JOHE

Journal of Occupational Health and Epidemiology

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Studying the toxicity of molybdenum trioxide nanoparticles in male Wister rats

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Citation: Akhondipour M, Faghihi Zarandi A, Amiri A, Gommnami N, Vazirinejad R. Studying the toxicity of molybdenum trioxide nanoparticles in male Wistar rats. JOHE. 2018; 7(4):233-9.

Article Info

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Article history
Received: Jan, 2018
Accepted: Dec, 2018

doi 10.29252/johe.7.4.233

Print ISSN: 2251-8096
Online ISSN: 2252-0902

Peer review under responsibility of Journal of Occupational Health and Epidemiology

Abstract

Background: With the spread of nanotechnology, various nanoparticles with new and emerging properties have been produced and the potential toxic effects of the majority of these particles remains still unknown. The present study was conducted to determine the toxicity of Molybdenum Trioxide nanoparticles in blood and body tissues of male Wistar rats.

Materials and Methods: Thirty Wistar rats with an average weight of 200±10 g were included in the present experimental study; the rats were divided into three groups of control, low dose intervention and high dose intervention. Nano-trioxide molybdenum was injected at 5 and 10 mg/kg body weight for 28 days; then, blood samples and rats organs were collected to measure the molybdenum content. Molybdenum concentration was measured by atomic absorption method. The collected data were analyzed using SPSS (Version 20) and appropriate statistical methods including one-way ANOVA were used in order to compare the mean of blood variables among the groups.

Results: The results showed that decreasing hematocrit ($p < 0.001$), hemoglobin ($p < 0.001$), and red and white blood cells ($p < 0.01$) in rates receiving 10 mg of Molybdenum trioxide nanoparticles was significantly higher than that among rates in the other two groups. The mean degradation of molybdenum trioxide nanoparticles in the liver and kidneys was significantly higher than the heart and stomach ($p < 0.05$).

Conclusion: The results of the study showed that molybdenum trioxide nanoparticles at high concentrations had a more toxic effect on blood and serum parameters in comparison with the low concentrations.

Keywords: Toxicity, Nanoparticles, Molybdenum Trioxide, Wistar Rats.

Introduction

Poisoning is one of the most common causes of admitting to emergency centers around the world (1); it is a major health problem and poses a threat to the general health and hospitalization of many people, leading to financial burden on patients and the health system in developing countries (2). The pattern of poisoning in a country is subject to various factors such as access to poisons, socioeconomic status, cultural status, and religious beliefs (3, 4).

In developed countries, household chemicals and prescription drugs are the most common cause of poisoning; but, agricultural chemicals such as pesticides have a greater role in poisoning in developing countries (5). According to the World Health Organization, approximately 193460 deaths occurred due to unintentional poisoning in 2012, 84% of which occurred in low-income countries (6). The rate of poisoning in the United States was 479 out of 100000 in 2011 and a death rate of 17 in 100000 was reported (7).

The use of nanoparticles and Nano-catalysts has increased considerably in domestic and industrial processes in recent years. These particles exhibit specific physical and chemical behavior due to the high ratio of surface to volume, small size and visual characteristics related to their size (8). Metal nanoparticles have certain catalytic properties (9, 10).

In addition, over time, the application of nanotechnology has become more focused on fields such as construction, color, medicine, food, cosmetics, electronics, and light. On the other hand, new departments and units have been set up at universities and research institutes to examine nanoparticles, and research budgets have increased significantly for nanotechnology; but at the same time, researchers have become increasingly worried about the environmental and toxic effects of Nano-oriented products (11, 12).

With the massive production of Nano-products, there is currently an urgent need to examine their potential toxic effects on the human body and the environment. More than 20 species of nanoparticles have recently been used in various medical applications and other species are getting tested on in developmental stages (13). The use of nanoparticles for drug and diagnostic uses may have toxic and harmful effects on various organs of the human body; so, the toxicity of nanoparticles must be necessarily considered before they are widely used (14).

