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The imbalance of oxidant/antioxidant enhanced the cytotoxicity andteratogenic effects of oral administration of silver nanoparticles in female albino rats

Research Article

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Abstract

Background: The aim of this study was to explore the effect of oral administration of silver nanoparticles (AgNPs) and identify potential role of alteration of antioxidant/oxidants and their effect on teratogenic effects in the female albino rat.

Methods: eighteen female rats were used to assess the acute effects of AgNPs. Rats in the treatment group were gavaged with 1 mL AgNPs, dissolved in di-ionized water, at doses of 0, 50 and 100 mg/ kg of body weight on 6th to 15th day of gestation of pregnant female albino rats. After 20 days of gestation, female rats were euthanized, examined grossly after dissection and feti were stored in ethyl alcohol and poin's solution for skeletal and visceral malformation detection.

Results: silver nanoparticles at dose dependent decrease significantly fetal body weight and length and enhanced skeletal malformation. The induction of skeletal abnormalities could be happen as imbalance of oxidant/ antioxidant levels as oxidant such as MDA was increased in liver in treated groups when compared to control one and also in placenta only at group 2. Nitric oxide, GSH and SOD1levels were increased significantly in liver of treated groups only. Notably, infiltration of neutrophil in placenta tissue could also affect placenta and fetal growth.

Conclusions: silver nanoparticles had a teratogenic effect in female albino rats that could be resulted from imbalance of oxidant/ antioxidant levels and pathological features in the placenta of dams.

Introduction

In the last ten years, nanoparticles have gained great attention and became part of many consumer products and nanotechnology has gained a significant of public interest due to increase the needs and applications of nanomaterials in many areas of human resources like as industry, agriculture, medicine and public health [1].

Silver nanoparticles were among of the most frequently used nanomaterial in consumer products [2] and were widely beneficial as antimicrobial materials that could be incorporated into plastics, textiles and food container [3]. It was estimated that approximately 320 tons per year of AgNPs were manufactured and used worldwide [4]. It was potent and effective antimicrobial agents due to their

protracted release of broad-spectrum bactericidal Ag cations [5].

The oral LD50 of silver nanoparticles were reported to be more than 5 gm/kg (6) which suggest safety and wide distribution in consumer products according hazardous classification of chemicals. In contrast to its safety, many authors found that the production of reactive oxygen species responsible of cytotoxicity of AgNPs [7-9].

The recent research's reported that silver nanoparticles had the toxicity and teratogenicity properties. Even there are many studies on teratogenic and cytotoxicity of silver nanoparticles in experimental animals, its mechanism still controversial. We explore the effects of oxidant/antioxidants status and correlation to teratogenic effects of silver nanoparticles with special reference to its median lethal toxicity.

Materials and methods

Experimental animals

Female albino rats purchased from experimental unit, Faculty of veterinary medicine, zagazig university; weighted from 150 to 200 gm. All rats were healthy and housed in plastic cages contain wood shaving as a bedding material. Animals were kept for 2 weeks before the experiment for accommodation and maintained on a balanced ration of feeds and water given ad libitium. The current experiment was done in accordance with guidelines of animal ethics committee in faculty of veterinary medicine, Mansoura university, Egypt

Chemicals

Silver nanoparticles was purchased from sigma-aldrich Cairo, Egypt

Experimental grouping and design

Eighteen Rats divided into three groups each one contains six rats weighted 150 to 200 gm; treated groups treated with silver nanoparticle orally, dissolved in di-ionized water, at doses level 0, 50, 100mg/kg (equivalent to 1/100 and 1/50 of the LD50) on 6th to 15th days of gestation of pregnant female rats. On 20th day of gestation [10].

Gross and skeletal examination

The abdominal wall of pregnant rat dams was opened and reflected over the thorax, the gravid uterus was grossly examined and the feti were separated. The resorption sites were examined. The separated feti were examined for live and dead ratio and also for any gross malformation. The fetal body weight and crown rump were detected also. The feti of each dam put into two groups the first group was kept in bouin's solution for visceral and histopathological examination and the second group was preserved in absolute solution of ethyl alcohol for skeletal malformation [11]. The procedures of preparation of feti such as skinning, evassieration and tissue dissolution and staining skeletal malformation were done according method described earlier [12].

