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EFFECT OF NANOPARTICLES Fe_2NiO_4 ON LEUKOCYTE - HEMATOCRIT AND LIVER ENZYMES IN VIVO CONDITION

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ABSTRACT

Immune and inflammatory responses can cause allergic reactions or autoimmunity, while suppressing the immune system cause maturation and proliferation of immune cells. Depending on the particular type of nanoparticle, there is a different report, both the excitation and the immune suppression. Aim of this study is effects of nanoparticles Fe2NiO4 on the number of leukocytes- HCT - platelet counts and liver enzymes in rats. Twenty four Wistar rat of male sex weighing 234± 43g were used in the experiments. Animals were randomly divided into groups, two Fe2NiO4 nanoparticle-treated rat groups (1,2) and one control group. Group 1 and 2 received. /5cc of solution containing 100, 200 ppm Fe2NiO4 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, serum glutamate oxaloacetat transaminase (SGOT) and serum glutamate pyrvate transaminase (SGPT) were evaluated at various time points (1, 2, 7 and 14 days).In four times, mean factors of SGOT, SGPT were similar between the four groups studied (PV> 0.05). On the seventh day of the study, mean of hematocrit between groups1and 2 (PV = 0.036) and groups 2 with 3 (PV = 0.017) showed a significant difference. Also, the average factor of Platelet in groups1and 2 were differed (increase) (PV =0.018). At day 14, the mean Neutrophil, in Group 1 with Group 2 (PV = 0.002) and group 3 (PV = 0.027) were difference (increase). Also on this day, the mean WBC in groups 1 and 2 (PV = 0.046) and mean

Lymphocyte in the first, second groups (PV = 0.036) and Group 1 with Group 3(PV = 0.023) showed a significant increase. The results of this study showed that the nanoparticles Fe_2NiO_4 (100,200 ppm) stimulate the immune system and inflammatory responses and has no effect on liver toxicity.

Keywords: Fe_2NiO4-SGPT-SGOT

INTRODUCTION

Today, nanoparticles, due to their new properties, such as large surface area and high reactivity, have largely been considered **[1, 2]**. Due to the rapid development of nanotechnology, nanoparticles are prepared with different shapes and diameters and are used in products and industrial goods **[3-5]**.

Risk to human health, no absolutely is related with production volume and the likelihood of exposure to nanoparticles, but also with the reactive power and the impact on biological systems such as the immune system is associated. So engineered nanoparticles (ENM) produced in lower quantities, which have a strong influence on the immune system, such as nickel and cobalt nanoparticles can have significant risks to human health **[6, 7]**.

Immunotoxicity is any adverse effect on immune system that may result from exposure to a toxic substance and may stimulate or suppress the immune system **[8]**. Stimulate of immune system can cause allergic reactions and inflammatory responses or autoimmunity; While immune suppression, can cause maturation and proliferation of immune cells that eventually will lead to increased susceptibility to infectious diseases or cancer growth.

There is different reports depending on the specific type of engineered nanoparticles, both the excitation and immunosuppression. However, the impact of engineered nanoparticles on the immune system, depending on the method of exposure to them as well. One of the nanopaticles in this study is nickel oxide that used as a catalyst for ceramics and also storage battery.

In studies of *in vivo*, in the lung, inflammatory responses induced by these nanoparticles have been reported. The presence of large particles of nickel along with nanoparticles of this article, cause increasing lung damage and inflammation.

The specific surface area of Ni nanoparticles, not only plays a role in damaging, but also, nickel ions have a significant effect on the toxicity of living cells **[9]**. On the other hand, we know that iron oxide nanoparticles used in this study, have many medical uses,

including: MRI, Diagnosis - cancer treatment - medical uses - Gene Therapy.

These nanoparticles accumulate in the liver cells and causes to product oxidative stress and free radical **[10]**.

In this study, the effects of intraperitoneal injection of different doses of nanoparticles Fe2NiO4 (100,200 ppm) on the number of leukocytes (neutrophil - lymphocyte) - HCT - platelet counts and liver enzymes in Wistar rats was studied.

