

RESEARCH PAPER

Investigation the effect of Fe_3O_4 nanoparticles on liver and stress oxidative parameters at the presence of magnetic field in rat

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ABSTRACT

Objective(s): This study was designed to evaluate the effect of Fe_3O_4 nanoparticles at presence of a constant magnetic field on rat liver and some stress oxidative parameters.

Materials and Methods: Fe_3O_4 nanoparticles were synthesized by co-precipitation method using iron chloride (III) and iron sulphate (II). The nanoparticles properties were studied by XRD and TEM. Fourty male wistar rats were randomly divided into four groups. First group was injected with normal saline (control). Second group was injected with Fe_3O_4 nanoparticles (100 mg/kg). Third group was treated under a constant magnetic field and fourth group was treated with both of Fe_3O_4 nanoparticles injection and constant magnetic field (all injections are intra peritoneally). Liver parameters (ALT, AST, Total protein, Bilirubin) and some stress oxidative parameters such as SOD and GPX were measured for all groups, 15 and 30 days post injection.

Results: The size of the synthesized nanoparticles was determined 14 nm. The crystalline structure of the nanoparticles was spinel. Serum concentration of ALT and AST were changed in some groups compared with the control group. At the presence of constant magnetic field and iron oxide injection, the amount of total protein and bilirubin significantly increased compared with that of control group. The enzyme activity of SOD and GPX haven't changed compared to the control group.

Conclusion: The results of this investigation show that this concentration of iron oxide nanoparticles (100 mg/kg) have not irreversible toxic effects at the level of liver parameters. Also, they have not any serious effects on the SOD and GPX enzyme activity even at presence of a constant magnetic field.

Key words: Fe_3O_4 , Liver parameters, Stress oxidative

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INTRODUCTION

Nanotechnology is the research and development of technology at the level of atomic, molecular, and macromolecular surfaces with approximate dimensions of 1 to 100 nm which is intended to provide a fundamental understanding of phenomena at the nano-scale materials. Also it is applied to build and use structures, components and systems that it makes new functions and properties due to their small size [1].

When particle size reduces to nano scale, they show unique mechanical, chemical, electrical and thermal properties. The increasing of surface area is the most important effect of particles size reduction. Overcoming the behavior of atoms

located at surface of the materials to the behavior of inner atoms, is the result of size reduction (increasing surface to volume ratio) [2, 3].

According to the increasing use of nanoparticles in various sciences including biology, medicine, clinical biochemistry and industry, exposing to nanoparticles will increase in the very close future. On the other hand, normal human environment has changed intensely due to the extensive range and expanding the electromagnetic field [4]. Nowadays electromagnetic fields are inseparable element of human life [5]. So, it seems that, investigation of nanoparticles effects on human health at the presence of magnetic fields is important.

Iron oxide (Fe_3O_4) nanoparticles contains a bivalent iron atom and two trivalent iron atoms.

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These nanoparticles have unique physicochemical, mechanical, electrical and magnetic properties owing to their small size which they can freely enter to the cells and interfere in their natural process. Therefore, material dimensions make nanotechnology distinguished from other technologies [6].

Fe₃O₄ nanoparticles are superparamagnetic. These magnetic property used for biomedical applications such as targeted drug delivery and gene therapy, repairing damaged tissue, magnetic resonance imaging and magnetic thermotherapy [7-8]. One of the goals of nanotechnology is to mount the drugs on the carrier materials (nanoparticles) and then send and drop them into the target cell which is called targeted drug delivery. Using magnetic nanoparticles and creating a magnetic field can send smartly a drug into the target tissue and has improved texture without damage to other tissues [9]. The act of magnetic nanoparticles on biological systems is based on magnetic drug targeting including strong interaction between ligand and receptor or through magnetic attraction of certain tissues [10-12].

ALT (alanine aminotransferase) and AST (aspartic aminotransferase) are important liver parameters and one of the best clinical evaluations of liver is obtained through the examining of these enzymes activity changes [13-16]. Bilirubin is final products of hemoglobin breakdown. Unconventional increasing of bilirubin is a sign of damage or disorder in the liver or gall bladder [17-19].

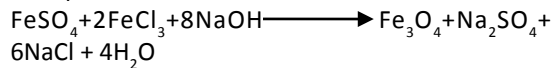
SOD (Superoxide dismutase) is one of key enzymes for antioxidant defense against damage caused by free radicals. This enzyme accelerates conversion reaction of superoxide anion to hydrogen peroxide and molecular oxygen. Superoxide dismutase exists in all aerobic tissues and the cytosol and mitochondria of cell [20]. GPX (Glutathione peroxidase) is the main component of antioxidant enzymes which acts not only for the removal of oxygen free radicals but also as a substrate for several peroxidases [21]. It is naturally created in the human liver by combining three amino acids (glutamic acid, cysteine and glycine) [22, 23].

