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Investigation of haemato- and hepatotoxic effects of titanium dioxide nanoparticles in male sprague dawley rats

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Abstract

This study investigated the toxic effect of TiO_2 NPs on blood and liver tissues of male Sprague Dawley rats. For this purpose, 25 adult male Sprague Dawley rats of 200±5g weight were procured from the animal house of Government College University Faisalabad, Pakistan. Rats were acclimatized for 7 days before the start of study in ventilated cages at (25±2°C) with the approval of ethical committee of Government College University Faisalabad. These rats were randomly divided in to 5 groups. First control (C) with no treatment, 2nd placebo injected with normal saline (S) and three treated groups (G1, G2 and G3). The treated groups were intraperitoneally injected with TiO_2 NPs @ 80 or 120 or 160 mg/kg BW of rats for 28 days on alternate day. Animal's mortality, hematology and liver histology were evaluated. During the experiment no mortality was found while TiO_2 NPs exposure showed highly significant pathological changes in haematological parameters and liver function test ($p<0.05$). The histological alterations were found severe in medium and high dose treated groups as compared to low dose while Control (C) and (S) saline treated groups showed normal histology. Treated groups showed different alterations such as vacuolation, damaging epithelial lining, karyolysis, sinusoids and neutrophilic and lymphocytic infiltration were highly significant in both G2 and G3 treated groups. This study explored the TiO_2 -NPs caused toxicity in blood and hepatic tissues. It is concluded from present study that selected dose of titanium dioxide has negative impact on the health of living organisms.

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Introduction

Titanium dioxide nanoparticles (TiO_2 NPs), among the most common metal oxide NPs, have received attention due to their low production cost, ability to form diverse structures and use in food, medicine, personal care products and other industries (Vance *et al.*, 2015. During the last ten years a lot of research work is going on using Titanium dioxide nanoparticles (TiO_2 NPs) for many applications such as air filter, soil filter, optical filters, photo catalysts for solar energy conversion, biosensors and use as photodegradation of organic pollutants as well as different biological purposes like drug delivery and immune-modulatory agent. High surface area and size make nanoparticles unique with respect to physicochemical properties. Nano particles show different colors on the absorption of light due to variety in their photolytic properties. They are widely used in domestic and commercial level due to their size, shape and structure (Khan *et al.*, 2017; Rabhi *et al.*, 2019). Nanotechnology also gives solutions for certain ecological problems (Younes *et al.*, 2015; Dudefoi *et al.*, 2017; Hong *et al.*, 2017; Shah *et al.*, 2017; Lim *et al.*, 2018).

Now a days different industrial sectors are using the TiO_2 NPs in different fields like, environmental protection and cosmetics (Hong *et al.*, 2017; Lu *et al.*, 2018) as fertilizers in agriculture fields to enhance the soil fertility for better growth of plants and crops and as pesticides to protect the crops from pests (Lim *et al.*, 2015). TiO_2 NPs are used in biomedical sciences like pharmaceuticals and medical equipment (Pandey and Prajapati, 2017). On one side, the nanotechnology improves the lifestyle of the people and on the other side causes the contamination of environment and becomes the serious threat to living organisms. NPs are chemically more reactive than heavy metals because of their small size, shape and concentration (Thomas *et al.*, 2018; Giorgetti, 2019).

TiO_2 NPs are one of the most used nanoparticles. TiO_2 NPs are considered amongst the top five nanoparticles worldwide (Baldini *et al.*, 2019). The applications of these particles depend upon their size including ultrafine particles (size ranging from 1 to 100nm) (Swidwinska-Gajewska and Czerczak, 2014). TiO_2 NPs occur in three different forms: rutile, anatase and brookite while in comparison of their photocatalytic activity anatase has higher potential than rutile and brookite (Allen, 2016; Samat *et al.*, 2016). Effectiveness of rutile-to-anatase of $TiO_2$was also checked in aqueous solution in many studies and found that anatase were more reactive (Shakeel *et al.*, 2018; Rabhi *et al.*, 2019).

Nanoparticles of TiO_2 have different properties, like the solid oxidation potential and photocatalytic activity. Materials composed of TiO_2 NPs and procedures on a nano scale have an area of broad research because of extensive variety of potential applications in the optical, electronic and biomedical field.

To avoid the toxicity, biosafety of nanotechnology is going to be the second most priority of nanotechnology that needs to be properly addressed (Jasim *et al.*, 2017; Shah *et al.*, 2017; Hou *et al.*, 2018, 2019). TiO_2 NPs have genotoxicity and induce inflammation and oxidative stress. TiO_2 NPs can reach in the hepatic tissues by crossing all the barriers due to their small size.TiO_2 NPs have supplementary capability of UV blocking and undoubtedly that is why these are used in the production of sunscreen lotions (Chen *et al.*, 2014; Magdolenova *et al.*, 2014; Shinohara *et al.*, 2009).

TiO_2 NPs are very toxic because of their reactive properties and specifically affect the liver, lungs, kidney, spleen, heart and brain. TiO_2 NPs can enter the brain through the olfactory bulb and can accumulate there in the hippocampus region which causes oxidative stress and mitochondrial damages. They can cause alterations in cells and organs of living organisms (Krawczk *et al.*, 2016; Yu *et al.*, 2015; Gate *et al.*, 2016; Pujalte *et al.*, 2016).

The detoxifications and recovering functions of liver tissue are affected by the penetration of TiO_2 NPs and the NPs can cause severe damages. Keeping in view

the hazardous potential of NPs on living organisms, toxicity of TiO_2 NPs was examined in the current study. The present study was planned to examine the noxious effects of varied sub-lethal doses of TiO_2 NPs on hepatic and blood profiles of male Sprague-Dawley rats by histological and haematological profiles (Azim *et al.*, 2015; Abu-Dief *et al.*, 2015;Hou *et al.*, 2018).

