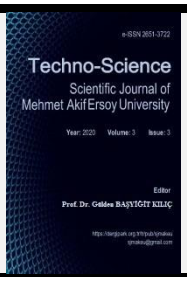

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DETERMINATION OF THE IMPACTS OF TITANIUM DIOXIDE NANOPARTICLES ON A NUMBER OF XENOBIOTIC-METABOLIZING ENZYMES IN RAT LIVER

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ABSTRACT

Nanotechnology techniques are used in many applications, such as cancer treatment, radiological imaging methods, and pharmaceutical industry, as well as in the microbiology field, tissue regeneration, injury healing, treatment of some chronic diseases, and production of vaccines. Whereas products of nanotechnology have a lot of benefits mentioned in our life, they also have some systemic, genetic and toxic effects in organisms. This study's goal was to reveal the impacts of titanium dioxide (TiO_2) nanoparticles on a number of xenobiotic-metabolizing enzymes in the rat liver fraction. In the current research, adult Wistar albino rats having a weight of approximately 150-200 g and fed under normal conditions were utilized. The incubation of four various concentrations of TiO_2 nanoparticles (0.5, 1, 5, and 10 ppm) was performed in the liver fractions. We studied the effects of TiO_2 nanoparticles on some enzymes identified in the microsomal fraction, such as N-nitrosodimethylamine demethylase (cytochrome P4502E1), NADPH cytochrome c reductase, NADH cytochrome b5 reductase, and other enzymes found in the cytosolic fraction, e.g. glutathione-S-transferase (GST), glucose-6-phosphate dehydrogenase (G6PDH), and glutathione level (GSH). GST, G6PDH, NADH-cytochrome b5 reductase, and NADPH cytochrome c reductase levels decreased statistically significantly, whereas the GSH level increased significantly in comparison with controls ($p < 0.05$). Cytochrome P4502E1 induction did not change in comparison with controls ($p > 0.05$). Accordingly, in this study, we have shown that TiO_2 nanoparticles are capable of inhibiting xenobiotic-metabolizing enzymes. Therefore, this inhibition can affect the detoxification system negatively.

1. INTRODUCTION

Nanotechnology is one of the most rapidly developing research fields in the world. It is a modern technique, which leads to reducing the use of other technologies and contributes to creating more sensitive products. Nanotechnology enables the efficient use in many fields, such as preparing pharmaceutical forms, medicine, biotechnology, agriculture, chemistry, physics, engineering, and many other industries. In recent days, nanomaterials of different sizes and properties have been synthesized for use in medical and biotechnological fields. The chemical and biological activities of synthesized nanoparticles can increase association to change in the surface area to volume ratio [1-4]. Changes in properties and the increased production due to the widespread use of nanomaterials have caused a lot of questions whether these products are harmful to the environment and human health or not [5, 6]. A study by Hagens et al. demonstrated that nanoparticles spread to the lungs, bone marrow, colon, spleen, liver and lymphatic system after intravenous injection, whereas they were distributed in the kidney, spleen, liver, brain, lungs and gastrointestinal (GI) system pathway after oral administration [7]. Some nanoparticle types can pass through the GI channel, and at the same time, these nanoparticles

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may be absorbed into the systemic circulation through the GI barrier. However, some nanoparticle systems have been found to accumulate in the liver [8].

Titanium dioxide (TiO_2) is one of the nanoparticles, which is generally used with a size of 1-100 nm in paints, plastics, paper, pigments, cosmetics, and personal skin care products, especially in water treatment systems, and as a bactericidal agent because of its stability and anti-corrosion properties. Therefore, TiO_2 nanoparticles have become one of the most produced nanomaterials in the world with 10,000 tons per year [9, 10]. Due to the increase in its production and use, it increases the uncontrolled destruction and release into nature with each passing day. Once TiO_2 nanoparticles enter the living system, they accumulate in different organs because there is no way the body can eliminate them. Since they are nano-sized, they can go all over the body, even through cells, and affect all cellular mechanisms[11]. The toxicity effects of TiO_2 nanoparticles on algae, bacteria, plants, animal and human cancer cells have been demonstrated in many studies[12-17]. Jani et al. (1994) reported that rutile TiO_2 nanoparticles taken orally could be absorbed through the gastrointestinal tract and pass from mesenteric lymph sources and lymph nodes to the liver [18]. It has been shown that ultrafine TiO_2 particles accumulate mostly in the liver, then pass to the kidney, where these particles are gradually excreted[19]. Trouiller et al. (2009) demonstrated that TiO_2 nanoparticles could break both single and double strands of DNA, damage chromosomes, and cause inflammation [11]. Studies have revealed that TiO_2 nanoparticles can promote oxidative stress, which occurs by high reactive oxygen species (ROS) production in cells or on the surface of cells[20, 21]. In a study on mice, TiO_2 nanoparticles were exposed to drinking water and were detected to cause genetic damage after the fifth day [11]. Therefore, it is important to understand that organisms may be affected by TiO_2 nanoparticles (NPs), so the mechanisms of their actions on organisms should be studied. In our previous studies, we have shown that TiO_2 nanoparticles are toxic in both healthy and cancer cell lines [22-24]. The present research aimed to determine the impacts of TiO_2 nanoparticles on a number of xenobiotic-metabolizing enzymes in rat liver fractions.

