Toxicity of Silver Nanoparticles after Injected Intraperitoneally in Rats

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Abstract: Metallic nanoparticles (NPs) offer a great promise in biomedicine.The purpose of the present study was to evaluate the toxic effects of Different doses (5,10) lg/kg/day)of nm SNPs up on intra peritoneal(i.p) Administration in rate every day for (30)days. The silver level sin blood did not increase with the dose administered, where as in all the organs examined there was a proportional increase on silver ,indicating efficient tissue uptake. No evidence of toxicity was observe dinany of the diverse studies performed, including survival, behavior, animal weight, organ morphology, blood biochemistry and tissue histology.
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Introduction:

Nanoparticles (NPs) offer a great possibility forbiomedical application, not only to deliver pharmaceutics, but also to be used as novel diagnostic and therapeutic approaches **[1].**

The small sizes of NPs imply that they could get close to a biological target of inter-est. Furthermore, metallic NPs can be made to resonantly respond to atime-varying magnetic field, with advantageous results related to the transfer of energy tothe particles **[2,3].** This leads to its use as a hyperthermic agent, thereby delivering toxic amounts of thermal energy to targeted bodies such as tumors **[4–7].**

Silver NPs (SNPs) shows several features thatmake them well suited for biomedical applications, including straightforward, synthesis, stability.

In most studies, systemically administrated SNPs were primarily taken up by liver in a large quantity and small amounts distributed in the kidney after single administration. However, little is known about biodistribution, and toxicity of SNPs after repeated administration.

Synthesis silver nanoparticles

The synthesis procedure shown here was adapted by Steve Ng and Chris Johnson from a procedure developed by L. Mulfinger **[8].**

Add 30 mL of 0.002M sodium Borohydride (NaBH4) to an Erlenmeyer flask. Add a magnetic stir bar and place the flask in an ice bath on a stir plate. Stir and cool the liquid for about 20 minutes. Drip 2 mL of 0.001M silver nitrate (AgNO3) into the stirring NaBH4 solution at approximately 1 drop per second. Stop stirring as soon as all of the AgNO3 is added.

The formation of silver nanoparticles can be observed by a change in color since small nanoparticles of silver are yellow.

Characterization of silver nanoparticles

Colloidal particles were also imaged with transmission electron microscopy (TEM) and scanning electron microscope (SEM) are shown in the fig(1),(2) respectively. The Ag nanoparticles are spherical in shape with a smooth surface morphology. The diameter of the nanoparticlesrange in size from(< 50) nm . TEM image also shows that the produced nano particles are more or less uniform in size and shape.

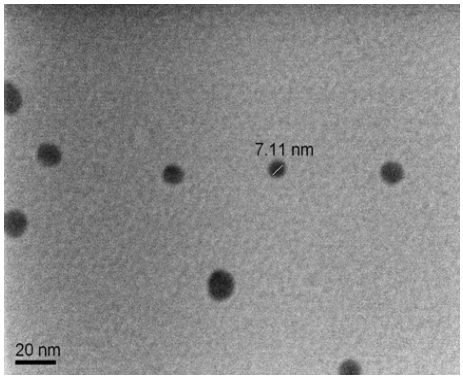


Fig (1) TEM image of silver nanoparticles, scale bar is 20nm.

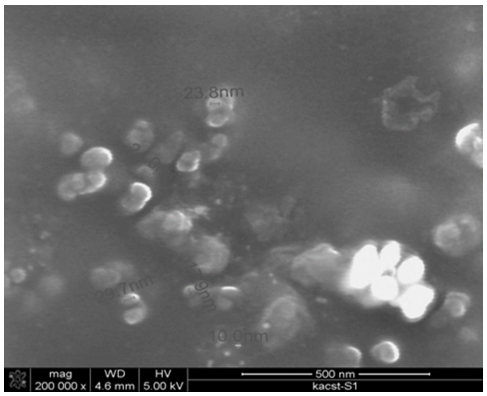


Fig (2) SEM image of silver nanoparticles, scale bar is 500nm.

