Role of Salvia officinal's Silver Nanoparticles in Attenuating Renal Damage in Rats Exposed to Methotrexate(Part I)

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Summary

The aim of the present study was to investigate the protective role of Salvia officinal's silver nanoparticles as antioxidant on nephrotic damage induced by methotrexate in adult rats. Green silver nanoparticles were synthesized using alcoholic extract of salvia officinal's leaves, and were characterized by UV-spectrophotometry and scanning electron microscope. The mixing of the plant extract of Salvia. officinal's with silver nitrate solution (1mM), lead to changing of the reaction mixture color to yellowish within one hour and to dark brown after 8 hours, indicating the generation of Salvia officinal's silver nanoparticles , due to the reduction of silver metal ions silver (Ag+) into Nano silver particles via the active compounds present in the S. officinal's plant extracts. Changing in color after the reduction of Ag+ to Salvia officinal's silver nanoparticles. The reduction rate and formation of nanoparticles can be increased further by increase in incubation time. Silver nitrate conversion to Nano silver particles was found to be successful as suggested by the change in color of the solution to brown. For studying the protective role of Salvia officinal's silver nanoparticles , twenty eight adult Wister albino rats were randomly assigned and divided in to four groups as follows T1, T2, T3, and T4, They were treated intramuscularly (twice per week) for 45 days as follows; T1:animals in this group, were given Salvia officinal's (150mg /Kg/.B.W), T2: animals in this groups were given Salvia officinal's Silver nanoparticles (150mg/Kg B.W.); T3:animals of this groups were given both Methotrexate (0.25mg/kg/ B.W.) and Salvia officinal's silver nanoparticles (150mg/Kg/B.W); T4: animals in this groups were given methotrexate (0.25mg/Kg B.W.) for 45 days . The animals of all groups were considered as control group at day zero and injected only doubled distilled water Intramuscala. Fasting blood samples were collected at 0, 15, 30 and 45 days of experimental periods from anesthetized rats using retro-orbital sinus technique and cardiac puncture technique, then sera was isolated for measuring: malondialdehyde, glutathione in serum, creatinine, and blood urea nitrogen and uric acid concentrations. The results showed that animals received methotrexate (group T4) caused a case of oxidative stress manifested by significant decrease grower in , elevation in malondialdehyde concentrations, renal dysfunction as documented by significant elevation in serum creatinine, urea and uric acid concentrations. On the other hand, the protective role of salvia officinal's and Salvia officinal's silver nanoparticles given concurrently with methotrexate was clarified in groups T2and T3 ,where there was alleviation of renal damage through correction of the previous mentioned parameters and correction of antioxidant status. In conclusion, the current study documented the antioxidant activity and reno protective effects of Salvia officinal's silver nanoparticles against damaging effects of methotrexate in rats.

Introduction

Nano particles are cluster of atoms range between 1-100nm size that showed totally different or improved properties depending on their distribution, size and morphology (1 and 2). Now aday nanosilver particles(SNPs) are widely used in many biological application (3)such as drug delivery treatment, diagnosis, medical device coating as well as personal health care (4 and 5). Biological methods for

synthesis of nanoparticles have paid great attention using reducing and stabilizing agent (enzymatic or non-enzymatic) including: enzymes (6 and 7), microorganism (8) fungus (9) as well as plant extracts (10) Green synthesis is preferable comparing to physical and chemical method as it is Eco friendly, easily handled with no need to high temperatures, pressure, energy or toxic chemicals (11 and 12).

Different plant extracts were used as reducing agent in green methods such as Nigella arvenus (13), Nigella sativa (14), Crocus sativus (15), Alliumm sepa(16), Lantana camara (17), Saccharum officinarum(18) as well as Salvia officinal's (7). Silver nanoparticles possess a wide spectrum range of antimicrobial activities (19 and 20), antiviral, antifungal as well as anti-inflammatory activity (21 and 22). In human study wound dressing containing SNPs prompted the healing of leg ulcer,acting as anti-bacterial and anti-inflammatory agent (23 and 24).Several investigators reported the anticancer activity of SNPs in vitro and in vivo studies (25-28). It is assumed that the highest antioxidant activity of green nanoparticles might be due to the preferential adsorption of antioxidant material from plant extract on to the surface of the nanoparticles (19). It should be mentioned that SNPs act as pro inflammatory at high doses and anti-inflammatory in low doses (29)

According to available literatures, there were few literatures (30) concerning the usage of Saliva officinals as reducing agent in green synthesis SNPs as well as, their systemic uses in different species. In this investigation alcoholic extract of Salvia officinal's (SO) leaves was used for green synthesis of SNPs and the possible hepatic and renal protective effect of SO and SNPs were studied in methotrexate treated rat. .

