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Evaluation of iron oxide nanoparticles effects on tissue and enzymes of liver in rats

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ABSTRACT

Background: Iron oxide nanoparticles can be used for medical imaging, disease diagnosis, drug delivery, cancer treatment, gene therapy and other cases. These particles accumulate in liver cells and lead to oxidative stress with generation of reactive oxygen species. Aim: This study investigates the effect of iron oxide nanoparticles on liver tissue and enzymes [alanine aminotransfere (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP)] in male rat. Methods: 40 adult male rats of wistar strain, weighing 250-300 g were used for this study. The rats were randomly assigned to four groups. One group was control and other three groups were fed with iron oxide nanoparticles at 20, 50 and 150 µg/kg concentrations respectively for 15 days. Venous blood was taken to measure liver enzymes at the end of treatment. Liver of rats removed for histological experiments. Results: Significantly enhanced ($P<0.05$) AST level obtained as well as ALT and ALP level with used of maximum concentration of iron oxide nanoparticles (150 µg/kg) as compared to normal group. Conclusion: Based on our result, using high concentration of iron oxide nanoparticles could be caused undesirable effects on liver with damage to hepatocyte and level elevation of liver enzymes.

Introduction

In recent years, nanotechnology has been cause of dramatic developments in various industries (Kuzma and Besley, 2008; Yangetal., 2008; Bystrzejewska-Piotrowska et al., 2009). In betweenthem,the role of nano-materials and nano-powders in thisgreat transformation is undeniable (Dresselhaus et al., 1996). Nanoparticlesconsisting of elements such as iron, nickel and cobaltandexhibitingmagnetic properties are called ‘‘magnetic nanoparticles’’(Gupta andGupta, 2005; Mirkovic et al., 2010). Magneticnanoparticles have been used commonly for various biomedical circumstances (Pankhurstetal.,2003; Saiyed et al., 2003; Gupta and Gupta, 2005). Iron oxide is one of the most popular magnetic nanoparticles in medicine and biotechnology (Ramchand et al., 2001). The application of small iron,oxide particles in *in vitro* diagnostics has been practised for nearly,40 years (Gilchrist et al., 1985). Superparamagnetic Iron Oxide, Nanoparticles (SPIONs) have been the most extensively investigated,due to their excellent biocompatibility and ease of synthesis formultifunctional biomedical applications such as cellulartargetingand drug delivery, tissue repair, magnetic resonance imaging (MRI) and magnetofection (Sun et al., 2008; Shubayevet al., 2009; Thoreket al., 2006; Gupta and Gupta, 2005; Bhaskaret al., 2010). The liver is amajor source of active chemical cells that have a high metabolicrateand 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 functionsare performed (Golik et al., 1990; Bahmaei et al., 1997; Amini et al., 1999). All the materials that absorbed through the intestines are transported to liver by the liver portal vein (Jain et al., 2008). Liver is the best location in the circulatory system to collectand accumulate metabolites and also to remove and neutralize toxins (Lomer et al., 2002). Iron nanoparticles accumulate in cells and cancerous tissues thus, for rapid diagnosis in early stages, has manyapplications (Handy and Shaw, 2007). Typically, magnetic nanoparticles,distribute to the liver (80-90%), spleen (5-8%) and bone marrow (1-2%). Their surfaces may interact with extracellular matrix components,and the plasma cell membranes of macrophages, endothelial cells, skin epithelium, respiratory depending on the route of administration And, particle size (Shubayevet al., 2009).SPIONs are mostly used as a MRI contrast agent in examinations of the liver (Yu et al., 2008) and have, traditionally been used in MRI for the detection and delineation of focal liver lesions (Wang et al. 2001). SPIONs are potentially capable to generate reactive oxygen species leading to oxidative stress, which, can be measured by lipid peroxidation (Jones and Grainger, 2009). Iron nanoparticles accumulate in the liver and affect on the liver, stem cells and fibroblast cells that decrease of mitochondrial activity,and lead to morphological changes. In addition, another effects of Iron nanoparticles are reduction of glutathione liver cells, increase shock and oxidative reactions in liver cells (Handy and Shaw, 2007).

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According to the medical application of iron oxide nanoparticles andits influence on liver, the aim of this study was to assess effect of iron oxide nanoparticles on tissue and enzymes of liver (ALT, ASTand ASP) in rats.

