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Specialty: Toxicology

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In silico and *in vivo* study of the susceptibility of adult rats to oral toxicity induced by titanium dioxide nanoparticles

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Abreviations List

- **Characters**

AST: aspartate aminotransferase

Apaf-1: Apoptic protease-activating factor

ALP: alkaline phosphatase

ALT: alanine aminotransferase

BAL: Bronchoalveolar lavage

BAX : BCL2-associated X protein

BBB: Blood–Brain Barriers

Bcl 2 : B cell lymphoma

BUN: Blood urea nitrogen

Caco-2 : Colon adenocarcinoma cells

CK: Creatine kinase

CAT: Catalase

CNS : Central Nervous System

Cr : creatinine

CRPI : Industriel Technology research center

Cyp: cytochrome p

DNA : Deoxy rebo Nucleic Acid

FPs: Fine particles

GIT: Gastro-intestinal

GPx: Glutathione peroxidase

GSHPx: Glutathione Peroxidase

H2O2: Hydrogen peroxide

Hb : hemoglobin

H2SO4: Sulfiric acid

HaCaT: human keratinocyte cells

HBDH: alpha-hydroxybutyrate dehydrogenase

HepG2: Hepato cellule

hERG I/II : human eather-a-go-go-related gene

IARC: International Agency for Research on Cancer

KCl : Chlorure de Potassium

LD50: Lethal dose at 50% concentration

LDH: lactate dehydrogenase

LOAL: lowest observed adverse effect level

logBB : logarithm Brain Barrier

logPS : logarithm Permeability surface

MCH : Mean Cell Hemoglobin

MCHC : Mean Cell Hemoglobin Concentration

MCV : Mean Cell Volume

MDA: 3,4-methylenedioxyamphetamin

mRNA : Messenger ribonucleique acid

Mrna : Messenger ribonucleique acid

N.M: not mentioned

NETs: Neutrophils extracellular traps

NIOSH : National Institute for Occupational Safety and Health

NOAEL:no observable adverse effect level

NPs: Nanoparticles

OCT2 : Organic Cation Transporter

OECD: Organization for Economic Co-operation and Development

OS: Oxidative stress

P53: Protein53

PDT: photodynamic therapy

pkCSM : predicting small-molecule pharmacokinetic properties using graph-based signatures

Pgp : P-glycoprotien

ROS: reactive oxygen species

SD : Sprague Dawley

SDM : Standard Deviation of the Mean

SOD: Superoxide dismutase

SPSS : Statistical Package for the Social Science

TGE : Thermoelectric Group

TiCl4: Tetrachloride

TiO_2 NPs: Titanium dioxide NPs

UA : Uric acid

UF-TiO2: Ultrafine TiO2

UV: Ultraviolet radiation

VDss : The volume of distribution

- **Units** :

> : greater than

% : percent

< : less than

= : equality

± : both plus and minus operations

g : gramme

mg : milligram

ml : milliliter

Kg : kilogram

Rmp : Round per minute

Mg/kg : Milligrams per kilogram

Min : minute

° C : degree Celsius

h : hour

TwA : Time weighted Average

Bw : body weight

µm: micrometer

nm: nanometer

General introduction

Nanotechnology is one of the fastest growing sectors of the high-tech economy. Several consumer products currently use nano-materials containing particles below 100 nanometers (Younes et al., 2019). These products have personal, commercial, medical, and military uses (Brumfiel G, 2006; Griffitt RJ et al., 2007). The unique physicochemical and electrical properties of engineered nanoparticles (NP) make them highly desirable in a variety of applications (Manke A et al., 2013).

Among the most commonly used nanomaterials is Titanium dioxide nanoparticles (TiO_2-NP). TiO_2-NPs are the earliest industrially produced Nanomaterials (Baan R et al., 2006). According to the U.S. National Nanotechnology Initiative, they are one of the most highly manufactured in the world (Liang G et al, 2009) (Iavicoli I et al., 2011).

TiO_2-NPs have become a mutual additive in cosmetics and sunscreens products and foodstuffs due to their properties which stem only from significantly decreased particle size (additive E171) (Piccinno, F et al., 2012; Weir A et al, 2012) (Ziental, D et al., 2020). Because of their excellent optical performance and electrical properties, TiO_2-NPs have a wide range of applications in many fields.

TiO_2-NPs often considered to be physiologically inert to humans. Nanoscale TiO_2 particles also has interesting photocatalytic properties, such as the ability to mediate photodegradation of pharmaceuticals, bacteria inactivation, the photooxidative killing effect on cancer cells, energy storage, as well as air and water purification (Deng, D et al., 2009; Weir, A et al., 2012; Musial J et al., 2020).

However, some recent studies have reported that nano-sized TiO_2 may generate potential harm to the environment and humans (HAO Linhua et al, 2009). Oral, inhalation and dermal interaction, during a short or long contact period with TiO_2-NPs, in humans and experimental animals has shown distinct health effects. Recent studies have shown that exposure to TiO_2-NPs can lead to deterioration of endocrine function, respiratory, hepatic, renal and cardiac (Shi H et al., 2013), may also have difficulty with the reproductive and central nervous system (HAO Linhua et al., 2009).

The objective of this paper is to investigate the *in silico* and *in vivo* acute and subacute toxicity in adult rats induced by nano-sized TiO_2 particles.

The Master's thesis consists of a general introduction, bibliographic synthesis, experimental studies, general conclusion and a list of bibliographical references.

