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Full Length Research Paper

The evaluation of teratogenicity of nanosilver on skeletal system and placenta of rat fetuses in prenatal period

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Nanosilver, comprising silver nanoparticles, is attracting interest for a range of biomedical applications owing to its potent antibacterial activity. It has recently been demonstrated that nanosilver has useful anti-inflammatory effects and improves wound healing, which could be exploited in developing better dressings for wounds and burns. The teratogenicity potential for intraperitoneally dose of nanosilver had not previously been evaluated. Despite the widespread use of nanosilver products, relatively few studies have been undertaken to determine the biological effects of nanosilver exposure. The purpose of this study was to evaluate the teratogenicity of nanosilver in rat fetuses. This study was performed on 30 pregnant rats that were divided into five groups. Control group received normal saline and test groups received nanosilver (0.4 and 0.8 mg/kg) intraperitoneally at 8 and 9th day of gestation, respectively. Fetuses were collected at 20th day of gestation. After determination of weight and length; the fetuses were stained by Alizarin red-Alcian blue method. Also, placenta were weighed, and width and volume were measured and examined macroscopically. The mean of weight of animals' fetuses that received nanosilver (0.4 and 0.8 mg/kg) in 8th day and weight and length (0.8 mg/kg) in 9th day was significantly decreased in comparison with normal saline group. The weight, volume and width of placentas in treated animals were lesser than in control group. No macroscopic anomalies were seen in all the groups. Thus, nanosilver had no effect on skeletal system of rat fetuses and it is necessary to determine the association between the period of exposure and histopathologic changes with different doses over different time periods.

Key words: Nanosilver, pregnancy, placenta, teratogenicity, rat.

INTRODUCTION

Some chemical agents and drugs can induce teratogenic effects and abortion (Finnell, 1999). Developmental defects are major health problems as in the USA, 3 to 5% of fetuses have congenital abnormality (Giavini and Menegola, 2004). It is estimated that 7 to 10% of human anatomic anomalies result from the disruptive actions of drugs, viruses and other environmental factors (Moore and Persaud, 2008). De Sanntis et al. (2004) also estimated that defects attributable to drug therapy represent about 1% of congenital defects of known etiology (De Santis et al., 2004).

There is increasing use of manufactured nanoparticles; the potential for exposure among manufactures and consumers is also increasing. Nanomaterials, defined as particles ranging from 1 to 100 nm in at least one dimension, have become widely utilized because of their unique physicochemical properties (Hood, 2004). Many of these nanomaterials, including gold nanorods (Eghtedari et al., 2007), composite nanodevices (Balogh et al., 2007), quantum dots (Azzazy et al., 2007) and nanosilver (Fu et al., 2006) have been used in biomedical settings.

The medical use of silver dates back centuries. Many of the industrial silver compounds, including nitrate, chloride, bromide, acetate, oxide, sulfate and cyanide (Weast et al., 1988) can be released in to the environment

from various sources (Rosenman et al., 1979). Silver can be found in low levels in many tissues (Rosenman et al., 1979; Wan et al., 1991; Hollinger, 1996; Sue et al., 1991) but without any clear physiologic function (Stokinger, 1981).

Nanosilver has been used since ancient times for jewelry, utensils, monetary currency, dental alloy, photography, explosives, etc. (Chen and Schluesener, 2008). Until the introduction of antibiotics, it was also used for its antiseptic activity, specifically in the management of open wound and burns. Due to its antimicrobial properties, silver has also been incorporated in filters to purify drinking water and clean swimming pool water (Agency for Toxic Substances and Disease Registry [ATSDR], 1990). Importantly, these properties are retained when silver is synthesized in nanoparticulate formulations, such as silver-containing multilayer film for surface modification (Fu et al., 2006), silver-loaded bone cement (Alt et al., 2004) and silver complexes in nanofiber mats (Melaiye et al., 2005).

