THE EUKARYOTE CELL INTERACTION WITH DOPED TiO_2 NANOPARTICLES

G.C. CORNEANU[1, 2], C. CRĂCIUN[3], M. CORNEANU[4], C. LAZĂU[5], I. GROZESCU[5]
S. TRIPON[3]

[1]University of Craiova, Genetics Dept., 200585-Craiova, Romania, gabicorneanu@yahoo.com; [2]West University *Vasile Goldiş* Living Sciences, Arad, Romania; [3]*Babeş-Bolyai* University, Electron Microscopy Center, 400006-Cluj-Napoca, Romania, ccraciun@hasdeu.ubbcluj.ro, tripon_septimiu@yahoo.com; [4]University of Agronomical Sciences and Veterinary Medicine of Banat, Genetics Engineering Dept., 300645-Timisoara, Romania micorneanu@yahoo.com ; [5]I.N.C.-D.E.M.C., 300860-Timisoara, Romania, carmenlazau@yahoo.com; grozescu@icmct.uvt.ro

Abstract. *The experiment was performed in Mus musculus, being analyzed the liver ultra-structural features, as well as the nanoparticles interaction (single TiO_2 nanoparticles of 10-20 nm, or conjugate with a metal, TiO_2-Fe, TiO_2-Pt or TiO_2-Ag) with the hepatic cell. The small nanoparticles can penetrate in hepatocyte, their effect being dependent on the conjugate metal kinds. The TiO_2-Pt and TiO_2-Ag nanoparticles induced reversible effect, while the TiO_2-Fe nanoparticles alter the hepatocyte ultra-structure. Depending on the metal kinds, the TiO_2-Me nanoparticles can be in relation with smooth and rough endoplasmic reticulum and with mitochondria, or in lipid droplets, being also present in the Kupffer cell.*

1. Introduction

Initially TiO_2 nanoparticles were considered an inert material, because it is formed of very small size. Subsequent, was point that the TiO2 nanoparticles can induce lesions at the chromosomes and DNA, as well as at the ultrastructural level. They can migrate in different organs, and induce oxidative stress and cell death. As result of the studies performed by International Agency for Research on Cancer (IARC), was re-revaluated the TiO2 toxicity, they being moved in the **2B** group of materials, as possible carcinogen for men [1]. The biological reactivity of the TiO_2 nanoparticles is dependent on different features: their crystallization shape (anatase, rutile or broklite), amount, size, a/o. Recently, were obtained TiO_2 nanoparticles doped with different chemical elements, as well as nanoparticles included in liposome. From the noble metals, the mostly used were silver, gold and platinum, as well as other metals (iron, copper, a/o). The noble metals are resistant at oxidation process. In many studies, the silver was deposited on TiO_2 surface, being more reactive than gold or platinum, having the photocatalitic effect proper to TiO_2 nanoparticles, as well as the ability to prevent bacteria development [2].

In the human or animal body, the research was performed to establish an eventual cytotoxic effect depending on different parameters: the nanoparticle size [3], their single presence or doped with other element [4], activation or not by UV rays [5], [6], encapsulated or not in different bioactive substances, a/o. The cyto- and genotoxicity analysis of the ultrafine TiO2 nanoparticles, in human lymphoblast cell, evidenced that these can induce cyto- and genotoxicity in a significant mode [7]. The analysis of the biological effects of three kinds of nano-TiO_2 of different size (10-20 nm anatase; 50-60 nm anatase and 50-60 nm rutile) on DNA and cell ultrastructure of the 293T and CHO cells, reveal that the all three kings of nano-TiO_2 manifest a higher toxic effect on tumor cells than on normal cells [8]. Also, the nano-TiO_2 from anatase kinds, were observed in the cytoplasm of CHO cells, indifferent on this size (50-60 nm or 10-20 nm).

The biological investigation point out different biological effects of the TiO_2-Me nanoparticles, depending on the metal type (silver, golden, copper or platinum). Thus, the use of the TiO_2-Pt and TiO_2-Au nanoparticles, are preferable in comparison with TiO_2-Ag or TiO_2-Cu doped nanoparticles [9].

There are a few studies regarding the TiO_2 nanoparticles interaction with the eukaryote cell. In a synthesis from 2006 [10], there wasn't present any paper regarding the interaction between the eukaryote cell with the TiO_2 nanoparticles. In previously researches, our groups point out the relation between TiO_2 nanoparticles with eukaryote animal cell [11], or vegetal cell [12]. In other paper [8], was underlined that the nano-TiO_2 from anatase kinds, were observed in the cytoplasm of CHO cells, indifferent on this size (50-60 nm or 10-20 nm).

In this paper the interaction of titanium dioxide nanoparticles with animal cell (hepatocyte from *Mus musculus*), depending on the chelate metal (single or chelate with silver, platinum, or iron) was analyzed.

2. Materials and methods

2.1. The preparation of titanium dioxide nanoparticles

Undoped and doped titanium dioxide nanocrystals were synthesized by the sol-gel route, using the precursors: titanium isopropoxide, isopropyl alcohol, distilled water, nitric acid, hexachloroplatinate acid, silver nitrate and ferrous nitrate. Over 30 ml of isopropyl alcohol, 5 ml of titanium isopropoxide were added in drops, under continuous stirring with the magnetic stirrer. After a few minutes of stirring, distilled water was added, continuously controlling the solution pH with nitric acid in order to avoid the precipitation. In the case of platinum-doped and iron-doped TiO_2 ions, after the adjustment of the pH (pH=2.5 for Pt and Ag, pH=5 for Fe), the previously prepared solutions, of were added hexachloroplatinate acid for doped with Pt, silver nitrate for doped with Ag and ferrous nitrate for doped with Fe under continuous stirring. In all cases, the gel was dried and washed in order to remove the secondary reaction products. The calcinations was achieved in the oven, at a temperature of 250ºC for undoped TiO_2 and 500ºC for Pt, Ag or Fe-doped TiO_2, for 3 hours.

The obtained materials were characterized by X-ray diffraction (XRD), scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDAX). XRD spectra were recorded at room temperature with a BRUKER D8 ADVANCE X-ray diffractometer using Cu Kα radiation in θ:2θ configuration. The SEM images were made in an Inspect S scanning electron microscope coupled with EDAX device.

2.2. Biological material

The experiments were performed on young females of *Mus musculus*, intra-peritoneal injected with a suspension of titanium dioxide. The animals were intraperitoneal injected (five injections of 0.5 ml each, one two days) with a 0.01% TiO_2 or TiO_2-Me suspension. A day after the last injection, the animals were sacrificed through the carotid artery section, and their liver were used for the electron microscopy investigations.

2.3. Electron microscopy investigation

The pieces of about 1 mm^3 of liver, were prefixed in a 2.7% glutaraldehyde solution (2 ½ hours), postfixed in a 1% Millonig solution (1 ½ hour) and then included in Epon 812. The seriated sections of about 60 nm thickness, were contrasted with uranyl acetate and lead citrate, and then analyzed at a TEM JEM JEOL-1010 electron microscope in Electron Microscopy Centre, *Babes-Bolyai* University (Cluj-Napoca, Romania).

3. Results and discussions

3.1. TiO_2 particles feature

The XRD patterns (Fig. 1) analyses present the crystallization as *anatase* form of the undoped / Pt, Ag or Fe doped TiO_2, even if the calcinations temperatures for the TiO_2 doping surpass the value of 500ºC. The presence of the dopant in the crystalline network of the titanium dioxide tripped the phase transition from *anatase* to *rutile*. From the diffraction spectra it is noticed that the dopants did not present separate peaks, which means that this is distributed uniformly in the crystalline network.

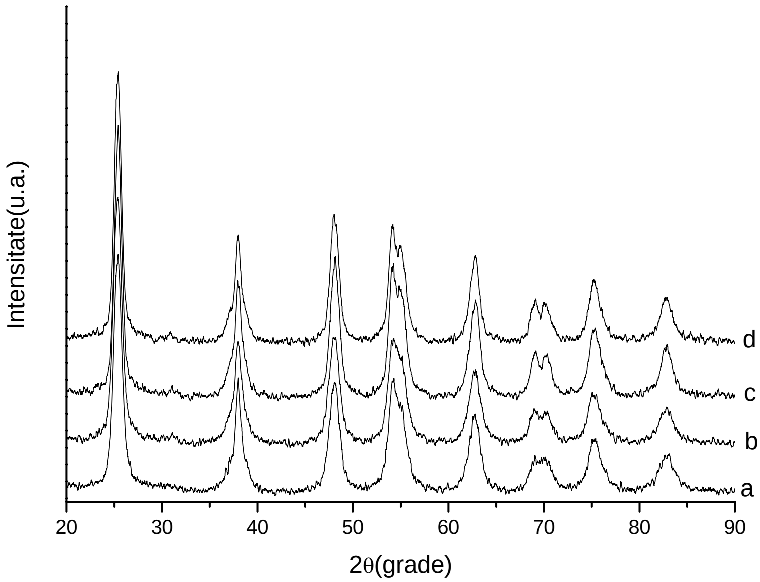

Fig. 1. XRD pattern for: (a) TiO_2 undoped; (b) TiO_2-Fe; (c) TiO_2-Pt; (d) TiO_2-Ag.

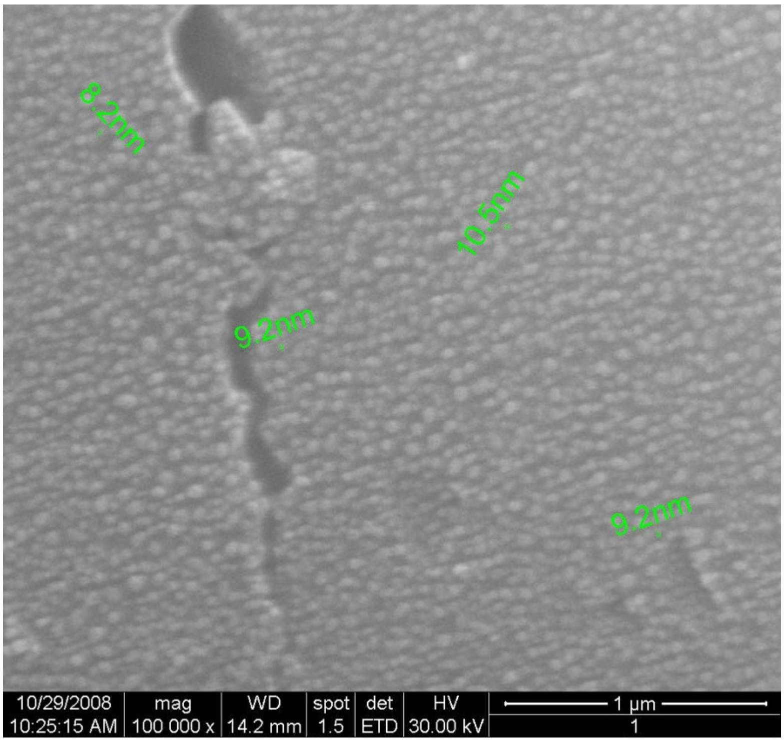

Fig. 2. SEM morphology for TiO_2 undoped.

