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Histobiochemical Effects of Dust Microparticles of Asalouyeh Area (South of Iran) on the Liver and Renal Tissues in the Pregnant Female Rats

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Abstract

Objective: Due to industrial development and mechanization of societies, air pollution has devastatingly affected people's lives which have become a very significant issue for researchers. Given that oil and gas production and surface facilities have made Assaluyeh to be one of the world's most polluted areas, the aim of this study was to investigate the toxic effects of air micro-dust in Assaluyeh on histological changes in the liver, kidney and liver enzymes.

Materials and Methods: Twenty-one adult female rats were divided in to 3 groups of control, negative control (exposed to micro-dust of a clean area free of oil contaminants) and treatment (exposed to the micro-dust contaminated with petroleum hydrocarbons in Assaluyeh). All animals had synchronized reproductive cycles. After 21 days of treatment, histological changes in liver and kidney, as well as serum levels of hepatic enzymes of alanine transaminase, aspartate transaminase, alkaline phosphatase (ALP), lactate dehydrogenase (LDH) were examined and measured. The serum levels of heavy metals of lead, cadmium, arsenic and mercury were measured using atomic absorption.

Results: The levels of heavy metals of lead, cadmium, arsenic and mercury in animals in the treatment group were significantly increased compared to the negative control group and the control group ($P \leq 0.05$). Serum levels of liver enzymes in the treatment group were not significantly different from the control group. No cell death was observed in the tissue sections. Cell irregularities were observed in the tissue sections of the treatment groups.

Conclusion: Micro-dust of Assaluyeh air did not show high toxicity on liver and kidney tissues.

Keywords: Dust, Liver, Kidney, Liver enzymes, Assaluyeh

Introduction

Industrial development and mechanization of human life in societies have never been without negative consequences. Air pollution has become of particular interest to researchers as the increase in air pollution in cities pose serious threat to the health of its residents. Although nanotechnology makes available products more efficient and effective, the size of the particles, which is their important characteristic, could threaten health and the environment. The particles are smaller than pollen and normal allergens and can cause allergy. These particles can attack human immune system. Harmful effects of particle pollutants in the air are mainly related to the concentration of particles smaller than 100 nm and do not depend on the mass concentration of larger particles. Potential risks of nanoparticles dispersed in the air, namely aerosols, are more important. This is due to their high mobility and the possibility of their absorption through the lungs, which are the easiest entry is to the (1).

Mineral and metal compounds play an important role in living organisms. However, excessive exposure to mineral and metal compounds has toxic effects (2). Heavy metals are persistent and extensive contaminants and affect the structure and function of multiple organs through generation of oxidative stress (3). Heavy metals generate reactive oxygen species (ROS) (4), and cause oxidative stress through different mechanisms. ROS causes cell damage and death. Excessive generation of ROS can cause changes in the trans-cellular structures. These changes include changes in the structure of proteins and DNA, lipid peroxidation of unsaturated fatty acids and changes in antioxidant system cells. Cells can create antioxidant and detoxification responses to heavy metals' effects. Antioxidant enzymes such as glutathione peroxidase, glutathione S-transferase, superoxide dismutase and catalase play a role in protecting cells from oxidative stress (4). Many previous studies have shown that heavy metals can cause histopathological changes in many tissues (5).

Today, it is known that oxidative stress induction is the main mechanism involved in the toxicity of nanoparticles.

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Oxidative stress is a process whereby the production of ROS exceeds the antioxidant capacity (6). The liver is one of the reticuloendothelial organs and is very sensitive to oxidative stress due to high blood flow (7). Through oxidative stress and lipid peroxidation, nanomaterials are involved in the destruction of membrane resulting in cell death (8).

When the production of ROS exceeds the antioxidant capacity and oxidative stress, the liver becomes extremely sensitive to oxidative stress due high blood stream (9). On the other hand, nanomaterials, through oxidative stress and lipid peroxidation, play an important role in the destruction of membrane resulting in cell death (8). Kidneys are the main organs protecting the body's internal environment and are susceptible to damages caused by drugs and environmental chemicals. Heavy metals can have toxic effects on the kidney cells. Renal toxicity of metals has been studied by some researchers (10,11).

