Mac Vet Rev 2013; 36 (1): 25–31

Available online at
www.macvetrev.mk

Original Scientific Article

UDC: 616-092-06:620.3]:599.323.4.084

ESTIMATE OF TOXICALLY INFLUENCE OF SILICON CARBIDE NANOPARTICLES ACCORDING TO HISTOPATOLOGYCAL CHANGES

Ristoski Trpe[1], Vasilevska Violeta[2], Mladenovska Kristina[3], Grozdanov Anita[4]

[1]*Faculty of Veterinary, University "Ss. Cyril and Methodius" in Skopje,*
[2]*Clinic of surgery diseases "St. Naum Ohridski*", Skopje,
[3]*Faculty of Pharmacy, University "Ss. Cyril and Methodius" in Skopje,*
[4]*Faculty of Technology and Metallurgy, University "Ss. Cyril and Methodius" in Skopje,*

Received 23 May 2013; Received in revised form 27 June 2013; Accepted 5 July 2013

ABSTRACT

Taking in consideration a very wide application of nanoparticules in different industrial sectors due to their remarkable properties for implementation in different products, very important part for future development of nanotechology is following a histopatologycal changes provoke of this material.
Silicon carbide (SiC) as ceramic material with high thermal conductivity, high stability, good wear resistance and small thermal expansion coefficient is very applied in ceramic's industry, power electronics, biomaterials, pharmaceutics etc. Histopathological changes of SiC particles were investigate on 4 weeks old female Wistar rats divided into four groups (two control and two experimental groups), sacrificed 2, 7 and 14 days after treatment. Histopathological diagnosis was performed on heart, liver, spleen, kidneys, lung, brain, gastrointestinal tract, using standard Hematoxilin-eosin staining methods. The main toxicological influences of SiC were observed on liver, lungs and gastrointestinal tract.

Key words: nanoparticules, SiC, toxicity, histopathology

INTRODUCTION

Last decades, the research work on new nano-structured materials and new nanotechnologies has been intensively performed. Nanostructures have shown remarkable properties such as high thermal stability, excellent mechanical and electrical characteristics which allowed them to have a wide application in many areas. Due to their nano-dimensions, nanoparticles are showing environmental and health impacts. There are many controversial papers that describe interactions of the NPs with the biological systems and rice concern that intentional or unintentional human exposure to certain types of engineered nanoparticles may lead to significant health i.e. toxicological effects (10, 11).

Corresponding author: Ass. Prof. Trpe Ristoski, PhD

E-mail address: tristoski@fvm.ukim.edu.mk
Present address: Faculty of Veterinary Medicine
Department of Pathology,
Lazar Pop Trajkov, 5-7, 1000 Skopje Macedonia
tel: +389 2 3240 787; fax: +389 2 3114 619

Development of the industrial sector based on application of engineered nanoparticels -ENPs and nanotechnologies is a fast-growing activity. Scientific achievements in many areas of nanotechnology have been successfully transferred into numerous practical applications in the electronics, food, (bio) pharmaceutical, chemical, cosmetic industry, etc. (1-4). However, due to their unique size and other physicochemical characteristics, they have increased the solicitude for the influence on the human health and ecological systems(2-4). Researches in this field are with high priority and several reports on the toxicological properties of the ENPs already exist with many documented deleterious effects, particularly in animals(6-9).

The specific physicochemical properties that make nanomaterials useful are the same that may hazard the human body and the environment. Particle size distribution, surface area, porosity, shape, charge density, electrophoretic mobility, strength, flexibility, crystallinity/solubility,

stability/(bio)degradability, bio(muco)adhesivity, bio-compati-bility, etc., they all can affect the biodistribution of the NPs and determine their health i.e. toxicological effects. Due to the direct contact with the environment, the main entries for NPs into the human body are skin, intestinal tract and lungs (17, 18).

Human exposure can be direct or indirect through contamination of the environment. Even very low concentrations of nanosized materials in the air represent very high particle number concentrations. In addition, the development of the nanotechnology has led to development of various strategies for controlled and targeted delivery of drugs, vaccines and diagnostics, which includes administration of nano-sized delivery systems into the human body by different routes (5, 12). On the other hand, the different physicochemical properties of the engineered nano-sized particles (compared with larger sized particles of the same components) have raised substantial concerns about the safety of the nano-sized materials, intentionally or unintentionally entered into the body, because the nanosized particles have been considered as the most dangerous fraction(13-16).