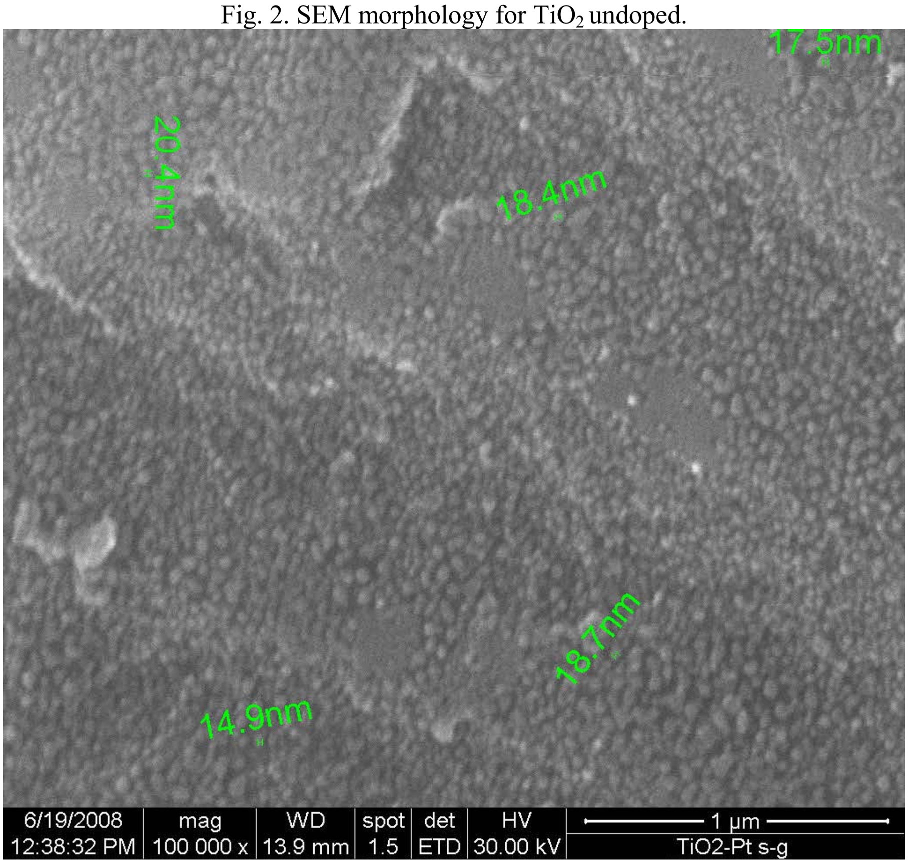

Fig. 3. SEM morphology for TiO2-Pt

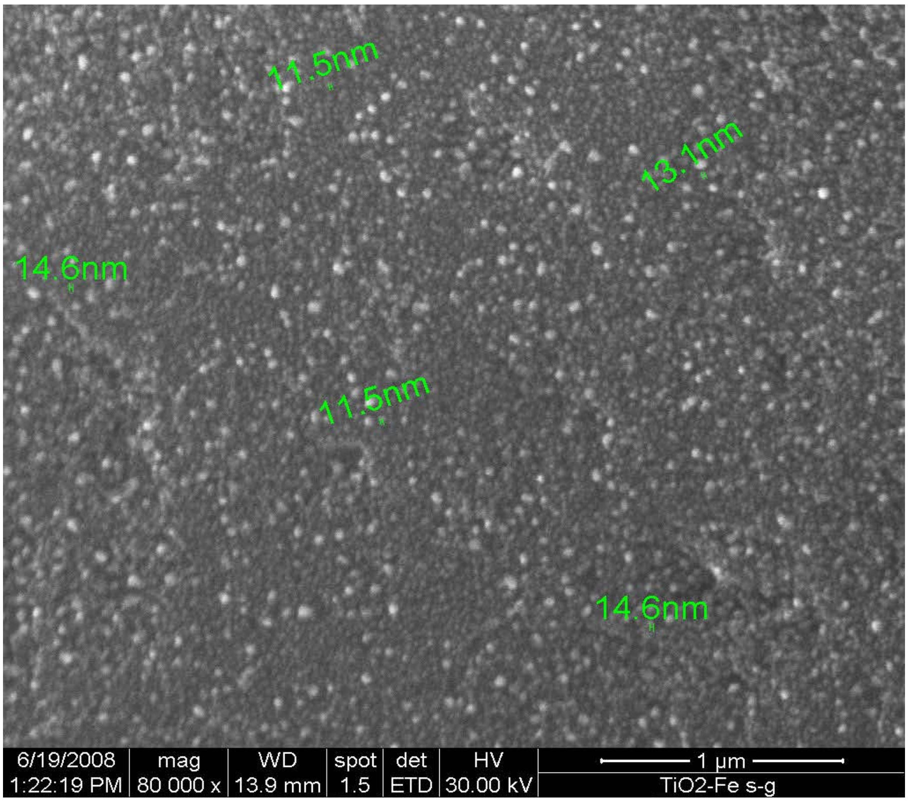

Fig. 4. SEM morphology for TiO2-Fe

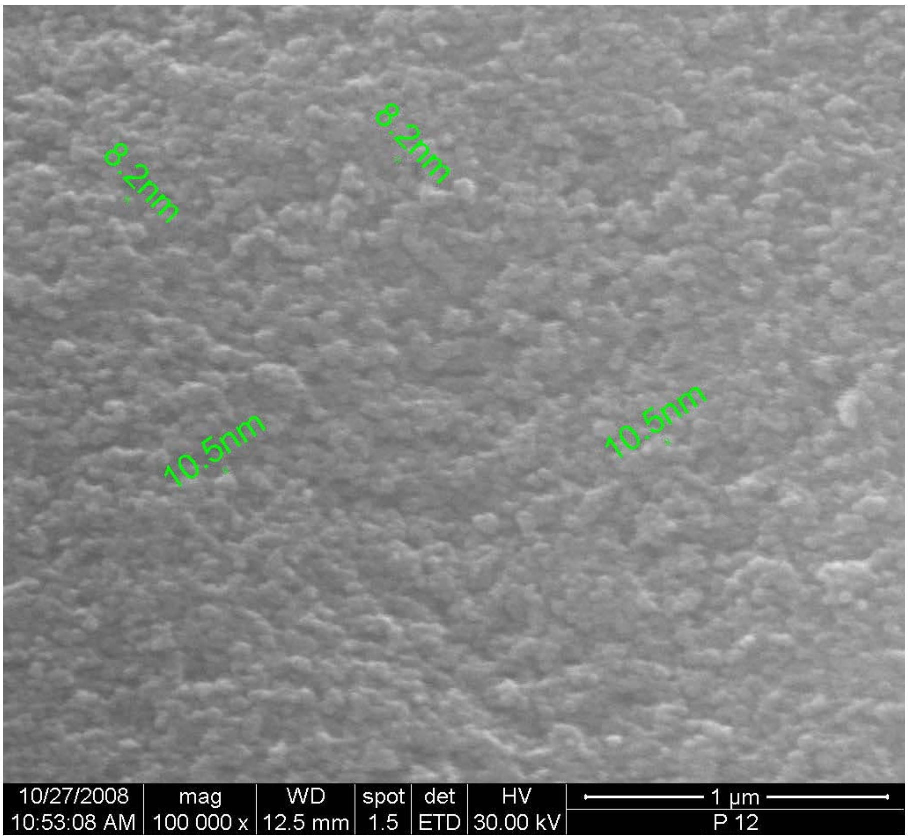

Fig. 5. SEM morphology for TiO_2-Ag

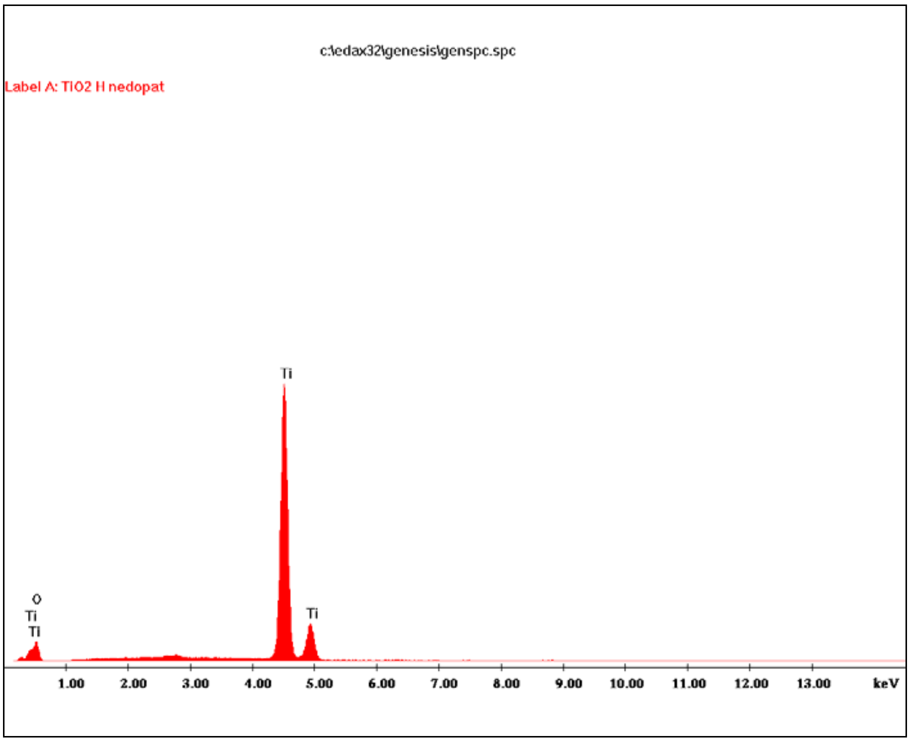

Fig. 6. EDX spectrum for elemental analysis of undoped TiO_2

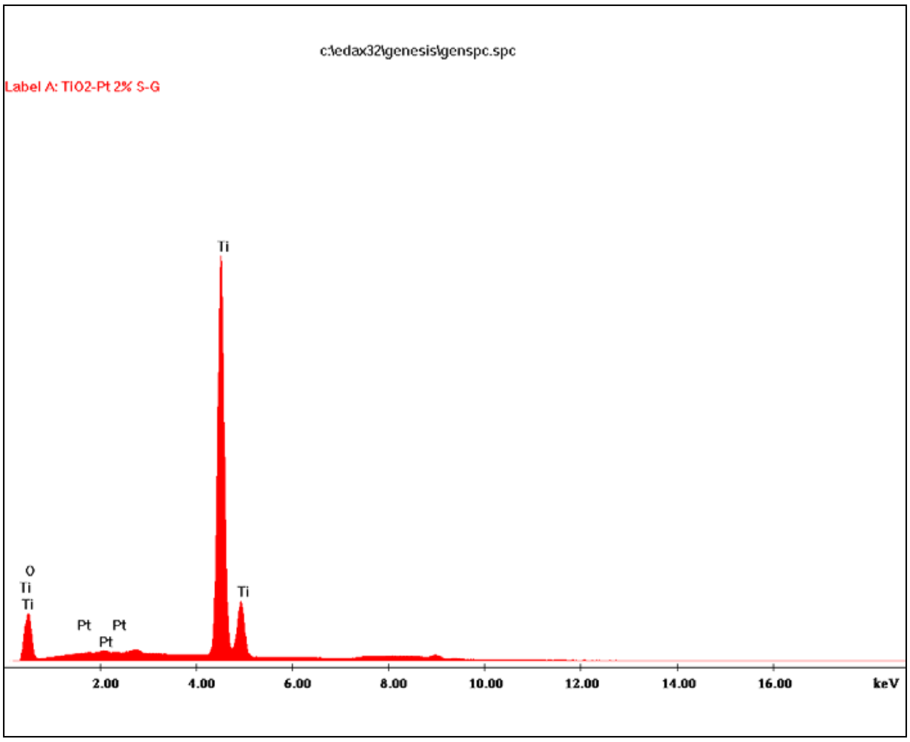

Fig. 7. EDX analysis for elemental analysis of TiO2-Pt

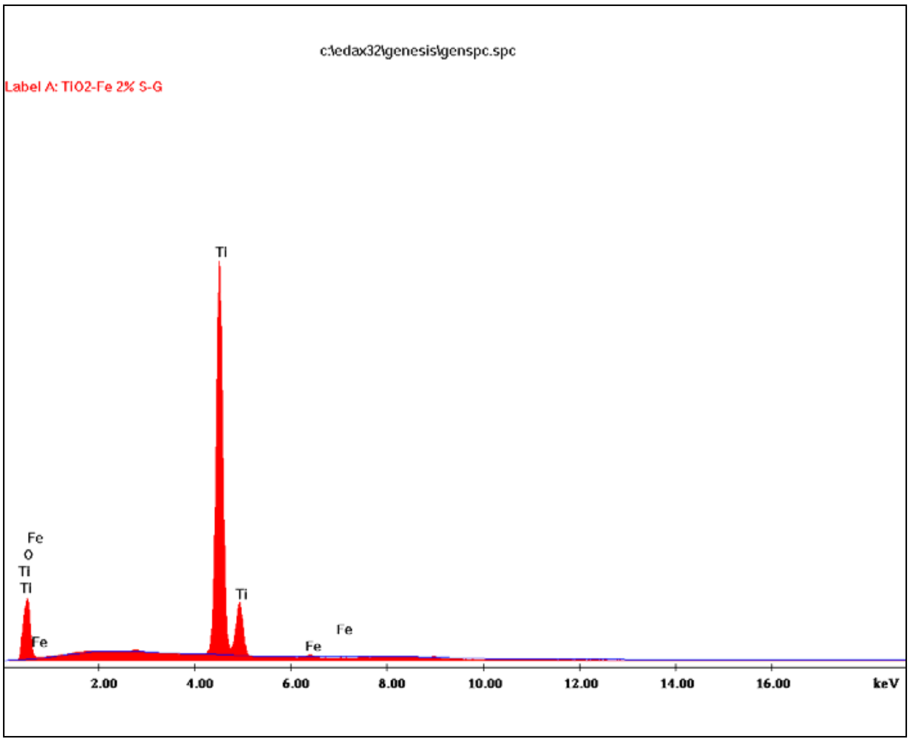

Fig. 8. EDX analysis for elemental analysis of TiO2-Fe

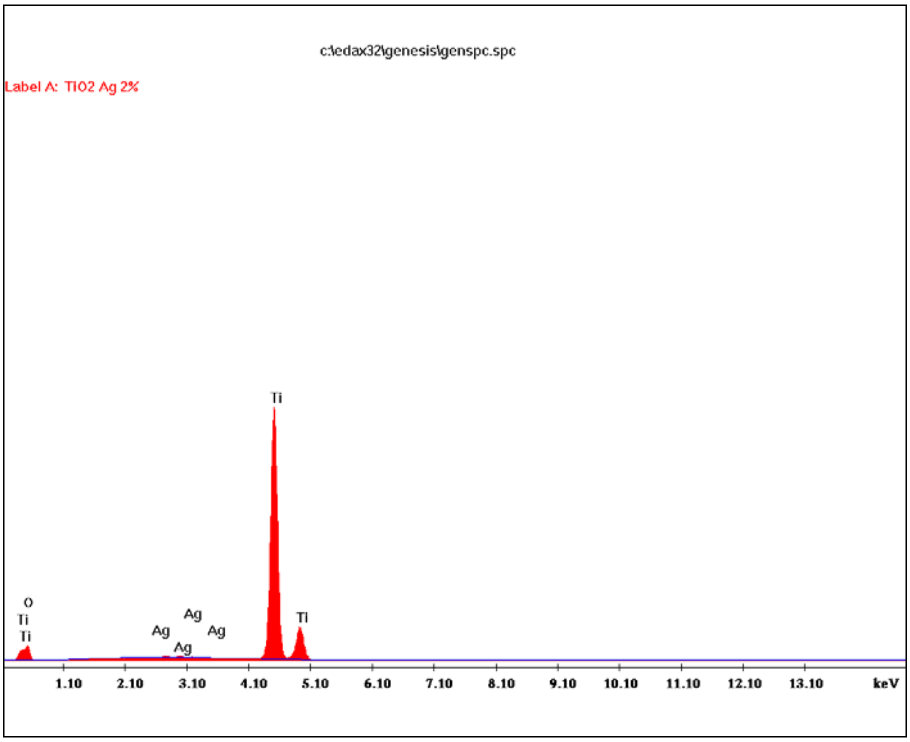

Fig. 9. EDX analysis for elemental analysis of TiO_2-Ag

From the surface morphology (SEM) it can be observed that the TiO_2, as well as TiO_2-Pt, TiO_2-Ag and TiO_2-Fe nanospheres dimensions range between 10-20 nm, having a spherical shape, indifferent of the chelated metal (Figs. 2 - 5). EDAX analysis presents undoped TiO_2 nanoparticles, as well as the nanoparticles with Pt, Ag and Fe ions in titanium dioxide structure (Figs. 6 - 9).

3.2. TiO_2 nanoparticles interaction with the eukaryote cell

