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ORIGINAL RESEARCH PAPER

Histopathological effects of zinc oxide nanoparticles (ZnO) on skin and muscle tissues of rats

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ABSTRACT

Although ZnO nanoparticles possess novel properties that make them available to a wide range of applications, the questions regarding their safety may arise when they come in direct contact with biological systems (such as skin, lungs, and tissues). In this study, we evaluated the possible toxic effects of different dosages of zinc oxide nanoparticles (25, 50 and 100 mg/kg) in three treatment groups in four weeks on skin and muscle tissues of treated rats. For toxicological assessments, male rats weighing 150 to 200 g were exposed to three different concentrations of zinc oxide nanoparticles (25, 50 and 100mg/kg) in an acute study. Toxic responses were assessed by clinical and histopathologic parameters. In all experimental animals the sites of exposure were scored for any type of dermal toxicity and compared with the negative control group. All changes were compared with the negative control group and the results were analyzed by one-way analysis of variance (ANOVA).

Results have been indicated that the mean levels of the histopatological injuries were scored in experimental groups and showed no significant difference with control group that mean,the number of vacuole degeneration showed significant increase in high dose group ($p<0.05$). The results showed that the topical application of zinc oxide cannot make remarkable effects on the skin and skeletal muscle tissue of the rats in low and medium doses. Although, we did not find any harmful effects on the use of low and medium dosages of zinc oxide nanoparticles on the skin and musculoskeletal system, we cannot ignore the observations regarding the sensitivity of cells and tissues to the potential cytotoxic effects this kind of nanoparticles. Therefore, it is suggested to conduct further researches on the complex toxicity mechanism of zinc oxide nanoparticles in living organisms.

Keywords: *Rats, Skin and Tissues Histopathology, Toxicity, Zinc Oxide Nanoparticles*

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INTRODUCTION

Nanotechnology has become the forefront of research in the past decade. With the advent of this emerging field, wide varieties of nanoparticles with exciting characteristics are manufactured and used for a broad range of applications especially in health, medicine and food branches [1]. Because of the special properties including small size and high specific surface area, the biological safety of the nonmaterial has received wide attention.

Zinc oxide nanoparticles have been used in a variety of products including semiconductors, catalysts, and paints. These particles are increasingly found in consumer products, such as sunscreen, because of their strong UV absorption especially in case of ZnO [8, 11]. Products containing ZnO nanoparticles may release Zn ions, which may be translocated from the environment into the human

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blood circulation and accumulate in skin, muscle tissues, potentially leading to toxicity in human organs [14] but limited studies have dealt with the potentially dangerous aspects of nanoproducts on ecosystem. One major reason for this scarcity is difficulty of identifying the effects of nanoparticles using currently available test methods [17].

Although nanoparticles possess novel properties that make them available in a vast range of applications, the questions regarding their safety arise when it comes in contact with the biological systems [2]. Despite the fact that these compounds are safe, studies reported under certain conditions adverse effects occurred cells. It is well documented that toxic effects of nanoparticles can be dramatically increased by reduction of their size and increasing their concentrations [17] thus it is essential to study toxicity of these nanocarriers in living organisms before any types of health and medical applications [2, 5]. In one study conducted by Ho *et al* (2014) oxidative stress were observed after bronchoalveolar lavage of ZnO nanoparticles in rats but the results didn't confirm the association between the number of doses and the level of lung inflammation. They found that both mass and surface area were effective as metrics for the toxicity of ZnO nanoparticles, although only surface area was previously indicated to be an effective metric. Their results are also consistent with recent study on ZnO nanoparticles which showed the role zinc ions as the major mediators of the nanoparticles toxicity [4].In other study conducted by Fubini *et al*., (2010) the application of zinc oxide nanoparticles in three different dosages (75, 180, and 360 mg/kg) on dermal collagen content of rats skin during 28 days were analyzed. The results showed that passing of zinc oxide nanoparticles through placenta is possible and damage caused by the particles could lead to the deformity or developmental retardation of the fetus [3].