In this paper , the bibliographic synthesis part includes 3 chapters: TiO_2-NPs (generalities, physiochemical properties, source and production, characteristics use), TiO_2-NPs and Toxicity (toxicokinetic and ADMET system, types of toxicity, TiO_2-NPs and stress oxidative, mechanism of toxicity and evaluation methods of stress oxidative and toxicity) and Health effects of TiO_2-NPs (genetic, mutagenic, carcinogenic, hepatic, renal, respiratory, hematological, cardiovascular and neurological effects and also their effects on the endocrine system and reproduction).

Although the experimental studies part presents the *in silico* and *in vivo* investigations which comprises materials, methods, results and discussion.

We end this work with a general conclusion which summarizes the main results of this work.

Bibliographic synthesis

Chapter I: Titanium dioxide nanoparticles (TiO_2-NPs)

1. Generalities

Titanium dioxide nanoparticles (nano-TiO_2) are one of the most widely used engineering nanomaterials, and are commonly used in food and personal care products (Weir et al., 2012), medical treatments (Pelgrift and Friedman, 2013), building materials (Bergamonti et al., 2014), environmental waste cleaning (Formoso et al., 2016), among others.

It has been estimated that in 2020, approximately 2.1 million tons of nano-TiO_2 will be produced, and the estimated annual usage of nano-TiO_2 will exceed 2.5 million tons in the United States by 2025 (Robichaud et al., 2009).

Titanium dioxide known as titanium oxide titanic acid anhydride, titania, titanic anhydride, or Ti white (Shi et al., 2013). With the molecular formula TiO_2, belongs to the family of transition metal oxides, has been used widely as a white pigment (United States EPA 2009), and when used as a food colorant it has the E number E 171.

Almost 90 % of the total production is used for paints, coatings, plastic, rubber and paper. Minor end-use sectors are textiles, food, leather, pharmaceutical and cosmetics (Gitte S et al., 2014).

In addition, titanium dioxide can be used as a catalyst in photocatalytic processes to generate electricity or to degrade specific organic compounds (causes stress oxidative later) (Gitte S et al., 2014).

2. Physicochemical properties of TiO_2-NPs

TiO_2-NPs, an odorless white powder with a molecular weight of 79.9 g/mol, constitutes the naturally occurring oxide; contain impurities such as iron, chromium, vanadium or zirconium that confer a spectrum of different colors.

Pure TiO_2 assembles in three crystal structures (fig 01) anatase, rutile (with tetragonal coordination of Ti atoms) and brookite (with rhombohedral coordination of Ti atoms). But only anatase/rutile or mixtures of these two polymorphs are employed in food sector and have natural and industrial importance. While the brookite is rarely used (Shah S. N.A et al., 2017).

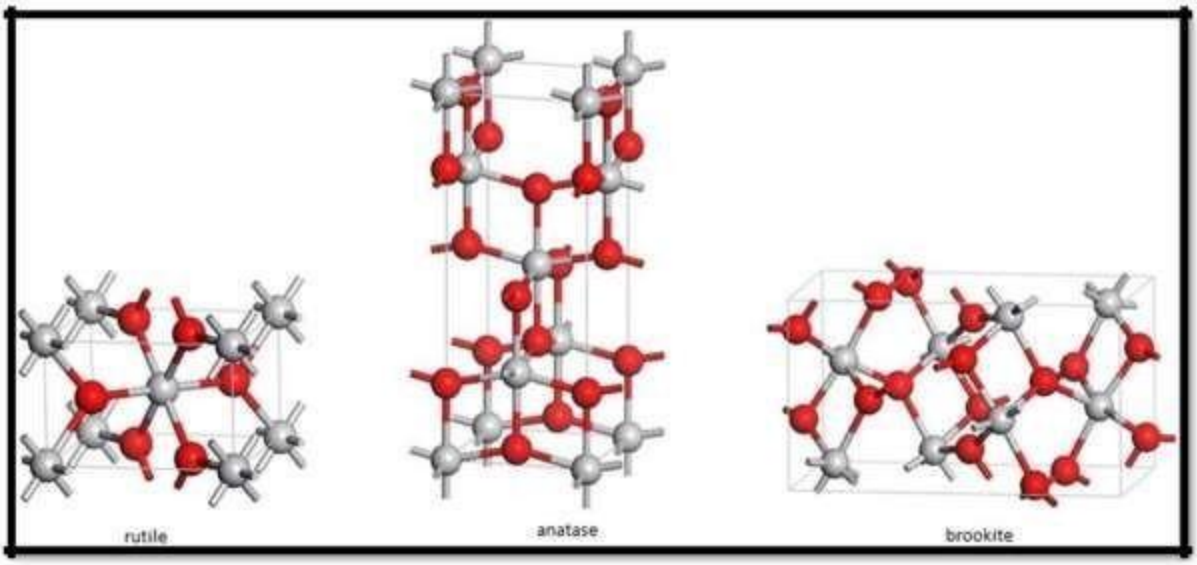

Figure 1: Tetragonal structures of crystalline forms of rutile, anatase and brookite Titanium dioxide nanoparticles. Spheres red: Oxygen, spheres grey: Titanium (Samat et al., 2016).

All TiO_2 particles are insoluble in water, organic solvents, hydrochloric acid and dilute sulfuric acid. They are highly stable to heat and remain unaffected by food processing. Only minimally degraded or dissolved under conditions, including low pH, which mimic the gastrointestinal milieu (Winkler C et al., 2018).

The essential physicochemical properties of TiO_2-NPs are as mentioned in table (01).