No comprehensive research has been conducted on the effects of pollution in the Assaluyeh area on rats. On the other hand, heavy metals such as zinc, copper, iron and nickel exist in soil and dust of the Assaluyeh area. Several oil and gas production facilities are also constructed in the area; therefore, this area is one of the most polluted regions of the world. The aim of this study was to evaluate the effect of the micro-dust in Assaluyeh on the histological changes in liver and kidney and liver damage serum factors in adult rats.

Materials and Methods

Statistical Population and Sample

Thirty Sprague-Dawley of female rats at the age of 100-120 days and weighs of approximately 150-200 g were purchased from laboratory animals breeding center of Bushehr University of Medical Sciences.

Keeping Conditions

Animals were kept in cages under controlled temperature conditions (22 ± 3°C), humidity of 60% to 65%, alternating lighting of 12 hours of light and 12 hours of darkness for 2 weeks under free regimen in order to cope with the conditions and get used to the new environment. Special compact foods (pellets) were produced by Livestock and Poultry Feed Company (Pastor, Iran). Water and food were provided for the animals during the entire treatments period without any restrictions.

Synchronization and Mating

A few days before the start of treatment, 100 µg of estradiol valerate (Orion Pharma, UK) solved in 0.2 mL of olive streel oil was intramuscularly injected. Forty-two hours after administration of estradiol valerate, 50 mg of progesterone (Gestone 50 mg/mL & 100 mg/2 mL; Nordic Pharma Limited, UK) (solved in 0.2 mL olive oil) was injected intramuscularly to female rats. After waiting for a few hours for progesterone to have its effects, female rates were mated. Vaginal smear was used to confirm pregnancy in all animals.

Grouping

After synchronization of their reproductive cycles, rats were randomly divided into 3 equal groups (each group consisted of 10 rats) as follows:

1. First group (control) was kept in a dust-free environment (clean room) for 3 weeks.
2. The second group (negative control) was kept for 21 days and every day for 8 hours (8 to 12 PM and 4 to 8 PM) in an environment contaminated with dust collected from dust-contaminated environments but free of petroleum contaminants in a simulated environment that was designed by a research team in Bushehr University of Medical Sciences, Iran (BPUMS.E-415). A system fan inside the glass aquarium constantly circulated dust in the environment.
3. Third group (treatment) was exposed to the dust collected from the polluted area of Assaluyeh, where the largest oil installations of Iran are located in. This city is located in the south of Iran and was contaminated with petroleum aromatic hydrocarbons for the same period of time.

Treatments

After confirming the pregnancy, animals were treated until delivery (21 days). Animals (group I and II) have been put inside special cage and then were transferred to the machine (dust air pollution maker made by researcher in Bushehr University of Medical Sciences and were exposed to dust). Animals have been treated for 21 days (8 hours in a day, 4 hours in the morning and 4 hours in the afternoon).

Blood Sampling

Upon delivery, the animals were removed from the device and kept fasting for 12 hours and then anesthetized and sacrificed. Blood samples were taken directly from the left ventricle. About 3 mL of blood was drawn from each animal. Blood samples were gently collected in test tubes and placed for 1 hour at room temperature to clot. Samples were then centrifuged at 3000 rpm for 5 minutes to obtain serum.

Atomic Absorption Spectroscopy

Atomic absorption spectroscopy (AAS) was used to measure the serum levels of lead, cadmium, arsenic and mercury. The device consists of a radiation source, sample holder, wavelength selector, detector, and signal and stability processor.

Biopsy and Histological Study

Samples were taken at end of treatment after anesthetizing the rats by ketamine and xylazine mixture (3:1) (50 mg/kg I.M). It was performed by sterilized surgical set from declared area of tissues (kidney and liver). Specimens were fixed in 10% formaldehyde solution and transfered to histopathological lab. For the light microscopic study, samples were dehydrated and embedded in alcohol (ethanol) and paraffin, respectively. They were cut in

to 3 microns thicknesses by a rotary microtome and stained normally (H & E). The microscopic slide photos were taken by microscope equipped with a Moticam camera model A352 (The Netherlands) in a high magnification ion (×100). The measurable parameters such as means diameter of renal corpuscles, distal and proximal convoluted tubules, collecting duct, thick and thin segments of Henle loop were evaluated using photomicrographs with ImageTool software version 3. The obtained results involved the observed changes on histopathological changes such as congestion and tissue damage in different groups.

