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 bio-active substances such as tannins and phenolic compounds, exhibiting an antioxidant activity. The green synthesis of silver nanoparticles (SAgNP) is an eco-friendly and cost-effective method of synthesis. In the present study the effect of Sumac Nano Silver particles on lead pharmacokinetics was studied. The green synthesis of silver nanoparticles was achieved by the addition of 25 ml of sumac extract to 200 ml of 1mM silver nitrate solution under hot stirring. The male albino rats (21 no.) were taken for the experiment and divided randomly in to 3 groups, each containing 7 rats, 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 nano sumac extract orally followed by lead acetate). In the present study it was exhibited that the SAgNP were formed successfully with number of advantages and there was found significant reduction ($p \leq 0.05$) in the blood lead concentration, after 6 hours suggesting the possibility of using SAgNP to reduce the lead concentration of the body. The SAgNP were more effective rather than the crude extract to reduce the blood lead concentration. 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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1. 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 a number of components, such as hydrolysable tannins, anthocyanins, phenolic acids and flavonols, but also organic acids, such as citric, tartaric and malic acids (Kapoor et al., 2018). Furthermore, the antioxidant materials contained in the sumac, such as tannins and phenolic compounds are found in high concentrations. It is reported that sumac extracts have antioxidant, hypoglycemic and antibacterial activities (Nasar- Abbas & Halkman, 2004). Nanotechnology may be defined as the science dealing with the phenomena of the various materials at nano levels. It is a promising science for the 21st century and heralds a new era of progress 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, Chandrapratap et al. 2024, Bhuneshwari et al. 2023, Lakhan et al. 2023). 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). The rapidly increasing interest in the biomedical applications of plant based silver nanoparticles (AgNP) is due to their antioxidant, anticancer and antibacterial effects, as well as their eco-friendly and economic properties. (Ahn & Park, 2020). There are reports in the literature which indicate that the plant metabolites, such as flavanoids, alkaloids, terpenes and tannins have an important part to play in the conversion of Ag to AgNPs. (Ovais et al., 2016) Moreover the use of plant extract in the synthesis of AgNPs gives rise to a cumulative effect due to the combined effect of both the action of the plant extract and AgNPs. (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.

2. Material and Methods

2.1 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.

2.2 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.

2.3 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.

2.4 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.

2.5 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

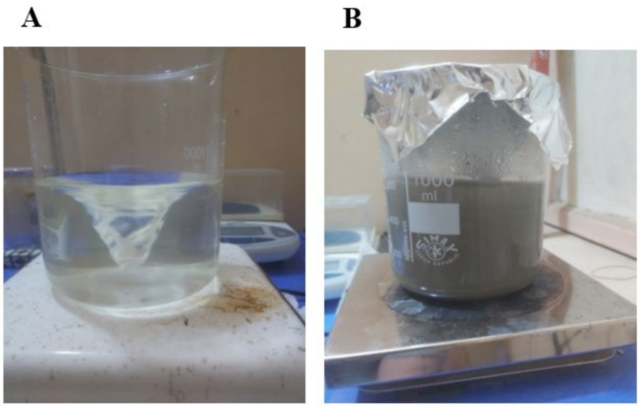

Figure 1. (A) synthesis of SAgNP in the beginning of the process, (B) synthesis of SAgNP in the ending of the process.

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.

2.6 Statistical Analysis

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

3. Results

3.1 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.

3.2 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.