Table 1: Proprieties physicochemical of Titanium dioxide nanoparticles (New Jersey Department of Health, 2016; Jargot DB et al., 2013; Shi H et al., 2013).

<table>
<tr><td>Chemical formula</td><td colspan="2">TiO_2</td><td>Melting point</td><td colspan="2">1843°C</td></tr>
<tr><td>Molar mass (g / mol)</td><td colspan="2">79 ,9 g/mol</td><td>Boiling point</td><td colspan="2">2972°C</td></tr>
<tr><td>Density (g / cm3)</td><td colspan="2">4.26 g/cm^3 at 25°C.</td><td>Density gas / vapor</td><td colspan="2">4,5g/cm^3</td></tr>
<tr><td>synonyms</td><td colspan="2">Rutile; Anatase; Brookite</td><td>Appearance</td><td colspan="2">white
odorless powder</td></tr>
<tr><td rowspan="2">Size</td><td>Ultrafine</td><td><100 nm</td><td rowspan="2">Solubility</td><td colspan="2" rowspan="2">Soluble in sulfuric acid alkalis; insoluble in water</td></tr>
<tr><td>Fine</td><td>0,1 et 0,4 µm</td></tr>
<tr><td rowspan="4">Explosive limits (NIOSH)</td><td rowspan="2">Fine TiO_2 (particles)</td><td rowspan="2">2,4 mg/m^3TWA</td><td rowspan="4">CAS (chemical abstract service) number</td><td>TiO_2</td><td>13463-67-7</td></tr>
<tr><td>Anatase</td><td>1317-70-0</td></tr>
<tr><td rowspan="2">Ultrafine TiO_2 (nanoparticles)</td><td rowspan="2">0.3mg/m^3
10-hr/TWA (time weighted average)</td><td>Rutile</td><td>1317-80-02</td></tr>
<tr><td>Brookite</td><td>121-88-41-9</td></tr>
</table>

3. Sources and production

The main sources of industrial extraction of TiO_2 are mineral and ore deposits. Rutile and anatase mineral deposits may contain up to 95% of TiO_2. However, these minerals are difficult to extract from primary rocks and never leach out. They can be extracted only from sands in which they are associated with other minerals, and such deposits are rare. Significant rutile deposits of such quality have been found in Australia and South Africa, whereas anatase is common in Brazil (Jovanovic B, 2015).

The majority of the TiO_2 pigment used in consumer products is being extracted from ilmenite ore ($FeTiO_3$) and leucoxene ore (TiO_2_xFeO_yH_2O), either by sulfate or chloride processing. Under natural conditions, TiO_2 is the least soluble common constituent on the

planet, and geochemical balances are constructed assuming TiO_2 is immobile (E Force, University of Arizona, Phoenix, AZ, personal communication) (Jovanovic B, 2015).

In the sulphate process, the mineral ilmenite ($FeTiO_3$) by treating it with sulphuric acid (H_2SO_4), causes the titanium to be dissolved as titanyl sulphate ($TiOSO_4$), which can be isolated and hydrolyzed at elevated temperature and dried to produce TiO_2.

The chloride process is a refinement of different polymorphs of titanium dioxide and titanium slag which is converted to liquid titanium tetrachloride ($TiCl_4$), then separated and oxidized at elevated temperature (calcinations) to form pure rutile TiO_2 (Figure 2) (Gitte S et al., (2014).

The synthetic methods leading to TiO_2 in general as well as to titania NPs include a series of techniques, with sol-gel synthesis and hydrothermal methods being the most frequently applied Noman M.T et al., 2019 and Chen, X et al., 2007, green chemistry and microwave methods are on the rise (Muniandy S.S et al., 2017 and Falk G.S et al., 2018).

By careful design and modification of the process parameters (substrates used, ratio of solvents, temperature, process time), it is possible to obtain the desired materials with varying specific physicochemical properties (surface area, form, size, crystallinity, photoactivity) (Ziental D et al., 2020).

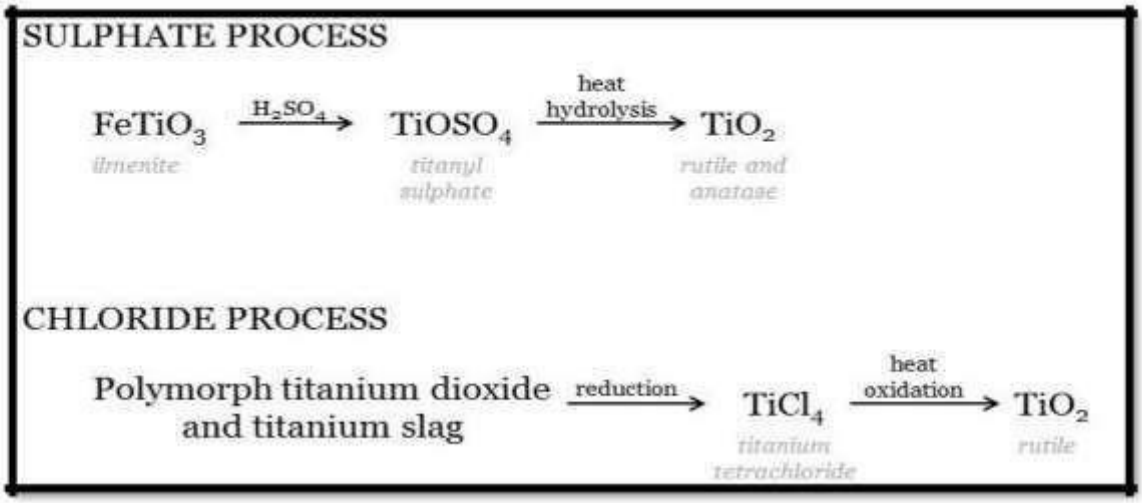

Figure 2: Scheme of production of Titanium dioxide via the sulphate and chloride process (Gitte S et al., 2014).

