Disturbances of some blood biomarkers caused by titanium dioxide nanoparticles and mitigating role of garlic oil

Alteraciones de algunos biomarcadores sanguíneos causadas por nanopartículas de dióxido de titanio y papel mitigador del aceite de ajo

Nahla Kamal Asaad College of Dentistry, University of Kirkuk, Kirkuk, Iraq. Email: a.p.nahlaasaad@uokirkuk.edu.iq
Kasim Sakran Abass Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Kirkuk, Kirkuk, Iraq. Email: Kasim_abass@uokirkuk.edu.iq
Received: 02/26/2021 Accepted: 05/15/2022 Published: 06/25/2022 DOI: http://doi.org/10.5281/zenodo.7221832

Abstract

Employing nanoparticles in many consumer industries makes them questionable in terms of safety for human health. The purpose of this experimental study was to investigate the harmful effects of titanium dioxide nanoparticles on some hematological parameters, and then evaluate the ameliorated role of garlic oil. This study was conducted on 28 male rats, they were divided into 4 groups as follows: Con, which included healthy animals used as control. Tio2-N, which included rats poisoned with titanium dioxide nanoparticles. Tio2-N + Gar contained intoxicated rats with a combined treatment with garlic oil. Gar, included rats supplied with garlic oil only. After collecting blood from the sacrificed animals, blood tests were performed. Rats dosed with Tio2-N showed a clear reduce in red blood cell count, hemoglobin concentration, mean body size, mean muscle hemoglobin, mean muscle hemoglobin concentration, hematocrit value, and platelet count, versus a significant increase in white blood cell count compared to the control animals. However, with the co-administration of garlic oil with Tio2-N, a clear improvement in blood disorders was observed. Thus, garlic oil demonstrated its attenuating effect against Tio2-N -induced hematological changes in a rat model.

Keywords: Hematological parameters, nanoparticles, garlic oil.

Resumen

El empleo de nanopartículas en muchas industrias de consumo las hace cuestionables en términos de seguridad para la salud humana. El propósito de este estudio experimental fue investigar los efectos nocivos de las nanopartículas de dióxido de titanio en algunos parámetros hematológicos y luego evaluar el papel mejorado del aceite de ajo. Este estudio se realizó en 28 ratas macho, se dividieron en 4 grupos de la siguiente manera: Con, que incluía animales sanos utilizados como control. Tio2-N, que incluía ratas envenenadas con nanopartículas de dióxido de titanio. Tio2-N + Gar contenía ratas intoxicadas con un tratamiento combinado con aceite de ajo. Gar, incluidas las ratas suministradas solo con aceite de ajo. Después de recolectar sangre de los animales sacrificados, se realizaron análisis de sangre. Las ratas a las que se administró Tio2-N mostraron una clara reducción en el recuento de glóbulos rojos, la concentración de hemoglobina, el tamaño corporal medio, la hemoglobina muscular media, la concentración de hemoglobina muscular media, el valor de hematocrito y el recuento de plaquetas, frente a un aumento significativo en el recuento de glóbulos blancos en comparación con los animales testigos. Sin embargo, con la coadministración de aceite de ajo con Tio2-N, se observó una clara mejoría en los trastornos sanguíneos. Así, el aceite de ajo demostró su efecto atenuante contra los cambios hematológicos inducidos por Tio2-N en un modelo de rata.

Palabras clave: Parámetros hematológicos, nanopartículas, aceite de ajo.

Introduction

Nanoparticles (1-100 nm) have unique properties that made them quickly enter the biomedical world[1,2]. It has been proven that titanium dioxide nanoparticles (TiO2-N) are among the best types of nanoparticles used in consumer industries[3]. TiO_2 nanoparticles are widely used in personal care products as well as in the manufacture of sunscreens to safeguard the skin from ultraviolet rays due to their broad-spectrum alleviation properties[4,5]. Moreover, they have been used in water handling to deteriorate chemicals through their catalytic action[6]. Since nanomaterials are being produced on a large