Metallic oxides nanoparticles are widely used in the industry and Molybdenum Trioxide Nanoparticles have attracted much attention. These nanoparticles are used predominantly in the industry, glass and as cracking catalysts, hydrogenation catalysts, and the production of refractory alloys. Due to the high toxicity of these nanoparticles, they can significantly threaten human health [15]. The small size of the nanoparticles makes them able to overcome the body's defenses and these particles may have toxic and undesirable effects on the growth, proliferation, and survival of the cells. (16)

In a similar study conducted by Mohseni kouchesfehani et al. In 2014, the effect of molybdenum trioxide nanoparticles on testicular histology changes and spermatogenesis process was investigated in adult Wistar rats (17). A study by Pandey et al. (2002), aimed at determining the effects of molybdenum on fertility in male rats, found that instantaneous swallowing of molybdenum may affect sperm morphology (18).

In a similar study by Mohseni kouchesfehani et al in 2014, molybdenum trioxide nanoparticles turned out interfere with testicular tissue due to having multiple oxidative states and dose-free radical production; this process would, in turn, reduce the different sex cells and the spermatogenesis process in adult Wistar rats (17).

Considering that few studies have been conducted on the toxicity effects of molybdenum trioxide nanoparticles on different tissues of the body (17, 19), the probabilistic effects of these particles on different systems and organs of mammals in different doses is considered necessary; therefore, the present study was conducted to investigate the toxicity effects of molybdenum trioxide nanoparticles on various tissues of male Wistar rats.

Materials and Methods

30 male Wistar rats, weighing 200±10 gr, prepared from Kerman Neuroscience Research Center were analyzed in the present experimental study. Animals were kept in special cages under 12 hours of light and 12 hours of darkness at 23±2 ° C and relative humidity of 55%. Meanwhile, all rats had free access to water and food. Animal feed was prepared from the Khorasan spp animal feed and poultry and placed in compact plates. The rats were randomly divided into three groups of ten. The cages were numbered and then, in accordance with the number written on the cages, the numbers were written on paper and placed inside a pack. Then, one of them was taken out of the pack and randomly assigned to the control group, the intervention group with low dose and the intervention group with high doses until the number of rats in each group reached 10. Groups, included one control and two intervention were as follows:

1. Control group: In this group, normal male rats were fed with normal diet for 10 days, and after that, the physiological serum of 5 and 10 mg/kg was injected intra-protaneally every other day for 28 days (17).
2. Low Dose Intervention (ILD) group: In this group, healthy male rats were fed normal diet for 10 days followed by the administration of 5 mg/kg body weight of molybdenum trioxide nanoparticles soluble in the physiological serum 3 every other day for 28 days.
3. High Dose Intervention (IHD) group: In this group, healthy male rats were fed normal diet for 10 days followed by the administration of 10 mg/kg body weight of molybdenum trioxide nanoparticles soluble in physiological serum every other day for 28 days. (17). It should be noted that the reason for using 5 and 10 mg/kg body weight of the body was that molybdenum nanoparticles caused apoptosis at concentrations above

25 µg / ml and necrosis in a number of cells at concentrations below 50 µg / ml (20).

The rats were poisoned according to the protocols presented in Mohseni kouchesfehani research (17); 20 rats in the intervention group used drinking water and compressed food. After 10 days, 10 µg of molybdenum trioxide nanoparticles (U.S.A Nano) was prepared prior to injection in ….ml of saline physiologic saline. In order to achieve the best distribution of nanoparticles in physiological saline, the Stokes solution was exposed to a sonication device (UP200H, Germany) belonging to Hiescher Company for 15 minutes; then, 5 and 10 mg/kg body weight of molybdenum trioxide nanoparticles was injected intraprotaneally every other day for 28 days; 28 days after the injection of molybdenum trioxide into the body of the experimental rats, they showed signs of lethargy, loss of hair in the back area and weight loss. These results indicates poisoning in laboratory rats. After the completion of 28 days, the rats were injected with ketamine sulfate (90 mg / kg) (4.5 mg / kg) and Xylasein for ethical consideration. / kg); then, they were anesthetized with CO2 gas and their blood samples were immediately collected through the neck and one part of the blood was poured into a falcon tube and about 5 cc of concentrated nitric acid was added to each falcon tube. (17, 21)