4. Characteristics use

TiO_2 has been used in micrometric powder form for its opacifying properties and whitened health. It represents about 70% of the world production of pigments, ahead of carbon black and iron oxide; he has been also used in the form of a nanometric powder, in particular for its capacity for absorbing ultraviolet rays.

Fine TiO_2 used as a white pigment in (Paints, lacquers, varnishes and coatings, printing inks, sleeping bath solutions for the paper industry plastics, rubber and leather, food coloring.

Fine non-pigmentary TiO_2 most application in coating of rods and welding flux manufacture of vitrified enamels, electronic components (semiconductors, miniature ceramic capacitors, resistors, varistors, etc.), medicaments as a carrier for certain active principles, shades as an opacifying agent, synthetic fibers, various titanium compounds (titanium carbide, titanates ...).

Ultra-fine TiO_2 also has many applications, particularly in the cosmetics industry, architecture, the food industry and purification air: manufacture of cosmetic products, in particular sun protection products, as an ultraviolet filter; manufacture of cements and glasses, due to its photocatalytic properties which allow the decomposition of organic materials, inorganic and microorganisms. These cements and glasses thus acquire self-cleaning and anti-

pollution properties, manufacture of photocatalytic media for air purifiers for improving air quality; coating and glazing of food products (confectionery, etc) (Jargot D. B et al., 2013).

TiO_2-NPs are widely used in paints, printing ink, rubber, paper, cosmetics, sunscreens, car materials, cleaning air products, industrial photocatalytic processes, and decomposing organic matters in wastewater (Figure 3) (Wang J et al; 2008).

Human exposure occurs either by oral, dermal or inhalation routes (Shakeel M et al., 2016). Evaluated as potential photosensitizers for use in photodynamic therapy (PDT) (Szacilowski K et al., 2005). Also show antibacterial properties under UV light irradiation (Yuan Y et al., 2010; Montazer M et al., 2011).

TiO_2-NPs are produced abundantly and used widely because of their high stability, anticorrosive and photocatalytic properties (Riu J et al., 2006).

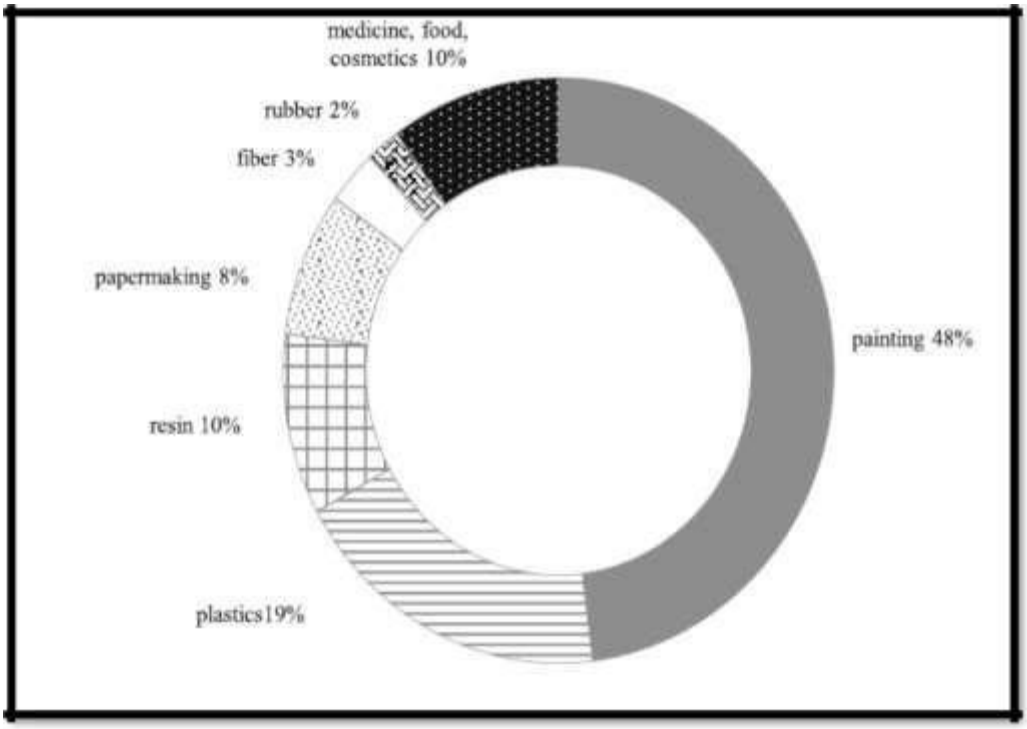

Figure 3: Application of Titanium dioxide nanoparticles (%) in industry (Hong et al, 2017).

Chapter II:
TiO_2-NPs and Toxicity

1. Toxicokinetic /ADMET system

1.1. Absorption

Following deposition of NPs at the initial site of exposure, absorption and translocation to systemic sites is a critical step in Toxicokinetic. It is often defined as migration of the NP to distal organs. For instance, at what rate are TiO_2-NPs absorbed through the gastrointestinal, the skin (dermal), pulmonary system, or other exposure sites, as with intravenous exposure, intra-peritoneal exposure, or subcutaneous exposure (Shi H et al., 2013).

