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EFFECT OF FE_4NIO_4ZN NANOPARTICLE ON INFLAMMATORY CYTOKINES: IL6 AND TNF MALE WISTAR RAT

DOUDI M[1], SETORKI M[*2], NAFISEH ESMAEIL[3] MOZHGAN TOODOOEI[1] AND TANNAZ ZABIHI[1]

1: Department of Microbiology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran

2: Department of Biology, Izeh Branch, Islamic Azad University, Izeh, Iran

3: Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

***Corresponding Author's E Mail: doctor.setorgi@gmail.com**

ABSTRACT

Today, detailed studies on the mechanisms Nanotoxicology according to the operating system free radicals of oxigen, have been conducte (through the release of proinflammatory factors, cytokines in organs. Aim of this study is the effects of different doses Fe4NiO4Zn nanoparticles on inflammatory cytokines.

Twenty four Wistar rat were used in the experiments. Animals were randomly divided into groups, two Fe4NiO4Zn nanoparticle-treated groups (1, 2) and control group. Group 1 and 2 received. /5cc of solution containing 100, 200 ppm Fe2NiO4 for 7 successive days. Then parameters such as IL-6, Tumor necrosis factor (TNF) were evaluated at (1, 2, 7 and 14 days). After 14 days, the tissue of lung investigated.

Mean IL-6 levels, 7 and 14 days after the intervention, in groups 1, 2 and group 1 with 3 were significantly greater than the control group. On day 7 of the study, the average factor of TNF, in groups 1 and 2, groups 1 and 3, as well as 2 and 3 significant difference were observed. On day 14 of the study, the mean factor of TNF, in groups 1 and 2 and also in groups 1 and 3 were significant. Histology results indicated pathological changes in Group 2: the thickened air sacs - fibrous and multiple congestion and lack of pathological changes in blood vessels in Group 3.

The results showed that intraperitoneal injection of Fe4NiO4Zn nanoparticles caused oxidative stress and inflammation in the lungs and low dose buildup severe pathological changes in the lung tissue.

Keywords: TNF, IL-6, Fe4NiO4Zn

INTRODUCTION

Oxidative stress can lead to membrane damage - death - inflammation or expression of selected genes depending on the dose used **[1]**.

Potential to cause oxidative stress is a feature of many pathogenic particles **[2]**.

Oxidative stress in cells that are exposed to pathogenic particles, it is recognized as a key pathogenic events that can lead to death and inflammation **[3]** and genotoxity **[4]**.

Cellular oxidative stress can cause from free radicals generated on the surface of particles or soluble components such as metals or organic compounds that are associated with nanoparticles **[5]**.

Free radical activity and type of study could reflect the activity of free radicals inside.Also, sometimes within the cell, Particles can, through their effects on cells that have not been stimulated by the radicals within cells (example: the effects that cause reduce antioxidants or stimulate cellular respiration of mitochondrial and oxidative stress, indirectly) cause oxidative stress **[6]**.

Researches show that zinc oxide nanoparticles are used in many fields, such as cosmetics - Biosensors - Food Additives - pigments - Resin Production and drug delivery,…

Also due to their anti-bacterial medicines, there are possibility of its use in dentistry and preventative medicine against microorganisms **[7]**.

ZnO nanoparticle uptake into cells by endocytosis and entry into lysosome, by the enzymes hydrolyzed in acidic PH, free zinc ions is caused by nanoparticles.

The toxicity of ZnO nanoparticles is related to free ions of zn in the solution that increases concentration of these ions in the cells, but toxicity of iron oxide may be related to its high uptake in cells **[8]**.

While the iron oxide interest as a non-hazardous compound but homeostasis imbalance will cause toxic effects. Iron oxide nanoparticles are capable to pass through skin and blood barrier of lungs and brain after intraperitoneal injection or breathing.

Before application of magnetic nanoparticles such as additional absorption of iron or transmission of drug conjugate, reviews toxic and non-essential structures of nanoparticles is necessary **[9]**.

Studies on biological tissues in conditions of in vivo revealed that in the lungs of rats treated with ZnO nanoparticles, the number of leukocytes influences into the alveolar compartment and can accumulate and cause lung inflammation and damage.

