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Original Article

# *In-vivo* Titanium Dioxide ($TiO_2$) Nanoparticles Effects on Chromosomal Abnormalities and Lactate Dehydrogenase Activity

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## ABSTRACT

In this study we investigated effect of $TiO_2$ nanoparticles on chromosomal abnormalities and lactate dehydrogenase activity and for this purpose nano anatase of titanium dioxide in four dosages of 10, 50, 100, 500, 800mg/Kg BW during intraperitoneal injection of male mice Balb/C were treated. After 24 hours, micronucleus test was carried out on the samples of bone marrow and determination of the Level of Lactate Dehydrogenase (LDH) level test performed on the samples of peripheral blood. Result showed that nano anatase of titanium dioxide in low dosage of 10, 50, 100mg/Kg BW, had not toxicity effects on bone marrow but in high dosages of 500, 800mg/Kg BW caused induced toxicity effect with increase micronucleus. In dosage of 10mg/Kg BW of nanoparticles showed LDH enzyme is blocked and a reduced in LDH level. The results of this study will be providing useful information for researcher to design of nanotechnology products

with minimal side effects. This study can be very useful in reducing the complications of nanoparticle toxicity.

**Keywords**: Titanium dioxide ($TiO_2$) nanoparticles, chromosomal abnormalities, micronucleus, lactate dehydrogenase (LDE), micro nucleated polychromatic erythrocytes (MNPCE)

## INTRODUCTION

Utilization of materials at the nano scale, due to it is growing unique chemical properties in different fields of science, especially medicine. Titanium dioxide ($TiO_2$) having various features such as robust oxidation, biocompatibility and mechanical properties of nanoparticles that are acceptable in a wide range of sciences, including pharmaceuticals, cosmetics, medicine and engineering is frequently used. In addition, $TiO_2$ nanomaterials due to their photocatalytic properties have attracted the attention of medical researchers. To date, despite numerous studies in the field of Titanium dioxide ($TiO_2$) nanoparticles used in science, few studies have been conducted on the toxicity of nano-$TiO_2$ in biological systems. $TiO_2$ is used for the removal of organic contaminants such as polychloro biphenyls, surfactants, pesticides and organic dye also the hydrophobic self-cleaning products, sunscreen cream especially as photo catalyst in wastewater treatment and air cleaning (Chen *et al*., 2007; Warheit, 2008; Lewinski *et al*., 2008; Toyooka *et al.*, 2009; Vishwasa *et al*., 2010)[1-5].

Johnson Cancer Center during the first comparative studies in America has shown that titanium dioxide nanoparticles cause genetic damage in mice systemic. They have been reported that titanium dioxide nanoparticles accumulate in different organs because they are too small and can be transported to all parts of the body (Irwin, 2009)[6]. Reduction of titanium dioxide nanoparticles breaks DNA single-stranded and double stranded. It cause to chromosomal abnormalities and inflammation, which all increases the risk of cancer (Irwin, 2009)[6]. Studies on the biochemical mechanism have been found to be affect titanium dioxide metabolic cycles, carrier performance and blood biochemical factors (Ma *et al.*, 2009)[7].

## MATERIALS AND METHODS

Titanium dioxides ($TiO_2$) nanoparticles have been prepared by sol-gel method. These small nanoparticles with three different XRD, TEM and FT-IR method have been characterized. According to the data, the particles 98.8% purification, size of 10nm, spherical shape and were in anatase phase. The nano-particles dissolved in distilled water by ultrasonic for 5min. this action due to prepared a homogenous suspension for injections.

### Animals and Group Treatments

A total of 32 male mice Bulb/C in the range of 6 to 8 weeks of age and ranged in weight from 20 to 30g were used. These mices divided 7 groups with 4 mice. Then the animals were transferred to a maintenance room for one week in environment with 21-25°C and humidity 44-50 and lighting for 12hours of light and 12hours dark period were compatible. Seven groups of animals used in this study included: control group, treated with sterile water, treated with nano anatase titanium dioxide for 24hours at 4 dosages of 10, 50, 100, 500, 800mg/Kg BW.

### Micronucleus (Mn) Assay

After the injection, and after 24hours of treatment, mouse bone marrow was extracted using the RPMI 1640 Glutamax medium. The bone marrows made smears on slides and then fixed in 85% methanol for 5min and were stained with 8% Giemsa solution for 20min. In each experiment group, the animals treated for a total of 200 cells in polychromatic erythrocytes (PCE) normal counts the same period, the number of cells with micronucleus (MNPCE) been identified.