- Oral absorption is generally reported to be low (Onishchenko et al. 2012). NPs, before they are absorbed in the human body, must pass through the gastrointestinal tract (GIT) regions which can alter their properties and change their potential toxicity (McClements DJ et al, 2016).
- Dermal absorption the open literature, demonstrating that nanoparticles do not penetrate into the viable epidermis or dermis cells of healthy skin and skin exposed to UV-B radiation. SCCS (2013) it was demonstrated that sunlight facilitates nanoparticles penetration deeper into the skin and possibly into the viable layers of the skin (Bennett *et al.* 2012). This has however not been confirmed by other identified studies.
- Pulmonary absorption by inhalation, Muhlfeld C et al., 2007 suggested that a small fraction of TiO_2- NPs (20 nm; 1 and 24 h) are transported from the airway lumen of adult male rats to the interstitial tissue and subsequently released into the systemic circulation.

1.2. Distribution

After the initial absorption of TiO_2-NPs, the systemic circulation can distribute the particles to all organs and tissues in the body. These NPs are translocated into the blood, interact with plasma–proteins, coagulation factors, platelets and red or white blood cells, retained in the liver and lymphatic system, distributed to other organs and tissues (Deng ZJ et al., 2009). After intranasally administration of TiO_2-NPs in serum (5 or 10 mg/kg), it's most found in liver, then rate, lung and kidney in rats (Fabian E et al., 2008; Xie G et al., 2011).

1.3. Metabolism

Liver is an organ with major metabolic function in the body and plays an important

part in the metabolism and biotransformation of toxic substances (Casotti, V, 2019; Mossa, A.T.H et al., 2013). In a study investigating the metabolic effects of titanium dioxide on HaCaT human keratinocyte cells, 268 biochemical metabolites, most of which were associated with cellular stress response, were detected, and 85 of them were found to be significantly altered (Tucci et al., 2013). Although metabolism of TiO_2-NPs still unknown.

1.4. Excretion

Elimination of TiO_2-NPs has two potential pathways for clearance kidneys-urine and bile-feces, most ingested TiO_2-NPs are excreted with urine. It is possible that not all of these particles will be eliminated from the body. After continuous exposure, accumulation of TiO_2-NPs may take place in some organs like the lungs, alimentary tract, liver, heart, spleen, kidneys and cardiac muscle (Shi H et al., 2013).

1.5. Toxicity

The most frequently investigated exposure routes in the Toxicokinetics studies of TiO_2-NPs were pulmonary, lung inhalation, dermal and oral administrations. TiO_2-NPs can be absorbed into the body through the lung and gastrointestinal tract; TiO_2-NPs have not the ability to penetrate through the intact skin into the human body under normal conditions. TiO_2-NPs injected intravenously or intra-peritoneally were found in different organs, such as liver, spleen, kidneys, lung, lymph nodes, and brain (Shi et al., 2013). Also, anatase nanoparticles display a stronger adjuvant activity than rutile, NPs in an allergy model based on the intranasal sensitization of mice with ovalbumin (Winkler C et al., 2018).

DL_{50} >5 000 mg/kg were determined in female rats exposed with different doses of TiO_2 -NPs (size 140 nm, mixture of rutile/anatase) (Warheit DB et al., 2007).

Studies concerning toxicity after oral administration to rats show a low level of toxicity at NOAEL > 1000 mg/kg bw/24 h (NOAEL—no observable adverse effect level) (Warheit DB et al., 2015).

Based on results from a subchronic 60 days oral (gavage) study in mice exposed to anatase titanium dioxide nanomaterials (primary particle size 5 nm, the SCCS concludes that a LOAEL of 5 mg/kg b.w/d (SCCS, 2013), Results from another subchronic 30 days oral(gavage) study in mice exposed to anatase TiO_2 -NPs with a primary particle size of 5 nm, a NOAEL of 62.5 mg/kg b.w/d (SCCS, 2013).

Toxicokinetics and accumulation sites of TiO_2-NPs are as summarized in figure 4.