Materials and methods

This study was carried out at the Muhammad Ali Research Laboratory of the Department of Zoology, Government College University Faisalabad, after the approval of ethical committee of Government College University Faisalabad, Pakistan.

Animals

Twenty-five healthy adult male Sprague-Dawley rats weighing 200±5g were procured from the animal house of Government College University Faisalabad. The rats were kept in steel cages in the animal house of Government College University Faisalabad. Rats were randomly divided into five groups having 5 rats in each group. Animal were given water and rodent feed (KENT 16% proteins) and water ad libtium. Moisture (35±5) temperature (25 ± 2°C) maintained in day and night cycle (12:12) were maintain during the course of study.

Toxicity assay for the TiO_2 nanoparticles

Animals were acclimatized for 7 days before the start of experiment. The rats of similar body weights (BWs) were randomly divided into 5 groups, each having five rats (Table 1). Control group (C) fed with usual food and water, placebo group (S) was intraperitoneally injected with 1ml normal saline for equivalency of shock that may be gained by the injection. Treated groups G1, G2 and G3 were intraperitoneally injected with TiO_2 NPs@80, 120 and 160 mg/kg BW, respectively on alternative days for 28 days (Table 1).

Sample collection

At the beginning and after 28 days of exposure, blood samples were collected for haematological examination. At 28th day animals were fasted overnight, blood samples were taken from the caudal vein of individual rat in tubes (EDTA anticoagulant tubes) and were analyzed by using haematology autoanalyser (Shanghaic Drawell Scientific Instrument Co., Ltd and DW-TEK5000 Automated Blood Hematology Analyzer). These samples were subjected for assessment of haematological variables. Rats were anaesthetized with administering ketamine hydrochloride (30 mg/kg BW) and sacrificed. The hepatic tissues were taken and weighed with Sartorius weighing balance, then separately dipped in fixative for further process of histology (Shakeel *et al.*, 2016).

Histological examination

Animals were sacrificed, and fresh portions of their liver tissues from its lateral lobes of liver were taken. These tissues were then dipped in (alcohol 55-60%, formaldehyde 30-35% and glacial acidic acid 10-15%) for 5–8 hr. After that, tissues were dehydrated in different ethanolic grades (60, 70, 80, 90 and 100%). After dehydration, liver tissues were placed in cedar wood oil until become transparent and clear. When tissues were clear, embedded in benzol and then benzol+paraplast at 50°C for 12 hrs. After that, embedded tissues were moved from molted wax in a block. After removing the bubbles paraffin wax became hardened. With the help of knife or scalpel, blocks were trimmed from paraffin wax and after that, tissues were fixed on wooden block for sectioning. Sections of 5 µm were cut by microtome (MR-2258 Rotary Microtome Histoline Laboratories Milan ITALY).After cutting, ribbons along with tissues were stretched and then fixed to already clean albumenized glass slides on Fischer slide warmer at 60°C.

Then placed these glass slides in incubator at 65°C about 12 hours for removal of bubbles if any bubble were left.Then stained by the haematoxylin–eosin staining technique (Shakeel *et al.*, 2018).

These stained tissues of control and exposed animals were observed under the light microscope (Nikon ECLIPSE Ci-200 with digital camera fixed with microscope and monitor) and images were taken for histological investigation (Shakeel *et al.*, 2016).

Statistical analysis

The data were statistically analyzed by means of Minitab 17 software through ANOVA in general linear model to find out the effect of TiO_2 NPs on different parameter of hematology.Tukey test was performed to compare the mean of different groups at P< 0.05.The histological alterations in treated groups were compared with the control and treated groups with the conventional grading system (-- no abnormality, + less abnormality, ++ moderate abnormality, +++ severe damage (Noureen *et al*., 2019).

Results

General observation

The general behavior, health condition and change in body weight of animals were observed during the study. The control and placebo group animals were normal in behavior and showed usual weight gain during the course of study. It was also observed that animals treated by medium and high doses (@120mg/kg and @160mg/kg of TiO2-NPs) after two weeks of exposure showed abnormal behavior cannibalism. No significant changes were observed in the body weight of rats in all groups (control and treated) during first two weeks (P> 0.05), while significant (P< 0.05) reduction in body weight was observed in medium (@120mg/kg BW) and high doses (@160mg/kg BW) during 3rd and 4thweeks of exposure compared with control, placebo and low dose treated (@80mg/kg BW) groups. Moreover, the significant differences in hepato-somatic index were also obtained in a doses dependent manner, least weight of liver was observed at high dose as compare with body weight in the control and treated groups (Table 2).

Table 1. Division of rats into different treatment groups.

Groups	Treatments
Control (C)	(no treatment)
Placebo (S)	(injected with Normal saline intraperiotionally)
G1	TiO_2 NPs @80mg/kg BW(intraperiotionally injected)
G2	TiO_2 NPs @120 mg/kg BW (intraperiotionally injected)
G3	TiO_2 NPs @160 mg/kg BW (intraperiotionally injected)

Table 3 shows the means ofhematological parameters of Sprague Dawley rats in control and treated groups after 28 days of trial. There was a dose-dependent alteration in various hematological parameters in rats treated with different doses of TiO_2 NPs. In current study RBCs, WBCs, LYM, Hb, MCH, MCV, HCT and PLT were significantly increased (P< 0.05) in Group G2 and Group G3 treated with medium and high doses of TiO_2 NPs (@120mg/kg BW and @160mg/kg BW), respectively as compared to the control group (Group C) and placebo group (Group S) as well as Group G1 (treated with the low dose, i.e., @80mg/kg BW). The highest mean values for RBCs, WBCs, LYM, Hb, MCH, MCV, HCT and PLT were observed in G3 (treated with 160mg/kg BW of TiO_2 NPs) as 12.40±0.04, 10.05±0.06, 89.91±2.05, 19.06±0.54, 22.86±0.74, 64.47±3.47, 26.50±1.97 and 990.9±60.46, respectively as compared to control groups (Group C and S).