Normal liver ultrastructure. The liver has a normal ultrastructure, similar to other reported data [13], [14]. The hepatocytes present one sfaerical nucleus (sometimes two), with a regulated outline and heterochromatine disposed in a small electrondense masses, usually under inner envelope (Fig. 10). In cytoplasm there are numerous mitochondria spherical or slightly elongated with cristae disposed transversally, rough endoplasmic reticulum well represented, numerous glycogen particles (Fig. 11), Golgi complex, smooth endoplasmic reticulum (SER) with a discrete presence, having a bigger concentration in the areas with glycogen, a/o. The lipid droplets are very rarely in cytoplasm, as small anelectrondense granules, especially at the vascular pole of the hepatocyte in the vicinity of Ito cell (Fig. 12).

The Kupffer cells are in a normal activity (Fig. 13), having accumulated a small amount of phagocytated material.

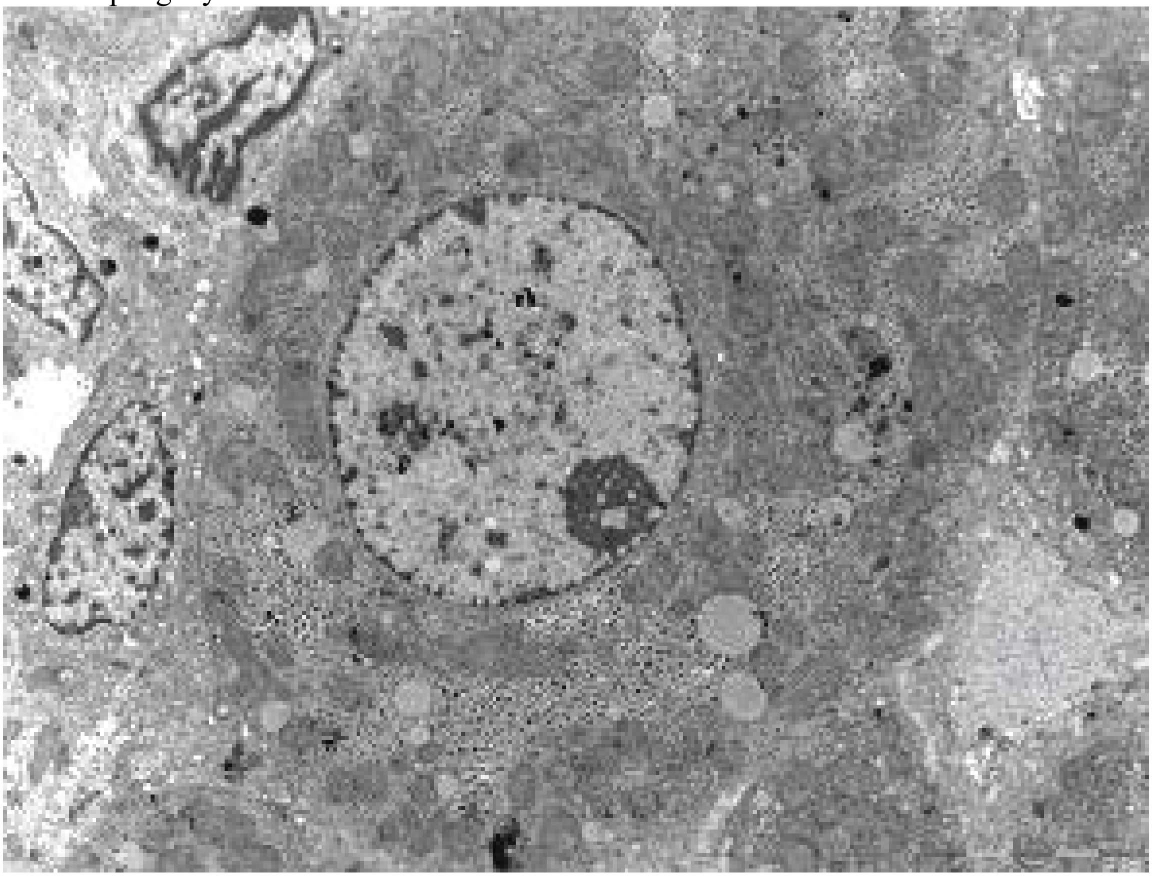

Fig. 10. Control. Hepatocyte with the normal ultrastructure.

