Effects of ZnO nanoparticles and $ZnCl_2$ solution on rat liver and kidney

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ABSTRACT

Recent studies have shown that Zn^{2+} is released from the surface of ZnO-NPs when they are suspended in an aqueous state. The aim of this study was to evaluate the effects of ZnO nanoparticles (ZnO-NPS) and $ZnCl_2$ in solution on plasmatic biochemical parameters, tissues trace elements and their possible cytotoxicity in rat liver and kidney. Rats were treated orally with doses of (ZnO-NPs) or ($ZnCl_2$) (10 mg/Kg) for 5 consecutive days,control group received a dose of 0.9% sodium chloride. Sub-acute exposure to ZnO-NPs or $ZnCl_2$ showed no significant change either in body weight, kidneys and liver weight. Oral administration of ZnO-NPs or $ZnCl_2$ increased the plasmatic transaminase activity (AST and ALT). However, the creatinine, uric acid and blood glucose concentrations remained unchanged. Rats received ZnO-NPs or $ZnCl_2$ solution showed a no significant increase of zinc content in the liver and kidney. Morover, the concentration of trace elements were slightly modulated after ZnO-NPs or $ZnCl_2$ exposure. Histological analysis in the liver and kidney showed signs of discreet cytotoxicity (Inflammatory response, Vascular congestion, edema formation) in ZnO-NPs and $ZnCl_2$ treated-rats compared to control group. Oral administration of ZnO-NPs or $ZnCl_2$ showed no obvious toxic effect in rat liver and kidney.

Keywords: ZnO Nanoparticles, $ZnCl_2$, liver, kidney, rats.

1 INTRODUCTION

Nanoparticles are defined as small objects that have at least one dimension in the range of 1–100nm. Compared to particles in micro scale, nanomaterial's showed different degrees of biological effects [1]. ZnO nanoparticles have been used in many applications in our daily life, such as drug carriers and cosmetics [2]. The recent wide application of nanomaterials in various fields has led us to address the potential risks of these materials to human health [3]. Previous studies have demonstrated that ZnO-NPs are toxic to microorganisms, cells, plants, aquatic biota and rodents, release of metallic cations Zn^{2+} are the main causes of toxicity [4]. Increasing numbers of studies demonstrated that many types of nanoparticles have toxic effects mainly on liver and kidneys tissues [1;4]. In the present work we evaluated the sub-acute toxicity of ZnO nanoparticles (30nm) and zinc chloride administered via the oral route in rat liver and kidney. For this purpose, we have studied the effects of the particle exposure on plasmatic biochemical parameters and the accumulation of Zn in rat liver and kidney. The histopathological changes in rat tissues have been examined as well.

2 MATERIALS AND METHODS

2.1 Characterization of NPs

The morphology of the ZnO NPs used in the study were assessed using transmission electron microscopy (TEM) and The crystalline phase of ZnO NPs was analysed by X-ray diffraction in the Laboratory of Physics of Materials and Nanomaterials applied at environment, Faculty of Sciences of Gabes, Tunisia. The TEM images showed that nano-ZnO were within the sizes 20-30nm [5]. ZnO nanoparticles were suspended in NaCl 0.9% at a concentration of 10 mg/ml and probe sonicated for 10 min to ensure nanoparticle dispersion and to prevent nano-ZnO deagglomeration and reagglomeration [6].

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2.2 Animals and treatments

Male Wistar rats (SIPHAT, Tunisia), weighing at the beginning of the experiment 150–200 g were randomly divided into control rats (n = 6), ZnO NPs (10 mg/kg body weight) treated rats (n = 6), zinc (10 mg/kg body weight) treated rats (n = 6). Animals were housed in groups of six in cages at 25°C, under a 12:12 light/dark cycle, with free access to basal diets and water. Animals were cared for in compliance with the Tunisian code of practice for the Care and Use of Animals for Scientific Purposes. The experimental protocols were approved by the Faculty Ethics Committee (Faculty of Sciences of Bizerte, Tunisia).

All animals were weighed at the beginning and end of each treatment. After the treatments, the animals were sacrificed and organs were taken.