With increased application of nanosilver products, however, comes the inevitable possibility of effects on the health of humans exposed to the products. Previous *in vitro* studies of nanosilver toxicity reveal toxic effects of nanosilver through reduced cell viability, damage to cell membrane and other biological effects on the organism. Nanosilver has been reported to be among the most toxic nanomaterials in some studies (Hussain et al., 2005; Soto et al., 2007; Bar-Ilan et al., 2009). Similarly, minimal toxicity was induced in HL-60 cell grown in direct contact with a metal silver plate (Yamazaki et al., 2006). However, accumulating evidence suggests that nanosilver formulations may be cytotoxic, as indicated by recent studies using rat liver cells (Hussain et al., 2005) and mammalian germline stem cells (Braydich-Stolle et al., 2005).

The risk of teratogenic effect of nanosilver on skeletal system and placenta in rat fetuses was evaluated, because of the maternal accidental exposure to nanosilver and the lack of report of the evaluation of nanosilver on teratogenicity, in the present study.

MATERIALS AND METHODS

Male and female healthy rat of Wistar strain, 3 to 4 month old, weighing 200 to 250 g were purchased (Joundishapour laboratory animal center, Ahvaz, Iran) and housed individually (males) or at 10 per polycarbonate cage (female) for a 2-week acclimation period. Rats were fed *ad libitum* by standard laboratory pellet (Pars khurakdam, Tehran, Iran) and tap water. A 12 h light:12 h dark was mentioned. Room temperature was at 23 ± 2°C with a relative humidity of 45 to 55%.

Females were mated overnight with males. Pregnancy was ascertained the next morning by the presence of a vaginal plug, and this time was designated as gestational day (GD) 0. Pregnant rats (n = 30) were randomly divided into five groups (24 pregnant rats in treatment groups, 6 pregnant rats in control group) and were treated as follows: Group 1 (Control group): Normal saline in equal volume of nanosilver was injected to pregnant rats for inducing similar condition (injection and handling) to other groups. Groups 2 and 3 (nanosilver groups): Nanosilver (0.4 and 0.8 mg/kg) was administered intraperitoneally at 8th day of gestation, respectively. Groups 4 and 5 (nanosilver groups): Nanosilver (0.4 and 0.8 mg/kg) was administered intraperitoneally at 9th day of gestation, respectively.

The animals were sacrificed by euthanized and cervical dislocation at 20th day of gestation. Following laparatomy, the uterus was exteriorized and the number and location of fetuses and resorption were noted, then their weight and length (crown- rump length) were measured. Individual fetuses were examined carefully for external anomalies then fetuses were stained by Alizarin red-Alcian blue method (Kimmel and Trammekl, 1981) and investigated by stereomicroscope (Nikon, SMZ200, Japan) for skeletal malformations. Then, the placenta were weighed, measured and examined macroscopically. The incidence of skeletal malformations was determined and was compared in the groups.

Statistical significance between groups was determined using SPSS program and compared by one way analysis of variance (ANOVA) and Post hoc least significant difference (LSD). The minimum level of significance was $P < 0.05$.

RESULTS

Fifty fetuses were obtained from six rats of control group. No macroscopic anomalies were observed in the control animals. In the control group, palatal closures of fetuses were normal at gestational day 20 (that is, palatal shelves had grown vertically on the sides of the tongue, then horizontally to meet and fuse). There were not any aborted fetuses from the total groups.

The mean of weight of animals' fetuses that received nanosilver (0.4 and 0.8 mg/kg) in 8th day was decreased significantly in comparison with the normal saline group (Figure 1). The mean of weight and length of animals' fetuses that received nanosilver (0.8 mg/kg) in 9th day, decreased significantly in comparison with other groups (Figures 1 and 2).

The mean of weight, volume and width of fetuses' placenta that received nanosilver (0.4 and 0.8 mg/kg) in 8 and 9th days decreased significantly in comparison with normal saline group. The mean of weight of fetuses' placenta that received nanosilver (0.8 mg/kg) in 8th day decreased significantly in comparison with other groups except fetuses placenta that received nanosilver (0.8 mg/kg) in 9th day (Figures 3 to 5).

DISCUSSION

Since there are no available data on the teratogenicity of nanosilver in consumer products of rat embryos, the teratogenic effects of nanosilver on skeletal system and placenta in rat embryos was evaluated in the present study for the first time.

