



## **Hepatorenal Effects of Silver Nanoparticles In In-Vivo Postnatal Model of Toxicity and In HepG2 Cell Line**

**MAHMOUD M. ELALFY<sup>1\*</sup>, MAMDOUH ABOUELMAGD<sup>1</sup>,  
EMAN A. ABDELRAHEEM<sup>1</sup> and MONA G. ELHADIDY<sup>2</sup>**

<sup>1</sup>Forensic and toxicology department, faculty of veterinary medicine, Mansoura University, Egypt.

<sup>2</sup>Medical physiology department faculty of medicine, Mansoura university, Egypt.

### **Abstract**

Silver nanoparticles (Ag-NPs) had many uses in medicine, household and industry. To better understand the postnatal toxicity of Ag-NPs in lactating female rats and its offspring's, 18 female rats after delivery were divided into three groups and dams received orally the AG-NPs at doses of 0, 50, 100 ppm daily for 21 days. After the end of treatment, all rats were euthanized and blood and tissues were separated for evaluation of biochemical and histopathology in dams and its pups. The Ag-NPs had no effect on the dam's weight while the reduction of rats' pups weight was noticed after first week only after the treatment. Notably, Ag-NPs had toxic effects in rat's pups, as well as its dam with evidence of elevation of liver enzymes, urea, creatinine and reduction of serum protein, albumin and globulin and considered the first report explained the toxicity in the rat's pups. Moreover, rats' pups revealed histopathological changes in liver and kidney as well as its dams. Notably, the nano-silver is considered cytotoxic for HepG2 cell line as well as mouse liver cell line. In conclusions, the Ag-NPs considered toxic in offspring as well as dams and had immunosuppressive effects in the postnatal model of toxicity as well as cytotoxicity to hepatic cells lines.



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Ag-Nps;  
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### **Background**

Ag-NPs are promising material for drug, antiseptics, household and anticancer therapy.<sup>1</sup> Its side effects

are lasting but still have no approval of widespread uses as its toxicity for cells of human, animal and environment are detected.<sup>2,3</sup>

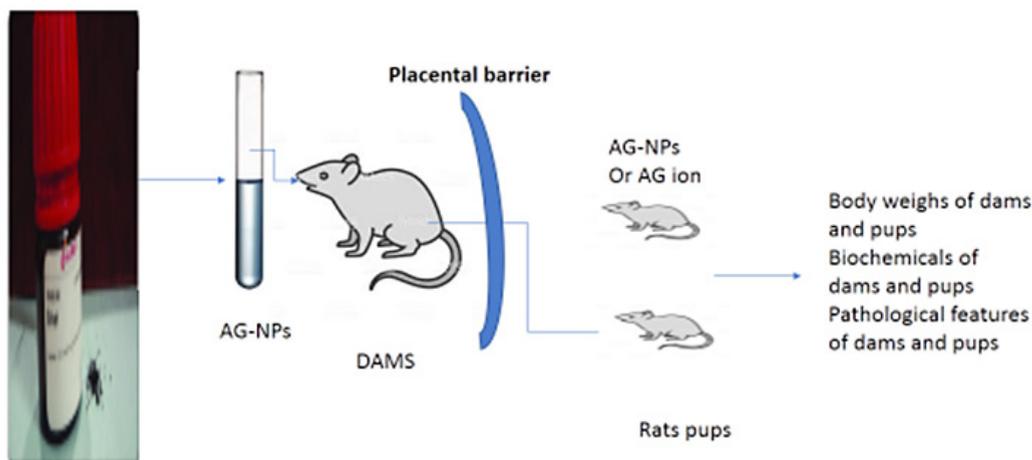
**CONTACT** Mahmoud M. Elalfy ✉ mahmoudelalfy@mans.edu.eg 📍 Forensic and toxicology department, faculty of veterinary medicine, Mansoura University, Egypt.



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**Illustrated graphical abstract of Ag NPs toxicity in female albino rats**

There were many studies on Ag-NPs on prenatal studies on both pups and dams in different animals' species. These studies evoked that Ag-NPs are widely distributed in all tissues and can cross the placental barrier and transfer to pups through milk suckling.<sup>4-6</sup>

The route of administration of Ag-NPs is considered a determinant factor in severity of toxic effects of Ag-NPs as it previously recorded that 4 to 18% only absorbed through GIT in mammals while it can absorb from other routes with high levels.<sup>7</sup> Even the low-level Ag-NPs reach blood circulation after oral administration, still evidence that Ag-NPs were distributed in different tissues of rats' dam.<sup>8</sup> Also type of Ag-NPs could also be another factor affecting its toxicity as chemically synthesized Ag-NPs expect to have more toxic complications than that produced by green means by use of plants extracts.<sup>9</sup>

The mechanism of toxicity of oral exposure Ag-NPs to rat dams and pups may be due to induction of oxidative stress and apoptosis in the liver of their offspring.<sup>10</sup> The rationale of this study to investigate the cytotoxicity of Ag-NPs in female rats and its pups in postnatal model of toxicity.

