Original Article

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A Comparative Study of the Effects of Sodium Arsenite and Nanoparticles of Sodium Arsenite on the Apparent and Skeletal Malformations in Rat Embryos

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ABSTRACT

BACKGROUND AND OBJECTIVE: Arsenic causes congenital anomalies in humans and animals, and nanoparticles of sodium arsenite are highly capable of inducing apoptosis. Since the effects of nanoparticles of sodium arsenite on fetal malformations have not been evaluated yet, this study aims to compare the effect of sodium arsenite and nanoparticles of sodium arsenite on skeletal malformations in rat embryos.

METHODS: This in-vitro study was performed on four groups of pregnant rats (n= 23 rats). Mating was confirmed by observation of vaginal plug. On the tenth day of gestation, pregnant rats in different groups received intraperitoneal normal saline (n=7 rats), sodium arsenite (11 mg/kg) (n=5 rats), nanoparticles of sodium arsenite (1 mg) (n=5 rats) and nanoparticles of sodium arsenite (11 mg) (n=7 rats). All the rats were euthanized on the twentieth day of pregnancy and their embryos were removed, their weight and length were measured, and then were stained with alizarin red and alcian blue. The skeletal system abnormities of embryos such as cleft palate, malfunctions or malformations in ribs, vertebrae, spine, sternum, arms, legs, fingers and reduction of ossification were evaluated by a stereomicroscope, and then compared with the control group.

FINDINGS: Sodium arsenite and nanoparticles of sodium arsenite reduced fetal weight from 5 g in the control group to 2 g ($p \leq 0.033$) and also, decreased fetal length from 38 cm to 28 cm in the control group ($p \leq 0.023$). The weight and length of fetuses were significantly reduced in nanoparticles of sodium arsenite group (11 mg), as compared to sodium arsenite group ($p \leq 0.033$). There were no skeletal malformations in the control group, while the percentage of anomalities was between 3% and 47% in the intervention group.

CONCLUSION: Nanoparticles of sodium arsenite, as compared to sodium arsenite, were more effective in reducing fetal length and weight and in diminishing the rate of skeletal malformations such as cleft palate.

KEY WORDS: *Fetal Rats, Skeletal Anomalies, Sodium Arsenite.*

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Introduction

Embryo's exposure to chemical substances including chemical drugs may lead to congenital malformation or miscarriage (1-3). The drugs such as valproic acid (VPA) (1), cyclophosphamide (CY), N-methyl-N-nitrosourea (MNU), phenytoin and arsenic are the best-known teratogenic drugs in humans and animals (4). Arsenic poisoning and exposure can also endanger human health (5-7). Based on the epidemiological studies, exposure to arsenic can lead to impaired memory, learning and nerve function in children and adolescents alike (8). Arsenic affects the oxidant-antioxidant system and leads to formation of free radicals and facilitates oxidation reactions (9, 10).

Arsenic is known as a teratogen which leads to fetal defects in humans and animals, especially laboratory animals. The anomalies caused by arsenic include exencephaly, anophthalmia, open eye, vertebrae sticking, twisted tail, short or no tail, short jaw, weight loss, as well as limb and rib anomalies (11-13). Nanoparticles of sodium arsenite have more advantages over the conventional medications, some of which are as follows: targeted drug delivery to the tissue, higher purity, higher drug absorption, higher efficiency and requiring lower doses of drugs with reduced frequency. Higher surface-area-to-volume ratio is the most important property of nanomedicines, which helps to increase the rates of solubility, absorption and ease of drug transmission through the blood-brain barrier (14).

However, complications and toxicity of nanomedicines must be considered while using them. According to a study done by Najafzadeh et al., the nanoparticles of sodium arsenite had higher ability to induce apoptosis in rat liver cells, as compared to sodium arsenite (15). In another study, teratogenic effects of sodium arsenite on mouse embryos were investigated, it was found that this material can cause fetal deformity in skeletal axial and ossification process (16). In the current study, we aim to compare the teratogenic effects of sodium arsenite nanoparticles with arsenate on the apparent and skeletal malformations in rat embryos.

Methods

In this in-vitro study, a total of 23 Wistar rats were provided from laboratory animal research center of Ahvaz Jundishapur University of Medical Sciences. Male and female rats were kept apart for two weeks in order to adapt to the environment. All the rats were kept at 20-22°C and in a 12 h light/12 h dark cycle, under equal environmental and nutritional conditions (they were given water and compressed food manufactured at Animal Feed of Shushtar). The samples were approximately aged 12 weeks, and their average weight was between 180-200 g.