In this study, the effects of intraperitoneal injection of different doses (100,200 mg / kg) Fe4NiO4Zn nanoparticles on inflammatory cytokines TNF, IL-6 and lung tissue of Wistar rats were studied.

MATERIALS AND METHODS

Characterization of Fe_4NiO_4Zn Particles

Twenty five g Fe4NiO4Zn nanoparticles which was provided from Yasa Teb Co. (Iran) that imports nanoparticles from Sigma (Germany). In order to make sure of the size of the nanoparticles, 1 g of them was sent to the department of Materials Engineering of the Islamic Azad University (Najafabad branch), and that center confirmed the validity of the nanoparticles size using X-ray tests **(Figure 1)**. Specifications of this nanoparticle is:

<100nm particle size,>99% trace metal basis, linear formula: Fe_4NiO_4Zn; form: nanopowder; CAS number: 12645-50-0; molecular weight: 411, 46; density: 2, 81 gr/ml at 25°C.

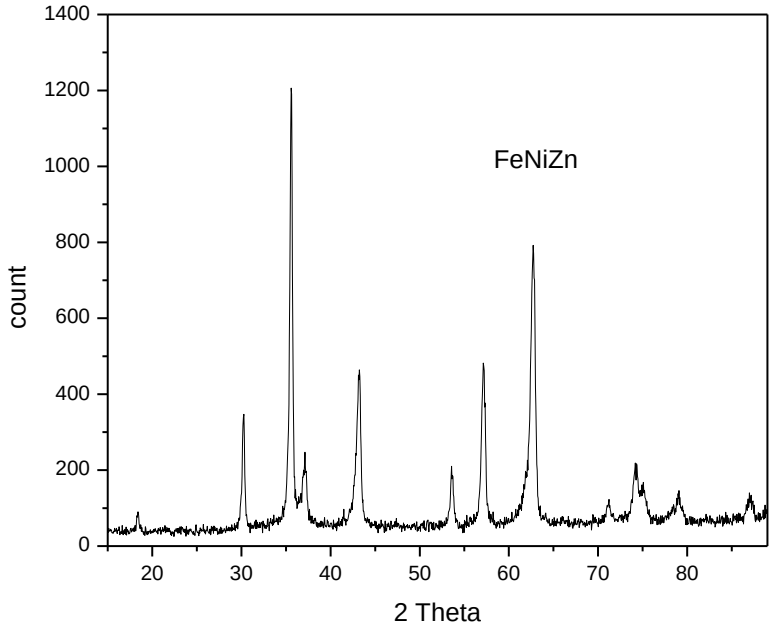

Figure 1: X-ray of Fe4NiO4Zn nanoparticle :d=0.9*1.504/(0.31*3.14/180)*cos17.77=262A=26 nm

Preparation of Mother Solution

To determine the nanoparticle concentration Fe_4NiO_4Zn, two mother of solution was provided:

Concentration of 100 ppm (mother solution of 1):

Amount of 50 mg of Nanoparticles desired in 1 mL of distilled water (100mg/1ml), what can be achieved with a concentration of 100 ppm of the nanoparticle.

Concentration of 200 ppm (mother solution of 2):

Amount of 100 mg of Nanoparticles desired in 2 mL of distilled water (200mg/1ml), what can be achieved with a concentration of 200 ppm of the nanoparticle.

Animal Preparation

This experimental study was performed on 24 Wistar male rats. These animals were purchased from the University of Medical Sciences Shahrekord that kept at the University of Nest Animals of Falavarjan in preparation for a trial period of two weeks.

Animals, expressed in terms of the degree of appropriate laboratory (the temperature of 2 ± 22 ° C) and and were kept well lit room (12 hours light and 12 hours darkness).

Animals had a mean weight of 225 ± 25 g and were divided into 3 groups of 8.

Control (group 1) and treated groups (2, 3) including: concentration 100nm, 200nm Fe4NiO4Zn, respectively. Nanoparticle and saline injections for 7 consecutive days was performed.

The effects of Fe4NiO4Zn nanoparticles on serum levels of inflammatory cytokines IL-6 (interleukin-6) and tumor necrosis factor) TNF using biochemical kits (co sigma) and Elisa methods were evaluated at 2nd, 7th and 14th days after the treatment.