Biochemical oxidant and antioxidant detection in tissue homogenates

Liver and placenta were separated from female pregnant dams and washed by normal saline, after that 0.5 gram of these tissue was homogenized in 4.5 ml of ice cold phosphate buffer saline (PBS) pH 7.4. Then the homogenized tissues were centrifuged at 3000 rpm for 15 minutes at 4oc, and lastly the supernatant were separated in eppendorf tubes and were stored at -20oc until estimation of biochemical tests.The Supernatant of tissues were testedforMalondialdehyde [13], nitric oxide [14], SOD1 [15], and glutathione-s-transferase [16].

Histopathological examination

Parts of from liver and placenta of female pregnant dams were separated and stored in 10% neutral buffer formalin. Sections of 5 micron thickness of processed tissue were prepared from collected samples, stained by hematoxyline and eosin and then examined by light microscope [17].

Statistical analysis

The raw data analyzed by SPSS statistical software, version 13. Descriptive statistics of frequency was computed to assess the occurrence of skeletal and visceral malformation and oxidant/antioxidant levels in dams' tissues [18].

Results

Chemical synthesized AgNPs at doses of 0, 50, 100 mg/kg were tested for its teratogenicity in female albino rats. AgNPs had resulted in reduce the body weight of rat dams non significantly when compared to control group (Table 1).

The feti body weight and length of treated groups with AgNPs at highest dose was significant decreased when compared with control group (Table 1 and Figure 1).

Table 1: shown the feti body weight of treated groups with AgNPs.

Groups	Doses of AgNPs	Feti body weight	Feti length
Group 1 (control)	di-ionized water 0.5 ml	3.6816±0.042[a]	5.3523±.042 [a]
Group 2	1/100 AgNPs =50 mg/kg	3.2406±0.045[b]	4.9929±.021[b]
Group 3	1/50 AgNPs =100 mg/kg	3.1865±0.049[b]	4.8370±.059[c]

A, b,c significant when $p \leq 0.05$

Table 2: show total skeletal abnormalities of

	Control group	Group 2	Group 3
Absence of parietal and interparietal bones	Zero	zero	38.80%
Absence of carpal and tarsal bones	Zero	83.30%	100%
abnormalities of phalanges	Zero	50%	53.30%
Abnormalities of ribs	zero	100%	72.20%
Abnormalities of sternum	zero	66.70%	50%
Abnormalities of coccygeal vertebrates	100%	100%	100%

Figure 1: The feti treated with silver nanoparticles shown a significant decrease of length of compared to control group non significantly when compared to control group.

Skeletal malformation assessment in female albino rats

Silver nanoparticles at dose of 50 mg/kg showed 83.3% of Absence of carpal and tarsal bones, 50% of abnormalities of phalanges and 100% of rats shown abnormalities of ribs and coccygeal vertebrates when compared to control group (Figure 2 and Table 2). While skeletal abnormalities of silver nanoparticles at dose of 100 mg/kg were 38.8% absence of parietal and interparietal bones, 100% Absence of carpal and tarsal bones and abnormalities of coccygeal vertebrates, 50% sternum segments abnormalities, 53.3% abnormalities of phalanges and 72.2% of fused ribs or absence when compared to control groups (Figure 3 and Table 2).

Oxidant/antioxidant levels

The oxidant as MDA was increased in liver in treated groups when compared to control one and also in placenta only at group 2. Nitric oxide, GSH and SOD1 were increased significantly in liver of treated groups only (Table 3).

Table 3 show the oxidant/antioxidant levels in liver and placenta of treated groups with AgNPs at different doses compared to control groups.

