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# Synthesis and dose interval dependent hepatotoxicity evaluation of intravenously administered polyethylene glycol-8000 coated ultra-small superparamagnetic iron oxide nanoparticle on Wistar rats

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ABSTRACT

Superparamagnetic iron oxide nanoparticles are being used in medical imaging, drug delivery, cancer therapy, and so on. However, there is a direct need to identify any nanotoxicity associated with these nanoparticles. However uncommon, drug-induced liver injury (DILI) is a major health concern that challenges pharmaceutical industry and drug regulatory agencies alike. In this study we have synthesized and evaluated the dose interval dependent hepatotoxicity of polyethylene glycol-8000 coated ultra-small superparamagnetic iron oxide nanoparticles (PUSPIOs). To assess the hepatotoxicity of intravenously injected PUSPIOs, alterations in basic clinical parameters, hematological parameters, hemolysis assay, serum levels of liver marker enzymes, serum and liver lipid peroxidation (LPO) levels, enzymatic antioxidant levels, and finally histology of liver, kidney, spleen, lung, brain, and heart tissues were studied in control and experimental Wistar rat groups over a 30-day period. The results of our study showed a significant increase in the aspartate transaminase (AST) enzyme activity at a dose of 10 mg/kg b.w. PUSPIOs twice a week. Besides, alanine transaminase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transferase (γGT) enzyme activity showed a slender increase when compared with control experimental groups. A significant increase in the serum and liver LPO levels at a dose of 10 mg/kg b.w. PUSPIOs twice a week was also observed. Histological analyses of liver, kidney, spleen, lung, brain and heart tissue samples showed no obvious uncharacteristic changes. In conclusion, PUSPIOs were found to posses excellent biocompatibility and Wistar rats showed much better drug tolerance to the dose of 10 mg/kg b.w. per week than the dose of 10 mg/kg b.w. twice a week for the period of 30 days.



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## 1. Introduction

Though the history of nanomaterials dates back to late seventies and eighties, only in recent years it has been much researched on biological systems (Shavel et al., 2007). With rapid development of nanotechnology, magnetic iron oxide nanoparticles hold immense potential in numerous fields such as biomedical applications (Silva et al., 2013), drug delivery (Corot and Warlin, 2013; Unsoy et al., 2014), magnetic resonance imaging (Jain et al., 2005), effective elimination of cancer cells through hyperthermia (Sadhukha et al., 2013), *in vivo* tracking of stem cells (Li et al., 2013), cell separation, enzyme immobilization, cell mechanics (Dilnawaz et al., 2010; Zigeuner et al., 2003), tissue engineering (Ito and Kamihira, 2011), targeted delivery of drugs or genes, magnetic transfections (Singh et al., 2010), cell apoptosis (Wang et al., 2012), bacterial pathogen detection (Pera et al., 2010), diabetes onset prediction (Fu et al., 2012), waste water treatment (Koehler et al., 2009), and magnetic immunoassay (MIA) (Bruls et al., 2009). Although potential applications of nanoparticles are limitless, of late some critical commentaries were posted regarding the future of nanomedicines, including "Why so many papers and so few drugs?" (Sengupta and Kulkarni, 2013; Venditto and Szoka, 2013), One of the main grounds which can be attributed to this is biosafety of nanomaterials and rapid clearance by reticuloendothelial system (RES). Several protein–PEG conjugates were already approved by Food and Drug Administration (FDA) to treat diseases in humans (Steevens et al., 2011). So polyethylene glycol (PEG) is considered as an encapsulation material in this study due to its wide clinical use and outstanding safety profile. PEG reduces clearance of nanoparticles by reticuloendothelial system by preventing the binding of plasma proteins (opsonization), which thereby results in increased circulation time (Roberts et al., 2002; Sengupta and Kulkarni, 2013). Chemopreventive role of PEG-8000 on azoxymethane induced colon carcinogenesis has also been documented (Roy et al., 2006).