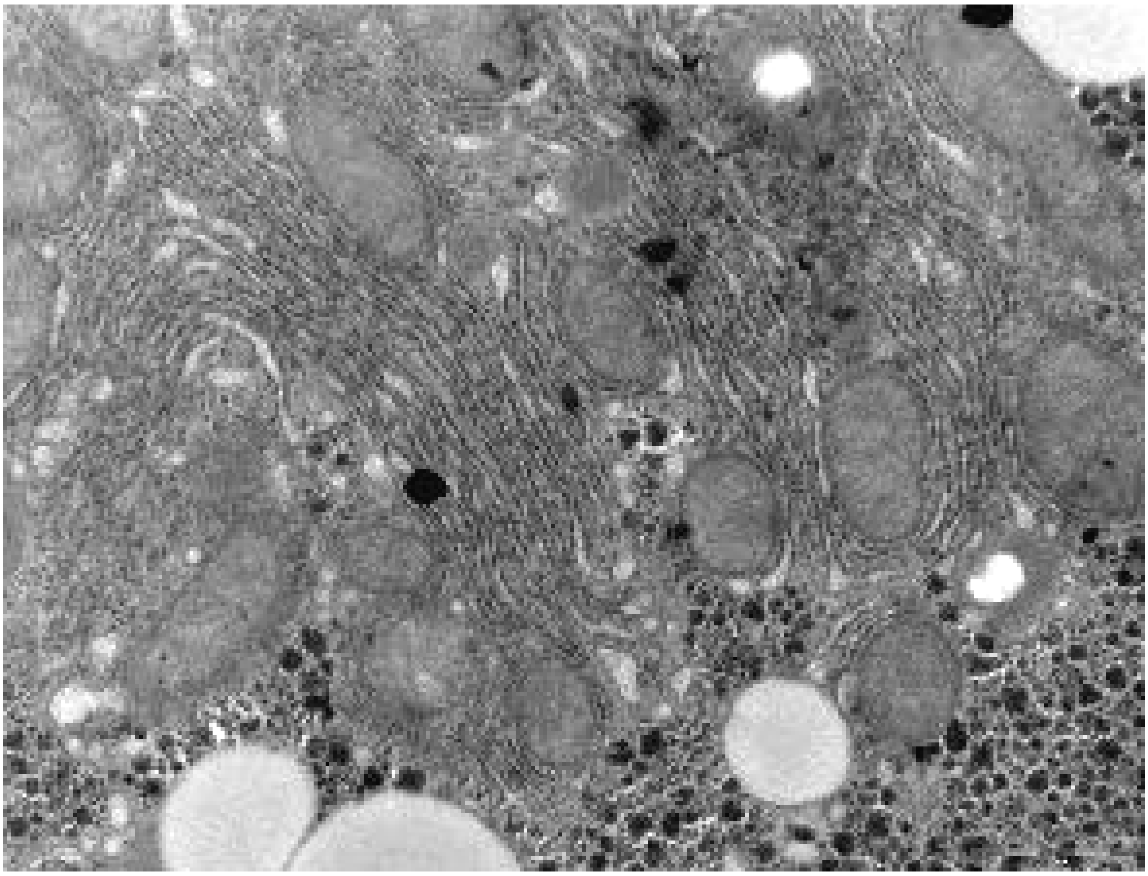

Fig. 11. Control. Cytoplasma with cellular organelles and glycogen

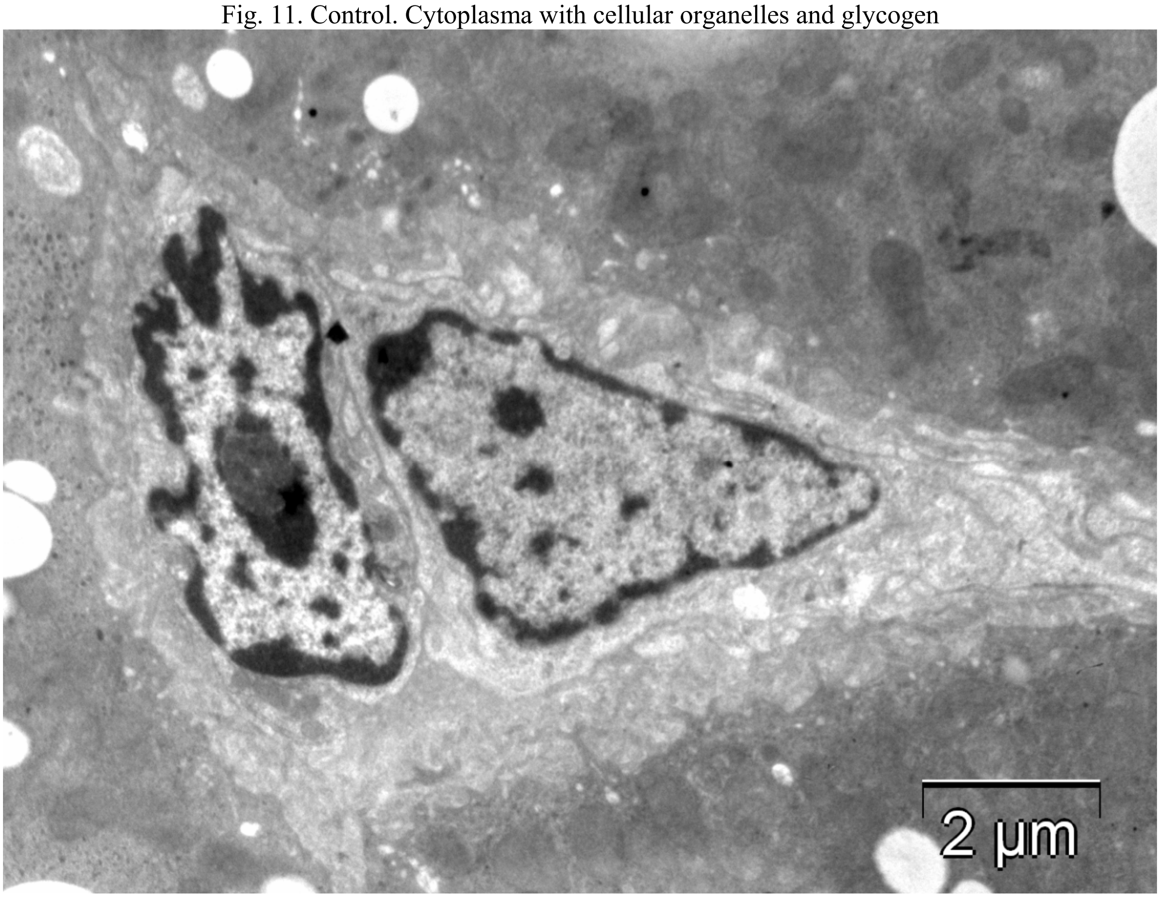

Fig. 12. Control. Vascular pole of hepatocytes and Ito cell.

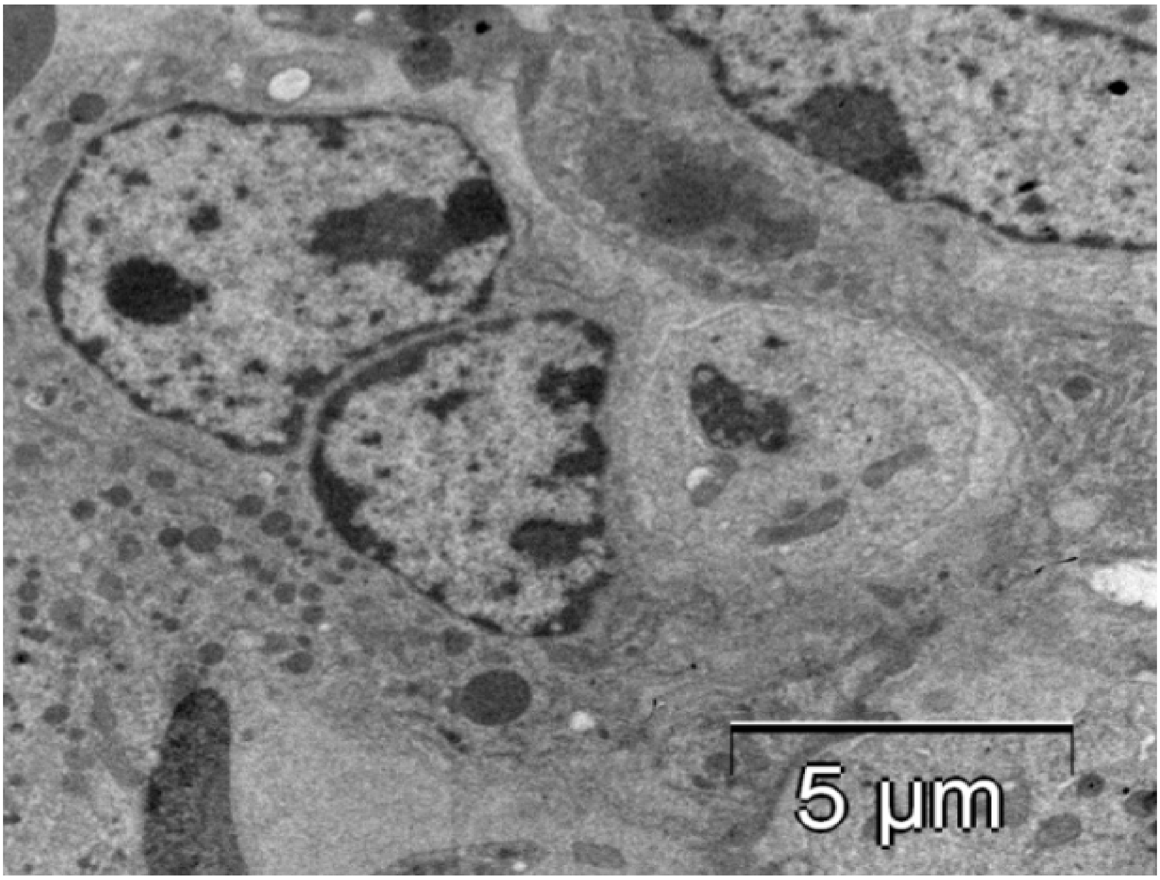

Fig. 13. Control. Kupffer cell in activity.