4. Discussion

4.1 Characterization of SAgNP

SAgNP was characterized through UV-vis, XRD and SEM

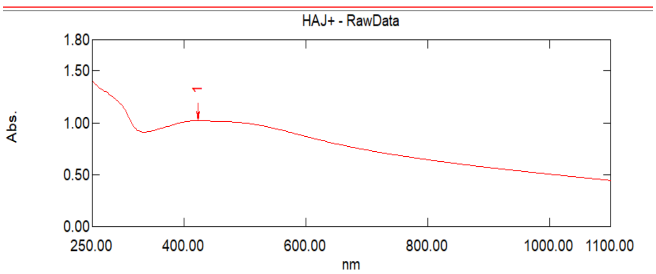

Figure 2. UV-vis of SAgNP

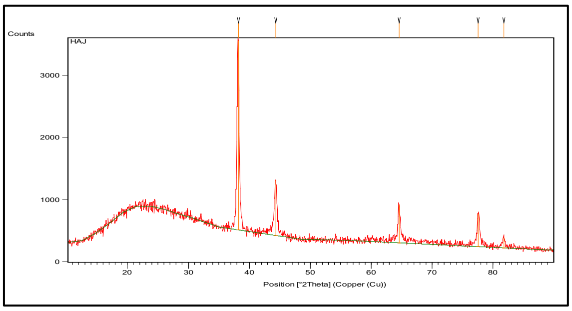

Figure 3. XRD pattern of SagNP

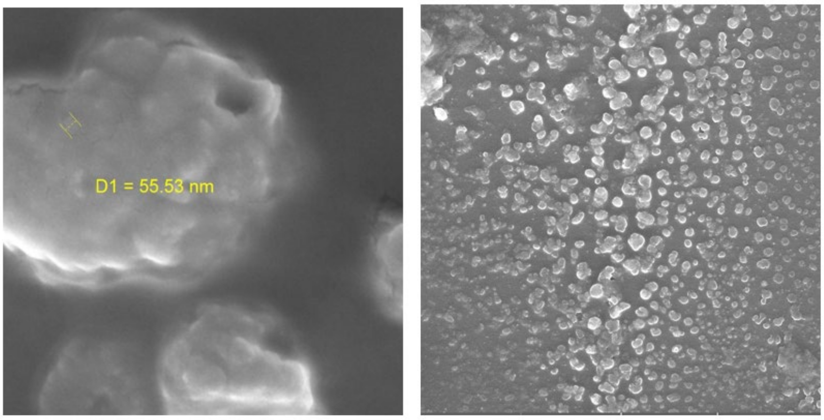

Figure 4: SEM of SAgNP (particle size and distribution)

techniques. The color change from colorless to dark gray indicated the formation of SAgNP. The color change is due to coherent oscillation of electrons present in the silver nanoparticles which are in resonance with the light waves to cause surface plamson oscillations (Dubey et al., 2010). The UV-vis analysis (Figure 2) showed the spectrum ranged from 250 to 1100 nm with a peak at 424 nm indicating the formation of SAgNP. This finding is consistent with the findings of Zheng et al. (2018) and Jebril et al. (2020) who found bridging and necking regions around 300 and 400 nanometers respectively. The TEM images have shown SAgNP with the diameter of approximately 55nm which was found to be in accord with the results obtained by Ibrahim et al. (2020). XRD analysis showed the characteristic peaks at 38.19°, 44.33°, 64.54°, 77.52°, and 81.76° which corresponded to 111, 200, 220, 311 and 222 Bragg's reflections of face–centered cubic (fcc) crystalline silver. These results confirm the formation of crystalline SAgNP and are in agreement with the results reported by Sajadi et al. (2019).

4.2 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.

Due to its antioxidant properties which sequester reactive oxygen species (ROS), sumac performs its effect by substances such as gallic

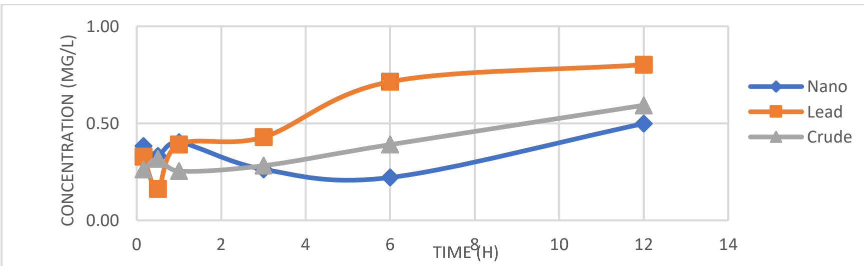

Figure 5. Concentration of lead in different treated groups

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}

acid, flavanols, phenolic acids, hydrolyzed tannins, anthocyanins and organic acids (Gulbagça et al., 2022). The activity of SAgNP increased with time of exposure, due to, probably, greater accumulation in the cell surface, interacting and protecting them (Rai et al., 2014). The size, shape and dose of the nanoparticles control their activity considerably (Periasamy et al., 2012). It was observed that Ag-NPs exert their effect by rupturing the membrane of the bacterial cells, increasing permeability, which reports in the death of the cell (Rai et al., 2014). In the group of the nanoparticles applied, it was observed that after a period of 6 h in lead, the concentrations suffered a drastic decrease with respect to the crude solution; possibly this is due to the synergy by the phenomenon which occurs in the presence of plants and AgNP (Periasamy et al., 2012).

5. Conclusion

The study showed that SAgNP had more effect than the crude extract in decreasing blood lead level. Green synthesis method used to synthesize silver nanoparticles was effective, safe and cheap as well as environmental friendly. Therefore, formulation of sumac silver nanoparticles (SAgNP) by means of green synthesis was effective which has been proved through analysis by UV-vis, XRD and SEM studies. Accordingly, SAgNP have shown characteristic color changes, crystalline character and spherical morphology in results, which accounted for the formation and commercial values of SAgNP. The pharmacokinetic studies showed that the absorption of lead is of irregular character and is influenced by many factor with accumulation of lead mainly in red blood cells with binding to intracellular proteins. The sumac extract has shown antioxidant properties, while show the SAgNP enhanced activities with time, possibly because the accumulation increased in the cell surfaces. The sum of plant extract plus AgNP has beneficially reduced the blood lead concentrations synergistically after 6 hours which indicates the modern preparation in bio medical applications. Further studies are necessary to elucidate, the mechanistic working of this and studies are also necessary for the synthesis and application of SAgNPs for the management of lead toxicity.

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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