After the anatomy and isolation of the liver, kidney, stomach and heart tissue of the mouse and weighing them, the tissues were placed in the test tube separately and placed in an oven at 60 ° C after adding the acid for 3 days. After complete digestion, the samples were strained and distilled into distilled water at 10 ml moles. Then, 5 cc was pulled out by pipetting and dispensed in 100 ml balloons with distilled water. These tissues, together with blood serum, were stored in a refrigerator of the FC100, the Fraj-Taji engineering company in Iran, to be used to measure six-membered molten iron ions. To measure the molybdenum concentration in the sample, the Atomic Absorption Spectrophotometer (AAA) of the Varian Spectra AA 220FS of the United States of America was used. It should be noted that mortality was measured 48 hours after injection and analyzed by the Spearman software and the LD50 rate was determined (22).

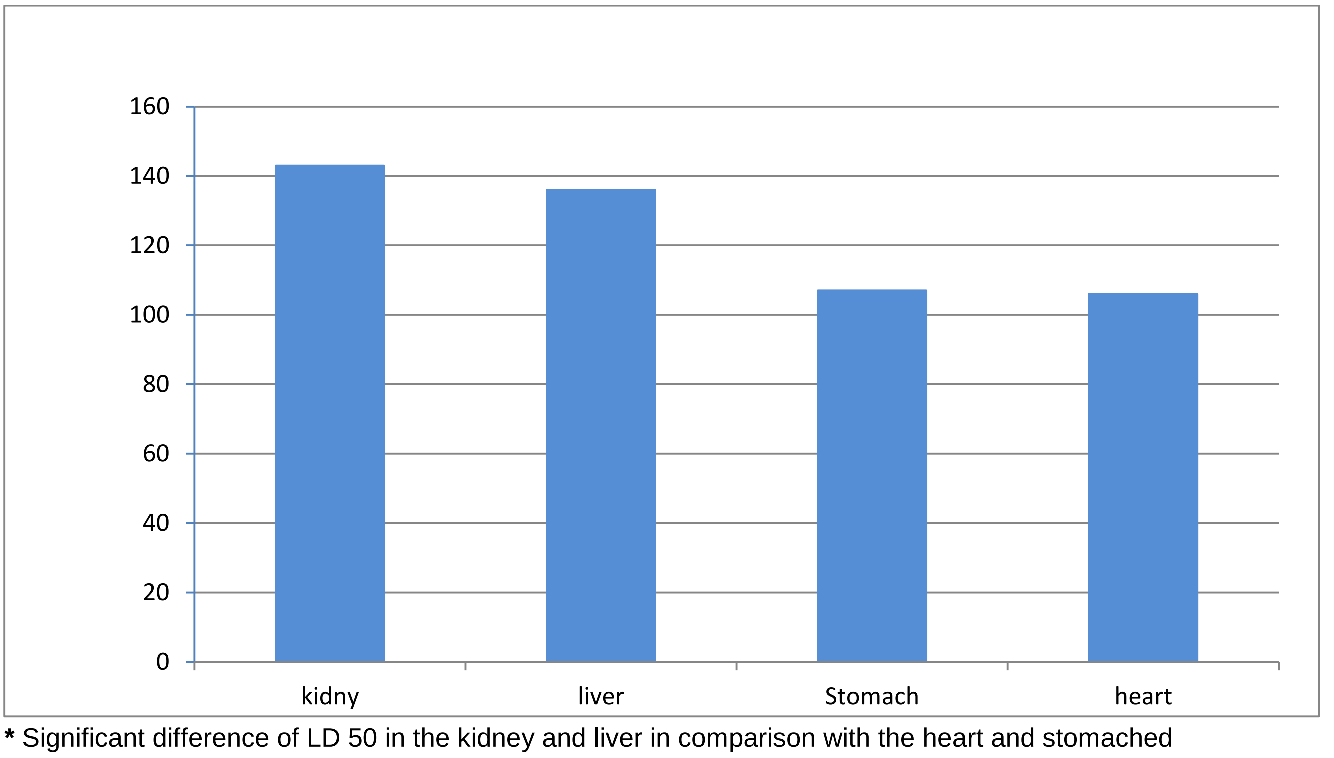
In this research, SPSS 20 was used to analyze the data obtained from all measurements. In order to compare the quantitative variables between groups, one-way ANOVA with a significant level of 0.05 standard deviation (Mean±SEM).