Moreover, at 20 pm, each three female rats were placed beside one male rat to breed, and the next day was considered the day-zero of pregnancy. Mating was confirmed by observation of vaginal plug. Pregnant rats were randomly divided into four groups, and were kept apart. In the first group (n= 7 rats), pregnant rats received intraperitoneal saline with the same amount of sodium arsenate on the tenth day of gestation (control group). The second group (n=5 rats), the rats were administered intraperitoneal sodium arsenite (11 mg) (Merck co, Germany) on the tenth day of gestation (16). Finally, on the tenth day of gestation, the rats received intraperitoneal nanoparticles of sodium arsenite (1mg) in the third (n= 5 rats) and intraperitoneal nanoparticles of sodium arsenite (11 mg) in the fourth (n=7 rats) groups, respectively. Nanoparticles of sodium arsenite were produced by Isfahan University of Technology using mechanical milling.

Particle size (about 50 nm) was confirmed via X-ray diffraction measurements. All the rats were euthanized through ether inhalation on the twentieth day of pregnancy, and the embryos were removed from uterus after opening the abdominal cavity and uterine incision. The fetuses were

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evaluated in terms of apparent anomalies in different parts of body, and their weight and length were measured using scale and caliper; moreover, the number of dead, alive and absorbed fetuses were determined.

After staining the samples with alizarin red and alcian blue, their skeletal malformations (e.g., appendage and axial skeletal deformities) were evaluated by means of a stereomicroscope, and then compared with the control group. The mean differences in weight and length of embryos in the experimental and control groups were determined performing ANOVA, LSD and independent t student's test. $p \leq 0.05$ was considered significant.

Results

In total, 181 embryos were obtained from pregnant rats, out of which, 55, 23, 26 and 33 were assigned to the control, arsenite conventional, and 1 and nanoparticles of sodium arsenite (11 mg) groups, respectively. The percentage of absorbed embryos in the control, arsenite conventional, 1 and nanoparticles of sodium arsenite (11 mg) groups were 3.51%, 54%, 16.13% and 23.26%, respectively. The mean weight of the embryos were 4.99±0.08, 2.24±0.24, 1.86±0.07 and 1.742±0.17 g in the control, sodium arsenate, nanoparticles of sodium arsenite (1 mg) and nanoparticles of sodium arsenite (11 mg/kg) groups, respectively. The mean fetal weight was significantly greater in the control group, as compared to the groups receiving sodium arsenite and nanoparticles of sodium arsenite ($p \leq 0.033$), this difference was larger between the control and the nanoparticles of sodium arsenite (11 mg) groups.

In addition, there was a significant difference in the mean weight of the group receiving sodium arsenite and the nanoparticles of sodium arsenite (11 mg) group, but the mean weight of the control group was even larger ($p=0.023$) (fig 1). The mean length of embryos in the groups receiving saline, sodium arsenate, arsenite nanoparticles (1mg/kg) and arsenite nanoparticles (11mg/kg) were 38.18±0.27, 26.13±1.4, 28.34±0.44 and 23.51±1.12, respectively. The mean length of fetuses in the control group was significantly larger than the groups receiving sodium arsenite and nanoparticles of sodium arsenite ($p<0.05$) (fig 2).

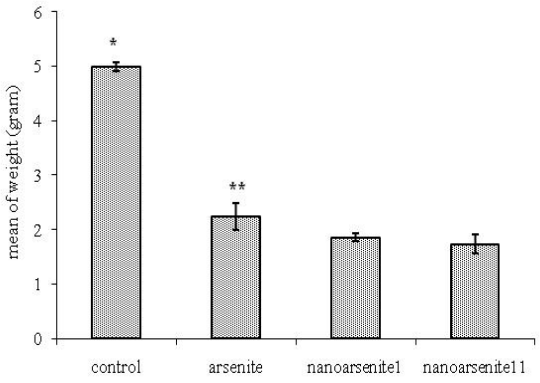

Figure 1. The comparison of the mean weight (g) of rat's embryos in the four groups

*Significant difference between the control and experimental groups,

**Significant difference between the groups receiving sodium arsenite and receiving sodium arsenite nanoparticles (11 mg/Kg)

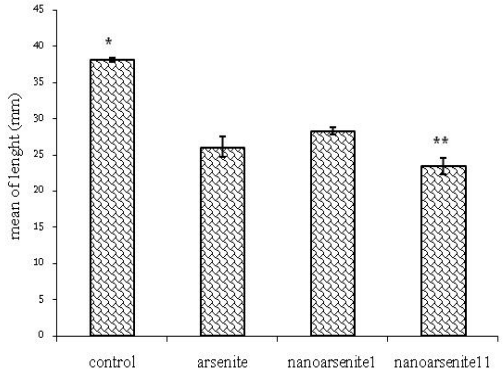

Figure 1. The comparison of the average length (mm) of rat's embryos in different groups