Materials and Methods

Biosynthesis of SNPs Using salvia officinal's plant:Collection and preparation of ethanolic extract of SO :well dried leaves of SO plant were collected in March 2016, from Baghdad market, Plant has been send to diagnosis. Twenty grams of SO Leaves has been powdered and sieved with fine mesh. The ethanol extracts of SO. was prepared by

mixing10 g of the dry sample with 100 ml of ethanol and shaken well and stayed for 24 hours and then filtered through a Whatman filter paper No. 1 (pore size 125 mm). The supernatant (filtrate) was further filtered through a Whatman filter paper No. 1. Heat treatment for the prepared sample was done at 80°C for concentrating the extract and removing the effect of the ethanol then kept at 4°C for further analysis. Then it was taken and extracted with soxohlet apparatus by using ethanol 70%. The extracted solvent was applied under reduced pressure in a rotary evaporator until it become completely dry. The residue was kept at 4°C for further usage (31).

Synthesis of phyto SNPs by using SO Plant Extract: Adding 1500mg of SO plant leaf extracts to 10 ml of (1mM) aqueous silver nitrate solution and kept at the room temperature for 8hrs to produce SNPs. The changing of solution color was measured at every one hour and for eight hours. The changing in the color intensity after the reduction of Ag+ to SNPs by salvia officinal's plants leaves extracts with increasing time of reaction was noticed.

Separation and Identification of Silver Nanoparticles:
spectrophotometery:The optical absorbance of the synthesized silver nanoparticles 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 first diluted 5 times with distilled water and used for UV-visible analysis. Absorbance of this solution was measured at 1hr interval for 1-8hrs and the changing in the color was observed gradually as it turned dark brown at the end of 8hrs (32) .

Silver nanoparticles solution was centrifuged at 10,000 rpm for 30 minutes. The pellets were washed 3 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 SNPs. The centrifugation process were done at time 1-8hrs.This was done in order to know the effect of time incubation on amount of SNPs synthesized (33). After the centrifugation of SNPs solution at different incubation time interval (1-8hrs), the samples were dried and weighing after washed three times with 20 ml

of de-ionized water to get rid of the free proteins/ enzymes that are not capping the SNPs.

The biomasses had settled in the base of the cone like carafes and the suspension over the accelerate was tested for scanning electron microscopy (SEM) perception. SEM of the aqueous solution samples of SNPs prepared by setting a one drop of the solution on the carbon-covered copper grids and the films on the SEM system permitted standing for two minutes, after that there is removing of the extra solution by using a blotting paper and drying the grid. The size appropriation of the subsequent nanoparticles evaluated on the basis of SEM micrographs (34 and 35) and this work was done in the nanotechnology center of Technology University.

This study has been conducted on 28 adult Wistar albino rats (aged 3 months and weighted 200±10g). They were adopted after acclimatization (for two weeks) in the animal house of College of Veterinary Medicine-University of Baghdad during the period extended from march, 2016 to august, 2016.They were housed in a well-ventilated rooms inside plastic cages (5 rats /cage) and feed on standard pellet diet and drinking water ad libitum during the experiment. The room temperature was kept at 23±2ºC and 12 hrs. light/dark cycle with light on from06:00 p.m. to 06:00 a.m. along the experiment period.

Twenty eight adult rats were randomly selected and equally divided in to four groups as follows T1, T2, T3, and T4,They were treated(twice per week) intramuscularly(IM) for 45 days as follows; T1: group, was given (SO)(150mg /Kg/.B.W) , T2: animals in this groups were given Salvia official's Silver nanoparticles(SOSNP) (150mg/Kg B.W.); T3, animals of this groups were given both Methotrexate (MTX)(0,25mg/kg B.W.) and SOSNP (150mg/ Kg /B.W). ,T4, animals in this groups were given MTX(0,25mg/Kg B.W.)for 45 days IM.

The animals of all groups were considered as control group at day zero and injected only doubled distill water IM.

