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Disposition Kinetics and Tissue Distribution of Titanium Dioxide (TiO_2) Nanoparticles Following Single Exposure in Male Wistar Rats

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Abstract

Titanium Dioxide (TiO_2) nanoparticles are one among the several environmental contaminants essentially due to their widespread applications in food, medicine, cosmetics, electronics, and several other applications. The current experimental study was undertaken to determine the toxicokinetic variables and tissue distribution profile of titanium (Ti) following single intraperitoneal (*i.p*) administration of titanium dioxide nanoparticles (TiO_2 NPs) in male Wistar rats. The $T_{max(obs.)}$, $C_{max\ (obs.)}$, the elimination half-life ($t_{1/2k10}$), the area under the curve (AUC_{0-336}), and $AUC_{0-\infty}$ of TiO_2 following single *i.p* administration of TiO_2 NPs in whole blood was 0.5h, 0.26 ± 0.03 µg.ml^{-1}, 486.31 ± 39.66 h, 48.81 ± 0.54 µg/ml*h and 128.28 ± 7.17 µg/ml*h, respectively. The mean Titanium (Ti) concentration ratio for tissue(s)- to whole blood measured at 336 h as well as the $C_{max(obs.)}$ was in the order of liver > spleen > lung > kidney > testis > brain following single *i.p* administration. The elimination half-life ($t_{1/2k10}$) was in the order of spleen > kidney > liver > lung. The toxicokinetic and tissue distribution profile TiO_2 NPs thus derived would serve as baseline data to execute long-term studies with toxicoepidemiological relevant concentration so as to re-visit safety pharmacology governing TiO_2 -nanoparticles exposure from various sources including pharmaceuticals.

Keywords: Nanoparticles, Toxicokinetics, Titanium Dioxide (TiO_2), Wistar Rats

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1. Introduction

Nanoparticles (NPs) represent a class of organic or inorganic substances between 1-100 nm in size, which may be of a natural origin produced by human activities or engineered for specific purposes[1]. The essence of nanotechnology involves the synthesis of Engineered Nanoparticles (ENPs) that exhibit characteristics, such as small size, large surface area to mass ratio, shape, crystallinity, surface charge, reactive surface groups, dissolution rate, state of agglomeration or dispersal that confer them properties substantially different from those of the bulk particles of the same composition[2].

The production and use of ENPs have been increasing rapidly, both in terms of development and commercial implementation in applications such as drug delivery systems, antibacterial materials, cosmetics, biosensors, tissue engineering, and electronics, yielding thousands of consumer-based products already available in the market[3]. Among ENPs, Titanium Dioxide (TiO_2) ranks as one of the most highly manufactured and consumed candidates, from the perspective of consumer products and research applications[3-5]. The worldwide production of TiO_2 accounts for 70% of the volume of pigments and is reported to be one of the most commonly used materials in various commercial products, including food, drugs, cosmetics, sunscreens, varnishes, coatings, and paints[3,6]. Hence exposure to TiO_2 NPs in daily life is inevitable and it can occur through various routes *viz.,* inhalation, oral, dermal, or through various drug delivery systems.

It has been reported continuous or long-term exposure to TiO_2 NPs, especially through inhalation can pose health risks[7]. Further, the ability of TiO_2 NPs to cross the intestinal epithelial barrier following oral exposure can lead to its accumulation in major organs over the long term[5]. In the past TiO_2 NPs have been considered as inert, however, several studies have shown that they were cytotoxic, and genotoxic due to the production of Reactive Oxygen Species (ROS) and activation of signaling pathways leading to inflammation and cell death[8,9]. The toxicokinetic profile of NPs varies significantly according to size, shape, particle agglomeration, and surface coating, thus establishment of their toxicokinetic variables and bio-distribution profile upon various modes of exposure become important. Studies on the biokinetics of TiO_2 NPs are limited hence, the current study was undertaken primarily to establish the toxicokinetic and tissue distribution profile of TiO_2 NPs following single systemic (*i.p*) exposure in Wistar rats.

2. Materials and Methods

2.1 Chemicals and Reagents

Titanium dioxide [Titanium (IV) oxide], *anatase* (TiO_2; MW: 79.87 $g.mol^{-1}$) nanopowder (< 25 nm particle size; 99.7% purity on trace metal basis) was procured from Ms. Sigma-Aldrich, St. Louis, MO, USA.