Histological examination

Histopathological features of silver nanoparticles displayed sever lymphocytic infiltration and degeneration in hepatocytes of dams in highest dose of AgNPs (100 mg/kg) while only degeneration of hepatocytes of dams at low

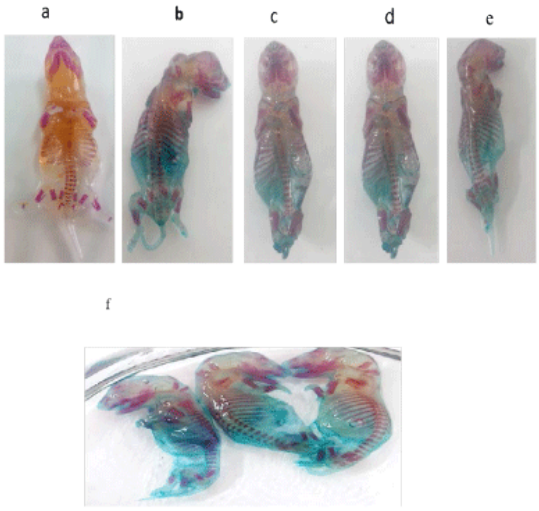

Figure 2: Skeletal abnormalities of silver nanoparticles at dose of 100 mg/kg a-showed control negative group b-showed absence of parietal and interparietal bones c-showed abnormalities of sternum vertebras d-showed absence of phalanges, carpal and tarsal bones e-showed abnormalities of ribs f-general abnormalities of 1/50 AgNPs

Table 3: show the oxidant/antioxidant levels in liver and placenta of treated groups with AgNPs at different doses compared to control groups.

Groups	MDA(nm/g)		Nitric oxide(µm/l)		GSH(u/g)		Sod1(iu/gm)	
	Liver	Placenta	Liver	Placenta	Liver	Placenta	Liver	Placenta
Group 1 (control)	14323±611	20789±714.46	38.66±5.30[a]	10.16±3.01[b]	32.57±1.63	4.9665±0.43	1939.5±194[a]	438±85
Group 2	15294±1586	20856±1342.78	19.45±3.46[b]	16.90±1.75[b]	29.66±3.71	5.5780±0.47	1257.7±89[b]	427±66
Group 3	15305±391	18709±1068.12	28.61±2.63[ab]	24.79±2.64[a]	34.19±.80	5.9330±0.54	2093.3±105[a]	402±65

A, b,c significant when $p \leq 0.05$

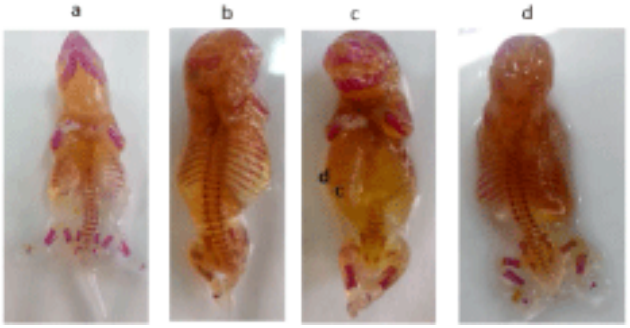

Figure 3: show the skeletal abnormalities of silver nanoparticles at dose of 100 mg/kg a- control group show normal skeletal formation b-showed abnormalities of ribs c-showed abnormalities of sternum vertebras d-showed abnormalities of tarsal and phalanges in 1/100 AgNPs group.