Undoped TiO_2 nanoparticles effect.

The presence of undoped TiO_2 nanoparticles, has a drastic affect under the metabolism and ultrastructure of the hepatocyte (Figs. 14-17). The nucleus, usually has 3-4 nucleoli, and an unregulated outline with invaginations, the heterochromatin having a parietal disposition, or spread as fine blocks in euchromatine (Fig. 14). The mitochondria are swelled, with matrix and crista rarefied, sometimes being in amitotic division (Fig. 15). The TiO_2 nanoparticles are accumulated in a big amount in the lipid droplets which became electrondense (fig. 15). Their transit from hepatocyte is practically absent, because they are absent in the Ito cell (Fig. 17). The glycogen microparticles are absent in the cytoplasma of hepatocytes. The cell response at the stress factor, is represented through presence, in a great number, of the vesicles of the smooth endoplasmic reticulum (SER), for counteract of the TiO_2 negative effect (Fig. 16). Also, the Kupffer cell is in metabolic activity.

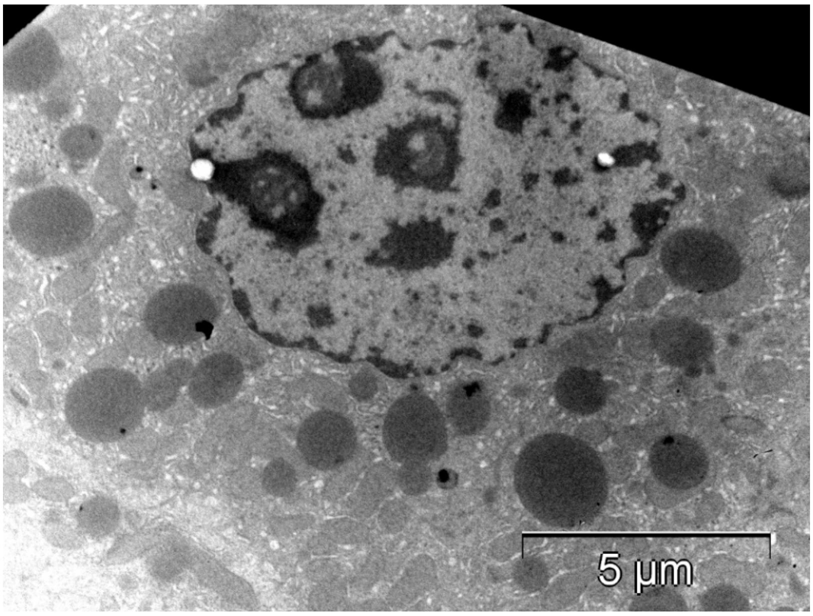

Fig. 14. TiO_2. Aterated nucleus and many SER vesicles.

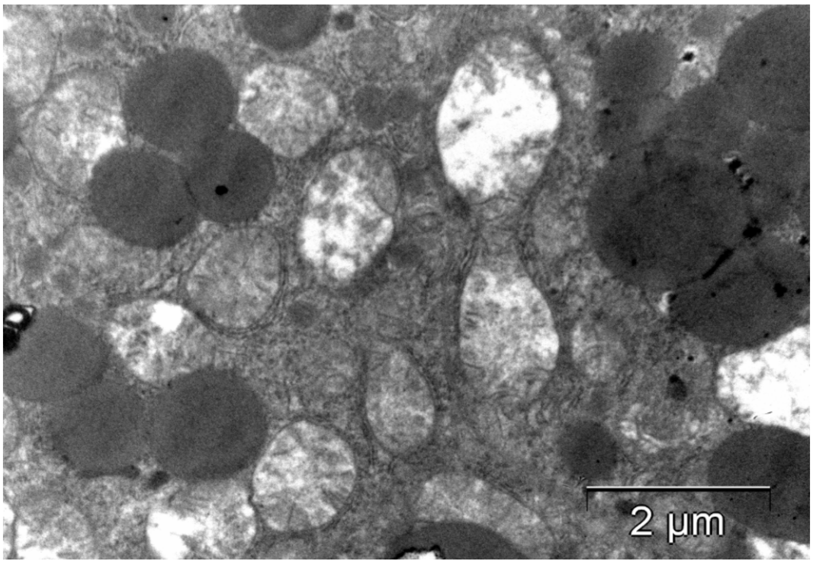

Fig. 15. TiO_2. Affected mitochondria, in amitotic division

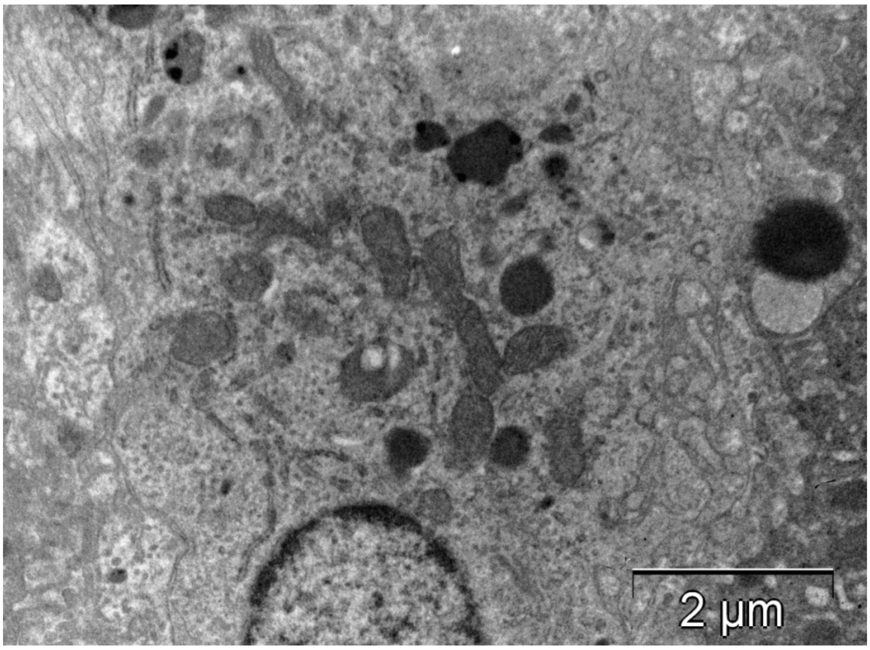

Fig. 16. TiO_2. The cell reacts to stress factor.

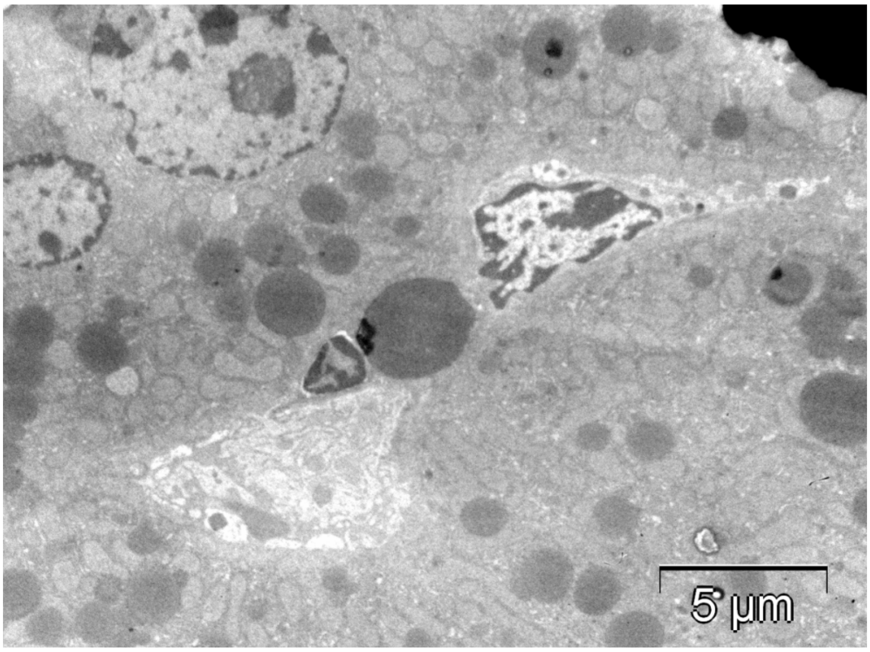

Fig. 17. TiO_2. Ito cell, without lipid droplets.

The TiO_2 – Ag nanoparticles effect.

The TiO_2-Ag nanoparticles induce reversible modifications in hepatocytes. The nucleus, with 1-3 nucleoli, presents the heterochromatine with parietal disposition or as fine blocks in its inner (Fig. 18). As result of the exogenous nanoparticles presence, the lipid metabolism is altered, hepatocytes having a big amount of lipid droplets (Fig. 18). Lipid droplets contain a moderate amount of TiO_2-Ag nanoparticles (Fig. 18). In some mitochondria, the TiO_2-Ag nanoparticles are massive accumulated (Fig. 19). As response of this stress factor, the vesicles of smooth endoplasmic reticulum is well represented in proximity (Fig. 19) Also ispresent Golgi complex. Practically, the glycogen microparticles are absent from hepatocytes.

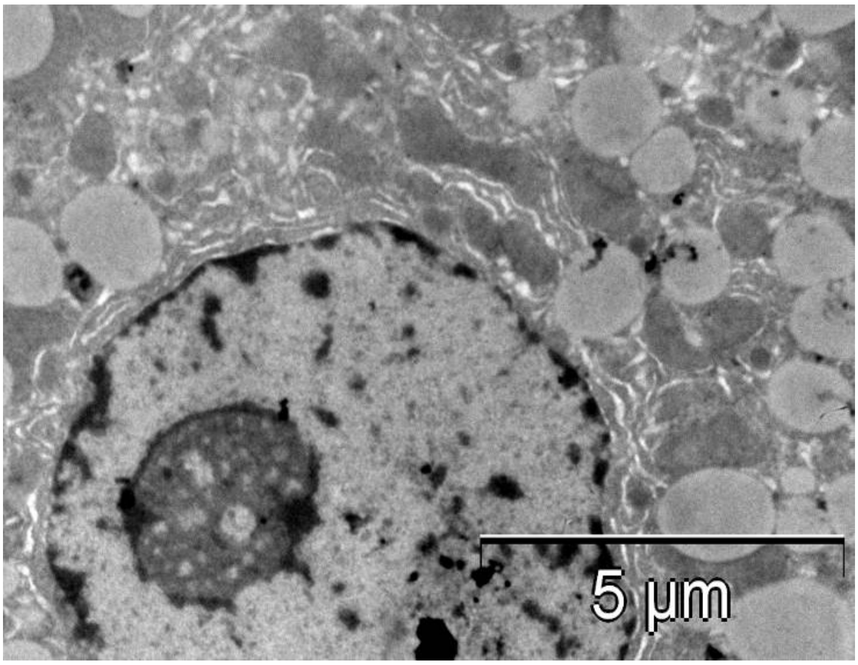

Fig. 18. TiO_2-Ag. Reversible alterations of the hepatocyte ultrastrucure.