MATERIALS AND METHODS

Characterization of Fe_2NiO_4Particles

Ten g Fe2NiO4 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. Specifications of this nanoparticle is: <50nm particle size (APS),>98% trace metal basis, linear formula: Fe_2NiO_4; form: nanopowder; CAS number: 235-335-3; molecular weight: 234, 38; density: 5, 36 gr/ml at 25°C; color:dark brown to very dray brown.

Preparation of Mother Solution To determine the nanoparticle concentration Fe2NiO4, two mother of solution was provided:

1 - 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.

2- 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.

Preparation of Animals

Twenty four Wistar rat of male sex (were purchased from the Animal Center of Falavarjan University), weighing 234± 43g were used in the experiments. 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 complete food. All animal experiments were performed in compliance with the local ethics committee.

Animals were randomly divided into groups, two Fe2NiO4 nanoparticle-treated rat groups and one control group. Group 1 and 2 received. /5cc of solution containing 100, 200 ppm Fe2NiO4 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 points (1, 2, 7 and 14 days). The blood was carried out from the corner of the eyelids 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 CBC) and other for testing of enzyme liver (that blood samples has been centrifuged for 15 minutes (3000RPM / Minute) and serum and plasma were separated.

Statistical Analysis

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 study entered into the model, as dependent variables and variable group as independent variables. The Tukey test was used to evaluate significant pairs.

RESULTS

In four times, mean factors of SGOT, SGPT were similar between the four groups studied (PV> 0.05).

On the seventh day of the study, mean of Hematocrit between groups1and 2 (PV = 0.036) and groups 2 with 3 (PV = 0.017) showed a significant difference. Also, the average factor of Platelet in groups1and 2 were differed (PV =0.018).

At day 14, the mean Neutrophil, in Group 1 with Group 2 (PV = 0.002) and group 3 (PV = 0.027) were difference (increase). Also on this day, the mean WBC in groups 1 and 2 (PV = 0.046) and mean Lymphocyte in the first, second groups (PV = 0.036) and Group 1 with SGroup 3(PV = 0.023) showed a significant increase **(Figures 1-7)**.

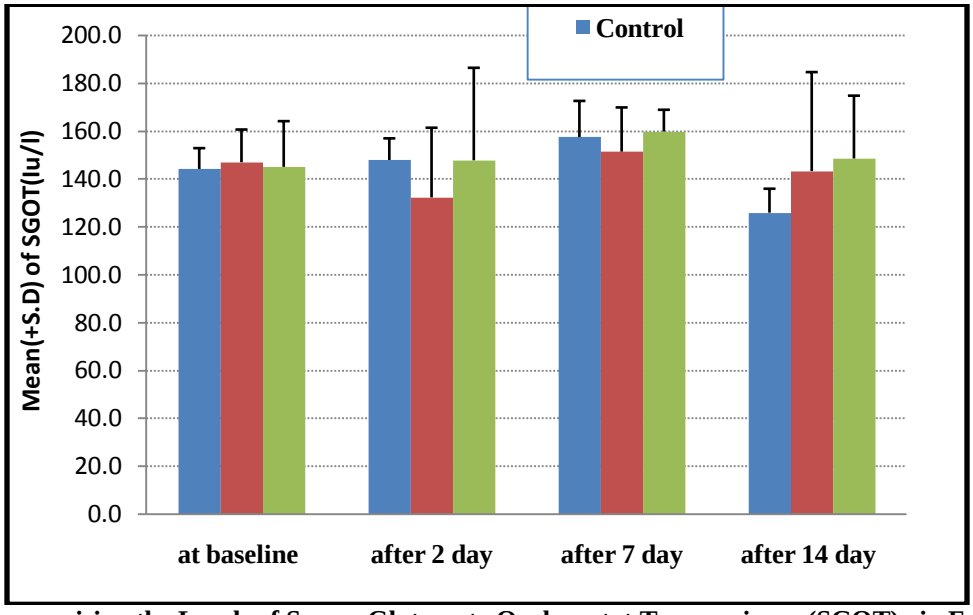

Figure 1: Comparision the Levels of Serum Glutamate Oxaloacetat Transaminase (SGOT) in Four Groups: (Fe2NiO4 Nanoparticle100, 200 ppm and Control Group)