## Materials and Methods

### Laboratory Animals

Female albino rats obtained from the experimental unit, Faculty of veterinary medicine, Zagazig

University, weighted from 180 to 200 gram. Animals were apparently healthy and housed in plastic cages containing wood shaving as a bedding material. Animals accommodated for 2 weeks before the experiment and maintained on a balanced ration also feed and water given ad libitum. We follow all guidelines of animal ethics in faculty of veterinary medicine, Mansoura University, Egypt.

### Tested Chemicals and Cell Culture

Nano-Silver, its size less than 100 nm, (USA, MKCD7246) and HepG2 cell line were purchased from Sigma Aldrich.

### Experimental Grouping and Design

Eighteen pregnant female rats divided into three groups each one contained six rats weighed 150 to 200 g and kept until delivery. In the current study, Silver Nanoparticles (Sigma Aldrich, USA) given orally, dissolved in di-ionized water, at dose level 0, 50, 100 ppm (equivalent to 1/50 and 1/100 of the LD50 recorded earlier<sup>11</sup> on first day of lactation till 21 days and control group intubated with di-ionized water as control. Dams weighted before dosing to maintain constant dose throughout the experimental period. On 21<sup>st</sup> day of lactation, all rats were sacrificed by over dose of thiopental, blood of dams and pups were collected for serum separation and different tissue samples were collected. Tissues of pups were separated and fixed in buffered formalin for histopathological examination.

### Histopathological Examination

Different tissues from dams and pups were fixed in 10% neutral buffered formalin. Fixed tissue where procedures were described before in detail for classical eosin and hematoxylin staining, imaging and reading for pathology detection.<sup>12</sup>

### Biochemical Analysis

Sera of all dams and pups of treated or control groups were tested for liver and kidney function tests for evaluation the postnatal toxicity of AG-NPs.

### Cytotoxicity of Silver Nanoparticles on of HepG2 cells

The stock solution of Ag NPs (100 mmol/L) was prepared in di-ionized water and stored at 4°C. Working solutions were prepared by dissolving the stock solution in the culture medium. We exposed HepG2 cells and the primary mouse liver cell line to different concentrations (0, 10, 100, 200 ppm/mL) of Ag NPs for 24 hours to determine its toxic effects. The HepG2 cells and mouse liver primary cell line were cultured according to methods described earlier.<sup>13</sup>

### Cell Viability and Oxidative Stress Tests

The viability of cells was detected by quantification of formed formazan salt. In this regard, HepG2

cells ( $2 \times 10^5$  cells) were seeded in 96-well plates. Later 24 h, the medium solution was changed with other medium containing different concentrations (0, 10, 100, 200 ppm) of silver nanoparticles and di-ionized water were added for 24. MTT (50 µg/mL) was added to each well. After 4 h incubation at 37°C, the later solution was discarded and formed formazan crystals was dissolved in DMSO (100 µL). The color developed was measured at 570 nm using a multiplate reader (Synergy HT, Bio-Tek, Winooski, Vermont). Additionally, the cell extract was centrifuged (10000 g, 10 min, 4°C) and supernatant was used for oxidative stress assays such as glutathione (GSH), superoxide dismutase (SOD), and MDA.

Statistical Analysis was carried out using t-test and Oneway Anova. and  $P < 0.05$  was considered significant (Spss version 13).<sup>14</sup>

## Results

### Dams and Pups Weights

There was no effect on body weight of rat dams while the pups had reduced body weight only after one week of treatment when compared to control group. (Table 1)

**Table 1: showed the postnatal effect of AgNPs on body weight of rat pups during the treatment**

Control	First week of treatment	Second week of treatment	Third week of treatment
Group 1	12.80±0.46212	18.52±0.76 <sup>c</sup>	34.39±1.032 <sup>a</sup>
Group 2	12.52±0.28196	23.05±0.51 <sup>b</sup>	35.59±0.77 <sup>a</sup>
Group 3	15.30±0.27750	28.19±0.60 <sup>a</sup>	36±0.83 <sup>a</sup>

a, b, c significant at  $\leq 0.05$

### Biochemical Effects of Silver Nanoparticles on Dams and its Pups

Here, we are the first report that recorded the effects of the toxicity of Ag-NPs in rat pups in postnatal model of toxicity especially biochemical alteration in the liver and kidney tissue. The hepatorenal toxicity of Ag-NPs was based on significant increase of activity of liver enzymes (AST, ALT) and levels of

creatinine, urea and cholesterol in dose dependent manner when compared to control groups. Also, it enhanced the reduction of total protein, albumin and albumin/globulin ratio when compared to the control group especially at low dose of silver nanoparticles treatment. Additionally, AG-NPs could have immunosuppression effects as it reduced the globulin level (table).