In this study, an attempt is to investigate the effects of iron oxide nanoparticles at the presence of magnetic field on the variation of liver parameters, SOD and GPX in rat.

EXPERIMENTAL

Synthesize of Fe₃O₄ nanoparticles

In this research, iron oxide nanoparticles was prepared throughout co-precipitation method that is one of the chemical methods of making nanoparticles



According to the above reaction, the amounts of 2.78 g from FeSO₄.7H₂O (0.1 mol), 3.24 g from FeCl₃ (0.2 mol) and 3.2 g from NaOH (0.8 mol) were prepared separately in the Erlenmeyer flask (100 ml distilled and deionized water). The appropriate amounts of masses were obtained from the molar ratio 1: 2: 8, respectively.

The solutions were dissolved with magnetic stirrer and heated up to boiling point. First of all, the FeCl₃ and FeSO₄ solutions were mixed together and then NaOH solution was added to them completely and suddenly. The black precipitate was formed immediately. The obtained solution was washed with distilled water several times in order to remove excess salts. After that, the product was placed in the oven for several hours at 40 °C temperature.

TEM (Transmission electron microscopy) and XRD (X ray diffraction) were used in order to investigate the structural properties.

Animal study

40 male Wister rats were purchased from the Medicine department of Isfahan University. They were kept in the animals' house of Falavarjan Azad University (natural light, 20 to 25°C temperature). They were divided randomly into 4 groups of 10 rats (weight range around 250 grams) and placed in separate cages.

The first group was treated by normal saline injection (control group) and the second group was treated by Fe₃O₄ nanoparticles injection with a concentration of 100 mg/Kg (100 milligrams per kilogram of body weight). The third group was affected by the magnetic field throughout the test. Group IV was treated under both of constant magnetic field (0.3 T at 1 cm distance) and Fe₃O₄ injection (100 mg/Kg). All injections were intra peritoneally.

Blood samples were collected 15 and 30 days post injection from eyes and heart, respectively. Blood serum were prepared and isolated after centrifugation and the liver parameters and SOD and GPX were determined (Cobas c311, Rosch

Germany).

For statistical analysis, the SPSS statistics (version 19.0) software, one-way ANOVA and Duncan tests were used. Charts were plotted by Excel software. The significant level of P < 0.05 was considered.

FINDINGS

Structural studies

One of the most effective and accurate methods of determining particle size is TEM. Based on TEM micrographs investigation, the size of the synthesized Fe₃O₄ nanoparticles was estimated about 14 nm (Fig 1). It can be seen that, the particles have uniform size distribution. Fig 2 indicates the size distribution of 8 to 20 nm for synthesized Fe₃O₄ nanoparticles which is obtained from TEM results. This form of size distribution is normal but most of the particles are around 14 nm.

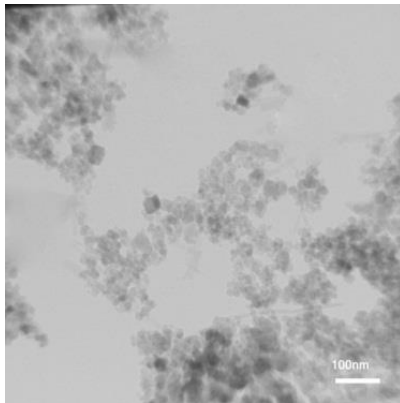


Fig 1. Transmission electron microscope image of Fe₃O₄ nanoparticles

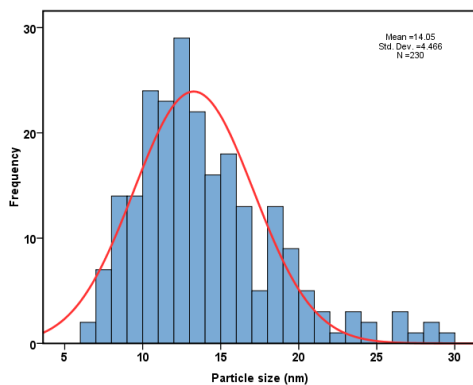


Fig 2. Size distribution of Fe₃O₄ nanoparticles (from TEM results)

