Original Article

Adverse Impact of Titanium Dioxide Nanoparticles on Hepato-Renal Functions and Improved Role of *Rosmarinus Officinalis*

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Abstract

The expansion of nanotechnology applications in the medical and health fields coincides with the growing concern about its potential toxicity. This study aims to examine the deleterious impact of titanium dioxide nanoparticles (TiO_2NPs) on some hepato-renal biomarkers and the potential attenuating efficacy of *Rosmarinus officinalis* (RO) in rat model. Twenty-eight male laboratory rats were enrolled in this experiment and were distributed into four groups. The rats in control group (CO) were not given any dose, while those in TiO_2NPs group were treated orally with TiO_2NPs by gastric intubation. In addition, the rats in TiO_2NPs+RO group were provided with both TiO_2NPs and *R.officinalis* extract orally. The rats in RO group were supplemented only with *R.officinalis* extract. After the animals were autopsied, the sera were obtained and biochemical tests were performed. Dosing experimental animals with TiO_2NPs led to a significant increase in serum levels of the studied biomarkers, however the data also indicated that RO effectively reduced the adverse action of TiO_2NPs on liver and kidney functions. In conclusion, *R.officinalis* extract can be considered defensive against potential nanotoxicity and may be adopted to prevent possible hepato-renal impairment caused by exposure to these toxins.

Keywords: Nanoparticles, laboratory rats, toxicity.

Introduction

The development of nanotechnology has led to the growing applications of nanoparticles (NPs), in many areas, including biomedicine, cosmetics, food industry, drug delivery, diagnostic devices and others. This is mainly due to the unique physical and chemical properties of these particles with a size between 1-100 nanometers.[1-4] Titanium dioxide nanoparticles (TiO_2NPs) belong to a group of metallic nanoparticles and are most commonly used in consumer products particularly pharmaceutical, cosmetic, optical and other commercial products, due to their high stability and outstanding photocatalytic features.[5-7] This extensive use of TiO_2NPs increases significant concerns about potential nanotoxicity,[8] as their penetration in the body may cause cytotoxicity and inflammation as well as damage to organs like liver and kidneys due to oxidative stress.[9,10] Hepatic biomarkers including Alanine transaminase (ALT), aspartate aminotransferase (AST) and total protein (TB) indicate the health of liver. ALT is an enzyme within hepatocytes that converts proteins into energy while the enzyme AST helps metabolize amino acids. Both enzymes are naturally present in the blood at low levels, and an increase in their levels is a vital indicator of hepatocytes' damage and dysfunction.[11] Total protein (TB) represents the total concentration of albumin and globulin in the blood serum and its lower levels can be used as a marker of liver damage.[12] Likewise, renal biomarkers including creatinine (CR), blood urea (BU) and uric acid (UA) indicate the health of kidneys. The CR is commonly used for monitoring the severity of renal dysfunction. The BU is commonly used as an important indicator of the glomerular filtration rate. Whereas, UA is generally the end product of purine metabolism, and functionally

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impaired kidneys are unable to filter most of the UA from the blood.[13,14] It is necessary to highlight the potential negative effects of TiO_2NPs on health in order to establish a scientific principle for its safe implementation and the sustainable development of nanotechnology.[15] Besides the use of medicinal plants by some people for treatment and prevention of health problems, there is a global trend to explore their beneficial effects to develop new drugs with fewer side effects.[16,17] *Rosmarinus officinalis* L (RO), commonly called as rosemary, is an evergreen wild medicinal herb with a distinct aroma. It belongs to the *Lamiaceae* family and possesses various health-promoting medicinal properties including antioxidant, anti-inflammatory, anti-diabetic, anti-infectious as well as anti-tumor.[18] Nowadays, RO is a common source of bioactive naturalistic compounds like phenolic compounds, terpenes and essential oils.[19] This study was conducted to estimate the toxic potential of TiO_2NP on some liver and kidneys' serological biomarkers in a rat model along with the protective role of RO.

