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The Histological and Biochemical effects of Titanium Dioxide Nanoparticle (TiO_2) on the liver in Wistar Rat

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Abstract

Nanoparticles have wide spread application in all aspects of modern life because of unique features of their as small size and high surface area. Their features especially high surface area cause it is very reactive and toxic. They can damage human and animal cells by increasing oxidative stress mechanism. Nanotitanium dioxide (TiO_2) having different capabilities such asrobustoxidation, biocompatibility, photocatalytic properties frequently used in a wide range of sciences, including pharmaceuticals, cosmetics, medicine and engineering. Wide application of this materials resulted wide exposing of human and animals to this, so analysis of its toxicity and distribution in the body is important. This study investigates the effects of TiO_2 nanoparticle on the concentration of hepatic hormones such as AST, ALT and ALP. Results showed significant alteration in ALT and ALP concentration, also histological studies showed any important changes in liver tissue that indicate toxic effects of nanoTiO_2 on liver health.

Keyword: TiO_2 nanoparticles, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, liver tissue.

Introduction

Nanoparticles have unique physical and chemical properties of size, shape and high surface to volume ratio that these properties have appropriated for biological, medical widespread applications. Unfortunately the dose of nanoparticles had been used in vitro and in vivo researches was high dose. Although in some cases the lowest dose was used, but it was so low and negligible[1]. So because of the new aspects of the nanoparticles, the development of the nanotechnology has inevitably risks which we can determine and avoid them[2]. Titanium element is intermediate metal with low density, high stability and Glossy surface[3]. More than 90 % of Titanium derivatives are TiO_2[4]. In recent years the uses of semiconductors as photocatalyst have widespread application for degradation of organic pollutants. Because of optical and electrical properties, low price, high photocatalystic activity, chemical stability, non-toxicity, abundance, availability and no corrosion in the light the titanic is used as a common photocatalyst[5]. So TiO_2 nanoparticles have several application in the medicine, medical and engineering sciences and it used to removing pollutions like anorganic toxic and the heavy metal from the sewerage, filtration of air and water[6]. Until now, there are a lot of studies about the application of TiO_2nano particles in different sciences, but there are little studies about the toxic effects of nanoparticles in live systems. The recent studies showed when the nanoparticles and nanofibers of TiO_2 enter the living cells, they trigger cell death (apoptos) and make free oxygen radicals (ORS) in the nucleus and injured the DNA and caused the toxicity, changing in the function and finally death of cell[7]. This study investigated the effects of TiO_2 nanoparticles in the in vivo system. Different studies with different doses and sizes, resulted different data. Therefore we studied the effects of 11 times injection in different doses (30, 50, 70 mg/kg) of TiO2 nanoparticles in hepatic enzymes concentration 21 days after injection.

Material and Methods

Preparation TiO_2 nanoparticles and injection: TiO_2 nanoparticles prepared from Spain andthe Notrino Company (Tehran-Iran). The result of x-ray studies (XRD) show that TiO_2 nanoparticle which used in this research isAnatasa, crystal phase, approximate size 18 nm (figure 1). Also with use of ICP-MS test we can measure the purity of TiO_2 nanoparticles as 99.986%. Table 1 showed some of features of these nanoparticles.

Table-1
Physical parameters of nano TiO_2 used in present study, These features is more important in chemical and biological properties of TiO_2 nanoparticles

Color	With
Morphology	Spherical
Crystallinephase	78.8 % Anatasa,21.2 % Rutilo
Specificsurfacearea	100-150 m^2/g
Density	3.84 g/cc
Size	10-15 nm
purity	99.986 %

Animals: This study is an experimental effort that carried out on animals and we used adult male Wistar rats weighing 300±30 gr were estimated from the animal house of martyr portal was developed. Animals whit average age of 2 months selected.

Testing carried out at temperature of 20-25 centigrade degree that day duration was 12 hours and 12 hours dark lighting. Municipal tap water was used adjusted drinking water and eating animals for food by rats (feed compression) that the company prepared feed was barking in this study. Experimental animals were randomly divided into 5 groups (6 rats in each group) as follows for first phase of experiment: fist control group feed by usual water and food. Second control group that referred to Placebo, injected by 1 ml physiological serum every other day intraperitoneally for equivalency of shock that obtained by intraperitoneally injection. Other groups from 3rd, 4th and 5th injected by 1 ml TiO_2 nanoparticles in 30, 50 and 70 mg/kg doses, injection repeated every other day intraperitoneally. This continued until 21 day. TiO_2 nanoparticles resolved in physiological serum in 20 min by sonication method to producing a stable suspension.

