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# Titanium-Induced Histological and Immunohistochemical Alterations in Liver, Spleen, Lung and Kidney in Male Albino Rats

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**Abstract** Different metals are increasingly used to manufacture implants, especially in the field of dentistry. Metallic implants of titanium are used therapeutically in biomedicine because of their high corrosion resistance and excellent biocompatibility when compared to more conventional stainless steels and cobalt-based alloys. However, no metal or alloy is completely inert. Thus the aim of the present study was to determine the histopathological and immuno-histochemical effects in some target organs of adult male albino rats induced by titanium exposure. This work was carried out on two groups: control group which included 10 rats and treated group which included 20 rats that received intraperitoneal injection of suspension of titanium dioxide ($TiO_2$) in a dose of 150 mg/kg body weight (BW) per day for 45 days. Samples of liver, spleen, kidney, and lung were processed for histological examination. Cryostat sections of spleen samples from each group were stained with common lymphocytic antigen (CLA) for lymphocyte detection. Results revealed histopathological changes in the liver, spleen, lung and kidney of the treated group. The CLA staining of the spleen in the treated group revealed toxic alteration within the spleen, indicating that the immune system may be affected and so interfering in the body defense mechanism.

## Introduction

Metallic biomaterials available for orthopedic purposes become essential to perform important physical activities due to their low cost and excellent mechanical properties. In addition, they are frequently used in dentistry. The manufacturers of different systems of implants strive to achieve an adequate design. Their choice of materials is aimed at guaranteeing minimum degradation, corrosion, dissolution, deformation and fracture among other properties (Ferreira et al., 2003).

Titanium has excellent physicochemical properties, such as good fatigue strength, resistance to corrosion, biocompatibility, whitening and photocatalysis, as well as excellent optical performance and electrical properties (Chen et al., 2009, Morishige et al., 2010). However, a corrosion phenomenon of such devices is the main problem resulting in subsequent spreading of the elements through the whole body via lymph and blood. The metal and the organic fluids interact releasing metallic products as a result of electrochemical processes (Olmedo et al., 2003). An important property of titanium is that on exposure to air or liquids, it rapidly develops a layer of oxide that reduces its reactivity. In fact, this layer of oxide that interacts with the tissues. The titanium do not corrode in the body; however, metal ions slowly diffuse through the oxide layer and accumulate in the tissue (Gotman,1997).

Titanium dioxide (TiO2) nanoparticles have a tendency to rapidly agglomerate into larger□sized particles when introduced into biological systems (Wang et al., 2007; Duan et al., 2010).

This work was performed to determine the histopathological and immuno-histochemical effects of

titanium exposure in the liver, spleen, lung and kidney of adult male albino rats.

## Materials and methods

This work was carried out using 30 adult healthy male albino rats weighing 200-250 gm each. They were kept in clean properly ventilated cages and had free access to food and water throughout the experimental period. They were acclimatized to their environment at least two weeks before starting the experiment.

The animals were divided into two main groups:

I. **Control group**: Included 10 rats that were given 5 ml saline solution by intraperitoneal injection to evaluate the effect of the vehicle.

II. **Treated group**: Included 20 rats that received $TiO_2$ (Sigma Chemical Company), 150 mg/kg BW. Suspensions were given to rats by an intraperitoneal injection every day for 45 days in 5 ml saline solution. All animals were sacrificed at 6 months by cervical dislocation after ether inhalation.

Systemic autopsies of all animals were performed. Samples of liver, spleen, kidney, and lung were obtained. The tissues were taken and immediately fixed in 10% buffered neutral formalin. Then the tissues were dehydrated in ascending grades of alcohol and then cleared in xylol. Impregnation was done in pure soft paraffin for two hours at 55˚C followed by embedding in hard paraffin. Sections of 6 microns thickness were cut by the microtome and preserved at room temperature (Detafield, 1989). They were stained with Haematoxylin and Eosin stain to study the general histological features by light microscope (Bancroft & Cook, 1994).

Cryostat sections of spleen samples from each group were stained with common lymphocytic antigen (CLA) as an immuno-histochemical technique for lymphocyte detection.

All ethically approved conditions used for animal housing and handling were considered. The experimental protocol used followed the regulations for administration and painless scarification of experimental animals.

## Results

### I-Histological results (Hematoxylin & Eosin stain)

#### A-The control group

Sections of the studied organs obtained from control rats showed the normal histological structure. The lung showed the normal appearance of alveoli, alveolar ducts and bronchioles (Figure1). The kidney showed the normal appearance of glomeruli, capsular space and tubules (Figure 2). The Liver showed the normal appearance of central vein and hepatocytes (Figure 3). The spleen showed the normal appearance of red pulp and white pulp (Figure 4).

#### B-The treated group

Sections of the studied organs obtained from this group revealed alterations in their structure as compared with those of control group. The lung showed thickening of alveolar septa and thrombosis of pulmonary vessels, while the lung macrophages were laden with brownish pigment and there was congested blood vessels and inflammatory cellular infiltrations (Figures 5 and 6). The kidney showed congestion and thrombosis in cortex vessels and atrophy of some glomeruli (Figure 7).

Dark brown particles were observed in the liver parenchyma around portal tract. The Kupffer cells were laden with brownish particles and the portal vessels were congested (Figures 8 and 9). The spleen showed dark brown particles in the marginal zone around the lymphoid follicle, and by higher magnification showed abundant macrophage cells containing brownish particles (Figures 10 and 11).

