Defense Effect of Ganoderma lucidum Against Zinc Oxide Nanoparticles Induced Nephrotoxicity

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ABSTRACT

Zinc oxide nanoparticles are growing in use in many fields, especially cosmetics. However, its potency effects on renal tissue are still predominately unknown. The purpose of this study is to confirm the toxicity of these particles on the kidneys of experimental rats and to assess the role of Ganoderma lucidum (GL) against ZnONPs-induced nephrotoxicity. This study was conducted on thirty male rats divided into three experimental groups (each group includes ten) as follows: CON group without any treatment, ZnONPs group in which rats were intoxicated with zinc oxide nanoparticles orally, and ZnONPs+ GL group which co-received GL poisoned rats. After nephrotoxicity was evaluated, the results showed a significant increase in serum renal function biomarkers including BUN, CR, and URIC in ZnONPs -treated rats compared to CON rats. Moreover, ZnONPs led to lower levels of GSH and SOD with higher levels of MDA in kidney tissues. Combined administration of GL has shown an improved effect against nephrotoxicity, as it has the role of modulating the level of parameters of renal function and oxidative stress induced by ZnONPs. It is suggested that it is desirable to employ GL as a salutary reno-defense nutrient against ZnONPs -induced toxicity.

Keywords: Nephrotoxicity, biochemical markers, oxidative stress.

Introduction

Zinc oxide nanoparticles (ZnONPs) can be considered as the key metal oxide nanomaterial due to their different medical, biological, and cosmetic implementations [1-3]. ZnONPs have anti-inflammatory, anticancer, antimicrobial, antidiabetic, antifungal, drug delivery, and bio-imaging properties [4,5]. On the other hand, *in vivo* and in *vitro* studies have demonstrated that nanoparticles have toxic effects, including cytotoxicity, genotoxicity and oxidative stress [6-9], which requires attention and careful control of exposure to these particles. Induction of oxidative stress is the powerful mechanism for the toxicity of NPs, as metal ions are released and reactive oxygen species (ROS) are produced [10,11]. Those excessive productions of ROS may result in oxidative modification of proteins, DNA deterioration, and increased efficacy of inflammatory signals that stimulate apoptosis, necrosis, and genotoxic effects [12]. After these nanoparticles enter the body by inhalation, ingestion or injection, they translocate to the blood, causing upsetting biological reactions in many organs [13] . ZnONPs can be considered potent nephrotoxins, as they can accumulate in kidney tissues and affect their structure and function [14,15]. Medicinal herbs are nowadays gaining increasing attention all over the world for their ability to enhance immunity and health with minimal side effects [16]. Many thousands of years ago, *Ganoderma lucidum* (GL); known as reishi mushroom; was recognized by Chinese medical professionals as a valuable remedy. They also describe it as a

medicine of kings. In addition, GL has a diversity of bioactive ingredients that possess a potent renal protective action chiefly by its anti-oxidant and free radical rummaging effect [17]. Since ROS occupy a key role in the pathophysiological processes of kidney disease. Therefore, antioxidants are likely to reduce the susceptibility of kidneys to oxidative inducing problems [18]. In the current era, there is an increase in the incidence of kidney injury as a result of exposure to toxins. The results of this study may be useful in making this mushroom acceptable among people as a good food for preventing kidney damage. So that, this study was designed to evaluate the renal defensive effect of GL on the renal toxicity prompted by zinc oxide nanoparticles in male laboratories rats.

Materials and Methods

Chemicals

Zinc oxide nanoparticle dispersion (white colored liquid) was purchased from Nanoshel LLC Company (Wilmington DE, USA). Molecular formula: ZnO, molecular weight: 81.37 g/mol, average particle size (APS): 30-40 nm, purity: 99, 9%. Ganoderma lucidum powder was purchased from ALTERNI, which is an Australian healthcare company providing natural health supplements.