Blood from the corner of the eyelids of animals was carried out with the help of capillary tube. The sample was centrifuged for 15 minutes (3000RPM / Minute), and serum was separated. All animals (at 14th day) were anesthetized by ether and sacrificed for histological assessment. The tissue of lung was collected and investigated.

All animal handling and manipulation procedures were performed according to the guideline of the Animal Welfare Act and the experimental protocols were approved by the Office of Research Ethics Committee of University of Shahrekord.

Histopathological Examination

Histological observations were performed according to the standard laboratory procedures. A small piece of lung or liver fixed in formalin 10% (v/v) was embedded in a paraffin block, sliced into 5 μm thicknesses and then placed onto glass slides. The section

was stained with hematoxylin-eosin (HE) and examined by light microscopy.

Statistical Analysis

Data analysis was conducted for each factor on based model MANOVA. Data analysis was conducted for each factor on based model MANOVA. In each of the models, factor variables in the first, second, seventh and fourteenth entered into the model, as dependent variables and variable group as independent variables. The Tukey test was used to evaluate significant pairs.

RESULTS

Biochemical Factors

Interleukin 6 At 7 and 14 days, averages of IL-6 between the Group 1 with 2 (PV <0.0001) and Group 1 with 3 significant differences were showed, respectively (PV <0.0001) **(Figure 2)**.

TNF (Figure 3):

In baseline and the second day, the mean factor of TNF were similar between the three groups (P.V>0.05.)

On day 7 of the study, according to the Tukey test, the average factor of TN, in groups 1 and 2 (PV <0.0001), groups 1 and 3 (PV <0.0001)) as well as 2 and 3 (PV = 0.041) significant difference were observed.

On day 14 of the study, according to the Tukey test, the mean factor of TNF, in groups 1 and 2 (PV <0.0001) and also in groups 1 and 3 (PV <0.0001) were significant.

Lung Tissue Sections from Control and Treated Rats for Coloring

Control group

This group does not have any pathological changes of lung tissue elements such as air bags - have normal blood vessels in sight.

Treatment group 2:

In this group, the pathological changes that show fibrous and thickening of the air sacs are being seen - multiple congestion in the blood vessels can be seen.

Treatment group 3

Pathological changes does not seen - bronchioles and air bags are natural - blood vessels; do not indicates hyperemia, clearly **(Figure 4-6)**.

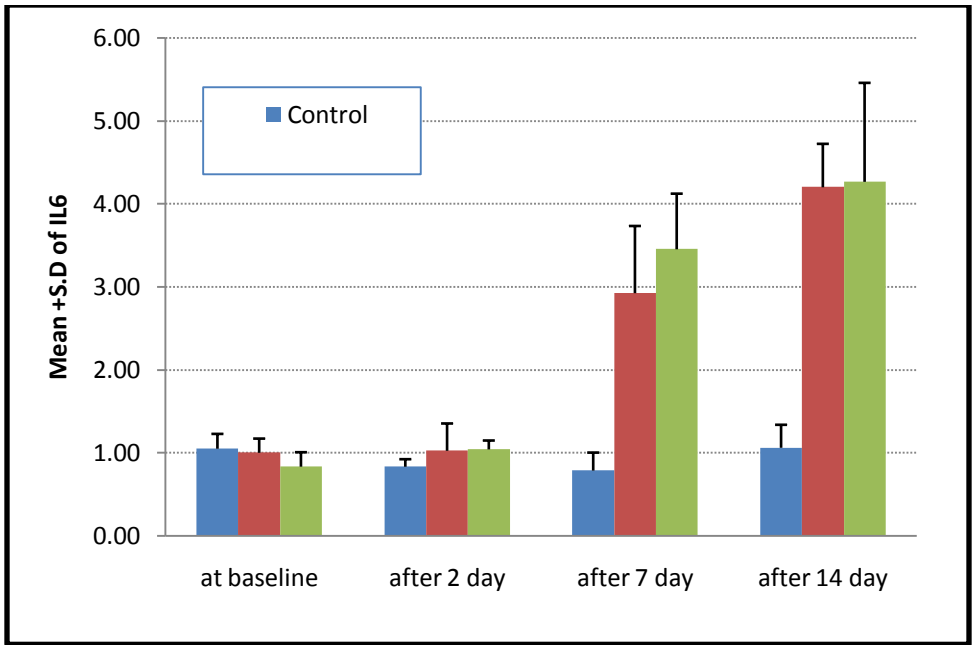

Figure 2: Comparison factor of IL-6 (ng/l) between group1, 2 and 3 (control, Fe4NiO4Zn:100 ppm, Fe4NiO4Zn:200 ppm, respectively) at baseline, 2, 14 days

