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Original Article

Role of *Salvia officinalis* Silver Nanoparticles in Attenuation Renal Damage in Rabbits Exposed to Methotrexate

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Abstract

Nanomaterials are now considered in an extensive range of applications in various fields such as biotechnology and biomedicine. The present study aimed to investigate the protective role of *Salvia officinalis* Silver Nanoparticles (SOSNPs) as an anti-oxidant on nephrotic damage induced by methotrexate (MTX) in adult rabbits. Green silver nanoparticles were synthesized using alcoholic extract of *Salvia officinalis* (*S. Officinalis)* leaves and were characterized by UV-spectrophotometry and scanning electron microscope. The mixing of the plant extract of *S. Officinalis* with silver nitrate solution leads to the change of the reaction mixture color to yellowish within 1 h and dark brown after 8 h. For studying the protective role of SOSNPs, a total of 28 adult Wistar albino rabbits were divided into four groups and treated intramuscularly (twice per week) for 45 days as follows: T1: *S. Officinalis* (150 mg/kg B.W), T2: SOSNPs (150 mg/kg B.W); T3: MTX (0.25 mg/kg B.W) and SOSNPs (150 mg/kg B.W); T4: MTX (0.25 mg/kg B.W). Blood was collected at 0, 15, 30, and 45 days using retro-orbital sinus and cardiac puncture technique, and the serum factors including malondialdehyde (MDA), glutathione (GSH) in serum, creatinine, as well as blood urea nitrogen and uric acid concentrations were measured at the next step. The results indicated that MTX (T4) caused a case of oxidative stress by a significant decrease in GSH and MDA as well as an increase in serum creatinine, urea, and uric acid concentrations. On the other hand, the protective roles of *S. Officinalis* and SOSNPs given concurrently with MTX were clarified in T2 and T3 groups, where there was the alleviation of renal damage through the correction of the previously mentioned parameters as well as the correction of anti-oxidant status. Finally, the present study documented the anti-oxidant activity and renal protective effects of SOSNPs against the damaging effects of MTX in rabbits.

Keywords: Methotrexate, Renal Functions, *Salvia officinalis*, Silver Nanoparticles

1. Introduction

Nanoparticles are clusters of atoms ranging between 1-100 nm in size, showing different or improved physicochemical properties depending on their distribution, size, and morphology (1, 2). Nowadays, silver nanoparticles (AgNPs) are extensively used in many biological applications (3) such as drug delivery, treatment approach, diagnosis, medical device, and protocol of health care (4, 5). The biological methods for the synthesis of nanoparticles have attracted researchers' attention using the reduction and stabilization of some enzymatic or non-enzymatic agents, including enzymes (6, 7), microorganisms (8), fungus (9), as well as plant extracts (10). Green synthesis is preferable to the physical and chemical methods as it is eco-friendly and easily handled with no need for high temperatures, pressure, energy, or toxic chemicals (11, 12).

Different plant extracts were used as reducing agents in green methods such as *Nigella arvenus* (13), *Nigella*

sativa (14), *Crocus sativus* (15), *Alliumm sepa* (16), *Lantana camara* (17), *Saccharum officinarum* (18), and *Salvia officinalis* (7). The AgNPs have an extensive range of antimicrobial (19, 20), antiviral, as well as antifungal and anti-inflammatory activities (21, 22). Some studies revealed that wound dressing containing AgNPs prompted the healing of leg ulcers in humans, acting as an anti-bacterial and anti-inflammatory agent (23, 24). The anti-cancer activity of AgNPs has also been reported in the *in vitro* and *in vivo* studies (25-28). The assumption is that the highest anti-oxidant activity of green nanoparticles might be due to the preferential adsorption of anti-oxidant activity from plant extract onto the surface of the nanoparticles (19). It is noteworthy that AgNPs act as pro-inflammatory at high doses and anti-inflammatory at low doses (29).

There were few studies (30) concerning the use of *S. Officinalis* as a reducing agent in the green synthesis of AgNPs and their systemic applications. In this investigation, alcoholic extract of *S. Officinalis* leaves was used for green synthesis of AgNPs, and the hepatic and renal protective effects of *S. Officinalis* and AgNPs were studied in MTX treated rats.

2. Materials and Methods

2.1. Biosynthesis of Silver Nanoparticles Using *Salvia Officinalis* Plant

2.1.1. Collection and Preparation of Ethanolic Extract of *Salvia Officinalis*

The well-dried leaves of the *S. Officinalis* plant were collected in March 2020 from the Baghdad market in Iraq. After differential diagnosis, an amount of 20 g of *S. Officinalis* leaves has been powdered and sieved with fine mesh**.** The ethanol extracts of *S. Officinalis* were prepared by mixing 10 g of the dry sample with 100 ml of ethanol, shaken well, kept for 24 h, and then filtered by employing a Whatman filter paper No.1 (125 mm pore size). The supernatant was s filtered again using the mentioned filter paper, and heat treatment for the prepared sample was performed at 80℃ for concentrating the extract and removing the residual ethanol. Afterward, it was taken and extracted with Soxhlet apparatus using ethanol 70%, and at the next step, the extracted solvent was exposed under reduced pressure in a rotary evaporator until it became completely dry, and then it was kept at 4℃ for analysis (31).

2.1.2. Synthesis of Phyto-Silver Nanoparticles

An amount of 1500 mg of leaf extracts of *S. Officinalis* plant was added to 10 ml of 1 mM aqueous silver nitrate solution and kept at room temperature for 8 h to produce AgNPs. The changes in the solution color were measured every 1 h and for 8 h. The changes in the color intensity were noticed after reducing Ag+ to AgNPs by leaf extracts of *S. Officinalis* plants with increasing reaction time.