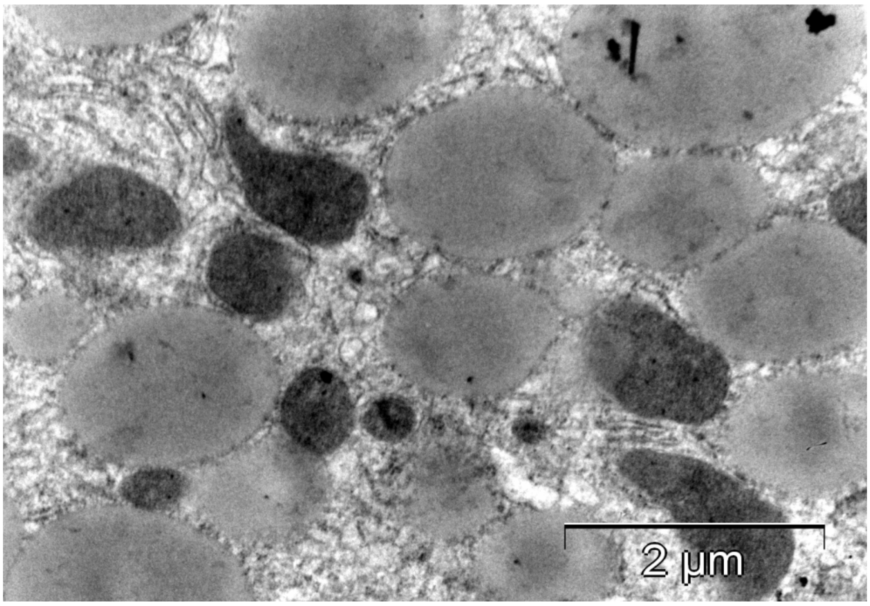

Fig. 19. TiO_2-Ag. Lipid droplets and mitochondria with TiO_2-Ag nanoparticles incorporated

Other reported researches [13] was evidenced an enhanced photocatalitic reactivity, in comparison with undoped TiO2 nanoparticles, and the doped TiO_2 nanoparticles not induced severe alterations in hepatocyte, similar to those induced by undoped TiO_2 nanoparticles.

The TiO_2 – Pt nanoparticles effect.

The TiO_2-Pt nanoparticles induced minor and reversible ultrastructural modifications. Some nuclei has an undulated outline (Fig. 20), the profiles of rough endoplasmic reticulum (RER) are slightly dilated and the vesicles of the smooth endoplasmic reticulum are slightly proliferated (Fig. 21).

Also, the hepatocytes do not present collagen fibers and cellular destructions. The mitochondria present a normal ultrastructure (Fig. 21). The small TiO_2-Pt nanoparticles penetrate in hepatocytes under shape of electrondense masses, being in strong relation with rough endoplasmic reticulum profiles, smooth endoplasmic reticulum vesicles and especially with mitochondria (Figs. 22, 23). There are mitochondria with a different amount of TiO_2-Pt nanoparticles, respectively much (Fig. 22), or little (Fig. 23). There were not evidenced any ultrastructural alterations of the cell organelles, induced by TiO_2-Pt. Also, in some Kupffer cells, there are many electrondense corpuscles, which probably contain TiO_2-Pt aggregates (Fig. 24).

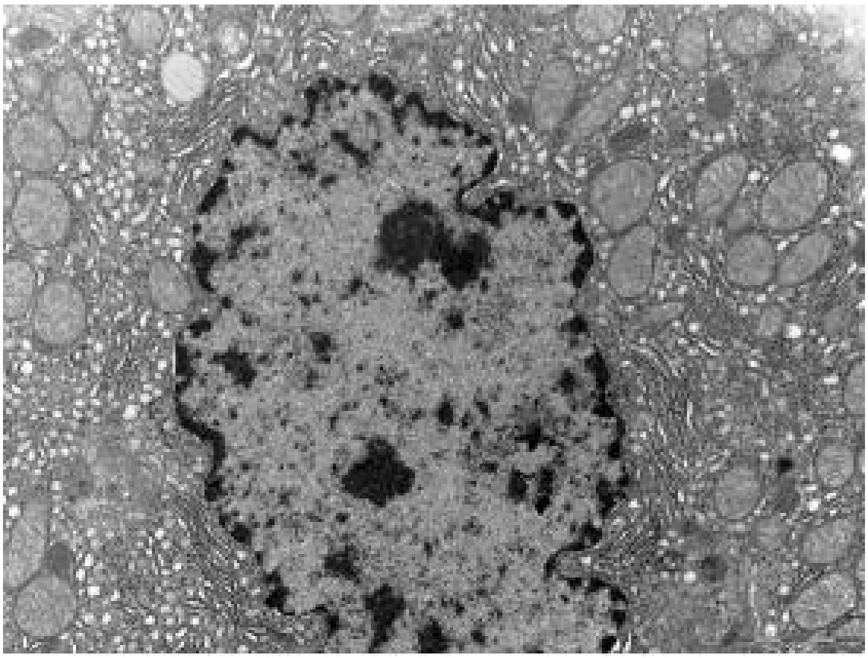

Fig. 20. TiO_2-Pt. Nucleus with unregulated outline.

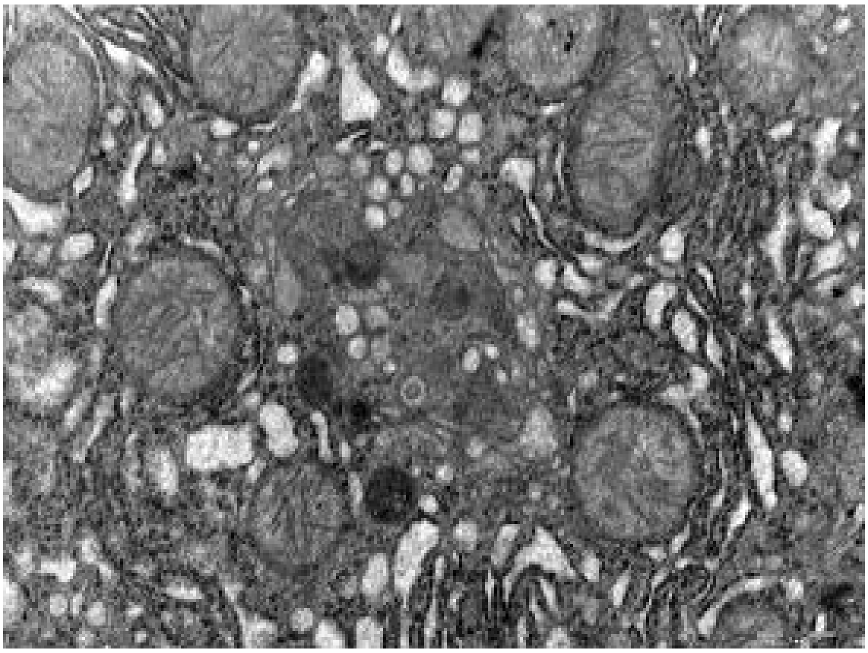

Fig. 21. TiO_2-Pt. Cellular organelles with slightly modifications.

In the hepatocyte of mouse, the TiO_2-Pt nanoparticles, manifest a high reactivity, in comparison with TiO_2-Ag nanoparticles. In the first case, the TiO_2-Pt nanoparticles penetrate in mitochondria and endoplasmic reticulum, while the TiO2-Ag nanoparticles are present in a moderate amount in the lipid droplets and massive accumulated in some mitochondria. The analysis of the biological effects of the TiO_2 doped with different metals (silver, golden, copper or platinum), reveals that the TiO_2-Pt and TiO_2-Au chelate nanoparticles, manifest an enhanced effects in comparison with TiO_2-Ag or TiO_2-Cu chelate nanoparticles [9]. Also, the chelated nanoparticles with some metals induced better biological effects in comparison with the unchelated TiO_2 [9].

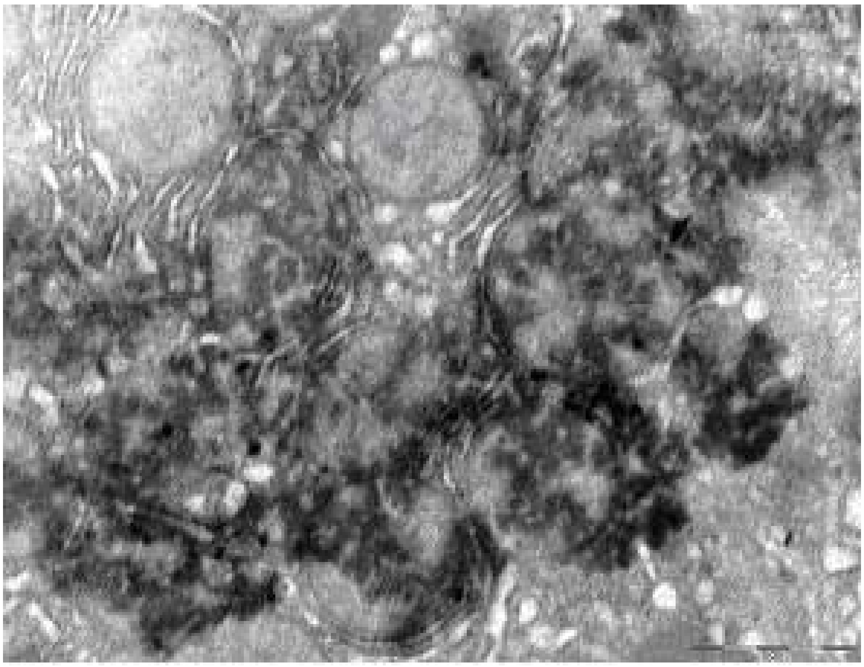

Fig. 22. TiO_2-Pt. Nanoparticles in strong relation with mitochondria and RER.