Nanosilver has become one of the most widely used nanomaterials in consumer products because of its antimicrobial and antiseptic properties. Because of public concern over the potential adverse effects of nanosilver (Benn et al., 2010), Sawosz et al. (2009) reported

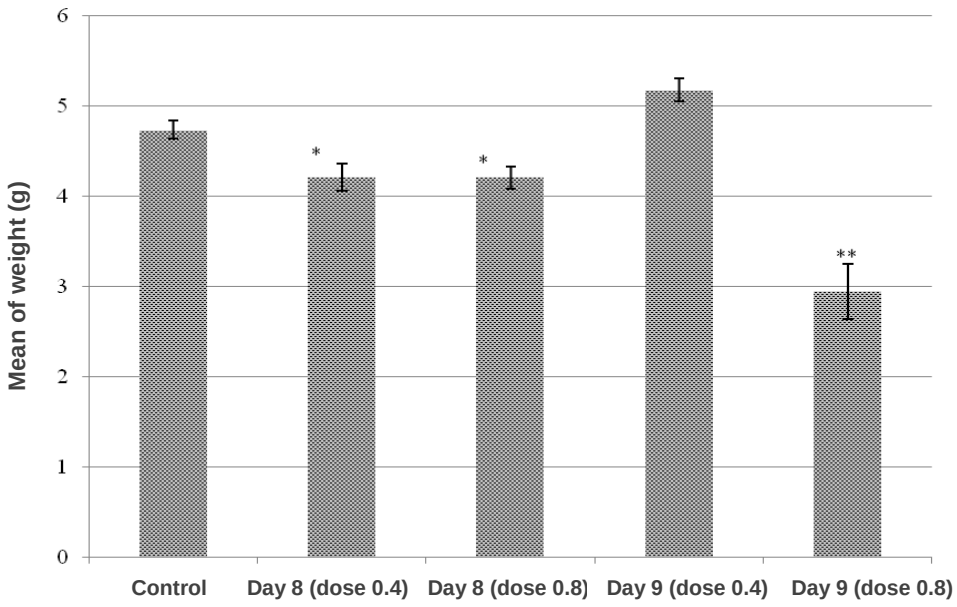

Figure 1. Weight (mean ± SEM) of fetuses in different groups. *Significant difference with normal saline group; **Significant difference with other groups (P < 0.05).

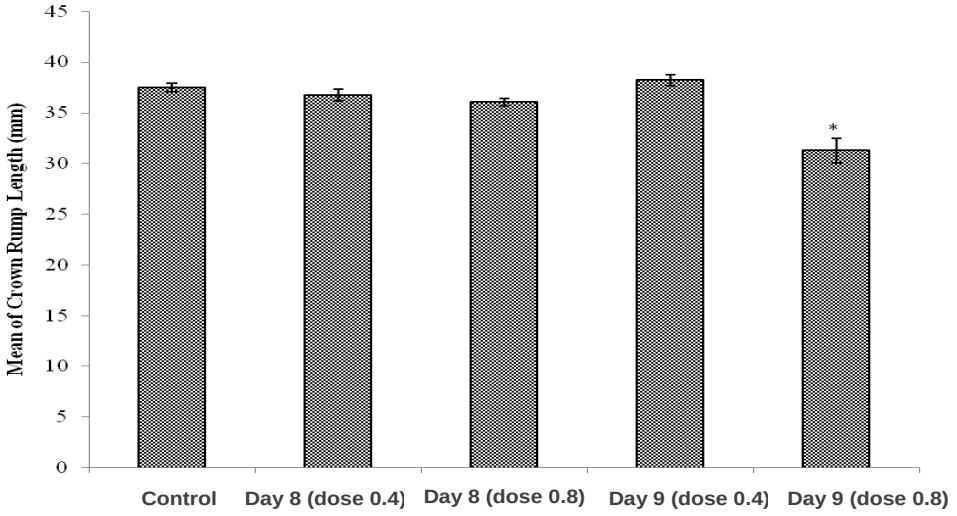

Figure 2. Crown rump length (mean ± SEM) of fetuses in different groups. *Significant difference with other groups (P < 0.05).

administration *in ovo* of 50 ppm hydrocolloids of nanoparticles of silver and alloys of silver with copper did not influence mortality, growth and development of 48 h and 20 days old chicken embryos. In previous studies, animals exposed to nanosilver showed minimal pulmonary inflammation or cytotoxicity following subacute exposure, but longer-term exposures with higher body burdens of nanosilver are needed to ensure that there are no chronic effects and to evaluate possible translocation of nanosilver to other organs (Stebounova et al., 2011; Kim et al., 2010).