2.2. Separation and Identification of Silver Nanoparticles

2.2.1. Spectrophotometry

The optical absorbance of the synthesized AgNPs was performed using a UV-visible spectrophotometer (Perkin-Elmer lambda 750 spectrophotometers) between the wavelengths of 300 and 800 nm at a resolution of 1 nm. The reaction mixture was diluted five times with distilled water and used for UV-visible analysis. The absorbance of this solution was measured at a one-hour interval for 1-8 h, and the changes in the color were gradually observed as it turned dark brown at the end of 8 h (32).

2.2.2. Centrifugation

Silver nanoparticles solution was centrifuged at 10,000 rpm for 30 min. The pellets were washed three times with 20 ml of distilled water and finally dried at 60°C to dispose of the free proteins/catalysts that are not topping the AgNPs. The centrifugation process was performed at different time intervals of 1-8 h to recognize the effect of time incubation on the amount of synthesized AgNPs (33). After the centrifugation of AgNPs solution at a different incubation time interval (1-8 h), the samples were washed and weighed three times with 20 ml of non-ionized water to remove free proteins/enzymes in AgNPs.

2.2.3. Scanning Electron Microscopy

The biomasses had settled in the base of the cone-like carafes, and the suspension was tested for

scanning electron microscopy (SEM) perception. The scanning electron microscopy of the aqueous solution samples of AgNPs was prepared by setting one drop of the solution on the carbon-covered copper grids, the films on the SEM system were permitted to stand for two min after, and the extra solution was then removed using a blotting paper and drying the grid. The size appropriation of the subsequent nanoparticles was evaluated based on SEM micrographs (34).

2.3. Animals and Housing

The present study was conducted on 28 adult Wistar albino rabbits (aged three months and weighted 1000±100 g), which were adopted after two-week acclimatization in the College of Veterinary Medicine, Baghdad University Iraq, from March 2020 to August 2020. Moreover, they were housed in well-ventilated rooms inside plastic cages (five rabbits/cage) and fed on a standard pellet diet and drinking water *ad libitum* during the experiment. The room temperature was kept at 23±2ºC and ventilated for 12 h. In addition, the light/dark cycle with a light on from 06:00 p.m. to 06:00 a.m. was provided along the experiment period.

2.4. Experimental Design

A total of 28 adult rabbits were randomly selected, equally divided into four T1, T2, T3, and T4 groups, and treated (twice per week) intramuscularly for 45 days as follows: T1: animals received *S. Officinalis* (150 mg/kg B.W) by intramuscular injection; T2: animals received SOSNPs (150 mg/kg B.W); T3: animals were injected both MTX (0.25 mg/kg B.W) and SOSNPs (150mg/kg B.W) intramuscularly; T4: animals received MTX (0.25 mg/kg B.W). The rabbits of all groups were considered the control group at day zero and injected only doubled distill water intramuscularly.

Blood samples were collected at day zero and every 15 days of the experiment, and at the end of the experiment, blood samples were collected via retro-orbital sinus technique and cardiac puncture technique. At the same time, the rabbits were anesthetized by intramuscular injection of xylazine (40 mg/kg B.W) and ketamine (90 mg/kg B.W) using a disposable syringe. Moreover, blood samples were kept in tubes (non-heparinized) and let for 30 min for standing, then serum was gained by centrifugation for 15 min at 3000 rpm, and tightly stopper tubes were kept frozen at -20°C for following chemical analysis; then, it was used for measuring serum concentrations of MDA (Merck, Germany) (35), GSH (Cypress Diagnostics-Belgium Kit), creatinine and uric acid (Cypress Diagnostics-Belgium Kit), and urea (HCUSABIO-China Kit) (36).

2.5. Statistical Analysis

Data have been analyzed statistically using Statistical Package for the Social Science (SPSS) software (version 20). Moreover, statistical analysis of data was conducted based on Two-Way Analysis of Variance (ANOVA) using a significant level of $P<0.05$ and specific group differences were determined using Least Significant Differences (LSD) as presented by (37).

3. Results and Discussion

3.1. Synthesis and Identification of Silver Nanoparticles

The biogenic synthesis of AgNPs using *S. Officinalis* leaf extracts was carried out in the present investigation. The mixing of the plant extract of *S. Officinalis* with silver nitrate solution (1 mM) led to changing of the reaction mixture color to yellowish within 1 h and also dark brown after 8 h (Figure 1), indicating the generation of AgNPs, due to the reduction of silver metal ions (Ag+) into AgNPs via the active molecules present in the *S. Officinalis* plant extracts.

The formation of AgNPs was monitored with color change and a UV-Vis spectrophotometer. The color of the reaction mixture started to change to yellowish within 1 h and to dark brown color after 8 h. The absorption spectra of AgNPs solution consist of a single sharp surface plasmon resonance (SPR) band at 400 nm (Figure 2 and Table 1). Moreover, the most

characteristic part of the silver solution is a narrow plasmon absorption band observable in the 350–600 nm regions, and the distinct visible peak was observed at a wavelength of 430 nm.

The changes in pellet weight (mg/time) indicated a correlation between incubation time and the amount of synthesized AgNPs, presented in table 2. After 1 h and 2 h of incubation times, there was a decrease in pellet weight compared to 6 h, 7 h, and 8h of incubation times. The amount of 1 ml of *S. Officinalis* plant extract mixing with 50 ml of 1 mM $AgNO_3$ solution provided a deep dark color pellet (Figure 1).