In a study, in order to compare the absorption rate of nano and micro-sized particles after oral administration, Zn levels in serum of rats were investigated. The results showed that nano-sized particles were higher compared to the micro-sized treated group in serum [5]. While numerous studies has been carried out on the toxic effects of ZnO on living tissues of animals, studies regarding histopathological effects of this substance on skin and muscle tissues of rats is limited. Thus, as a result of the substantial amount of production and usage of ZnO nanoparticles in toxicological branches, it is necessary to evaluate their potential toxic effects on skin and muscle tissues in biological conditions. Moreover, investigations regarding the possibility of susceptible subgroups are also critical to better understand the neurotoxicity of ZnO nanoparticles, as well as to guide exposure standards.

On the basis of above reasons, we aimed in this study to investigate the effects of three different dosages of ZnO nanosolution (25, 50 and 100 mg/kg) of on skin and muscle tissues of rats in a 28 days study.

MATERIALS AND METHODS

Preparation of nanoparticle solution

The nanoparticle used in this experiment was purchased from Nanosany Company (Mashhad, Iran). Their specification was a nearly spherical white crystalline with particles in the range of 10 to 30 nm with a purity percentage of 99%. Then, the amount of 25, 50 and 100 mg of the solution was prepared by diluting with 0.9% normal saline solution in Milli-Q water. The characteristics and the image of its electron microscope (TEM) are presented in Table 1 and Fig. 1, respectively.

Experimental animals

Rats were taken from the Animal Breeding Center, located in the North of the country. The rates weigh were in the range of 150–200g and treated in a 12-hr light/dark cycle at a temperature of 22±1 °C with free access to standard pellet diet and water. Animal experiments were carried out according to the guidelines of Institutional Animal Ethics Committee regulations approved for the purpose of control and supervision of the experiments on rats. Individual rats were identified with picric acid marks. In addition to this, each rat

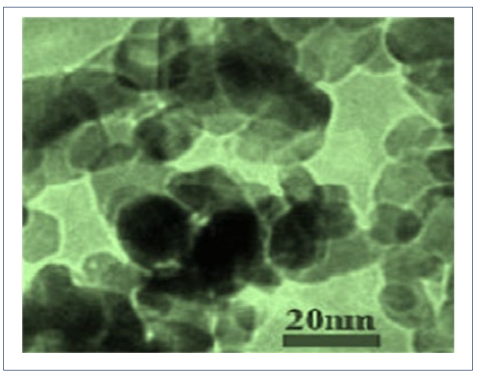

Fig. 1. TEM image of ZnO nanoparticle.

cage was identified by labels having details such as experimental number, name, animal numbers and date of experiment. All the animals were acclimatized for a period of 5 days before initiation of experiment.

Treatments with ZnO nanoparticles

The rats were randomly divided into 4 groups as below:

Control group (A): In this group, the rats fed normally with water and food and did not receive any nano solution or drug.

Low dose group (B): Rats in this group received 0.5 ml of 25 mg/kg of zinc oxide solution in the form of intraperitoneal, dermal and oral intravenous injection twice a week.

Medium dose group (C): Rats in this group received intraperitoneal, dermal and oral injections of 0.5 ml of 50 mg/kg nanoparticle solution twice a week.

High dose group (D): Rats in this group received intraperitoneal, dermal and oral injections of 0.5 ml of 100 mg/kg nanoparticles solution twice a week.

All rats were examined for the onset of any immediate signs of toxicity and body changes during our 28 days continuous study. At the end of all experiments, their skin and muscle tissues were undertaken and transferred to the laboratory for digestion, slide preparations and histopathologic tests.

Skin and muscle tissue sampling

Skin and muscle tissues sampling were collected to assess histological changes. Other pre-treatments (tissue collection from the rats, fixation, special processes such as decalcification, tissue trimming, cassetting, processing, embedding, sectioning, deparaffinization and rehydration, staining, and coverslipping) were performed in according to the method of Samrot *et al.*, (2017) [13].

Statistical Analysis

Values were expressed as percent per population or as the mean ±standard deviation (SD).To assess the association between variables and clinicopathological data nonparametric chi square test was used. Statistical differences between the control and treated samples were examined with student t test followed by SPSS 18.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Size of the nanoparticles is one of the critical factors responsible for nanoparticle properties and their mechanism of cellular interaction. Thus in the present study, the nanoparticles size has been studied by TEM analysis. Although the data available from the manufacturer indicated that the primary particle size of the ZnO nanoparticles was< 50 nm, the characterization of ZnO nanoparticles was also detected in our laboratory. The TEM images demonstrated that ZnO nanoparticles were within 10–30 nm in size (Table 1), and the majority of the particles comprised a polygonal shape with smooth surfaces (Fig. 1).