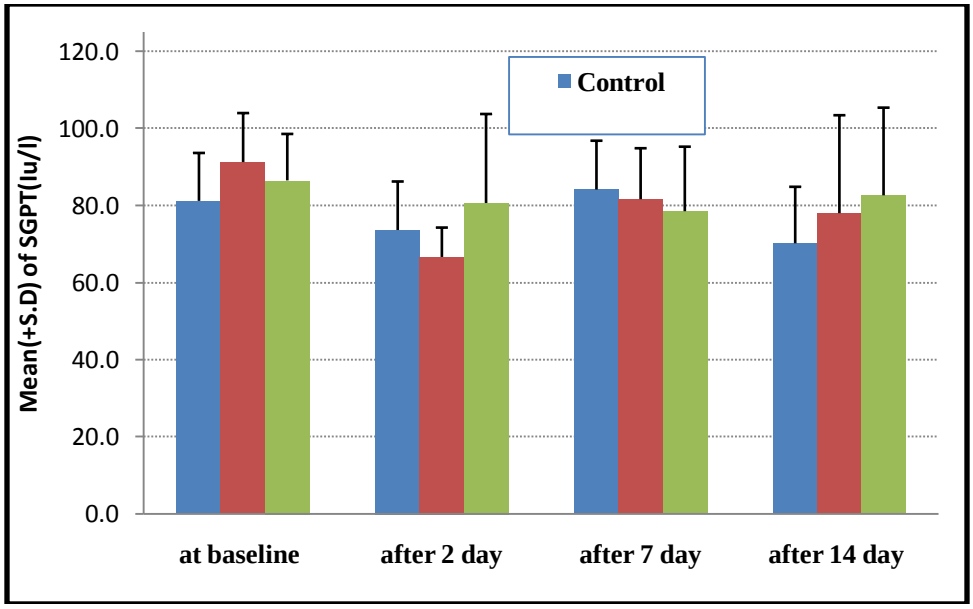

Figure 2: Comparision the Levels of Serum Glutamate Pyrvate Transaminase (SGPT) in Four Groups: (Fe2NiO4 Nanoparticle100, 200 ppm and Control Group)

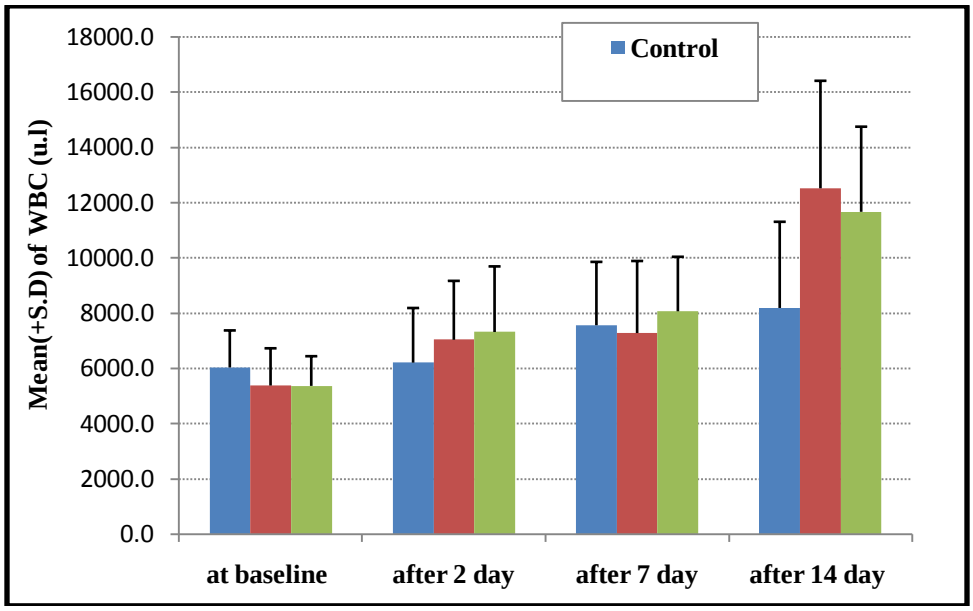

Figure 3: Comparision the Levels of White Blood Cell (WBC) in Four Groups: (Fe2NiO4 nanoparticle100, 200 ppm and control group)