2.3 Zinc content analysis in tissues

The concentrations of zinc in the liver and the kidney were determined by Atomic Absorption Spectrophotometry (Perkin Elmer 306) in an air acetylene flame. Zinc concentration was measured using Perkin Elmer intensitron lamp Norwalk CT (USA). The standard solution of zinc used in this assay resulted by the dissolution of $ZnCl_2$ in distilled water. Fractions of liver or kidney tissue were lyophilized, weighed and digested in 2 ml of concentrated HNO_3 in pressurized Teflon containers at 160°C for 3 h. After cooling at room temperature, samples were diluted with 10 ml of deionized water [7]. Zinc concentration was calculated in μg/g of the dry mass of tissues.

2.4 Biochemical assays in plasma

Control and treated rats were sacrificed 24 h after the last exposure. Blood was collected in heparinized chilled tubes and immediately centrifuged. Aliquots of plasma were frozen and stored at –80°C prior to biochemical analysis, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), creatinine and uric acid concentrations were measured using the enzymatic methods according to manufacturer instructions (Biomaghreb).

2.5 Histopathological techniques

A small piece of liver, kidney was fixed by 10% formalin and then embedded into paraffin, sectioned for 5–6-mm thick, and mounted on the glass microscope slides using standard histopathological techniques. The sections were stained with hematoxylin eosin and examined by light microscopy.

3 STATISTICAL ANALYSIS

The data were expressed as mean ± standard deviation. For statistical analysis, the experimental values were compared to their corresponding control ones. A one-way analysis of variance (ANOVA) in STATISTICA (Version 5) was used to illustrate the significant difference between the experimental group and the control. The significant difference was considered to be $P < 0.05$.

4 RESULTS

Oral administration of ZnO-NPs or $ZnCl_2$ induced no significant changes in the body weight and the coefficient of organs (as the ratio of tissues wet weight (mg) to body weight (g)) between the exposed rats and the control group(Table1).

Table 1. Effect of ZnO-NPs and $ZnCl_2$ on Body Weight and coefficient of liver and kidney.

	Body Weight (g)	**Liver Weight (%)**	**KidneyWeight (%)**
CT	144.33±5.57	3.82±0.10	0.76±0.09
ZnO-NPS	133.5±6.99	3.74±0.07	0.80±0.02
$ZnCl_2$	144.83±2.98	3.80±0.11	1.01±0.07

The determination of zinc by atomic absorption in the liver and kidney shows no significant variations in the concentration of this element in the treated groups compared to the control group (Fig.ure 1).

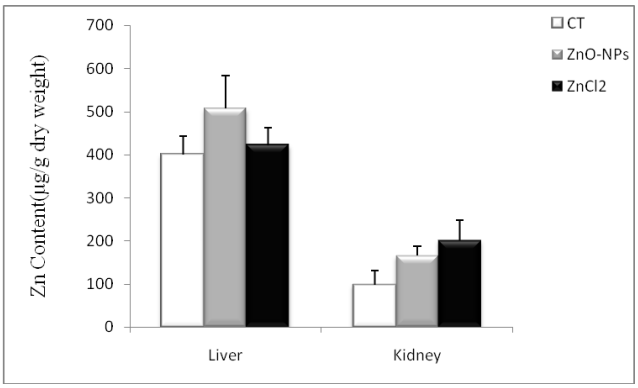

Figure. 1. Effects of ZnO nanoparticles and zinc chloride treatment on zinc content in rat liver and kidney. CT: Control group.

Plasmatic biochemistry analysis was shown in Table 2. Oral administration of ZnO-NPs or $ZnCl_2$ increased the plasmatic transaminase activity (AST and ALT).The level of AST in serum is often tested along with ALT to whether the liver is damaged. Uric acid and creatinine levels remained unchanged.

Table 2. Effects of ZnO nanoparticles and zinc chloride treatment on plasmatic biochemical parameters

Parameters	Experimental group		
	CT	ZnO-NPs	$ZnCl_2$
AST (U/l)	38.85±5.19	116.9±24.83*	144.02±15.89*
ALT (Ul/l)	12.6±1.92	23.27±2.48*	25.2±4.02*
Creatinine (mg/dl)	0.67±0.06	0.67±0.057	0.53±0.04
Uricacid (mg/dl)	6.34±0.38	7.01±0.40	7.54±0.60

aspartate aminotransferase (AST), alanine aminotrasferase (ALT) CT: Control.
*<0.05 compared to control group.

