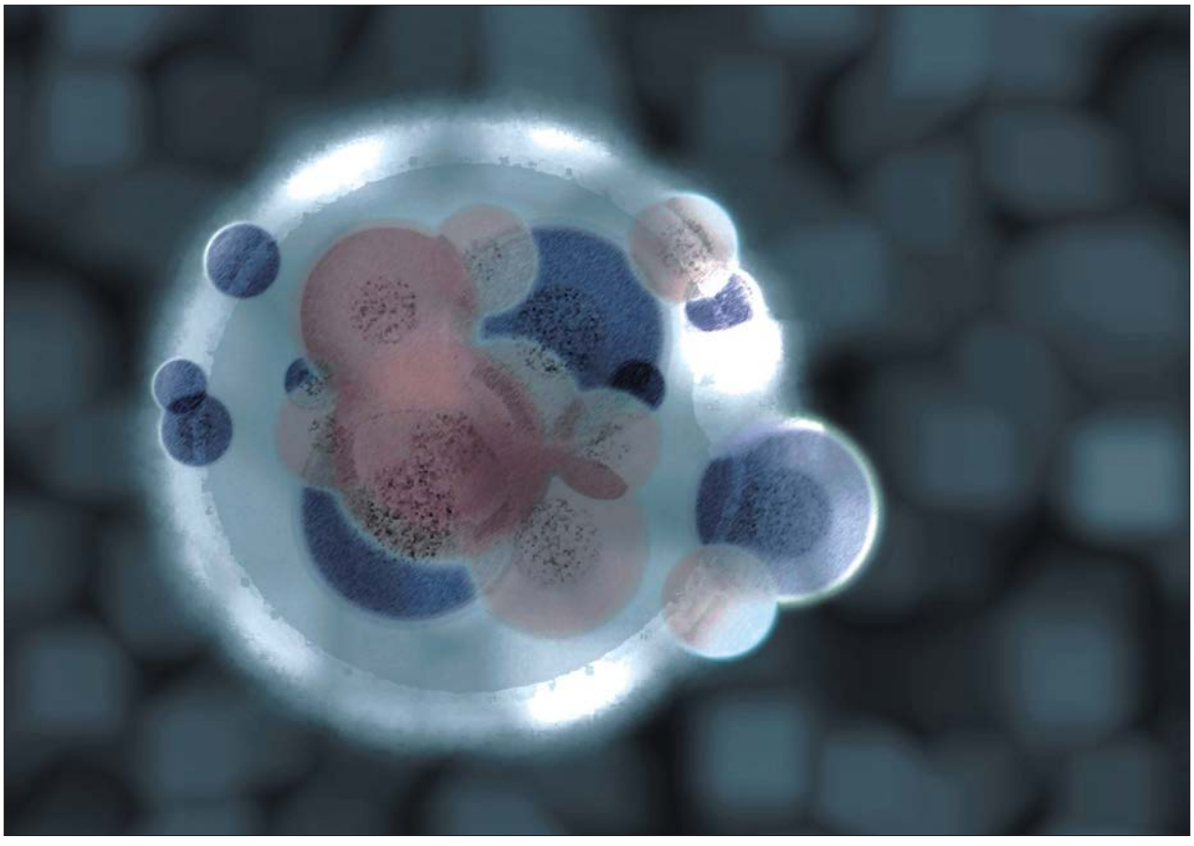

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Effect of Nanoparticles Fe_4NiO_4Zn on Liver Enzymes-White Blood Cell and Hematocrit in Wistar Rat