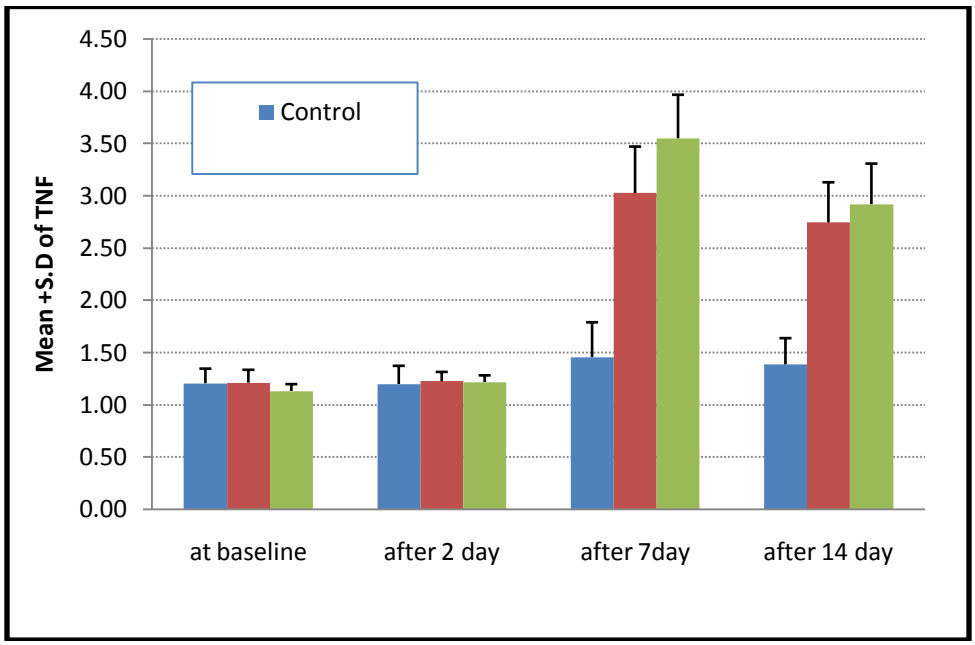

Figure 3: Comparison factor of TNF (ng/l) between group1, 2 and 3 (control, Fe4NiO4Zn:100 ppm, Fe4NiO4Zn:200 ppm, respectively) at baseline, 2, 14 days

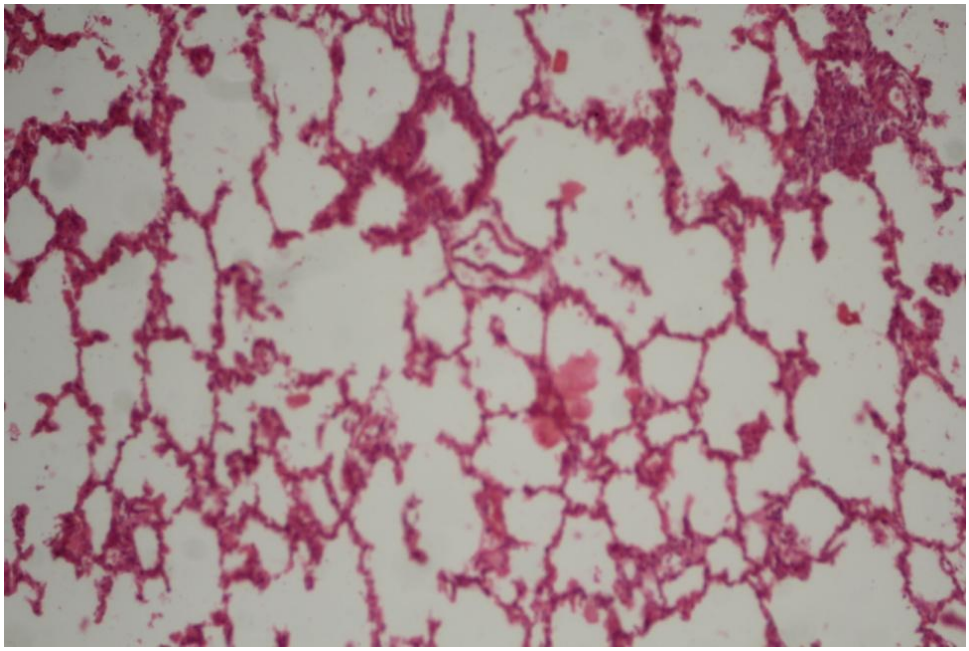

Figure 4: Light micrographs of sections in the lung of control group every day for 7 successive days (group 1)