### II- Immunohistochemical results (Common Lymphocytic Antigen stain)

#### A-The control group

Spleen samples from control animals showed T cells around central arteries, which stained positive with common lymphocytic antigen (Figure 12).

#### B-The treated group

Sections obtained from this group showed the treated spleen that stained negative with common lymphocytic antigen (Figure 13).

Table (1) showed the distribution of the intensity of Common Lymphocytic Antigen (CLA) staining in the control and treated groups.

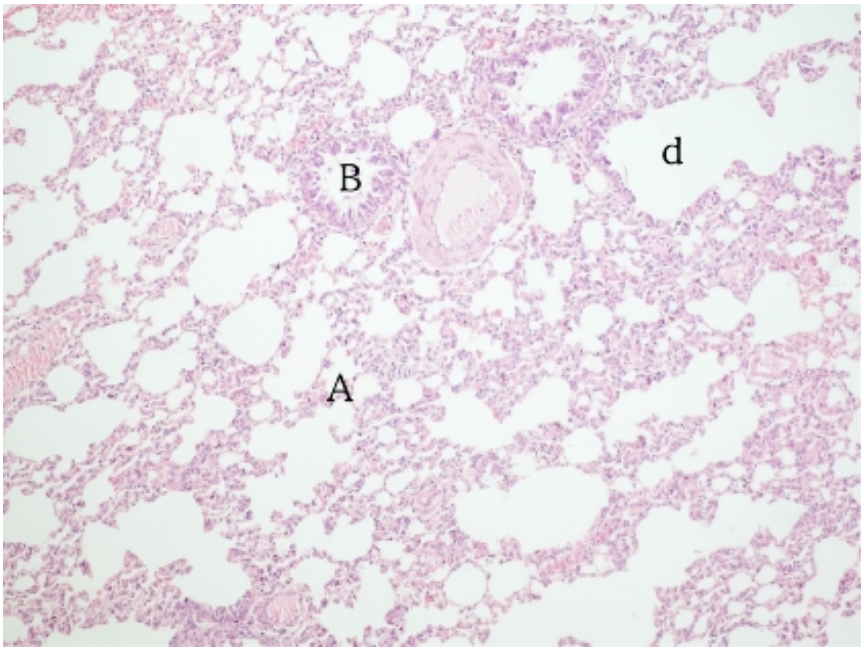


**Figure (1): A photomicrograph of control rat lung showing normal appearance of alveoli (A), alveolar ducts (d) and bronchioles (B). (H&E X400)**

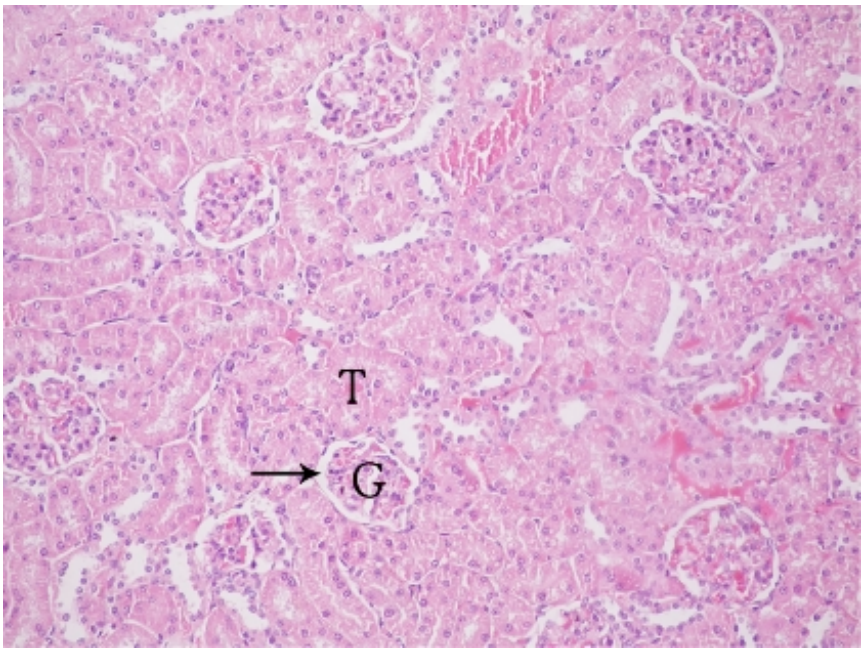


**Figure (2): A photomicrograph of control rat kidney showing normal appearance of glomeruli (G), capsular space (→) and tubules (T). (H&E X400)**