Materials and Methods

Nanoparticles and Plant Extract

Aqueous TiO_2NP dispersion (15 wt%) was purchased from Research Nanomaterials, Inc (US). Its basic characteristics are shown in table 1 and figure 1. Regarding the herb Rosmarinus officinalis (RO), a liquid extract of certified organic rosemary was obtained from Herb Pharm Company (US). This extract is fast absorbing, gluten-free and non-GMO

Table 1: Basic characteristics of TiO2 NP suspension

Appearance	Crystal	Assay TiO_2	Average particle size	Solvent	TiO_2 Purity
Milky white liquid	Rutile	≥ 15.5%	5-15 nm	85% water	99.9%

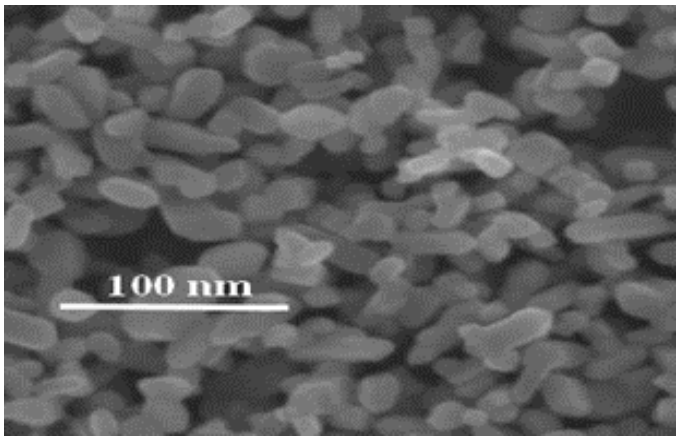

Figure 1: TiO_2NP particle size according to SEM assay.

Experimental Groups

In total, 28 male albino rats, 17 to 23 weeks old with 190-225gr of weight were obtained from laboratory animal centers located in Kirkuk University, Iraq. These animals were placed in suitable cages kept under all standard laboratory conditions with the availability of water and diet. Moreover, the rats were accustomed to the laboratory conditions for seven days prior initiating the experiments. The rats were divided into 4 groups, each containing 7 rats. The groups were made according to the different doses of TiO_2NPs and RO extract as illustrated in table 2.

Table 2: Dosage of TiO2NPs and RO extract

Groups	Dosing for 14 consecutive days
CON	Untreated rats.
TiO_2NPs	Suspension of TiO_2NPs (300 mg/kg) was given using gastric intubation [20].
TiO_2NPs+RO	After being dosed with nanoparticles, rats were given RO (220 mg/kg) orally [21].
RO	Rats were provided only with RO extract (220 mg/kg) orally.

The dosing period for all groups except the control group lasted for fourteen consecutive days. On the fifteenth day, all rats were dissected under general anesthesia and blood was taken through the puncture of hearts and placed in sterile tubes. Thereafter, the sera needed for biochemical assessments were separated by centrifuging the blood samples for several minutes.

Ethical Issues

This experiment was conducted according to the scientific research and ethical guidelines, in Iraqi universities, related to the consumption of animals in laboratory research, and also in accordance with the protocols approved by the US National Institutes of Health issued in 1978.

Data Analysis

The obtained data were analyzed using GraphPad Prism (version 9) and the results are presented as mean and standard error (M±SE). Besides, one-way analysis of variance (ANOVA) followed by Tukey's test was applied to find variation among the experimental groups. A p-value <0.05 was considered statistically significant.

Results

Figure 2 shows the levels of hepatic biomarkers in the sera of all experimental groups used in this study. Dosing rats with TiO_2NPs significantly (P<0.05) increased AST (319.18±2.16), ALT (212.29±3.22), and TB (5.18±0.28) levels, when compared to control rats (122.42 ±1.88; 88.28±1.66; 2.55±0.26, respectively). However, rats co-treated with TiO_2NPs and *R. officinalis* showed a significant decrease in these biomarkers (202.72±4.11; 123.67±2.21; 4.02±0.27, respectively) compared to rats exposed to TiO_2NPs only. Moreover, no significant variation in their levels was observed between the control and *R. officinalis* groups.