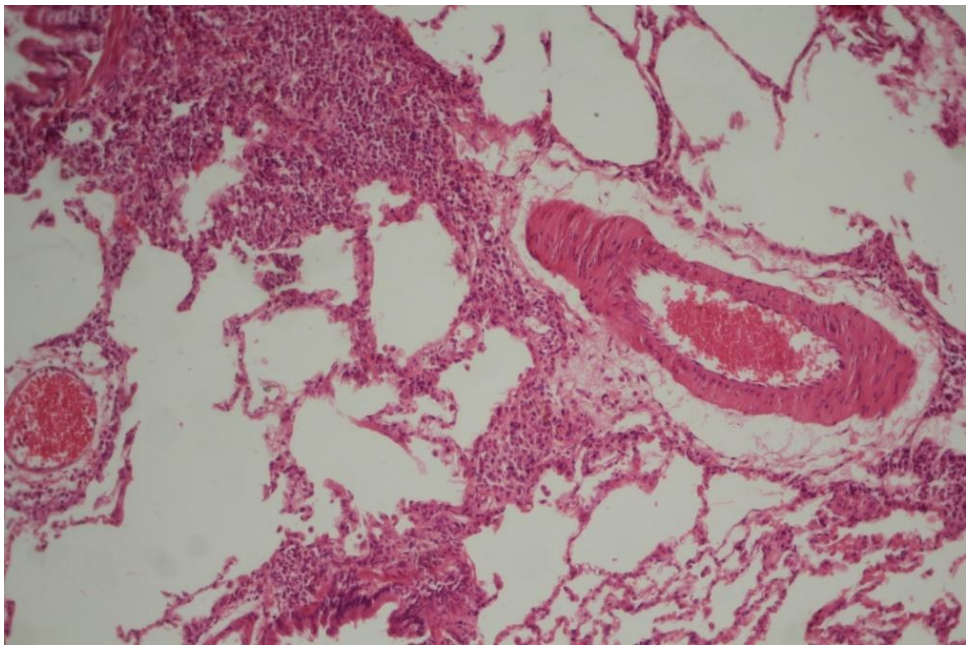

Figure 5: Light micrographs of sections in the lung of Fe4NiO4Zn -treated rat received 100 ppm every day for 7 successive days (group 2)