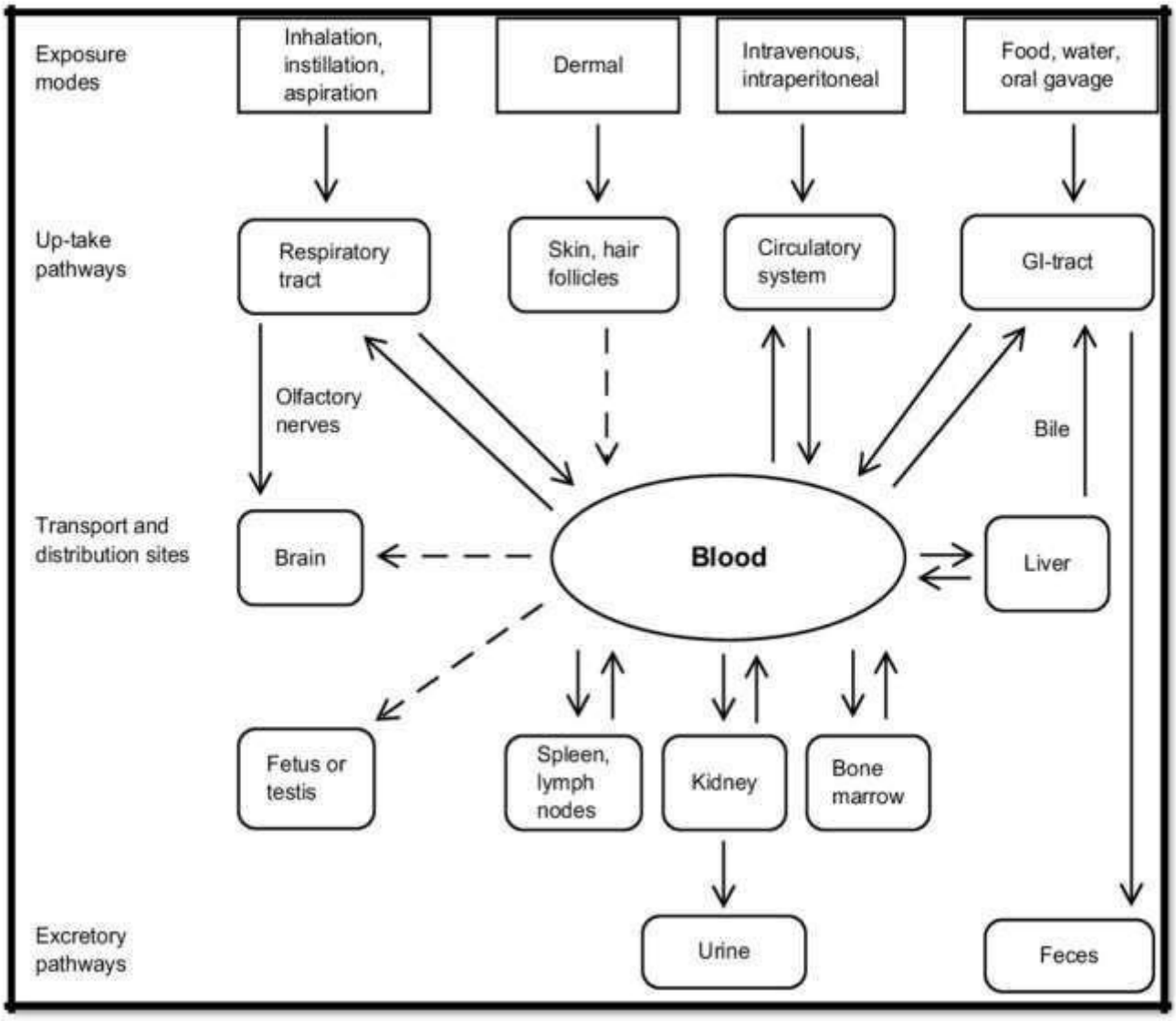

Figure 4: Toxicokinetic and accumulation sites of Titanium dioxide nanoparticles. (The arrows in dotted lines represent uncertainties) (Shi et al, (2013).

2. Types of toxicity

The toxic effects of test substances are usually measured in terms of acute, sub-acute, sub-chronic or chronic exposure conditions. Studies with a maximum of 2 weeks (14 days) study duration are normally referred to as acute toxicity studies. Sub-acute toxicity studies last for a maximum of 4 weeks (28 days), sub-chronic toxicity studies for a maximum of 13 weeks (90 days) and chronic toxicity studies last longer than 4 months.

The toxicity of TiO_2-NPs will be discussed in terms of these types of studies (Shi H et al., 2013). Although the whole metabolic pathways of TiO_2 are not known, NP has been shown to cause oxidative stress and cell toxicity (Gültekin F, 2019). According to the Type of toxicity of TiO_2-NPs, it can be:

2.1. Acute toxicity

No mortality or adverse signs resulted from an acute exposure by single oral gavage administrations of TiO_2-NPs particles according to the OECD (Organization for Economic Co-operation and Development) test guideline 420 (Wang J et al., 2007).

No acute oral toxicity studies with the anatase form alone have been identified. Results from studies submitted by the applicant with anatase/rutile mixtures and titanium dioxide (85 % anatase and 15 % rutile) (Gitte S et al., 2014).

2.2. Subacute

A subacute exposure was carried out in male albino mice with anatase particles (size of 20–50 nm, for an overview of oral toxicity studies). Daily doses of 10, 50 and 100 mg/kg body weight were applied for 14 consecutive days (Shukla RK et al., 2013). At the highest dose, this treatment induced significant increase of liver weight and histologic changes of mononuclear cells to the vicinity of sinusoids accompanied by angiectasis(Winkler C et al.,2018).

2.3. Chronic

Chronic lung inhalation studies have shown that TiO_2-NPs can cause bronchoalveolar adenomas and cystic keratinizing squamous cell carcinomas at high doses (Lee KP et al., 1985) and alveolar/bronchiolar adenoma (Trochimowicz HJ et al., 1988).

Chronic lung inhalation studies that exposed pigs or rats, have reported findings of pulmonary pathology such as increased incidences of pneumonia, squamous metaplasia, sustained pulmonary responses, enhanced proliferation of pulmonary cells, defects in macrophage function, alveolar epithelial metaplasia, progressive fibroproliferative lesions and accumulation of macrophages in interalveolar septa (Shi et al., 2013).

2.4. Subchronic

The systemic toxicity following repeated dose exposure to titanium dioxide nanoparticles seem to be restricted to the organs where particles accumulate over time. Impaired lung

clearance and lung overload is observed in particular in rats at high dose levels associated with chronic inflammation, pulmonary damage and lung tumors (Gitte S et al., 2014).

A subchronic inhalation study comparing pulmonary responses to TiO_2-NPs in several species were performed (Bermudez E et al, 2004). Female rats, mice, and hamsters were exposed to aerosol concentrations of 0.5, 2.0, or 10 mg/m3 TiO_2-NPs (6 h/day, 5 days/week,
for 13 weeks).