Male Wistar rats (N = 48) of 8 weeks of age and weighing 185.98±1.04 g were obtained from an authorized vendor (Biogen® Laboratory Animal Facility, Attibele, Bengaluru – 562107, Karnataka, India). They were housed in the Small Animal facility of Veterinary College, Bengaluru (CCSEA Registration No.493/GO/ReBiBt-S/Re-L/01 dated 16.09.2016) in polypropylene cages and maintained under standard management practices including light: dark cycle of 12h each. Animals were fed with standard rodent chow (Amrut®, Ms. Pranav Agro Industries Ltd, M.S, India) and given free access to water ad *libitum*.

2.2 Experimental Animals

After one week of acclimatization, the experimental rats were randomly divided into twelve sub-groups of four rats in each (n = 4). Freshly prepared TiO_2 NPs were suspended in carboxy methyl cellulose (0.2% w/v) and subjected to ultrasonic vibration by using an ultrasonic cleaner for 30 min and stirred over a vortex agitator for about 5 min in order to obtain a homogenized suspension. A single dose of TiO_2 NPs (100 $mg.kg^{-1}$) suspension was administered (*i.p*) to all the experimental rats grouped for the toxicokinetic studies.

2.3 Blood and Tissue Samples

Blood and tissue samples *viz*., liver, spleen, kidney, lung, testis, and brain tissues were collected at 0.083, 0.5, 1, 2, 3, 6, 12, 24, 28, 72, 168, and 336 h post-administration (*i.p*) of TiO_2 NPs from experimental rats. Four rats at each time interval were sacrificed under anesthesia (Ketamine hydrochloride @50 $mg.kg^{-1}$ + Xylazine hydrochloride @10$mg.kg^{-1}$) while withdrawing blood samples.

2.4 Sample Preparation

All the glass wares and acid-resistant labware were cleaned with nitric acid (10% v/v HNO_3) followed by rinsing in deionized water and drying. A known quantity (0.5 ml of blood or 500 mg of liver, spleen, kidney, lung, testis, or brain tissues) was digested using 5 ml of HNO_3

and 0.5 ml of hydrogen peroxide (H_2O_2). After digestion, the solutions were filtered through Whatman filter paper (# 1) and the filtrate was made up to 50 ml of deionized water and stored until analysis at room temperature.

2.5 Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)

The Titanium (Ti) concentration in samples (in duplicate) thus prepared was subjected to Inductively coupled plasma optical emission spectrometry (ICP-OES; Prodigy XP; Make-Teledyne Leeman Labs, U.S.A). The calibration for the analysis method was performed by using multi-element standard solution 6 (100 ppm) (*Trace* CERT; Ms. Sigma-Aldrich, Switzerland) under the following conditions, *viz.*, radiofrequency power: 1.1 kW; emission wavelength: 334.941 nm; plasma argon flow rate: 18 $L.min^{-1}$, sheath argon (auxiliary) flow rate: 0.3 $L.min^{-1}$, nebulizer pressure: 40 psi, pump flow rate: 1.4 $ml.min^{-1}$, sample flow rate 1.4 $ml.min^{-1}$ and read delay 45 sec. and rinse delay 45 sec.

The Limit of Detection (LOD) and Limit of Quantification (LOQ) for 'Ti' in blood or tissues were 0.000528 ppm and 0.0016 ppm, respectively. Two types of blanks were used for the analysis of prepared samples. The calibration blank was used to establish the analytical curve and the method blank was used to identify possible contamination resulting from either the reagents (acids) or the equipment used during sample processing, including filtration.

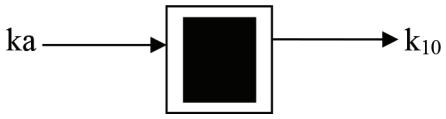
The 'calibration blank' was prepared by acidifying reagent water to the same concentrations of the acids found in the standard or samples. A sufficient quantity of calibration blank was prepared to flush the system between standards and sample runs. The calibration 'blank' was also employed for all initial 'calibration blank' as well as intermittent 'calibration blank' determinations. The 'method blank' was prepared with all of the reagents in the same volumes as used in the processing of samples. The method blank was carried through the complete procedure and contains the same acid concentration in the final solution as the sample solution used for analysis. The concentrations of 'Ti' in blood and tissue samples were expressed as $\mu g.ml^{-1}$ of blood or $\mu g.g^{-1}$ of tissue, respectively.

2.6 Toxicokinetic Analysis

The whole blood concentration of Titanium (Ti) *vs* time curve was plotted for individual experimental rats (n=4) and the mean concentration of Ti vs time plot was examined. The toxicokinetic parameters were derived by using a menu-driven *add-in* program for Microsoft Excel written in Visual Basic (PK Solver; version 2.0) and validated previously[10].