Statistical Analysis

The collected data was analyzed using SPSS 17.0. Charts were drawn using Excel. Descriptive statistics for the studied quantitative variables were presented as mean and standard deviation (SD). The Kolmogorov-Smirnov test confirmed normal distribution of data. Independent *t* test was used to compare mean quantitative factors in each of the test groups with control groups. One-way analysis of variance (ANOVA) was used to compare mean variables between groups. Pearson correlation test was used to investigate the correlation between quantitative variables. $P<0.05$ was considered significant in all analyzes.

Results

Heavy Metals Measurement

Table 1 shows the amounts of heavy metals (plumbum, arsenic, cadmium and hydrargyrum) measured by using atomic absorption techniques. As it is shown, the mean amount of plumbum in standard rate, control and test groups (T1 and T2) is 0.01 ± 0.005, 0.01 ± 0.002, 0.02± 0.005 and 0.08 ± 0.002 respectively. Data analysis showed that the mean amount of plumbum increased significantly just in T2 group (Assaluyeh- petroleum air pollution) compared to other groups ($P < 0.05$).

The mean amount of cadmium in standard rate, control and test groups (T1 and T2) is 0.004 ± 0.0007, 0.005± 0.0003, 0.006 ± 0.0052 and 0.028 ± 0.001 respectively. Data analysis showed that the mean amount of cadmium was increased significantly just in T2 group (Assaluyeh-petroleum air pollution) compared to other groups ($P < 0.05$).

The mean amount of arsenic in standard rate, control and test groups (T1 and T2) is 0.004 ± 0.0007, 0.008± 0.001, 0.010 ± 0.001 and 0.041 ± 0.001 respectively. Data analysis showed that the mean amount of arsenic increased significantly just in T2 group(Assaluyeh- petroleum air pollution) compared to other groups ($P < 0.05$).

The mean amount of hydrargyrum in standard rate, control and test groups (T1 and T2) is 0.0007 ± 0.0027, 0.0013 ± 0.001, 0.0017 ± 0.001 and 0.0046 ± 0.0002 respectively. Data analysis showed that the mean amount of hydrargyrum increased significantly just in T2 group (Assaluyeh- petroleum air pollution) compared to other groups ($P < 0.05$).

The results of Serum Enzymes Measurement

Table 2 shows the amounts of enzymes including alanine, aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) in control and test groups (T1 and T2). Data analysis showed that the mean amounts of enzymes did not have any significant difference in T2 group (Assaluyeh- petroleum air pollution) compared to other groups ($P < 0.05$).

Histopathological Findings

Morphology Study

Figure 1 shows the light micrographs sections of hepatic tissues in control and test (T1 and T2 groups). As it

Table 1. Mean of Atomic Absorption Parameter in Different Groups

Heavy Metals	Standard Rate & Groups			
	Standard Rate	Control (Non-air Pollution)	Non petroleum Air Pollution (T1)	Petroleum Air Pollution (T2)
Plumbu (ppm)	0.01 ± 0.005	0.01 ± 0.002	0.02 ± 0.005	0.08 ± 0.002*
Cadmium (ppm)	0.004 ± 0.0007	0.005 ± 0.0003	0. 006 ± 0.0052	0.028 ± 0.001*
Arsenic (ppm)	0.004 ± 0.0007	0.008 ± 0.001	0.010 ± 0.001	0.041 ± 0.001*
Hydrargyrum (ppm)	0.0007 ± 0.0027	0.0013 ± 0.001	0.0017 ± 0.001	0.0046 ± 0.0002*

Data were analyzed with *t* test and f test method. Values were expressed as the mean ± standard deviation (SD). Significant difference with control group (*); $P < 0.05$; n=10.

Table 2. Enzyme Assessment of Serum in Different Groups

Groups	Control (Non-air Pollution)	Non-petroleum Air Pollution (T1)	Petroleum Air Pollution (T2)
LDH, IU/L	778.8±351.83	951.4±430.02	784 ±333.29
ALP, IU/L	65.68±19.4	72.31±11.51	75.14±22.37
AST, IU/L	40.34±18.73	43.53±12.31	48.42±17.65
ALT, IU/L	57.32±9.31	52.7±6.86	60.47±8.49

Data were analyzed with *t* test and f test method. Values were expressed as the mean ± standard deviation (SD).