scale with extensive use of them, this raises concerns about their toxic effects, especially on human health. Therefore, evaluation of their potential adverse effects in vivo may provide valuable information for the safe use of these particles[7,8]. Body exposure to TiO_2-N by inhalation, absorption, or ingestion is very common. It has been shown that after penetration into the body, cytotoxicity may occur. Also, it should be noted that the potential toxicity of TiO_2-N is due to oxidative stress, whereby reactive oxygen species are formed on the surface of TiO_2-N in the presence of ultraviolet light[9-11]. Blood parameters are important indicators in diagnosing the physiological state in the body, as the bioavailability of chemical compounds at toxic levels can alter blood parameters[12]. Medicinal plants have a great role in the prevention and even treatment of various diseases, as many of them have proven to enhance human health since ancient times[13,14]. Garlic (*Allium sativum* L.) is a common spice that has many health benefits, due to its diverse bioactive compounds. It has important antioxidant as well as anti-inflammatory properties[15]. Garlic has been used historically for relief from influenza, fever, food poisoning, and as a mild anticoagulant, among others[16]. The purpose of the current study is to confirm the harmful changes induced by titanium dioxide nanoparticles on hematological parameters as well as to note the enhanced effect of garlic oil in a rat model.

Materials and Methods

Chemicals

Research grade Titanium Dioxide Nano Powder (TiO2) was obtained from Ultrananotech Private Limited, India. The product specifications are as follows: purity: 99.9%, particle size: 30-50 nm, color: white, crystal form: anatase, surface specific area: 30-50 m2/g, density: 3.8 g/cm3. As for garlic oil (100% pure and organic), it was obtained from Chiltan Pure® Pakistan.

Experiment design

Twenty-eight male albino rats, aged from 6 to 8 weeks, and weighing 200-265 g were obtained from animal houses affiliated with academic institutions in Iraq. They were placed in plastic cages designated for them in a room with ideal specifications in terms of ventilation, temperature, relative humidity and even lighting. They were also allowed to obtain food and water easily. Animals were habituated for 7 days to laboratory conditions before commencing the experiment. Rats were distributed into four experimental groups, seven in each as illustrative in table 1.

Table 1: Dosing of experimental groups.

Groups	Treatments over a two-week experiment period
Con	Healthy rats without any treatment, used as control.
Tio_2-N	Rats received titanium dioxide nanoparticles 300 mg/kg orally [17].
Tio_2-N + Gar	Tio_2-N intoxicated rats were treated with 100 ml/kg garlic oil via gastric tube [18].
Gar	Rats were given only garlic oil at a dose of 100 ml/kg.

Blood Biomarkers

One day after the last treatment dose, all animals used in this study were anesthetized and then dissected. Blood samples were taken after puncture of the heart and placed in tubes with anti-coagulants used to measure blood parameters. An automated blood analyzer was used to measure the following hematological parameters: red blood cell count (RBC), hemoglobin concentration (HB), packed cell volume (PCV), platelet count (PLT), and white blood cell count (WBC).

Data analysis

Using (IMB) SPSS statistics (version 26), analyzes of the study data was conducted. By one-way analysis (ANOVA) differences were found between the experimental groups, followed by Tukey's post- hoc interpretation test. The data were displayed as mean ± standard deviation. $p \leq 0.05$ was considered to be a statistical value.

Results

The results of this experimental study demonstrated obvious changes in the blood profile of Tio2-N rats as compared to Con. A significant lower value in RBC count (5.26±0.64), HB concentration (10.49±0.43), percentage of PCV (33.76±1.19), and PLT counts (548.47±44.91) was observed, versus a significant higher value in WBC count (13.32±0.60) after Tio2-N treatment compared to the control group (7.05±0.45,13.41±0.721, 42.91±0.77, 711.89±17.72, and 10.14±0.76 respectively). When these poisoned animals were supplied with garlic oil, a significant improvement was observed in their blood parameters as shown in figures 1-5.