The toxicokinetic variables for Ti following single extravascular (*i.p*) administration (100 $mg.kg^{-1}$) of TiO_2 NPs were derived after subjecting the data of whole blood concentration of 'Ti' *vs* time intervals into one -compartment model with extravascular input (no *lag* time) and one-order elimination as described in the following equation:

$$C(t) = A^*[EXP(-k10^*t) - EXP(-ka^*t)]$$



The PK-Solver program calculates the Area Under the Concentration-time curve (AUC) based on the linear trapezoidal rule. The following variables were derived to characterize the toxicokinetics of TiO_2 NPs in blood or tissue samples (*viz.*, liver, spleen, kidney, lung, testis, and brain) after single extravascular (*i.p*) administration by subjecting the data into a one-compartment model[11] and following toxicokinetic variables were derived.

k_a = Absorption rate constant

$t_{1/2ka}$ = Half-life of absorption

k_{10} = Elimination rate constant

$t_{1/2k10}$ = Elimination of half-life in blood or tissue

T_{max} = Time to reach maximum concentration in blood or tissue

C_{max} = The maximum concentration in blood or tissue

$AUC_{(0-t)}$ = The area under the titanium concentration in blood or tissue *vs* time-curve from time '0'- 336 h

$AUC_{(0-\infty)}$ = The area under the titanium concentration in blood or tissue *vs* time- curve from time '0'- to '∞'

MRT = Mean Residence Time

Vd_{ss} = The steady-state volume of distribution

Further, the Area Under the Curve (AUC) ratio for tissue-to-whole blood was determined as $AUC_{(tissue)}/AUC_{(whole\ blood)}$ at various time intervals (*viz.*, 24, 48, 72, 168, and 336 h) was also determined.

3. Results

3.1 Blood and Tissue Concentration of Titanium (Ti)

The Titanium (Ti) concentration in whole blood ($\mu g.ml^{-1}$) or tissues ($\mu g.g^{-1}$) at various time intervals following

a single *i.p* administration of TiO_2 NPs (100 mg.kg^{-1}) is shown in Table 1.

The mean (±SE) peak concentration [$C_{max(obs.)}$] of Ti in whole blood (0.26±0.03 µg.ml^{-1}) was observed at post 0.5 h administration (*i.p*) of TiO_2 NPs. The mean (±SE) $C_{last\ (obs.)}$ of 'Ti' in whole blood was 0.06±0.00 µg.ml^{-1} (Table 2; Figure 1).

The mean (±SE) peak concentration [$C_{max(obs.)}$] of Ti in liver (642.27±17.46 µg.g^{-1}), spleen (352.60±15.48 µg.g^{-1}), kidney (4.08±0.13 µg.g^{-1}) and lung (40.51±3.76 µg.g^{-1}) (Table 3; Figure 2) were observed at 24h post administration of TiO_2 NPs. Whereas, the mean (±SE) peak concentration of Ti in testis (3.68±0.20 µg.g^{-1}) and brain (2.92±0.17µg.g^{-1}) (Table 3; Figure 3) was observed at 1 and 2 h post-administration, respectively.

3.2 Toxicokinetic Variables

The toxicokinetics (in whole blood or tissues) of TiO_2 NPs following single extravascular (*i.p*) administration of TiO_2 NPs (100 mg.kg^{-1}) in male Wistar rats was best described by a one-compartment model. The toxicokinetic variables of TiO_2 NPs in whole blood following single *i.p* administration in male Wistar rats are presented in Table 2. The mean (±SE) absorption half-life ($t_{1/2ka}$), and elimination half-life ($t_{1/2k10}$) of TiO_2 NPs in whole blood were 0.02±0.001 h and 486.31±39.66 h, respectively. The mean (±SE) area under the Ti concentration-time curve from time '0' h to '336' h [$AUC_{(0-336)}$] and the area under the Ti concentration-time curve from time '0' h to 'infinity' [$AUC_{(0-\infty)}$] for TiO_2 NPs in whole blood were 48.81±0.54 µg/ml*h and 128.28±7.17 µg/ml*h, respectively. The Mean Resident Time (MRT) and steady-state volume of distribution (Vd_{ss}) for TiO_2 NPs in whole blood were 701.62±57.21 h and 3.78±0.59 L.kg^{-1}, respectively.