Table 1: Effect of 5 and 10 mg molybdenum nanoparticles on hemoglobin, hematocrit, red blood cells, white blood cells and blood platelets

	Control group (n=10)		5 mg dose intervention group (n=10)		10 mg dose intervention group (n=10)		p-value Comparing the control group and the 5 mg dose group (Independent t)	p-value Comparing the control group and the 10 mg dose group (Independent t)	p-value Comparing low dose and high dose intervention groups (Independent t	p-value Comparing the control group and 5 and 10 mg dose group	p-value Overall p value One-way variance analysis
	means	SEM	means	SEM	means	SEM					
Hemoglobin (WB)	11.660	0.154	10.980	0.246	10.140	0.168	0.143	0.893	0.195	0.171	0.000
Hematocrit (percentage)	34.980	0.463	32.940	0.738	30.420	0.504	0.143	0.893	0.195	0.171	0.000
Red blood cell count (million per ml)	6.811	0.117	6.480	0.092	4.290	0.100	0.730	0.684	0.902	0.833	0.005
White blood cell count (1000 ml)	4410.000	121.518	4710.000	181.628	3950.000	132.706	0.087	0.931	0.131	0.066	0.004
Platelet count (100,000 ml)	444.600	13.682	444.600	8.155	426.900	7.782	0.266	0.234	0.918	0.199	0.122

Results

Table 1 compares the effects of 5 and 10 mg/kg body weight of molybdenum trioxide nanoparticles on hemoglobin, hematocrit, red blood cells, white blood cells and blood platelets; it, also, compares these values with those of the control group that did not receive any significant interventions. The results of the study, based on one-way ANOVA, showed significant differences between the groups for all parameters except for blood platelets; this value was p <0.001 for blood hemoglobin and hematocrit, p=0.005 for the red blood, p = 0.004 for white blood cell, and p=0.122 for platelet. Figure1 compares median lethal dose (LD 50) of molybdenum trioxide nanoparticles in vital organs. The mean lethal dose (LD50) of molybdenum trioxide nanoparticles was 106±73.79 for the heart, 136±99.42 for the liver, 143±44.48 for the liver and was 107 ± 59.5 for the stomache. The results of this study showed that the mean degradation of molybdenum trioxide nanoparticles in the liver and kidneys was significantly higher than the heart and stomach. (p <0.05).



***** Significant difference of LD 50 in the kidney and liver in comparison with the heart and stomached

Figure1: LD50 Nanoparticles of Molybdenum Trioxide in Vital organs of male Wistar rats

Discussion

The present study was conducted to determine the toxicity of molybdenum trioxide nanoparticles in male Wistar rats. In terms of nature and objectives, the present study is experimental. In this study, 30 male rats were randomly divided into 3 groups of 10. The groups included a control group and two intervention groups.

In order to evaluate the changes in blood levels, the present study investigated the effect of molybdenum trioxide nanoparticles at concentrations of 5 and 10 mg / kg. The results showed that nanoparticles cause changes in blood parameters, one of which is the increase in white blood cell count in a low dose of nanoparticles; this is consistent with the results of Kim et al. and Razmara et al. studies both conducted in 2014 (23, 24). But the number of white blood cells was greatly reduced in 10 mg dose. In this regard, studies by Ryu et al in 2014 showed that the levels of white blood cells and lymphocytes decreased in the high concentrations of nanoparticles (25). The reason for such a finding might be the following point; the increase in white blood cell count can be due to the high concentration of nanoparticles, which, due to more contact surfaces and a greater effect on the membrane of the cells, penetrates the white blood cell mitochondria and changes the activity of their enzymes (24). It also reduces cellular activity, stimulates oxidative stress and reduces the antioxidant activity of the cell, thus reducing the amount of white blood cells. (26)