Blood samples were collected at day zero and each fifteen days of experiment and at end of experiment, blood samples were drawn via retro-orbital sinus technique, and cardiac puncture technique while the rats were anesthetized by intramuscular injection of xylazine (40mg/kg B. W.) and ketamine (90mg/kgMB.W.) using disposable syringe. Blood samples were kept in tubes (non-heparinized) and let for 30 minutes for standing, then serum were gained by centrifugation for 15 minutes at 3000 rpm and kept tightly stopper tubes frozen for next chemical analysis at -20 °c. Then it was used for measuring serum concentrations of the following parameters : Malondialdehyde(MDA). using MDA kit (Merck-Germany) according to (36) , GSH using growth stimulating hormone(GSH) kit (Cypress diagnostics-Belgium) according to (37), Creatinin and uric acid using creatinine and uric acid kits (Cypress diagnostics-Belgium) according to (37) and urea using urea kit (HCUSABIO-China) according to (37). Data have been analyzed statistically using the Microsoft Program of Statistical Package for the Social Science (SPSS) version 20. Statistical analysis of data was conducted on the basis of Two-Way Analysis of Variance (ANOVA) utilizing a significant levels of (P<0.05). Specific group differences were determined using Least Significant Differences (LSD) as portrayed by (38).

Results and Discussion

The biogenic synthesis of SNPs using SO. leaf extracts was carried out in this study. The mixing of the plant extract of S. officinal's with silver nitrate solution (1mM), lead to changing of the reaction mixture color to yellowish within one hour and to dark brown after 8h, (Fig.1) indicating the generation of SNPs, due to the reduction of silver metal ions Ag+ into SNPs via the active molecules present in the SO. plant extracts.

Figure,1: Changing in color after the reduction of Ag+ to SNPs by Salvia officinal's plant leafs extract at (1-8hrs).

The formation of SNPs was monitored with color change and UV-Vis spectrum. The color of the reaction mixture started changing to yellowish within 1hr and to dark brown after 8h. The absorption spectra of SNPs solution consists a single sharp surface plasmon resonance band at 400 nm (Fig.2 and Table,1). The most characteristic part of silver solution is a narrow plasmon absorption band observable in the 350 – 600 nm regions. The distinct visible peak was observed at wavelength 430nm .

The changes in pellet weight (gm. / time) gave an evidence of correlation between incubation time and the amount of synthesized SNPs, as in (Table,2). After 1hs and 2hrs of incubation time, there was a decrease in pellet weight when they were compared with 6, 7 and 8h of incubation time. One ml of SO. plant extract mixing with 50 ml of 1mM $AgNO_3$ solution gave a deep dark color pellet (Fig.1).

Table, 1: Absorbance values obtained by a spectrophotometer at wavelength 434nm in different times.

| Duration time(hrs.)	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.	7 hrs.	8 hrs.
Absorbance at 434nm(peak)	0.1456	0.190	0.240	0.272	0.333	0.418	0.431	0.46
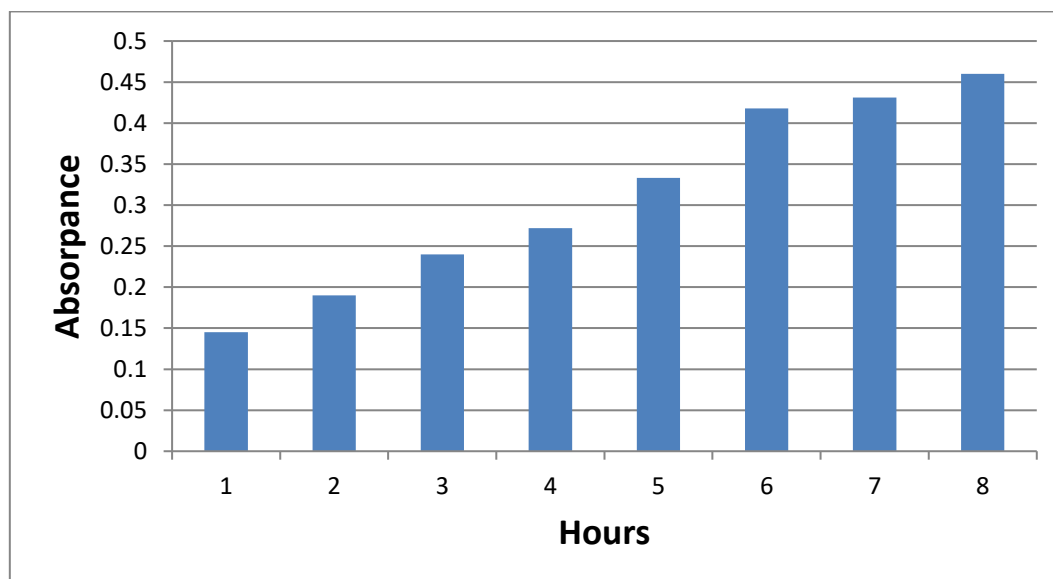
Figure, 2: Silver nanoparticles a diagram showing the absorbent rates of SOSNPs at different times.