Laboratory animals and experimental design

Thirty- healthy adult male albino rats aged (16-22) weeks and weights ranging from 185-225 g were used in this experiment obtained from Laboratory Animal Centers. Rats were housed in moisture, ventilated, temperature-controlled cages with twelve hr light–12 hr dark cycles, and no obstacle to go into water and diet. Rats were adapted to laboratory conditions for a week before beginning of the experiment. All rats were distributed into three groups (n = 10 in each group), as shown in Table 1

Table 1: Designing treatments for experimental animals

Groups	Treatments and Dosages
CON	Rats without any treatments
ZnONPs	Rats were intoxicated with 50 mg/kg ZnONPs for 14 days [19] via oral gavage.
ZnONPs +GL	ZnONPs intoxicated rats were treated orally with 500mg/kg [20] GL, lasts 14 days.

Samples collection and preparation

Twenty-four hours after the last day of treatments, all rats were euthanized and sacrificed. Animals' hearts were punctured to take blood samples and put them in sterile tubes to separate the serum. Then, centrifugation was performed at 3500 rpm for 10 minutes to separate the serum, for biochemical renal function assessments. A midline incision technique was used to harvest and collect kidney tissues.

Kidney function markers

Serological parameters including: creatinine (CR), blood urea nitrogen (BUN), and uric acid (URIC) were determined using Randox diagnostics kits (UK), by auto biochemical analyzer.

Oxidative stress biomarkers in kidney tissues

Malondialdehyde (MDA) concentration in kidney tissues was measured by spectrophotometric method. The reaction of MDA with thiobarbituric acid led to the formation of a colored compound with an absorption peak of 535 nm, while, superoxide dismutase (SOD) activity had been tested by employing the Marklund and Marklund method [21]. Reduced glutathione (GSH) level was performed enzymatically according to the procedure for the previous study [22].

Glutathione was evaluated by comparing the absorption of the test solution to the glutathione standard.

Statistical Analysis

Using the statistical package (SPSS version 26), the data were analyzed. All parameters were listed as mean ± standard deviation and critical variance was resolved using analysis of variance (ANOVA), and subsequently Duncan's numerous range post hoc-test. Results were taken into consideration to be significant at p value < 0.05.

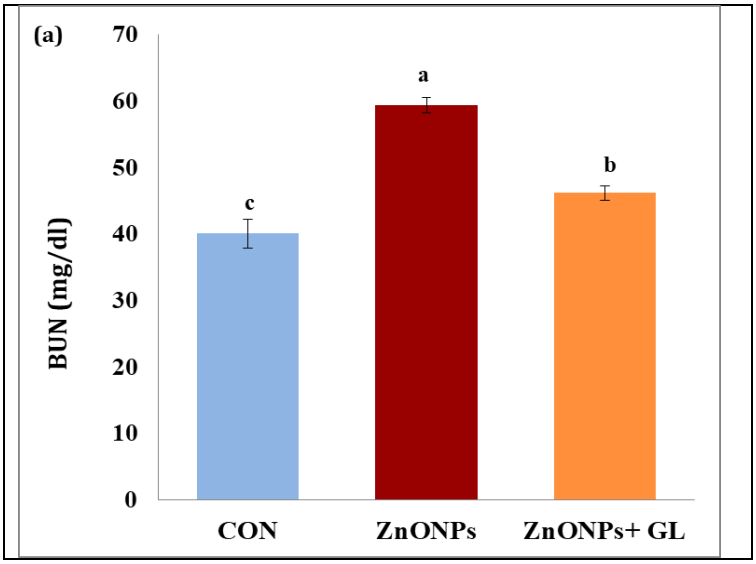
Results

Effect of GL on serological biomarkers

Serum blood urea nitrogen, creatinine, and uric acid levels were measured to determine protective effect of *G. lucidumon* on renal function disturbed by the toxicity of zinc oxide nanoparticles. Rats dosed with ZnONPs significantly increased BUN , CR, and URIC levels compared to CON rats. However, GL marked decreased serum levels of those three parameters in the ZnONPs +GL group contrasting with the ZnONPs group (Fig. 1).