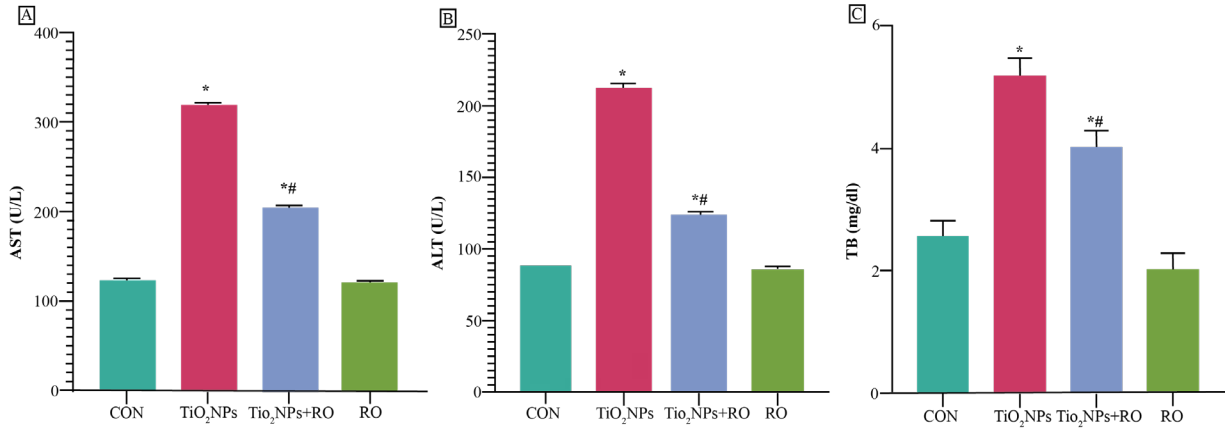

Figure 2: The values of the hepatic biomarkers: a) AST, b) ALT, and c) TB, for experimental rats. Data were represented as mean ± SE. The mark*mentions the variance with CON group, while the mark#mentions the variance with TiO_2NPs group.

According to Figure 3, exposing rats to TiO_2NPs induced a clear increase (p<0.05) in the indices of CR (2.12±0.21), BU (41.56±1.80), and UR (6.98±0.45) versus control groups (0.79±0.06; 20.71±0.77; 3.27±0.26, respectively). However, when TiO2NPs intoxicated rats were co-treated with *R. officinalis,* there was a marked decrease in the levels of renal biomarkers (1.17±0.17; 29.39±2.18; 4.78±0.51, respectively) compared to TiO_2NPs rats. On the other hand, no remarkable difference was reported between CON and RO groups.

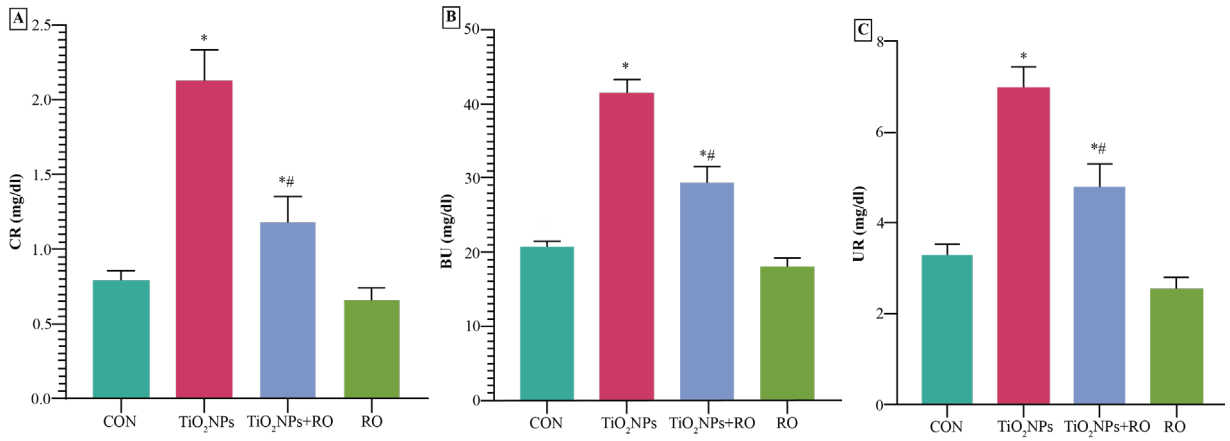

Figure 3: The values of renal biomarkers: a) CR, b) BU, and c) UR, for experimental rats. Data were represented as mean ± SE. The mark*mentions the variance with CON group, while the mark#mentions the variance with TiO_2NPs group.