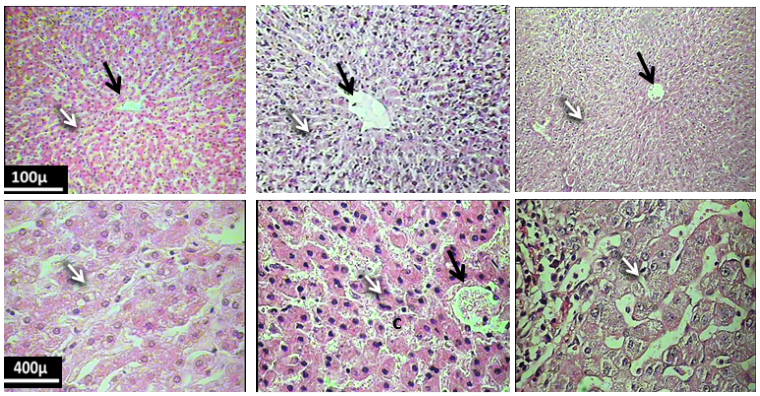

Figure 1. Light Micrographs Sections of Hepatic Tissues in Control and Test (T1 and T2) Groups. As seen, in T2 group (Asalouyeh- Petroleum air pollution) compared to other groups, hepatocytes lost their regular arrays and many abnormal spaces with congestion were seen between liver cells. Meanwhile, liver cells highly lost their cellular arrays; cellular nucleus was highly heterochromatic and pyknotic and seen some lymphatic infiltration in T2 group (Asalouyeh- Petroleum air pollution) compared to other groups.

was observed in T2 group compared to other groups, hepatocytes lost their regular arrays and even an irregular confined space with congestion was seen among liver cells. Meanwhile, liver cells lost their cellular arrays significantly; cellular nucleus was extremely heterochromatic and pyknotic; and some lymphatic infiltration was seen in T2 group (Assaluyeh- petroleum air pollution) compared to other groups.

Also a lot of pathological changes such as congestion, irregularity, nuclear density, dispersion tissue were seen in renal tissues in T2 group (Assaluyeh- petroleum air pollution) compared to other groups (Figure 2).

Morphometry Study

For assessing Petroleum air pollution on renal tissues, the morphometric of light micrographs such as mean diameter of renal corpuscles, distal and proximal convoluted tubules, collecting duct, thick and thin segments of Henle loop were analyzed using ImageTool software.

The diameter of kidney tubules was measured (Table 3). As it was shown, the mean diameter of renal corpuscles in control and test groups (T1 and T2) is 151.10 ± 11.08, 166. 20 ± 8.11 and 197.56 ± 12.20 respectively. Data analysis showed that the mean diameter of renal corpuscles was increased significantly just in T2 group (Assaluyeh- petroleum air pollution) compared to other groups ($P < 0.05$).

The mean diameter of proximal convoluted tubules in control and test groups (T1 and T2) is 73.45 ± 9.21, 71.07 ± 6.46 and 98.46 ± 13.37 respectively. Data analysis showed that the mean diameter of proximal convoluted tubules was increased significantly just in T2 group (Assaluyeh- petroleum air pollution) compared to other groups ($P < 0.05$).

The mean diameter of distal convoluted tubules in control and test groups (T1 and T2) is 75.66 ± 1.27, 80.12 ± 14.16 and 101.14 ± 08.11 respectively. Data analysis showed that the mean diameter of distal convoluted tubules increased significantly just in T2 group (Assaluyeh- petroleum air pollution) compared to other groups ($P < 0.05$).

The mean diameter of collecting duct in control and test groups (T1 and T2) is 133.01 ± 15.43, 139.12 ± 02.21 and 171.02 ± 2.13 respectively. Data analysis showed that the mean diameter of collecting duct increased significantly just in T2 group (Assaluyeh- petroleum air pollution) compared to other groups ($P < 0.05$).

The mean diameter of loop of Henle, the thick segment in control and test groups (T1 and T2) is 63.11 ± 17.02, 68.37 ± 12.07 and 71.18 ± 9.13 respectively. Data analysis showed that the mean diameter of loop of Henle, the thick segment increased significantly just in T2 group (Assaluyeh- petroleum air pollution) compared to other groups.