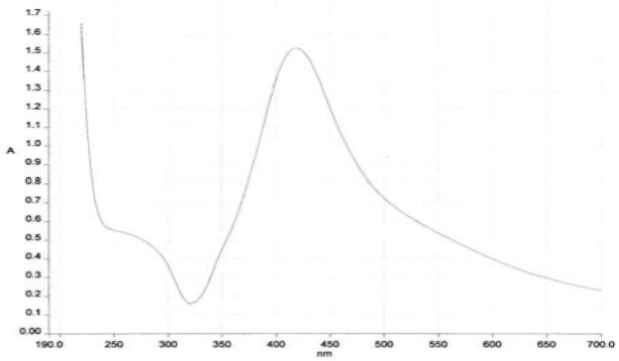
UV/ Vis Spectroscopy Analysis:

In metal nano particles such as in silver, the conduction band and valence band lie very close to each other in which electrons move freely. These free electrons give rise to a surface plasmon resonance (SPR) absorption band **[9-11],** occurring due to the collective oscillation of electrons of silver nano particles in resonance with the light wave **[12].** Classically, the electric field of an incoming wave induces a polarization of the electrons with respect to much heavier ionic core of silver nanoparticles. As a result a net charge difference occurs which in turn acts as a restoring force. This creates a dipolar oscillation of all the electrons with the same phase.

When the frequency of the electromagnetic field becomes resonant with the coherent electron motion, a strong absorption takes place, which is the origin of the observed colour. Here the colour of the prepared silver nanoparticles is yellow. This absorption strongly depends on the particle size, dielectric medium and chemical surroundings **[9,10].** Small spherical nano particles (<50nm) exhibit a single surface plasmon band **[13].** The UV/Vis absorption spectra of the silver nano particles dispersed is shown in the fig(3).

The absorption peak (SPR) is obtained in the visible range at 420 nm. With the above mentioned concentration. The stability of silver nanoparticles is observed for 4 months and it shows a SPR peak at the same wavelength.

Absorbance



Wavelength (nm)

Fig. 3.-UV–Vis absorption spectrum of silver nanoparticles obtained.

Solution was prepared

To obtain the desired doses, 10 mL of the 1 nM solution was centrifuged at 13,500 rpm for 10 min and the obtained pellet was resuspended in sodium citrate 1.2 mM to obtain the following concentrations: 5 nM (55 lg of silver/ml)and 10 nM (110 lg of silver /ml). The solutions were a dark yellow color with a maximum of absorbance centered at 420 nm.

Animals and GNPs treatment

Thirty Male Wister Albino Rats of 12 weeks, weighing 150-250 gm were used fortheexperi-ments. The rats fed with commercial pelleted diet obtained

from King Fahad Medical Research Center in Jeddah. weremain-tained on a 12h dark/light cycle ina room with controlled temperature (25± 2 C). The duration of the experiment is 30 days.

The rats were randomly divided into 3 groups, each group containing 10 rats. The first group acted as control. The second and third groups were injected intraperitoneally once a day by 5 and 10 mg (Nano Ag) /Kg body weight per day, respectively.

Injections of SNPs solution

Adjusting the final volume with the animal weight for the given dose)at doses of 5,10 lg/kg/day daily for (30) days. The doses used were chosen based on in vitro studies of SNPs toxicity inneuro-blastoma cells in culture (data not shown)showing that metallic NPs did not produce significant toxicity upto10nMconcentration, which correspond to the quantity on the highest dose. Control group drinking water. The body weight of animals and their behavior were carefully re- cordeddaily during the course of the experiment. One day after the injection in (30),2 mice were sacrificed, and the blood, liver, kidney, were collected immediately. Serumfrom mouse blood was isolated by centrifugationat3000rpm for 10 min. A part of the organs was stripped and immediately fixed in a 10% formal in solution for further histopathological evaluation.

Statistical analysis

Statistical analysis was performed with SPSS (Version 16). Statistical evaluation was performed by analysis of two-tailed Student's t-test or analysis of variance (ANOVA) following multiple comparison tests with Duncan's method. The level of statistical significance was set at $p < 0.05$