Materials and methods

Experimental Animals and Diet 40 adult male rats of wistar strain, weighing 250-300 g were used for this study. They were housed individually in stainless steel mesh-bottomed cages and were acclimatized before start of the experiments at suitable conditions of temperature and light for a period of two weeks. The environmental conditions were set at a temperature of 20-25°C relative humidity of 55±5% and a 12 h light/dark cycle. This study was carried out according to the guidelines approved by the Animal Care and Use Committee.

Experimental Design

The rats were randomly assigned to tow groups. First (control) group with 10 animals received 1 ml/day distilled water and second group were further subdivided into three groups that were fed with 1 ml/day distilled water containing iron oxide nanoparticles at 20, 50 and 150 µg/kg concentrations respectively for 15 days with used of gavage tubes. The iron oxide nanoparticles (30 nm), synthesized from Center of Science and Nanotechnology Institute, Payam-e-Noor University of Yazd, were prepared.

Fig 1. Morphometric changes after treatment with high dose of iron oxide nanoparticles. A: Normal liver of rat as a control; B: Damaged liver of rat after high dose tereatment iron oxide nonaparticles.

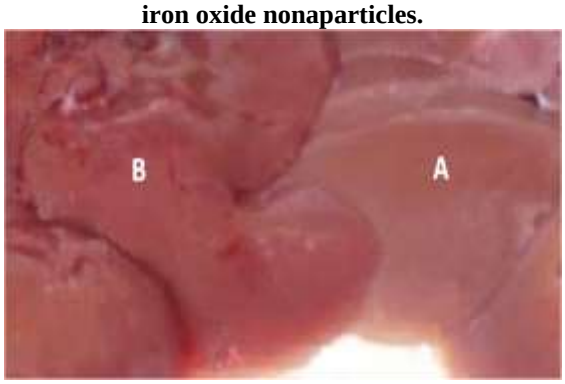


Fig 2. Phatological sections after treatment with different doses of iron oxide nanoparticles. A: Normal liver of rat as a control; B, C & D: Experimental groups were treated with 20 µg/kg (B), 50 µg/kg (C), 150µg/kg (D). The sections were stained with H & E dye. The empty spaces represent elimination of hepatpcytes especially around of central veins. 20× magnification (A-C) & 40× magnification for D.

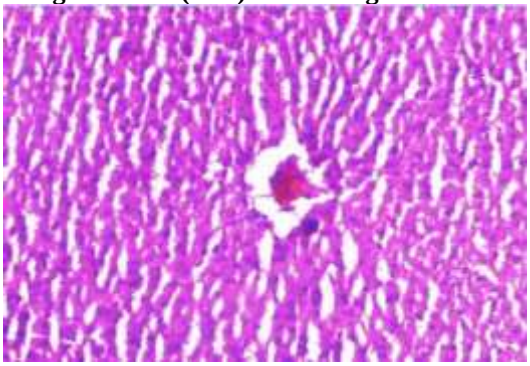


Fig 3. Serum concentration of ALP in experimental groups were treated by different doses of iron oxide nanoparticles. Experimental groups; group 1: control, group 2: 20 µg/kg, group 3: 50 µg/kg, group 4: 150µg/kg. Histograms show mean values (± SD, n=3; P < 0.05). *: Significant difference with other groups.

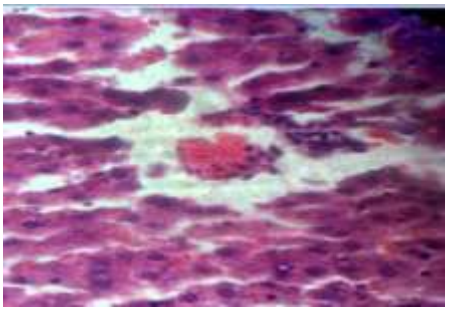


Fig 4. Serum concentration of ALT in experimental groups were treated by different doses of iron oxide nanoparticles. Experimental groups; group 1: control, group 2: 20 µg/kg, group 3: 50 µg/kg, group 4: 150µg/kg. Histograms show mean values (± SD, n=3; P < 0.05). *: Significant difference with other groups.