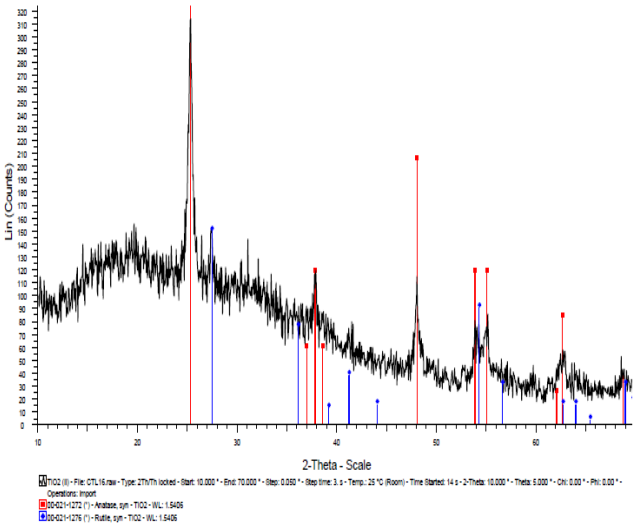

Figure-1
XRD diffraction pattern of TiO_2 nanoparticles

One day after the last injection, blood sample of all animals prepared from neck veins. After clotting, samples were centrifuged at 3000 rpm for 20 minutes. Serum is poured into a container cap and stored in freezerand then used for hepatic enzymes measurement. Alanineaminotransferase, aspartate aminotransferase, alkaline phosphatase in serummeasured by photometricmethod.

The results (hormone concentrations) analyzed based on the statistical program SPSS and analyzed by ANOVA and Tukey test was the difference in the level P <0.05 was considered significant.

Histological examination: One day after the last injection after blood sampling, rats anesthetized the testes separated and were immersed in 10% formalin. Samples were divided into 5 micrometer and stained by hematoxylin and eosin method and studied by light microscope.

Results and Discussion

Hepatic hormones concentration and response to TiO_2 treatment: Hepatic enzymes are good indicator for liver health status therefore after 11 injections of TiO_2 nanoparticle intraperitoneally, blood sample prepared and blood serum separated. After that hepatic enzymes concentration such as AST, ALT and ALP measured (table 2). Results showed ALP hormone level significantly increased in TiO_2 treated rats rather than control and placebo rats (P< 0.0001). ALT level of rats' serum that treated with nano TiO_2 enhanced significantly rather than controls. Last hepatic enzyme (ALP) doesn't show significant improved with nano TiO_2 injection in all three doses (F=1.550, P<0.01).

Table-2
Measurement of hepatic factors, Asterisk symbol showed significant increase and a symbol showed significant decrease rather than control groups

Groups	ALP (U/lit)	ALT (U/lit)	AST (U/lit)
Intact control	318.87± 31.90	48.00 ± 5.31	113.12± 45.22
Placebo control	357.62 ±37.83	53.00±16.37	155.75± 49.41
30 mg/kg dose	231.75± 54.54 [a]	78.14± 30.65 *	322.37± 225.80
50 mg/kg dose	228.12± 85.68 [a]	101.12± 31.61 *	355.50± 225.85
70 mg/kg dose	287.62± 67.08 [a]	73.00± 15.29 *	302.62± 314.96
F	3.41	5.12	1.57
P	<0.01	<0.001	0.01

The results of histological studies of TiO_2 treated rats: Hepatic tissue pieces that stained by hematoxylin and eosin and studied by light microscope. The result of the survey of the histopathologic liver in A, D groups show the Central venous congestion tubular, the little Hepatocytenecrosis (with Infiltrating lymphocytes and notarial in that region), the little plethora of microphages or the kupffer cells. Also we see hydro pic degeneration changes in the B, E groups and in the number of centrilobular vein hepatocytes of the liver, the comparative Macrophages or the kupffer cells, infiltrating of sinusoids and centrilobular vein hepatocytes of the center of the liver and in the little necrosis or hepatocytes dots of F, C groups. We show the difference of the result as figure 2-5.

The weight changes of rats in different groups: Injection of TiO_2intraperitoneally may be causes physiological changes such as changes of weight in treated rats. Results analyzed by T-test were the difference in the level P <0.05 was considered significant. Rats weighing results showed no significant increase as table 3 (F=0/251, P=0/604).