Figure 1. Changes in color after the reduction of Ag+ to AgNPs by *S. Officinalis* plant leaf extract at 1-8 h

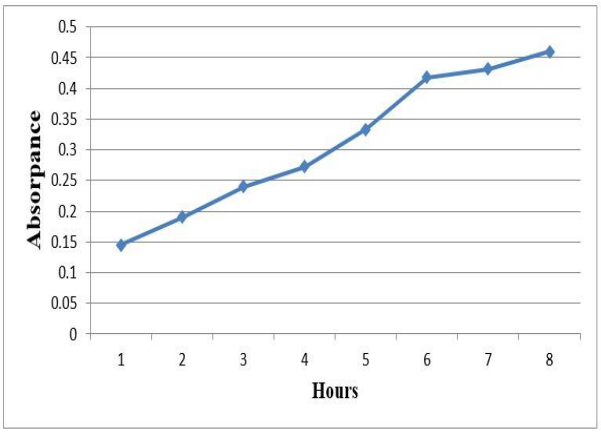

Figure 2. A diagram of the absorbent rates of SOSNPs at different times

Table 1. Values of absorbance obtained by a spectrophotometer at a wavelength of 434nm at different times

Duration (h)	1 h	2 h	3 h	4 hr	5 h	6 h	7 h	8 h
Absorbance at 434nm (peak)	0.1566	0.1930	0.2430	0.272	0.3323	0.4018	0.4300	0.4587

Table 2. Amounts of AgNPs synthesized by *S. Officinalis* extract

Time (h)	1 h	2 h	3 h	4 h	5 h	6 h	7 h	8 h
SOSNPs (g)	0.0149	0.185	0.248	0.265	0.362	0.422	0.438	0.457
QTY (ml)	50	50	50	50	50	50	50	50

Scanning electron microscopy images of AgNPs solution are shown in (Figure 3), which indicate the adsorption and/or deposition of AgNPs onto the surface of roughly sphere-shaped polydispersed particles. The silver nanoparticles that emerged in the images have a variety of shapes such as spherical, triangle, and irregular. As shown in figure 4, the presence of ring patterns in the selected area of electron diffraction reveals the single face-centered cubic crystalline nature of the spherical nanoparticles with a preferential growth direction along the Ag+. The shape evolution of protomorphic AgNPs was indicated in electron microscope images of samples prepared at different times (38). Moreover, the average size of AgNPs was between 20-50 nm, with a few larger particles exceeding 80 nm only in the case of *S. Officinalis* leaf extract at the longer reaction times (Figure 4).

The present study provided evidence that the *S. Officinalis* leaves and flowers were excellent sources for synthesizing stable SOSNPs in shorter times. The color change was due to the reduction of silver ions, indicating the formation of AgNPs. The mechanism by

which the SOSNPs could be synthesized by plant extract may be explained by the high total content of phenols and flavonoids (39, 40). The mechanism with high reduction capacity (41, 42).

These nanoparticles exhibited yellowish-brown color in an aqueous solution due to the excitation of surface plasmon vibrations in AgNPs, which is consistent with the results of some previous investigations (8, 43). The *S. Officinalis* leaf appears with organic compounds such as tannins, terpenoids, steroids, glycosides, and benzenoids present (44), which are responsible for the efficient stabilization of nanoparticles and reduction of metal ions.

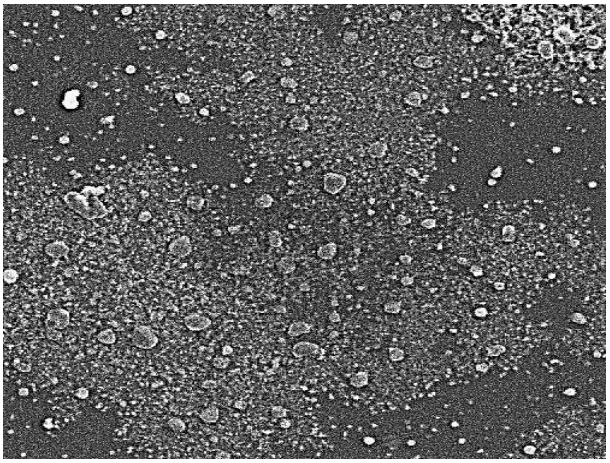

Figure 3. Crystalline clusters of AgNPs obtained by scanning electron microscopy (2000X)

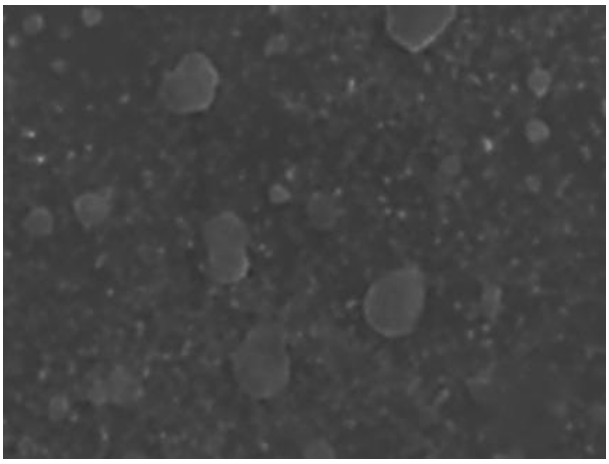

Figure 4. Crystalline clusters of AgNPs obtained by scanning electron microscopy (4000X)

3.2. Separation of *Salvia officinalis* Silver Nanoparticles

It is renowned that AgNPs exhibit dark brown color, depending on the intensity and the size of nanoparticles; the colors appear due to the excitation of SPR of AgNPs (18). The spectra absorption of AgNPs solution consists of a single sharp SPR band at 400 nm; this result is consistent with the findings of some other studies (45-47).

It was observed from the spectra that the SPR peak of AgNPs occurs at 434 nm with a high absorbent; this peak is due to the typical SPR of AgNPs, indicating that the particles were well-dispersed without aggregation and it was extremely specific for AgNPs. These findings are in line with some previous studies (48-50), in which, reduction of silver was indicated as well, showing that using the UV method, the reduction of silver is faster than that using the conventional method.

The SEM image was taken after seven days following the completion of all reactions; even after seven days, the particles did not accumulate. This observation confirmed that *S. Officinalis* extract might act both as a reducing and a stabilizing agent in synthesizing SOSNPs (51).