Table 2. Mean±SE of weekly body weight (g) and hepato somatic (H-S) index of rats in control and treated groups.

	Groups				
Duration	Control	Placebo	G1 @80 mg/kg	G2@120 mg/kg	G3@160 mg/kg
Initial weight	$201.4\pm.97^{A}$	$201.60\pm.81^{A}$	$201.80\pm.86^{A}$	$201.80\pm.96^{A}$	$201.80\pm.91^{A}$
1st week	$214.8\pm.58^{A}$	$215.12\pm.717^{A}$	$215.07\pm.85^{A}$	$215.10\pm.871^{A}$	$215.40\pm.60^{A}$
2nd week	$246.80\pm.37^{A}$	$246.60\pm.871^{A}$	$246.20\pm.73^{A}$	$246.80\pm.734^{A}$	247.00 ± 1.048^{A}
3rdweek	$276.62\pm.38^{A}$	$277.10\pm.74^{A}$	$276.69\pm.892^{A}$	$229.40\pm.927^{B}$	$227.60\pm.812^{B}$
4th week	$293.48\pm.281^{A}$	$293.68\pm.680^{A}$	285.87 ± 1.130^{A}	$218.20\pm.583^{B}$	$213.96\pm.678^{C}$
H-S index	1.75^{B}	1.75^{B}	1.78^{B}	2.31^{A}	2.32^{A}

(Means bearing the different latters in rows are significantly different P<0.05).

Table 4 shows the mean ± SE ofliver function profile of Sprague Dawley rats in control and treated groups after 28 days of trial. In this study alterations in various liver function profiles in rats treated with different doses of TiO_2 NPs were observed. In current study ALT, AST, LDH and ALP were significantly increased (P< 0.05) in Group G2 and Group G3, treated with medium and high doses of TiO_2 NPs (@120mg/kg BW and @160mg/kg BW), respectively as compared to the control group, placebo group and low dose treated group (Group 1).

The highest mean values for ALT, AST, LDH and ALP (treated with 160mg/kg BW of TiO_2 NPs) was observed as 141.064±0.40, 55.48±0.37, 2635.91±23.11 and 61.33±0.26, respectively (Table 4).

Table 3. Mean ± SE of Hematological parameters of Sprague Dawley rats in control and treated groups after 28 days of trial.

	Blood Parameters							
Groups	RBC ($X10^6$/M)	WBCs ($X10^3$/µl)	LYM %	Hb (g/dl)	MCH (pg)	MCV (fl)	HCT (%)	PLT ($X10^3$/µl)
C	$7.57±0.74^C$	$6.06±1.30^C$	$77.61±0.62^C$	$13.47±0.60^D$	$15.44±1.10^D$	$55.88±0.76^C$	$26.43±1.23^D$	$406.6±78.78^E$
S	$7.85±0.61^C$	$6.01±1.30^C$	$77.57±0.66^C$	$13.60±0.65^D$	$15.67±0.63^D$	$55.76±0.32^C$	$26.50±0.59^D$	$486.9±76.27^D$
G1	$7.79±0.59^C$	$5.95±0.55^C$	$77.72±0.47^C$	$14.51±0.39^C$	$16.51±0.20^C$	$56.58±0.37^C$	$27.49±0.84^C$	$575.1±26.61^C$
G2	$9.70±0.31^B$	$8.77±1.98^B$	$85.69±1.07^B$	$16.81±0.60^B$	$20.64±0.26^B$	$60.64±0.60^B$	$36.68±0.77^A$	$857.2±80.68^B$
G3	$12.40±0.04^A$	$10.05±0.6^A$	$89.91±2.05^A$	$19.06±0.54^A$	$22.86±0.74^A$	$64.47±3.47^A$	$26.50±1.97^B$	$990.9±60.46^A$

(Means bearing different letters in Columns are significantly differefent at P< 0.05.).

Histology results

Fig. 1 represents the histology of liver tissues of Sprague Daweley rats. In current study a dose dependent alterations in liver histology was found (Table 5). Liver histology in Control (C) and Placebo group (S) showed normal histology with central vein (CV), G1 group treated with low dose of TiO_2 NPs @80 mg/kg BW showed vacuolation (V), epithelial line damages (ELD), dilation of central vein (DCV) and multi nuclei (MN). G2 Group exposed with medium dose of TiO_2 NPs @120mg/kg BW showed hemorrhage (H), epithelial line damages (ELD), lymphocytic infiltration (INF), mild neutrophils (MN), vacuolation (V) and dilation of central vein (DCV). G3 grouptreated with high dose of TiO_2 NPs 160 mg/kg of BW showed sever alterations like vacuolation (V), hemorrhage (H) and degeneration of central vein (DCV), pyknosis (P), severe necrosis (N) and apoptosis (A) lymphocytic infiltration (INF) karyorrhexis and karyolysis(KRL), karyolysis (K) and multi nucleated (MN) cells.

Table 4. Mean ± SE ofliver function profile of Sprague Dawley rats in control and treated groups after 28 days of trial.

