Investigation of The effect of Fe$_2$O$_3$ nanoparticles on the blood cells and liver enzymes in male rat

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ABSTRACT

By using of nanotechnology ,the Hopes to use metal Nanoparticles such as iron oxide in industrial applications ,medical imaging, Disease diagnosis ,drug delivery ,cancer treatment ,gene therapy and other cases is rapidly expanding and progressing. Given the strength of iron nanoparticles penetrate cell membranes and the accumulation of these particles after entering to body in tissues such as liver ,many researchers believe that The small size of iron nanoparticles as an important and useful properties are in the industry, Could be endangering human health. In this study ,Nanoparticles of iron oxide in doses of 25 and 50 and 100 and 200 mg/ kg that in 1 ml distilled water was dissolved, for 21 day's duration with oral to fifty male rats that they placed in five groups of ten each ,were fed. A group was considered as control group that received 1ml distilled water simultaneously with experimental group per day. After this duration, Blood samples were taken from rats that was first part of the blood sample to measure a number of factors And the second part after separating the serum, was used to measure the enzymes. The results indicate significant changes in certain factors such as blood platelets and white blood cells. And in discussed enzymes, the significant changes were observed. The results indicate the effectiveness of the immune system and mechanism blood centers of the iron oxide (Fe$_2$O$_3$) nanoparticles that in the long- term changes in blood cells as well as other parameters can be seen, of which, reduced immune function in rats exposed to nanoparticles which is statistically lower immune function, is associated with reduced blood cells and As well as iron oxide nanoparticles In addition to being Reduce the body's immune system has been with Cells damaged hepatocytes and with their effects on the liver cell membrane permeability, altered serum enzymes Concentration, that increase The arrival of enzymes into blood plasma, liver damage can be proved.

Key words: iron oxide, nanoparticles, blood cells, liver enzymes, rat

Introduction

In recent years, Nanotechnology has been cause of dramatic developments in various industries[1-6]. In between them, the role of Nano materials and Nano-powders in this great transformation is undeniable[4]. These Nano particles Due to having Magnetic properties, thermodynamically, mechanical, optic electronically, electrical and optical, quite unique have been and are of considerable importance that among these nanomaterial, which can be pointed to the magnetic particles of iron Which have very broad applications in sectors such as agriculture and industrial and medical Such as soil and nutrient enrichment and catalysts, sensors and transfer controlled drug in the body and etc. [7-8]. These compounds are mainly physical and mechanical properties due to the unique magnetic properties that are used[9-11]. Therefore, familiarity with various methods of synthesizing compounds that lead to generate of a granulated, phase percent and will be different shapes and properties, is of great importance[12]. The magnetic iron oxide nanoparticles have unique properties due to nanometer scale such as injection ability, biological compatibility, being stable in body

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physiological conditions, such as PH and accumulation in organs, have particularly applications in medical such as Targeted drug delivery (as a carrier for certain drugs), cancer therapy, tissue engineering (cell classification) and magnetic resonance imaging[13]. The Liver is a major source of active chemical cells that have a high metabolic rate and it metabolic systems, from viewpoint of energy and substrate are shared with each other and processes several materials that are carried to other parts of the body and many other metabolic functions are performed[14-16]. All the materials that absorbed through the intestines are transported to liver by the liver portal vein, except complex lipids (chylomicrons) that are transported by the lymphatic vessels[17-19]. Liver is the best location in the circulatory system to collect and accumulate metabolites and also to remove and neutralize toxins. This removal and evacuation through bile (an exocrine secretion that is important in fat digestion) occurs[20].Liver of viewpoint detoxification and metabolism of endogenous and exogenous chemicals is important and plays an important role in neutralizing toxins and carcinogens and non-activating compounds and steroid drugs[21]. Extensive research about effect of iron nanoparticles in increase magnetic resonance images contrast Performed and the current is applied[22-23]. The role of iron nanoparticles in targeted drug delivery, much research has been done and to deliver targeted anticancer drugs has been significant progress in breast and brain cancers and etc. also because Iron nanoparticles accumulate in cells and cancerous tissues Thus, for rapid diagnosis in early stages, has many applications. Given that iron nanoparticles into the bloodstream, they accumulate in the liver, the effect of iron nanoparticles on the liver and stem cells and fibroblast cells of rats has been studied that decrease of mitochondrial activity and morphological changes in rat liver cells has been. Also Causes Reduction glutathione lives cells and increase Shock and oxidative reactions in liver cells[24]. These Oxidative reactions cause interaction of nanoparticles and cells are created and cause to create free radicals that can cause cell death [25-26]. Following inhalation of iron particles by rat, the presence of nanoparticles in rat brain has been determined. Although no toxic effect on nerve cells of iron nanoparticles is not clear [27]. The iron nanoparticles react with thiol of proteins and of enzymes and cause to alter their structure that increased leakage and necrosis and finally it will death[28]. On the other hand, because sperm has a large number of mitochondria, the experimental in INVITRO conditions on spermatozoid cells done and the effects of iron nanoparticles on the sperm, due to infertility sperm [29].