The toxicokinetic variables of TiO_2 NPs in the liver, spleen, kidney, and lung following single *i.p* administration of TiO_2 NPs in male Wistar rats are presented in Table 3. The mean (±SE) elimination half-life ($t_{1/2k10}$) of TiO_2 NPs in the liver, spleen, kidney, and lung were 306.55±12.77 h, and 571.26±62.70 h, 366.84±38.76 h and 215.02±54.19 h, respectively. The mean (±SE) area under the Ti concentration-time curve from time '0' h to '336' h [$AUC_{(0-336)}$] and the area under the Ti concentration-time curve from time 0 h to infinity [$AUC_{(0-\infty)}$] for TiO_2 NPs in liver 166321.16±992.85 µg/g*h and 421991.70±5108.89 µg/g*h, in spleen 96861.18±3643.50 µg/g*h and 518277.80±191060.87 µg/g*h, in kidney 1031.57±7.12 µg/g*h and 2795.09±58.27 µg/g*h and lung were 9103.36±375.55 µg/g*h and 18297.41±6153.56 µg/g*h, respectively.

Similarly, the toxicokinetic variables of TiO_2 NPs in the testis and brain following single *i.p* administration of TiO_2 NPs in male Wistar rats are also presented in Table 3. The mean (±SE) elimination half-life ($t_{1/2k10}$) of TiO_2 NPs was 47.86±4.48 h in the testis and 125.74±41.06 h in the brain. The mean (±SE) area under the Ti concentration-time curve from time '0' h to '336' h [$AUC_{(0-336)}$] and the area under the Ti concentration-time curve from

Table 1. Mean (±SE) titanium concentration in whole blood (µg.ml^{-1}) or tissues (µg.g^{-1}) following intraperitoneal administration of TiO_2 NPs in male Wistar rats (n = 4)

Time (h)	Whole blood	Liver	Spleen	Kidney	Lung	Testis	Brain
0.083	0.05±0.001	0.92±0.01	0.92±0.02	0.06±0.003	0.73±0.05	0.07±0.002	0.08±0.001
0.5	0.26±0.03	475.69±16.09	157.81±10.64	2.63±0.17	27.68±4.61	2.79±0.03	2.81±0.24
1	0.13±0.01	563.33 ±17.74	256.96±11.16	3.23±0.19	28.30±4.52	3.68±0.20	2.84±0.15
2	0.13±0.01	562.01±16.36	264.73±12.95	3.27±0.25	29.86 ± 2.28	3.46±0.11	2.92±0.17
3	0.14±0.003	577.62±9.93	267.74±6.65	3.52±0.15	32.61±3.90	3.24±0.12	2.66±0.22
6	0.16±0.002	588.65±14.18	275.26±15.88	3.68±0.17	33.62±5.66	2.90±0.14	1.75±0.08
12	0.18±0.004	614.13±16.31	323.87 ± 12.86	3.84±0.12	38.42±3.57	2.81±0.08	1.42±0.15
24	0.20±0.01	642.27±17.47	352.60±15.48	4.08±0.13	40.51±3.76	2.02±0.06	1.08±0.05
48	0.21±0.005	634.38±11.30	338.74±9.53	3.95±0.15	38.01±3.30	1.85±0.01	1.08±0.02
72	0.20±0.004	615.24±14.63	331.52±14.66	3.53±0.08	35.69±3.88	0.92±0.01	1.04±0.04
168	0.19±0.000	556.09±8.27	307.78±13.90	3.16±0.10	32.78±2.97	0.89±0.04	1.02±0.03
336	0.06±9.35E-05	312.54±4.62	236.27±14.86	2.23±0.04	11.82±2.32	0.85±0.04	0.96±0.05

Note: Titanium concentration was measured by ICP-OES

Table 2. Toxicokinetic variables of titanium dioxide nanoparticles in whole blood following intraperitoneal administration of TiO_2 NPs (100 $mg.kg^{-1}$) in male Wistar rats (n = 4)

Variables	Unit	Rat #1	Rat #2	Rat #3	Rat #4	Mean±SE
ka	1/h	45.92	34.79	32.90	38.47	41.43±2.88
$t_{1/2ka}$	h	0.02	0.02	0.02	0.02	0.02±0.001
k_{10}	1/h	0.001	0.001	0.001	0.001	0.001±0.000
$t_{1/2k10}$	h	396.51	499.19	496.95	590.67	486.31±39.66
$T_{max(obs.)}$	h	0.5	0.5	0.5	0.5	0.5
$C_{max(obs.)}$	$\mu g.ml^{-1}$	0.32	0.29	0.22	0.21	0.26±0.03
$AUC_{(0-336)}$	µg/ml*h	50.04	49.31	47.73	48.05	48.81±0.54
$AUC_{(0-\infty)}$	µg/ml*h	112.65	132.27	127.58	147.48	128.28±7.17
MRT	h	572.06	720.21	716.98	852.18	701.62±57.21
Vd_{ss}	$L.kg^{-1}$	2.57	3.92	4.01	5.44	3.78±0.59
$C_{last(obs.)}$	$\mu g.ml^{-1}$	0.06	0.06	0.06	0.06	0.06±0.00