Recently, many classes of manufactured nanoparticles are known. *Carbon nanotubes:* Multi-walled carbon nanotubes (MWCNTs) and single-walled carbon nanotubes (SWCNTs), discovered in 1991 by Iijima, are constituted by a unique graphitic sheet or two or more sheets nested together in a tubular multilayer structure(1).They posses extraordinary properties: high electrical and thermal conductivity, great strength and rigidity, energy storage and field emission(2).

Due to the nanoscale size and carbon backbones, harmful and pathogenic effects from the nanotubes can arise due to their ability to enter the respiratory tract, deposit in the lung tissue, redistribute from their site of deposition, escape from the normal phagocytic defenses and modify the structure of the proteins. In this way, CNTs can potentially activate inflammatory and immunological responses, affecting normal organ functions.

Inorganic nanoparticles of metal and their oxides (Au, Ag, Co, Cr, ZnO, TiO_2, CeO_2, CrO_2): Insoluble inorganic NPs can be composed of pure metals or their oxides, or various inorganic products and alloys (Au, Ag, Co, Cr, ZnO, TiO_2, CeO_2, CrO_2,). Only their nanometric dimensions distinguish them from the same products normally found on a larger scale. Because of their unique properties related to their nanometric scale, these particles are precisely produced. At this scale, they display mechanical, electrical and other properties that do not exist when in larger dimensions(9).

Other groups are Quantum dots and semiconductors (CdSe, CdTe, ZnSe), Zero-valent metals (Fe(II)salts), and Insoluble organic nanoparticles.

MATERIALS AND METHODS

Nano-sized Silicon Carbide (SiC) particles were used in this experiment. A 0,5% hydroxypropylmethylcellulose K4M (HPMC, K4M) was used as a suspending agent. A 1,5 g of the particles powder was dispersed into the surface of 0,5%, w/v HPMC solution (10 ml), and then the suspending solutions containing SiC particles were treated by ultrasonic for 15-20 min and mechanically vibrated for 2-3 min. The size of the particles was determined using laser diffractometry (Mastersizer Hydro-2000S, Malvern Instruments Ltd., UK). The size of the nanoparticles was 10-20 nm.

Animals and treatment

Four weeks old female Wistar rats (average body mass 276$\pm$28,38 g) were acclimated during the 2 week period before starting the experiment. The rats were fed rodent diet and filtered water *ad libitum*. At 5th week, the rats were divided into four groups: 2 control groups, one control, not-treated group (n=3) and the second one, vehicle-control group (n=9), treated with adequate volume of the solution of HPMC in water (0,5% m/v), and 2 experimental groups treated with different dose of nanoparticles, one (n=9) with a dose of 1 g/kg body weight and the second one (n=9) treated with a dose of 5 g/kg body weight. After vigorous stirring, suspension of SiC nanoparticles containg the correspondent dose of nanoparticles was given to rats as a single dose via intragastric tube in a minute (according to OECD procedure). Food and water were provided 2 h later.

The symptoms and mortality were observed and recorded carefully during the whole period of study. No rat died during the study, and they all were active and non-anorectic.

2, 7 and 14 days later, animals from the vehicle-control and 2 experimental groups were sacrificed (n=3 per group at each study period). The tissues of

heart, liver, spleen, kidneys, lung, brain, small and lower intestine were taken and immediately fixed in a 10% formalin solution for histopathological diagnosis.