*Significant difference between the control and experimental groups,

**Significant difference between the group receiving sodium arsenite and the group receiving nanoparticles of sodium arsenite (11 mg/Kg)

The mean length of embryos in the group receiving nanoparticles of sodium arsenite (11 mg)

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was significantly lower than the other groups ($p<0.05$). The mean length of embryos in the group receiving sodium arsenite was significantly more than the nanoparticles of sodium arsenite (11 mg); however, the mean length of embryos in the control group was greater ($p=0.033$).

The mean length of embryos in the nanoparticles of sodium arsenite (1 mg) group was more than the group receiving sodium arsenite, but this difference was not significant. After taking sodium arsenate, the only observed change was embryo absorption. The percentage of fetal viability in the group receiving saline, sodium arsenate, nanoparticles of sodium arsenite (1mg) and nanoparticles of sodium arsenite (11 mg) were 96.49%, 46%, 83.87% and 76.74%, respectively. We observed a number of abnormalities including cleft palate, spina bifida, cleft spine, two-part sternum, sticking sternal vertebra, sticking ribs, incomplete rib and reduction of ossification of hands and feet by stereomicroscope (table 1). However, apparent and skeletal anomalies were not observed in the control group. There was no significant difference between the groups receiving sodium arsenite and nanoparticles of sodium arsenite in reduction of fetal ossification. There was a significant difference in rates of fetal absorption and cleft palate between the sodium arsenite and nanoparticles of sodium arsenite groups, while there were no significant differences between the groups receiving nanoparticles of sodium arsenite. Spina bifida, lack of ossification in sternal vertebra, incomplete rib and two-part sternum were observed in the sodium arsenite and nanoparticles of sodium arsenite groups, but there was no significant difference between the two groups.

Sticking ribs were observed only in the group receiving sodium arsenite. In nanoparticles of sodium arsenite groups, the mean length of embryos and the incidence of anomalies were significantly reduced with the increase in drug dosage ($p<0.05$); however, there was a significant difference among the groups regarding fetal weight. Also, there was no significant difference between the nanoparticles of sodium arsenite groups regarding the other anomalies.

Table 1. Ferequency of observed fetal anomalies in the four groups

Anomalies / Groups		Control (N=55)	11 mg Sodium Arsenite (N=23)	1 mg Nanoparticles of Sodium Arsenite (N=26)	11 mg Nanoparticles of Sodium Arsenite (N=33)
Live Fetus N(%)		55(96.49)	23(46)	26(83.87)	33(76.74)
Absorbed Fetus N(%)		2(3.51)	27(54)	5(16.13)	10(23.26)
Fetal Anomalies N(%)	Cleft palate	0(0)	9(39.13)	20(76.92)	25(75.76)
	Spina Bifida	0(0)	11(47.82)	8(30.77)	10(30.30)
	Two-part Sternum	0(0)	1(4.34)	1(3.85)	1(3.03)
	Sticking Sternal Vertebra	0(0)	11(47.82)	13(50)	15(45.45)
	Incomplete Rib	0(0)	6(26.08)	7(26.92)	7(21.21)
	Sticking Ribs	0(0)	5(21.74)	0(0)	0(0)
	Reduction of Ossification of Hands and Feet	0(0)	9(39.13)	11(42.31)	13(39.39)

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Discussion

In total, the incidence percentage of the main important anomalies caused by sodium arsenate, including cleft palate, cleft spine, sticking-ribs and reduced ossification of arms and legs, were 39.13%, 47.8%, 21.74% and 39.13%, respectively, which changed to 76.92%, 30.77%, 0% and 42.31% when exposed to nanoparticles of sodium arsenite at a dose of 1 mg. Moreover, the incidence percentages of the aforementioned anomalies were 75.76%, 30.30%, 0% and 39.39%, respectively, while exposed to nanoparticles of sodium arsenite at a dose of 11 mg. Arsenic, as a poison, can have various effects on the enzymes and proteins' activities. Arsenic disrupts the activities of the mitochondrial energy through inhibition of nicotinamide adenine dinucleotide and competing with phosphate during oxidative phosphorylation. Also, it affects the mitochondrial enzymes and impairs respiratory membrane, which seem be associated with the cytotoxic effects of arsenic (9, 10). In addition, arsenic inhibits the enzyme succinate dehydrogenase and leads to isolation of oxidative and phosphorylation processes (9, 10). Since oxidative stress and free radical production play an important role in the incidence of fetal malformations, it seems that arsenic is involved in developing fetal skeletal anomalies in rats. According to a study conducted by Najafzadeh et al., sodium arsenite (11 mg/Kg) in pregnant rats on the tenth day of gestation led to anomalies in the 55% of embryos (16). Exposure to different doses of sodium arsenite in mouse embryos was studied by Hood et al., and it was reported that doses of 10 or 12 mg/Kg sodium arsenite were teratogenic and phototoxic. They also observed anomalies such as short lower jaw, open eye, limb anomalies, short or no tail, twisted tail and vertebrae sticking (17). However, the observed anomalies were different in our study, which may be as a result of using salt arsenic (arsenate) and different types of animals (Syria mice) in Hood et al. study. Also, according to a study performed by Machado et al., administration of sodium arsenite at doses of 5, 10 and 15 mg/Kg to Syria mice on the eighth day of pregnancy caused malformations in 9.3%, 45% and 15% of mice embryos, respectively (11).