Parameters	Groups				
	C	S	G1	G2	G3
ALT	$13.45±.17^D$	$13.46±182^D$	$46.71±.241^C$	$134.29±.547^B$	$141.064±.406^A$
AST	$44.32±.19^D$	$46.58±.32^C$	$47.63±.41^C$	$52.35±.38^B$	$55.48±.37^A$
LDH	$1337.99±2.26^D$	$1336.17±.83^D$	$1442.56±1.88^C$	$2128±11.68^B$	$2635±23.11^A$
ALP	$46.17±.102^D$	$46.64±.066^D$	$54.36±.25^C$	$58.07±.20^B$	$61.33±.26^A$

(Means bearing the different latters in rows are significantly different P<0.05).

Discussion

Studied Parameters

The present study was designed to evaluate the toxic effects of anatase TiO_2 NPs on mortality, behavioral changes, blood parameters (CBC and liver functions test), hepatosomatic index and liver histology of male Sprague Dawley rats. TiO_2 NPs were administered to rats at doses of 80 mg/kg, 120 mg/kg and 160 mg/kg for 28 days on alternate days. Results showed zero mortality rate among the rats of all groups but

behavioral changes were observed e.g., cannibalism, loss of appetite and aggressive behavior in G2 (120 mg/kg) and G3 (160 mg/kg) while G1 (80 mg/kg) group showed normal behavior. The behavioral changes of present work were similar to the observance of Rihane *et al.* (2015) and Amara *et al.* (2015), who examined pathophysiological and emotional changes in rats induced with TiO_2 NPs.

Table 5. Comparison of histological alterations in control and TiO_2 NPs treated male Sprague Dawley rats after 28 days of trial.

Parameters	Groups				
	S	C	G1	G2	G3
Vacuolation (V)	-	-	-	++	+++
Central vein dilation (CVD)	-	-	+	++	+++
Multi nuclei (MN)	-	-	+	++	+++
Hemorrhage (H),	-	-	-	++	+++
Pyknosis (P),	-	-	-	+	++
Necrosis (N)	-	-	+	++	+++
Necrosis (N) and apoptosis (A)	-	-	-	++	+++
Degeneration of central vein (DCV),	-	-	-	+	+++
Karyolysis (K)	-	-	-	++	++
Lymphocytic infiltration (INF)	-	-	-	+	+++
Epithelial line damages (ELD),	-	-	-	++	+++

Growth Parameters

In current study, body weight of rats was decreased by enhancing concentration of TiO_2 NPs but hepatosomatic index was increased. Our results were in accordance with the findings of Shrama *et al.* (2015) who reported effect of intravenously administered TiO_2 NPs on rats. Similar findings were evaluated by Amara *et al.* (2015) who worked on reproductive systems and emotional behavior with biochemical changes in rats, respectively.

The body weight reduction might be due to interruption of exposed chemical in the metabolism and absorption of essential nutrients. Similarly, Hu *et al.* (2010) investigated effect of studied nanoparticles on neurons and spatial memory and also observed increase in hepatosomatic index. Xu *et al.* (2013) reported that increase or decrease in hepatosomatic index was caused by accumulation or excretion of TiO_2 NPs which leaded to liver damages.

Hematological Studies

In current study hematological analysis showed enhancement in WBCs, RBCs, LYM, HB, MCH, MCV, HCT and PLT. Our results are in accordance with the findings of Xu *et al.* (2013) and Grissa *et al.*, 2016 who also assessed acute toxicity caused by TiO_2 NPs and noted increment in all above blood parameters. Wang *et al.* (2016) investigated the effect of TiO_2 NPs on bone replacement and repair and noted similar increase in blood parameters. Shakeel *et al.* (2016) demonstrated that increment in WBCs was due to stress condition which regulated immune system while MCV enhancement was due to disturbance in mitotic division. Younes *et al.* (2015) also evaluated subacute toxicity by TiO_2 NPs and recorded same changes in hematological parameters.

Liver Function Studies

Liver function test showed significant enhancement in ALT, AST and ALP enzymes in dose dependent manner which is similar to results of Abbasi-Oshaghi *et al.* (2019). They reported oxidative stress and apoptosis in liver and intestines of rats induced by TiO_2 NPs. Vasantharaja *et al.* (2015) and Amara *et al.* (2013) also demonstrated that increment in these enzymes were due to rupture of liver membrane and leakage of enzymes in blood. Alarifi *et al.* (2013) evaluated apoptotic and histological changes in hepatic tissues by TiO_2 NPs and noticed enhancement in liver enzymes similar increment was also reported by Morgan *et al.* (2018).