Table ,2: Amount of the Silver nanoparticles (SOSNP) (g.) that synthesized by Salvia officinal's extract (ml)

Time(hrs.)	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.	7 hrs.	8 hrs.
SOSNP(gm.)	0.01	0.14	0.20	0.27	0.33	0.41	0.43	0.46
QTY(ml)	50	50	50	50	50	50	50	50

Electron Microscopy images of SNPs solution are shown in (Fig.3). These observations indicate the adsorption and/or deposition of SNPs onto the surface of roughly sphere-shaped poly dispersed particles. The SNPs that emerged in the images have a variety of shapes: spherical, triangle and irregular. As can be seen in (Fig.4) typical example, presence of rings patterns in the selected area 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 photomorphic SNPs was seen in electron microscope images of samples prepared at various times (34). The average size of the silver nanoparticles ranged between 20 and 50 nm with a few larger particles exceeding 80 nm only in the case of SO. leaf extract at the longer reaction time (Fig. 4).
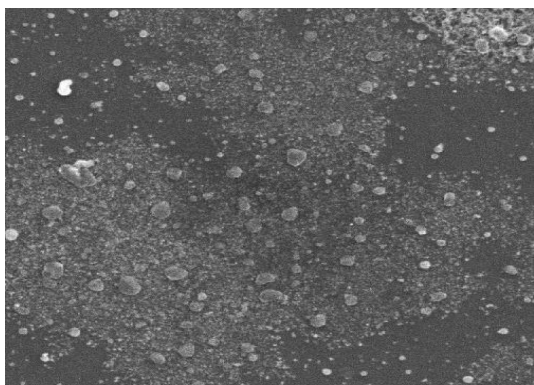
Figure, 3: Crystalline clusters of SNPs obtained by scanning electron Microscopy (2000X)
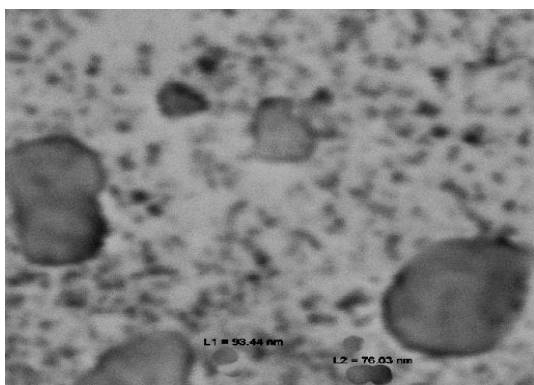
Figure, 4: Crystalline clusters of SNPs obtained by scanning electron Microscopy (4000X)

This study provided evidence that the leaves and flowers of the SO. were good sources for synthesizing stable SONPs in lesser time. The change in color was due to reduction of silver ions indicating formation of SNPs. The mechanism by which the plant extract could be synthesized SONPs may be explained by the high total content of phenols and flavonoids (39 and 40), with have high reduction capacity (41 and 42) .

These NPs. exhibited yellowish brown color in aqueous solution due to excitation of surface Plasmon vibrations in SNPs, and this result has been previously obtained by several investigators (43 - 45). The plant leaf SO appears with organic compounds such as tannins, terpenoids, steroids, glycosides and benzenoids present (46) are responsible for efficient stabilization of nanoparticles and reduction of metal ions.

Separation and Identification of *SONPs*:It is renowned that SNPs exhibit dark brown color, depending on the intensity and the size of Nano particles; the colors arise due to the excitation of surface plasmon resonance (SPR) of the SNPs (47). The absorption spectra of SNPs solution consists a single sharp surface plasmon resonance band at 400 nm. This result is in agreement with (48 - 50).