Note: ka = Absorption rate constant; $t_{1/2ka}$ = Half-life of absorption; k_{10} = Elimination rate constant $t_{1/2k10}$ = Elimination half-life; $T_{max\ (obs.)}$ = Observed time to reach maximum concentration; $C_{max\ (obs.)}$ = Observed maximum concentration; $AUC_{(0-t)}$ = The area under the titanium concentration-time curve from time '0' h to 336 h; $AUC_{(0-\infty)}$ = The area under the titanium concentration-time curve from time '0' h to '∞'; MRT = Mean residence time; Vd_{ss} = Steady-state volume of distribution derived as $AUMC/(AUC)^2$ x dose ($mg.kg^{-1}$).; $C_{last\ (obs.)}$ = Last observed concentration.

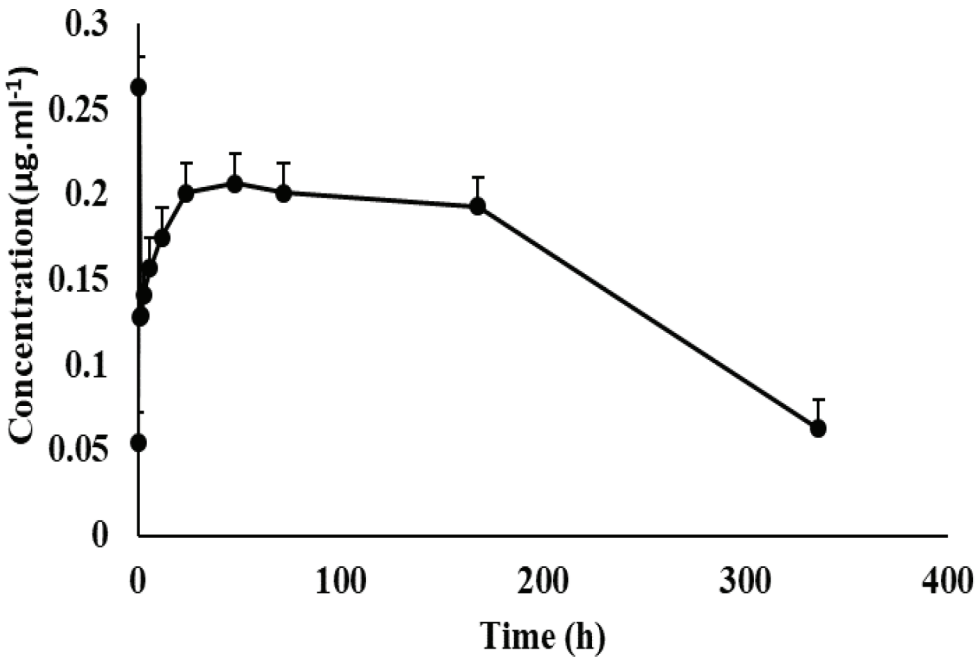


Figure 1. Semi-logarithmic plot of time *vs* concentration of titanium in whole blood following intraperitoneal administration of TiO_2 NPs (100 $mg.kg^{-1}$) in male Wistar rats (n = 4 at each time interval).

time '0' h to 'infinity' [$AUC_{(0-\infty)}$] for TiO_2 NPs in testis 227.94±9.02 µg/g*h and 229.48±9.63 µg/g*h and in brain 124.51±21.46 µg/g*h and 124.62±22.34 µg/g*h, respectively.

The Ti concentration ratio for tissue(s)-to whole blood following single *i.p* administration of TiO_2 NPs (100 $mg.kg^{-1}$) is enlisted in Table 4. At different time intervals (24h to 336h), the order of Ti concentration ratio for tissue(s)-to whole blood was in the order of liver > spleen > lung > kidney > testis- > brain. In a similar pattern, the $AUC_{(0-336)}$ ratio for tissue(s)-to whole blood was also observed (Table 5).

