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Original article

Protective effects of thymoquinone and avenanthramides on titanium dioxide nanoparticles induced toxicity in Sprague-Dawley rats

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ABSTRACT

The protective effect of thymoquinone (TQ), the major active ingredient of *Nigella sativa* seeds, and avenanthramides (AVA) enriched extract of oats on titanium dioxide naonparticles (TiO_2 NPs) induced toxicity and oxidative stress in Sprague-Dawley (SD) rats was investigated. Sixty rats were divided into 6 equal groups. The first, second, third, fourth and fifth groups received TiO_2 NPs, TiO_2 NPs and TQ, TiO_2 NPs and AVA, TQ only, or AVA only for 6 weeks. The sixth group served as the control. Exposure to TiO_2 NPs resulted in increased liver enzyme markers, oxidative stress indices, tumor necrosis factor alpha (TNF-α) and DNA damage. Histopathological alterations were also observed in the liver, brain, lung, kidney, heart and testes. Co-administration of TQ and AVA with TiO_2 NPs decreased the level of liver enzymes, oxidative stress, TNF-α and DNA damage. Furthermore, TQ and AVA increased the total antioxidant and glutathione (GSH) levels. In conclusion, TiO_2 NPs induce hazardous effects in different organs and are closely related to oxidative stress. TQ and AVA have antioxidative and anti-inflammatory effect against the detrimental effect of TiO_2 NPs.

1. Introduction

Titanium dioxide nanoparticles (TiO_2 NPs) are used in widespread applications, such as cosmetics, food colorants and white pigments, and in the sanitization of air, soil and water. Everyone has been exposed to nano size TiO_2, particles because as we inhale them with every breath and consume them with every drink [1]. The small size of NPs may be the main factor that makes them harmful to human health [2]. The oxidative stress and cellular damage caused by NPs in the liver cells are related to the particle size and chemical composition of NPs. When nanoparticles when enter the livers of rats, induce local oxidative stress [3]. TiO_2 enhances the activities of liver damage related enzymes. Nanosized-TiO_2 induced apoptosis and DNA cleavage occurs in the hepatocytes of mice [4]. Moreover, Okaya et al. [5] reported that aggregated TiO_2 particles were clearly obsreved in the liver sinusoids and were phagocytized by Kupffer cells. In an acute and subacute toxicity study, Wang et al. [6] reported that mice treated with TiO_2 NPs showed histopathological alterations in hepatocytes including hydropic degeneration, fatty change, necrosis, apoptosis and hepatic fibrosis.

Nanosized-TiO_2 causes an inflammatory response in the airways of mice [4]. Oberdörster et al. [7] studied TiO_2 induced toxicity in the lung after inhalation of TiO_2 in rats and hamsters. They found heavy infiltration of neutrophils and macrophages in the bronchoalveolar lavage (BAL) fluid and NP deposition in the lung.

Currently, medicinal plants are a key resource for drug synthesis [8]. *Nigella sativa* (NS) Linn. is an annual flowering plant of the Ranunculaceae family. The plant grows in different countries near the Mediterranean Sea, Pakistan, India and Iran [9]. Thymoquinone (TQ) is the major active ingredient of the volatile oil of NS seeds [10]. TQ has antioxidant and anti-inflammatory actions. TQ protects gastric mucosa from mucosal injury induced by alcohol through its antioxidant effect [11] and TQ is an effective anti-inflammatory agent in an asthmatic animal model [12]. TQ also has anti-hypertensive, anti-diabetic, anti-bacterial, anti-tumor and immunomodulatory activities [13].

Avena sativa (oats) have also been used as a traditional medicine for centuries. Oats contain many antioxidants, such as flavonoids, non-flavonoid phenolic acids and vitamin E. The phenolic-rich fractions of oats have antioxidant activity. Diets with oat by-product cause an increase in the serum alpha-tocopherol concentration [14]. Avenanthramides, nitrogen-containing phenolic compounds,

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are anti-inflammatory agents that inhibit NF-kappa B [15]. Avenanthramides also have antioxidant activities [16]. Liu et al. [17] indicated that oats AVAs may reduce the risk of atherosclerosis by inhibiting vascular smooth muscle cell proliferation.

The mechanisms associated with the toxic effects of titanium dioxide nanoparticles (TiO_2) are not completely understood. This study evaluated the histopathological changes, oxidative stress and different mechanisms related to the effects of chronic oral administration of TiO_2 NPs on the parenchymatous organs of male Sprague-Dawley (SD) rats and evaluated the protective effects of thymoquinone (TQ) and avenanthramide (AVA).

2. Materials and methods

2.1. Chemicals

Titanium dioxide nanoparticles (TiO_2 NPs) (nanopaticle size 21 nm) and; thymoquinone (TQ) were purchased from Sigma-Aldrich, Germany. The AVA-enriched extract of oats was obtained from Shannix, China, USA. ELISA kits for tumor necrosis factor alpha (TNF-α) were provided by KOMABIOTECH, Seoul, Korea. Kits used for the determination liver function and oxidative stress biomarkers were purchased from Spectrum, Germany. The testosterone enzyme immunoassay test kit was obtained from Immunometrics Ltd., London, UK.

