Preliminary histological studies on the influence of glycerol-iron-oxide nanoparticles

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This paper reports structural and morphological studies as well as *in vivo* evaluation of glycerol-iron-oxide nanoparticles (GIO-NPs) in order to investigate their influence on different organic tissues. Transmission electron microscopy (TEM) studies confirm the structure characteristic to iron oxide nanoparticles and their ellipsoidal shape. The histopathological examinations indicated that the studied nanoparticles induce many morphological alterations to the liver, kidneys and spleen tissues 24h, 48h and 72h exposure.

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1. Introduction

In the last decades the life expectancy has increased in the developed countries. For example, in United States alone, the life expectancy at birth increased with 0.2 between 2009 and 2010, from 78.5 years to 78.7 years according to a study made in 2014 [1]. This tendency could be the result of the advances of modern medicine. Nowadays, researchers and doctors worldwide are trying to improve the quality of life of the patients treated in hospitals around the world by developing new treatment techniques and by perfecting the existing methods of treatment and diagnosis. In this context, the field of nanotechnology has been widely used for improving the diagnosis and therapeutic methods [2]. Numerous multifunctional nanoparticles have been studied for different applications such as drug deliveries, biomolecules carriers or optical dyes [2-7]. Another reason that determined researchers to study nanoscale materials is their interaction at a molecular level with various biological systems which allows them to influence the functions of the biological systems [2-6]. On the other hand, in order to administrate the correct treatment, a good diagnosis must be made in the earliest stages of the disease. To this end, many diagnosis instruments have been developed. One of the most important diagnosis machines presently used in most hospitals worldwide is the magnetic resonance imaging (MRI). This instruments allows doctors to perform a noninvasive examination of the living human body, obtaining three-dimensional images of the biological tissues [8-10]. The most commonly used contrast agents for obtaining MRI images are based on gadolinium. Although these compounds have been proven to be adequate, only millimolar concentrations that must be injected in the human body prior to the examination have been demonstrated to be effective [8, 10-11]. In this context, an alternate material has been provided by the field of nanotechnology. Previous studies have shown that lower concentrations of iron oxide nanoparticles could be better contrast enhancement agents due to their superparamagnetic and biocompatible properties [12-20]. Furthermore, these properties have made iron oxide nanoparticles ideal candidates for a number of biomedical applications such as targeted drug delivery systems [21-23], tumor hyperthermia [24], magnetic separation of immune cells [21, 25] or proteins [21, 26] and biosensors [8, 27]. However, in order to be used in biomedical applications, several obstacles must be overcome. The nanoscale sizes and high ratio between their surface area and volume provide the appropriate conditions for aggregation and interaction with the proteins found in plasma which in

terms may lead to their release by the endothelial system [28-29]. For this reason, generally, iron oxide nanoparticles are embedded in a biocompatible polymer that improves their dispersity and stability [8, 30-34]. Glycerol is a biocompatible and nontoxic natural polymer which has been approved by the US Food and Drug Administration (FDA) to be used in certain amounts for clinical use [35]. Among numerous applications, glycerol is currently used for reducing the eye pressure for glaucoma patients, or for reducing the brain pressure when administered intravenously to patients suffering from strokes, meningitis or encephalitis [35-37].

In this study we aim to synthesize a compound based on iron oxide nanoparticles embedded in glycerol, our goal being to combine the properties of both materials in order to obtain an improved compound able to be used in different biomedical applications.

2. Synthesis of glycerol-iron-oxide nanoparticles

The synthesis of glycerol-iron-oxide-nanoparticles nanoparticles was conducted according to the method reported by S.L. Iconaru et al. [38].

3. Characterizations of glycerol-iron-oxide Nanoparticles

3.1 TEM studies

The obtained nanoparticles were investigated by transmission electron microscopy (TEM). A FEI Tecnai 12 microscope (operating in BF, SAED and HRTEM modes) was used. For this studies the nanoparticles were embedded in an epoxy resin. The specimens used for TEM were prepared by ultramicrotomy method.

3.2 Animals

Male Brown Norway rats (weighing ~ 200 ± 10g) were purchased from the National Institute of Research and Development for Microbiology and Immunology "Cantacuzino", Bucharest. The rats were housed in an environment controlled for temperature (22 ± 2°C), light (12 h light/dark cycles) and humidity (60 ± 10%). The animals were maintained under specific pathogen free conditions in accordance with NIH Guide for the Care and Use of laboratory Animals.