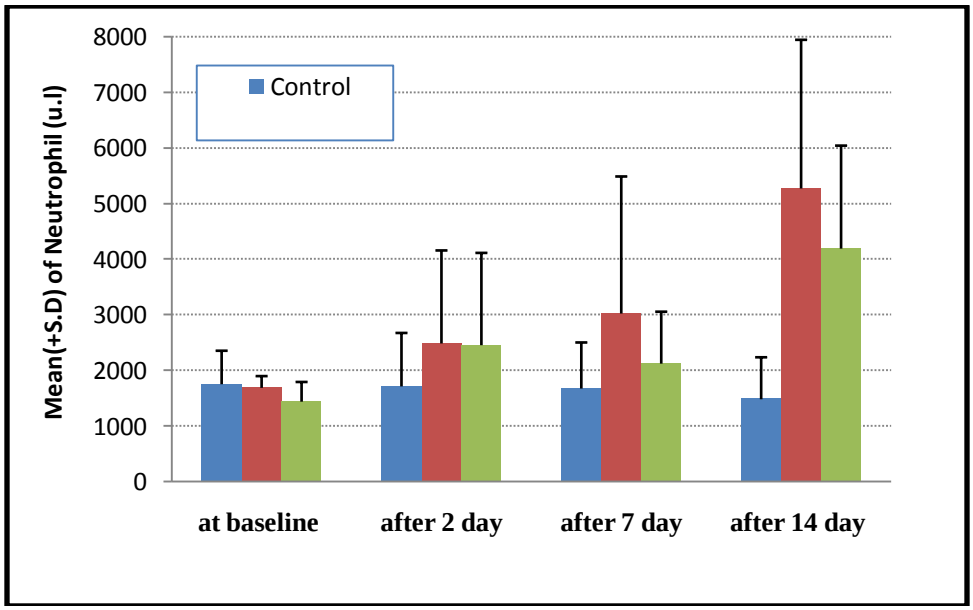

Figure 4: Comparision the levels of Neutrophil in four groups: (Fe2NiO4 nanoparticle100, 200 ppm and control group)

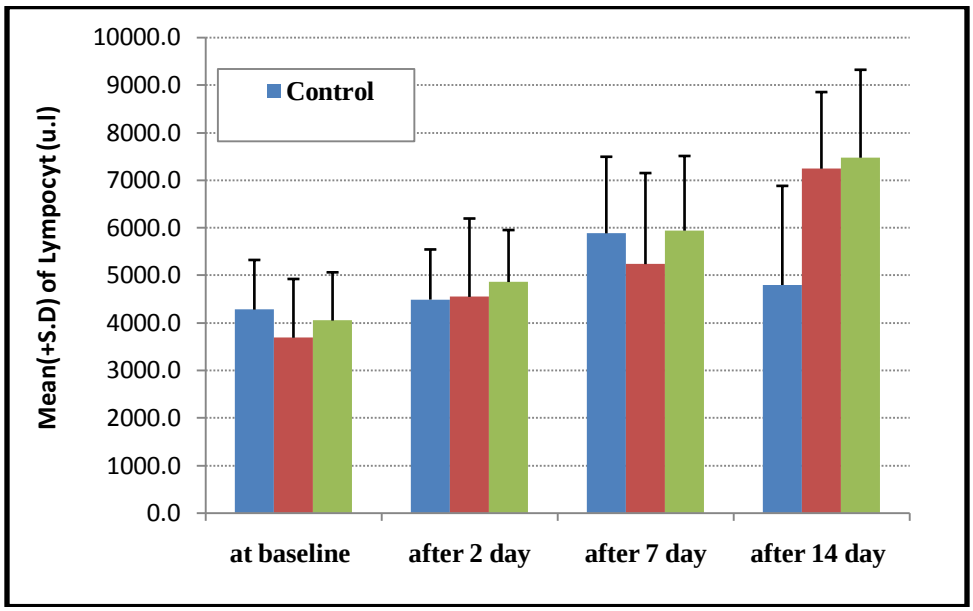

Figure 5: Comparision the levels of Lymphocyt in four groups: (Fe2NiO4 nanoparticle100, 200 ppm and control group)