Table 3. Toxicokinetic variables of titanium dioxide nanoparticles in liver, spleen, kidney, lung, testis and brain following intraperitoneal administration of TiO_2 NPs (100 $mg.kg^{-1}$) in male Wistar rats (n = 4)

Variables	Unit	Liver	Spleen	Kidney	Lung	Testis	Brain
$t_{1/2k10}$	h	306.55±12.77	571.26±62.70	366.84±38.76	215.02±54.19	47.86±4.48	125.74±41.06
$T_{max(obs.)}$	h	24	24	24	24	1	2
$C_{max(obs.)}$	$\mu g.g^{-1}$	642.27±17.46	352.60±15.48	4.08±0.13	40.51±3.76	3.68±0.20	2.92±0.17
$AUC_{(0-336)}$	µg/g*h	166321.16±992.85	96861.18±3643.50	1031.57±7.12	9103.36±375.55	227.94±9.02	124.51±21.46
$AUC_{(0-\infty)}$	µg/g*h	421991.70±5108.89	518277.80±191060.87	2795.09±58.27	18297.41±6153.56	229.48±9.63	124.62±22.34
$C_{last(obs.)}$	$\mu g.g^{-1}$	312.54±4.62	236.27±14.86	2.23±0.04	11.82±2.32	0.85±0.04	0.96±0.05

Note: $t_{1/2k10}$ = Elimination half-life; $T_{max\ (obs.)}$ = Observed time to reach maximum concentration; $C_{max\ (obs.)}$ = Observed maximum concentration; $AUC_{(0-336)}$ = The area under the titanium concentration-time curve from time '0' h to 336 h; $AUC_{(0-\infty)}$ = The area under the titanium concentration-time curve from time '0' h to '∞'.

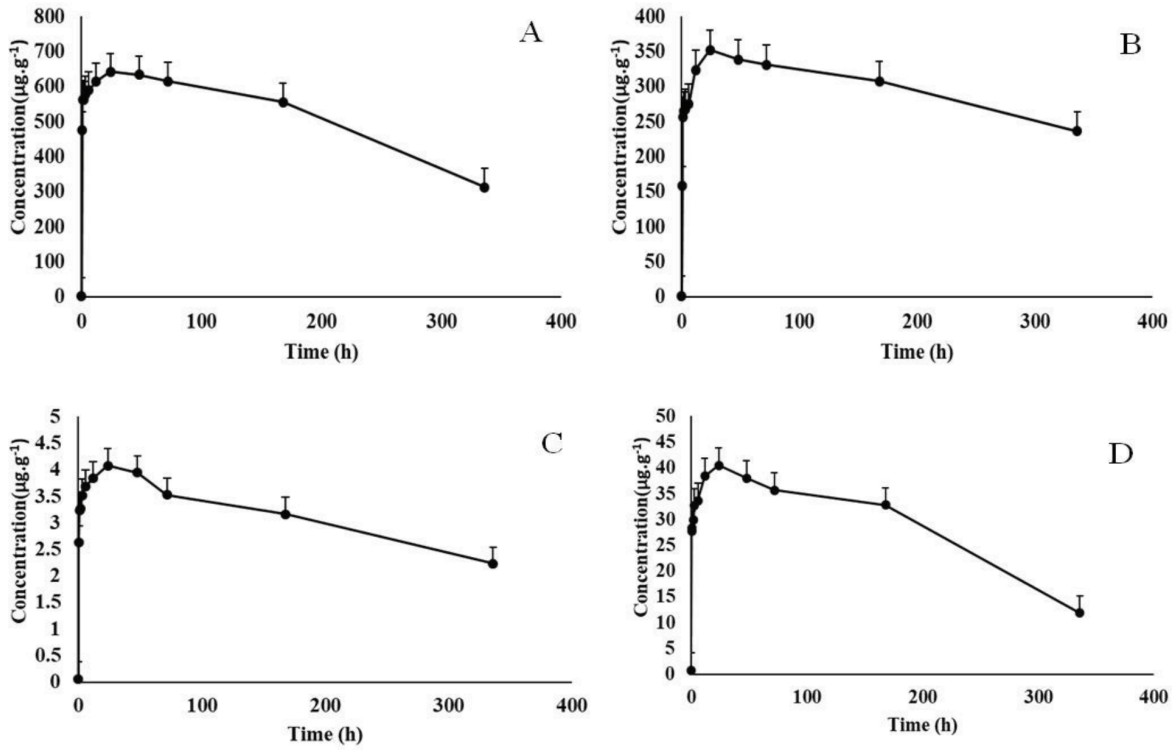


Figure 2. Semi-logarithmic plot of time *vs* concentration of titanium in liver **(A)**, spleen **(B)**, kidney **(C)** and lung **(D)** following intraperitoneal administration of TiO_2 NPs (100 $mg.kg^{-1}$) in male Wistar rats (n = 4 at each time interval).