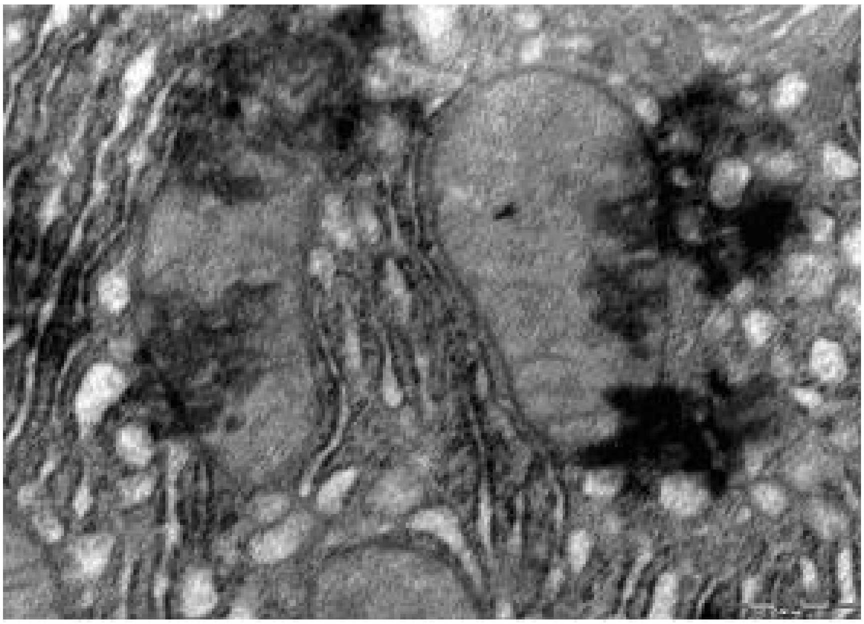

Fig. 23. TiO_2-Pt. Nanoparticles in close relations with mitochondria.

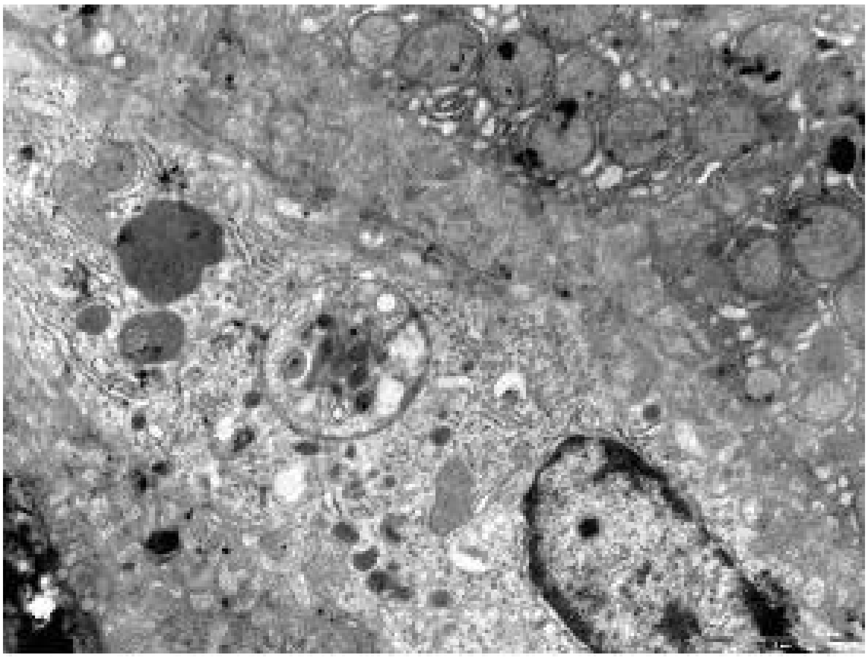

Fig. 24. TiO_2-Pt. Kupffer cell with electrondense granules.

In some researches performed with TiO_2-Pt and TiO_2-Au nanocomposites, point out that these manifest also an enhanced of the photocatalitic reactivity [14]. Also, in other experiment was established that, the chelated nanoparticles with some metals, induced better biological effects in comparison with the unchelated TiO_2 [9]. Similarly, at comparison of the biological effects of the TiO_2 doped with different metals (silver, golden, copper or platinum), the TiO_2-Pt and TiO_2-Au chelate nanoparticles, manifest enhanced effects in comparison with TiO_2-Ag or TiO_2-Cu chelate nanoparticles [9].

TiO2-Fe nanoparticles effect.

The TiO_2-Fe nanoparticles induce complex modifications at the hepatocyte level. The nuclei present deep incisures, having an unregulated outline (Fig. 25), the rough endoplasmic reticulum is poor represented, but instead the vesicles of smooth endoplasmic reticulum present a proliferation, as response to this stress factor (Fig. 26). Also, the mitochondria present the matrix and the mitochondrial crista rarefied, with slightly tendency to vacuolization (Fig. 26). The TiO_2-Fe nanoparticles are present in some hepatocytes, in relation with cellular organelles and nucleus (Figs. 27, 28). The Kupffer cells manifest an increased activity, having in their cytoplasm numerous electrondense granules represented by TiO_2-Fe and cell remnantes (Fig. 29).

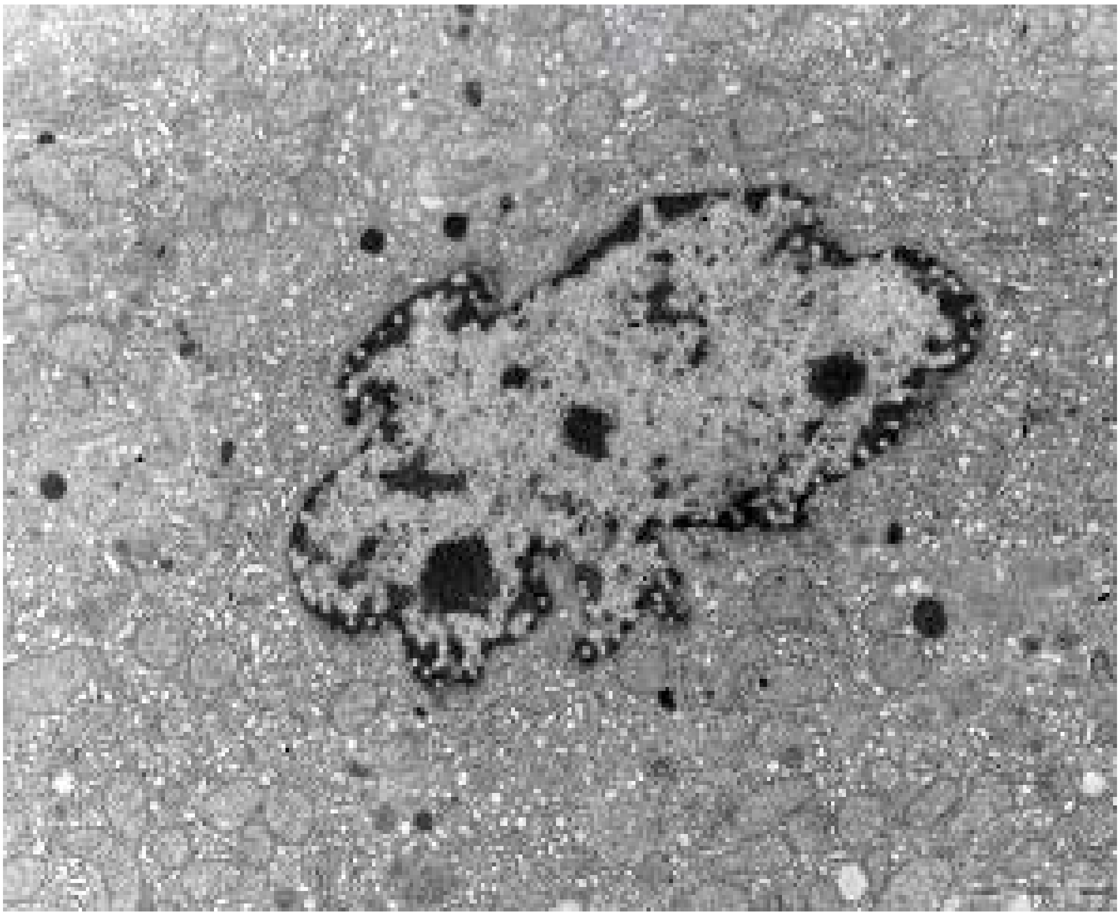

Fig. 25. Nucleus has deep incisures and heterochromatine blocks.

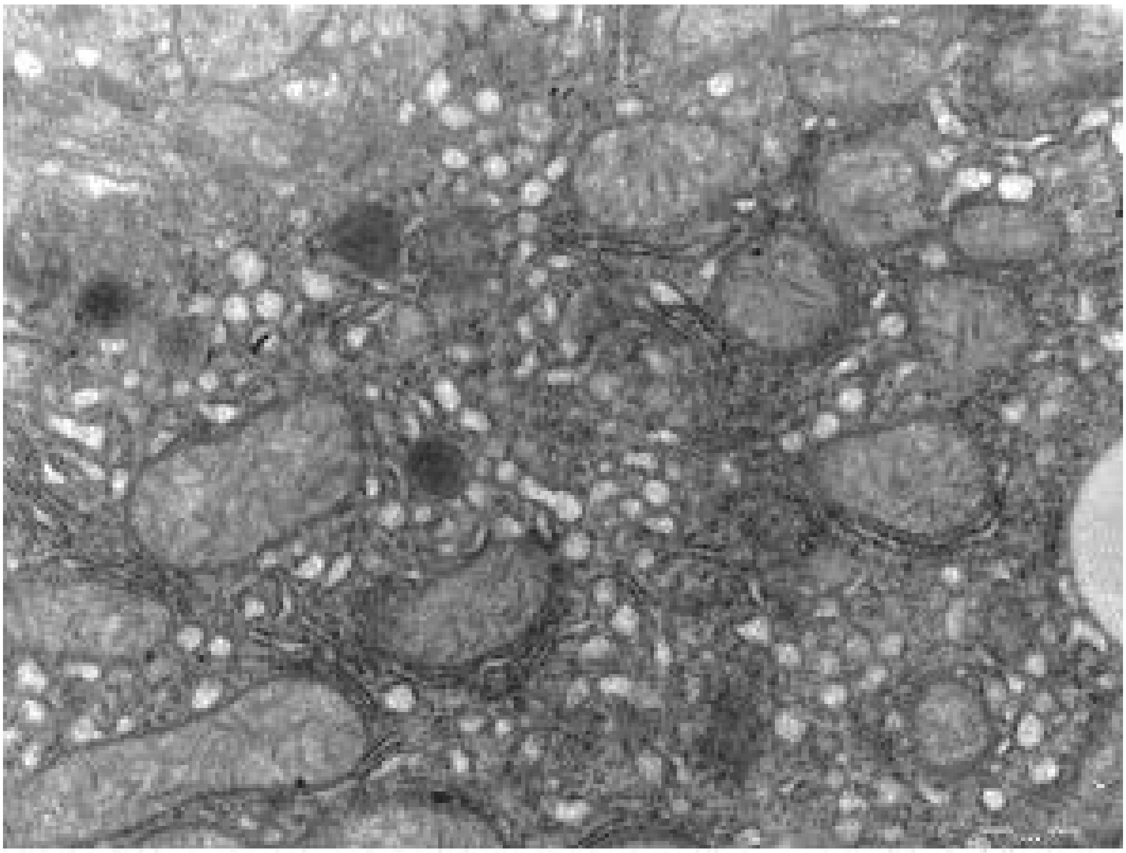

Fig. 26. Cytoplasm with vesicular SER and mitochondria.