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Abstract: Pathogenic mechanisms initiated by nanoparticles, has been demonstrated by the effects of inflammation such as fibrosis-oxidative stress and DNA damage. Aim of this study is, to determine the effects of nanoparticles Fe_4NiO_4Zn on the number of leukocytes, HCT, platelet counts and liver enzymes in rats. Twenty four male Wistar rat weighing 234±43 g were used in the experiments. Animals were randomly divided into groups, two Fe_4NiO_4Zn nanoparticle-treated rat groups (1, 2) and one control group. Group 1 and 2 received 5 cc of solution containing 100, 200 ppm Fe_4NiO_4Zn via IP injection for 7 successive days. The control group was treated with 5 cc normal saline with same procedure. Then, several biochemical parameters such as number of leukocytes (neutrophil-lymphocyte), HCT (hematocrit), platelet counts, Serum Glutamate Oxaloacetat Transaminase (SGOT) and Serum Glutamate Pyrvate Transaminase (SGPT) were evaluated at various time intervals (1, 2, 7 and 14 days). Average SGOT, 14 days after the intervention, in the control group with group 1 ($p<0.0001$) and group 2 ($p = 0.007$), average SGPT, 2 days after the intervention, in the control group with group 1 ($p = 0.040$) and group 2 ($p = 0.036$) and 7 days after intervention, in the control group with group 1 ($p<0.0001$) and group 2 ($p<0.0001$) indicates statistically significant difference (reduction). In the second phase, a significant difference between the control group and group 1 ($p = 0.033$) and group 2 ($p = 0.002$) was observed in conjunction with platelet factor. Average WBC, 2 days after the intervention in control group with group 1 ($p = 0.046$) and 14 days after intervention in the control group with group 2 ($p = 0.004$) showed statistically significant differences (increasing). Neutrophil average of 14 days in control group with group 1 ($p = 0.006$) and group 2 ($p<0.0001$) indicates a significant difference (increasing). In four cases, the mean hematocrite and lymphocyte factors among three groups were identical ($p>0.05$). The Fe_2NiO_4 nanoparticles stimulate the immune system and inflammatory responses and cause reduction of liver enzymes (*in vivo* condition). Perhaps the most important reason for the lack of toxic effects of nanoparticles are rapidly removed from the circulation by the reticuloendothelial system in the liver-spleen and lymph nodes. Of course, the rapid elimination occurs after the phenomenon of opsonisation (accumulation of blood proteins in the particles) that causes stimulate the immune system and disposal of nanoparticles.

Key words: Fe_4NiO_4Zn, nanoparticles, HCT, rat

INTRODUCTION

Pathogenic mechanisms initiated by nanoparticles, has been demonstrated by the effects of inflammation such as fibrosis-oxidative stress and DNA damage. This issue is caused by inflammation, toxicology tests are objectives (Borm *et al*., 2006; Lu *et al*., 2009; Nel *et al*., 2006). Inflammation is a complex and coordinated responses (although defense against infection, while chronic, by environmental stimuli, such as induction of inhaled particles may be harmful (Mroz *et al*., 2008; Donaldson *et al*., 2006; Nel *et al*., 2009).

Type-risks and result of inflammation, depends on: Inflammation of the nature of the stimulus starter, damaged tissue, cell secretory nature, chronic and genetic susceptibility of the person. Inflammatory can not be done in conditions of *in vitro*, because the inflammation depends on: The vascular system healthy and a massive collection of cellular interactions and hormone. Although, *in vitro* studies with nanoparticles could prove claims of proinflammatory effects, but such studies can not be anything more than a general indication entitled "Inflammation" to prove. They can not predict inflammatory form, speed stability and continuity. Magnetic nanoparticles are important materials that have unique magnetic properties due to reduced particle size below 100 nm (Oberdorster *et al*., 2005).

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In recent years, nanoferrites are used at high levels (due to abnormal magnetic properties). In this study, the effects of intraperitoneal injection of different doses of nanoparticles Fe_4NiO_4Zn (100, 200 ppm) on the number of leukocytes (neutrophil-lymphocyte)-HCT-platelet counts and liver enzymes in Wistar rats were studied.

MATERIALS AND METHODS

Characterization of Fe_4NiO_4Zn particles: Twenty five gram Fe_4NiO_4Zn nanoparticles which was provided by Yasa Teb Co. (Iran) that imports nanoparticles from Sigma (Germany). In order to make sure about the size of the nanoparticles, 1 g of sample was sent to the department of Materials Engineering of the Islamic Azad University (Najafabad branch) and they confirmed the validity of the nanoparticles size using X-ray tests. Specifications of this nanoparticle is:

- Less than 100 nm particle size, >99% trace metal basis, Linear formula: Fe_4NiO_4Zn; Form: Nanopowder; CAS number: 12645-50-0; Molecular weight: 411,46 g mol^{-1}; Relative density: 2,81 g mL^{-1} at 25°C

Preparation of standard solution: To determine the nanoparticle concentration (Fe_4NiO_4Zn), two standard solution were prepared:

- **At 100 ppm concentration (solution 1):** Amount of 50 mg of nanoparticles desired in 1 mL of distilled water (100 mg/1 mL), what can be achieved with a concentration of 100 ppm of the nanoparticle
- **At 200 ppm concentration (solution 2):** Amount of 100 mg of nanoparticles desired in 1 mL of distilled water (200 mg/1 mL), what can be achieved with a concentration of 200 ppm of the nanoparticle

Preparation of animals: Twenty four wistar male rat (were purchased from the Animal Center of Falavarjan University), weighing 234±43 g were used in the experiment. They were acclimated in the controlled environment (temperature: 22±1°C; humidity: 60±10% and light: 12 h light/dark cycle) with free access to water and a commercial laboratory food. All animal experiments were performed in compliance with the local ethics committee.