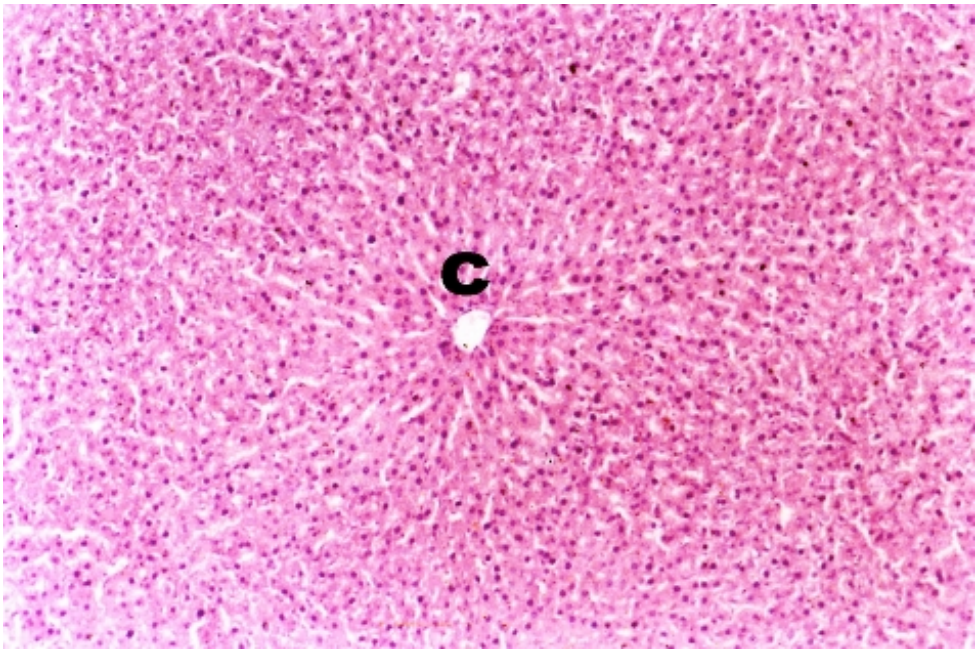


**Figure (3): A photomicrograph of control rat liver showing normal appearance of central vein (C) and hepatocytes. (H&E X400)**

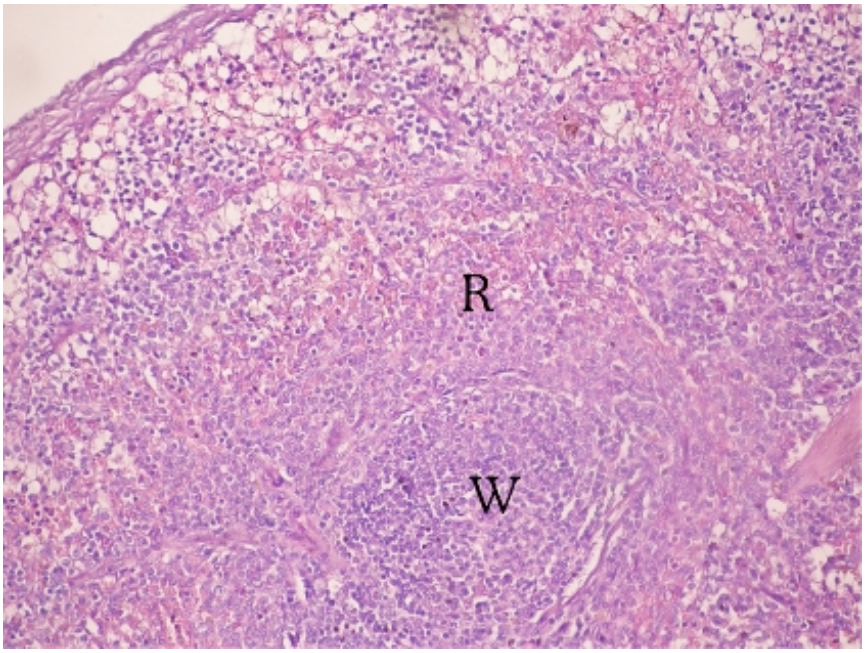


**Figure (4): A photomicrograph of control rat spleen showing normal appearance of red pulp (R) and white pulp (W). (H&E X400)**

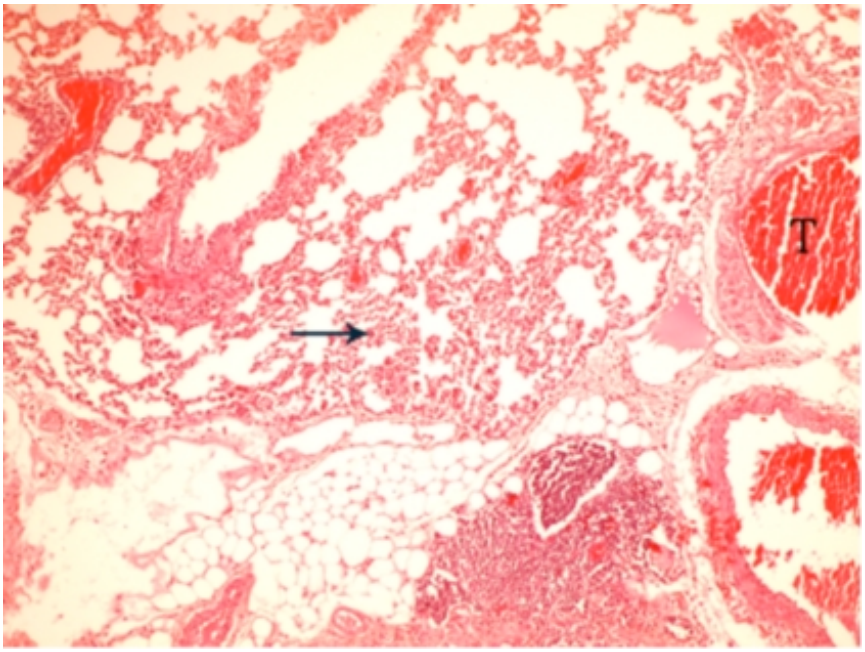


**Figure (5): A photomicrograph of rat lung in titanium treated group (II) showing thickening of alveolar septa (→) and thrombosis of pulmonary vessels (T). (H&E X400)**