In the current study, typical arsenic (11 mg/kg) was administered, which led to fetal absorption in 54% of the cases. While it was 16.13% and 23.26% in nanoparticles of sodium arsenite (1 mg/kg) and nanoparticles of sodium arsenite (11 mg/kg) groups, respectively. In a study by Wlodarczyk et al., arsenic was regarded as an important risk factor of embryonic anomalies. They explained that fetal complications are caused by differences in metabolite profiles. According to that study, exencephaly was the main anomaly caused by arsenate (18).

However, this complication was not observed in this study, which might be due to the differences in the type of animal, salt arsenic, and time of arsenic prescription during pregnancy. Additionally, Hills et al. mentioned to skeletal malformations and weight loss as the major effects of arsenic on embryos. They also observed many other anomalies such as exencephaly, rib (such as rib sticking) and vertebral anomalies (such as vertebrae sticking), as well as wrist and ankle malformation (12). However, exencephaly was not observed in our study, but cleft palate, spina bifida, as well as vertebral and rib malformations were found. These differences may be due to diverse arsenic exposure times. For instance, exencephaly is caused by the neural tube defects which are formed on the seventh to ninth days of pregnancy in mice. According to a study by Liu et al, sodium arsenite administration in rats' drinking water on the eighth to eighteenth days of pregnancy led to changes in fetal adrenal function (19).

Another study showed that the intraperitoneal injection of different doses of arsenic on the ninth day of pregnancy caused developmental defects to the brain, visual system and organs (20). Also, in another study, it was suggested that arsenate led to fetal growth retardation and neural tube defects

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(20), these results were in agreement with ours regarding fetal growth and weight in rats, although the defects of the neural tube was not assessed in the present study. The amount of arsenic in drinking water and its relationship with pregnancy and fetal complications in humans were assessed by Akhtar et al. According to their observations the rates of miscarriage, stillbirth and preterm delivery in pregnant women, who were exposed to arsenic, were significantly higher than the pregnant women who were not exposed to it (22). Moreover, arsenic sulphide nanosuspension was shown to have a highly toxic effect on human melanoma cell lines (23). It seems that as the size of nanoparticles is reduced, their behavior change because the particle size reduction leads to the emergence of new properties such as increased reactivity, absorption, and physical interactions on the particle surface, reduced melting temperature and different electrical properties (14). We also found that increase in the amount of medicine can significantly expand the number of anomalies. Based on a study carried out by Zhang et al., the size of the nanoparticles plays an important role in their transmission through the biological membranes and in the interactions of biological membranes (24). Sonavane et al. have propounded that the release of small gold nanoparticles in blood and tissues such as liver, kidney, spleen and passing them through the blood-brain barrier (BBB) is more likely than the larger particles (25). In Najafzadeh et al. study it was demonstrated that nanoparticles of sodium arsenite were more capable of inducing apoptosis than the typical sodium arsenite. The rates of apoptosis at doses of 40 µmolar and 100 µmolar for typical sodium arsenite were 30/67% and 57/34% and for nanoparticles of sodium arsenite were 42/67% and 86/67%. In our study, administration of typical sodium arsenite and nanoparticles of sodium arsenite led to higher rates of fetal anomalies, as compared to the control group, and there was a significant difference in the fetal length and weight of the groups receiving nanoparticles of sodium arsenite (11 mg/kg) and those receiving sodium arsenite (11 mg/kg). Besides, there was a significant difference in the incidence of cleft palate between the nanoparticles of sodium arsenite and the sodium arsenite groups. Although the rate of fetal absorption was higher in the sodium arsenite group, as compared to the nanoparticles of sodium arsenite group.

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