**Table 2: The hepatorenal effects of oral administration of Ag-NPs treatment in postnatal toxicity in rat dams**

Urea	Creatinine (mg/dL)	Cholesterol (mmol/L)	Urea (mmol/L)	Creatinine (mg/dL)	Cholesterol (mmol/L)
Group 1	7.80±1.511 <sup>b</sup>	29.89±2.06 <sup>b</sup>	79.73±2.70 <sup>b</sup>	39.4155±2.87 <sup>b</sup>	4.23±.33 <sup>b</sup>
Group 2	26.67±2.42 <sup>a</sup>	47.88±5.08 <sup>a</sup>	98.81±5.96 <sup>a</sup>	44.01±1.05 <sup>ab</sup>	4.4977±.27 <sup>b</sup>
Group 3	28.49±3.126 <sup>a</sup>	52.92±5.28 <sup>a</sup>	105.07±8.67 <sup>a</sup>	45.46±.97 <sup>a</sup>	6.1482±.74 <sup>a</sup>

a, b, c significant at ≤ 0.05

The fetal toxicity of Ag-NPs in rat pups was evidenced by increased activity of liver enzymes (ALT, AST) and levels of urea, creatinine, and cholesterol in significant manner and dose dependent effect when compared to control groups. Also, the Ag-NPs had

no effect on total protein and globulin levels of pups. Notably, albumin and albumin/globulin ration were reduced in significant manner when compared to control groups.

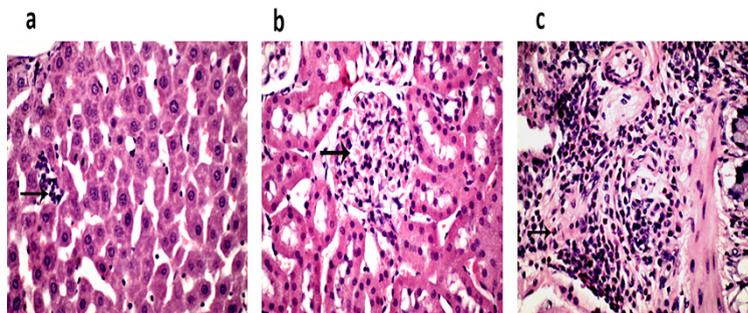
**Table 3: show the effects of oral administration of AG-NPs treatments in protein metabolism of rat dams**

	Total protein	Albumin	Globulin	Albumin / globulin ratio
Group 1	11.41±0.39162 <sup>a</sup>	5.85±0.22369 <sup>a</sup>	5.56±0.45	1.11±0.09
Group 2	9.36± 0.45469 <sup>b</sup>	4.90±0.23192 <sup>b</sup>	4.45±0.50	1.18±0.20
Group 3	10.34±0.60785 <sup>ab</sup>	5.43±0.10869 <sup>ab</sup>	4.90±0.58	1.20±0.14

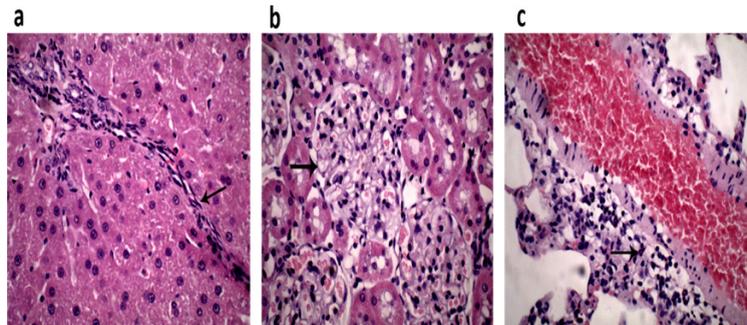
a, b, c significant at ≤ 0.05

Pathological finding of Ag-NPs treatment in rat dams and its pups. The group treated with Ag-NPs at dose of 50 ppm shown mild lymphocytic infiltrate into hepatic tissue, congestion of the glomerular capillaries and proliferation of peribronchiolar lymphoid tissue and neutrophilic recruitment. While group treated with

100ppm of AG-NPs displayed hepatic intralobular fibroblastic proliferation, congestion of the glomerular capillaries and proliferation of the renal glomeruli and congestion of interlobular blood capillaries and neutrophilic recruitment into pulmonary tissue (figure 1, 2).