Histopathological dermal effects of ZnO nanoparticles

The natural structure of the rat skin was visible in four groups and its building was preserved in all three layers of epidermis, dermal and hypodermic. During the study period, skin treatment with ZnO nanoparticles did not cause any adverse effects because no statistically significant differences in three layers of skin gain were observed between the ZnO-treated and control rats (Table 2). Further, no abnormal skin clinical signs and behaviors

Table 1. The ZnO nanoparticle characteristics

Nano-particle	Nano-particle size	Specific surface	Percentage of purity	Appearance color	Real density
ZnO nanoparticle	10-30 nm	20-60 m^2	99%	milky white	5.606 g/cm^3

Table 2. Percentage rate of histopathological findings in test groups.

Group	Control	ZNP-1	ZNP-2	ZNP-3	Statistical significance level
Necrosis cell (%)	10.9 ± 3.78[a]	11.6 ± 2.01[a]	11.75 ± 1.21[a]	11.8 ± 1.83[a]	0.074[ns]
Hyperaemia	-	-	-	+	0.92 [ns]
inflammation	-	-	+	++	0.108 [ns]
Hyaline Casts	-	-	+	++	0.85 [ns]
Vacuole degeneration	-	+	++	+++	0.122

[a] Similar lowercase letters did not represent significant differences at the 5% probability level. Ns; not significant.
Natural structure (without injury) (-); Mild injury (+); Moderate injury (++); Sever injury (+++)

were detected in both the control and treated groups. Exposure to the most dosages of ZnO nano particles solution for 28 days generally didn't cause any significant changes in the skin of rats (Fig. 2).

The histopathological effects on muscle tissues

The effect of feature changes of muscle tissues was determined in different dosages of ZnO nanoparticles. As shown in Fig. 3, no change in statues was observed in tissues sample of treated rats following of the low and high dosage of ZnO nanoparticles solution. In all of the groups, muscle cells were healthy and their dark and clear lines were visible. In these groups, the nuclei were located below the sarcolemma and their dark and clear lines were visible (see Fig. 3).

As ZnO nanoparticles are used increasingly in various commercial products as well as in biological and medical applications, it is more important than ever to study their possible toxicological effects on humans. Our study showed that the use of various dosages of (25, 50 and 100 mg/kg) zinc oxide nano particles in treated rats did not cause any harmful effect on their skin and skeletal muscle tissues other than vacuole degeneration indexes).

Recently, a few in vivo toxicity tests of ZnO nanoparticles showed that toxicity appeared only in relatively high-dosage treated groups when rats were treated by oral administration [12, 14]. Other exposure routes including skin or inhalation produced different results according to the exposure routes. When rats were treated orally with 50 or 300 mg/kg of ZnO nanoparticles once a day for 14 days, ZnO nanoparticles accumulated in liver and led to cellular damage [4, 15].A study showed that some treated male wistar rats with zinc oxide nano particles presented tissue necrosis. These complications were attributed to improper injection techniques or inaccurate after-treatment management, besides the intrinsic characteristics