It was observed from the spectra that the SNPs SPR peak occurs at 434 NM with high absorbent. This peak, due to the surface plasmon resonance typical of silver nanoparticles and indicates that the particles were well dispersed without aggregation and it is very specific for silver nanoparticles and this agreed with previous studies (51 - 53) ,which is an indication of reduction of silver. This indicates that by UV method, silver gets reduced in a faster way than the conventional method.

The SEM image was taken after 7 days following the completion of all reactions. Even after 7 days, the particles did not agglomerate. From this observation, it was confirmed that SO extract might act both as reducing and stabilizing agent in synthesizing SONPs (54).

Effect of (SO and SOSNP) on antioxidant / oxidant status in normal and MTX treated rats: along experimental period significant elevation ($p < 0.05$) in serum GSH was observed in groups T2 and T3 after l/M injection of green NP alone or concurrently with MTX comparing 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 comparing to the value in other treated groups (Table,3). the results also showed significant ($p < 0.05$) decrease in serum MDA concentration in groups T1, T2 andT3 comparing to the value in MTX treated (T4) groups along experimental period. Green nanoparticle (SOSNP) and MTX- SNP groups showed highest significant ($p < 0.05$) decrease in serum MDA concentration (group T2 andT3) compared to the value in other treated groups (Table, 4).

Table, 3: Effects of *Salvia Officinal's* (SO) and *Salvia officinal's* Silver Nanoparticles (SOSNP) on serum reduced glutathione (GSH) concentration (µmol/L) in normal and methotrexate (MTX) treated rats
LSD= 12.90

Treatment Time	T1	T2	T3	T4
0 control	123.00±2.56 A a	121.00±3.33 A b	118.20±3.44 A b	121.00±1.82 A a
15	124.60±1.73 C a	156.20±5.11 A a	140.00±2.75 B a	105.20±4.61 D b
30	119.80±2.52 CD a	153.40±6.90 A a	147.20±4.70 B a	114.40±3.32 C ab
45	124.20±1.32 C a	154.20±1.32 A a	148.40±0.93 B a	106.40±2.98 D b

Values are expressed as mean ± SE, n= 7 rats 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 ,4: Effects of Salvia Officinal's(SO) and Salvia officinal's Silver Nanoparticles (SOSNP) on Malonydialdehyde (MDA) concentration (µmol/L) in normal and methotrexate (MTX) treated rats

Treatment Time	T1	T2	T3	T4
0	456.00±6.80 A a	448.00±7.86 A a	444.00±12.2 A a	470.00±6.34 A b
15	418.00±4.07 B a	348.00±4.37 C b	354.00±5.80 C b	515.00±25.2 A a
30	450.00±10.7 B a	343.00±7.37 C b	338.00±6.46 C b	519.00±19.0 A a
45	443.00±13.0 B a	361.00±12.2 C b	355.00±11.6 C b	543.00±18.3 A a

Values are expressed as mean ± SE, n= 7 rats 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). LSD= 44.96

Significant decrease in Serum MDA and elevation in GSH concentration was observed in SO treated group. The antioxidant activity of SO was documented by many investigations (55 and 56), which attributed to their flavonoid content with their free radical scavenging activity .

The result is also in accordable with (57), where rats received different concentration of SO (0.3-13.33mg/ml in water) has antioxidant protection through increase glutathione content and elevation in glutathione peroxidase activity. A number of phyto compounds, such as polyphenols, flavonoids, tannin, sugars, alkaloids and triterpenoids/steroids have been reported to be present in the SO are responsible for potent antioxidant, anti-inflammatory, larvicidal and other medicinal properties (46).

Salvia official's Silver Nano particle (SOSNPs) injection to rats caused correction of antioxidant status disturbance (elevation in MDA and depression in GSH) that caused by MTX. The free radical scavenging potential of SNPs are documented (16, 19 and 58). Its manifest strong antioxidant properties reaching 98.6% (59). An elevation of activities and mRNA expression levels of GSH-pX and GSH-RD, SOD, catalase and total antioxidant capacity with a significant decrease in MDA in normal compared to diabetic rats were determined after exposure to 10mg/kg /B.W. of SNP per so (60). It enhanced antioxidant enzymatic activities and provide cell protection mechanism against oxidative stress induced membrane damage by catalyzing the elimination of peroxide (61). It has been assumed that the highest antioxidant activity of nanoparticles might be due to preferential adsorption of antioxidant material of plant extracts on the surface of nanoparticles (19). It should be declare that large size SNPs are more effective as antioxidant than smaller size SNPs (62 - 64).