Insufficient evidence is available presently to show that administration of silver or ionisable silver compounds in pregnancy is a cause of infertility, impaired foetal growth, or abnormal development in any species. Silver nitrate (1%) administered by intrauterine injection to 13 cynomolgus monkeys between 27 and 43 days of pregnancy caused early vaginal bleeding and termination

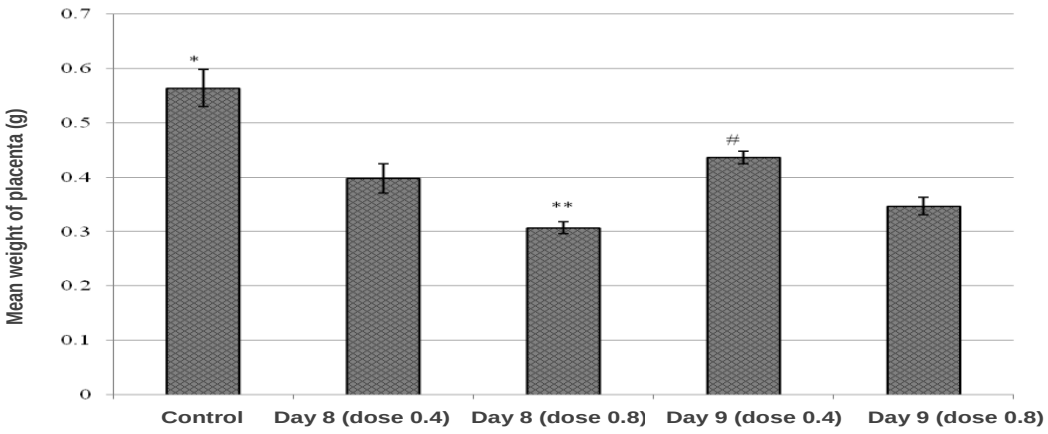

Figure 3. Weight (mean ± SEM) of placenta in different groups. *Significant difference with other groups (P < 0.0001); **Significant difference with other groups except group 5 [day 9 (dose 0.4)] (P < 0.0001); #Significant difference with group 5, (P = 0.036).

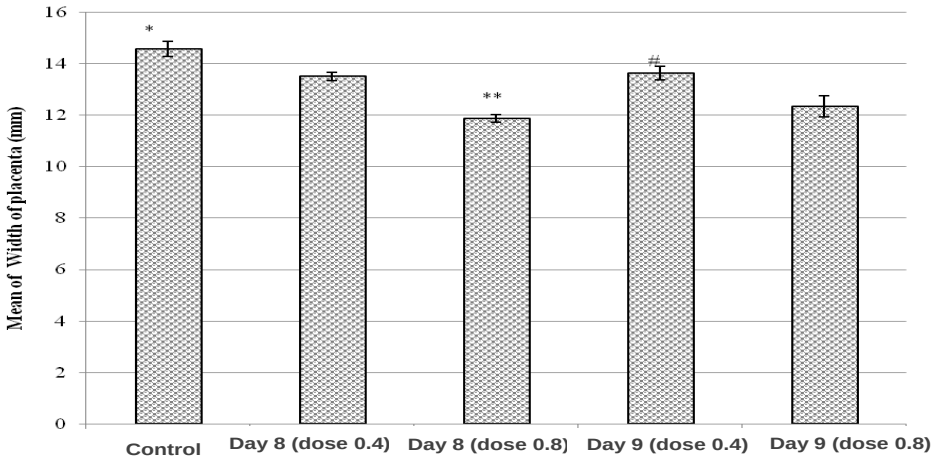

Figure 4. Width (mean ± SEM) of placenta in different groups. *Significant difference with other groups (P < 0.0001); **Significant difference with other groups except group 5 [day 9 (dose 0.4)] (P < 0.0001); #Significant difference with group 5, (P = 0.002).