The mean diameter of loop of Henle, the thin segment

Table 3. Morphometric Assessment of Renal Tubule and Corpuscle Diameter in Different Groups

Groups	Control (Non-air Pollution)	Non-petroleum Air Pollution (T1)	Petroleum Air Pollution (T2)
Renal corpuscle	151.10 ± 11.08	166. 20 ± 8.11	197.56 ± 12.20*
Proximal convoluted tubule	73.45 ± 9.21	71.07 ± 6.46	98.46 ± 13.37*
Distal convoluted tubule	75.66 ± 1.27	80.12 ± 14.16	101.14 ± 08.11*
Collecting duct	133.01 ± 15.43	139.12 ± 02.21	171.02 ± 2.13*
Loop of Henle, thick segment	63.11 ± 17.02	68.37 ± 12.07	71.18 ± 9.13
Loop of Henle, thin segment	23.10 ± 2.14	25.10 ± 2.24	28.10 ± 13.09

Data were analyzed with *t* test and f test method. Values were expressed as the mean ± standard deviation (SD). Significant difference with control group (*); $P < 0.05$; n=10.

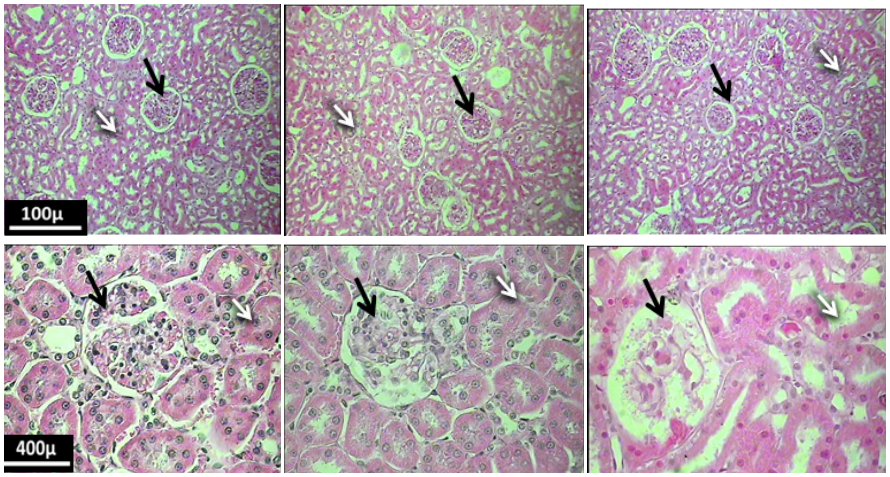

Figure 2. Light Micrographs Sections of Renal Tissues in Control and Test (T1 and T2) Groups. A lot of pathological changes such as congestion, irregularity, nuclear density, dispersion tissue were seen in renal tissues in T2 group (Asalouyeh- Petroleum air pollution) compared to other groups.

in control and test groups (T1 and T2) is 23.10 ± 2.14, 25.10 ± 2.24 and 28.10 ± 13.09 respectively. Data analysis showed that the mean diameter of loop of Henle, the thin segment increased significantly just in T2 group (Assaluyeh- petroleum air pollution) compared to other groups.

Discussion

In recent decades, researchers have investigated the interaction of nanoparticles with cells and organisms by studying the relationship between size, shape and cellular uptake (12), biodistribution (13), toxicity mechanism (14) and even how nanoparticles might react to toxicity tests. Results have revealed the harmful effects of nanoparticles through the changes in indices involved in toxicity measurement. Nanoparticles are transferred to different parts of the body through the blood stream (12). Carbon nanoparticles interact with cells, proteins, tissues, organs and may retain or metabolize their structure depending on their physico-chemical properties (15). Although the 3 organs of the skin, lung and small intestine are known as the main entries of particles into the body due to direct contact with environmental factors, cardiovascular and circulatory systems, brain, liver, etc are indirectly influenced by the presence of nanoparticles. Little information is available on the effects of nanoparticles on organs such as the liver, kidneys, and spleen. It can be said that transport and accumulation of nanoparticles in these organs, their destructive reactions with the cells, and cell cytotoxic can cause disease. Diseases with unknown source are associated with the presence of nano- and microparticles in the liver and kidney. In this study, the effects of air micro-dust in Assaluyeh on histological changes in liver and kidney and liver enzymes of female rats were studied to evaluate the toxicity of micro-dust of Assaluyeh.