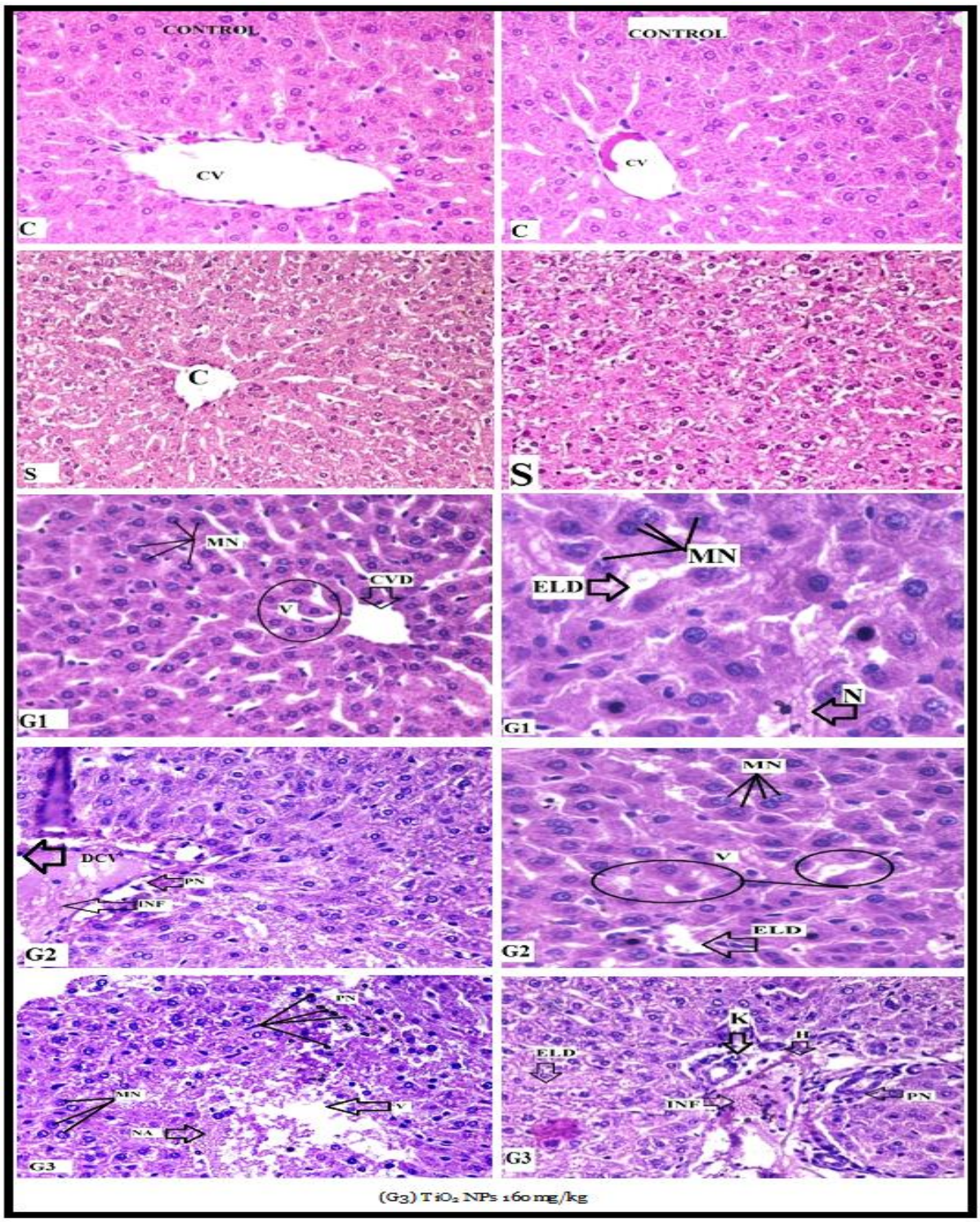

Fig. 1. A photomicrograph (40X) of haematoxyline-eosin stained sections of liver tissuesof Sprague Daweley rats. Liver tissue sections in C and S groups showed normal histology with central vein (CV), G1 group treated with low dose of TiO_2 NPs@80 mg/kg BW showed vacuolation (V), epithelial line damages (ELD), dilation of central vein (DCV) and multi nuclei (MN), G2 Group exposed with medium dose of TiO_2 NPs@120mg/kg BW showed hemorrhage (H), epithelial line damages (ELD), lymphocytic infiltration (INF), mild neutrophils (MN), vacuolation (V) and dilation of central vein (DCV). G3 group treated with high dose of TiO_2 NPs 160 mg/kg of BW and found the high injury of vacuolation (V), hemorrhage (H) and degeneration of central vein (DCV), pyknosis (P), severe necrosis (N) and apoptosis (A) lymphocytic infiltration (INF) karyorrhexis and karyolysis(KRL) karyolysis (K) and multi nucleated (MN) cells.

The capacity of TiO_2 NPs to cross blood barrior and distributed in liver, lungs and spleen. TiO_2 NPs produced severe effects in liver of rats and raised the level of ALP, AST, ALT enzymes and LDH activity also caused change in WBC and produced histological alterations in hepatic tissues of rats like inflammation of hepatic tissues, necrosis, apoptosis and vacuolation (Elgrabli *et al.*, 2015; Heringa *et al.,* 2016; Morgan*et al.*, 2018).

Histopathological Studies

In current study, histological changes in liver e.g., vacuolization, central vein damage, multinucleation, hemorrhage, pyknosis, necrosis, apoptosis, dilated central vein, sinusoidal dilation, karyolysis and epithelial damages were observed in G2 and G3 group which were more severe at 160 mg/kg dose while low dose group showed only necrosis, central vein damages and multinucleation. The results of current study is in agreement with Shakeel *et al.* (2016) who investigated effect of TiO_2NPs and bulk salt on liver histology and observed necrosis, hemorrhage, vacuolization, sinusoidal dilation and karyolysis in a dose dependent manner. Another study by Abu-Deif *et al.* (2015) also revealed similar changes in liver e.g., sinusoidal dilation, infiltration of inflammatory cells and apoptosis in TiO_2 NPs treated rats and also investigated prophylactic effect of milk thistle seeds. Valentini *et al.* (2019) also noticed hepatotoxicity and renotoxicity by TiO_2 NPs and exhibited necrosis, pyknosis and lymphocytes infiltration which is also similar to our results. Some previous scientists also found similar damages in liver hepatocytes and confirmed that TiO NPs are toxic for the normal histology (Shrivastava *et al.*, 2013; Elgrabli *et al.,* 2015; Fadda *et al.,* 2018). In current study swelling of the cells may be due to leakage of lysosomal hydrolytic enzymes (Sharma *et al.*, 2014).