3.3 Histological examination

In order to test the toxicity of glycerol-iron-oxide nanoparticles under *in vivo* conditions, the rats (n=2 per group) were treated with normal saline and glycerol-iron-oxide nanoparticles with a concentration of 1.7 ml/kg via intraperitoneal injection. The selected tissues (liver, kidney and spleen) were studied by histopathological examinations. For the histopathological studies the selected organs were prelevated from rats and fixed in 10% formalin. The organs were prepared as paraffin-embedded glass slides stained with hematoxylin and eosin. The morphological changes were observed by microscope examination [20, 39].

4. Results and discussions

Information about the structure and morphology of the glycerol-iron-oxide nanoparticles were obtained by TEM. In Fig. 1 are presented the BF (Bright field), SAED (Selected Area Electron Diffraction) and HRTEM (High-resolution transmission electron microscopy) images obtained on the studied powders.

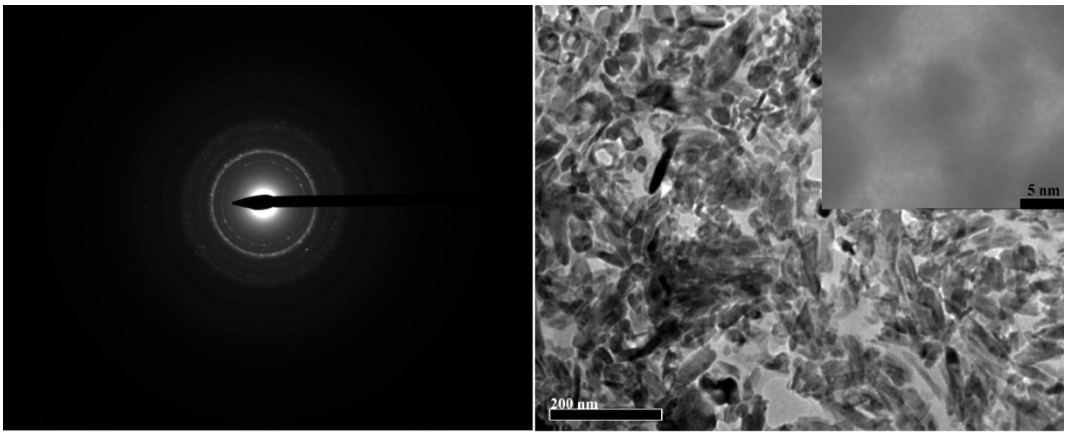

Fig. 1. Bright field TEM image (left), SAED pattern (right) and HRTEM image (inset) of glycerol-iron-oxide nanoparticles

The TEM images show that the glycerol-iron-oxide particles were synthesized at nanometric scale with uniform distribution and ellipsoidal morphology. The rings from the SAED pattern confirms the structure of iron oxide. Moreover, the interplanar distance deduced from the HRTEM image could be indexed to the structure of iron oxide.

4.1 Histological examination

Lately, modification of the nanoparticle surfaces has led to acheving an impressive number of theoretical and experimental studies.

In this paper are presented the *in vivo* studies obtained after the administration of 1.7 ml/kg solution containing glycerol-iron-oxide nanoparticles. The concentration injected in the rats was chosen accordingly to our previous studies [20] thus allowing us to evaluate the influnece of the iron oxide surface from the histopathological point of view.

The *in vivo* toxicity study (24 h) was performed with glycerol-iron-oxide nanoparticles administered by intraperitoneal injection with a concentration of 1.7 ml/kg. The animals survived after the administration of glycerol-iron-oxide nanoparticles, but they showed severe symptoms such as: lethargy, vomiting or diarrhea during the experiment. The histopathological assessment of the liver, kidney and spleen tissues have been performed.

After 24 h from intraperitoneal injection we observed significant macroscopic histopathological modifications in the case of the liver, kidney and spleen tissues compared to the reference group.