2. MATERIALS AND METHODS

2.1. Chemicals

The TiO_2 nanoparticles synthesized according to the sol-gel method were used. The selection of the TiO_2 NPs concentrations (0.5, 1, 5, and 10 ppm) that were utilized in tests was carried out in a careful way in accordance with the findings acquired from a preliminary concentration response study.

2.2. Synthesis of TiO_2 Nanoparticles

In our research, TiO_2 nanoparticles were utilized, and deionized water (DIW) was selected as the base fluid. We prepared TiO_2 nanoparticles by the sol-gel process [25, 26].

2.3. Animal Material

Adult male Wistar albino rats, weighing 150-200 g (2-month-old male) and nourished under normal conditions at the Cumhuriyet University Experimental Animal Laboratory, Sivas were used. Rats were divided into 2 groups of 8 animals each and the animals were kept under room conditions (20–22°C). Liver tissues were obtained from rats determined by ethics committee approval (Sivas Cumhuriyet University Faculty of Medicine Ethics Committee (B.30.2.CUM.0.01.00.00-50 / 60). Study in liver tissues was done *in vitro*.

2.4. Preparation of Tissue Homogenates and Microsomal/Cytosolic Fractions

At the end of the implementation of the study protocol were taken from the rats under light ether anesthesia. We removed the liver, weighed it, and perfused with cold 0.9% saline to separate small pieces. The livers that were extracted from the rats before homogenization were washed using ice-cold phosphate-buffered solution, and the cleaning of the fat and collagen tissues was performed simultaneously. Phosphate buffered solution (pH: 7.4) was used for the homogenization of liver tissues under external cooling in ice water, and their centrifugation was carried out at 40,000xg for a period of 60 min at a temperature of 4°C. For measuring G6PDH, GST, and GSH activities and protein levels, we transferred supernatants into Eppendorf tubes. The centrifugation of the liver supernatants that had not been utilized yet was performed at 105,000xg at a temperature of 4°C for a period of 60 min again, and the cytoplasmic microsomal fraction was acquired for the analysis of hepatic chemical metabolizing parameters. The determination of the N-nitrosodimethylamine demethylase (cytochrome P450 2E1), NADPH cytochrome c reductase, and NADH cytochrome b5 reductase activities was carried out in the microsomal fraction. All specimens were stored at a temperature of -80 °C until analysis [27].

2.5. Protein Determination

The determination of microsomal and cytosolic protein concentrations was performed as described by the Coomassie brilliant blue method. Bovine serum albumin was utilized as a protein standard [28].

2.6. Analysis of Xenobiotic-Metabolizing Enzymes

1-chloro-2,4-dinitrobenzene was utilized as a substrate to determine cytosolic GST activity when GSH was present by monitoring the increase in absorbance at 340 nm and presented as units/mg protein [29]. The measurement of GSH levels was made in accordance with Beutler's method [30]. The measurement of G6PD was also carried out by employing Beutler's method [31]. The determination of cytochrome P4502E1 (N-nitrosodimethylamine N-demethylation) activity was performed as a result of the measurement of formaldehyde formation by utilizing Nash's reagent [32]. The measurement of NADPH cytochrome *c* reductase was carried out at 550 nm using cytochrome *c* as an electron acceptor. The millimolar extinction coefficient was utilized (ε = 0.021 m*M*-1 cm-1) for calculation [33]. Potassium ferricyanide was used as a substrate to determine NADH cytochrome *b5* reductase activity when NADH was present by monitoring the decrease in absorbance at 420 nm. The millimolar extinction coefficient was utilized (ε = 1.02 m*M*-1 cm-1) for calculation [34].