The vacuolated portion in cytoplasm of hepatic tissues of rats may be due to the exposure of TiO_2 NPs. The apoptotic and necrosis effects in hepatocytes in treated rats increased in the dose-dependent way. Necrosis was also observed in TiO_2 NPs treated rats tissues that might be caused by oxidative stress (Gui *et al.*, 2013). After the entrance of TiO_2 NPs in the liver it become difficult to clean it from the body. Clinically it was found that TiO_2 NPs caused infection to hepatic tissue due to rising activities of serum enzyme. TiO_2 NPs after entrance in circulatory system by inhalation, intravenous, dermal and can reached different organs like kidney, liver, spleen, heart, ovary and brain (Bamidele *et al.*, 2013). It is known that TiO_2 NPs of 25 to 80 nm caused histopathological alterations like damage central vein, necrosis, apoptosis and hydropic (Wang *et al.*, 2015).The finding of this experimental study are comparable with the results of other research works where exposure of TiO_2 NPs intraperitoneally caused cancer and study is also in good agreement with other studies who observed the same findings after exposing living organisms to TiO_2 NPs (Alarifi *et al.*, 2013; Fakhar-e-Alam *et al.*, 2014). Apoptosis was also found in rats treated with TiO_2 NPs. Damaged central vein, dilation of central blood vessel, vacuolization and blood sinusoids, showed that TiO_2 NPs may alter the membrane penetrability and affected the endothelial lining of blood vessels.

At high dose more histopathological alteration were observed. Degeneration of liver tissue, necrosis was also reported. Some others scientist found the similar results which supported this work (Liu *et al.*, 2014; Cui *et al.*, 2014). Histological alteration due to NPs has been found in number studies (Fadda *et al.*, 2018; Shakeel *et al.*, 2018; Fadda *et al.*, 2019).

It was revealed that the TiO_2 NPs administration in hepatic tissues persuaded certain oxidative stress. TiO_2 NPs have shown to cross through cell membranes and intermingled with hepatic tissues. The exposure of TiO_2 NPs induced significant alterations in blood parameters and histopathological effects.

Conclusion

It was concluded that exposure of TiO_2 NPs induced hemato- and hepatotoxicity in male Sprague-Dawley rats in a dose-dependent manner. TiO_2 NPs decreased the physical activities and increased the

haematological and histological alterations. Current study revealed the significant harmful effects of TiO_2 NPs on liver function, hepatic tissues and blood parameters of rats.

References

Abbasi-Oshaghi E, Mirzaei F, Pourjafar M. 2019. NLRP3 inflammasome oxidative stress and apoptosis induced in the intestine and liver of rats treated with titanium dioxide nanoparticles: in vivo and in vitro study. International journal of nanomedicine **14,** 1919.
https://doi.org/10.2147/IJN.SI92382

Abu-Dief EE, Khalil KM, Abdel-Aziz HO, Nor-Eldin EK, Ragab EE. 2015. Histological Effects of Titanium Dioxide Nanoparticles in Adult Male Albino Rat Liver and Possible Prophylactic Effects of Milk Thistle Seeds. Life Science Journal **12(2),** 115-123.

Alarifi S, Ali D, Al-Doaiss AA, Ali BA, Ahmed M, Al-Khedhairy AA. 2013. Histologic and apoptotic changes induced by titanium dioxide nanoparticles in the livers of rats. International Journal of Nanomedicine **8,** 3937–3943.
http://dx.doi.org/10.2147/IJN.S47174

Allen R. 2016. The cytotoxic and genotoxic potential of titanium dioxide (TiO_2) nanoparticles on human SH-SY5Y neuronal cells in vitro. The Plymouth Student Scientist **9,** 5-28.

Amara S, Khemissi W, Mrad I, Rihane N, Ben Slama I, Mir LE, Sakly M. 2013. Effect of TiO_2 nanoparticles on emotional behavior and biochemical parameters in adult Wistar rats. *General Physiol.* Biophysics **32(2),** 229-234.
https://doi:10.4149/gpb_2013015

Azim SAA, Darwish HA, Rizk MZ, Ali SA, Kadry MO. 2015. Amelioration of titanium dioxide nanoparticles-induced liver injury in mice: possible role of some antioxidants. Experimental and Toxicological Pathology **67(4),** 305-314.
http://dx.doi.org/10.1016/j.etp.2015.02.001

Bamidele FP, Ajibade AJ, Oyewo EB, Hannah AO. 2013. A study of some effects of aqueous extract of neem (*Azadirachta indica*) leaves on the lead acetate induced neurotoxicity in the superficial layers of superior colliculus of adult Wistar rats (*Rattus norvegicus*). British Journal of Pharmaceutical Research **3(2),** 217–231.

Baldini E, Palmieri T, Pomarico E, Auböck G, Chergui M. 2019. Electron Dynamics in Anatase TiO2 Nanoparticles by Ultrafast Broadband Deep-Ultraviolet Spectroscopy. In EPJ Web of Conferences (205, p. 05017).
https://doi.org/10.1051/epjconf/201920505017

Cui Y, Chen X, Zhou Z, Lei Y, Ma M, Cao R, Sun T, Xu J, Huo M, Wen C, Che Y. 2014. Prenatal exposure to nanoparticulate titanium dioxide enhances depressive-like behaviors in adult rats. Chemosphere **96,** 99-104.
http://dx.doi.org/10.1016/j.chemosphere.2013.07.051.