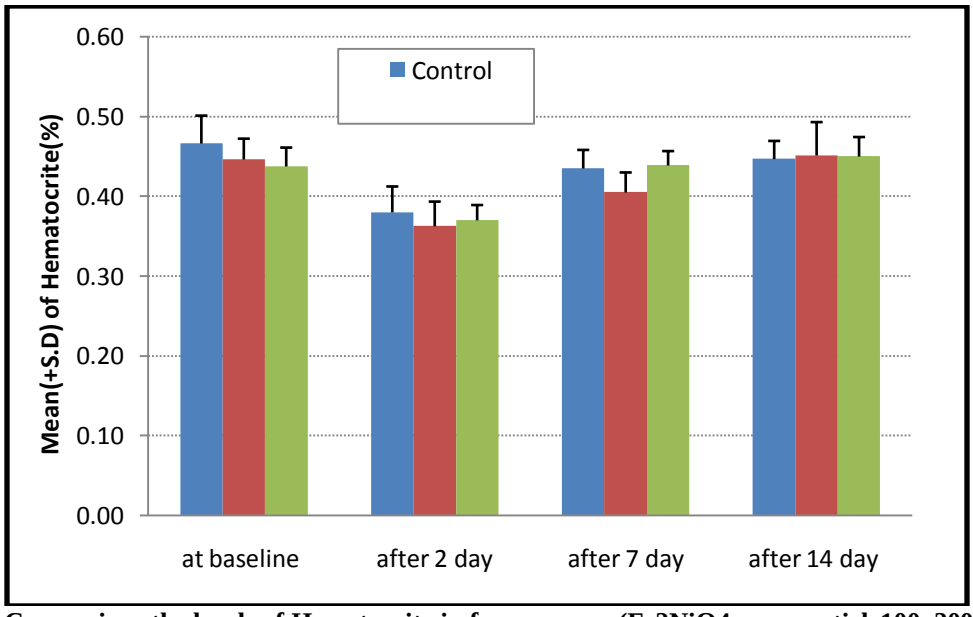

Figure 6: Comparison the levels of Hematocrite in four groups: (Fe2NiO4 nanoparticle100, 200 ppm and control group)

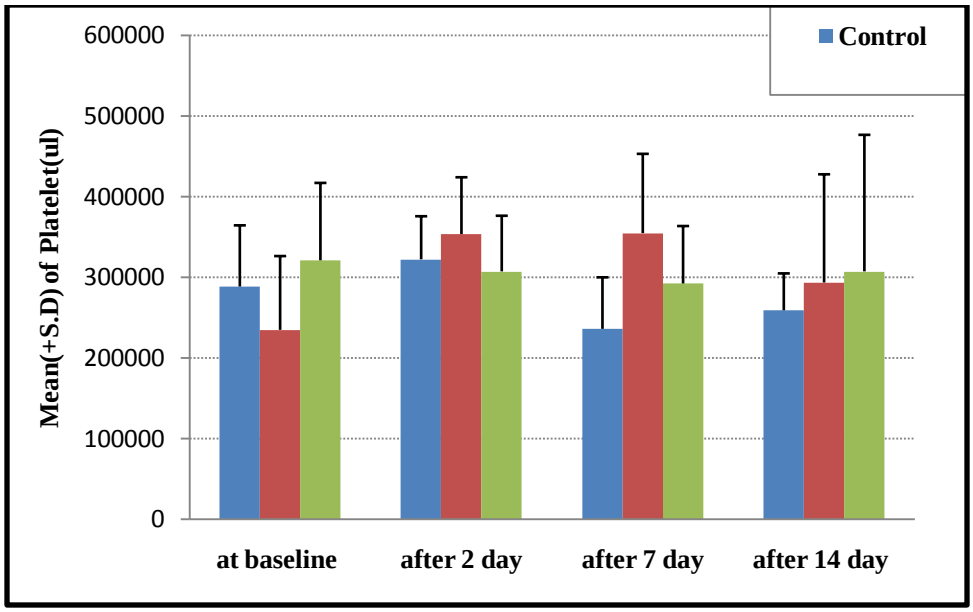

Figure 7: Comparision the Levels of Platelet in Four Groups: (Fe2NiO4 Nanoparticle100, 200 ppm and Control group)

DISCUSSION

The results of this study showed that mean neutrophils on days 2-7 and 14, levels of WBC and lymphocyte at 2 and 14 days, the rate of platelet at days 7 and 14 and hematocrite at day 14 in both the treatment groups (Fe_2NiO_4 nanoparticle 100, 200 ppm) compared to control group showed an increase .Also, In four times, mean factors of SGOT, SGPT were similar between the four groups studied (PV> 0.05). These factors were increased, but this increasing was not significant.