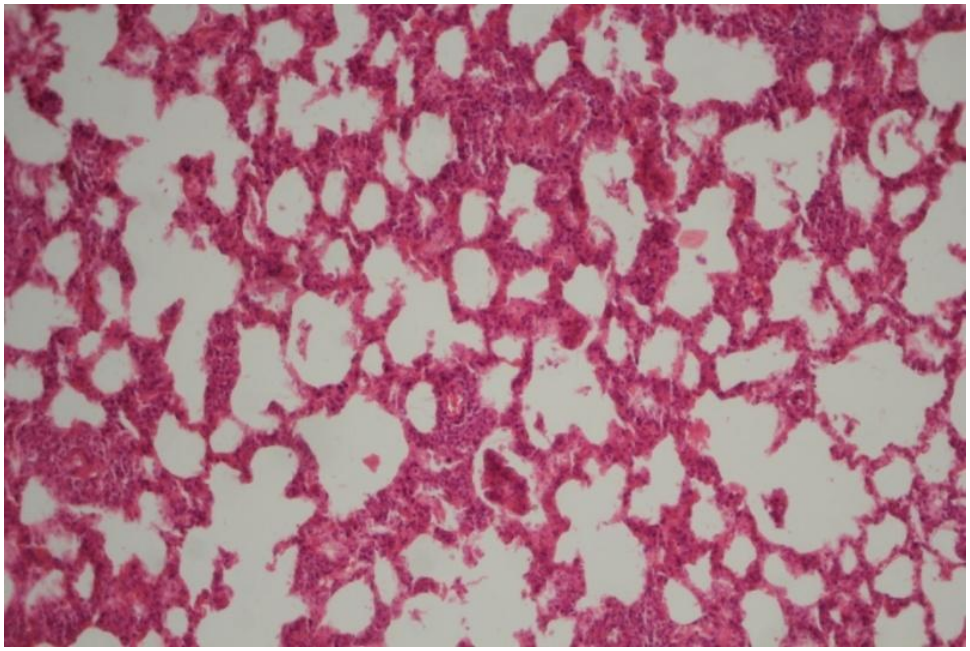

Figure 6: Light micrographs of sections in the lung of $Fe4NiO_4Zn$ -treated rat received 200 ppm every day for 7 successive days (group 3)

DISCUSSION

The results showed that intraperitoneal injection of $Fe_4NiO4Zn$ nanoparticles caused oxidative stress and inflammation in the lungs and low dose buildup severe pathological changes in the lung tissue.

It is believed that the nanoparticles through their surface activity are created the electron donor and recipient sites and can be able to product superoxide free radicals and cause problems in the chain of the electron transport.

In a multi-step process, free radicals generated by the nanoparticles, transcript the factors involved in the antioxidant defense system, such as Nrf-2 and cause further expressed as 200 times the antioxidant enzymes such as SOD (superoxide dismutase), CAT (catalase) and hemoxygenase 1.

If this process is completed, protective systems by MAPK (mitogen activatedprotein kinase) and NF-KB, through intracellular signals, is replaced and chemokine and proinflammatory cytokines are released that are initiating inflammatory responses and occurs apoptosis in the third stage. Today, detailed studies on the mechanisms Nanotoxicology according to the operating system ROS, have been conducte (through the release of proinflammatory factors, cytokines and MAPK in respiratory system - Gastroenterology - Hematology &... .)

A series of events Molecular continuous in three-phase of oxidative stress are including release of the oxygenase 1 and TNF. Study on

the shape - and size distribution of nanoparticles demonstrate relationship between toxicity and their physicochemical characteristics in phenomenon of inflammatory response **[10]**.

Berardis and colleagues found that nanoparticles of zinc oxide, can cause increasing of hydrogen peroxide and hydroxyl radicals - reducing the amount of molecular oxygen and reduced glutathione and interleukin-8 (IL-8) (a means of signaling for releaseing of proinflammatory mediators) **[11]**.

John M and Associates were examined inflammatory response in mice following inhalation of iron nanoparticles. Their results showed that acute exposure to nanoparticles, caused increasing of inflammation in treated mice compared with controls **[12]**.

Andrea Gojova and colleagues were investigated inducing of inflammation metal oxide nanoparticles (particles combined) in vascular endothelial cells.

Their studies showed that exposure to zinc oxide nanoparticles directly and acute significantly, lead to increase mRNA levels of inflammatory markers such as IL-8, ICAM-1, MCP-1, but iron oxide nanoparticles had not such an effect **[13]**.

At the highest concentration, ZnO nanoparticles (50 μg / mL), causing significant toxicity to cells and inflammatory responses. Nanoparticles of zinc oxide and iron oxide are examples of metal oxides that are associated with environment and career.

Katharina et al, genotoxity of metal nanoparticles were investigated in terms of in vivo.

Animals used in this study, were hyperlipidemic and without adequate. apoprotein E levels. The animals received 5 nm nanoparticles of nickel hydroxide. The results of this study showed systemic and pulmonary inflammatory reactiones - atherosclerosis (as a long-term effect) and the high rate of mitochondrial DNA damage **[14]**.

Hori et al were studied nanoparticle impact Nio (size 20 nm) of lungs rats. The results showed that the levels of lactate dehydrogenase and lipid peroxidation dehydrogenase increased (24 h after inhalation.) Survey results indicated induction of oxidative stress and damage to the lung **[15]**.

Ogami et al biological studied effects of nickel nanoparticles in the lungs of rats (size 20 nm -4 weeks). The results showed that after 4 days of inhaled nanoparticles, a temporary increase in the number of inflammatory cells lungs were observed, but at the end of the first month, such an increase was not observed **[16]**.

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