Though ultra-small superparamagnetic iron oxide nanoparticles (USPIOs) are synthesized by numerous methods very few reports are available on its *in vivo* characteristics, and not many reports are available on its dose interval dependent toxicity which is a crucial yardstick in cancer chemotherapy (Gu et al., 2012; Jain et al., 2008; Szalay et al., 2012). To ensure the biosafety and to facilitate safe and fast clinical translation of nanoparticles, it is important to systematically study the hepatotoxicity of nanoparticles introduced into the body. Due to its portal blood supply, liver is the first major organ to be exposed to ingested chemicals. It is the most common site prone to toxicity. In assessment of risks, study of the liver has been warranted and continues to be important in understanding of toxicity. More than 900 drugs have been implicated in causing liver injury and it is the most common reason for a drug to be withdrawn from the market. DILI is responsible for 5% of all hospital admissions and 50% of all acute liver failures (Pandit et al., 2012; FDA, 2009; Lee and Senior, 2005). The liver plays an astounding array of vital functions; so, considering the importance of drug-induced hepatotoxicity, in this present study, ultra-small superparamagnetic iron oxide nanoparticle formulation with polyethylene glycol-8000 as encapsulation material was synthesized, characterized and its *in vivo* hepatotoxic characteristics were investigated. *In vivo* characteristics of PUSPIOs on serum liver biomarker enzymes, serum and liver lipid peroxidation (LPO) levels, and enzymatic antioxidant levels were evaluated and effect on various collected organs (liver, kidney, spleen, lung, brain, and heart) over a 30-day period was investigated.

## 2. Materials and methods

### 2.1. Materials

Iron (II) chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$), iron (III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$) and polyethylene glycol (PEG, Mw = 8000) were obtained from Sigma–Aldrich, USA. Ammonium hydroxide (25–28%) was obtained from Sisco Research Laboratory (SRL), India. All the other chemicals used in this study were of analytical reagent grade.

### 2.2. Methods

#### 2.2.1. PUSPIOs synthesis

Magnetite nanoparticles were synthesized by the chemical co-precipitation of $Fe^{2+}$ and $Fe^{3+}$ under alkaline conditions, as already reported in literature (Pan et al., 2009; Kuo et al., 2012). As a typical procedure, 0.1 M of $FeCl_3{\cdot}6H_2O$ was dissolved in 50 mL of distilled water and 0.05 M of $FeCl_2{\cdot}4H_2O$ was dissolved in 50 mL of distilled water. The $Fe^{2+}$ solution was added into the $Fe^{3+}$ solution under constant stirring. 28% of aqueous ammonium hydroxide ($NH_4OH$) solution was added drop wise until the pH of the solution attained 8. The color of the solution changed from light brown to black, indicating the formation of $Fe_3O_4$ nanoparticles, and then 0.30 M of PEG-8000 solution was added into the iron solution. The solution was allowed to crystallize completely for another 60 min at 80 °C under rapid stirring. The resultant product was washed by repeated cycles of centrifugation and redispersed in distilled water. Washing was performed several times in distilled water. Then, the final product was dried at 100 °C in vacuum for 2 h, and the $Fe_3O_4$ nanoparticles were finally achieved.

#### 2.2.2. Characterization of PUSPIOs

The crystal pattern of the nanoparticles was acquired with an X-ray Diffractometer (XRD) GE Inspection Technologies, TT3003 – Germany. High resolution scanning electron microscopy (HR SEM) images were recorded in FEI Quanta FEG 200. Transmission electron microscope (TEM) images were verified using Philips Morgagni 268 electron microscope. Fourier transform infrared (FT-IR) spectra of PUSPIOs were documented by the potassium bromide pellet method in Bruker, Germany at the range of 400–4000 $cm^{-1}$. Measurement of magnetization of the PUSPIOs was supported with a Vibrating Sample Magnetometer (VSM), Lakeshore, VSM 7410.

#### 2.2.3. In vivo studies on Wistar rats

Male Wistar strain albino rats weighing about 140–150 g were obtained from the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) approved Breeder. All the experiments were designed and conducted