Biochemical indicators

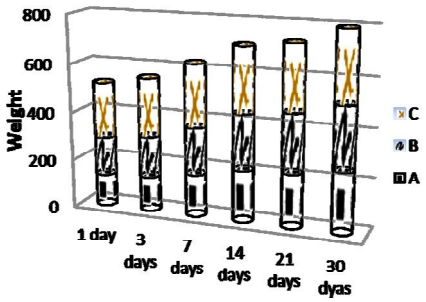
We tested if SNPs treatment produces sub-acute toxicity inmice during the course of the study. We observed no mortality or any gross behavioral changes in mice receiving SNPs at the doses studied. There was effect either of SNPs treatment on the body weight Fig(4).but tissue size, color, and morphologies remain unchanged after treatment with SNPs. No evidences of atrophy, congestion, orinflammation were observed different doses of SNPs as compared to controls showed statistically significant differences between the group(A,B,C) of the parameters tested(Table 1). Additional hematological studies were done to assess changes on the levels of Na (mmol/L),Sodium; K (mmol/L), Potassium;. BUN (mg/dL), Blood urea nitrogen; CRE(mg/dL), Creatinine; CHO(mg/dL), Total cholesterol; TP(g/dL), Total protein; ALB (g/dL), Albumin; ALP (IU/L, Alkaline phosphatase LDH (IU/L), Lactate dehydrogenase CPK (U/L), Creatine phosphokinase;) triacylglyceride transposition of the great arteries (TGA).

None of these parameters showed any statistical difference between the control and the experimental mice groups (data not shown).

Results

Animal Observation in the Effect on Body Weights

A very highly significant increase in body weight on 1, 3, 7 days in Group (C), also Significant increases in the body weight on the 1day in Group (B), day 14, 21, 30 in Group (C) compared with Groups (A).



Fig(4). The body weight

Table (1): Serum values for rats (30-day) after injected intraperitoneally of silver nanoparticles (mean ± S.D.)Dose (mg/Kg)

	A	B	C
	Mean±S.D	Mean ± S.D	Mean ± S.D
Na mmol/l	139±1	142±1	141.7±0.6
K mmol/l	4.9±0.2	4.8±0.6	4.5±0.05
BUN mmol/l	3.8±0.3	7.1±0.6***	6.7±0.4***
Crea µmol/l	54.3±1.6	37.3±1.5***	36.3±2.5***
Chol mmol/l	1.7±0.2	1.2±0.2	1.5±0.1
TGA mmol/l	1.5±0.5	1±0.3	0.6±0.3
TPg/l	68±2.6	61.7±1.5*	60±1***
ALBU g/l	68±2.6	14±1***	12±1***
ALP U/l	134.7±1.5	256.7±14.2***	248.2±***
CPK U/l	207±40.7	240±19.1	168.3±9.1
LDH U/l	136.3±12.1	243.3±31.5***	193.7±13.9

*** The mean difference is very highly significant at $p< 0.001$. * The mean difference is significant at $p<0.05$, N.S Not significant.

There appeared to be increase, Sodium Na (mmol/l), for rats in the low and high-dose(B),(C) groups statistically not significant (Table1).and decrease Potassium K (mmol/l) in the low and high-dose(B),(C) groups statistically not significant .

However, there was a significant increase ($P < 0.001$) in Blood urea nitrogen BUN (mmol/l), for the low and high-dose(B),(C) groups.

A significant decrease ($P<0.001$) in Creatinine Crea(μmol/l)was also found in the low and high-dose (B),(C) groups .no significant decrease in total cholesterol Chol (mmol/l)andtriacyl glyceride transposition of the great arteries (TGA) were also found in the low and high-dose(B),(C)groups.

A significant decrease in Total protein TP (g/l), was found in the low dose($P < 0.05$) and high-dose($P < 0.001$) group. .

A significant decrease in Albumin ALBU (g/L), , was found in the low doseand high-dose ($P < 0.001$)group.

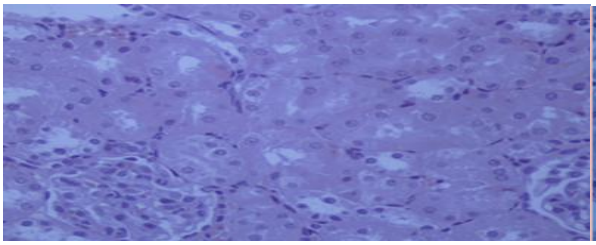
A significant increase ($P < 0.001$) in Alkaline phosphatase ALP (U/L)were also found in the low and high-dose (B),(C) groups no significant (increase ,decrease) respectively in Lactate dehydrogenase LDH(IU/L), also found in the low and high-dose(B),(C)groups.