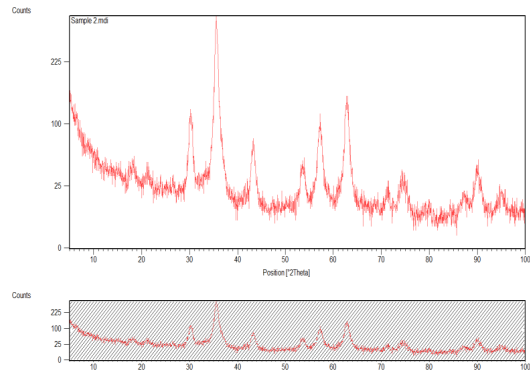


Fig 3. X-ray diffraction diagram of Fe₃O₄

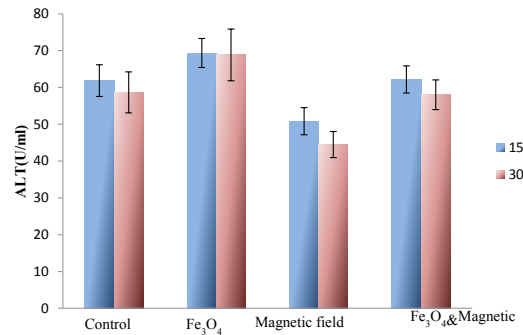


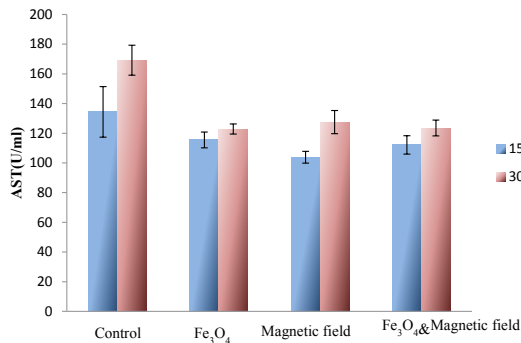
Chart 1. ALT mean value in all groups 15 and 30 days post injection

Analysis of the Fe₃O₄ crystal lattice structure was studied by XRD which is shown in Fig 3. As can be seen, the structure of the sample is coincided with spinel crystalline structure. According to Debye- Scherrer equation, the size of the Fe₃O₄ nanoparticles was estimated around 10 nm. The estimated size difference between TEM and XRD is related to their accuracy. By considering the fact that TEM is more accurate and Debye- Scherrer equation just approximately determined the particle size, it can be easily concluded that TEM size result is more reliable.

In vivo studies in rat

Charts 1 and 2 indicates the mean values of ALT and AST of all groups 15 and 30 days post injection. As can be seen, in both 15 and 30 days post injection, significant increase in ALT and significant reduction in AST (P < 0.05) is indicated for group received Fe₃O₄ nanoparticles (100 mg/kg) compared with the control group. The constant magnetic field group, 15 and 30 days post

injection showed a significant reduction in ALT and AST (P<0.05) compared with the control group. Intra peritoneal injection of Fe₃O₄ nanoparticles at the presence of constant magnetic field, 15 and 30 post injection indicated significant reduction in AST compared to the control group. At the same condition, the serum levels of alanine aminotransferase did not show any significant difference in comparison with the control group.