Influence of GL on oxidative stress biomarkers in renal tissue

When evaluating the influence of GL on ZnONPs -induced oxidative stress in tissues of the kidney, it was observed that ZnONPs caused a considerable raise in the concentration of MDA metabolites in the ZnONPs group was taken into comparison to CON group ($P < 0.05$). Co-administrated rats with GL significantly decreased the concentration of MDA metabolites in the ZnONPs +GL group was taken into comparison to the ZnONPs group ($p < 0.05$). In addition, ZnONPs produced a substantial decrease in SOD and GSH activity in the ZnONPs group taken into comparison with the control group ($P < 0.05$). GL-dosed rats revealed a considerable increase in SOD and GSH levels in the ZnONPs +GL group compared to the ZnONPs group (Fig. 2).



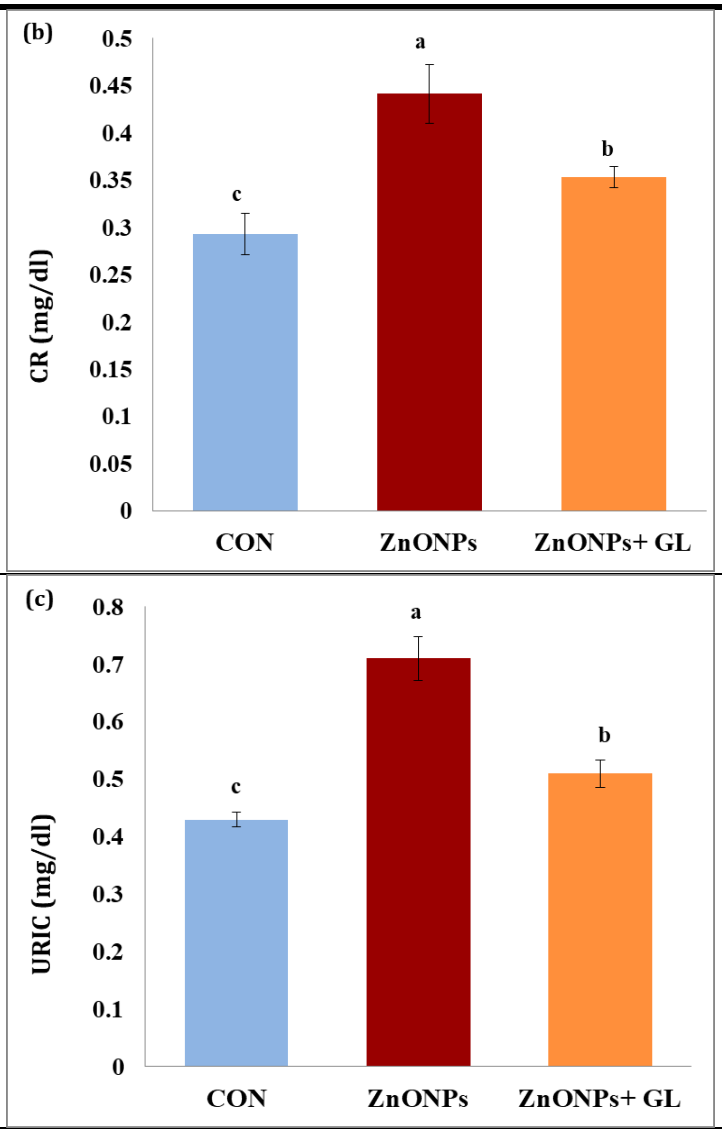
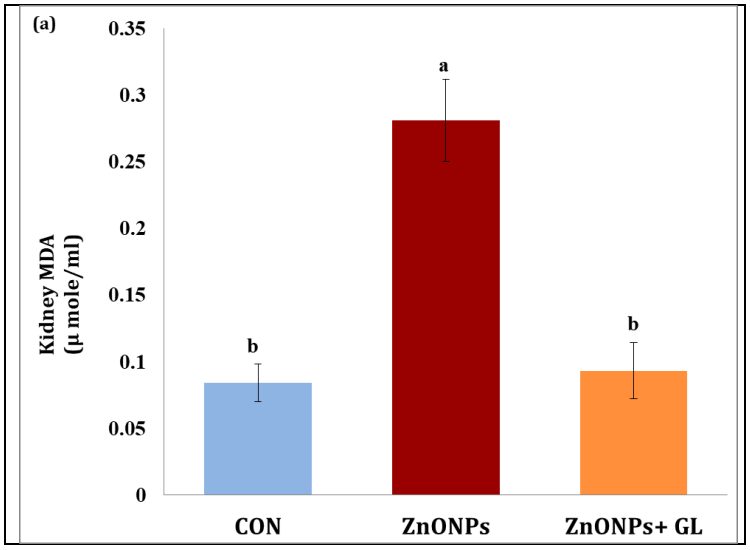