Animals were randomly divided into 3 groups, two Fe_4NiO_4Zn nanoparticle-treated rat groups and one control group. Group 1 and 2 received, 5 cc of solution containing 100, 200 ppm Fe_4NiO_4Zn via IP injection for 7 successive days, respectively. The control group was treated with/5 cc normal saline with same procedure. Then, several biochemical parameters such as number of leukocytes (neutrophil-lymphocyte), HCT (hematocrit), platelet counts with SYS mex K-1000 and liver enzymes: Serum Glutamate Oxaloacetat Transaminase (SGOT) and Serum Glutamate Pyrvate Transaminase (SGPT) with kit of Pars Azmoon Company (Iran), (Hitachi Automatic Analyzer 902, Roche) were evaluated at various time intervals (1, 2, 7 and 14 days). The blood was carried out from the corner of the eyelid of animals with the help of capillary tube.

Two blood samples were taken: One for testing of CBC (that blood samples were drawn into EDTA tubes) and other for testing of enzyme liver (that blood samples has been centrifuged for 15 min at 3000 rpm and serum and plasma were separated.

Statistical analysis: Data was analysed for each factor by one way ANOVA. In each of the models, factor variables in the first, second, seventh and fourteenth study entered into the model, as dependent variables and variable group as independent variables. The Tukey test was used to evaluate significant pairs.

RESULTS

Average SGOT, 14 days after the intervention, in the control group with group 1 ($p<0.0001$) and group 2 ($p = 0.007$)-Average SGPT, 2 days after the intervention, in the control group with group 1 ($p = 0.040$) and group 2 ($p = 0.036$) and 7 days after intervention, in the control group with group 1 ($p<0.0001$) and group 2 ($p<0.0001$) indicates statistically significant difference (reduction). In the second phase, a significant difference among the control, group 1 ($p = 0.033$) and group 2 ($p = 0.002$) was observed in conjunction with platelet factor. Average WBC, 2 days after the intervention in control group with group 1 ($p = 0.046$) and 14 days after intervention in the control group with group 2 ($p = 0.004$) showed statistically significant differences (increasing). Neutrophil average of 14 days in control group with group 1 ($p = 0.006$) and group 2 ($p<0.0001$) indicates a significant difference (increasing). In four cases, the mean Hematocrite and Lymphocyte factors among the three groups were identical ($p>0.05$), (Fig. 1-7).

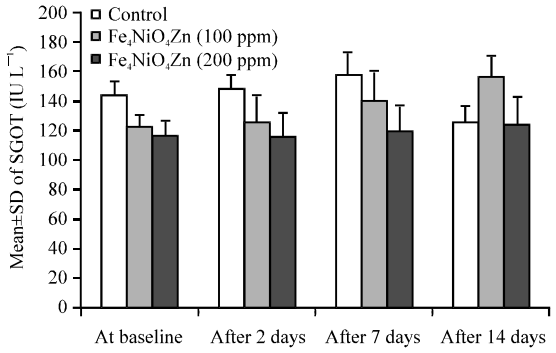

Fig. 1: Comparison of the levels of Serum Glutamate Oxaloacetat Transaminase (SGOT) in four groups at different concentrations

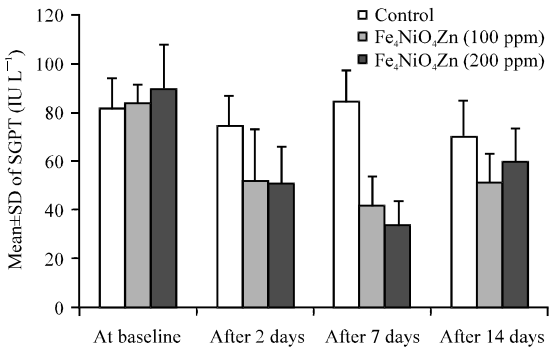

Fig. 2: Comparison of the levels of serum glutamate pyrvate transaminase (SGPT) in four groups at different concentrations