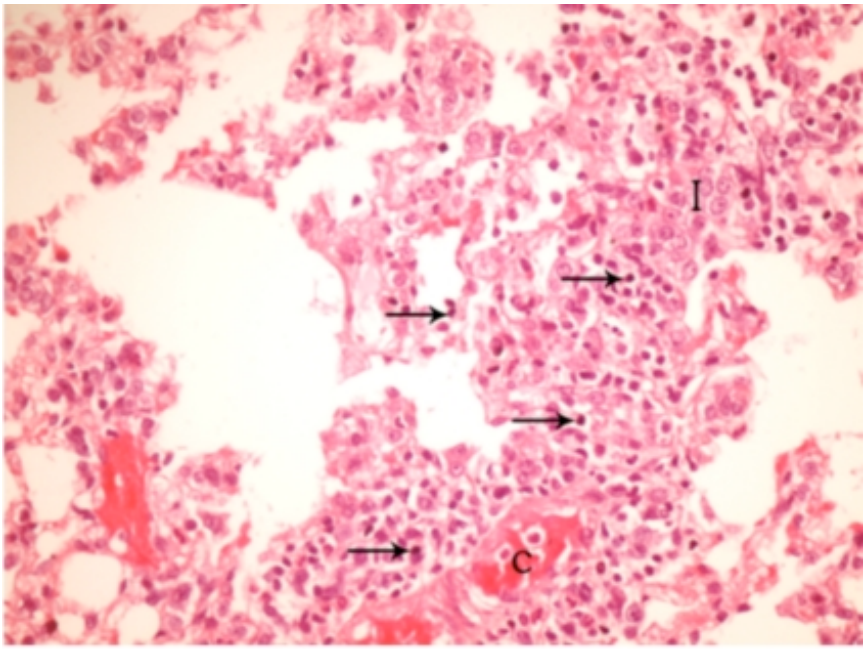


**Figure (6): A photomicrograph of rat lung in titanium treated group (II) showing macrophages laden with brownish pigment (→). Notice congested blood vessels (c) and inflammatory cellular infiltrations (I). (H&E X600)**

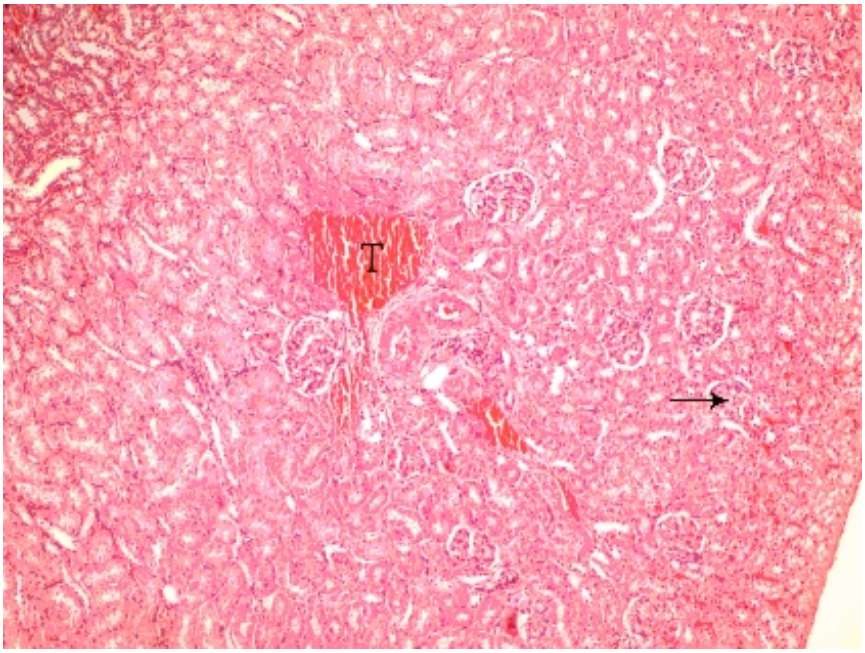


**Figure (7): A photomicrograph of rat kidney in titanium treated group (II) showing congestion and thrombus in cortex vessels (T) and atrophy of some glomeruli (→). (H&E X400)**