3.3. Effects of *Salvia officinalis* and *Salvia officinalis* Silver Nanoparticles on Control and Methotrexate Treated Rabbits

During the experimental period, a significant increase (P<0.05) in the serum GSH was observed in the T2 and T3 groups after intramuscular injection of green nanoparticles alone or concurrently with MTX compared to the value in T4 and T1 groups. Highest significant (P<0.05) increase in GSH was observed in group T2 at the end of the experiment in comparison with the value in other treated groups (Table 3). The results also indicated a significant (P<0.05) decrease in serum MDA concentration in T1, T2, and T3 groups

compared to the value in MTX treated (T4) group along the experimental period. A type of green nanoparticles (i.e., SOSNPs) and MTX-AgNPs groups indicated the highest significant (P<0.05) decrease in serum MDA concentration (T2 and T3 groups) compared to the value in other treated groups (Table 4).

Table 3. Effects of *S. Officinalis* and SOSNPs on reduced GSH concentration (µmol/L) in normal and MTX treated rabbits

Treatment Time	T1	T2	T3	T4
0	120.00±2.16^{a}	119.00±4.03^{b}	117.20±4.41^{b}	120.00±2.02^{a}
15	123.40±2.13^{a}	155.10±4.1^{a}	139.10±2.75^{a}	103.11±4.6^{b}
30	118.60±1.92^{a}	154.30±6.70^{a}	139.10±4.70^{a}	111.60±3.32^{ab}
45	124.20±1.22^{a}	155.30±1.2^{a}	146.30±0.93^{a}	100.10±2.98^{b}

Table 4. Effects of *S. Officinalis* and SOSNPs on MDA concentration (µmol/L) in normal and MTX treated rabbits

Treatment Time	T1	T2	T3	T4
0	346.00±4.80^{a}	337.00±8.06^{a}	354.00±11.2^{a}	360.00±5.14^{b}
15	308.00±5.07^{a}	259.00±4.77^{b}	244.00±6.08^{b}	415.00±23.1^{a}
30	340.00±10.6^{a}	298.00±6.17^{b}	228.00±7.46^{b}	418.00±17.0^{a}
45	333.00±11.11^{a}	267.00±10.12^{b}	266.00±13.6^{b}	440.00±17.3^{a}

LSD= 10.85

Values are expressed as mean±SE, n=7 rabbits in each group; T1: animals received *S. Officinalis* extracts (150 mg/kg B.W); T2: animals received SOSNPs (150 mg/kg B.W); T3: animals received both SOSNPs and MTX (150 mg/kg B.W and 0.25 mg/kg B.W, respectively); T4: animals received MTX (0.25 mg/kg B.W). The capital letters refer to significant differences among various groups at (P<0.05). The small letters refer to significant differences among various groups vs. time 0 at (P<0.05).

A significant decrease in serum MDA and a significant increase in GSH were observed in *S. Officinalis* treated group. The anti-oxidant activity of *S. Officinalis* was documented by many investigations (52, 53), which attributed to their flavonoid content with their free radical scavenging activity. This result is also in line with (54), where *S. Officinalis* concentration in rabbits (0.3-13.33 mg/ml in water) has anti-oxidant protection through raising the GSH content and increasing the glutathione peroxidase (GPx) activity. Many phytocompounds, such as polyphenols, flavonoids, tannin, sugars, alkaloids, as well as triterpenoids/steroids, have been reported to be present in the *S. Officinalis*, which are responsible for potent anti-oxidant, anti-inflammatory, larvicidal, and other medicinal properties (44).

The injection of SOSNPs to rabbits caused a correction in the disturbance of anti-oxidant status (increase in MDA and decrease in GSH) caused by MTX. The free radical scavenging potential of AgNPs is documented in (7, 16, 19), and its observed strong anti-oxidant properties have been evaluated to be 98.6% (55). An increase in the activities and mRNA expression levels of glutathione peroxidase (GSH-pX) and glutathione reductase (GSH-RD), superoxide dismutase (SOD), catalase (CAT), as well as total anti-

oxidant capacity with a significant decrease in MDA in normal compared to diabetic rabbits were obtained after exposure to 10 mg/kg B.W of AgNPs (56). Anti-oxidant enzymatic activities were enhanced, and a cell protection mechanism was provided against membrane damage induced by oxidative stress by catalyzing peroxide elimination (57). It has been assumed that the highest anti-oxidant activity of nanoparticles might be due to preferential adsorption of anti-oxidant material of plant extracts on the surface of nanoparticles (19). It is noteworthy that large-size AgNPs are more effective anti-oxidants than smaller ones (58-60).

A significant decrease in GSH and a significant increase in MDA were observed in MTX treated group, indicating oxidative stress. Nuclear factor-erythroid 2 related factor 2 (Nrf2) is a transcriptional activator that can act as a sensor for oxidative stress and has an important role in the regulation of defensive genes activation and induction of anti-oxidant enzymes as SOD, CAT, and GPx that participated in the suppression of injury evoked by reactive oxygen species ROS and protection of cells against oxidative stress injurious effects (61, 62). It has been found that MTX induced a decrease in mRNA of Nrf2 as well as Nrf2 binding capacity, which can partially explain the reduction of the anti-oxidant status in MTX treated mice (63).

3.4. Effects of *Salvia Officinalis* and *Salvia Officinalis* Silver Nanoparticles on Serum Kidney Function in Control and Methotrexate Treated Rabbits

A significant decrease (P<0.05) in serum urea concentration was observed in the T1, T2, as well as T3 groups after intramuscular injection of *S. Officinalis*, green nanoparticles, and MTX, throughout the experimental period compared to the values in group T4, in which MTX was given (Table 5). It was demonstrated in table 6 that intramuscular injection of *S. Officinalis* (T1) or green nanoparticles alone (T2), in combination with MTX (T3), caused a significant decrease in serum uric acid concentration in the T1, T2, and T3 groups during the experimental period compared to the values in group T4 (P<0.05). With exception to the value in T4, which demonstrated significant differences in creatinine concentrations, non-significant differences were observed over time in other experimental groups during the experimental period compared to zero time (P>0.05) (Table 7).