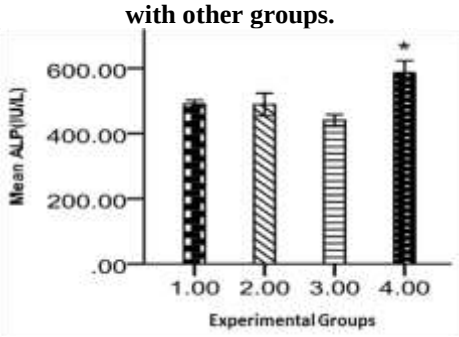
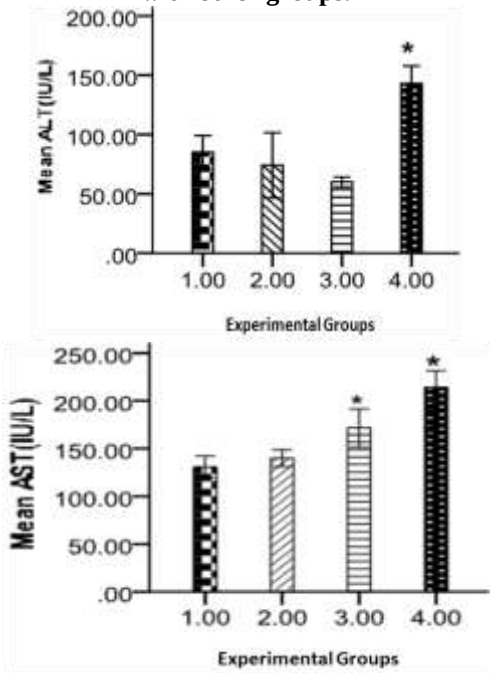


Fig 5. Serum concentration of AST in experimental groups were treated by different doses of iron oxide nanoparticles. Experimental groups; group 1: control, group 2: 20 µg/kg, group 3: 50 µg/kg, group 4: 150µg/kg. Histograms show mean values (± SD, n=3; P < 0.05). *: Significant difference with other groups.



Biochemical experiments and parameters measurement

For hematologic and chemistry analyses, venous blood from the orbital sinus at the inner corner of eye rats was collected to measure factors at the end of the study from all animals. Serum by centrifugation at rate 3000 RPM for 15 min was isolated for measured concentrations of liver enzyme [alanine aminotransfere (ALT) and aspartate aminotransferase (AST) and alkaline phosphatase (ALP)] were given to the auto-analyzer. Liver enzymes were measured using enzymatic kits (Pars Azmoon Co, Iran) with IFCC method.

Histological experiments

At the end of study, rats were anesthetized with chloroform and liver of the scarified rats were dissected, removed, washed with physiologic serum and put in 10% formalin solution. In the future stage, specimens were dehydrated and blocked then prepared microtomic sections and stained with Hematoxylen and Eosin (H and E) (Mohamed et al., 2009). Ishak scoring system (Ishak et al., 1995) were used for histology analysis.

Statistical analysis

Statistical evaluations were conducted by SPSS13.0. ANOVA and Tukey test was performed to investigate level of liver enzymes. *P* values <0.05 were considered statistically significant.

Results

The effects of different concentrations of ironoxide nanoparticles on the factors studied after 15 days. Morphometric changes was shown in experimental groups compered to control group. These changes included hepatotrphic effects; hepatocyte damages (Fig. 1). 150 µg/kg iron oxide nanoparticles consumption was leaded to significantly (P<0.05) rise ALP level (Fig. 1) as well as ALT (Fig. 2) levelin comparison with normal group. Non-significant difference was observed in these enzymes level between fed groups with 50 and 20 µg/kg iron oxide nanoparticles with normal group. As shown in the Fig. 3, AST level significantly (P<0.05) enhanced with used of maximum concentration of iron oxide nanoparticles (150 µg/kg) as compared to normal group. Using tow other concentration of iron oxide nanoparticles (50 and 20 µg/kg) had not significant effect on AST level in comparison with normal group.