### Determination of the Level of Lactate Dehydrogenase (LDH)

In this study, after 24hours all treatments, with used of non-heparin tube hematocrit were gained blood from mouse eyes. Then separation serum of blood carried out by centrifuged at 1500rpm for 5min. using diagnostic kits and devices LDH out analysis determined the level of enzyme activity in IU/L.

### Statistical Analysis

The experimental design was a randomized complete block with three replicates. Analysis of variance (ANOVA) was calculated using SPSS v. 11.5 and differences between treatment means were compared using Duncan's multiple range test at $\alpha < 0.05$.

## RESULTS AND DISCUSION

### Effects of Titanium Dioxides Anatase Nano on the Chromosomal Abnormalities in Bone Marrows

Results showed that micronucleus changes in mices control group were not significant differences with treated group by sterile water thus sterile water no effect on the production of MNPKHs. Dosages of 10, 50 and 100mg/kg BW titanium dioxides anatase nano were not significant differences with control group and treated group by sterile water, but in 500, 800mg/Kg BW micronucleus changes were significant differences with other control treated groups. This results showed that low dosages of $TiO_2$ nanoparticles had not toxicity effects but in high dosages toxicity specified with increase % micronucleus (MN) (Figure-1). After being dispersed nanoparticles of titanium dioxide can act as a precursor ROS. Also interaction between nanoparticles and cellular content can be led to ROS production. ROS can cause to damage the cell membrane and the product surface it is more antioxidant, can lead to toxicity and eventually cause cell apoptosis (Jin *et al*., 2011)[8].

### Effects of Titanium Dioxides Anatase Nano on the Activity of The Enzyme Lactate Dehydrogenase

Enzyme levels in the group treated with distilled water and also in dosages of 500, 800mg/Kg nano anatase not significant effects with control group. Zaqout *et al* (2012) reported that decrease in level activity of enzyme is depending on type of $TiO_2$ nanoparticles crystal form *in-vitro*[9]. These nanoparticles often attached in anatase and morph phase to enzyme that this bond will led to change in secondary structure or active form of protein. Our result showed that LDH had more tendencies to $TiO_2$ nanoparticles in low dosages, whereas this tendency had significant at high dosages of $TiO_2$ nanoparticles (Figure-2).

## CONCLUSION

Titanium dioxide, also known as titanium (IV) oxide or titania, is the naturally occurring oxide of titanium, chemical formula $TiO_2$. When used as a pigment, it is called titanium white, Pigment White 6, or CI 77891.

Generally it is sourced from ilmenite, rutile and anatase. It has a wide range of applications, from paint to sunscreen to food colouring. When used as a food colouring, it has E number E171. Anatase is one of the three mineral forms of titanium dioxide, the other two being brookite and rutile. It is always found as small, isolated and sharply developed crystals and like rutile, a more commonly occurring modification of titanium dioxide, it crystallizes in the tetragonal system; but, although the degree of symmetry is the same for both, there is no relation between the interfacial angles of the two minerals, except in the prism-zone of 45 and 90°C. It has capacity to oxidize the DNA strands of chromosomes on the A-T, G-C base of nucleotides by direct action on three hydrogen bonds of purine moiety and two hydrogen bonds of pyrimidine moiety, so the increasing concentration of TN shows the increasing value of MNPCE. The same event happens in LDE enzyme activity on nanoparticles of titanium dioxide. Since enzyme is macromolecular protein entity made by the genomics of chromosomes of nucleotides which get oxidized by $TiO_2$. The base pair of nucleotide of both MNPCE and LDE gets oxidized by its transition metal property.

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**Table-1:** The MNPCEs frequencies (%) in bone marrow and LDH activity (IU/L) of peripheral blood after intraperitoneal injection with Nano-anatase $TiO_2$ suspension for 24 h. Different letters indicate significant differences ($P < 0.05$) according to Duncan's multiple range tests.

| Observation | Control | Nano-anatase (mg/kg BW) | | | | | |
|---|---|---|---|---|---|---|---|
| | | St.W | 10 | 50 | 100 | 500 | 800 |
| MNPCEs/PCEs (%) | 0.118±0.01$^a$ | 0.113±0.00$^a$ | 0.119±0.00$^a$ | 0.121±0.01$^a$ | 0.124±0.00$^a$ | 0.48±0.02$^b$ | 0.49± 0.01$^b$ |
| LDH activity (IU/L) | 1449±0.28$^a$ | 1445±0.46$^a$ | 834±0.04$^b$ | 830±0.01$^b$ | 839.5±0.04$^b$ | 1450 ±0.42$^a$ | 1459±0.09$^a$ |