2.2. Animals

Male Sprague-Dawley rats, weighing 160–200 g was obtained from the animal house of the Faculty of Medicine and were used in this study. All experimental procedures in the present study were approved by the Standing Committee for Biochemical Research Ethics in Jazan University (SCBRE). Animals were maintained under standard conditions with 12–h light/dark cycles, 22 °C, and 60% humidity. Food in the form of dry chow pellets consisted of (29% protein, 13.4% fat, 56.5% carbohydrate and 1.1% salt mixture) and water were available ad libitum. A total of 60 adult male SD rats were used.

2.3. Treatment protocol

After 2 wks of acclimatization, the rats were randomly divided into 6 equal groups of 10 rats each. The first, second, third, fourth and fifth groups received TiO_2 NPs, TiO_2 NPs and TQ, TiO_2 NPs and AVA, TQ only, and AVA only, respectively for 6 wks. TiO_2 NPs (150 mg/kg b.w.) in 1% Tween 80 [6] was administered daily as an oral dose using a gastric tube. TQ was administered daily at a dose of 20 mg/kg b.w. in corn oil by gastric tube [18]. AVA was administered daily at a dose of 20 mg/kg b.w. by gastric tube [19]. The sixth group served as the control and received 1% Tween 80 daily by gastric tube (0.5 ml/rat).

2.4. Sampling

At the end of experiment, the rats were deprived of food overnight and then; anesthetized with diethyl ether. Blood was collected from the retro-orbital plexus into anticoagulant-free test tubes to determine hematological parameters, and the serum was removed and stored at −20 °C until the determination of AST, ALT, reduced glutathione (GSH), lipid peroxidation (LPO), total antioxidant, TNF-α and testosterone hormone was performed. The rats were then immediately sacrificed by cervical decapitation. The liver samples were divided into 2 portions. The first portion was stored in 10% buffered formalin for histopathology and the second portion was used for a single gel. Samples from the brain, kidney, lung, heart and testes were stored in 10% buffered formalin for histopathology.

2.5. Measurement parameters

2.5.1. Hematological examination

Blood samples with anticoagulant were used for the determination of hematological parameters. The complete blood count, including erythrocyte count, hemoglobin and total and differential leucocytes were performed for each sample [20].

2.5.2. Measurement of serum liver function enzyme activities

Activities of ALT and AST in the serum samples were measured colorimetrically using ALT and AST activity assay kits purchased from Sigma-Aldrich (Germany). ALT and AST activity assays depend on the transfer of an amino group from alanine and aspartate to α-ketoglutarate resulting in the generation of pyruvate and glutamate, respectively. The generated pyruvate and glutamate are proportional to the activity of ALT and AST in the serum and can be colorimetrically measured at 570 and 450 nm for ALT and AST, respectively [21].

2.5.3. Measurement of serum lipid peroxidation (LPO)

Malondialdehyde reacts with thiobarbituric acid reactive substance (TBARS) that can be measured spectrophotometrically at 520 nm and 535 nm [22].

2.5.4. Total antioxidant capacity

The determination of the antioxidative capacity is performed by the reaction of antioxidants in samples with a defined amount of exogenously provided H_2O_2. The antioxidants in the sample eliminate a certain amount of the provided H_2O_2. The residual H_2O_2 is then determined colorimetrically using an enzymatic reaction according to the method of Koracevic et al. [23].

2.5.5. Measurement of serum glutathione (GSH)

Glutathione (GSH) was determined using the method of Beutler et al. [24]. Briefly, in an Eppendorf tube, 200 μl of sample was added to 300 μl distilled water and 300 μl precipitating solution (1.67 gm glacial *meta*-phosphoric acid, 0.2 gm EDTA and 30 gm NaCl). The solution was incubated for 5 min and then centrifuged at 5000 rpm for 10 min. To a cuvette, 200 μl of the supernatant and 800 μl of phosphate solution (0.3 M Na_2HPO_4) and 100 μl DTNB [0.04 gm 5,5 dithiobis-(2-nitrobenzoeix acid)/100 ml 0.1% sodium citrate] was added and measured at 412 nm against a blank consisting of 1 ml phosphate solution, 250 μl diluted precipitating solution (3:2 distilled water) and 125 μl DTNB.

2.5.6. Measurement of serum TNF-α

TNF-α levels were measured using various commercially available rat enzyme-linked immunosorbent assay (ELISA) kits. Specifically, for TNF-α levels, the Invitrogen ELISA kit (catalog number KRC3014; Invitrogen, Carlsbad, CA, USA) was used.

2.5.7. Measurement of serum testosterone

Determination of the serum testosterone hormone level was measured using quantitative enzyme immunoassay with commercially formulated kits (Immunometrics Ltd., London, UK). The enzymes were detected by color and the absorbance was measured at 450 nm using an ELISA reader.

2.5.8. Detection of DNA damage by comet assay

The DNA damage was measured by single cell gel electrophoresis (comet assay) in the liver tissue. The Trevigen® Comet Assay™ Kit (# 4250-050-K) was used for alkaline electrophoresis. The kit