Discussion

The main finding of this study was significant increase (P<0.05) ALP, ALT and AST level with 150 µg/kg iron oxide nanoparticles consumptionas compared to normal group. An initial step in detecting liver damage is a simple blood test to determine the presence of certain liver enzymes in the blood. The activity of these enzymes is normally used to evaluate the liver function. Under normal circumstances, these enzymes reside within the cells of the liver. But when the liver is injured, these enzymes are spilled into the blood stream. Among the most sensitive and widely used of liver enzymes are theaminotransferases. They include AST and ALT. These enzymes are normally contained within liver cells. If the liver is injured, the liver cells spill the enzymes into blood, the level is increased in cases of liver cell death resulting from cases, such as shock or drug toxicity (Hutchinson et al., 2002; Sally et al., 2002). As a result, the rate of iron oxide metabolism in the liver is believed to be dependent upon the dose injected, percent initial dose taken up by the liver and the cellular distribution within the liver (Wisse et al. 1991; Dupas et al. 1999). In the study by Lijuan et al (2010), toxic effects of inhalation exposure to ferric oxide (Fe2O3) nanoparticles in rats. In their study, wistar rats were consecutively treated with Fe2O3 at 8.5 mg/kg body weight twice daily for 3 days and parameters in serum,and hispathological examinations were analyzed at 12 h and 36 h after the 3 day treatment. Iron (Fe) content in liver and lung tissues was significantly increased at 36 h. The levels of serum ALT, AST andALP in nanoparticle-exposed group were significantly decreased compared to the unexposed controls. Histopathological examination showed ferric oxide anoparticles caused severe damage in liver. Damaged liver cells develop leaky membranes, allowing for escape,of intracellular enzymes into the bloodstream (Pratt and Kaplan, 2000; American Gastroenterological Association, 2002; Green and Flamm,2002).Previous studies have indicated that Kupffer cells may be more efficient at metabolising iron oxide particles than liver endothelial cells (Wisse et al. 1991). The rate of metabolism in these cells is believed to be dependent upon the concentration of iron oxide particles taken up by each cell type (Wisse et al. 1991; Dupas et al.1999). Conflict of interest: None of the authors have any conflicts of interest to disclose and all authors support submission to this journal.

Reference

American Gastroenterological Association. American Gastroenterological Association Medical Position Statement: Evaluation of Liver Chemistry Tests. Gastroenterology 2002 ;123: 1364-6.

Amini M.; Zarghi A.; Vatanpour H. Sensitive high-performance liquid chromatographic method for determination of captopril in plasma. Pharm Acta Helv 1999; 73 (6): 303.

Bahmaei M.; Khosravi A.; Zamiri C.; Massoumi A.; Mahmoudian M. J. Determination of captopril in human serum by high performance liquid chromatography using solid-phase extraction. J Pharm Biomed Anal. 1997; 15 (8): 1181.

Bhaskar S.; Tian F.; Stoeger T.; Kreyling W.; Fuenteet J. M. de la.; Grazú V.; Borm P.; Estrada G.; Ntziachristos V.; Razansky D. Multifunctional nanocarriers for diagnostics, drug delivery and targeted treatment across blood-brain barrier: Perspectives on tracking and neuroimaging. Part Fibre Toxicol 2010; 7: 1-3.

Bystrzejewska-Piotrowska G.; Golimowski J.; Urban P. L. Nanoparticles: Their potential toxicity, waste and environmental management. Waste Management 2009; 29(9): 2587- 2595.

Dresselhaus M. S.; Dresselhaus G.; Eklund P. C. Science of Fullerenes and Carbon Nanotubes. Fullerene Science and Technology 1997; 5(3): 627-628.

Gupta A .K.; Gupta M. synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. Biomaterials 2005; 26 (18): 3995–4021.

Gilchrist R. K.; Medal R.; Shorey W. D.; Hanselman R. C.; Parrot J. C.; Taylor C. B. Selective inductive heating of lymph nodes. Ann Surg 1957; 146: 596–606.

Golik A.; Modai D.; Averbukh Z.; Sheffy M.; Shamis A.; Cohen N.; Shaked U.; Dolev E. Metabolism 1990; 39: 665.

Green R. M.; Flamm S. AGA Technical Review on the Evaluation of Liver Chemistry Tests. Gastroenterology 2002; 123: 1367-84.

Handy R. D.; Shaw B. J. Toxic effects of nanoparticles and nanomaterials: Implications for public health, risk assessment and the public perception of nanotechnology. Health, Risk & Society 2007; 9(2): 125-144.

Hutchison TA, Shahan, DR, Anderson ML (eds.): Drugdex System. Englewood, CO; Micromedex Inc. Expires: December 2000.