4. Discussion

4.1 Toxicokinetics and Tissue Distribution

The toxicokinetics in whole blood and tissue distribution of TiO_2 NPs following single extravascular (*i.p*) administration of TiO_2 NPs (100 $mg.kg^{-1}$) in male Wistar albino rats was best described by a one-compartment model (no *lag* time) with first-order elimination. The peak concentration [$C_{max(obs.)}$] of Ti in whole blood (0.26±0.03 $\mu g.ml^{-1}$) was observed at post 0.5 h administration (*i.p*) of TiO_2 NPs. In a study, Golasik *et al.*,[11] reported that Ti attained peak concentration in serum after 30 min of

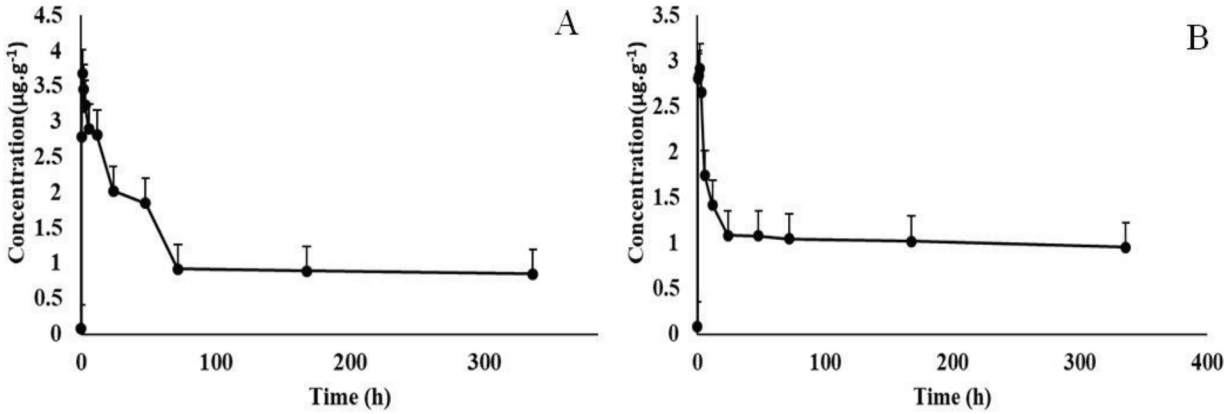


Figure 3. The semi-logarithmic plot of time *vs* concentration of titanium in testis **(A)** and brain **(B)** following intraperitoneal administration of TiO_2 NPs (100 mg.kg^{-1}) in male Wistar rats (n = 4).

Table 4. The Mean (±SE) titanium concentration ratio for tissue(s)-to-whole blood following intraperitoneal administration of TiO_2 NPs (100 mg.kg^{-1}) in male Wistar rats (n = 4 at each time interval)

Concentration ratio						
Time (h)	**Liver-to-Whole Blood**	**Spleen-to-Whole Blood**	**Kidney-to Whole Blood**	**Lung-to Whole Blood**	**Testis-to-Whole Blood**	**Brain-to-Whole Blood**
24	3196.57±145.95	1754.87±61.42	20.31±0.94	201.62±14.38	10.05±0.53	5.40±0.45
48	3070.57±82.88	1639.61±60.16	19.10±1.09	184.00±18.06	8.95±0.20	5.22±0.12
72	3060.15±100.95	1648.97±64.34	17.54±0.59	177.51±21.18	4.56±0.08	5.19±0.27
168	2882.41±41.77	1595.35±69.47	16.40±0.56	169.91±15.45	4.62±0.21	5.29±0.17
336	4992.72±68.69	3774.25±242.62	35.59±0.67	188.76±36.99	13.60±0.61	15.26±0.84

Table 5. The Mean(±SE) $AUC_{(0\text{-}336)}$ ratio for tissue(s)-to-whole blood following intraperitoneal administration of TiO_2 NPs (100 mg.kg^{-1}) in male Wistar rats (n = 4 at each time interval)

$AUC_{(0\text{-}336)}$ ratio					
Liver-to-Whole Blood	**Spleen-to Whole Blood**	**Kidney-to-Whole Blood**	**Lung-to Whole Blood**	**Testis-to-Whole Blood**	**Brain-to-Whole Blood**
3407.23±38.75	1984.28±64.29	21.13±0.15	186.49±9.48	4.67±0.16	2.55±0.46

intravenous (*i.v*) administration of ionic Ti (6 mg.kg^{-1}) in male Wistar rats. However, it is important to note that the comparison of toxicokinetic variables between TiO_2-nanoparticles and the ionic form of titanium cannot be equated.