Discussion

According to the results of this study, TiO_2NPs clearly caused harmful change in the levels of serum markers by disrupting hepato-renal function. Exposure of rats to TiO_2NPs significantly elevated ALT, AST, TB, BU, CR and UR levels compared to the untreated rats in control group. High levels of BU, CR and UR in serum are signs of renal dysfunction due to low glomerular filtration rate.[22] Since the liver and kidneys are the primary target organs for toxins and various chemicals, therefore they can be considered as important targets for nanoparticle toxicity.[23] In general, after NPs enter the body and reach the digestive system, they are distributed through the blood and settle in the body's essential organs, which may cause oxidative stress and cytotoxicity.[24] The toxic potential of TiO_2NPs may be induced by the degradation of molecules mediated by reactive oxygen species.[25] These results were consistent with previous studies conducted on the toxicity of titanium dioxide in laboratory animals. In a previous study by Shukla and colleagues, exposing mice to these particles orally for 14 consecutive days caused a clear change in the levels of liver enzymes in sera. These results were attributed to oxidative stress, as high accumulation of TiO_2NPs in hepatic tissues may have induced apoptosis.[26] In 2018, Morgan et al. observed a significant increase in hepatic enzyme levels in mice poisoned with these particles (100 mg/kg) for up to 2 months,. They observed an increase in the oxidative stress through increased lipid peroxidation and decreased concentrations of antioxidants in the hepatic tissue. Moreover, histopathological examination of the liver tissue showed a rise in their biochemical results as the lesions of programmed cell death were clear.[27] A recent study of Bakour and colleagues on male rats demonstrated that sub -acute administration of TiO_2NPs caused hyperglycemia and elevated liver enzymes, accompanied by an alteration in lipid profile and renal indicators.[28] In another study on adult male Wistar rats, the ability of TiO_2NPs (acute toxicity) to cause kidney damage was observed.[29] Likewise, another group of researchers exposed laboratory adult male rats to TiO_2NPs for 14 consecutive days. Their results confirmed the incidence of negative changes in the serum levels of hepatic and renal biomarkers with oxidative damage in these tissues.[30] Hence, it can be stated that oxidative stress and apoptosis are the main elements of TiO_2NP-induced cytotoxicity. Induction of lipid peroxidation may lead to changes in the cell membrane, causing disruption of vital cellular functions. In addition, these changes lead to disruption of the ionic balance of cells, which affects the physiology of body organs.[31] However, the results of present study demonstrated clear reduction in this toxicity upon co-dosing with RO. The results showed that the animals in the TiO_2NPs+RO group had better hepato-renal function than those exposed only to TiO_2NPs.The levels of biochemical indicators studied in the sera of TiO_2NPs+RO rats were found lower as compared to TiO_2NPs rats. It is worth noting, that natural antioxidants have become the focus of scientific research, especially with regard to preventive medicine, and R. officinalis is considered one of the natural antioxidants due to its high content of polyphenols.[32] This antioxidant activity of RO is due to its main compounds including carnosol, carnosic acid, rosmanol, rosmarinic acid, oleanolic acid, ursolic acid, epirosmanol and the essential oils. These bioactive compounds inhibit lipid peroxidation through a mechanism of scavenging free radicals of lipids. Due to its ability to neutralize reactive oxygen species, it protects against cellular damage (functionally and structurally) as well as cell death that may result from constant exposure to free radicals in biological systems.[33] In a study, pre-supplementation of mice with RO extract significantly attenuated the toxic effects associated with CCl4 by inhibiting oxidative stress, stimulating cellular antioxidant synthesis, restoring renal function indicators to their normal level, and correcting renal structure.[34] Another study reported that the supplementation of *R.officinalis* in the diet of rats was able to prevent the severity of renal dysfunction caused by DETs toxicity.[35] Similarly, a study demonstrated RO as an effective antioxidant in modulating hepatotoxicity induced by hexavalent chromium. The restoration of hepatic biochemical parameters close to normal values was observed.[36] The findings of this research shows that RO contributes to the restoration of normal levels of hepato-renal biomarkers being a powerful antioxidant that reduces the toxicity caused by NPs.

Conclusion

This study confirmed the toxic effect of TiO_2NPs on serum hepato-renal indices by increasing their levels in a rat model. However, combining its dose with RO prevented this toxicity by restoring the levels of serum indicators. Thus, RO can be considered defensive against potential nanotoxicity and its adoption is recommended to prevent hepato-renal function disruption caused by toxin exposure.

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