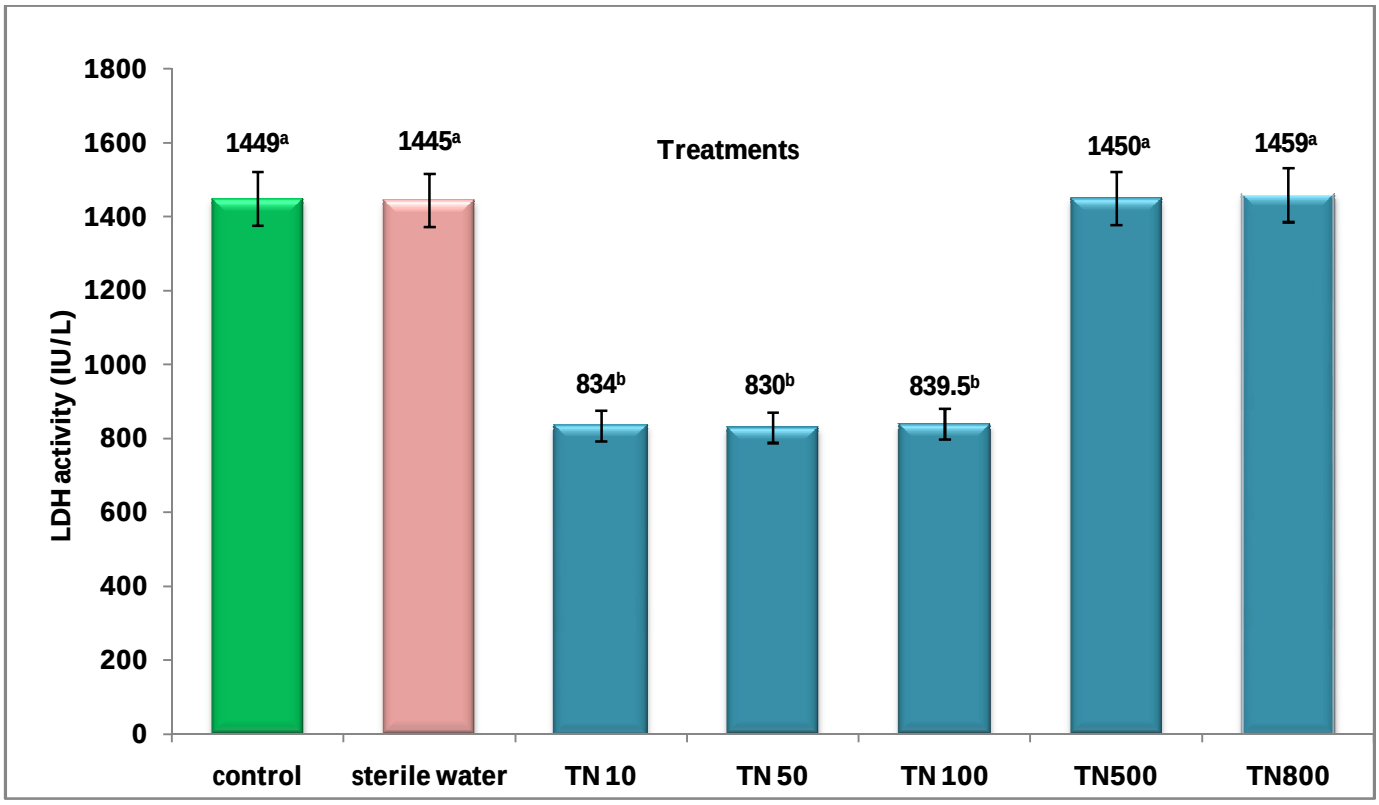


**Figure.1**. Micronucleus changes induced by different doses of anatase $TiO_2$. Different letters indicate significant differences ($P < 0.05$) according to Duncan's multiple range tests.