Figure 1: Levels of sero-markers of kidney function: (a) BUN, (b) CR, and (c) URIC for three experiment groups. Data were indicated as mean ± SD. Dissimilar letters (a-c) specify substantial within-column differences ($P < 0.05$).



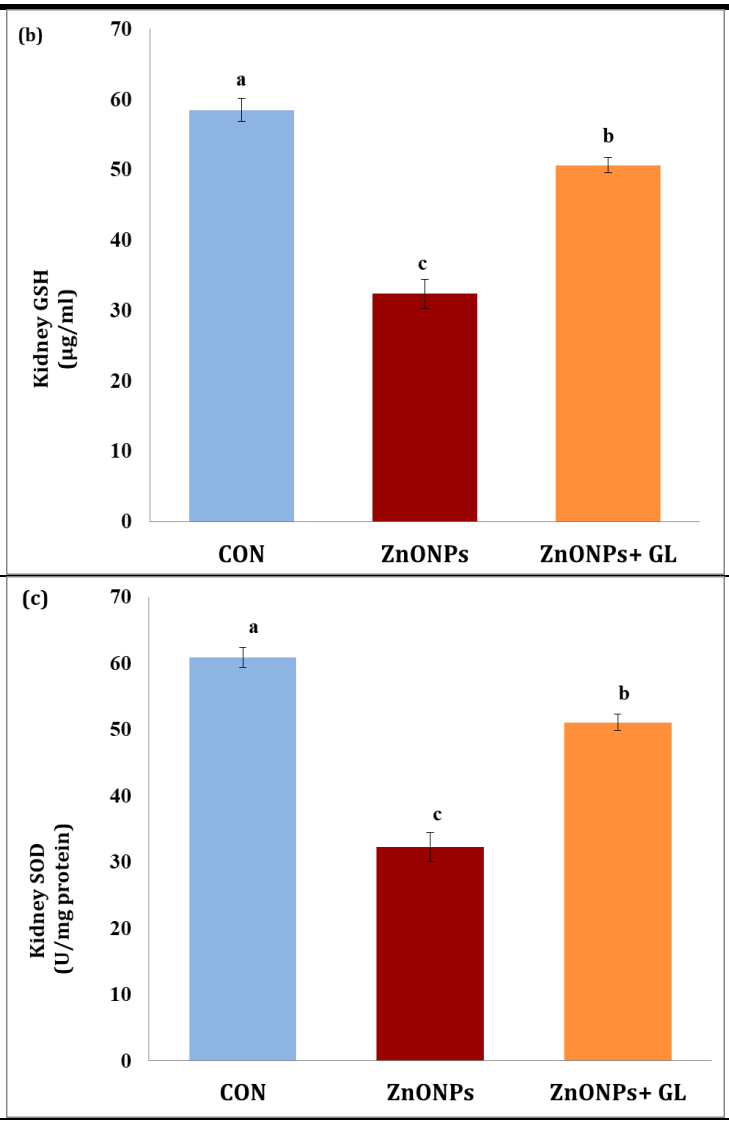


Figure 2: Levels of oxidative stress markres of kidney tissue: (a) MAD, (b) GSH, and (c) SOD for three experiment groups. Data were indicated as mean ± SD. Dissimilar letters (a-c) point to substantial within-column differences ($P < 0.05$).