There were significant species differences in the pulmonary responses to inhaled TiO_2- NPs. Under conditions where the lung TiO_2-NPs burdens were equivalent, rats developed a more severe inflammatory response than mice and developed progressive epithelial and fibroproliferative changes.

Clearance of particles from the lung was markedly impaired in mice and rats exposed to10 mg/m3 TiO_2-NPs, whereas clearance in hamsters did not appear to be affected at any of the administered doses (Bermudez E et al., 2004).

In the next table (02), some *in vitro* and *in vivo* investigations have been done on diverse species (human, animal, cell culture) on the toxicity of TiO_2-NPs were summarized according to the type of toxicity (acute, subacute, chronic and subchronic).

Table 2: Some papers have been investigated different types of toxicity of Titanium dioxide nanoparticles (TiO_2-NPs).

TiO_2-NPs Size / Form / Dose	**Types of Toxicity and Mode of administration**		**Species**	**Paper title**	**Reference**
0, 2, 10, 20,50 mg/m^3 For 6 h/day for 5 days	**Acute toxicity**	Inhalation	Rat	Development of a short-term inhalation test in the rat using nano-titanium dioxide as a model substance	Ma-Hock L et al.,2009
Anatase 5 nm Mixture of anatase and rutile 21 nm 4 hours		Inhalation	Mice	Inflammatory response of mice to manufactured titanium dioxide nanoparticles: Comparison of size effects through different exposure routes	Grassian et al.,2007

25 and 80 nm 5 g/kg via a single	**Acute toxicity**	Oral gavage	Rat	Gender difference in hepatic toxicity of titanium dioxide nanoparticles after subchronic oral exposure in Sprague-Dawley rats	Wang et al., 2007
25, 80 nm and 155 nm (on crystallinity) 5 g/kg bw, single dose		Oral gavage	Mice CD-1	Susceptibility of Young and Adult Rats to the Oral Toxicity of Titanium Dioxide Nanoparticles	Wang et al., 2007
50–100 nm 300 mg/bw/day 14-days repeated dose		Oral gavage in milli-Q water	Rats	Effect of glycyrrhizic acid on titanium dioxide nanoparticles-induced hepatotoxicity in rats	Orazizadeh, M.,2014
66 nm 100 mg/kg over 10 days		Oral gavage	Mice	Titanium dioxide induced inflammation in the small intestine.	Nogueira CM et al., 2012
80% Anatase, 20% Rutile 20 nm 300 mg/bw/day 14-days repeated dose		Oral gavage in bi-distilled water	Rats	Effects of vitamin A and vitamin E on attenuation of titanium dioxide nanoparticles-induced toxicity in the liver of male Wistar rats	Moradi, A et al.,2019
Anatase 80 nm and 100 nm 2 weeks Doses between 0 and 2593 mg/kg b.w		Intraperitoneal injection	Mice	In vivo acute toxicity of titanium dioxide nanoparticles to mice after intraperitoneal injection."	Chen J et al., 2009
0, 0.16, 0.4 and 1 g/ kg−1 once a day for 14 consecutive days		Intragastric	Rat	NMR-based metabonomic study of the sub-acute toxicity of titanium dioxide nanoparticles in rats after oral administration	Qian Bu et al.,2010

Anatase Rutile micron size 1 or 100 mg/kg 28 days	Subacute toxicity	Intragastric intubation	Rats male	Effects of Titanium Dioxide Nanoparticles on Small Intestinal Mucosa in Rats	Onishchenko et al., 2012
5 mg/kg/b.w for 3 days per week Repeated 3 weeks		Oral administration	Mice	Repeated administration of the food additive E171 to mice results in accumulation in intestine and liver and promotes an inflammatory status	Talamini, L et al.,2019
5, 25, 50 mg kg-1 at weekly interval for 30 days		Injected i.v	Rat	Oxidative stress mediated cytotoxicity of TiO2 nano anatase in liver and kidney of Wistar rat	Meena R ,Paulraj., 2011
5 nm 125 and 250 mg/kg b.w 30 days		Oral	Rat	Gender difference in hepatic toxicity of titanium dioxide nanoparticles after subchronic oral exposure in Sprague- Dawley rats	Duan et al., 2010
75 ± 15 nm 10, 50 and 200 mg/kg BW per day for 30 days		Oral	Rat		Wang et al., 2013
0, 10, 50, 200 mg kg 1 per day for 30 days		Oral	Rat	Susceptibility of Young and Adult Rats to the Oral Toxicity of Titanium Dioxide Nanoparticles	Yun Wang et al.,2012

5-6 nm 2.5 mg, 5 mg, and 10 mg/kg for 90 days	Subchronic Toxicity	Intranasally	Mice	Neurotoxicity and gene-expressed profile in brain-injured mice caused by exposure to titanium dioxide nanoparticles	Ze Y et al., 2014
0, 2, 10 and 50 mg/kg 30 and 90 days		N.M	Rat	Effect of titanium dioxide nanoparticles on glucose homeostasis after oral administration	Chen Z et al.,2018
5–6 nm 2.5,5,10 mg/kg Every day for 90 days		Intragastric administration	Mice	Molecular mechanism of kidney injury of mice caused by exposure To Titanium dioxide nanoparticles	Gui S et al., 2011
5-6 nm 1.25 mg, 2.5 mg and 5 mg/kg For 9 months		Intranasal exposure	Mice	TiO_2 nanoparticle-induced neurotoxicity may be involved in dysfunction of glutamate metabolism and its receptor expression in mice	Ze X et al., 2016
0, 2, 10 and 50 mg/kg 1 per day for 90 days		Oral exposure	Rat	Gender difference in hepatic toxicity of titanium dioxide nanoparticles after subchronic oral exposure in Sprague-Dawley rats	Zhangjian et al.,2018
0, 2, 10 and 50 mg/kg b.w Daily for 90 consecutive days		oral gavage	Sprague-Dawley Rat	Gender difference in hepatic toxicity of titanium dioxide nanoparticles after subchronic oral exposure in Sprague- Dawley rats	Zhangjian Chen et al.,2017
2.5, 5, and 10 mg/kg Every day, 90 day		Intragastric administration	Mice	The chronic spleen injury of mice following long-term exposure to titanium dioxide Np	Sang X, et al.,2012