Materials and Methods

Reagents and apparatuses:

The Iron oxide nanoparticles (30 nm), synthesized from Center of Science and Nanotechnology Institute, payam-e- noor University of Yazd, were prepared. And the Nano surface area, by AFM microscope, for use in this study was analyzed. Suspension of iron oxide nanoparticles obtained at the Institute, with ultrasound for 10 minutes was under the influence then move for 2 minutes and finally with different doses 25, 50, 100 and 200 mg / kg by gavage to rats was fed by mouth. To prepare a solution, iron oxide nanoparticles from Institute received and doses of 25, 50, 100 and 200 mg / kg were prepared, and then 10cc Deionized water (EC≤1) Add to each and every 24 hours 1cc to any rat tested, was given. Devices needed include centrifuges sigma 101, a digital scale with an accuracy of 0.001 mg, The H1 cell counter for counting blood cells[ sysmex k – 1000], Auto Analyzer system, NIKON light microscope, Incubator 72c°

Measurement Temperature, humidity and weight of rats:

To ensure optimal temperature and humidity environment, daily both temperature and humidity were controlled until Preserved Temperature at 22 ± 1 c° and Humidity at 60%. Also During the period, changes in weight of rats, regularly investigated by weighing the rats and obtained numbers, was recorded (control=Saline; Group 1=25 Mg/kg; Group 2 = 50Mg/kg; Group 3 = 100Mg/kg; Group 4 = 200Mg/kg).

Rat blood sample and serum preparation:

For Biochemical tests of blood, blood sampling was performed after 21 days. Blood sampling prepared by a hematocrit tube used stone method[30]. In this method venous blood from the orbital sinus at the inner corner of eye rats were collect by heparin hematocrit tube, that hematocrit tubes have a diameter of 75 mm and inner diameter 1.2 mm. To do this, after anesthesia by ether, rat for short time (for get blood sampling) is held by the thumb and index finger, Hairy end of the tube, slowly into the eye cavity and rotating, the end of the tube was inserted into the venous sinuses inside the eye[31]. Capillaries in this area are sensitive and are ruptured by pressure effect and blood will gush from the open side tube[32]. After leaving a few drops, hairy tube is removed and for blood collection a tube was slowly with eye contact. Blood samples taken from each rat was divided into two parts. Amount of blood to the test cell containing EDTA on CBC glass was poured and give to cell counter device. And blood slides were taken from them. The second part blood to separation of serum were inserted in the centrifuge especially tubes. Serum by centrifugation...
at rate 3000 RPM for 10 min was isolated and for measured concentrations of SGOT and SGPT and ALP were given to the auto-analyzer. To standardize auto analyzer device, to measurement of enzymes used of N terolap and U terocal that is calibrator. And level enzymes activation was measured Based on der liters global unite. To standardize the cell counter, controlled bloods that have a specific cells from eghlim Danesh Company is used. To clotting time test, blood samples were collected by a hairy tube, in the natural tube[33].

Blood cell count and slides investigation:

After collecting blood samples for CBC container is containing anticoagulant EDTA and prevents blood clots. CBC Samples was on the hematology mixer machine for ten minutes, until the mixture be homogeneous. Then, the samples were given to the cell counter device to count blood cells, some white blood cells, red blood cells, platelets, lymphocytes and neutrophils, hemoglobin, hematocrit. Prepared slides of blood samples, until investigate potential changes in the morphology of blood cells.