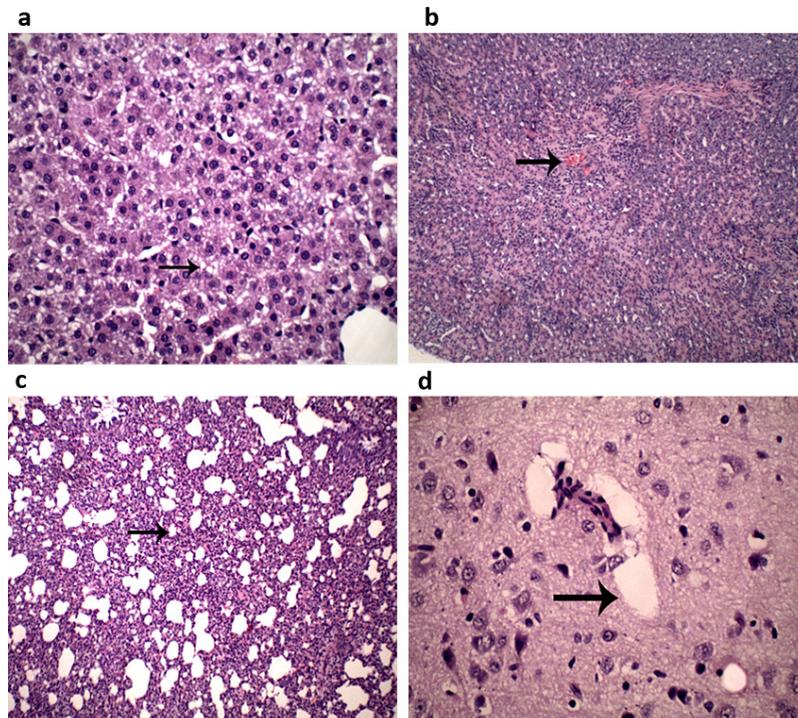
**Fig. 1: The depicted figures of dams treat with Ag-NPs at dose of 50 ppm displayed histological alteration as mild lymphocytic infiltrate into liver tissue (a), congestion of the glomerular capillaries and proliferation of peribronchiolar lymphoid tissue (b) and neutrophilic recruitment of lung tissue (c) when compared to control group**



**Fig. 2:** The depicted figures of dams treat with AG-NPs at dose of 100 ppm displayed intralobular fibroblastic proliferation in liver tissue (a), congestion of the glomerular capillaries and proliferation of the renal glomeruli (b) and congestion of interlobular blood capillaries and sever hemorrhage and neutrophilic recruitment into pulmonary tissue (c) when compared to control group

Notably, silver nanoparticle secreted in milk and enhanced histopathological changes at dose of 50 ppm in rat pups such as perivascular edema in brain parenchyma, neutrophilic recruitment into the renal glomeruli, local necrosis of hepatocytes and proliferation of interstitial tissue with pulmonary

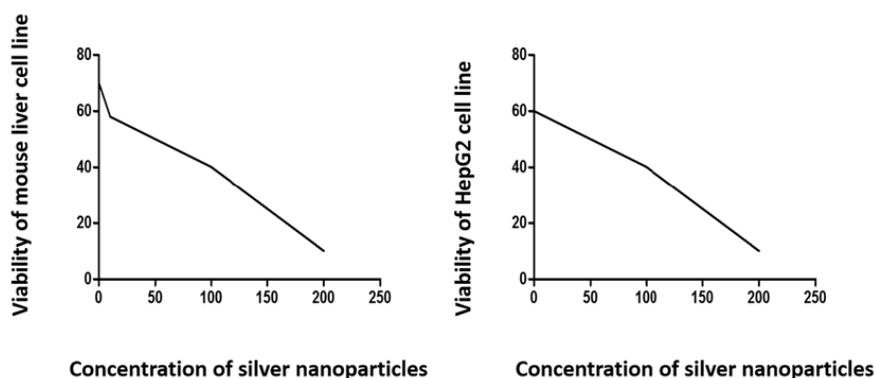
atelectasis and hemorrhage. While the AG-NPs at 100 ppm induced perivascular edema in brain parenchyma, congestion of interstitial blood capillaries of renal tissue, vacuolation of hepatocytes and proliferation of interstitial tissue and pulmonary atelectasis (figure 3).