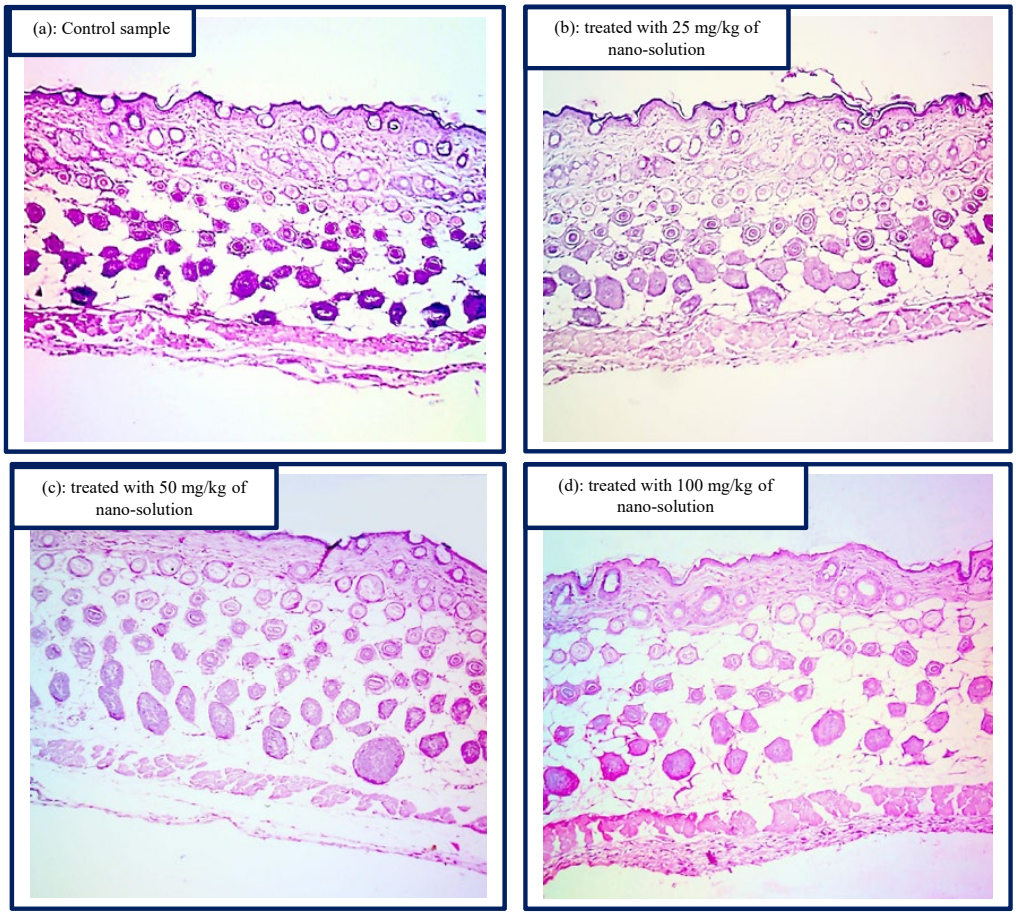

Fig. 2. Histopathological changes in skin of treated rats with different dosages of ZnO nanoparticles (25, 50 and 100 mg/kg, respectively) as compared with control sample (a) ×100. No harm was observed in any of these treatments.