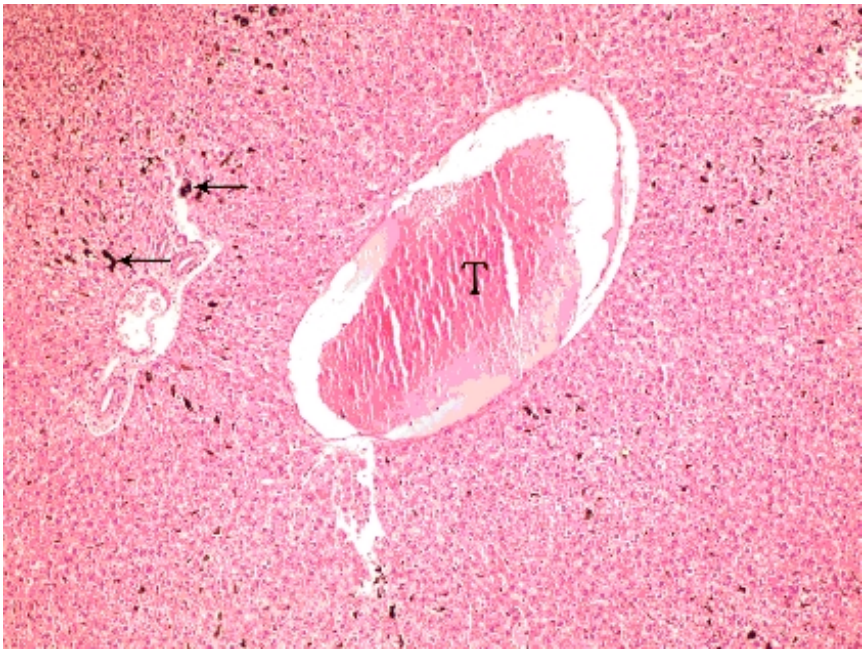


**Figure (8): A photomicrograph of rat liver in titanium treated group (II) showing thrombus in the central vein (T). Notice dark brown particles around the portal tract (→). (H&E X400)**

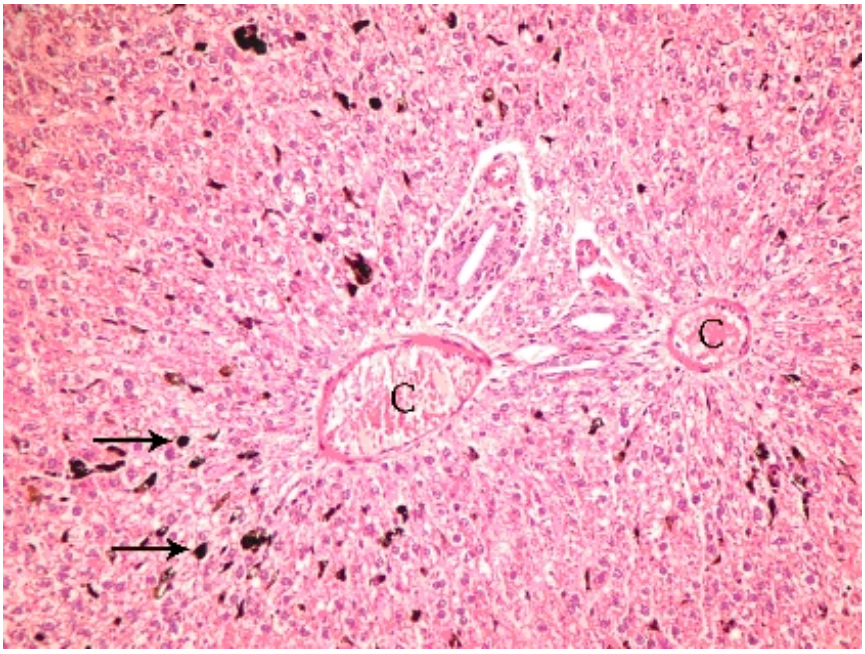


**Figure (9): A photomicrograph of rat liver in titanium treated group (II) showing the Kupffer cells laden with brownish particles (→) and the congested portal vessels (C). (H&E X400)**

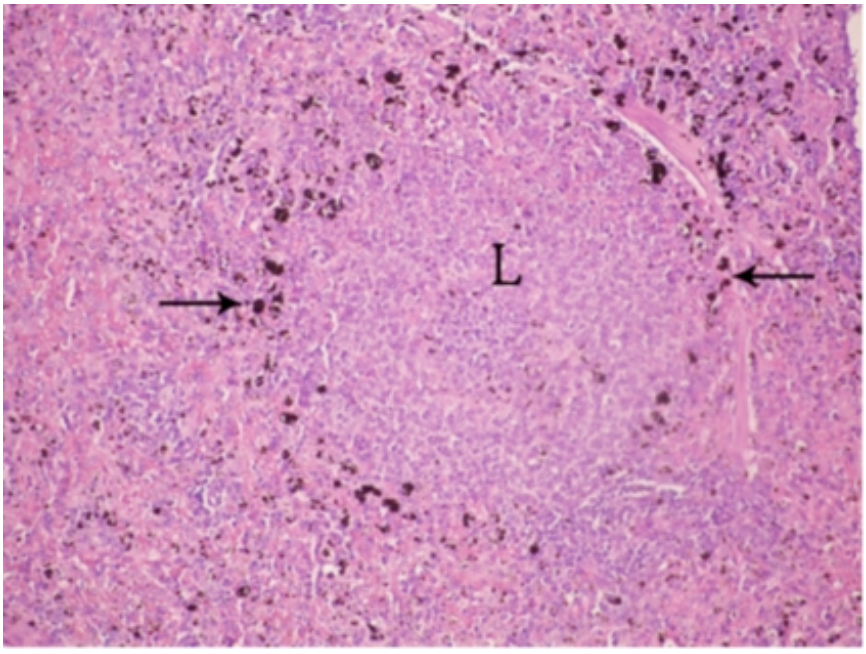


**Figure (10): A photomicrograph of rat spleen in titanium treated group (II) showing brown particles in the marginal zone (→) around the lymphoid follicle (L). (H&E X600)**