**Fig. 3:** The depicted figures of feti liver of dams treated with AG-NPs (50 ppm) shown vacuolation of hepatocytes (a), congestion of interstitial renal blood capillaries (b), proliferation of interstitial tissue and pulmonary atelectasis (c). perivascular brain edema (50 ppm of AG-NPs)

Notably, the viability percentage measured by MTT assay on primary liver cell of mice or HepG2 cells exposed to 0, 1, 10, 100 and 200 ppm of AgNPs for 24 h displayed a dose-response pattern as shown

in Figure 4. Also silver nanoparticles enhanced oxidative stress in HepG2 cells based on increase MDA and reduction of GSH and GST (table 4).



**Fig. 4: Pattern effect of Silver Nanoparticles on cell viability in mouse liver cell line and HepG2 cell line**

**Table 1: Showed effect of oxidative stress in HepG2 cell line**

Concentration of Silver Nanoparticles (ppm)	GSH mg/g tissue	SOD U/g tissue	MDA nmol/g tissue
0	20.69±1.095 <sup>a</sup>	8.42±0.054 <sup>a</sup>	14.97±0.93 <sup>c</sup>
10	18.69±1.05 <sup>a</sup>	7.42±0.035 <sup>a</sup>	17.97±0.63 <sup>c</sup>
100	9.69±1.03 <sup>b</sup>	4.42±0.054 <sup>b</sup>	53.97±0.93 <sup>b</sup>
200	5.91±1.042 <sup>b</sup>	2.08±0.53 <sup>b</sup>	75.96±1.90 <sup>a</sup>

a, b, c significant at  $\leq 0.05$

### Discussion

There was several evidence that AG-NPs presence in milk of lactating rats<sup>1</sup> or mice<sup>2</sup> was reported previously after treatment with different routes of administration.<sup>3-5</sup>

In the current research, there was no effect of silver nanoparticles on body weight of dams while the pups had effect on body weight only after one week of treatment when compared to the control group (table 1). In this regard, there was no difference on pup's weight among treated groups by silver nanoparticles reported by Rashno *et al.*,<sup>7, 15</sup>

The histopathological changes in rat pups such as edema in the brain and liver necrosis (figure 3) recorded also in several studies after

oral exposure to silver nanoparticles.<sup>7,8</sup> Also, Amiri *et al.*,<sup>8</sup> investigated the brain toxicity of Ag-NPs on pregnant female mice and it's offspring during and after pregnancy. Moreover, the exposure to AgNPs may enhanced apoptosis and necrosis via the intrinsic pathway in the offspring tissues of rats treated with 25 and 100 ppm of Ag-NP for 21 days.<sup>9</sup> The pathological alteration was prominent in the dam (figure 1,2) as well as in its offspring (figure 3) due to an immature immune system especially in early postnatal period, lack of protective enzymes, high metabolic rate, and low infiltration rate.<sup>10,11</sup> Also, another evidence recorded presence of distribution of Ag-NPs in kidney, lung and liver of rat's pups<sup>5</sup> and this accumulation of AG-NPs could release Ag ions that are responsible for toxicity induction<sup>16</sup> and similar studies revealed the reduction

of total protein and albumin (table 3) after silver nanoparticles treatments.<sup>17, 18</sup> While few studies reported that Ag-NPs elevated the total protein in non-significant manner as silver nanoparticle binding with albumin and retain its presence in serum after exposure to high dose.<sup>19, 20</sup> Moreover, the oral administration of Ag-NPs considered less toxic than intraperitoneal treatment as only 4-18 % of Ag-NPs were absorbed after oral treatments<sup>21</sup> So, the toxicity of silver nanoparticles could result in damage of liver parenchyma based on the reduction of serum protein and elevation of liver enzymes (figure 2)<sup>13, 14</sup> as well as our finding in rat's pups as they had immature liver. Notably, the silver nanoparticles had cytotoxic effects on HepG2 and primary mouse liver cell line<sup>19, 20</sup> thorough enhancement of oxidative stress and reduction of antioxidants (figure 4 and table 4).

In conclusion, the Ag-NPs considered to be toxic in offspring as well as dams and had immunosuppressive effects in the postnatal model

of toxicity. Also it had a toxic effect in HepG2 cell line mediates by oxidative stress.

#### Author contributions

Mahmoud elalfy, Eman Abdelraheem and Mona El-hadidy designed, carried out the experiment, analysis, write and approved the submission. Mamdouh abouelmagd supervised and approved the submission.

#### Acknowledgment

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#### Conflict of interest

All author declares that no conflict of interest

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