The levels of red blood cells, hemoglobin, mean corpuscular red blood cell, hemoglobin and hematocrit mean weight decreased significantly in high doses of molybdenum trioxide nanoparticles. In this regard, Sheydaei et al., in 2017, reported that zinc oxide nanoparticles cause changes in blood cells. In high concentration, nanoparticles increased some of factors such as white blood cells, hemoglobin, red blood cell and platelet levels;

therefore, nanoparticles at high concentrations are supposed to have higher toxicity than low concentrations (27), which is consistent with the results of the present study. The reason for such a result might be the fact that although the molecular mechanism involved in the toxicity effects of nanoparticles is still not fully understood, research has shown that active oxygen species play an important role in the toxicity of nanoparticles. Active oxygen species are involved in the cell signal and immune fields, causing severe damage to cell molecules, including proteins, lipids, and DNA, and harmful effects in the cell) 28-29).

Reduced number of platelets of the intervention group subjects compared to the control group, although this decrease was not statistically significant, is another finding of the present study (P> 0.05). The result obtained from the present research is consistent with the results presented by Ben-Slama et al. in 2015 (28). Rezaei-Zarchi reported that nanoparticles are responsible for altering the integrin's level of platelet and phosphotoprotein levels in the platelets (30). The researchers described the mechanism of nanoparticle effect on the platelet, a process whereby nanoparticles may penetrate into the platelet and occupy spaces and ecovolar granules, preventing the spread of hyaloplasmic and reducing platelet aggregation [31]. As for the present research, the levels of red blood cells and the mean volume of hemoglobin decreased significantly in high doses of molybdenum trioxide nanoparticles. Faiz and Razmara in their studies proved that free radicals produced by nanoparticles cause inflammation of red cells and, as a result, hemolysis (24, 32). Cheraghi et al reported that changes in the average volume of hemoglobin are probably due to delay in the mitotic period and DNA damage in the presence of nanoparticles (33).

To put it in a nutshell, the results of the present research showed that low doses of molybdenum trioxide nanoparticles (5 mg/kg of nanoparticle body weight) caused a significant decrease in the number of cells in blood parameters, whereas injection of high dose of nanoparticles of molybdenum trioxide (10 mg/kg body weight nanoparticles) caused a significant reduction in the levels of blood cells in the tested rats.

The results of the present research showed that median lethal dose of molybdenum trioxide nanoparticles in the liver and kidney was significantly higher than the heart and stomach. In this regard, the results of Frygal et al study in 2005 reported a median lethal dose of LD50 for 125 mg/kg body weight of molybdenum oxide (34). The median lethal dose (LD50) for sodium molybdenum was reported to be between 110-30000 mg / kg bodyweight in Leigh et al study (35), quite consistent with the results of present study in terms of median lethal dose. Of course, according to the EU criteria for harmful substances, the toxicity of molybdenum compounds is very small, because with increasing consumption of molybdenum, its urinary excretion increases, and about 36-90% of the total molybdenum is removed through the urine, more than 1% through the bile and a small amount is also excreted through feces (35). However, the present research had specific limitations, such as the provision of nanoparticles, as well as the toxic effects of these particles on the researcher's bodies. It is recommended to test the effect of other nanoparticles on the body and organs. However, it is recommended to study the effects of other doses of molybdenum trioxide nanoparticles on the human body and other organisms, as well as the effects of other nanoparticles on other organs of organisms.

Conclusion

The results of the present research showed that molybdenum trioxide nanoparticles at high concentrations (10 mg/kg) had a higher toxic effect on blood and serum parameters compared to low concentrations. The results of this study confirmed the toxicity of these nanoparticles, and it is likely that administration of these nanoparticles at two different concentrations is due to an increase in the leakage of alanine aminotransferase, alkaline phosphatase and aspartate aminotransferase enzymes from liver cells and their serum levels; it was, also, shown that poisoning the rats caused liver function impairment and damage to liver cells and liver damage, so exposure to large amounts of nanoparticles should be avoided. Finally, it is recommended to study the effects of other doses of molybdenum trioxide nanoparticles on the human body and other organisms, as well as the effects of other nanoparticles on other organs of organisms.

Acknowledgement

The present research was sponsored by Kerman University of Medical Sciences and Health Services; hereby, the researchers feel obligated to express their appreciation to all individuals who contributed to the completion of the study.

Conflict of interest: None declared.

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