Dudefoi W, Moniz K, Allen-Vercoe E, Ropers M-H, Virginia K. 2017. Impact of food grade and nano- TiO_2 particles on a human intestine community. Food and Chemical Toxicology **106**, 242-249.
https://doi:10.1016/j.fct.2017.05.050

Elgrabli D, Beaudouin R, Jbilou N, Floriani M, Pery A, Rogerieux F. Lacroix G. 2015. Biodistribution and clearance of TiO(2) nanoparticles in rats after intravenous injection. PLoS One **10(4),** e0124490.
https://doi:10.1371/journal.pone.0124490

Fadda LM, Ali HM, Mohamed AM, Hagar H. 2019. Prophylactic administration of carnosine and melatonin abates the incidence of apoptosis, inflammation, and DNA damage induced by titanium dioxide nanoparticles in rat livers. Environmental Science and Pollution Research, 1-9.
https://doi.org/10.1007/s11356-019-05059-4

Fadda LM, Hagar H, Mohamed AM, Ali HM. 2018. Quercetin and Idebenone Ameliorate Oxidative Stress, Inflammation, DNA damage, and Apoptosis Induced by Titanium Dioxide Nanoparticles in Rat Liver. Dose-Response, **16(4),** 1559325818812188. https://doi.org/10.1002/jbt.22040

Fakhar-e-Alam M, Abbas N, Imran M, Atif M. 2014. Apoptotic effect of TiO_2 in HepG2 cellular model. Journal of Optoelectronics and Advanced Materials **16,** 1481-1486.

Gate L, Disdier C, Cosnier F, Gagnaire F, Devoy J, Saba W, Mabondzo A. 2017. Biopersistence and translocation to extrapulmonary organs of titanium dioxide nanoparticles after subacute inhalation exposure to aerosol in adult and elderly rats. Toxicology letters **265,** 61-69. http://dx.doi.org/10.3390/ijms18122757

Grissa I, Guezguez S, Ezzi L, Chakroun S, Sallem A, Kerkeni E, Haouas Z. 2016. The effect of titanium dioxide nanoparticles on neuroinflammation response in rat brain. Environmental Science and Pollution Research **23(20),** 20205-20213. https://doi.10.1007/s11356-016-7234-8

Gui S, Sang X, Zheng L, Ze Y, Zhao X, Sheng L, Wang L. 2013. Retracted article: Intragastric exposure to titanium dioxide nanoparticles induced nephrotoxicity in mice, assessed by physiological and gene expression modifications. Particle and fibre toxicology **10(1),** 4. https://doi.org/10.1186/s12989

Hong F, Yu X, Wu N, Yu-Qing, Zhang YQ. 2017. Progress of *in vivo* studies on the systemic toxicities induced by titanium dioxide nanoparticles. Toxicological Research **6,** 115-133. https://doi.10.1039/c6tx00338a

Hou J, Wang L, Wang C, Zhang S, Liu H, Li S, Wang X. 2019. Toxicity and mechanism of action of titanium dioxide nanoparticles in living organism. Journal of Environmental Sciences (China) **75,** 40-53. https://doi.10.1016/j.jes.2018.06.010.

Hou X, Cai Y, Mushtaq M, Song X, Yang Q, Huang F, Wei Q. 2018. Deposition of TiO_2 nanoparticles on porous polylactic acid fibrous substrates and its photocatalytic capability. Journal of nanoscience and nanotechnology **18(8),** 5617-5623. https://doi.org/10.1166/jnn.2018.15426

Jasim B, Thomas R, Mathew J, Radhakrishnan EK. 2017. Plant growth and diosegenin enhancement effect of silver nanoparticles in fenugreek (Trigonellafoenum graecum L) Saudi Pharmaceutical Journal **25 (3),** 443-447. https://doi.org/10.1016/j.jsps.2016.09.012

Khan I, Saeed K, Khan I. 2017. Nanoparticles: Properties, applications and toxicities. Arabian Journal of Chemistry. https://doi.org/10.1016/j.arabjc.2017.05.011

Krawczyk M, Stanisz E. 2016. Ultrasound-assisted dispersive micro solid-phase extraction with nano-TiO2 as adsorbent for the determination of mercury species. Talanta **161,** 384-391. http://dx.doi.org/10.1016/j.talanta.2016.08.071

Lim JH, Sisco P, Mudalige TK, Sanchez-Pomales G, Howard PC, Linder SW. 2015. Detection and characterization of SiO_2 and TiO_2 nanostructure in dietary supplements. Journal of Agriculture and Food Chemistry **63,** 3144-3152. http://dx.doi.org/10.1021/acs.jafc.5b00392

Lu Z, Xie X, Zhou Y, Yu D, Deng Y, Ouyang J, Yang B, Luo D, Zhang D, Kuang H. 2018. Gestational exposure to titanium dioxide nanoparticles impairs the placentation through dysregulation of vascularization, proliferation and apoptosis in mice. International Journal of Nanomedicine **13,** 777-789. http://dx.doi.org/10.2147/IJN.S152400

Magdolenova Z, Collins A, Kumar A, Dhawan A, Stone V, Dusinska M. 2014. Mechanisms of genotoxicity. A review of in vitro and in vivo studies with engineered nanoparticles. Nanotoxicology **8(3),** 233-278.
https://doi.org/10.3109/17435390.2013.773464

Morgan A, Ibrahim MA, Galal MK, Ogaly HA, Abd-Elsalam RM. 2018. Innovative perception on using Tiron to modulate the hepatotoxicity induced by titanium dioxide nanoparticles in male rats. Biomedicine & Pharmacotherapy **103,** 553-561.
http://dx.doi.org/10.1016/j.biopha.2018.04.064