Histopathological examination

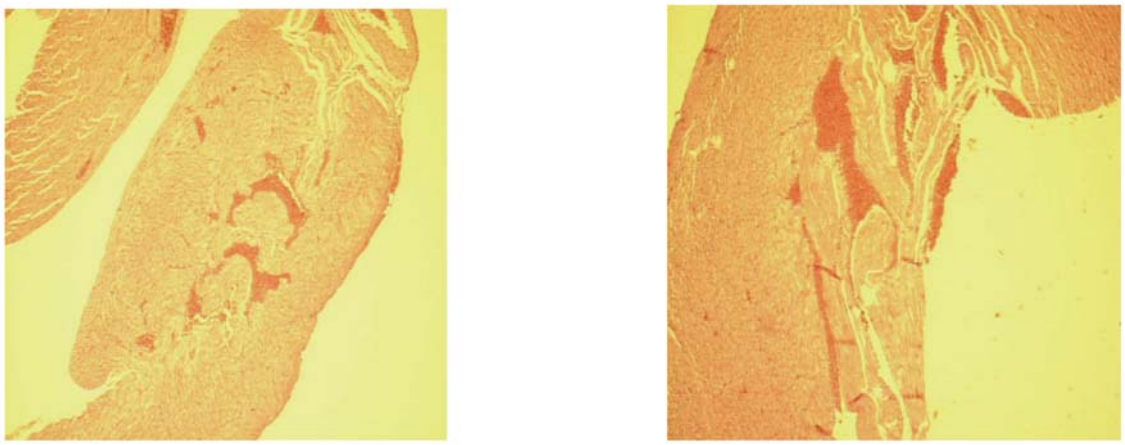
Histopathological examination was performed using standard laboratory procedures. The tissued were embedded in paraffin blocks, then sliced into 5 μm in thickness were cut. Hematoxylin-eosin staining was perform and optical microscope Nicon-Eclipsa 600 was use for microphotography.

RESULTS

Macroscopicaly no significant differences were recognized between control and treated groups.

Due to the influence of the nanoparticules, microscopical changes on the tissues of the treated groups (heart, liver, spleen, kidneys, lungs and gastrointestinal tract) are found with histopathological examination.

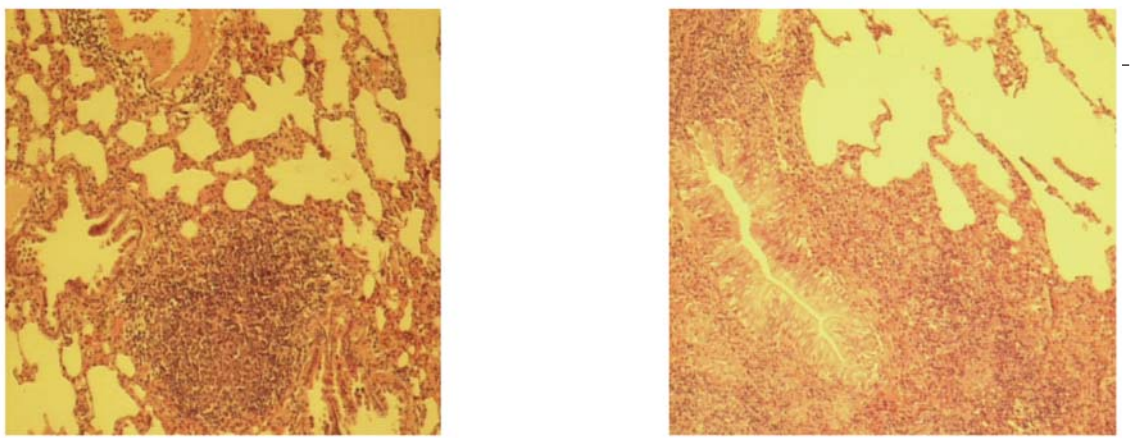
Characteristic changes registered at the heart of treated animals are shown on the Fig. 1. Congestion with diffuse haemorrhages are registered as consequence of nanoparticles presence.



(a) (b)

Figure1. Heard, treated animals, haemorrhages, (H.E. x10)

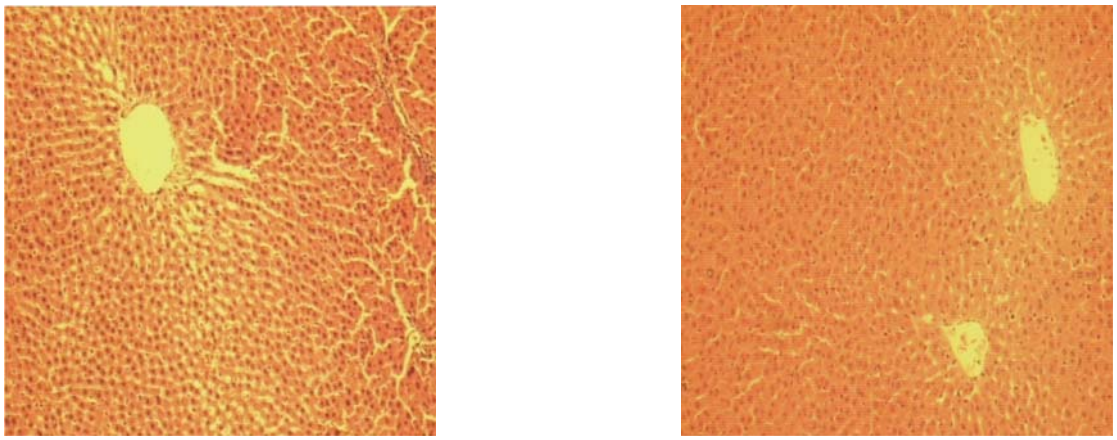
In Fig. 2, characteristic microscopic pictures of nanoparticles effects towards lungs are shown. Widen interstitial pneumonia with infiltration of inflammatory cells, was noted. Infiltration of lymphocytes, eosinophils, neutrophils and macrophages, mainly are located peribonchialy and perivasculary.