Table 5. Effects of *Salvia Officinal's* (SO) and *Salvia Officinal's* Silver Nanoparticles (SOSNP) on concentration (mg/dl) in normal and methotrexate (MTX) treated rabbits

Treatment Time	T1	T2	T3	T4
0	45.00±0.54^{a}	47.60±1.50^{a}	48.210±3.51^{a}	45.00±1.62^{c}
15	43.20±1.83^{a}	43.20±1.43^{ab}	46.60±1.80^{a}	52.00±2.14^{b}
30	45.30±1.4^{a}	38.40±0.80^{b}	43.10±1.44^{a}	66.10±3.00^{a}
45	44.40±1.86^{a}	43.10±1.80^{ab}	41.30±1.03^{b}	60.00±2.22^{b}

LSD= 6.09

Values are expressed as mean ± SE, n= 7 rabbits each group, T1: animals received SO extracts (150mg /Kg B.W) .T2: animals received SOSNP (150mg/Kg B.W.)T3: animals received both SOSNP and MTX (150mg/Kg B.W., 0,25mg/Kg B.W. respectively). T4: animals received MTX (0,25mg/Kg B.W.).The different capital letters refer to significant differences between different groups at (P<0.05).The different small letters refer to significant differences within groups VS 0 time at (P<0.05).

Table 6. Effects of *Salvia Officinal's* (SO) and *Salvia Officinal's* Silver Nanoparticles (SOSNP) on serum acid concentration (mg/dl) in normal and methotrexate (MTX) treated rabbits

Treatment Time	T1	T2	T3	T4
0	0.83±0.05^{a}	0.85±0.04^{a}	0.73±0.04^{a}	0.81±0.05^{b}
15	0.74±0.04^{b}	0.74±0.04^{b}	0.71±0.05^{a}	0.91±0.04^{a}
30	0.72±0.03^{c}	0.73±0.06^{b}	0.71±0.05^{a}	0.93±0.04^{a}
45	0.84±0.06^{a}	0.74±0.05^{ab}	0.68±0.06^{a}	0.93±0.03^{a}

LSD= 0.17

Values are expressed as mean ± SE, n= 7 rabbits each group, T1: animals received SO extracts (150mg /Kg B.W) .T2: animals received SOSNP (150mg/Kg B.W.)T3: animals received both SOSNP and MTX (150mg/Kg B.W., 0,25mg/Kg B.W. respectively). T4: animals received MTX (0,25mg/Kg B.W.).The different capital letters refer to significant differences between different groups at (P<0.05).The different small letters refer to significant differences within groups VS 0 time at (P<0.05).

Table 7. Effects of *Salvia Officinal's* SO) and *Salvia Officinal's* Silver Nanoparticles (SOSNP) on serum uric Creatinine (mg/dl) in normal and methotrexate (MTX) treated rabbits

Treatment Time	T1	T2	T3	T4
0	1.55±0.07^{a}	1.57±0.09^{a}	1.70±0.07^{a}	1.45±0.04^{c}
15	1.72±0.06^{a}	1.34±0.07^{a}	1.55±0.06^{a}	3.21±0.05^{b}
30	1.83±0.13^{a}	1.42±0.09^{a}	1.50±0.04^{a}	3.115±0.08^{ab}
45	1.77±0.03^{a}	1.49±0.10^{a}	1.69±0.09^{a}	3.15±0.17^{a}

LSD=0.395

Values are expressed as mean ± SE, n= 7 rabbits each group, T1: animals received SO extracts (150mg /Kg B.W) .T2: animals received SOSNP (150mg/Kg B.W.)T3: animals received both SOSNP and MTX (150mg/Kg B.W., 0,25mg/Kg B.W. respectively). T4: animals received MTX (0,25mg/Kg B.W.).The different capital letters refer to significant differences between different groups at (P<0.05).The different small letters refer to significant differences within groups VS 0 time at (P<0.05).

It has been found that small size and high dose of AgNPs caused renal damage by increasing the concentrations of serum creatinine, urea, and uric acid (64, 65); however, a low dose of AgNPs (4.8 mg/kg B.W), injected intramuscularly for 28 days in rats, caused renal protective activity; this finding was also demonstrated by (60, 66), in which the safeness of *in vivo* use of low doses of AgNPs with large sizes was recorded. The results also indicated significant variations in renal function tests that alter MTX exposure, indicating the renal toxicity; this finding was also documented by (67-69). It is believed that MTX induces renal injury, either by precipitation in, or a direct toxic effect on the renal tubules. Methotrexate and its metabolites are poorly soluble in acidic environments, promoting potential precipitation in acidic urine (70, 71).

A significant decrease in serum urea, creatinine, and uric acid levels was observed in rabbits exposed to SOSNPs, which indicates its re-protection. The high affinity of metal nanoparticles towards nitrogen-containing compounds and their capability to bind them (72) could be the reason behind the decrease in the concentration of renal biomarkers after treatment of

SOSNPs. Moreover, metal nanoparticle surface properties provide some functionalities with small organic molecules, including urea, uric acid, and creatinine, by their various functional groups (73), which lead to the reduction in their concentration.

Authors' Contribution

Study concept and design: M. A. S. and N. A. S.
Acquisition of data: M. A. S. and N. A. S.
Analysis and interpretation of data: M. A. H.
Drafting of the manuscript: L. F. G.
Critical revision of the manuscript for important intellectual content: M. A. S. and N. A. S.
Statistical analysis: M. A. S. and N. A. S.
Administrative, technical, and material support: M. A. S. and N. A. S.