Biochemical tests are frequently used in diagnosis of liver and kidney diseases. Cells' response to exogenous toxins can be detected using these tests. In humans, AST along with LDH and ALP is used to assess damage to the liver or liver diseases. When hepatic dysfunction is high, the level of these enzymes rises (16). In this study, the levels of enzymes of LDH, alanine, AST, ALP and ALT in the treatment group did not have a significant difference with the control group. Therefore, it seems that despite a significant increase in the levels of lead, cadmium, arsenic and mercury in the treatment group compared to the control group, renal and hepatic toxicity factors did not show a significant increase compared to the negative control group. Thus, in this study, liver and kidney tissue slides were examined. Liver tissue slides in the treatment group showed relatively unnatural order in hepatocytes (however, they still were regular), vacuolization of the cytoplasm and heterochromatic nuclei, and lymphoid infiltrates in some sections. Necrosis was not observed in any of the liver tissue sections. Therefore, obviously, the levels of LDH enzyme were not significantly changed. It is interesting that, at kidney histological examinations, non-renal tubules of the kidneys and renal corpuscle densities were also observed in the treatment group (similar to the negative control group) but no necrosis or death was observed in proximal renal tubules. This confirms the non-toxicity of micro-dust of Assaluyeh air on the kidneys. The same irregularities in spleen cells and low irregular density (in some tissue sections) and red pulp congestion were observed. However, cell death and tissue necrosis were not observed.

Heavy metals refer to those elements that have atomic masses greater than iron, which are highly absorbed by living tissues. They accumulate in the tissues and hardly exit them. They include zinc, cadmium, cobalt, copper, lead, nickel, arsenic, vanadium, and chromium (17). Levels of iron, copper, zinc and nickel in Assaluyeh's dust were measured by AAS (18). Iron and copper had the highest and lowest levels, respectively.

Some researchers showed that low doses of mercury and lead can cause oxidative stress on the kidneys and tissue damage and changes in serum biochemical parameters.

Previous studies have shown that heavy metals can cause liver damage in rats under different conditions (19). Mercuric chloride and lead nitrate can easily cross the blood-brain barrier and the placenta. Many researchers have reported that mercury and lead cause oxidative stress and stimulate ROS (20).

The lead absorbed either by ingestion or breathing accumulates in soft tissue. Studies on people who had been exposed to lead for a long time in their lives show that, after death, the maximum amount of lead (33%) is found in the body's soft tissues such as cortex and center of the kidney and the liver tissue (21).

Kidney is the main organ targeted by the toxicity of lead minerals (22). As a result of exposure to lead in animals, the epithelium of the renal tubules becomes the main site prone to cancer (23). Low levels of mercury can also cause oxidative stress, cytotoxicity, and increased beta-amyloid which is associated with neurodegeneration disorders such as Parkinson and Alzheimer. In addition, inorganic mercury can impair the renal tubular function. Its toxicity is due to GSH depletion and degradation of mitochondria. (24,25).

Liver's exposure to cadmium causes liver injury via the synthesis of metallothionein (26). Cadmium upregulates the expression of stress genes and disrupts the activity of different enzymes that are responsible for protection of the liver against oxidative stress. Liver indicators' disorder and increased oxidative stress enhance lipid peroxidation, DNA damage, and carbonylation of proteins (27).

Chronic exposure to low concentrations of arsenic is associated with diseases such as hepatocellular carcinoma, melanosis, hyperkeratosis, diabetes mellitus, hypertension, liver cirrhosis, liver fibrosis and destruction of parenchyma cells (28).

Thus, although serum concentrations of heavy metals of lead, cadmium, arsenic and mercury were significantly increased in the animals in the treatment group compared to the controls, histopathological examinations revealed that they had no toxic effect on the liver and kidney tissues of rats. This could be due to the lack of a chronic treatment period, the low-dose exposure to nanoparticles in animals, or the method of entry of nanoparticles into the body (through the air). In general, the results of the present study showed that air dust of Assaluyeh, which contains contaminated nanoparticles, are not highly toxic on the liver and kidneys of mature female rats (29,30).

Conflict of Interests

None to be declared.

Ethical Issues

Working with laboratory animals in all stages of research was in accordance with standards and regulations of ethical committee of Bushehr University of Medical Sciences.

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