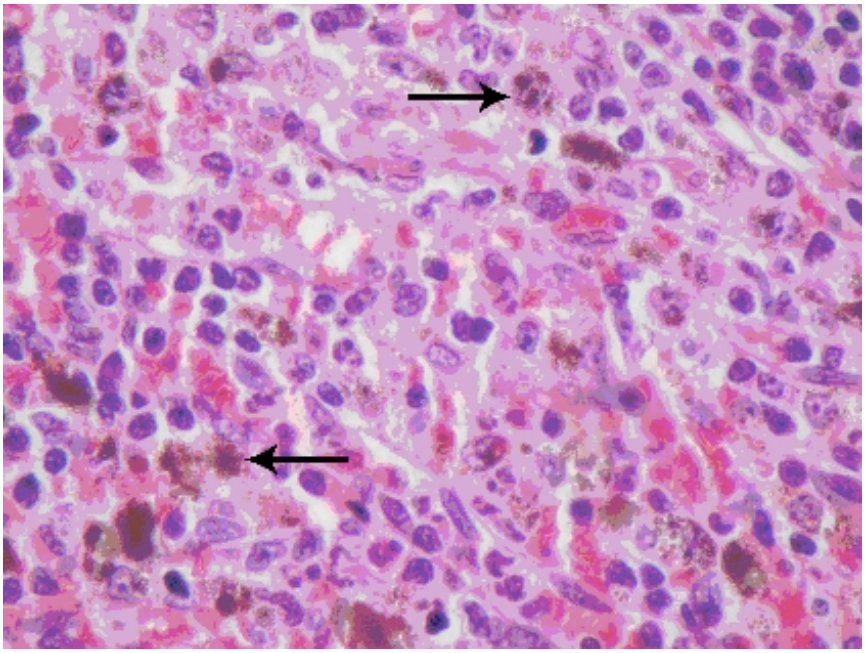

**Figure (11): A photomicrograph of rat spleen in titanium treated group (II) showing abundant macrophage cells containing brownish particles (→). (H&E X1000)**

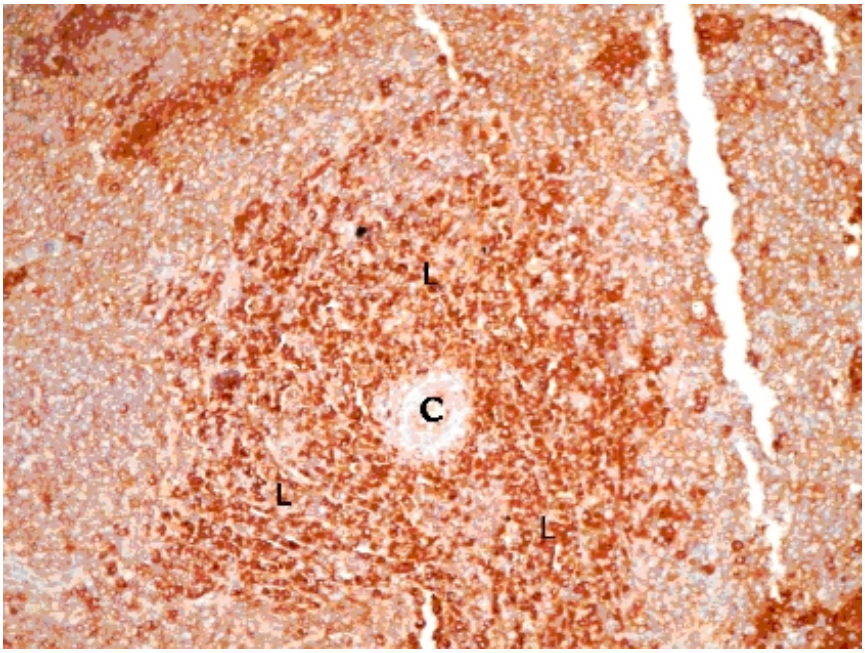


**Figure (12): A photomicrograph of rat spleen in control group showing (II) lymphocytes (L) around central arteriole (C) within the lymphoid follicle of the white pulp, stained positive with common lymphocytic antigen. (CLA X600)**

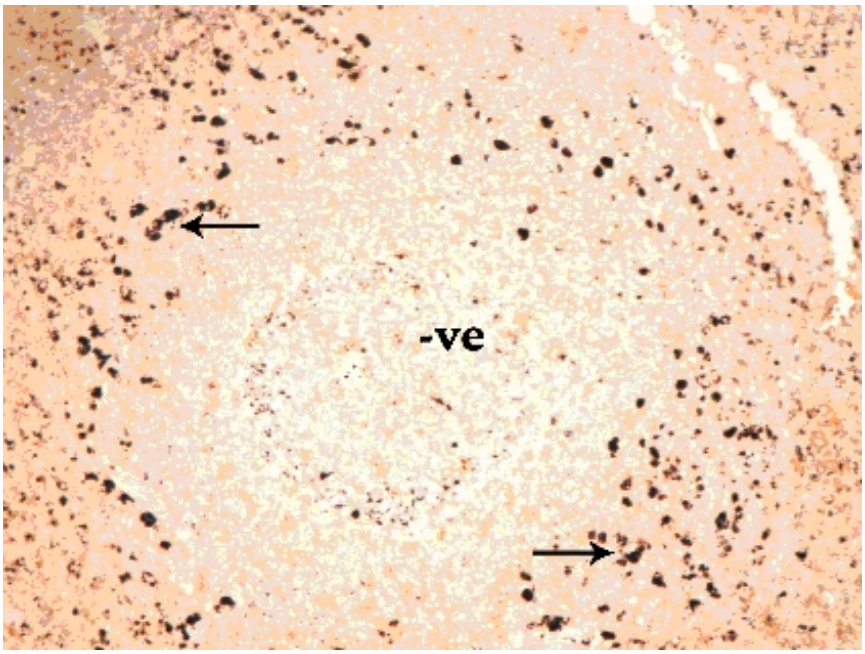


**Figure (13): A photomicrograph of rat spleen in titanium treated group (II) stained negative with common lymphocytic antigen (-ve). Notice brownish particles in the marginal zone around the lymphoid follicle (→).**
**(CLA X600)**