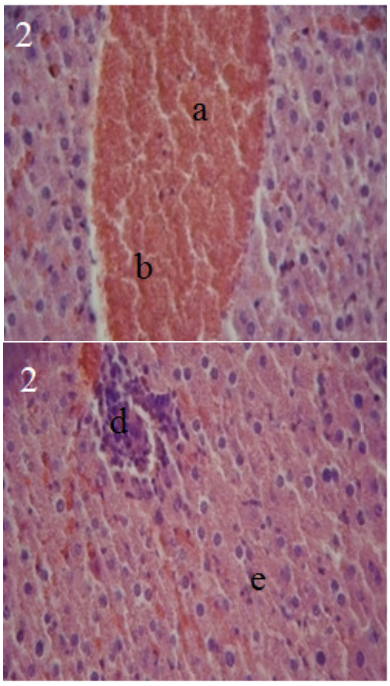

Figure-2
Treatment groups received dose of 30 mg/kg (A, D) consists of: a-venous congestion of centrilobular liver, b-lymphocytes, d - necrosis small hepatocytes with infiltration of lymphocytes and neutrophils in the area, e-cells Kupfer –

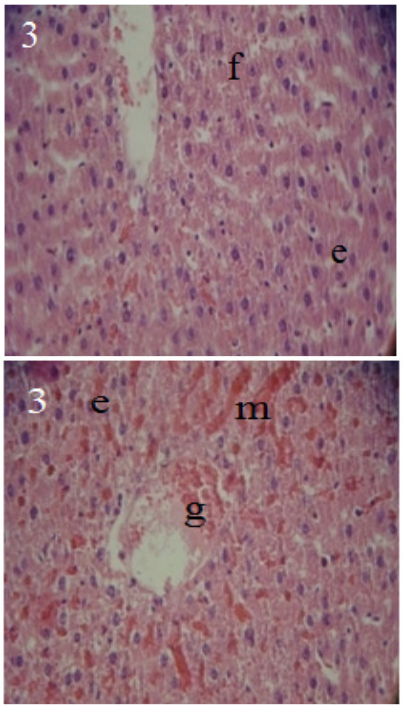

Figure-3
treatment groups received a dose of 50 mg/kg (B, E) consists of: f - Hydropic degeneration, e-cells Kupfer, g-congestion of centrilobular liver sinusoids, m-centrilobular hepatic venous congestion, e-cells Kupfer

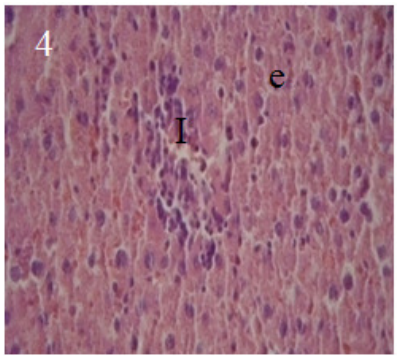

Figure-4
Treatment group receive a dose of 70 mg/kg (C, F) consists of: I - necroses small hepatocytes, e-cells Kupfer

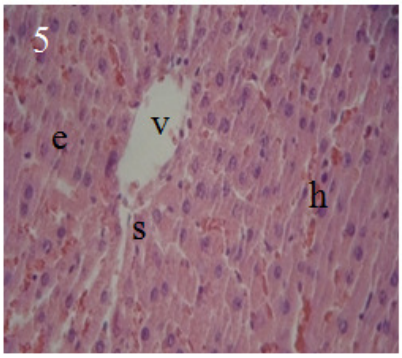

Figure-5
The control and placebo included: h- hepatocytes, s-sinusoids, v - vein centrilobular liver

Table-3
The weighting results of rats before of injection and blooding

Groups	Weight beforeinjection (gr)	Weight beforeblooding (gr)
Control group	251.37±22.17	213.50±27.86
Placebo group	224.12 ±49.91	228.25±29.38
Treated by 30 mg/kg of dose	226.87 ±18.69	226.75±15.69
Treated by 50 mg/kg of dose	236.87 ± 28.14	232.25±20.13
Treated by 70 mg/kg of dose	224.75±53.11	229.12±37.19

Discussion: Nanoparticles have very specific chemical and physical characteristics of size, shape and high surface area to volume ratio that facilitate its medical and biological applications. This material distributed in all of body rapidly after injection by circulation and reached to the all of the organs and tissues[8]. Before its application as medicine equipment, effects of nanoparticle on environment, biocompatibility and its toxic effects on human and animals should be assessed. These particles because of small size have high surface area and they are highly reactive, it is one important reason for its toxic effects[9]. TiO_2 nanoparticle due to optical, electrical and catalytic

properties, have very important applications in various industries, including industrial pigments, sun block, bioremediation, air and water filtration and cancer treatment[10]. Therefor because of widespread use of TiO_2 nanoparticle human and animal are exposing to this material. Aim of our study is investigation of TiO_2 exposing effects in sex hormones concentration so its negative role in productivity.

Also in this research, we assess about the toxic of the liver and survey an aspartateaminotransferase, Alanine aminotransferase and alkaline phosphatesserum and the liver tissue appearance.