The elimination half-life of TiO_2 NPs in whole blood was 486.31±39.66 h (~ 20.26 days) after single *i.p* administration. Elgrabli *et al.*,[12] observed a half-life of 12.6 days for TiO_2 NPs in blood after a single *i.v* administration of TiO_2 NPs in male Sprague Dawley rats. The higher elimination half-life of TiO_2 NPs in whole blood observed in the present study may be on account of the administration of relatively a large dose after single *i.p* administration in male Wistar rats. Golasik *et al.*,[11] reported a very short serum terminal half-life (2.91±1.97 h) of ionic Ti following a single *i.v* administration of titanium @ 6 mg.kg^{-1} in male Wistar rats.

Golasik *et al.*[11] reported a very small area under the concentration-time curve [$AUC_{(0\text{-}t)}$] following single *i.v* administration of ionic Ti in rats, however, $AUC_{(0\text{-}336)}$, $AUC_{(0\text{-}\infty)}$ for TiO_2 NPs in whole blood were 48.81±0.54 µg/ml*h and 128.28±7.17 µg/ml*h, respectively.

The mean resident time (MRT) in the present study was 701h (~29 days) following a single extravascular (*i.p*) route of administration, thus TiO_2 NPs spend more time in the kinetic space. The volume of distribution at steady state

(Vd_{ss}) was very large in the current study. Traditionally Vd_{ss} perceived as the least biased and most reliable indicator of the extent of distribution. Thus, it can be concluded that TiO_2 NPs possess wide distribution among various tissues. However, Vd_{ss} appears proportionally constant only during continuous *i.v* infusion or at a single instant in time after discrete dosing[13].

In the present study peak Ti concentration [$C_{max\ (obs.)}$] in tissues observed in the order of decreasing trend showed liver > spleen > lung > kidney > testis > brain. Our findings confirm previous studies wherein the liver, spleen, and lungs appear to be major organs of Ti accumulation[12,14-16]. In another study, Golasik *et al*.,[11] observed the highest concentration of Ti in the kidneys followed by the spleen and liver. The use of nanoparticles of metal (TiO_2 NPs) instead of ionic Ti might be one of the primary causes of difference in the target organ (liver) where in higher concentration of Ti was observed in our study.

The elimination half-lives for TiO_2 NPs in liver, spleen, kidney, lung, testis and brain following single *i.p* administration were 306.55±12.77 h (~12.77 days), 571.27±62.70 h (~23.80 days), 366.84±38.76 h (~ 15.29 days), 215.02±54.19 h (~ 8.96 days), 47.86±4.48 h (~ 1.99 days) and 125.74±41.06 h (5.24 days), respectively. In a similar study, Elgrabli *et al*.,[12] reported half-lives in the liver, spleen, and lung as 12.6, 68.3 and 72.1 days following exposure TiO_2 NPs (1.7 mg/rat; (*i.v*) in Sprague Dawley rats. On the contrary, Golasik *et al*.,[11] observed a very short half-life for ionic Ti in the kidney, spleen, and liver following a single *i.v* administration of Ti (6 $mg.kg^{-1}$) in Wistar rats. The discrepancy with respect to tissue half-lives might be primarily due to the nature of the chemical (TiO_2 NPs instead of ionic Ti) and the route of administration (*i.p*) adopted in the current study.

5. Conclusion

Toxicokinetic analysis of TiO_2 NPs following single intraperitoneal administration in male Wistar rats indicates rapid elimination from the central compartment (blood circulation) and spends more time in kinetic space with a large volume of distribution at steady state (Vd_{ss}). The $AUC_{(0\text{-}336\ h)}$ ratio for tissue(s) to whole blood following single *i.p* exposure was in the order of liver > spleen> lungs> kidney>testis> brain. Further, the time *vs* tissue concentration profile titanium in the liver, spleen, kidney, and lungs was best described by a one-compartment model. The characterization of the disposition profile of titanium thus derived would serve as baseline data to design suitable animal experimental studies to determine the molecular pathological consequences following long-term exposure to titanium dioxide nanoparticles.

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7. References

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