**Table (1): Distribution of the intensity of Common Lymphocytic Antigen (CLA) staining in the control and treated groups.**

| CLA intensity | Control group (n=10) | | Treated group (n=20) | |
|---|---|---|---|---|
| | No. | % | No. | % |
| 0 | - | - | 16 | 80 |
| + | - | - | 4 | 20 |
| ++ | - | - | - | - |
| +++ | 10 | 100 | - | - |

## Discussion

In this study, we evaluated the histopathologic effects of $TiO_2$ in albino rats following intraperiotneal injection. It was reported that titanium oxide ($TiO_2$) is transported in blood by phagocytic monocytes and deposited in organs such as liver, spleen, and lung six months after intraperitoneal injection (Olmedo et al.,2005).

In this study the lung showed congestion and thrombosis of pulmonary vessels which could be induced by the blocking of blood vessels with $TiO_2$ particles (Chen et al., 2009). In addition, there was thickening of alveolar septa due to diffuse deposition of titanium in the lung which was engulfed by macrophages in the interstitium and alveolar spaces. This coincided with the findings of (Chen et al., 2009) who observed interstitial pneumonia associated with alveolar septal thickening in animals exposed to high-dose titanium.

Lung macrophages in this study were laden with brownish pigment which is most probably $TiO_2$. This finding was described by Geiser et al., (2007), and was assumed to be due to phagocytosis or a nonendocytic process by passive uptake mechanisms (not triggered by receptor–ligand interactions) subsumed as ‘‘adhesive interactions’’.

Additionally, inflammatory cellular infiltrations were noticed in this study. The appearance of lymphocytic infiltration may suggest that $TiO_2$ interfere with the antioxidant defense mechanism, leading to reactive oxygen species (ROS) generation which, in turn, may imitate an inflammatory response (Abdelhalim, 2011).

Regarding the changes found in the kidney in this study, there was congestion and thrombosis in the cortex vessels and atrophy of some glomeruli. Wang et al., (2007) reported that nephrotoxicity and increased BUN level was observed in the experimental animals treated with titanium. Moreover, Chen et al., (2009) observed renal glomerulus swelling in groups treated with titanium high-dose.

This study revealed brownish particles in the liver parenchyma around the portal tract. The Kupffer cells were laden with brownish particles which are most probably $TiO_2$, and the portal vessels were congested. Wang et al., (2007) reported that the changes of serum biochemical parameters as alanine transaminase, aspartate aminotransferase, (ALT /AST),

and lactate dehydrogenase (LDH), the hydropic degeneration around the central vein and the spotty necrosis of hepatocytes of liver, indicated that the hepatic injury was induced after exposure to mass different-sized $TiO_2$ particles.

Furthermore, Wang et al., (2012) found that $TiO_2$ induced liver edema in young rats and only slight liver injury in adult rats. They reported that $TiO_2$ exposure can provoke reductive stress (i.e., increased reduced glutathione (GSH)/oxidized glutathione (GSSG) ratios) in plasma through enhancing the glucose and GSH levels in young rats or reducing the glutathione peroxidase acitivity and GSSG levels in adult rats. Cui et al., (2012) found that long-term exposure to $TiO_2$ resulted in obvious titanium aggregation in hepatocyte nuclei, an inflammatory response, hepatocyte apoptosis, and liver dysfunction. Moreover, Chen et al., (2009) observed hepatocellular necrosis and apoptosis together with hepatic fibrosis in high-dose groups.

Furthermore, Cui et al., (2012) showed striking microarray data changes in the expression of 785 genes related to the immune/inflammatory response, apoptosis, oxidative stress, the metabolic process, response to stress, cell cycle, ion transport, signal transduction, cell proliferation, cytoskeleton, and cell differentiation in $TiO_2$ exposed livers. In particular, a significant reduction in complement factor D (Cfd) expression following long-term exposure to $TiO_2$ NPs resulted in autoimmune and inflammatory disease states in mice.

Sharma et al., 2012a and Sharma et al., 2012b studied the possible mechanism through which ZnO nanoparticles exert their toxic effects on human liver cells, and found that ZnO nanoparticles accumulate in the liver and induce intracellular reactive oxygen species (ROS) generation. ROS trigger a decrease in mitochondrial membrane potential (MMP) with a simultaneous increase in the ratio of Bax/Bcl2 (Bax is a protein of the Bcl-2 gene) leading to mitochondrially mediated apoptosis. Moreover, ROS can also induce DNA damage. It seems that different nanoparticles exert their toxic effects on different tissues through similar oxidative mechanisms.