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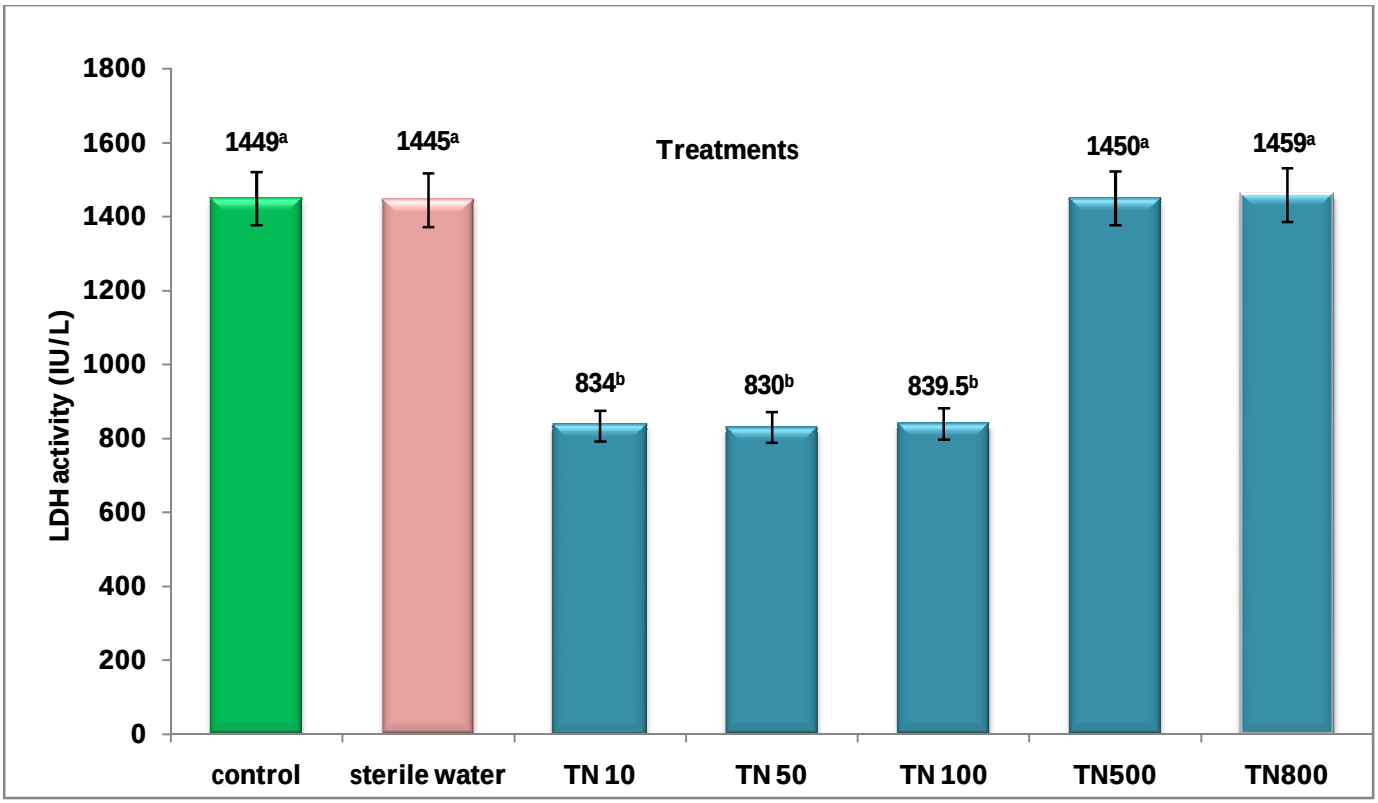


**Figure.2**. LDH activity changes induced by different doses of anatase $TiO_2$. Different letters indicate significant differences ($P < 0.05$) according to Duncan's multiple range tests.

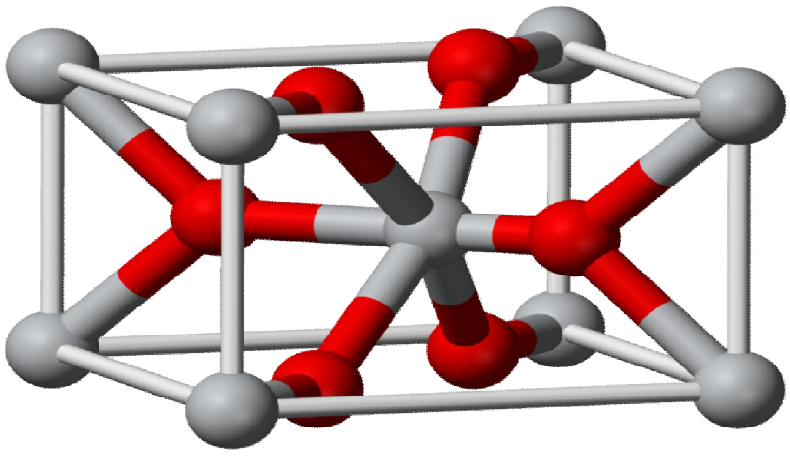

**Figure.3.** Tetragonal crystal lattice of titanium dioxide (Rutile unit)