(a) (b)

Figure 2. Lungs, treated animals, mononuclear inflammations (H.E. x10)

In Fig. 3, characteristic changes are registered at the liver of tested animals. In this organ, as consequence of the presence of nanoparticles, the stasis with microhaemorrhages are registered, then parenchimatous degeneration of hepatocites, sinus spaces is part narrowed, there is a presence of lymphocytes and eosinofiles in portal spaces, too.

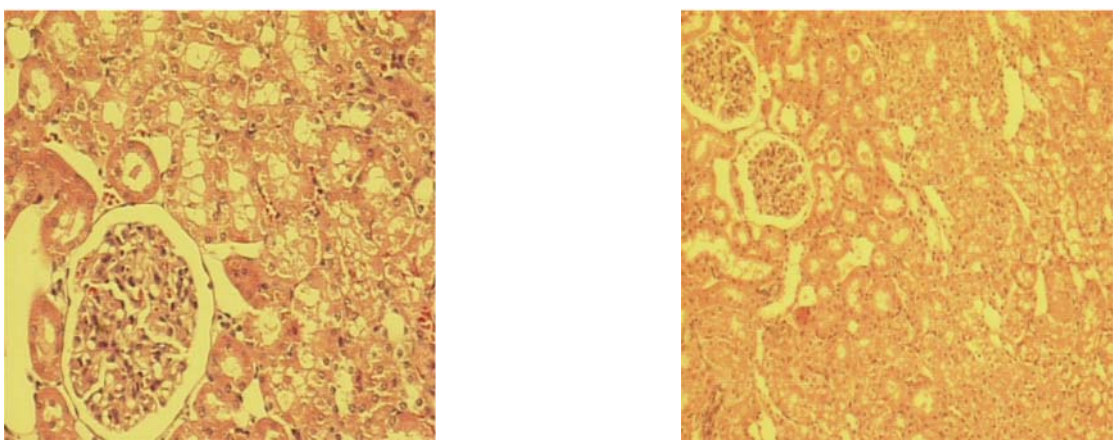


(a) (b)

Figure 3. Liver, control group (a), treated animals (b), parenchymatous degeneration, (H.E. x10)

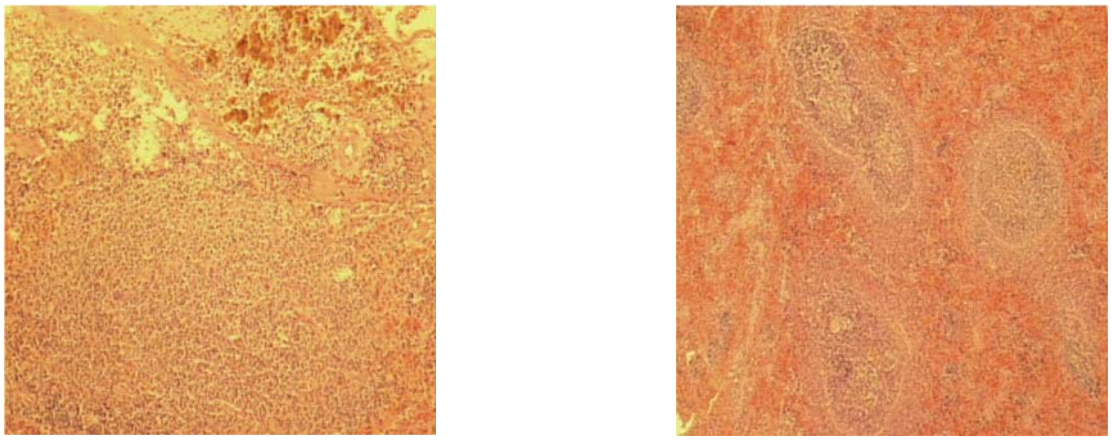
Histopathological changes are registered at the kidneys of treated animals on Fig. 4. At the kidneys, because of nanoparticles presence, stasis, haemorrhages, vacuolar degeneration of tubular cells with yellow-brown pigment, are noticed.