In Fig. 2 are presented the optical images of the rat liver after 24 h of exposure to glycerol-iron-oxide nanoparticles (1.7 ml/kg). The microscopic observations of the rat kidney injected with glycerol-iron-oxide nanoparticles (1.7 ml/kg) after 24 h are reported in Figure 3. Also, in fig. 4 are presented the microscopic observations of the rat spleen injected with glycerol-iron-oxide nanoparticles (1.7 ml/kg) after 24 h.

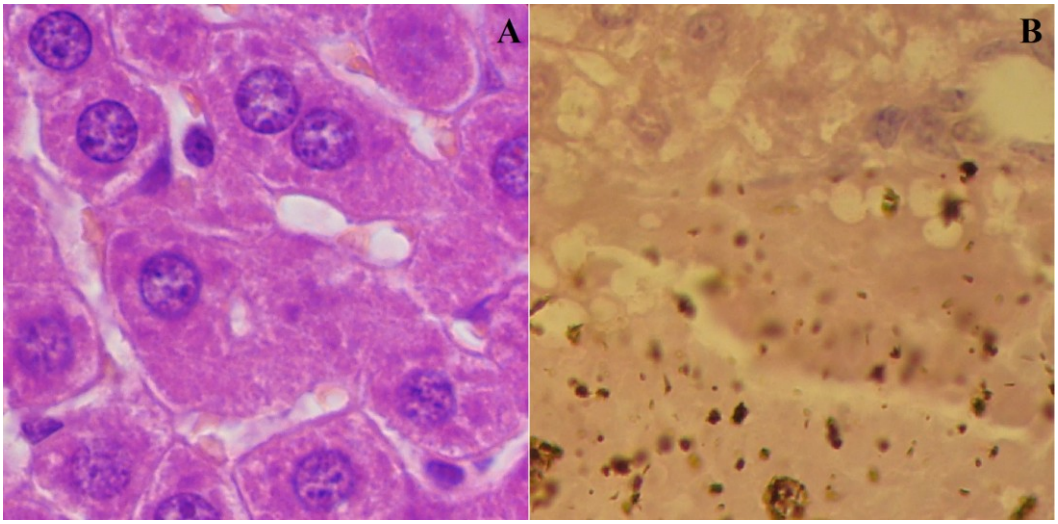

Fig. 2: Light optical images of the liver after 24h exposure to glycerol-iron-oxide nanoparticles at a concentration of 1.7 ml/kg (B) and reference specimen (A).

Pathological sections of liver after injection with a 1.7 ml/kg dose of glycerol-iron-oxide nanoparticles (Figure 2B) show that the architecture of the liver was strongly altered by the presence of the nanoparticles. The negative effects of the glycerol-iron-oxide nanoparticles are highlighted by the presence of hepatocytes with anisokaryosis, nucleoli, formation of chromocenters and focal intra hepatocyte cholestasis (HE, 600x) in the liver of treated rats (Figure 2B). According to the results reported in literature [42] this kind of liver modifications are possible (in a moderate manner) even in conventional housing conditions.

Moreover, it could easily be observed the presence of the granulo vacuolar cytoplasmic degeneration and microgranular brown pigment deposits in Kupffer cells (HE, 600x) (Figure 3B).

Fig. 3 (A and B) presents the kidneys pathological micrographs of rats before and after injection with a 1.7 ml/kg dose of glycerol-iron-oxide nanoparticles.

Figure 3B reveals that the kidney tissue does not preserve the structure of the reference tissue (Figure 3A) exhibiting significant modifications.

In the case of kidneys we observed that the tubular cells have marked cytoarchitectural distorsions and present anisochromia with formation of chromocenters. Also, a high granular cytoplasmic degeneration (HE, 600x, Figure 3B) was revealed. On other hand, the nuclei are enlarged and their contours are irregular. Furthermore, we observe an important deposition of microgranular brown pigment in the renal interstitium (HE, 600x).