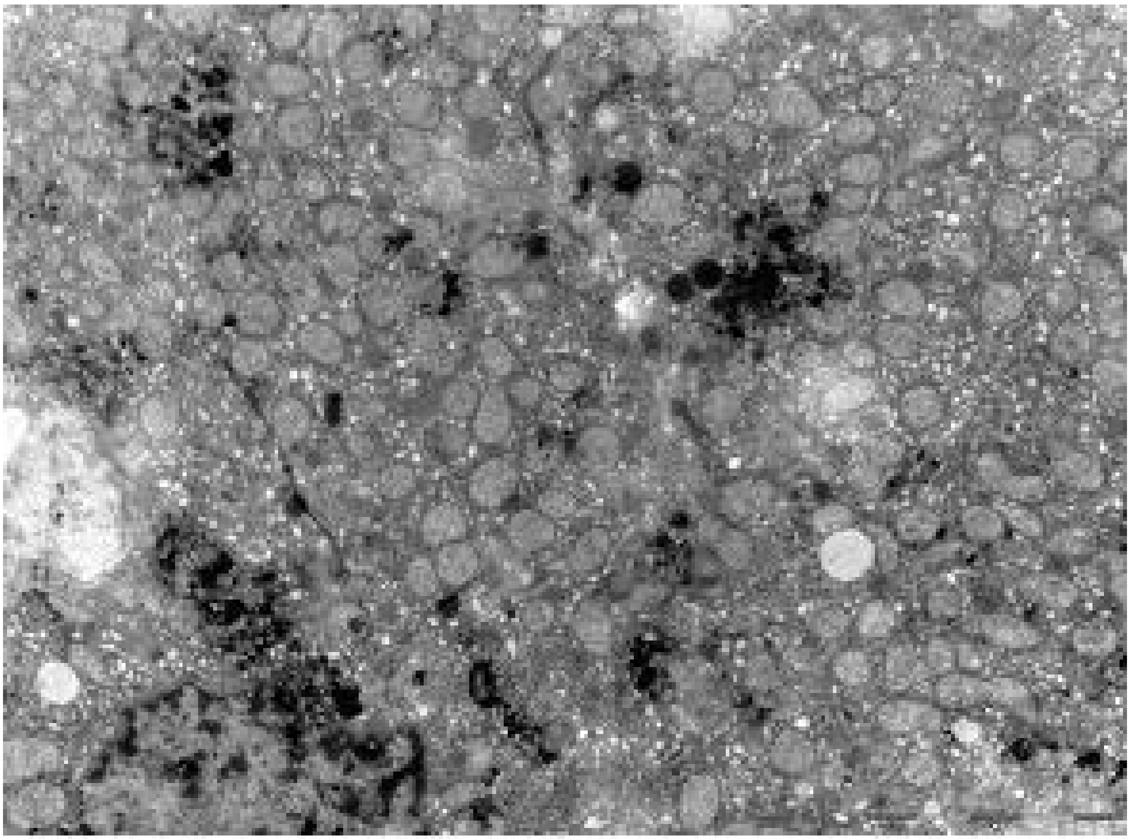

Fig. 27. TiO_2-Fe nanoparticles in hepatocytes.

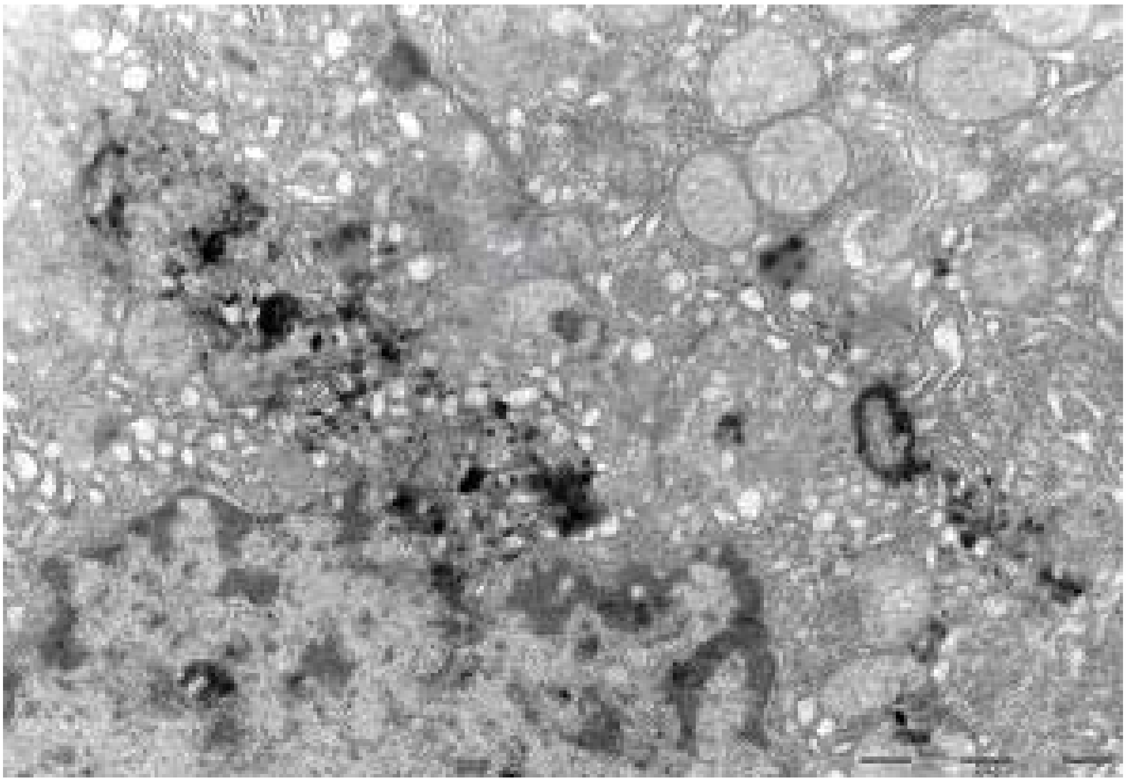

Fig. 28. TiO_2 nanoparticles in relation with nucleus and cellular organelles.

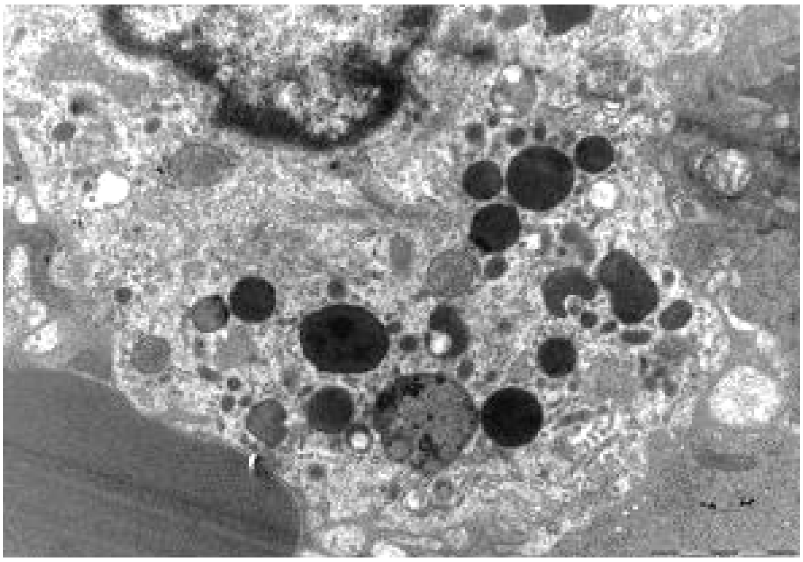

Fig. 29. Kupffer cell with intense activity of phagocytated electrondense bodies

Nanoparticles with iron or iron oxides, present a particular magnetic property (super-paramagnetism). In the medical area, they are used for mark and separation of biological materials, deliver of the drugs, analysis with magnetic resonance, a/o.

Conclusions

In this experiment was analyzed the TiO_2 nanoparticles effect (*anatase* crystallization form, size of about 10-20 nm), doped or not with a metal, at the liver level in *Mus musculus*, and their interaction with the cellular organelles. The titanium dioxide effect was dependent on the chelated metal. Also, depending on the chelated metal, the TiO_2 nanoparticles penetrate or not in hepatocyte, and there are in different relations with cellular organelles or cellular inclusions (lipid dropletss)

The TiO_2 nanoparticles, induced a drastic effect at the hepatocyte level, affecting the nucleus and some organelles ultrastructure of organelles (mitochondria especially), as well as the lipid metabolism. The TiO_2 nanoparticles are accumulated in the lipid droplets, as well as in some mitochondria.

The TiO_2-Ag nanoparticles, altered the lipid metabolism, and induced slightly, reversible modifications at the ultrastructural level (dilatation of the rough endoplasmic reticulum, the nucleus outline). In hepatocyte, the TiO_2-Ag nanoparticles, are moderate accumulated in lipid droplets, and massive accumulated in mitochondria.

The TiO_2-Pt nanoparticles, induced also slightly, reversible modifications. They penetrate in some hepatocytes, being in strong relation with rough and smooth endoplasmic reticulum, and with mitochondria. Their excess is accumulated and degraded in the Kupffer cell.

The TiO_2-Fe nanoparticles induced severe alteration of the hepatocytes ultrastructure. They penetrated in some hepatocytes being localized at the rough endoplasmic reticulum and mitochondria level. Their excess was accumulated in the Kupffer cell, as some electrondense bodies.

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