At the spleen, due to nanoparticles presence, stasis and yellow-brown pigment, is show on Fig. 5.



(a) (b)

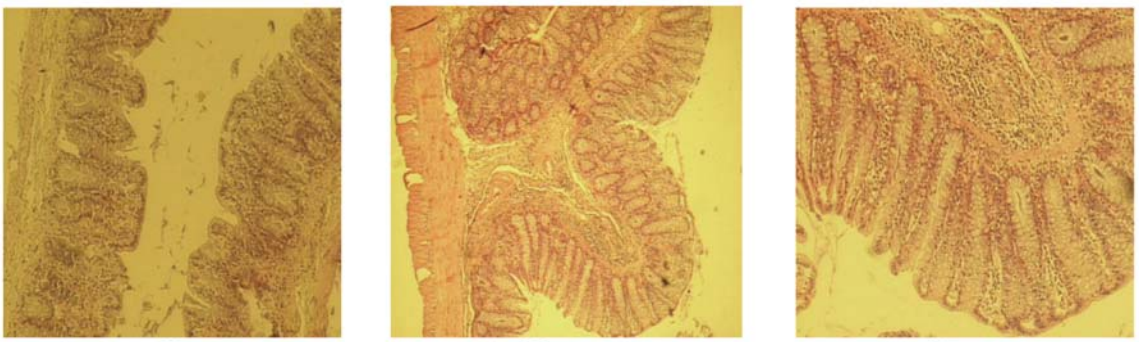
Figure 4. Kidney, treated animals, vacuolar degeneration, (H.E. x20)



(a) (b)

Figure 5. Spleen, treated animals, yellow-brown pigment (a) (H.E. x10)

In Fig. 6, changes are registered at the gastro-intestinal tract (GIT) of treated animals. At the GIT due to influence of nanoparticles, reaction of intestinal mucosa and submucosa are registered. Also, there are present of lymphocytes, eosinophils and plasma cells.



(a) (b) (c)

Figure 6. GIT, treated animals, colon (a), gaster (b,c), mononuclear infiltration, (H.E. x20)

DISCUSION

Currently, most NP-related cytotoxicity dates are generated by individual studies, where a few specific parameters have been monitored for a certain type of NP together with a certain cell type. The field of nanotoxicology is steadily gaining importance, leading to the generation of more and more date. Unfortunately, most date is generated from stand-along studies, in order to enhance understanding of NP induced cytotoxicity, there is an urgent need for standardization of the protocols used. Recently, emphasis has been placed into understanding the role of the route of particle administration as a potential source for toxicity and many researches are focused on elucidating the mechanistic underlying NP toxicity which is postulated to range from inflammatory cell infiltration and cellular necrosis to apoptosis. Among nanoparticles, the smallest, anatase, and spherical nanoparticles are consider that induced the highest cytotoxic effects (19-21).

In this paper, several tissues we pointed out which could help to optimize nanotoxicology studies and to improved understanding of the risks for toxicity and health effects of the NPs. We noted, due to the presence of nanoparticles,

the stasis and haemorrhages were registered on almost all investigated organs. Degeneration of hepatocytes and tubular cells of kidney, infiltration of lymphocytes, eosinfiles and other inflammatory cells, are main histopathologicaly founding.

Taking in considerations, that many authors have identified two main limitations for identify NP toxicity. First, is chronicity of NPs exposure and secondly, different studies apply different particles and formulations leading to conflicting and unreliable results. Consequently, for the future investigations more emphasis should be placed on defining the dose of NPs in relation to the route of administration. Well summarized results in this field is especially important for the industry and sectors where NPs are produced or used in terms of providing guidance on the assessment of exposure on this materials.

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