Oral administration of ZnO-NPs or $ZnCl_2$ modulates biochemical parameters related to liver function. This result was supported by the histopathological examination.The histopathological analysis of liver shows sinusoidal congestion and the formation of a lymphocyte clusters for both treated groups compared to control group (Figure.2).The histopathological examination of the kidney of treated rats with ZnO-NPs or $ZnCl_2$ showed intraparenchymal vascular congestion (Figure 3).

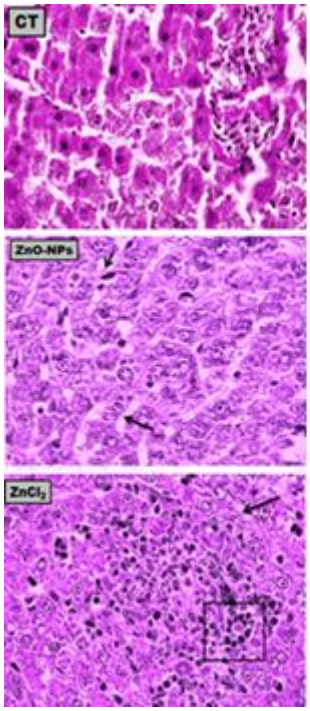

Figure .2 Sinusoidal congestion and edema in hepatocytes of the rats exposed to ZnO-NPs and $ZnCl_2$.Magnification =40,liver in control group(CT) showing normal structure. The arrows shows sinusoidal Congestion, lymphocyte clusters (square shape).

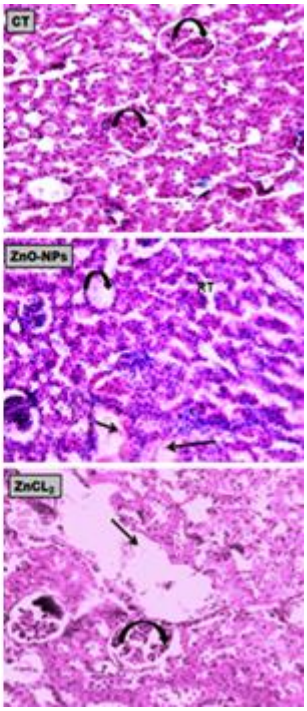

Figure .3. Renal pathological changes of the rats exposed to ZnONPs et $ZnCl_2$ compared to control group. Magnification =40, CT: Control group showing normal structure with their glomerulus (curved arrow) The arrows shows intraparenchymal vascular congestion, RT: renal tubular.

5 DISCUSSION

The oral route was selected as the route of exposure for rats in this study as the ZnO nanoparticles are being used in food packaging and may gain entry into the body directly [8]. Even when used in other consumer products like coating and dermatological applications, there is a risk of ingestion during use. Moreover, they may gain entry into the gastrointestinal tract after their accidental release into the environment [9]. In this study, rats were treated orally with moderate doses of ZnO-NPs or $ZnCl_2$ (10 mg/kg) and plasmatic biochemical assays, as well histopathological examinations of tissues were performed. The health and behavior of the animals were normal throughout the study. Biochemical analyses showed increased levels of transaminase activity (AST and ALT), indicating slight liver injury. The orally administrated ZnO nanoparticles could induce liver damage, as revealed by the histopathological examination which showed very limited hepatic necrosis. Landsiedel et al [10] reported that Zn^{2+}, which is present due to the solubility of ZnO NPs, seems to be responsible for inducing inflammatory responses and necrosis. These results support the findings of Wang et al [11] who observed liver damage in mice after an oral exposure of ZnO nanoparticles although at a higher dose of 5 g/kg. However, there were no changes in the creatinine and uric acid concentrations measured in blood samples, indicating that there was no detectable inflammatory response or kidney toxicity.Previous data showed that feeding the ZnO nanoparticle suspension through digestive tract at a dose of 0.6mg daily led to the damage to some primary organs (heart, lung, liver and kidney) of mice [2].

We conclude that oral administration of nano-size zinc oxide or $ZnCl_2$ at lower doses showed no obvious toxic effect in rat liver and kidney.

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