Ethics

All the procedures were approved by the ethics committee of the College of Veterinary Medicine, Baghdad University Iraq (No.: 2020-4789-12).

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Satyavani KS, editor Green Synthesis of Silver Nanoparticles by Using Stem Derived Callus Extract of Bitter Apple (Citrullus Colocynthis). 2011.
2. Sivaranjani K, Meenakshisundaram M, editors. Biological Synthesis of Silver Nanoparticles Using Ocimum Basillicum Le Af Extract And Their Antimicrobial Activity. 2013.
3. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv. 2009;27(1):76-83.
4. Naidu K, Govender P, Ada J. Bioedical application and toxicity of nanosilver. Med Technol. 2015;29(2):13-9.
5. Rai MK, Deshmukh SD, Ingle AP, Gade AK. Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria. J Appl Microbiol. 2012;112(5):841-52.
6. Gamazo C, Prior S, Concepcion Lecaroz M, Vitas AI, Campanero MA, Perez G, et al. Biodegradable gentamicin delivery systems for parenteral use for the treatment of intracellular bacterial infections. Expert Opin Drug Deliv. 2007;4(6):677-88.
7. Madureira AR, Nunes S, Campos DA, Fernandes JC, Marques C, Zuzarte M, et al. Safety profile of solid lipid nanoparticles loaded with rosmarinic acid for oral use: in vitro and animal approaches. Int J Nanomedicine. 2016;11:3621-40.
8. Quang HT, Van Quy N, Anh-Tuan L. Silver nanoparticles: synthesis, properties, toxicology, applications and perspectives. Adv Nat Sci Nanosci Nanotechnol. 2013;4(3):033001.
9. Prakash P, Gnanaprakasam P, Emmanuel R, Arokiyaraj S, Saravanan M. Green synthesis of silver nanoparticles from leaf extract of Mimusops elengi, Linn. for enhanced antibacterial activity against multi drug resistant clinical isolates. Colloids Surf B Biointerfaces. 2013;108:255-9.
10. Daoud S, Mona AMA, Dalal HM, Alkhalifah MME, Afrah EM. Biosynthesis of silver nanoparticles using salvia off Icinalis and assessment of their anti-bacterial activity. Int J Curr Res. 2015;7(10):21548-52.
11. Mie R, Samsudin MW, Din LB, Ahmad A, Ibrahim N, Adnan SN. Synthesis of silver nanoparticles with antibacterial activity using the lichen Parmotrema praesorediosum. Int J Nanomedicine. 2014;9:121-7.
12. Prasad R. Synthesis of Silver Nanoparticles in Photosynthetic Plants. J Nanopart. 2014;2014:963961.
13. AH S. Evaliuation the effect of silver nanoparticles and cefotaxim on healing of experimentaly induced infected fractured bones in rabbits: University of Baghdad 2016.
14. Amooaghaie R, Saeri MR, Azizi M. Synthesis, characterization and biocompatibility of silver nanoparticles synthesized from Nigella sativa leaf extract in comparison with chemical silver nanoparticles. Ecotoxicol Environ Saf. 2015;120:400-8.
15. Thamer N, Almashhedy L. Acute Toxicity of Green Synthesis of Silver Nanoparticles Using Crocus Sativud L. On White Albino Rats. Int J Phytopharm. 2016;7:13-6.
16. Gomaa EZ. Antimicrobial, antioxidant and antitumor activities of silver nanoparticles synthesized by Allium cepa extract: A green approach. J Genet Eng Biotechnol. 2017;15(1):49-57.
17. P PS, T KS. Antioxidant, antibacterial and cytotoxic