Blood clotting time measurement:

After the rat were anesthetized, by hairy tube Contact with the inner veins, and remove blood clots in the tube, approximately 1 ml were collected and immediately placed in a water bath at 37 degrees Celsius, Each minute, the tube, we examined the clotting time and clotting time are measured with a stopwatch.

Blood expanse and staining:

For preparation the expansion of blood, the wedge method was used[34]. In this case, during get blood sampling of rats, a drop of blood from each sample for 2 to 3 cm in diameter, at the end of a clean slide on a flat surface and free of dust that had been given, was located. Then by right hand thumb and index finger, the end of the second slide (spreader slide) with an angle of 30 to 45 degree and put it on the first slide, And pulled it back to make contact with the blood drop. Shortly thereafter, the blood is distributed in the angle between two slides. Then slide the player with average speed, on the first slide to move forward with a thin expanse of the average obtained. Then put them in the air, until the expanse surface is completely dry. After ensure of complete drying, the slide covered by the alcohol, or ethanol or inside in containing alcohol bowl[35]. After 5 minutes, add the alcohol poured away and will wait until the slides are completely dry. Then, the slides were fixed, placed inside in Giemsa solution for 10 min and after this period, we wash slides with water and after drying the slides, we will study them under a microscope.

Auto analyzer machine:

This device can be used for enzymatic and biochemical tests. This machine has high efficiency and can perform 200 tests per hour.

Results and Discussion

Different concentrations of iron oxide nanoparticles:

The effects of different concentrations of iron oxide nanoparticles on the blood factors studied, after 21 days.

On the number of white blood cells:

To evaluate the significance of the observed mean difference, Duncan and Tukey test results were used. Duncan and Tukey test results, proved that in Concentration of 200 mg / kg relative to the concentration of 25 mg / kg, the difference was statistically significant reduction in the level of 5%, but relative to control and other concentration reduce the concentration difference, is not significant, so the highest number of white blood cells was seen in concentration 25mg / kg and the lowest concentration in 200mg / kg, respectively. (Fig.1).

On the number of lymphocytes:

Analyses of iron oxide nanoparticles in concentrations of 25 and 50 and 100 and 200 mg/kg in rat, according to Tukey and Duncan proved that There are significantly decreased difference in 200Mg/kg concentrations in the 5% level ratio to concentration in 25,50 mg / kg and control group, but ratio to the concentration of 100mg / kg this difference is not significant reduction. And also between25, 50 and 100 mg / kg ratio to each other, there are significantly decreased difference but the difference reduction between 50mg/kg concentration and control group, is not significant, so that low dose of Iron oxide nanoparticles has been caused a nonsignificant increase in the amount of lymphocytes ratio to the control group (p <0.5). (Fig.2).

On the number of neutrophils:

Results Statistical analysis of the effect of iron oxide nanoparticles (Fe2O3) on number of neutrophils indicates that between concentration 200mg/kg, 25mg/ kg and control concentrations, there was significant decrease deference, But this difference were not significant in the 50 and 100 mg / kg experimental groups. But according to Duncan’s test between 25 mg / kg experimental group and 50, 100, 200 mg / kg experimental groups have decreased significantly at 5% level, that this difference only in the 200mg / kg experimental group on test tuky is significant. (Fig.3).
Fig. 1: Effect of the iron oxide (Fe₂O₃) nanoparticles on the number of white blood cells.

Fig. 2: Effect of the iron oxide (Fe₂O₃) nanoparticles on the number of lymphocytes.

Fig. 3: Effect of the iron oxide (Fe₂O₃) nanoparticles on the number of neutrophils.

On the number of monocytes:

According to Duncan's test between 200 mg / kg group with 25, 50, 100 mg / kg experimental groups and control group there was a significant reduction in the level of \( p \leq 0.05 \) So that this difference decreased with the test tuky 25, 50 and 100 mg / kg experimental groups was significant, but with control group this difference decreased is not significant. (Fig.4).

