutes later.

The dose of lead acetate represented 10% of the LD_{50} of lead according to Umar et al. (2019). Blood samples were collected at time intervals of 10 minutes, 30 minutes, 1, 3, 6, and 12 hours after dosing the animals using a mixture of ketamine and xylazine to anesthetize the animals (Flecknell, 2015). All animal procedures were conducted following the guidelines of scientific research ethics of the College of Veterinary Medicine, University of Fallujah.

Blood Sample Digestion Process:

For each sample, nine milliliters of a mixture of nitric-hydrochloric acids (65% nitric acid and 37% hydrochloric acid in a 1:3 ratio) were added. The mixture was then boiled in a water bath at 95°C for 4-5 hours until all samples were completely dissolved (Uddin et al., 2016). The concentration of lead in the blood was measured using an atomic absorption spectrophotometer (AAS) in the Department of Chemistry, College of Science, University of Al Anbar.

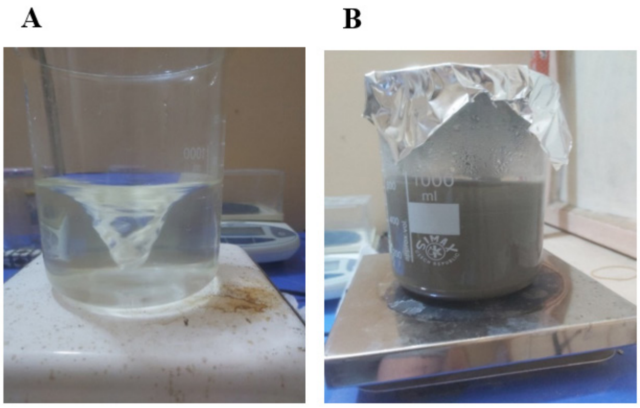

Figure 1. (A) synthesis of SAgNP in the beginning of the process, (B) synthesis of SAgNP in the ending of the process.

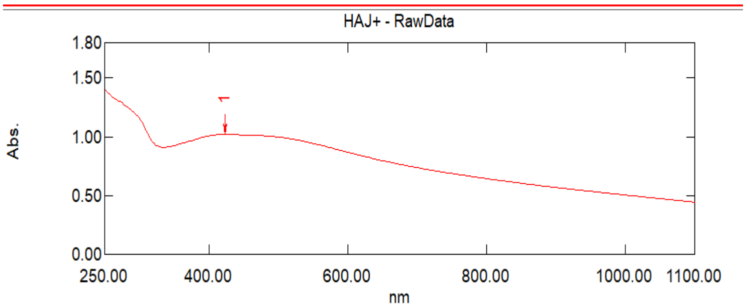

Figure 2. UV-vis of SAgNP

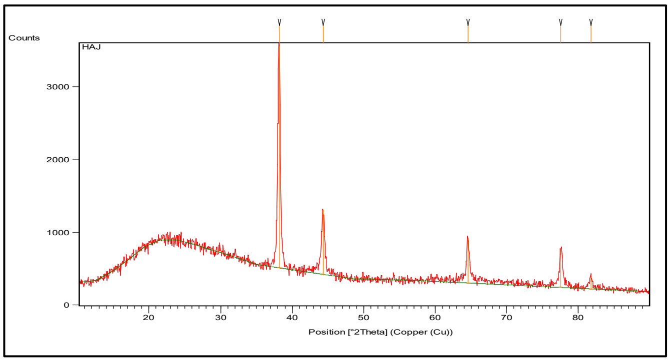

Figure 3. XRD pattern of SagNP

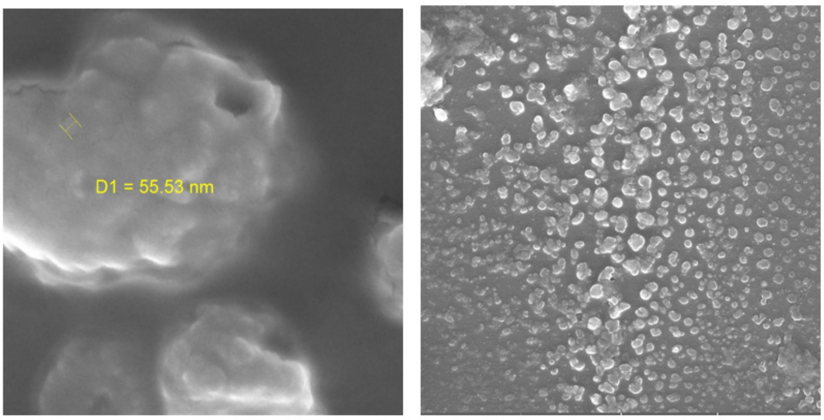

Figure 4: SEM of SAgNP (particle size and distribution)

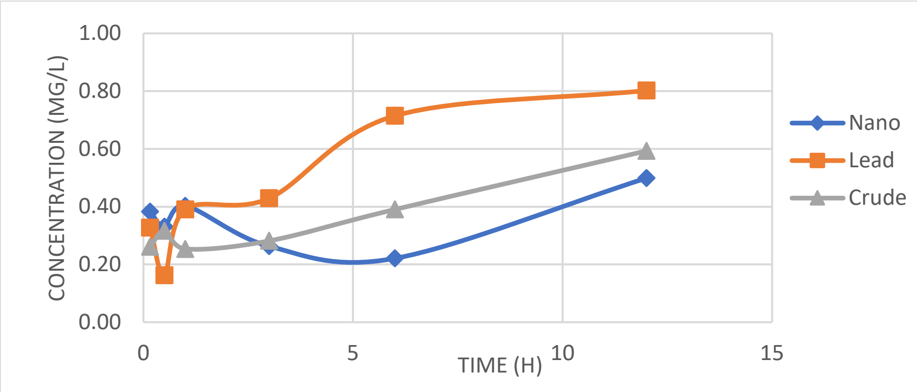

Figure 5. Concentration of lead in different treated groups

Table 1. concentration of lead (mg/l) in different treated group in rows Different litters refer to significant differences ≥ 0.05