We demonstrated that, An increases in Na , BUN but decrease in crea due to some side effect on the kidney , Also decrease in the level of K due to bulk of heart muscle contraction, also in the levels of Chol, TGA are important for low of lipids but increase inCPK and LDH in Group (B) but decrease in Group (C) and decrease in the levels of TP , AlBU but increase in ALP, may be some damage in the liver in all groups.From the above , high dose of Ag in Group (C) better conclusion in some biochemical parameters and similar with others copmpared with Group (B).

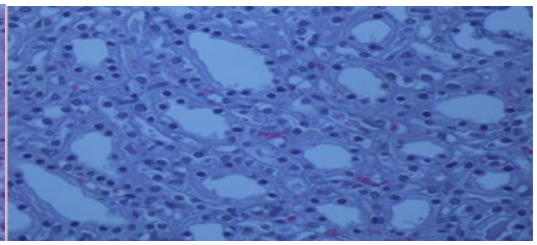
Liver and kidney histopathology

The liver of control rats showed a normal structure (Fig. 5A), which was influenced by the administration of silver nanoparticles (Figs 5B,) (low does). the trabecular structure of the lobules was slightly or distinctly blurred. The cytoplasm of hepatocytes, contained empty vacuole-like spaces, and were enlarged. Some sinusoids were overfilled with erythrocytes and the walls of most sinusoids showed numerous Kupffer cells. Locally, mononuclear cell infiltrates were observed, most frequently in the hepatocytes. In a few animals of this group, an increased density of nuclear chromatin and a very compact nuclear structure were noted . After exposure to (Fig. 5c) (high does), the trabecular liver structure was more seriously affected than after Ag nanoparticles administration.

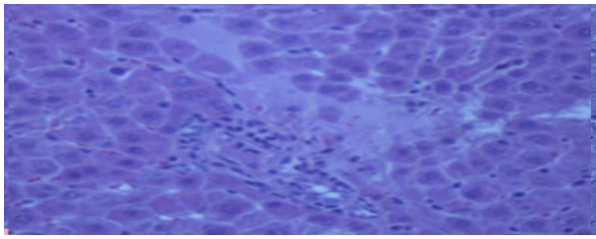
Normal structure of the cortex and medulla was observed in the kidney of control rats (Figs 5A). The animals exposed to silver nanoparticles (5,10)low and high does. showed similar changes, but of different intensity, in the renal tubules and glomeruli (Figs 5A). Hypertrophy of epithelial cells and degeneration of epithelia of renal tubules with infiltration of mononuclear cells, dilation of glomeruli as well as hyperaemia of medullary and cortical parts with mononuclear cell infiltrates were evident in all animals treated with silver nanoparticles (5,10)low and high does. Mononuclear cell infiltrates were observed in some places of the medullary part of the kidney, and at these sites the inflowing cells blurred the tubular structure (Fig. 5A).



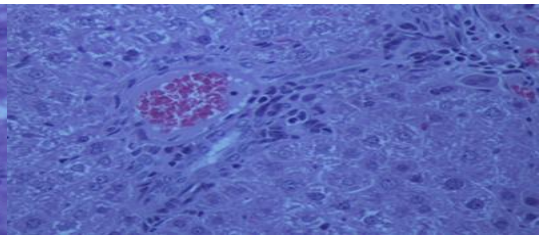
A (control) kidney



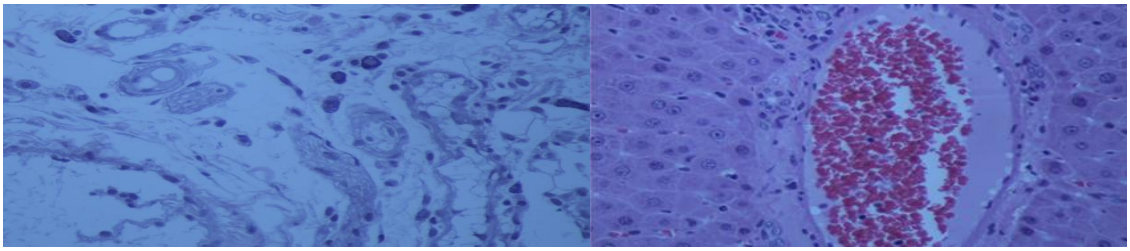
A (control) liver



B (low dose) kideny



B (low dose) liver



C (high dose) kidney **C (high dose) liver**

Fig(5). Histopathological findings in Kidney, liver: (A) control Kidney, liver (B) group (low does) (C) group (high does)

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