In this study, the spleen showed the appearance of brown particles in the marginal zone around the lymphoid follicle. Chen et al., (2009) showed by histopathological examinations that some $TiO_2$ particles had entered the spleen and caused its lesion. Umbreita et al., (2012) also identified $TiO_2$ particles agglomerates in the spleen 26 weeks after I.V. injection, indicating that tissue clearance is limited. In addition, redistribution within the histological micro-compartments of organs, especially in the spleen, was noted.

To understand the spleen injury, Wang et al., (2011) suggested that nanoparticulate $TiO_2$ caused congestion and lymph nodule proliferation of spleen tissue, which might exert its toxicity through oxidative stress, as it caused significant increase in the mouse spleen reactive oxygen species accumulations, subsequently leading to strong lipid peroxidation.

On the other hand, Fabian et al., (2008) reported that rats exposed to $TiO_2$ nanoparticles by a route that allows immediate systemic availability showed expected tissue distribution, no obvious toxic health effects, no immune response, and no change in organ function. Therefore, even with 100% bioavailability of the 5 mg/kg $TiO_2$ dose afforded by the intravenous route of administration, there were no remarkable toxic effects evident in the experimental animals. These results indicated that $TiO_2$ nanoparticles could be used safely in low doses.

Regarding the immune changes found in the study, the spleen of the control group showed that T cells around the central artery were stained positive with common lymphocytic antigen, while sections obtained from the treated group showed that the spleen stained negative.

Ferreira et al., (2003) reported several pronounced alterations in the spleen architecture, manifested by irregular features within the capsule and medulla, namely depletion of T4 and B cells. Altogether these results suggest toxic alterations within the spleen induced by titanium particles, indicating that the immune system may be hampered and so interfering in the body defense mechanisms.

Li et al., (2010) demonstrated that $TiO_2$ NPs had obvious accumulation in the mouse spleen, leading to congestion and lymph nodule proliferation of spleen tissue, and splenocyte apoptosis. $TiO_2$ NPs effectively activated caspase-3 and -9, decreased the Bcl-2 the levels of gene and protein, and increase the levels of Bax, and cytochrome c genes and their protein expression, and promoted ROS accumulation. This study also indicated that $TiO_2$ NPs-induced apoptosis in the mouse splenocyte via mitochondrial-mediated pathway. These findings provide strong evidence that the $TiO_2$ NPs can induce the spleen pathological changes and apoptosis, leading to the reduction of immunity of mice.

Furthermore, Moon et al., (2011) showed that $TiO_2$ nanoparticles may damage the development and proliferation of B- and T-lymphocytes, reduce the activity of macrophages, and decrease natural killer (NK) cell population levels, outcomes that appear to lead to an increase in tumor growth in situ. These studies allow us to suggest that $TiO_2$ nanoparticles might have the potential to enhance tumor growth through immunomodulation of B- and T-lymphocytes, macrophages, and NK cells.

## Conclusion

It is concluded that titanium induces different histological changes including liver, spleen, lung and kidney of male albino rats. The CLA staining of the

spleen in titanium treated group revealed toxic alteration within the spleen, indicating that the immune system may be affected.

## Recommendation

Further studies are recommended to study the titanium effects on human tissues and the immune system. Additionally, it is recommended that patients using titanium implants in dentistry or orthodontic appliances should be routinely screened for titanium toxicity.

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**الملخص العربي**

# التغيرات الهستولوجية والهستوكيميائية المناعية الناتجة عن أثر التيتانيوم على الكبد والطحال والرئة والكلى في ذكور الفئران البيضاء

**رباب الكيلاني[1] و خالد مصطفى[2] و غادة الميهى[3]**

تستخدم المعادن المختلفة على نحو متزايد في صناعة الأجهزة المزروعة بالجسم وخاصة في مجال طب الأسنان. وتستخدم رقائق التيتانيوم علاجيا في الطب وذلك لقدرتها العالية على مقاومة التآكل وقدرتها العالية بالمقارنة مع سبائك أخرى من الفولاذ المقاوم للصدأ (الاستانلس ستيل) والكوبالت. وبالرغم من ذلك فإنه لا يوجد [illegible] مرضية وفشل في بعض الأجهزة. لهذا فان الهدف من هذا البحث كان لدراسة السمية النسيجية والمناعية للتيتانيوم في بعض الأعضاء المستهدفة في ذكور الجرذان البيضاء البالغة.

تم تنفيذ هذا العمل على مجموعتين: مجموعة ضابطة وتتكون من 10 فئران، والمجموعة التي تم معالجتها بالتيتانيوم وتتكون من 20 [illegible] الطحال والكلى والرئة. وتم صبغ عينات من الطحال من المجموعتين بصبغة مضادات الليمفاوي المشترك لفحص الخلايا الليمفاوية. وقد أوضحت النتائج وجود تغيرات هستولوجية في نسيج كل من الكبد والطحال والكلى والرئة في المجموعة المتعرضة للتيتانيوم. وقد أوضحت صبغة مضادات الليمفاوي المشترك لنسيج الطحال للمجموعة المتعرضة للتيتانيوم تغيرات سمية في الطحال تفيد أن الجهاز المناعي قد تعرض للخطر.

1 قسم الطب الشرعي والسموم الإكلينيكية كلية الطب جامعة طنطا
2 قسم الهستولوجى كلية الطب جامعة طنطا
3 قسم تقويم الأسنان كلية الأسنان جامعة طنطا