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سنتز و اندزه گیری نانو ذرات سرب(II) سولفید در بافت های بدن موش های آزمایشگاهی به روش ICP-OES

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Synthesis and Determination of Lead (II) Sulfide Nanoparticles in Rat Organs by ICP-OES

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چکیده

تحقیقات جدید بیانگر اثرات نا مطلوب نانو ذرات سولفیدی فلزات است. در این کار تحقیقاتی، نانو ذرات سرب (II) سولفید تحت شرایط مختلف ساخته شد. تکنیک های میکروسکوپ الکترونی روبشی و پراش اشعه ایکس برای اندازه گیری قطر و شکل ذرات بکار رفت و توزیع، تجمع و خواص سمی این نانو ذرات در بدن موش های آزمایشگاهی به عنوان یک مدل بیولوژیک بررسی شد. نانو ذرات سرب (II) سولفید با قطر کمتر از ۵۰ نانومتر با دوزهای ۱۵ و ۶۰ میلی گرم بر کیلو گرم وزن بدن موش های آزمایشگاهی نژاد ویستار، بصورت شرب طی ۲۸ روز به آن ها خورانده شد و غلظت یون های سرب و آهن در بافت های بدن با استفاده از اسپکترومتری نشری پلاسمای جفت شده القایی اندازه گیری شد. غلظت سرب به ترتیب در کبد، طحال، کلیه و مغز بیشترین مقدار را داشت و در کلیه و مغز موش های سمی شده با دوز پایین نسبت به گروه کنترل معنی دار نبود. همچنین غلظت آهن در گروه های مسموم دارای بیشترین مقدار سرب، کاهش یافت که احتمالا" به علت تداخل سرب درمکا نیسم آهن است.

واژه‌های کلیدی
نانو pbs–؛ نانوذرات؛ توزیع بافت؛ موش آزمایشگاهی.

Abstract
Recent studies have reported that metal sulfide nanoparticles had potential adverse effects. In this research, lead (II) sulfide nanoparticles have been synthesized under different reaction conditions. Scanning electron microscopy and X-ray diffraction were used to characterize the particle size and morphology. Distribution, accumulation and toxic effects of nanoparticles on rats as a biological model were studied. Lead (II) sulfide nanoparticles (less than 50 nm in diameter) were administered orally in two doses (15 or 60 mg/Kg body weight/day) to Wister rats for 28 consecutive days. Lead concentrations and iron parameters in soft tissues were measured using inductively coupled plasma optical emission spectroscopy. The lead levels were highest in the liver, followed in decreasing order by the levels in the spleen, kidney and brain. There were no significant levels of lead in kidney and brain at low dose of administration. Iron concentration was lowest in the group that had the highest lead level, which is probably due to an interference that could take place by lead through iron uptake mechanism.

Keywords
Nano-PbS; Nanoparticles; Tissue Distribution; Rats.

1. INTRODUCTION

Nanotechnology refers to a wide range of technologies that measure, manipulate, or incorporate materials and/or features with at least one dimension between approximately 1 and 100 nm. Despite the fact that there are a number of publications concerning the undesirable side effects of nanotechnology, the health and safety aspects of nanotechnology have lagged far behind its development. Nanoparticles (NPs) have been shown to produce cytotoxic, inflammatory and oxidative stress responses in different mammalian cells *in vitro*. When nanoparticles get inside the body, they come into contact with different biomolecules, especially protein [1-9].

Lead is most abundant of the heavy metals in earth's crust, having widespread industrial applications. Exposure to low-levels of lead has

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been associated with behavioral abnormalities, learning impairment, decreased hearing, and impaired cognitive functions in humans and in experimental animals. On the other hand, at high levels it causes damage to almost all organs, and most importantly to the central nervous system, kidneys and blood, culminating in death [10-12]. Experimental evidences suggest that cellular damage mediated by free radicals can be involved in the pathology associated with lead toxicity. Few earlier studies indicated that the disruption of reducing status of tissue might cause formation of reactive oxygen species (ROS), which may damage essential biomolecules such as protein, lipids and DNA [13-14].

With increasing interest to their potential toxicity, adverse effects of NPs have been recently studied *in vivo* and *in vitro* [15]. However, despite the growing literature on nanomaterial's applications, the information about biological effects of lead sulfide nanoparticles (PbS-NPs) is still insufficient and often controversial.

2. EXPERIMENTAL

2.1. Materials

All the chemicals used in this work were of either analytical grade or of extra pure grade of highest purity available locally. Triton X-100, $Pb(NO3)_2$ and Na_2S were purchased from Merck Chemicals Co.

