RESEARCH ARTICLE

Effect of oral administration of silver nanoparticles on blood parameters and bone marrow cells of female albino rats

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Elalfy MM, Abdraheem EE, Abouelmagd V. Effect of oral administration of silver nanoparticles on blood parameters and bone marrow cells of female albino rats. Clin Pharmacol Toxicol Res.2019;2(2):1-3.

Background: The aim of this study was to investigate the effect of oral acute dosing with silver nanoparticles (AgNPs) and identify potential genotoxicity and their effect on blood parameters 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 of di-ionized water containing AgNPs at dose of 1/50 and 1/100 of LD50 weight on 6th to 15th day of gestation of pregnant female albino rats. Control rats received 1 mL of di-ionized water only. After 20 days of gestation, rats were euthanized, blood samples and bone marrow were collected to investigate for hematologic and genotoxicity of sliver nanoparticles.

Results and conclusion: AgNPs were enhanced hematotoxiciy in female albino rats at time of delivery and after end of treatment evidenced by reduced level of RBC, WBC, hemoglobin and calculated hemo-indices. Notably, AgNPs enhanced genotoxicity by increased chromosomal aberrations at dose dependent manner. While no change in micronuclei numbers when compared to control group. Taken collectively, AgNPs considered not safe as pharmacological agent during pregnancy.

Key Words: *Silver nanoparticles; Blood parameters; Genotoxicity; Hematologic*

INTRODUCTION

AgNPs have vital role as antibacterial, antiseptic, and anti-tumor effects based in previously recorded articles in literature.

AgNPs are used in many products, such as foods, cosmetics, and medicines [1,2]. Ag-nanoparticles are used also in personal care products, household products, and dietary supplements, due to their antibacterial effects [3,4].

Although the harmful effects of AgNPs has been reported *in vitro* and *in vivo,* mechanistic understanding of the toxicity particularly at the cell levels and safety of AgNPs remained unclear [5].

Previously, there many evidences that AgNPs have teratogenic effects especially on skeletal malformation [6] maternal toxicity [7] and also there is a recent record of presence of AgNPs in milk of female mice [8].

The rationale of this study to evaluates the safety of silver nanoparticles using during pregnancy in short term of toxicity model.

MATERIALS AND METHODS

Experimental animals

Female albino rats obtained from experimental unit, Faculty of veterinary medicine, zagazig university; weighted from 150 to 200 g. Animals were apparently healthy and housed in plastic cages contain 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 libitium. We follow all guidelines of animal ethics in faculty of veterinary medicine, Mansoura University, Egypt.

Experimental grouping and design

Eighteen Rats divided into three groups each one contains six rats weighted 150 to 200 g; treated groups intubated with silver nanoparticle orally dissolved in di-ionized water at dose level 1/50 and 1/100 of the LD50 [9] on 6th to 15th days of gestation of pregnant female rats and control group intubated with di-ionized water as control. Animals weighted before dosing to maintain constant dose throughout the experimental period. On 20th day of gestation, all rats injected intraperitoneally with colchicine (4 mg/kg body weight) 3 hours before sacrificing then femur removed immediately, and bone marrow samples were collected.

Chromosomal aberration detection: Rats intraperitoneally injected with colchicine (4 mg/kg body weight) 3 hours before sacrificing then femur removed immediately and bone marrow received in centrifuge tube by injection of 5 ml sodium citrate (0.6%) hypotonic solution then incubated for 20 min at 37°C followed by centrifugation for 2 min at 2000 rpm. and Supernatant discarded and 5 ml of cold fixative solution (methanol and glacial acetic acid with ratio 3:1 added to precipitate and left at room temperature for 5 min followed by centrifugation for 2 min at 2000 rpm and such technique repeated twice, then from the 75 cm by using Pasteur pipette suspension dropped on a clean, moisten and cold slide followed by air drying and staining with giemsa stain solution 5% and at least investigation of 1000 metaphase cells per each group examined for chromosomal aberration.

Mitotic index detection: Mitotic index detected according to Sehgal et al. [10], Where approximately 3000 cells for each group analyzed for the mitotic index (MI), calculated as the number of divided cells at metaphase per total number cells according to following formula: MI%=number of the divided cells × 100/total number of the calculated cells.

Micronuclei detection: Micronuclei assay detected according to Thomas et al. [11], where the bone marrow cells from the femur flushed by 5 ml saline solution by using syringe in a centrifuge tube and centrifuged at 4°C for 15 min, supernatant discarded, and the pellet was re-suspended in 100 µl then one drop applied to a glass slide followed by air drying then fixed in methanol solution 95% for 2 min and stained with giemsa stain 5% stock solution, for counting, At least 1000 examined for each group and number of micronuclei detected from the total number of cells. The chi-square test used to determine the significance and the total chi-square.

Hematological analysis

Hematological examination: Whole blood was collected on EDITA-vacuum tubes. Erythrocytes count and Hemoglobin (Hb) was determined and then Packed Cell Volume (PCV) was determined by conventional method. Erythrocytes indices as Mean Corpuscular Values (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC) and Mean Corpuscular Hemoglobin (MCH) were calculated. Total leukocytes and platelets count were also detected by automatic whole blood analyzer [12].