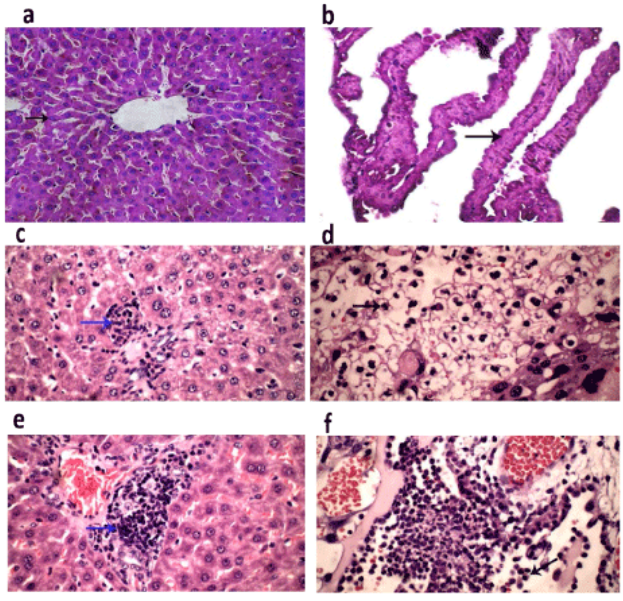

Figure 4: show pathological features of silver nanoparticles. A) normal liver b) normal placenta c. section of liver of group 2 displayed lymphocytic recruitment into the portal area (arrow). (HE, 400x) resulted from AgNPstreatment (arrow). (HE, 100x), d) placenta of groups 2 displayed edema and neutrophilic exudate resulted from AgNPstreatment (arrow). (HE, 400x), e) liver of group 3 displayed sever lymphocytic recruitment into the portal area resulted from AgNPstreatment (arrow). (HE, 400x) and f) placenta of group 3 displayed aggregations of neutrophils and plasma cells resulted from AgNPstreatment (arrow). (HE, 400x).

dose of AgNPs (50 mg/kg). Moreover, placenta of dams displayed edema and neutrophilic exudate resulted from AgNPs at lower dose while in highest dose of AgNPs (100 mg/kg) shown aggregations of neutrophils and plasma cells

Discussion

The NPs considered as effective drug delivery due to its unique physicochemical characters and various polymers had been used in drug delivery research because they could effectively develop drugs to the target site which increased the therapeutic benefits and minimized side effects [19]. Silver nanoparticles considered among nanoparticles that had focus of drug developer in recent years. Several studies were done in silver nanoparticles in experimental animals to evaluate the safety of them as therapeutics like genotoxicity [20,21], cytotoxicity [22]and teratogenicity [23-27] but the doses that used in most of these research's not relative to LD50 [6]. Here we conduct the experiment based on LD50 recorded earlier. According chemical classification of poison, silver nanoparticles expected to be safe but continuous release of silver ion [28-30] from that nanoparticle resulted in increase the oxidant production [31,32] and in same times consumed the antioxidant pathways like nitric oxide, GSH and SOD1 [33,34] to scavenger the free radicles at target tissue. The mechanism of teratogenicity of silver nanoparticles is not easy task but here we notice that silver nanoparticles enhanced neutrophil infiltration in placenta tissue of dams that could have a role in fetal developmental toxicity as neutrophil activation via the protease activated receptor system, resulted in the generation of toxic ROS molecules, thereby enhanced placental damage and subsequent fetal injury [35]. Moreover, neutrophil infiltration affect placenta growth and fetias depletion of neutrophil with either antibodies lead to a reduction in fetal resorption, and an increase in both fetal and placental mass [36].

Finally, there no one mechanism for teratogenicity of silver nanoparticle as induction of ROS lead to DNA damage, genotoxicity and cell injury of feti [37], infiltration of neutrophil in placenta could be indicator of placental damage and fetal injury. Notably, the increase of neutrophils counts promoted endothelial dysfunction after placental ischemia [38], and endothelial membrane damage resulted from neutrophil activation may be the causeof preeclampsia and intrauterine growth restriction [39,40] and Prenatal exposure of AgNPs can enhanced devastative and detrimental effect in the organogenesis stage of the developing embryos and fetuses.

On conclusions, imbalance of oxidant/antioxidant pathways and neutrophil infiltration enhanced damage in placenta and feti injury could resulted from silver nanoparticles treatment and explain its teratogenicity

All author declare that no conflict of interest

Author contributions

Mahmoud elalfy and emanabedalraheem designed, carried out the experiment, analysis , write and approved the submission. Mamdouh abouelmagd supervised and approved the submission.

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