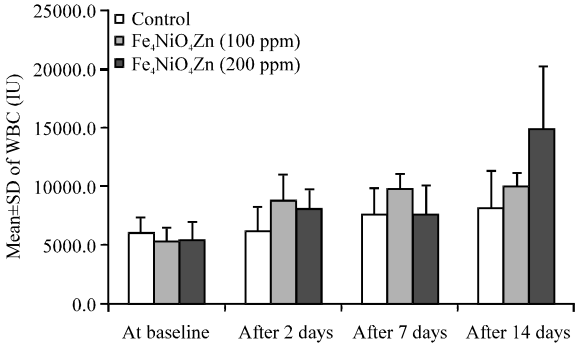

Fig. 3: Comparison of the levels of White Blood Cell (WBC) in four groups at different concentrations

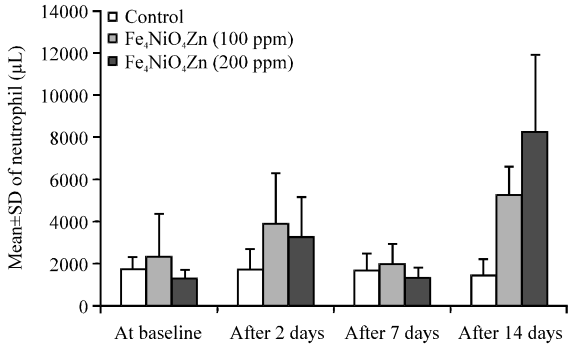

Fig. 4: Comparison of the levels of neutrophil in four groups at different concentrations

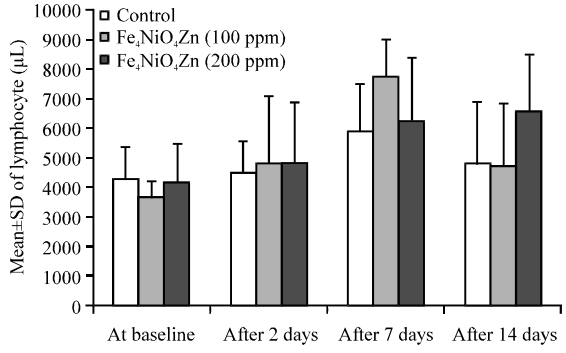

Fig. 5: Comparison the levels of Lymphocyte in four groups at different concentrations

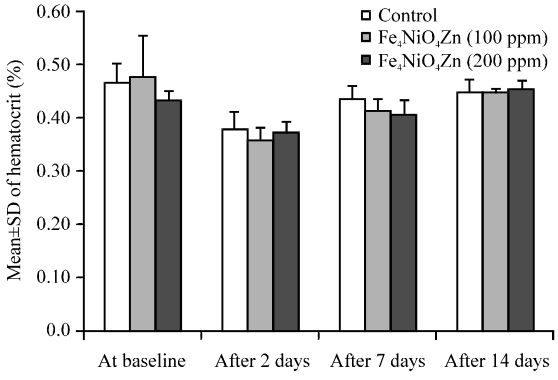

Fig. 6: Comparison the levels of hematocrit in four groups at different concentrations

DISCUSSION

The results showed that male wistar rats treated with nanoparticles Fe_4NiO_4Zn reduce the amount of liver enzymes (SGPT, SGOT). Recipients of these nanoparticles have increased WBC, neutrophil and lymphocyte, as a result, the immune system is stimulated.

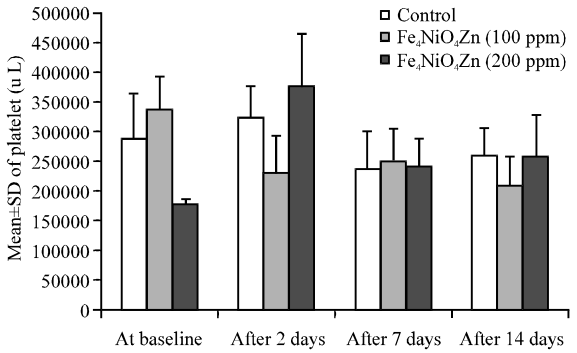

Fig. 7: Comparison of the levels of platelet in four groups at different concentrations

The advent of nanotechnology, which produces a wide range of engineered nanoparticles has transformed engineering. Since engineered nanoparticles have considered in the field of employment, consumer or the environment (due to their unpredictable biological and chemical reactions), therefore, threats to human health are predicted. Immune response, the key concern is the adverse effects of nanoparticles will lead to fibrosis or cancer (depending on the physical resemblance to the toxic fibers). While special provisions (due to the toxicity of nanoparticles) can be performed, the nature of construction of certain nanoparticles, predicting it difficult (but not impossible) that make up how some nanoparticles interact with cellular structures such as DNA-membrane proteins cytoskeleton (Willard *et al*., 2004).