Significant decrease of GSH and significant increase in MDA was observed in MTX treated group indicating oxidative stress. Nuclear factor erythroid 2-related factor 2(Nrf2) is a transcriptional activator that can serve as a sensor for oxidative stress. It has an important role in the regulation of defensive genes activation and induction of antioxidant enzymes as SOD, catalase (CAT) and glutathione peroxidase (GPx) that participated in suppression of injury evoked by ROS and protection of cells against oxidative stress injurious effects(65 and 66). It has been found that MTX induced a decrease in mRNA of Nrf2 and Nrf2 binding capacity. This can partially explain the depression of the anti-oxidant status in MTX treated mice.(67)

Effect of SO and (SOSNP) on serum kidney function in normal and methotrexate (MTX) treated rats: significant decrease (p<0.05) in serum urea concentration was observed in groups T1, T2, and T3 after I/m injection of SO, green NP and green NP and MTX, along experimental period comparing to the value in group (T4) that received methotrexate (Table,5). Table,(6) demonstrated that I/m injection of SO (T1) or green NP alone (T2), in combination with methotrexate (T3) caused significant (p<0.05) decrease in serum creatinine concentration in groups T1, T2 and T3 along the experimental period comparing to the value in methotrexate (T4) treated group. With exception to the value in T4 which showed significant differences (p<0.05) in uric acid concentration, within the time, non-significant (p>0.05) differences was observed in other experimental groups along experimental period comparing to zero time (Table ,7).

Significant decrease in serum urea, creatinine and uric acid was observed in SOSNP exposed rats indicating its reno protectively. High affinity of metal Nano particles towards nitrogen containing compounds and their ability to tagged them (68) could be the reason behind the decrease of renal biomarkers concentration after SOSNPs treatment. Besides, the surface properties of metal nanoparticle allow to functionalities with small organic molecules, including urea, uric acid and creatinine by their various functional groups(69) leading to depression in their concentration.

It has been found that small size and high dose of SNP caused renal damage, through elevation in serum creatinine, urea and uric acid concentration (70 and 71). While low dose of SNPs (4. 8 mg / kg B.W) injected I/m for 28 days in rat possessed Reno protective activity. This declaration also demonstrated by (64 and 72) whom they recorded the safeness of in-vivo use of SNPs of low dose and large size. The results also showed significant changes in renal function test alter MTX exposure indicated Reno toxicity, which was documented by (73-75). It is believed that MTX induces renal injury, either by precipitation in, or a direct toxic effect on the renal tubules. MTX and its metabolites are poorly soluble when in acidic environments, promoting potential precipitation in acidic urine (76 and 77).

Table, 5: Effects of Salvia Official's (SO) and Salvia Official's Silver Nanoparticles (SOSNP) on serum urea concentration (mg/dl) in normal and methotrexate (MTX) treated rats.

Treatment Time	T1	T2	T3	T4
0	47.00±0.55 A a	49.80±1.40 A a	50.20±3.51 A a	47.00±1.77 A c
15	44.40±1.33 B a	45.40±1.33 B ab	48.80±1.80 B a	55.20±2.04 A b
30	47.40±1.44 B a	40.60±0.93 C b	45.40±1.44 BC a	65.00±2.50 A a
45	46.60±2.16 C a	45.20±1.60 C ab	43.40±1.03 C b	59.00±2.13 A b

LSD= 5.89

Values are expressed as mean ± SE, n= 7 rats 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 Official's* (SO) and *Salvia Official's* Silver Nanoparticles (SOSNP) on serum creatinine concentration (mg/dl) in normal and methotrexate (MTX) treated rats.

Treatment Time	T1	T2	T3	T4
0	0.72±0.03 A a	0.76±0.02 A a	0.65±0.02 A a	0.71±0.03 A b
15	0.62±0.03 B b	0.67±0.04 B b	0.62±0.03 B a	0.80±0.02 A a
30	0.64±0.02 B c	0.64±0.05 BC b	0.60 ±0.03 C a	0.82±0.02 A a
45	0.73±0.05 B a	0.68±0.03 B ab	0.58±0.04 C a	0.84±0.01 A a

LSD= 0.097

Values are expressed as mean ± SE, n= 7 rats 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 acid concentration (mg/dl) in normal and methotrexate (MTX) treated rats.