potential of silver nanoparticles synthesized using terpenes rich extract of Lantana camara L. leaves. Biochem Biophys Rep. 2017;10:76-81.
18. Velu M, Lee JH, Chang WS, Lovanh N, Park YJ, Jayanthi P, et al. Fabrication, optimization, and characterization of noble silver nanoparticles from sugarcane leaf (Saccharum officinarum) extract for antifungal application. Biotech. 2017;7(2):147.
19. Phull A-R, Abbas Q, Ali A, Raza H, kim SJ, Zia M, et al. Antioxidant, cytotoxic and antimicrobial activities of green synthesized silver nanoparticles from crude extract of Bergenia ciliata. Future J Pharm Sci. 2016;2(1):31-6.
20. Skandalis N, Dimopoulou A, Georgopoulou A, Gallios N, Papadopoulos D, Tsipas D, et al. The Effect of Silver Nanoparticles Size, Produced Using Plant Extract from Arbutus unedo, on Their Antibacterial Efficacy. Nanomaterials (Basel). 2017;7(7).
21. Panda SK. Ethno-medicinal uses and screening of plants for antibacterial activity from Similipal Biosphere Reserve, Odisha, India. J Ethnopharmacol. 2014;151(1):158-75.
22. Hamedi S, Shojaosadati SA, Mohammadi A. Evaluation of the catalytic, antibacterial and anti-biofilm activities of the Convolvulus arvensis extract functionalized silver nanoparticles. J Photochem Photobiol B. 2017;167:36-44.
23. Syed B, M NN, B LD, K MK, S Y, S S. Synthesis of silver nanoparticles by endosymbiont Pseudomonas fluorescens CA 417 and their bactericidal activity. Enzyme Microb Technol. 2016;95:128-36.
24. Verma DK, Hasan SH, Banik RM. Photo-catalyzed and phyto-mediated rapid green synthesis of silver nanoparticles using herbal extract of Salvinia molesta and its antimicrobial efficacy. J Photochem Photobiol B. 2016;155:51-9.
25. Ge L, Li Q, Wang M, Ouyang J, Li X, Xing MMQ. Nanosilver particles in medical applications: synthesis, performance, and toxicity. Int J Nanomedicine. 2014;9:2399-407.
26. Patra JK, Das G, Baek KH. Phyto-mediated biosynthesis of silver nanoparticles using the rind extract of watermelon (Citrullus lanatus) under photo-catalyzed condition and investigation of its antibacterial, anticandidal and antioxidant efficacy. J Photochem Photobiol B. 2016;161:200-10.
27. Anand K, Tiloke C, Naidoo P, Chuturgoon AA. Phytonanotherapy for management of diabetes using green synthesis nanoparticles. J Photochem Photobiol B. 2017;173:626-39.
28. Venugopal K, Ahmad H, Manikandan E, Thanigai Arul K, Kavitha K, Moodley MK, et al. The impact of anticancer activity upon Beta vulgaris extract mediated biosynthesized silver nanoparticles (ag-NPs) against human breast (MCF-7), lung (A549) and pharynx (Hep-2) cancer cell lines. J Photochem Photobiol B. 2017;173:99-107.
29. Alessandrini F, Vennemann A, Gschwendtner S, Neumann AU, Rothballer M, Seher T, et al. Pro-Inflammatory versus Immunomodulatory Effects of Silver Nanoparticles in the Lung: The Critical Role of Dose, Size and Surface Modification. Nanomaterials (Basel). 2017;7(10).
30. Fierascu R, IONa R, Dumitriu I. Noble metals nanoparticles synthesis in plant extracts. J synthesis. 2010;1:22.
31. J.B. H. Phytochemical Methods. Methods of Plant Analysis. Dordrecht: Springer; 1984.
32. Vidhu VK, Philip D. Spectroscopic, microscopic and catalytic properties of silver nanoparticles synthesized using Saraca indica flower. Spectrochim Acta A Mol Biomol Spectrosc. 2014;117:102-8.
33. M Awwad A, Albiss B, L Ahmad A. Green synthesis, characterization and optical properties of zinc oxide nanosheets using Olea europea leaf extract. Adv Mater Lett. 2014;5(9):520-4.
34. Gliga AR, Skoglund S, Wallinder IO, Fadeel B, Karlsson HL. Size-dependent cytotoxicity of silver nanoparticles in human lung cells: the role of cellular uptake, agglomeration and Ag release. Part Fibre Toxicol. 2014;11:11.
35. Yoshikawa M, Yoneda T, Takenaka H, Fukuoka A, Okamoto Y, Narita N, et al. Distribution of muscle mass and maximal exercise performance in patients with COPD. Chest. 2001;119(1):93-8.
36. Burtis CA, Ashwood ER, Bruns DE. ietz textbook of clinical chemistry and molecular diagnostics. 3rd ed. Philadelphia, 1999.
37. Snedecore GW, Chochran WG. Statistical methods. 6th ed: The Iowa state University Press; 1973.
38. Huang J, Li Q, Sun D, Lu Y, Su Y, Yang X, et al. Biosynthesis of silver and gold nanoparticles by novel sundried Cinnamomum camphora leaf. Nanotechnology. 2007;18:104 -5.
39. DeKosky ST, Carrillo MC, Phelps C, Knopman D, Petersen RC, Frank R, et al. Revision of the criteria for

Alzheimer's disease: A symposium. Alzheimers Dement. 2011;7(1):e1-12.