Cho *et al*. (2010) studied the high volume of nanoparticles such as: Titanium-Zinc oxide, copper oxide, nickel oxide and selenium) on inflammation in the lungs of rats (due to increasing concerns about occupational exposure to nanoparticles (through inhalation)). The results showed that nanoparticles of nickel oxide and zinc oxide cause inflammation in high doses in the lungs.

After 24 h and four weeks inhaled nanoparticles, nickel oxide was associated with pattern of average cytotocxicity/neutrophilic, severe cytotocxicity ZnO/eosinophilic and copper oxide with severe cytotocxicity, neutrophilic/eosinophilic (Cho *et al*., 2010).

Ulich *et al*. (1991) and Wolpe *et al*. (1989) found that nanoparticles of zinc oxide and nickel (after 24 h), showed inflammation of the cytotoxic intermediate/neutrophilic with increased IL-1β, IL-13, MIP-2 (mediators of acute inflammation, neutrophilic).

Inhalation of zinc oxide nanoparticles (50-70 nm) and its micrometer (<1,000 nm) by rats, leading to cytotocxicity and neutrophilic inflammation (not eosinophilic), which reaches its peak at 24 h and fix after 4 week of inhalation (Sayes *et al*., 2007; Warheit *et al*., 2009).

Eosinophils plays a key role in mediating asthma and other allergic conditions in accordance with WHO (2010). About 300 million people suffering from asthma severity. Many of these people, perhaps, the workshops work constant exposure to nanoparticles, which may lead to the adoption of eosinophils by their lungs; it increases the risk of an asthma attack (WHO, 2010).

Another study, the acute toxicity of Zn powder in N-Zn nanoscale and microscale M-Zn at a concentration of 5 mg kg^{-1} of body weight on adult male and female rats *in vivo* was done. According to the findings, nano and micro powder placed on high-dose oral exposure may produce toxic effects on the hematopoietic systems, biochemical systems and a variety of tissues including the liver and kidney. Blood elements, the RDW-CV and PLT in group N-Zn significantly increased while the HGB (hemoglobin), HCT (hematocrite) mice in this group compared with the control group dropped (Kante *et al*., 1982).

The toxicity of zinc oxide nanoparticles is associated with the net release of ions in solution, which increases ion concentration in the cell, but the toxicity of iron oxide may be related to its high uptake in cells (Wang *et al*., 2008).

Fang *et al*. (2010) found that after the mice orally administered nanoparticles, SOD levels, MDA, glutathione in liver and kidney tissues showed significant changes compared to the control group.

Piao *et al*. (2003) found that intraperitoneal injection of ZnO nanoparticles decreased SGPT enzymes and lactate dehydrogenase. Reduce the level of this enzyme, possibly decreased synthesis of this enzyme and ultimately reduce the toxicity in cell due to the generation of zinc oxide.

CONCLUSION

Side effects of Fe_4NiO_4Zn nanoparticles in the present study, possibly because the nanoparticles injected, absorbed by the liver and excreted from the body gradually.

Results indicated that the most important reason for the lack of toxic effects of iron oxide nanoparticles on animals is the rapid elimination from the blood stream by the reticuloendothelial system in the liver-spleen and lymph nodes. Of course, the rapid elimination occurs after the phenomenon of opsonisation (accumulation of blood proteins in the particles) that stimulate the immune system and cause disposal of nanoparticles.

So, many nanoparticles injected into the blood stream quickly removed and only a small amount get opportunity to enter into various organs (Shubayev *et al*., 2009). Also, Sadauskas *et al*. (2007) demonstrated

that the cells of the liver Kupfer plays central role in the removal of nanoparticles from the blood stream.

Another reason that may explain the disappearance of the toxic effects of nanoparticles Fe_4NiO_4Zn is, the decaying properties of nanoparticles injected into the body. Because in the animals, many of nanoparticles, bind to organic molecules and agglomerates and lumps nodes. So many of their special properties in terms of size, the shape and surface charge have changed and the ability to influence many organs and tissues are lost (Gupta and Curtis, 2004).

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