On the number of RBC:

The results of the effect of iron oxide nanoparticles on the concentration of 25, 50, 100 and 200 mg / kg shows a dose-dependent decrease Garlic, that This difference decreases, in any of the experimental groups was not statistically significant, hence The statistical analysis(\( p > 0.05 \)) is between control and treated groups in all cases. (Fig.5).
On the concentration of Hb:

Using of tuky and Duncan's test determined that between average hemoglobin levels in all experimental groups, there is a dose-dependent decrease in overall, that this decrease is not significant in any of group (p> 0.05). (Fig.6).

On the Hematocrit value:

To evaluate the significance of the observed mean difference, Duncan and Tukey test results were in. according to these tests, concluded that there is a general decrease in all experimental groups, that this decrease is not significant(p>0.05). (Fig.7).

On the number of Platelets:

The results of statistical analysis by Duncan's test showed that the average number of platelets in 200mg/kg experimental groups was significantly decreased deference with all experimental groups and control group at levels (p <0.05) and 25mg/kg experimental groups (low dose) showed a significant increase compared with the control and experimental groups, but between 50 and 100 mg/ kg, a significant reduction was observed, Also according to the experimental test tukey25 mg/kg with control group increase in the number of platelets than the control group was observed that this increase is not statistically significant (p> 0.05). (Fig.8).

(ALT) SGOT:

The Statistical analysis of effect iron oxide (fe₂O₃) nanoparticles on the enzyme aspartate amino transferase concentrations, according to Duncan's test proves that there is no significant difference between The average SGOT level of all experimental groups and control groups (p>0.05), but between The experimental groups 25mg/kg compared to 200mg/ kg and the control group shows a significant difference (p ≤ 0.05). But according to the test tuky, 25mg/kg with 100 and 200 mg/kg experimental groups contain significant deference at (p ≤ 0.05) level, hence into control group indicate a non-significant decrease (p>0.05). (Fig.9).
Fig. 6: Effect of the iron oxide (Fe₂O₃) nanoparticles on the concentration of Hb.

Fig. 7: Effect of the iron oxide (Fe₂O₃) nanoparticles on the Hematocrit value.

Fig. 8: Effect of the iron oxide (Fe₂O₃) nanoparticles on the Hematocrit value.

(ALT) SGPT:

To determine significant differences observed for the mean concentration of the enzyme alanine aminotransferase Duncan and tuky tests were used. Duncan's test determined that the 200mg/kg experimental group with control and other concentrations tested showed significant deference at (p ≤ 0.05) level. Although other experimental groups with each other and with control group, have non-significant difference. The 200mg/kg experimental test tuky with control group and 25 and 50 mg/ kg experimental groups was significant deference, but with the 100mg/kg experimental groups, this difference is not significant (p>0.05). (Fig.10).

ALP:

The results of Duncan test for investigation average of alkaline phosphatase enzyme proved that the 200mg/kg experimental groups has been a significant increase compared to the other experimental groups, But this difference with the
control group is a non-significant. Hence the 25 and 50 and 100 mg/kg experimental groups which are significantly different with control group. According to Tuky test 100 and 200 mg/kg experimental groups based on significant deference, but 25 and 50 mg/kg experimental groups contains non-significant difference with control group. (Fig.11).

**Blood cell:**

The following image was related to the blood of control group rats, Distribution and density of white blood cells and erythrocytes were normal, and in total control of everything in the blood samples during the course of their, were routine.

**On average of MCV:**

The results of statistical analysis showed an overall decreasing trend in mean MCV in all experimental groups, ratio to group was seen. This decrease is not significantly different according to tuky and Duncan's test, hence statistical analysis was as the ($p > 0.05$). (Fig.12).

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**Fig. 9:** Effects of different doses of zinc oxide nanoparticles of the next investigated enzymes on AST after 21 days.

**Fig. 10:** Effects of different doses of zinc oxide nanoparticles of the next investigated enzymes on ALT after 21 days.

**Fig. 11:** Effects of different doses of zinc oxide nanoparticles of the next investigated enzymes on ALP after 21 days.
On Blood clotting time test:

The results of the effect of iron oxide nanoparticles on the blood clotting time shows that nanoparticles of iron oxide was cause to overall increase blood clotting time of the experimental groups ratio to the control group. This trend increased, dose-dependent and the analysis is not significant at 5% probability level (p>0.05). (Fig.13).

References