40. Rodrigues MR, Kanazawa LK, das Neves TL, da Silva CF, Horst H, Pizzolatti MG, et al. Antinociceptive and anti-inflammatory potential of extract and isolated compounds from the leaves of Salvia officinalis in mice. J Ethnopharmacol. 2012;139(2):519-26.
41. Muniyappan N, Nagarajan NS. Green synthesis of silver nanoparticles with Dalbergia spinosa leaves and their applications in biological and catalytic activities. Process Biochem. 2014;49(6):1054-61.
42. Sharma VK, Yngard RA, Lin Y. Silver nanoparticles: green synthesis and their antimicrobial activities. Adv Colloid Interface Sci. 2009;145(1-2):83-96.
43. Veera bN, Rama K, Rajkiran B, Jahnavi A, Manisha RD, Pratap RMP. Green Synthesis of plant-mediated silver nanoparticles using Withania somnifera leaf extract and evaluation of their antimicrobial activity. Int J Adv Res. 2013.
44. Tanamatayarat P. Antityrosinase, antioxidative activities, and brine shrimp lethality of ethanolic extracts from Protium serratum (Wall. ex Colebr.) Engl. Asian Pac J Trop Biomed. 2016;6(12):1050-5.
45. Stamplecoskie KG, Scaiano JC. Light emitting diode irradiation can control the morphology and optical properties of silver nanoparticles. J Am Chem Soc. 2010;132(6):1825-7.
46. Sulaiman GM, Mohammed WH, Marzoog TR, Al-Amiery AAA, Kadhum AAH, Mohamad AB. Green synthesis, antimicrobial and cytotoxic effects of silver nanoparticles using Eucalyptus chapmaniana leaves extract. Asian Pac J Trop Biomed. 2013;3(1):58-63.
47. Balavandy SK, Shameli K, Biak DR, Abidin ZZ. Stirring time effect of silver nanoparticles prepared in glutathione mediated by green method. Chem Cent J. 2014;8(1):11.
48. Saravanan M, Vemu AK, Barik SK. Rapid biosynthesis of silver nanoparticles from Bacillus megaterium (NCIM 2326) and their antibacterial activity on multi drug resistant clinical pathogens. Colloids Surf B Biointerfaces. 2011;88(1):325-31.
49. Ahmad N, Shree K, Srivastava M, Dutta R. Novel rapid biological approach for synthesis of silver nanoparticles and its characterization. Int J Pharmaco and Pharmaceutical Sci. 2014;1:28-31.
50. Chung IM, Park I, Seung-Hyun K, Thiruvengadam M, Rajakumar G. Plant-Mediated Synthesis of Silver Nanoparticles: Their Characteristic Properties and Therapeutic Applications. Nanoscale Res Lett. 2016;11(1):40.
51. Supraja S, Ali SM, Chakravarthy N, Jaya Prakash Priya A, Sagadevan E, Kasinathan MK, Sindhu S, Arumugam P. Green synthesis of silver nanoparticles from Cynodon dactylon leaf extract. Int J Chem Tech. 2013;5(1):271-7.
52. Ranjbar A, Ataie Z, Khajavi F, Ghasemi H. Effects of silver nanoparticle (Ag NP) on oxidative stress biomarkers in rat. Nanomed J. 2014;1(3)::205-11.
53. Safwat GM, Mohammed ET. Salvia officialis olis Improves the athreogenic index and cardiotoxicity in albino rabbits treated with 5-flouroracil. Int J Pharmacol Biosci. 2015;6(2):59-66.
54. Horvathova E, Srancikova A, Regendova-Sedlackova E, Melusova M, Melus V, Netriova J, et al. Enriching the drinking water of rats with extracts of Salvia officinalis and Thymus vulgaris increases their resistance to oxidative stress. Mutagenesis. 2016;31(1):51-9.
55. Barbinta-Patrascu ME, Bunghez IR, Iordache SM, Badea N, Fierascu RC, Ion RM. Antioxidant properties of biohybrids based on liposomes and sage silver nanoparticles. J Nanosci Nanotechnol. 2013;13(3):2051-60.
56. Afifi M, Abdelazim AM. Ameliorative effect of zinc oxide and silver nanoparticles on antioxidant system in the brain of diabetic rats. Asian Pac J Trop Biomed. 2015;5(10):874-7.
57. Prasannaraj G, Sahi SV, Ravikumar S, Venkatachalam P. Enhanced Cytotoxicity of Biomolecules Loaded Metallic Silver Nanoparticles Against Human Liver (HepG2) and Prostate (PC3) Cancer Cell Lines. J Nanosci Nanotechnol. 2016;16(5):4948-59.
58. AshaRani PV, Low Kah Mun G, Hande MP, Valiyaveettil S. Cytotoxicity and genotoxicity of silver nanoparticles in human cells. ACS Nano. 2009;3(2):279-90.
59. Rai M, Kon K, Ingle A, Duran N, Galdiero S, Galdiero M. Broad-spectrum bioactivities of silver nanoparticles: the emerging trends and future prospects. Appl Microbiol Biotechnol. 2014;98(5):1951-61.
60. Schluesener JK, Schluesener HJ. Nanosilver: application and novel aspects of toxicology. Arch Toxicol. 2013;87(4):569-76.
61. Kansanen E, Kuosmanen SM, Leinonen H, Levonen AL. The Keap1-Nrf2 pathway: Mechanisms of activation and dysregulation in cancer. Redox Biol. 2013;1:45-9.
62. Mukherjee S, Chowdhury D, Kotcherlakota R, Patra S, B V, Bhadra MP, et al. Potential theranostics application

of bio-synthesized silver nanoparticles (4-in-1 system). Theranostics. 2014;4(3):316-35.

63. Abo-Haded HM, Elkablawy MA, Al-Johani Z, Al-Ahmadi O, El-Agamy DS. Hepatoprotective effect of sitagliptin against methotrexate induced liver toxicity. PLoS One. 2017;12(3):e0174295.

64. Pani Jyoti P, Singh R, Pani S. Small Size Silver Nanoparticle's Corrosive and Hazardous Manifestations on Mature and Developing Kidney Following Accumulation in Pregnant Mice and Offspring's after Serial Oral Bolus Experimental Application: A New Chapter in Teratogenicity and Toxicity Search. J Cytol Histol. 2017;8:3.

65. Qin G, Song T, Shibin L, Haoliang L, Yanwu W, Peng Z, et al. Toxicological Evaluation of Silver Nanoparticles and Silver Nitrate in Rabbits Following 28 Days of Repeated Oral Exposure. Environ Toxicol. 2017;11:1-10.

66. Hunt PR, Marquis BJ, Tyner KM, Conklin S, Olejnik N, Nelson BC, et al. Nanosilver suppresses growth and induces oxidative damage to DNA in Caenorhabditis elegans. J Appl Toxicol. 2013;33(10):1131-42.

67. Widemann BC, Adamson PC. Understanding and managing methotrexate nephrotoxicity. Oncologist. 2006;11(6):694-703.

68. Ramamoorthy SK, Hephziba R. Acute renal failure post high dose methotrexate infusion successfully managed with high dose folinic Acid and high flux dialysis. Indian J Hematol Blood Transfus. 2013;29(2):90-2.

69. Garneau AP, Riopel J, Isenring P. Acute Methotrexate-Induced Crystal Nephropathy. N Engl J Med. 2015;373(27):2691-3.

70. Schwartz S, Borner K, Muller K, Martus P, Fischer L, Korfel A, et al. Glucarpidase (carboxypeptidase g2) intervention in adult and elderly cancer patients with renal dysfunction and delayed methotrexate elimination after high-dose methotrexate therapy. Oncologist. 2007;12(11):1299-308.

71. Harms J, Khawaja A, Taylor M, Han X, Mrug M. Recovery of methotrexate-induced anuric acute kidney injury after glucarpidase therapy. SAGE Open Med Case Rep. 2017;5:2050313X17705050.

72. Wahajuddin, Arora S. Superparamagnetic iron oxide nanoparticles: magnetic nanoplatforms as drug carriers. Int J Nanomedicine. 2012;7:3445-71.

73. Weingart J, Vabbilisetty P, Sun XL. Membrane mimetic surface functionalization of nanoparticles: methods and applications. Adv Colloid Interface Sci. 2013;197-198:68-84.