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Received: July 31, 2019, Accepted: August 13, 2019, Published: August 21, 2019

TABLE 1
Detection of chromosomal aberration in rat's bone marrow exposed to Ag-NPs at doses of 1/50 of LD50.

	Control negative	Ag-NP 1/50 of LD50	Ag-NP 1/50 of LD 100
HGB (gm/dl	11.7 ± 0.14	8 ± 0.15*	3.2 ± 0.2*
RBC	6.64 × 1012 ± 1.3	4.71 ± 1.3*	1.71 ± 1.2*
HCT %	35.5% ± 2	26.6 ± 2.5	9.6 ± 3.5*
MCV fl	53.6 ± 4	56.6 ± 4.1	56.3 ± 4.2
MCH pg	17.6 ± 3.3	16.9 ± 3.4	18.7 ± 3.4
MCHC d/dl	32.9 ± 2.1	30 ± 2.2	33.3 ±2.35
RDW-cv %	14.9 ± 2.1	13 ± 2.4	14.9 ± 1.9
RDW-sd fl	26.4 ± 2.4	25.6 ± 3.1	28.8 ± 3.3
PLT	167 × 109/l ± 10	102 ± 11.2*	42 ± 7.1*
MPV fl	8.6 ± 2.3	10.5 ± 2.1	10.6 ± 2.3
PDW	14.6 ± 2.3	15.9 ± 2.34	16.1 ± 2.4
PCT	0.156 ± 0.01	0.107 ± 0.02	0.044 ± 0.02
WBC	7 × 109/l ± 3.5	1.4 ± 0.5*	0.5 ± 0.2*

TABLE 2
Detection of chromosomal aberration in rats bone marrow exposed to Ag-NPs at doses of 1/100 of LD50.

	Total	Break	Fragment	gap	Ring	Chromosoal association	Acentromeric chromosome	Hypoploidy	Polyploidy
Control Gr.1	2.8 ± 0.36^{d}	1.15 ± 0.22^{c}	1.25 ± 0.25^{b}	0.38 ± 0.0.18^{c}	1^{b} ± 1.3	0^{c}	0^{c}	0^{b}	0^{c}
Gr.2 1/50	9.12 ± 0.63^{c}	2.1 ± 0.26^{b}	2.13 ± 0.44^{a}	1.38 ± 0.18^{b}	1.38 ± 0.18^{a}	0.63 ± 0.18^{b}	0.5 ± 0.18^{b}	0.75 ± 0.16^{a}	0.38 ± 0.18^{c}
Gr.3 1/100	13.8 ± 0.52^{b}	3 ± 0.32^{a}	2.5 ± 0.18^{a}	3 ± 0.32^{a}	1.88 ± 0.22^{a}	0.88 ± 0.13a^{b}	0.88 ± 0.13^{a}	0.75 ± 0.16^{a}	1 ± 0.18^{b}

Data management and data analysis

To test the hypotheses, the raw data analyzed by SPSS statistical software, version [13]. Descriptive statistics of frequency was computed to assess the occurrence of Micronuclei and chromosomal aberration by chi-square analysis with 95% confidence intervals (95% CI). For all statistical analysis α=0.05 was considered a significant level. While blood parameters were analyzed by one-way [14].

RESULTS

Sliver nanoparticles have no effect on final body weight at end of treatment at 15 days of gestation. Silver nanoparticles at doses 1/50 and 100 of LD50 were induced hematotoxicity evidence by decreased level of hemoglobin, RBCs, WBC and different calculated blood induces as shown in Table 1.

Chromosomal aberration was dose dependent with significant increase in all doses (0, 1/50 and 1/100 of the LD50) especially higher doses represented by structural abnormalities as chromosomal break, fragments, gap, association and a centromeric chromosomes beside numerical abnormalities as polyploidy and hypoploidy where the results in Table 2.

DISSCUSION AND CONCLUSION

Previously, it was found that low dose of silver nanoparticles considered safe. Also, according oral LD50 of Ag-NPs more than 5000 mg/kg, recorded earlier [9] proof safety of Ag-NPs at low doses but not for long exposure as well as recorded her in current study. Also, short exposure of high dose at 2000 mg/kg had no change in hematology in albino rats after 48 h of only two successful doses [12,13]. Notably, the oral dosing of Ag-NPs considers the safest one than other routs like intraperitoneal exposure as Ag-NPs could be excreted through GIT nearly 90% through feces while intraperitoneal rout enhanced bioaccumulation and distribution through different tissues. Orally administered silver has been found to be absorbed in a range of 0.4 –18% in mammals with a human value of 18% [14,15]. Teratogenic evidence of small doses of Ag-NPs [6] proof its geno-toxicity that as all genotoxic agents has potential induction of feti malformations. In similar report to current study proof increased the chromosome breakage and polyploidy cell rates also ensured the potential genotoxicity of AgNP [16]. The genotoxicity of AgNP has been investigated *in vitro* [17,18]. Moreover, the Ames test result of AgNPs was due to the bacterial being is incapable of endocytosis. On conclusion, the increase the oral exposure of silver nanoparticle enhanced both hematotoxicity and genotoxicity.

All authors have no conflict of interest.

ACKNOWLEDGEMENT

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