2.7. Statistical Analysis

All numerical data are expressed as the mean ± SE and the significance between means was assessed using student's *t* test. Hypotheses were tested at both the 0.05 and 0.001 levels. P<0.05 was taken as significant.

3. RESULTS

The TiO_2 NPs were successfully synthesized by the sol-gel method and characterization analyzes are shown in our previous articles [24, 25]. The GST, GSH level (GSH), and G6PDH levels were revealed in the cytosolic fraction, while N-nitrosodimethylamine demethylase (cytochrome P4502E1), NADH cytochrome b5 reductase, and NADPH cytochrome c reductase levels were found in the microsomal fraction. In our study, the GST level decreased significantly, while the GSH level increased significantly compared to controls (p<0.05). Similarly to the GST activity, G6PDH showed a decrease compared to the control group (p<0.05) (Figure 1). NADH-cytochrome b5 reductase and NADPH cytochrome c reductase increased significantly in comparison with controls (p<0.05). The cytochrome P4502E1 induction did not change compared to controls (p>0.05) (Figure 2).

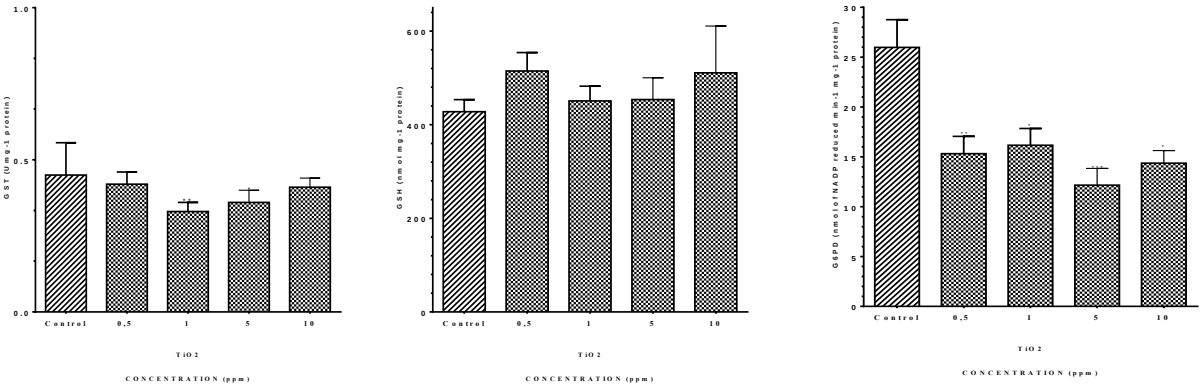

Fig 1. The diagram showing the effect of TiO_2 NPs on GST, GSH, and G6PDH protein. In vitro, it was treated at a concentration range of TiO_2 from 0.5 to 10. TiO2 was compared to the control. Represents the mean ± SEM of three separate experiments (*** p <0.0001, ** p < 0.001 and * p < 0.01).