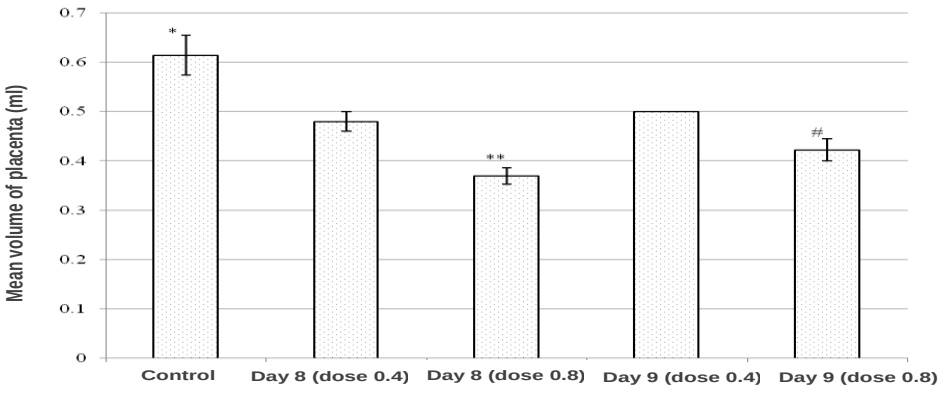

Figure 5. Volume (mean ± SEM) of placenta in different groups. *Significant difference with other groups (P < 0.0001); **Significant difference with other groups except group 5 [day 9 (dose 0.4)] (P < 0.0001); #Significant difference with group 5 (P = 0.036).

of pregnancy, but two of seven animals that re-mated became pregnant again and delivered healthy offspring (McCauley et al., 1994).

One of the classical toxic responses to the silver is argyria, which was reported for the first time by Blumberg and Carey (1934) in a woman who had ingested a total dose of 6.4 g silver nitrate over a 1-year period of time and showed argyria symptoms after the first six months of exposure. Rosenman et al. (1979) also reported respiratory irritation, abdominal pain and decreased night vision in workers exposed to silver nitrate and silver oxide dusts over one to ten years (Rosenman et al., 1979). Discoloration of the conjunctiva and cornea in some workers was reported after inhalational exposure (Moss et al., 1979). Williams (1999), in a case study of a 51-year-old man exposed to silver compounds, showed corneal and conjunctival. Chang et al. (2006) recorded a case study of a 59-year-old man who was distressed from dermal and face color change. He had ingested colloidal silver two to three times per year for two years and showed endocrine disruptions, such as hyperlipidemia, hypertension and diabetes as well as blue-grey facial signs (Chang et al., 2006). Neurologic symptoms are unusual consequence of silver toxicity which was recently reported in a 75-year-old man who had a history of self-medication with colloidal silver and was presented with myoclonic seizures (Tang and Xi, 2008).

Korani et al. (2011) showed that the toxic effects of nanosilver depend on the route of administration. Some reports have proved that many medical devices loaded with silver could release silver ions which could be translocated through the blood circulation and accumulate in some organs, such as the liver and kidney. It may induce hepatotoxicity or renal toxicity and may lead to death in some cases of extreme exposure to a certain dose of nanosilver (Stepien et al., 2009). Korani et al. (2011) showed that silver nanoparticles with properties similar to those of nanosilver could be translocated in the body and cause histopathologic changes in the liver and spleen unlike those caused by silver nitrate given by the same route of administration. In conclusion, the present study showed the effects of nanosilver for the first time in teratogenicity on skeletal system in rat. The present results indicate that exposure to 0.4 and 0.8 mg/kg of silver nanoparticles in 8 and 9th days of gestation of rat decrease the weight and length of embryos and did not have influence on the skeletal system. It is proposed that they can determine the association between the period of exposure and histopathologic changes with different doses over different time periods.

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