Discussion

Results of this experiment showed that ZnONPs significantly induces nephrotoxicity by disrupting renal function as well as generation oxidative stress in it. Since the kidney is the primary excretory organ, it is considered a main target of toxins [22,23]. Similarly, it is a secondary target for the toxicity of nanoparticles. NPs after being absorbed by the digestive system are distributed in the liver, kidney and spleen as major organs [24,25].It has been reported that ZnONPs are potent nephron-toxins. This cytotoxicity is attributable to membrane damage, mitochondrial injury, oxidative stress, and apoptosis [26]. ZnONPs significantly raised levels of BUN, CR and URIC taken into comparison with the control group. The levels of SOD and GSH in kidney tissues were significantly decreased, in contrast to MDA which increased significantly. All of this proved the nephrotoxic effect of zinc oxide nanoparticles on experimental rats. It is intuitive that the high level of serum CR and BUN are features of impaired kidney function as a result of a decrease in the glomerular

filtration rate. Besides, oxidative stress is another pathological cause that is considered to cause kidney impairment. It has been proven that lipid peroxidase has a detrimental effect on the glomerular basement membrane [27]. In many experimental models of toxins, it was revealed that high species of reactive oxygen and oxidative damage cause kidney damage. Depending on their unique physical and chemical properties, NPs are adsorbed, internalized, circulated and distributed in the renal system [28,29]. The toxicity of ZnONPs may result from the dissolution of particles leading to damage mediated by reactive oxygen species (ROS). In addition, intracellular ROS are mainly produced when solute Zn^{2+} enters cells [30]. The positively charged nanoparticles are much higher endocytosis than the negatively or neutrally charged NPs [31]. However, dropping within zinc oxide nanoparticles nephrotoxicity with *G. lucidum* was observed, renal dysfunction and oxidative stress rebalance were observed in rats given GL in combination with ZnONPs. Confirming this, rats in ZnONPs + AG group had better renal function than those treated with ZnONPs. Serum BUN , CR and URIC levels in ZnONPs + AG group had been inferior to the ZnONPs group. *G. lucidum* is of a series of different biological events, like anti-oxidant, anti-inflammatory, growth and metastasis which are anti-tumor, and more. It has been shown to play an important role in the prevention and treatment of many kidney diseases, as triterpenoids and polysaccharides are the key operative constituents of GI and have many pharmacological activities [32] . Results were agreement with the findings of previous study that demonstrated the bioactivity of Ganoderma polysaccharides in mitigating lipid oxidation, scavenging free radicals, and thus improving renal health. Moreover, administration of GL polysaccharides significantly improved impaired renal function and reduced structurally proximal tube-shaped mutilation [33]. In 2006, He et al. investigated the influence of *G. lucidum* on streptozotocin-induced type 1 diabetes in mice, and determined that *G. lucidum* polysaccharide (GL-PS) could protect diabetic nephropathy by improving metabolic disturbances, oxidative stress, and renal dysfunctions related to kidney lesions [34]. In another study by Pillay et al. (2011), they used cisplatin to inducting nephrotoxicity in mice to determine the renal protective activity of total terpenes sequestered from GL. Upon treatment with GL-terpenes, tube-shaped injury and released antioxidant activity were significantly improved by increasing serum levels of GSH, SOD, CAT, and GPX and reducing MDA [35]. The results of this research proved that G. lucidum contributed to the restoration of renal function in addition to being a powerful antioxidant that reduced the oxidative stress in ZnONPs-induced nephrotoxicity.

Conclusions

This experiment evaluated the nephrotoxic effects caused by the administration of ZnONPs, Co-administration of GL resulted in reduced nephrotoxicity, as indicated by decreased serum markers, elevated renal tissue levels of GSH and SOD, and decreased renal tissue MDA. Thus, *G. lucidum* can be considered to be defensive against nephrotoxicity and can be taken to prevent kidney damage caused by exposure to toxins.

Conflict of insert

The author declares that there are no conflicts of interests .

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