Figure 1: Effects of treating rats with Tio2 as well as Tio2-N + Gar on red blood cells. The upper asterisk* denotes the considerable variance when compared with the control group, while the two upper stars means the considerable variance when compared with Tio2 nanoparticles.**

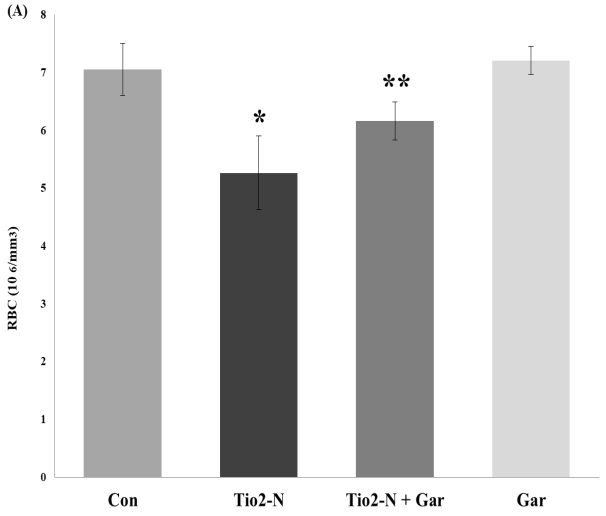


Figure 2: Effects of treating rats with Tio2-N as well as Tio2-N + Gar on hemoglobin concentrations. The upper asterisk* denotes the considerable variance when compared with the control group, while the two upper stars means the considerable variance when compared with Tio2 nanoparticles.**

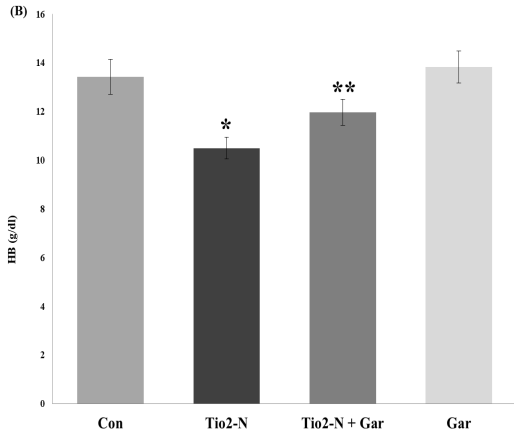


Figure 3: Effects of treating rats with Tio2 as well as Tio2-N + Gar on packed cells volume. The upper asterisk* denotes the considerable variance when compared with the control group, while the two upper stars means the considerable variance when compared with Tio2 nanoparticles**

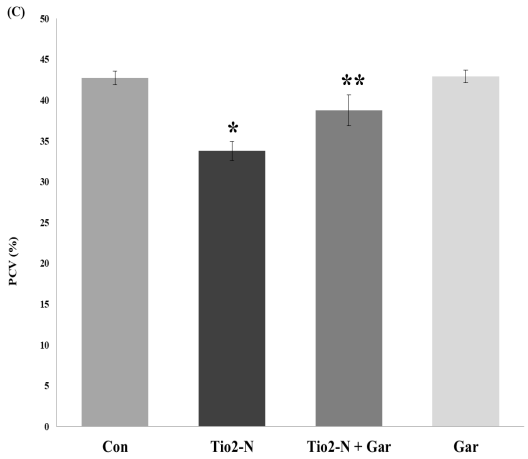


Figure 4: Effects of treating rats with Tio2 as well as Tio2-N + Gar on platelets count. The upper asterisk* denotes the considerable variance when compared with the control group, while the two upper stars means the considerable variance when compared with Tio2 nanoparticles.**