Cart 2. AST mean value in all groups 15 and 30 days post injection

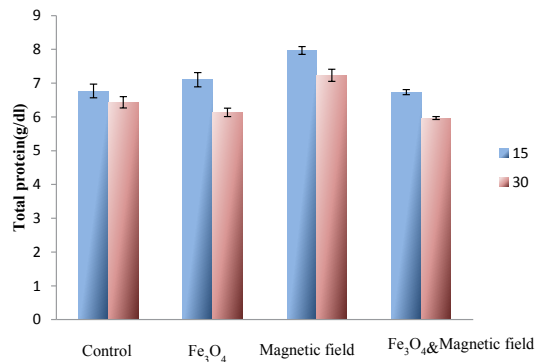


Chart 3. Total protein mean value in all groups 15 and 30 days post injection

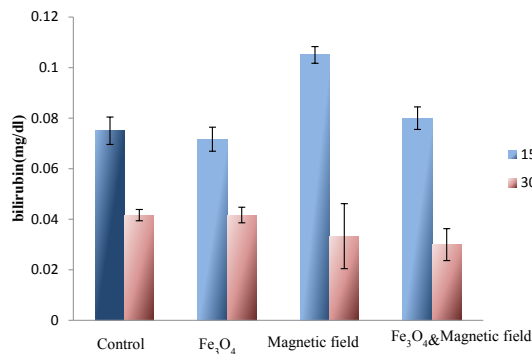


Chart 4. Bilirubin mean value in all groups 15 and 30 days post injection

Time causes significant increasing of aspartic aminotransferase serum concentration (P<0.05) but no significant difference was seen in serum ALT levels. According to the chart 3 and 4, intra peritoneal injection of Fe₃O₄ nanoparticles (100 mg/kg), 15 and 30 day post injection showed no significant difference in the amounts of total protein and bilirubin in comparison with the control group. The amount of total protein has increased significantly at the presence of constant magnetic field, 15 and 30 days post injection compared to control (P<0.01). The amount of bilirubin has enhanced significantly 15 days post injection compared with the control group (P<0.05) but there was not any significant difference in the amount of bilirubin 30 days post injection. Intraperitoneal injection of Fe₃O₄ nanoparticles (100 mg/kg) at the presence of magnetic field showed no significant difference in the amounts of total protein and bilirubin in both 15 and 30 days post injection. Also, time has caused significant decrease in the amount of total protein and bilirubin (P<0.01) compared to control group. According to the chart 5 and 6, the results showed that the intraperitoneal injection of Fe₃O₄ nanoparticles (100 mg/kg) at the presence of constant magnetic field has no effect on the enzyme activity of superoxide dismutase and glutathione peroxidase compared to the control group 15 and 30 days post injection. Antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase as confounding factors act and play important roles in modulating oxidative pressure to prevent a chain reaction of free radicals [16, 24]. Superoxide dismutase enzyme increases the rate of reaction of converting superoxide anion to hydrogen peroxide and molecular oxygen [20]. Time has no significant effect on the SOD and GPX enzyme activity.

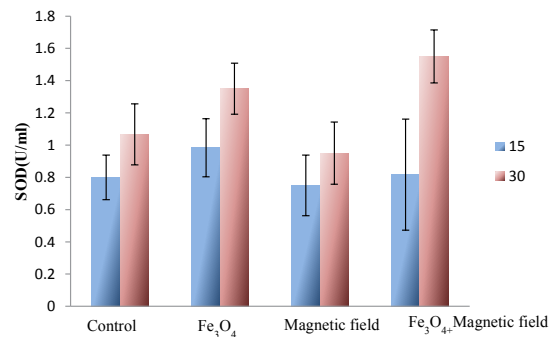


Chart 5. SOD enzyme changes in all groups 15 and 30 days post injection

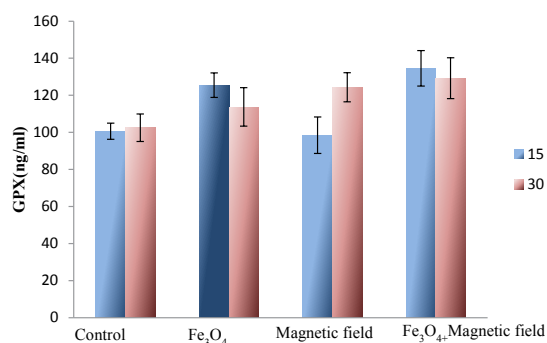


Chart 6. GPX enzyme changes in all groups 15 and 30 days post injection

DISCUSSION

Aspartic aminotransferase and alanine aminotransferase leakage from the liver cytosol to blood flow caused increasing of these enzymes activity [25-26] and it may be due to increasing of anabolism and reducing of catabolism [27]. A significant increasing of ALT and AST is a sign of liver damage and stress [28].

ALT enzyme is a more specific marker for liver damage [13]. Thus, increasing of the ALT enzyme shows damaging effect of iron oxide nanoparticles on liver cells.

Following the use of magnetic fields, a series of activities is started in the body that guides message from the cell membrane to the nucleus and also genetic content. The magnetic fields affect function of organs (heart, blood, brain, and nerves) and turns on alters cell membrane potential and ion distribution in the cell. These changes may affect the biochemical processes and change serum enzyme activity and biochemical parameters [29]. Several studies were performed related to the effect of electromagnetic fields on biological processes such as enzyme transaminase and different results have been reported [30]. The obtained results show influence of iron oxide nanoparticles on the activity of liver enzymes at the presence of magnetic field.

Iron oxide nanoparticles and magnetic field cause significant increasing of ALT and significant decreasing. Therefore, nanoparticles have toxic effect but magnetic fields may correct this effect to some extent. Furthermore, time (30 days post injection) has corrected some toxic effects of nanoparticles which it suggests that there were not any sever and serious liver damages while the effect of time and constant magnetic field corrected the ALT changes.

Iron oxide nanoparticles at the presence of constant magnetic field cause a significant reduction in AST activity and the effect of time on the AST activity was positive. In fact the liver over the time adapt itself to the new conditions.

Constant magnetic field has produced a significant increasing in total protein and time has caused significant correction, so certain disorders are not created in the liver. Iron oxide nanoparticles and magnetic field have proven no side effects on the amount of bilirubin and as a result, they are not created certain disorders in the liver.

CONCLUSION

The results of this investigation show that iron oxide nanoparticles even at the presence of constant magnetic field has affected reversible toxic effects on the liver activity and over time these effects had been corrected.

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