2.2 Methods

2.2.1 Preparation of PbS-NPs

PbS-NPs were synthesized by a simple precipitation method, in which a solution of analytical grade with high purity was made of $Pb(NO3)_2$ and Na_2S, one mole of each. In this solution, Triton X-100 and sodium hydroxide (0.1 N) was slowly added and solution was stirred for 2 h. The solution was filtered and the PbS-NPs were dried in an oven at 110 ºC. The PbS-NPs were further calcinized at 400 ºC for 2 h. Structural characterizations were done for confirmation of PbS-NPs. The powder-X-ray diffraction (XRD) analyses were performed on a Philips PW-1830 X-ray diffract meter and the morphologies of the samples were characterized using scanning electron microscopy (SEM) (JEOL 6300)

2. 2. 2 Maintenance of the Animals

Male Wistar rats (250 ± 20g) were obtained from animal house facility of Kerman Neuroscience Research Center (Kerman, Iran). The animals were kept under a controlled light: dark (12:12 h) schedule. The rats were housed in well-cleaned sterilized cages in an air-conditioned room with temperature maintained at 23 ± 1 ◦C and humidity 50% with water and a meal available *ad libitum*. Animal ethical committee of Kerman Neuroscience Research Center approved the protocols for the experiments.

2. 2. 3 Animals and Treatment

Male Wistar rats were treated orally with or without a suspension of PbS-NPs in saline serum for 28 consecutive days and were classified as follows:

- Group control (received normal food and distilled water to drink)
- Group PbO-NPs (Low Dose; LD) (15 mg/kg body weight, once daily)
- Group PbO-NPs (High Dose; HD) (60 mg/kg body weight, once daily)

All animals were sacrificed under light ether anesthesia, 48 h after the last dosing. Kidneys, spleen, liver and brain samples were weighed, dried and collected for determination of lead and iron concentration. The samples were put in an oven at 60^0C for 3 days. Then, 1 g of each samples were digested by 1 ml of HNO_3. After digestion, the solutions were vaporized with the addition of 0.5 ml of H_2O_2 under the hood. Afterwards, the fragment was diluted with distilled water to 10 ml volume. Determination of lead (Pb^{2+}) and iron (Fe^{2+} and Fe^{3+}) concentrations in samples were performed by using inductively coupled plasma optical emission spectroscopy (ICP-OES, Model: Varian VISTA-MPX) and biochemical parameters in serum were also assessed to determine potential pathological changes. Standards were prepared from 1000 and 10000 mg/L ICP standards purchased form Sigma-Aldrich. It was necessary to dilute the samples for the analysis of the elements to overcome the problems with ionization found in samples of this nature. The diluted solutions produced better precision. The determinations for Pb and Fe were performed in triplicate to obtain the best precision, because of the variation due to ionization.

2. 2. 4 Clinical Hematological Variables

Blood was collected by cardiac puncture in heparinized tubes and level of hemoglobin (Hb), platelets (PLT), red blood cell (RBC) count and white blood cell (WBC) count were measured using a Sysmex hematology analyzer (model K4500).

3. RESULTS AND DISCUSSIONS

At nanosize, materials may exhibit unique properties when compared with larger or bulk forms of the same material. However, the same properties that make nanomaterial desirable in these various applications have the potential to alter the biological properties that impact the environment, health, and safety of these materials.

Metal and metal oxide nanoparticles are also under development for antimicrobial, self-decontaminating and UV blocking functions for both military protection gear and civilian health products. Ag, TiO_2, ZnO and CeO_2 are among the nanomaterials most widely incorporated into market goods [12-14]. In this study, PbS-NPs have been synthesized. The SEM and XRD were used to characterize the particle size and morphology (Fig. 1).

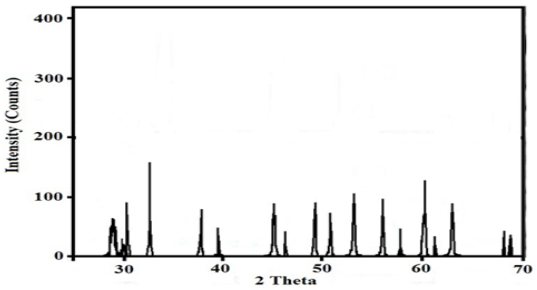

Fig. 1. X-ray diffraction for PbS-NPs

The XRD pattern for the PbS-NPs is compared to standard crystalline. These data show that the nanoparticles have the same crystal structure as that of bulk PbS. The diameters of individual particles were assessed by measurement of over 100 particles in the SEM image and yielded a primary particle size distribution of 42 ± 8 nm for NPs. Estimated from the Debye-Scherer formula for the calculation of particle sizes from the broadening of the XRD peaks ($D=0.891\lambda/\beta(\cos\theta)$), where D is the average grain size, λ is the X-ray wavelength (0.15405 nm), and θ and β are the diffraction angle and full width at half maximum of an observed peak, respectively the average size of the particles was found to be around 50 nm in diameter , which is in agreement with the value obtained from the SEM images (Fig. 2).