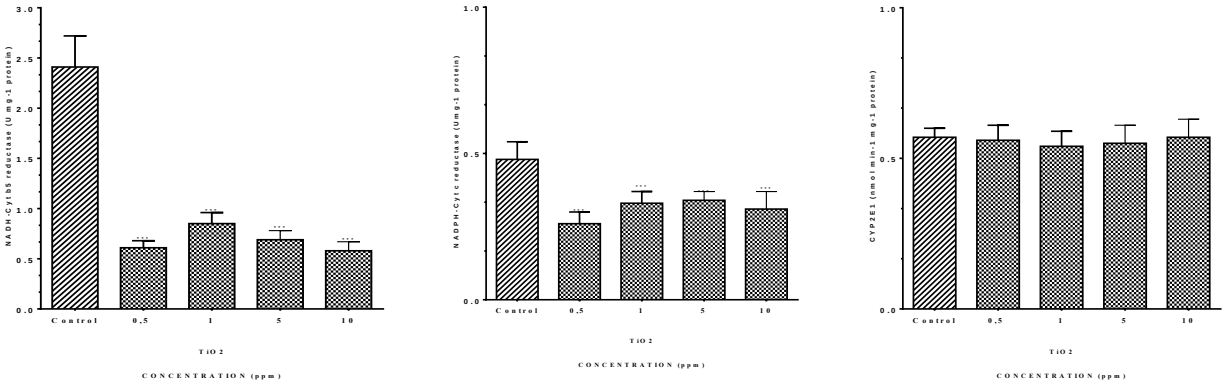

Fig 2. The diagram showing the effect of TiO_2 NPs on NADH-cytochrome b5 reductase, NADPH cytochrome c reductase, and cytochrome P4502E1 protein. In vitro, it was treated at a concentration range of TiO2 from 0.5 to 10. TiO2 was compared to the control. Represents the mean ± SEM of three separate experiments (*** $p < 0.0001$, ** $p < 0.001$ and * $p < 0.01$).

4. DISCUSSION

The unique quantum and chemical features of materials produced at or below the nanoscale are utilized by nanotechnology. During the last decade, rapid expansion has been experienced in the sector, and engineered nanoparticles (ENPs) are widely applied in the industry nowadays and are included in numerous consumer products [38, 39]. The majority of the nanoparticle toxicity studies carried out so far have addressed the determination of the risks related to the inhalation of metal oxide nanoparticles and their absorption through the skin. Thus, a lot of the *in vitro* systems utilized to screen nanoparticle toxicity have utilized terrestrial vertebrate cell lines, e.g. alveolar macrophages [40], bronchial epithelial cells [41], and pneumocytes and dermal fibroblast cell lines [42]. Until recent times, not significant attention has been attached to possible risks related to the exposure of aquatic organisms to NPs and the relevant requirement for the related in vitro models [43]. The metabolic activation site of xenobiotics takes a significant part in the ensuing toxic cellular responses. Considerable attention has been attached to the content of nuclear xenobiotic-metabolizing enzymes because of their proximity to cellular DNA, the suggested target of a lot of mutagens and carcinogens [44, 45]. Glutathione conjugating enzymes (GSTs) are found in various subcellular compartments, such as mitochondria, cytosol, endoplasmic reticulum, plasma membrane, and nucleus. There are implications of the regulation and function of GSTs in oxidative stress, cell growth, disease progression and prevention [46]. In this study, it was observed that TiO_2 nanoparticle had an inhibition effect mostly on the xenobiotic-metabolizing enzyme system. We think that this inhibition weakens the xenobiotic defense system. A lot of research have indicated possibly harmful impacts of nanoparticles on cells and tissues. Toxicological research conducted recently on NPs has proved that nanoparticles may lead to the induction of more significant levels of cellular oxidative stress [47]. Sereemaspun et al. have shown that metallic AgNPs have inhibitory impacts on cDNA expressed in human P450. However, some changes occur in the P450 activities of specific isozymes [48]. In a preliminary study, we determined that the HeLa cell line of Al_2O_3, TiO_2, and TiO_2+Al_2O_3 nanoparticles had anticancer impacts and caused the inhibition of cell growth. We revealed that the above-mentioned drugs exhibited higher activity in the HeLa cell line in comparison with the L-929 cytotoxic impacts on the HeLa cell line [49]. In the other study we conducted, it was shown that TiO_2 considerably reduced cell viability in the breast cancer cell line (MDA-MB–231 and MCF–7) in comparison with controls [24]. This study is important because it is the first article to investigate the interaction of TiO_2 nanoparticles with xenobiotic-metabolizing enzymes. The widespread usage of nanotechnology in everyday life and commercial products unavoidably causes nanomaterials to spread to the environment. Thus, the possibility of living things to be exposed to nanomaterials is increasing every day. It is evident that the further investigation of potential health risks at the molecular level will be useful in the development of new materials that do not pose a threat to future health.

In this study, the synthesis of TiO_2 nanoparticles was performed in a successful way by the sol-gel method by utilizing titanium isopropoxide and isopropanol. The characterization of the material synthesized was carried out by employing SEM, XRD, FTIR, and UV-Vis spectroscopy techniques. The findings of the current study show that titanium dioxide nanoparticles are capable of inhibiting xenobiotic-metabolizing enzymes. This inhibition may attenuate the detoxification system capacity in a negative way.

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Competing Interests: The authors declare no competing interests. We also declare that the article is not a multipart study output.

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