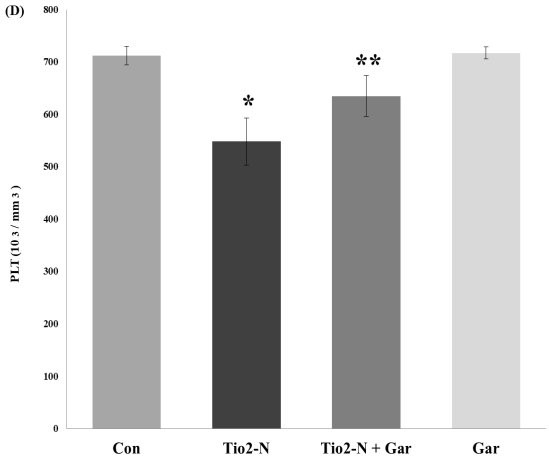
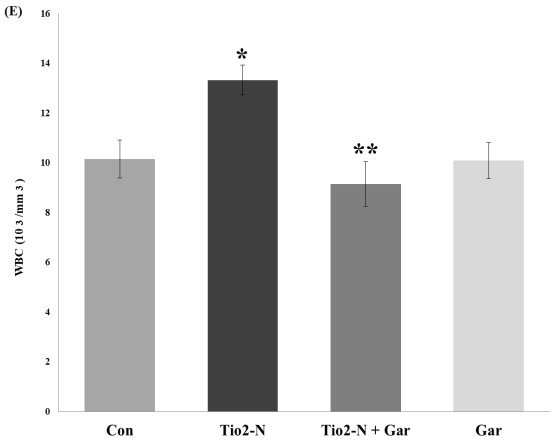


Figure 5: Effects of treating rats with Tio2 as well as Tio2-N + Gar on white blood cells. The upper asterisk* denotes the considerable variance when compared with the control group, while the two upper stars means the considerable variance when compared with Tio2 nanoparticles**



Discussion

The small size of nanoparticles makes them capable of penetrating different organs of the body, especially vital ones, after being ingested orally. Where these particles can cross the small intestine and reach the circulatory system[19,20]. The normal physiological properties of hematological markers may be disturbed by the presence of biochemical products in the bloodstream due to their interaction with components of the blood[21]. It has been proven that there is a strong correlation between measurement of blood parameters and prediction of toxicological risks in vivo[22,23]. The decrease in the number of red blood cells after exposure to Tio2-N may be due to the damage of those red cells and the reduced rate of red blood cell formation, caused by the deterioration of the hematopoietic system, resulting in the development of anemia, followed by hypoxia[24]. Our findings came in one line with a similar recent study conducted by Ramadhan & Ghareeb (2021), where they found that gold nanoparticles caused blood disorders that led to anemia in male albino rats[25]. Also, in a previous study by Heydrnejad and colleagues (2015), they confirmed the slight toxic effects of oral exposure to Ag-NPs on blood biomarkers in both male and female mice[26]. Toxins play the role of stimulating the lipid peroxidation system in tissue cells, causing significant cellular damage due to excess free radicals. This may explain the reduced erythrocyte survival by necrosis as a result of endothelial dysfunction[27]. In an animal experiment study conducted by Chen and colleagues (2020) to investigate oxidative stress in mice after oral exposure to TiO2-NPs at daily doses and at different concentrations for three months, they found that oxidative stress was the primary effect of TiO2-NPs through biological reaction[28]. Hemoglobin, the main intracellular protein in the red cell, is made in the bone marrow. So the decrease in its concentration is due to blood loss, immaturity of reticulocytes, or rapid destruction of blood cells, which leads to anemia[29]. Factors that negatively affect red blood cells also affect the

packed cell volume, which is a measure of red blood cell mass[30]. Chemicals in general may cause damage to marrow tissue and thus inhibit enzymes needed for blood formation[31]. Platelets have a major role in blood clotting, and a low platelet count (thrombocytopenia) is caused by either a defect in the bone marrow or anemia[32].

As for the white blood cells, they contribute to the body's immunity and fight against the invasion of foreign antigens. The usual inflammatory response is in the form of an accumulation of white cells to selectively remove cells and restore homeostasis[33].

On the other hand, the results proved the beneficial effects of garlic oil on the hematological toxicity of TiO2-NPs in laboratory rats. These came in line with the evidence of previous experiments on animals that studied the effects of garlic's protection from chemicals, as they proved that garlic can lower the toxic effects of several agents in the blood and vital organs of the body by radical screening and the effect of antioxidants as well as cell protection activities[34-36]. In a similar study conducted by Mahmoud and colleagues (2022), they concluded that ZnO nanoparticles led to obvious disturbances in blood indicators, but when combined with garlic oil, blood toxicity was significantly ameliorated[37].

Conclusions

The toxic potential of TiO2-NPs has been demonstrated at elevated concentrations in the blood profile. However, the use of garlic oil was efficient in reducing those toxic changes in blood biomarkers.

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