Noureen A, Jabeen F, Tabish TA, Ali M, Iqbal R, Yaqub S, Shakoor Chaudhry A. 2019. Histopathological changes and antioxidant responses in common carp (*Cyprinus carpio*) exposed to copper nanoparticles. Drug and chemical toxicology, 1-8.
http://dx.doi.org/10.1080/01480545.2019.1606233

Pandey RK, Prajapati VK. 2017. Molecular and immunological toxic effects of nanoparticles. International Journal of Biological Macromolecules **107**, 1278-1293.
http://dx.doi.org/10.1016/j.ijbiomac.2017.09.110

Pierzchala K. 2010. Oxidative stress on human cells in the presence of nano-sized titanium dioxide. 2010. École polytechnique fédérale de Lausanne EPFL: Lausanne.
http://dx.doi.org/10.5075/epfl-thesis-4906

Pujalte I, Dieme D, Haddad S, Serventi AM, Bouchard M. 2017. Toxicokinetics of titanium dioxide (TiO2) nanoparticles after inhalation in rats. Toxicology letters **265,** 77-85.
https://doi.org/10.1016/j.toxlet.2016.11.014

Rabhi S, Belkacemi H, Bououdina M, Kerrami A, Brahem LA, Sakher E. 2019. Effect of Ag doping of TiO_2 nanoparticles on anatase-rutile phase transformation and excellent photodegradation of amlodipine besylate. Materials Letters **236**, 640-643.
http://dx.doi.org/10.1016/j.matlet.2018.11.006

Samat MH, Ali AM M, Taib, MF M, Hassan, O.H, Yahya MZA. 2016. Hubbard U calculations on optical properties of 3d transition metal oxide TiO_2. Results in physics **6,** 891-896.
http://dx.doi.org/10.1016/j.rinp.2016.11.006

Shah SNA, Shah Z, Hussain M, Khan M. 2017. Hazardous effects of titanium dioxide nanoparticles in ecosystem. Bioinorganic chemistry and applications.
http://dx.doi.org/10.1155/2017/4101735

Shakeel M, Jabeen F, Iqbal R, Chaudhry AS, Zafar S, Ali M, Asghar MS. 2018. Assessment of titanium dioxide nanoparticles (TiO_2 NPs) Induced hepatotoxicity and ameliorative effects of Cinnamomum cassia in Sprague-Dawley rats. Biological Trace Element Research **182(1),** 57-69.
https://doi.org.10.1007/s12011-017-1074-3

Shakeel M, Jabeen F, Qureshi NA, Fakhr-e-Alam M. 2016.Toxic Effects of Titanium Dioxide Nanoparticles and Titanium Dioxide Bulk Salt in the Liver and Blood of Male Sprague-Dawley Rats Assessed by Different Assays. Biological Trace Element Research **2(173),** 405-426.
http://dx.doi.org/10.1007/s12011-016-0677-5

Sharma P, Singh R, Jan M. 2014. Dose-dependent effect of deltamethrin in testis, liver and kidney of Wistar rats. Toxicology International **21(2),** 131–139.
http://dx.doi.org/10.4103/0971-6580.139789

Shinohara N, Oshima Y, Kobayashi T, Imatanaka N, Nakai M, Ichinose T, Gamo M. 2014. Dose-dependent clearance kinetics of intratracheally administered titanium dioxide nanoparticles in rat lung. Toxicology **325,** 1-11.
https://doi.org/10.1016/j.tox.2014.08.003

Thomas S, Kumar Mishra P, Talegaonkar S. 2015. Ceramic nanoparticles fabrication methods and applications in drug delivery. Current pharmaceutical design **21(42),** 6165-6188.

http://dx.doi.org/10.2174/1381612821666151027153246

Valentini X, Rugira P, Frau A, Tagliatti V, Conotte R, Laurent S, Nonclercq D. 2019. Hepatic and Renal Toxicity Induced by TiO_2 Nanoparticles in Rats: A Morphological and Metabonomic Study. Journal of Toxicology. http://dx.doi.org/10.1155/2019/5767012

Vance ME, Kuiken T, Vejerano EP, McGinnis SP, Hochella Jr F, Rejeski D, Hull MS. 2015. Nanotechnology in the real world: Redeveloping the nanomaterial consumer products inventory. Beilstein journal of nanotechnology **6(1),** 1769-1780. http://dx.doi.org/10.3762/bjnano.6.181

Vasantharaja D, Ramalingam V, Aadinaath RG. 2015. Oral toxic exposure of titanium dioxide nanoparticles on serum biochemical changes in adult male Wistar rats. Nanomedicine Journal **2(1),** 46–53.

Wang Y, Chen Z, Ba T, Pu J, Chen T, Song Y, Wang H. 2013. Susceptibility of young and adult rats to the oral toxicity of titanium dioxide nanoparticles. Small **9(9-10),** 1742-1752. https://doi.org/10.1002/smll.201201185

Younes NRB, Amara S, Mrad I, Ben-Slama I, Jeljeli M, Omri K, El GJ, El ML, Rhouma K, Abdelmelek H, Sakly M. 2015. Sub-acute toxicity of titanium dioxide (TiO_2) nanoparticles in male rats: emotional behavior and pathophysiological examination. Environmental Science and Pollution Research **22(11),** 8728–8737. https://doi.org/10.1007/s11356-014-4002-5

Yu R, Fang X, Somasundaran P, Chandran K. 2015. Short-term effects of TiO_2, CeO_2, and ZnO nanoparticles on metabolic activities and gene expression of Nitrosomonas europaea. Chemosphere, **128,** 207-215. https://doi.org/10.1016/j.chemosphere.2015.02.002