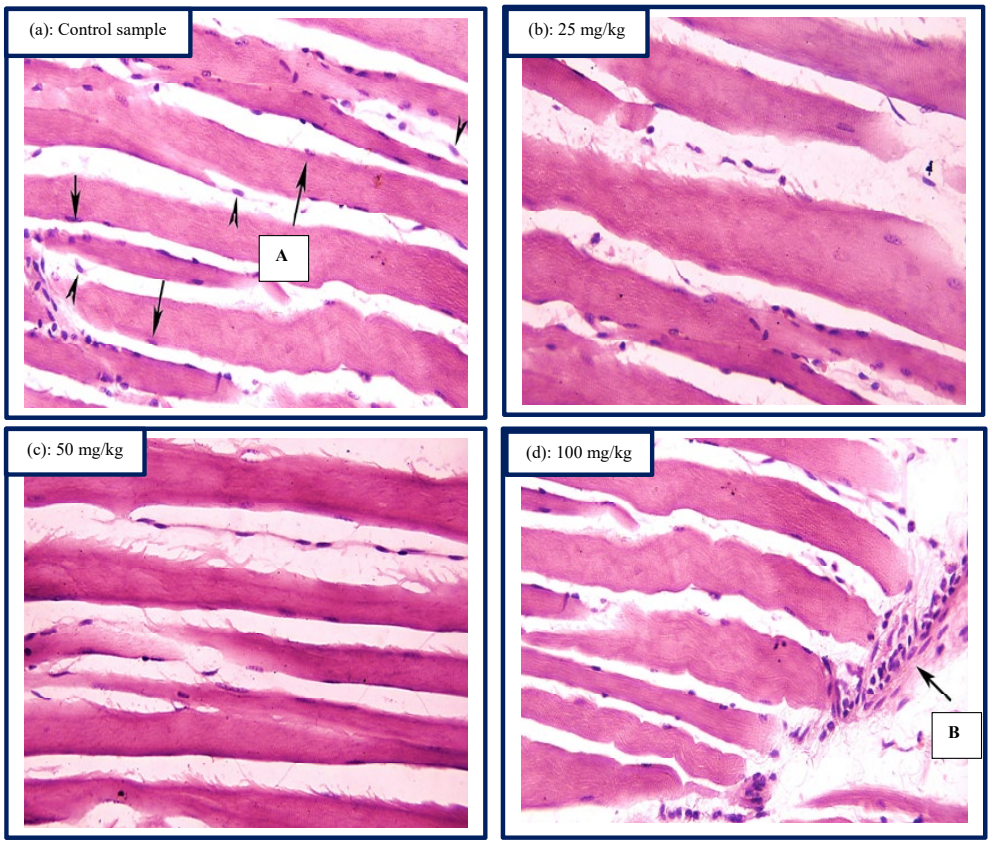

Fig. 3. Histopathological changes in muscle tissues of treated rats with ZnO nanoparticles. Rats were treated with 25, 50 and 100 mg/kg-ZnO nano particles, respectively as compared with control sample (a) ×400. A) Necrosis cells and B) Hypertension in muscle tissues. No harm was observed in any of these treatments.

of the local environment. The evidence of tissue necrosis was linked to external events whereas it could have been related to an endogenous reaction based on inflammatory response. The presence of inflammation after the intra testicular injection of 200 mM zinc oxide nanoparticles was reported, and anti-inflammatory drugs were prescribed to minimize this side effect in male wistar rats [6, 7].

The aim of this study was to evaluate the zinc-based nanoparticles dermal toxicity based on the OECD guidelines (OECD 402, OECD 410) that is currently used to evaluate the acute and repeated dose dermal toxicity of chemicals. Of course, in these treatments, application of different dosage of nanoparticles used was more than their ordered actual dose. It seems that since the surface of nanoparticles is high, their major effects depend on their surface to their molecular mass. Moreover, different nanoparticles with different physicochemical properties have different toxicological effects [7, 8].

Different studies on the toxicological effects of nanoparticles have indicated that these particles act with the similar oxidative toxicological mechanisms and these effects are dependent on their physicochemical properties such as specific surface area, the properties of the metal concerned, as well as nanoparticle size. Furthermore, different exposure route, such as oral administration, dermal contact, and inhalation have various specific toxicological impacts [7, 9, 16]. For example, in a study, a comparison was made between the effects of different dosages of nano titanium oxide particles in different sizes on health through long-term inhalation of these particles. The results showed that smaller nano titanium oxide particles tended to produce more pulmonary tonsils than coarser ones [16].

Finally, according to the obtained results of this study in three routs of administrations and three different dosages, we conclude that ZnO nanoparticles have no destructive effects on skin

and muscle tissues of the rats in evaluated doses but with regards to previous studies, it was confirmed that ZnO nanoparticles have toxic effects on some biological systems [9, 10]. Understanding the toxicity mechanism of ZnO nanoparticles in biological systems need to be more evaluated under different laboratory conditions.

Because ZnO nanoparticle is a complex system with no identifiable mechanism in different conditions, the best way to move forward is with a multidisciplinary approach incorporating results from emerging imaging techniques, biochemical, pharmacologic and genetic studies in order to better understand the molecular basis of this nanoparticle.

CONCLUSION

In current study, treated rats with various dosages of zinc oxide nanoparticles didn't exhibit significant histopathological changes regarding different histological parameters. In summary, histopathological observations found in our study indicated that ZnO nanoparticles have not cytotoxic actions on skin rats and don't induce damage to muscle tissue. Nowadays, due to the development of nanotechnology and wide uses of these nanoparticles in food and pharmaceutical industries, medicine and biology, there is wide exposure of this material to humans and the environment.

Therefore, these results provide useful information for designing proper metal nanoparticles in order to better understand their pathophysiological mechanisms for delivery of bioactive molecular and other nutraceuticals in food and pharmaceutical products.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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