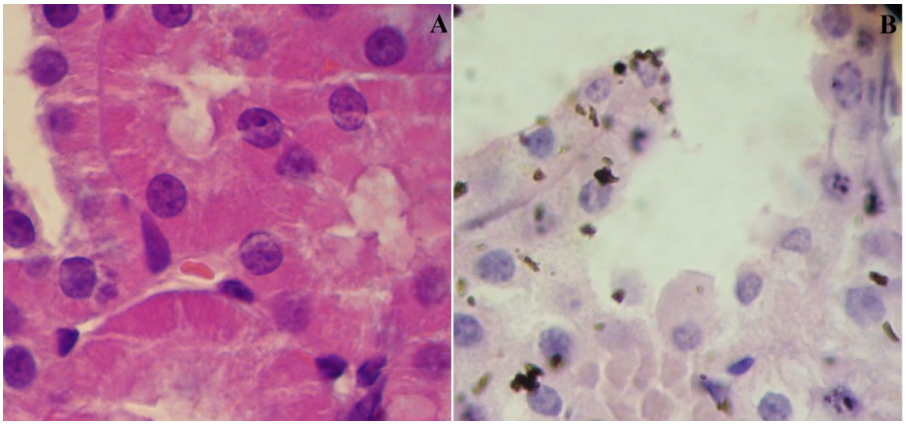

Fig. 3: Light optical images of the kidney after 24h exposure to glycerol-iron-oxide nanoparticles at a concentration of 1.7 ml/kg (B) and reference specimen (A).

The spleen pathological sections before and after injection with a 1.7 ml/kg dose of glycerol-iron-oxide nanoparticles are reported (Fig. 4A-B). Fig. 4B shows that the morphology and the structure of the spleen tissue was very affected by the glycerol-iron-oxide nanoparticles compared with the morphology of the reference (Fig. 4A).

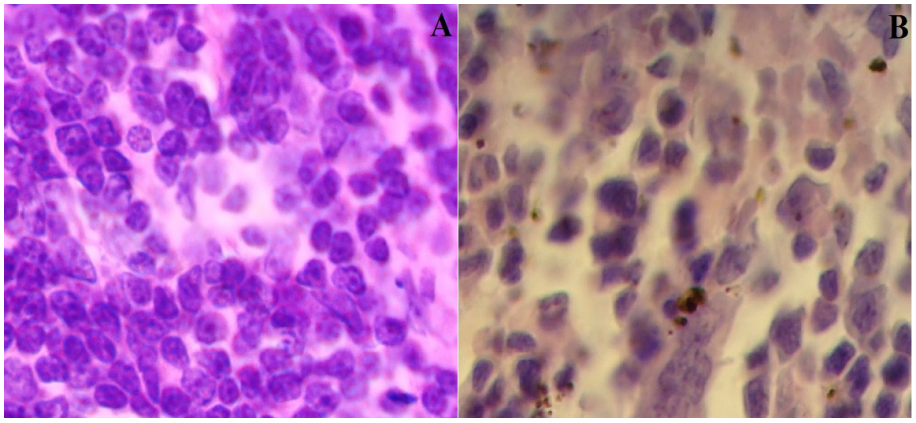

Fig. 4. Light optical image of the spleen after 24h exposure to glycerol-iron-oxide nanoparticles at a concentration of 1.7 ml/kg (B) and reference specimen (A).

The spleen red pulp was observed after injection with a 1.7 ml/kg dose of glycerol-iron-oxide nanoparticles (Figure 4B) along with an important number of monocytes. Furthermore, the nuclear contour presents asymmetry. Moreover, the anisochromia with focal chromocenter formation was observed in the spleen after injection of glycerol-iron-oxide nanoparticles.

In this study we have observed that the tested glycerol-iron-oxide nanoparticles induce many morphological alterations such as an increase of granulomas or tissue damage to the liver, kidneys and spleen. The presence of structural and morphological modifications of the studied tissues could be explained by the high concentration of glycerol-iron-oxide nanoparticles injected in the rats specimens.

5. Conclusions

In this paper we reported the structural, morphological and biological properties glycerol-iron-oxide nanoparticles synthesized by an adapted co-precipitation method. The TEM images showed that the obtained nanoparticles had an ellipsoidal morphology and were uniformly distributed. Also, the rings from the SAED pattern confirms the structure of iron oxide.

On the other hand, we have reported that the glycerol-iron-oxide nanoparticles induce many morphological alterations such as an increase of granulomas or tissue damage to the liver, kidneys and spleen. The presence of structural and morphological modifications of the studied tissues could be explained by the high concentration of glycerol-iron-oxide nanoparticles injected in the rats. However, the toxicity of iron-oxide-glycerol nanoparticles should be further investigated with other cells and other methods in order to understand the real threat that they can pose if used in biomedical applications.

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