In this study, nanoparticles Fe_2NiO_4 were increased blood lymphocytes and WBC. This is probably due to the body's production of white blood cells to fight the incoming particles.It is anticipated that the nanoparticles with increasing inflammation of lymph node, can cause increase involvement cells in inflammatory reactions and increase cell division S to G phase of transition lymphocytes Step 1 **[11, 12]**. Nanoparticles depending on concentration of particles and over time can cause either to reduce or increase white blood cells.

Many studies show that nanoparticles can accumulate in the liver - Spleen - lung - heart , brain and where they causes inflammatory responses **[13-20]**. For example, nanoparticles can cause progressive enzymatic activity and mRNA expression of cytokines during inflammatory responses in rats and mice **[17-20]**.

As is well known, NL recruitment towards the free surface of lower airways in response to the deposition of particles, NPs (nanoparticles) included, is quite often

described as "inflammation" and, thus, as a pathological phenomenon. We maintain, however, that this concept can be somewhat misleading. Beyond any doubt, enhanced recruitment of NLs is typical of acute and, to a lesser degree, chronic inflammatory processes induced by microbial or chemical agents.

Studies of Ahmed and colleagues showed that nickel nanoparticles with a size of 25 nm and a concentration of 25-100 µg / mL, induced cytotoxicity in the liver cells that dependent to concentration of nanoparticle.

The nanoparticles of nickel induced oxidative stress with a dose-dependent manner. This device has been demonstrated by generates oxygen free radicals, ROS (reactive oxygen species) and GSH (glutathione) depletion **[21]**.

Afkhami et al, effect of iron oxide nanoparticles on liver enzymes and thyroid hormones were studied. These results indicated that iron oxide nanoparticles at high concentrations (g/kgµ150) have toxic effects on the liver and thyroid **[22]**.

Nouri *et al*, effect of a single injection of different doses (50, 100, 200,300 mg/kg) iron oxide nanoparticles (Fe3o4) coated with DMSA (Dimercapto succinic acid) on liver and kidney function in response to absorption nanoparticles to these organs, (after four days of treatment) was evaluated. The results showed that short-term use DMSA-coated iron oxide magnetic nanoparticles do not provide specific toxicity in cases of medical and biological **[23]**.

Jian *et al* in 2008, with an intravenous injection of iron oxide nanoparticles in rats, a temporary increase in the rate of enzyme ALT, AST, ALP and total capacity of iron (total iron-binding capacity: TIBC) were observed (from 6 to 26 hours after injection). Changes in oxidative stress in different tissues increased as well as 3 days after treatment and then declined **[24]**.

Studies of Babadi and colleagues showed that the rate of liver enzymes SGPT, SGOT, ALP (alkaline phosphatase) in rats treated with high doses of iron oxide nanoparticles (150 µg / kg) compared to the group treated has increased substantially **[25]**.

The most sensitive and the most used liver enzymes including: aminotransferase SGPT, SGOT. Though liver damage, liver cells leak these enzymes into the blood and resulting in increased levels of these enzymes, leading to death liver cell **[26, 27]**. Research shows that the amount of metabolism iron oxide in liver, depends on: injected dose rate - the percentage of the starting dose received by the liver and the liver cell spreading **[28]**. Membranes of liver cells damaged permeable

which causes leakage of intracellular enzymes into the blood stream **[29, 30]**. Studies show that metabolic rate in kupfer cells depends on high levels concentration of iron oxide nanoparticles obtained **[31]**.

CONCLUSION

The results of this study showed that the nanoparticles Fe2NiO4 (100,200 ppm, <50nm particle size (APS),>98% trace metal basis, linear formula: Fe_2NiO_4; form: nanopowder; CAS number: 235-335-3; molecular weight: 234, 38; density: 5, 36 gr/ml at 25^0 c; color:dark brown to very dray brown) stimulate the immune system and inflammatory responses and has no effect on liver toxicity.

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