Ishak K.; Baptista A.; Bianchi L.; Callea F.; Degroote J.; Gudat F.; Denk H.; Desmet V.; Korb G.; Macsween R. N. M.; Phillips M. J.; Portmann B. G.; Poulsen H.; Scheuer P. J.; Schmid M.; Thaler H. Histological grading and staging of chronic hepatitis. J Hepatol 1995; 22(6): 696-9.

Jain T. K.; Reddy M. K.; Morales M. A.; Leslie-Pelecky D. L.; Labhasetwar V. Biodistribution, Clearance, and Biocompatibility of Iron Oxide Magnetic Nanoparticles in Rats. Mol Pharmaceutics 2008; 5(2): 316-327.

Jones C. F.; Grainger D. W. In vitro assesements of nanomaterial toxicity. Adv Drug Deliv Rev 2009; 61(6): 438-456.

Kuzma J.; Besley J. Ethics of Risk Analysis and Regulatory Review: From Bio- to Nanotechnology. Nano Ethics 2008; 2(2): 149-162.

Lomer M. C.; Thompson R. P.; Powell J. J. Fine and ultrafine particles of the diet: influence on the mucosal immune response

and association with Crohn's disease. Proc Nutr Soc 2002; 61: 123-130.
Mirkovic B.; LahTurnsek T.; Kos J. Nanotechnology in the treatment of cancer. Zdrav Vestn 2010; 79: 146–155.
Mohamed R. A.; Ramadan R. S.; Ahmed L. A. Effect of substituting pumpkin seed protein isolate for casein on serum liver enzymes, lipid profile and antioxidant enzymes in CCl4-intoxicated rats. Advanc in Biol Res 2009; 3(1-2): 9-15.
Pankhurst Q. A.; Connolly J.; Jones S. K.; Dobson J. Applications of magnetic nanoparticles in biomedicine. J Phys D ApplPhys 2003; 36: R167–81.
Pratt D. S.; Kaplan M. M. Evaluation of abnormal liver-enzyme results in asymptomatic patients. N Engl J Med 2000; 342: 1266-71.
Ramchand C. N.; Priyadarshini P.; Kopcansky P.; Mehta R. V. Applications of magnetic fluids in medicine and biotechnology. Indian J Pure ApplPhys 2001; 39: 683–9.
Saiyed Z. M.; Telang S. D.; Ramchand C. N. Application of magnetic techniques in the field of drug discovery and biomedicine. Biomagn Res Technol 2003; 1:2.
Sally A.; Tice R. P. H.; Dean Parry R. P. h. Medications that need hepatic Monitoring. Hospital Pharmacy 2001; 36 (4): 456-464.
Shubayev V. I.; Pisanic T. R.; Jin S. Magnetic nanoparticles for theragnostics. Adv Drug Deliv Rev 2009; 61: 467-477.
Sun C.; Lee J.; Zhang M. Magnetic nanoparticles in NMR imaging and drug delivery. Adv Drug Deliv Rev 2008; 60 (11): 1252-1265.
Thorek D. L.; Chen A. K.; Czypryna J.; Tsourkas A. Superparamagnetic iron oxide nanoparticle probes for molecular imaging. Ann Biomed Eng 2006; 34: 23-38.
Wang Y. X.; Hussain S. M.; Krestin G. P. Superparamagnetic iron oxide contrast agents: physicochemical characteristics and applications in MR imaging. EurRadiol 2001; 11: 2319–2331.
Lijuan W.; Lin W.; Wenjun D.; Fang Z. Acute Toxicity of Ferric Oxide and Zinc Oxide Nanoparticles in Rats. J N N 2010; 10 (12): 8617-8624(8).
Wisse E.; Doucet D.; Van Bossuyt H. A transmission electron microscopic study on the uptake of AMI-25 by sinusoidal liver cells. In: Wisse E (ed) Cells of the hepatic sinusoid, Kupffer Cell Foundation, the Netherlands 1991; 3: 534–539.
Yang W.; Peters J. I.; Williams R. O. Iii. Inhaled nanoparticles-a current review. Int J Pharm 2008; 356(1- 2): 239-247.
Yu M. K.; Jeong Y. Y.; Park J.; Park S.; Kim J. W.; Min J. J.; Jon S. Drug-loaded superparamagnetic iron oxide nanoparticles for combined cancer imaging and therapy in vivo. Angew Chem Int Ed Eng 2008; l47 (29): 5362-5.