Time (H)	Groups		
	Lead	Crude	Nano
0.16	0.33±0.12[a]	0.26±0.02[a]	0.38±0.8[a]
0.5	0.16±0.11[a]	0.32±0.02[a]	0.33±0.10[a]
1	0.39±0.08[a]	0.25±0.06[a]	0.40±0.4[a]
3	0.43±0.07[a]	0.28±0.05[a]	0.26±0.05[a]
6	0.71±0.15[a]	0.39±0.10[b]	0.22±0.05[c]
12	0.80±0.05[a]	0.59±0.05[b]	0.50±0.7[c]

Statistical Analysis:

All data were statistically analyzed using IBM SPSS Statistics V.26 with analysis of variance (ANOVA) and LSD (least significant difference).

Results and discussion

Characterization of SAgNP:

The SAgNP were characterized using UV-vis, XRD, and SEM analyses. Figure 1 depicted the color change from colorless to dark gray, indicating the formation of SAgNP.

XRD analysis confirmed the crystalline structure of the generated silver nanoparticles. Figure 3 displayed the diffraction pattern of AgNPs.

SEM results in Figure 4 revealed spherical and homogeneous distribution of SAgNP with an average size of approximately 55 nm.

Pharmacokinetics of Lead:

Lead absorption is influenced by various factors such as age, fasting, nutrition, calcium concentration, gastrointestinal tract state, and particle size, resulting in irregular absorption patterns as observed in our study (Figure 5) (Agency for Toxic Substances and Disease Registry, 2020).

Our study demonstrated that SAgNP significantly decreased the concentration of lead in blood compared to the control and crude groups, but only after 6 hours, not before. Refer to Figure 5 and Table 1 for details.

Discussions

Characterization of SAgNP:

UV-vis, XRD, and SEM analyses were employed to characterize the SAgNP. The observed color change from colorless to dark gray indicated the formation of SAgNP. This color change is attributed to the coherent oscillations of electrons in the silver nanoparticles, resonating with light waves and stimulating surface plasmon oscillations (Dubey et al., 2010).

UV-vis analysis (Figure 2) revealed a spectrum ranging from 250 to 1100 nm, with a peak at 424 nm, confirming the formation of SAgNP. This observation is consistent with findings by Zheng et al. (2018) and Jebril et al. (2020), who reported bridge and neck regions around 300 and 400 nanometers, respectively. TEM images depicted SAgNP with a diameter of approximately 55 nm, aligning with results reported by Ibrahim et al.(2020) .

XRD analysis showed characteristic peaks at 38.19°, 44.33°, 64.54°, 77.52°, and 81.76° corresponding to 111, 200, 220, 311, and 222 Bragg's reflections of face-centered cubic (fcc) crystalline silver. These results confirm the synthesis of crystalline SAgNP, consistent with findings by Sajadi et al.(2019) .

Pharmacokinetics of Lead:

Lead primarily accumulates in red blood cells (99%) and binds to various intracellular proteins. Albumin is the predominant ligand in plasma, while lead may also form complexes with other substances such as transferrin, γ-globulins, and sulfhydryl compounds like cysteine and homocysteine (Al-Modhefer et al., 1991; Bergdahl et al., 1997; Guo et al., 2014). The half-life of lead in blood is approximately 25 days (WHO, 2008), explaining the observed increase in lead levels over time.

Sumac exerts its effect through its antioxidant properties, scavenging reactive oxygen species (ROS) due to components like gallic acid, flavanols, phenolic acids, hydrolyzed tannins, anthocyanins, and organic acids (Gulbagça et al., 2022). The activity of SAgNP increased over time, possibly due to enhanced accumulation on cell surfaces, interacting and protecting cells (Rai et al., 2014). The size, shape, and concentration of nanoparticles greatly influence their activity (Periasamy et al., 2012). Research indicates that Ag-NPs disrupt bacterial cell membranes, increasing permeability and leading to cell death (Rai et al., 2014). In the nano group, lead concentrations decreased significantly after 6 hours compared to the crude group, possibly due to synergistic activity resulting from the combination of plant extract and AgNP (Periasamy et al., 2012).

Conclusion

The study revealed that SAgNP exhibited a more pronounced effect than crude extract in reducing blood lead concentrations. Moreover, the green synthesis method used for producing silver nanoparticles was found to be not only safe but also cost-effective and environmentally friendly. In conclusion, the synthesis of sumac silver nanoparticles (SAgNP) using green synthesis methods proved successful, as confirmed by UV-vis, XRD, and SEM analyses. The SAgNP exhibited a characteristic color change, crystalline structure, and spherical morphology, indicating their formation and promising properties. The pharmacokinetic study revealed that lead absorption follows an irregular pattern influenced by various factors, with lead primarily accumulating in red blood cells and binding to intracellular proteins. Sumac extract demonstrated antioxidant properties, while SAgNP showed enhanced activity over time, possibly due to increased accumulation on cell surfaces. The combination of plant extract and AgNP synergistically decreased lead concentration in blood after 6 hours, highlighting their potential for biomedical applications. Further research is warranted to explore the mechanisms underlying these effects and optimize the synthesis and application of SAgNP in lead toxicity management.

Author contributions

A.H.S., M.M.S. developed the Study design, and wrote, reviewed, and edited the paper.

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Competing financial interests

The authors have no conflict of interest.

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