Also we show in table 2 one day after the last infusion of the mean amount of alanineaminotransferaseserum in an attendance groups of A, B, C, there is the meaningful difference with other groups so the concentration of ALT in 3 groups become increased (P<0/001). Also with paying attention to the shapes of 5-2, the texture studies in these groups show difference changes in the number of the hepatocytes in centrilobularto the little necrosis and hepatitis dots, the kupffer cells increasing in the centrilobular. we know that an ALT is an enzyme which we can find more in the liver cells and the kidney and make in the liver via the liver hepatitis at the first degree and even though the destroying of the liver, ALT enter to the blood current and because of the increasing the level of cerium, we can point to the destroying the liver[11]. So in this research, we have an increasing of the mean density of an ALT cerium one day after the last infusion which show that the dots necrosis in the liver cause destroying the liver cells and increasing the density of an ALT enzyme in the cerium. So the possibility is because of an entering the Nano particles into the liver and of the result of the destroying the liver and the toxic of TiO_2 the Nano particles.

The result of this research in the first stage including an increasing of the density of an alanineaminotransferase, affecting on the proportion of ALT to the AST and destroying the liver hepatitis in around of the vein of centrilobular of the liver which agree to the result of the study of Wang and Ciu. Wang and et al see that Tio_2 Nano particle with 25, 80 nm sizes in 5 g/kg doze from the weight of the body, the toxic effect on the mature rats and biochemical parameters cerium like the proportion of alanine aminotransferase to the aspartate aminotransferase and the pathology of the liver. Because of being compact of this nanoparticle in the liver cells, we can see destroying the liver cells around of the centrilobularvein[12].

In another research via cuie and et al, prescription into the stomach TiO_2anatasanano (5nm) continuously in 60 days in 5, 10 mg/kg dozes cause the histopatalogic changes in the liver, Necrosis and influence the tumult cells and destroying the working of the liver and increase the amount of enzymes alanine, aminotransferase, aspartateaminotransferase and alkalinephosphatase in the serum[13].

Also, we can say when we can make toxic via TiO_2 nanoparticles even though the type of covering, the crystalline network and the form of nano and the size of TiO_2 nanoparticles are the key role so in the crystalline phase of anatasa if the size of the particles become decrease the amount of toxic of the cells became increase[14]. In the research with passing 21 days from the last infusion in an attendance groups D, E, F (after passing 21 days from an attendance) the density of an ALT in the cerium in every 3 dozes (30, 50, 70 mg/kg) go back to the normal state in the control group level because of the gradual exclusion of the collection nano particles in the liver so the disorders in the texture are not very high with passing 21 days from an infusion (shape 5-2). an amount of ALT come back to the control group level 21 days after the last infusion and lack of changes of the amount of AST show that the toxic of TiO_2 nanoparticles are return with mention doses in the cells and parenchymal liver cells with passing time.

Before studies show that Tio_2Nano particles have a good deal and influence in the liver, lien, kidney, lunges without the texture disorder and decrease in the mention organs with passing one month of an infusion so after the end of one month we have a certain difference in a measuring enzymes in the blood cerium and don't report any toxic in the organs. Then we can use from TiO_2nanoparticles in low doses[15].

In different reports we show that an important value of the nanoparticles after an infusion to the body absorb to the liver but the reticule endothelial system in this organ can exclusion the collection of nanoparticles from the body[16,17]. It seems that this phenomena cause destroying the temporary toxic effects of TiO_2 Nano particles in this research.

In other research researchers don't see any obvious toxic and death and histopathology changes in the heart, lunges liver and kidney after assessing in one week later and this work done via an infusion different types and size of Tio_2Nano particles in dose of 20 mg/kg to rats [18].In the Wei Han and et al view, TiO_2nanoparticles are exclude via the digestive and urinary system and don't collect any toxicity of this material in the body.

In this research in an anatomy time, the chugging of TiO_2 Nanoparticles in an abdominal hole space is a reason for the nontoxic effects or little toxic of TiO_2 nanoparticles, because in this time nanoparticles can't enter to the blood circuit and different textures. When we want to certificate this point, Garcia and et al say in 2005 in the body of animals, a lot of nanoparticles become persisting in the connection to an organic compounds and molecules. So the qualities of nanoparticles like the size and the form and the surface entrance are changed and lose the ability to influence to the most organs and texture of the body[17].

Conclusion

So with paying attention to the nanoparticle which are 5-20 nm have the most application in the medical and biological

contexts[19]. We conclude from the result if this research and reports from other researcher that the application of TiO_2 nanoparticles in vivo statement in little value are in the medical contexts and don't make any toxic and especial disorder in the body. So it is necessary to do more researches about the effect of these particles on organs and other blood factors in different time intervals.

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