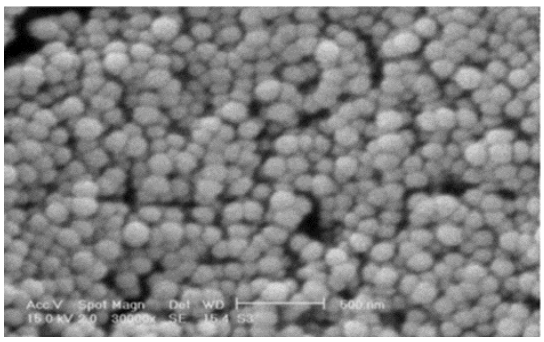

Fig. 2. Scanning electron microscopy PbS-NPs.

The effects of exposure to PbS-NPs in the various tissues, iron concentrations and some hematological variables are shown in Tables 1 and 2.

Table 1. Concentration of Pb^{2+} (mg/kg) in PbS-NPs intoxicated rats.

Group	Brain	Spleen	Liver	Kidney
Control	0.09±0.01	0.90± 0.05	0. 98 ± 0.05	0. 81 ± 0.04
PbS-NPs (L.D.)	0.15±0.16	2.15± 0.13*	3.02± 0.28*	1.02± 0.11
PbS-NPs (H.D.)	2.04± 0.14*	15.51± 0.51*	19.04± 0.68*	8.95± 0.31*

Values are mean (± SEM; n = 5); *Significant at $P < 0.05$ when compared with control.

Table 2. Hematological variables in PbS-NPs intoxicated rats

Group	WBC	RBC	Iron (μg/mL)	PLT
Control	7.48± 2.11	8.73± 0.54	6.95± 1.20	654.6± 52.4
PbS-NPs (L.D.)	7.4± 2.12	7.90± 0.36	6.28± 1.13	631.4± 51.3
PbS-NPs (H.D.)	8.9± 3.31*	7.59± 0.31	4.63± 1.08*	478.1± 42.1*

WBC = white blood cells as $\times 10^3/\mu L$; RBC = red blood cells as $\times 10^6/\mu L$; Hb = hemoglobin as g/L and PLT = platelet as $\times 10^3/\mu L$; Values are mean (± SEM; n = 5); *Significant at $P < 0.05$ when compared with control.

A significant accumulation of PbS-NPs compared with the control group has been shown in the organs at the dose of 60 mg/kg body weight. The lead levels were highest in the liver, followed in decreasing order by the levels in the spleen, kidney and brain. There were no significant levels of PbS-NPs in kidney and brain at low dose. There are reports on the association of protein with nanoparticles and the formation of a "protein corona". This could lead to altered properties of nanoparticles, thereby influencing their distribution and interactions with cells and biomolecules. Many reports using in vitro systems indicate that the mechanism of PbS-NPs toxicity involves the generation of reactive oxygen species (ROS). Several studies have shown that lead can accumulate in the brain when its concentration in the blood is elevated [16]. Blood parameters (WBC, RBC, PLT and iron) that are known to indicate an immune response in organ function were unchanged in the animals exposed to PbS-NPs at the dose of 15 mg/kg body weight but Platelet counts showed a decreased in high lead exposure group. No effect of lead on these variables in the present could be attributed to short duration of lead exposure. Iron concentration was lowest in the group that had the highest lead level, which is probably due to an interference that could take place by lead through iron uptake mechanism. The binding of protein with nanoparticles may trigger conformational changes in protein folding, altering its biological function and affecting the

signaling pathways activated by nanoparticles [17]. This study might be effective for preliminary testing of PbS-NPs toxicity.

4. CONCLUSIONS

In conclusion, high accumulations of Pb(II) in the animals exposed to PbS-NPs have been found in the liver, followed in decreasing order in the spleen, kidney and brain. Iron (Fe^{+2} and Fe^{+3}) concentration was lowest in the group that had the highest lead level, which is probably due to impairment of hemoglobin production and changes in the red blood cell membrane. It is known that the toxic behavior of NPs differ from their bulk counterparts. Hence, before NPs are commercially used it is most important that they be subjected to appropriate toxicity evaluation.

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