Treatment Time	T1	T2	T3	T4
0	1.49±0.08 A a	1.50±0.06 A a	1.64±0.09 A a	1.39±0.04 A c
15	1.66±0.08 C a	1.36±0.09 C a	1.49±0.07 C a	2.27±0.05 B b
30	1.70±0.13 B a	1.33±0.07 B a	1.45±0.02 B a	2.65±0.08 A ab
45	1.71±0.06 B a	1.43±0.11 B a	1.64±0.10 B a	2.85±0.17 A a

LSD=0.40

Values are expressed as mean ± SE, n= 7 rats 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).

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دور الفضه النانويه للميراميه في الأذى الكلوي المستحدث في الجرذان المعرضه للكرب التاكسدي (الجزء الاول)

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الخـــــلاصـــــه

أن الأهتمام المتزايد بالجسيمات النانوية ناتج عن زيادة تطبيقاتها في الهندسة والفضاء والمنتجات الاستهلاكية فضلا عن الاستخدامات الدوائية. صممت هذه الدراسة لمعرفة الدور الوقائي لجسيمات الفضة النانوية المصنعة باستعمال مستخلص الميرامية الكحولي كمادة مضادة للأكسدة للأذى الكبدي والكلوي الناتج عن أستخدم عقار الميثوتركسايت. صُنّع في هذه الدراسه دقائق الفضه النانوية الخضراء بواسطه المستخلص الكحولي للأوراق الميراميه التي وصِّفت باستخدام المطياف الضوئي بالأشعة فوق البنفسجية والمجهر الماسح الألكتروني. لمعرفة الدور الوقائي للفضة الناويه المصنعه من الميراميه ، قسمت 28 من الجرذان البيضاء الى أربع مجاميع عشوائية (T1 ,T2 ,T3, T4) تحتوي كل مجموعه على سبعة جرذان وعلى النحو الآتي: المجموعة الأولى حقنت بمستخلص الميرامية وبجرعة 150 ملغم /كغم من وزن الجسم فيما حقنت حيوانات المجموع الثانيه بمحلول جسيمات الفضة النانويه المحضره وبجرعه 150 مغلم/كغم من وزن الجسم أمّا حيوانات المجموعه الثالثة فحقنت بمحلول جسيمات الفضه النانويه المحضره وبجرعه 150 مغلم/كغم من وزن الجسم مع عقار الميثوتركسايت وبجرعه 0.25مغلم/كغم من وزن الجسم في حين حقنت حيوانات المجموعه الرابعه بعقار الميثوتركسايت وبجرعه 0.25 مغلم / كغم من وزن الجسم .استمرت التجربه لمدة 45 يوم وكانت المعالجه حقناً وبالعضله مرتين اسبوعًيآ. فيما اعتبرت جميع الحيوانات في التجربه مجموعه سيطره في اليوم 0 من عمر التجربه واعطيت الماء المقطر والمعقم حقناً وبالعضله . تم اخذ عينات الدم بعد تجويع الحيوانات في الأيام (0,15,30,45) يوم من عمر التجربه وبتقنيه الجيوب المداريه للعين . عُزلت الأمصال من عينات الدم لقياس تراكيز المالونلنيل ثنائي الالديهايد، جلوتاثايون الاختزالي ، تركيز الكرياتينين ، اليوريا وحامض البوليك في مصل الدم .أظهرت النتائج أن الحيوانات التي أعُطيت عقار الميثوتركسايت قد عانت إجهادًا تاكسدي تمثل بحدوث ارتفاع معنوي بتركيز MDA وانخفاض معنوي في تركيز GSH في مصل الدم مع خلل في وظائف الكليه تمثلت بحدوث ارتفاع معنوي بتراكيز اليوريا والكرياتينين وحامض اليوريك في مصل الدم . في حين ظهر الدور الوقائي لدقائق الفضه النانويه المحضره متمثلا بتخفيض حدة الأذى الكلوي من خلال تصحيح المعاير المذكوره سابقا وتصحيح مضادات الأكسده . نستخلص من هذه التجربه الدور المضاد للاكسده والدور الوقائي لدقائق الفضه النانويه المحضره ضد الأذى المستحدث باستخدم عقار الميثوتركسايت في الجرذان .

الكلمات المفتاحيه : الميراميه ، الفضه النانويه ، ميثوتروكسيت ، وظائف الكليه.