Size (N.M) 5 mg /kg b.w 5 days 10 weeks	**Subchronic Toxicity**	Oral	Mice	Food-grade titanium dioxide exposure exacerbates tumor formation in colitis associated cancer model	Urrutia-Ortega IM., 2016
Anatase 5–12 nm 100 and 200 mg/kg/b.w 60 days		Intragastrically	Rats	Titanium dioxide nanoparticles in food and personal care products	Weir, A et al.,2012
15- 40 nm 10 mg/m^3 for 2 years, followed by 6 months holding period	**chronic toxicity**	Lung inhalation	Rat	Chronic inhalation exposure of Wistar rats and two different strains of mice to diesel engine exhaust, carbon black, and titanium dioxide	Heinrich UF R et al., 1995
bulk TiO_2 dust Up to 250 mg/m^3 for 2 years, 6 h/day for 5 days/week)		Inhalation exposure	Rat	Pulmonary response of rats exposed to titanium dioxide (TiO2) by inhalation for two years.	Lee K P et al.,1985
TiO_2 1.25, 2.5 5 mg/kg Anatase Half a year		N.M	Mice	Toxicological effect of TiO2 nanoparticle induced myocarditis in Mice	Hong FS et al.,2015
Anatase (purity 99.90%) 29 _ 9 size 0, 2, 10, 50 mg/kg b.w/day	**N.M**	Oral gavage in ultra-pure water	Rats	Gender difference in hepatic toxicity of titanium dioxide nanoparticles after subchronic oral exposure in Sprague-Dawley rats	Chen, Z et al., 2019

5 mg/kg	**N.M**	Intraperitoneal injections	Rat	Hazardous Effects of Titanium Dioxide Nanoparticles in Ecosystem	Hussain S et al., 2009
0.77 mg/m^3	**N.M**	Inhaled	Mice	Inhalation exposure study of titanium dioxide nanoparticles with a primary particle size of 2 to 5 nm. Environ	Grassian V.H et al.,2007
1000 mg/kg/24 h	**N.M**	Oral	Rat	Effects of Titanium Dioxide Nanoparticles Exposure on Human Health: Review	Warheit DB, Donner EM.,2015
25, 80 ou 155 nm 5 000 mg/kg	**N.M**	Gavage	Mice	Nano-TiO Feasibility and challenges for human health risk assessment based on open literature. Pulmonary responses of mice, rats and hamsters to subchronic inhalation of ultra-fine titanium dioxide particles	Christensen FM et al.,2011 Bermudez E et al.,2004
Coated rutile 20 nm *in vivo*	**N.M**	Dermal exposure	Human particip-ants	Stratum corneum is an effective barrier to TiO_2 and ZnO nanoparticle percutaneous absorption	Filipe et al., 2009

20–30 nm 50–150 nm needle-shaped particles, water-in-oil commercial emulsion	**N.M**	Dermal exposure	*in vitro*, four specimens of domestic pig ear	Penetration study of formulated nanosized titanium dioxide in models of damaged and sun-irradiated skins: Photochemistry and photobiology	Miquel-Jeanjean C et al.,2012
20 mg /Kg Every two days	**N.M**	Intraperitoneal injections	Rat	Effects of Titanium Dioxide Nanoparticles (TiO_2) on Behavioral Parameters of Rats	Naima RIHANE B et al.,2016
25 mg/kg once on day 0, once on day 3 and once on day 6	**N.M**	Intraperitoneal injection	Rats	Effect of TiO_2 nanoparticles on emotional behavior and biochemical parameters in adult Wistar rats	Salem Amara et al.,2018
0, 10, 50, 200 mg/kg b.w	**N.M**	Oral exposure	Rat	Susceptibility of Young and Adult Rats to the Oral Toxicity of TiO_2 Nanoparticles	Wang et al., 2013
0.77 mg/m^3	**N.M**	Inhaled	Mice	Inhalation exposure study of titanium dioxide nanoparticles with a primary particle size of 2 to 5 nm. Environ	Grassian V.H et al.,2007
Anatase	**N.M**	Intratracheal instillation	Mice	Pulmotoxicological effects caused by long-term titanium dioxide nanoparticles exposure in mice	Sun Q et al., 2012

Anatase 100 mg/ kg	N.M	oral administration	Mice	Repeated administration of the E171 to mice results in accumulation in intestine and liver and promotes an inflammatory status.	Talamini L et al.,2019
3 nm repeatedly administrated	N.M	Intratracheal instillations	Mice	Nanoparticles and blood–brain barrier: the key to central nervous system diseases	Dominguez A et al.,2014

N.M: not mention

3. TiO_2-NPs and oxidative stress

Reactive oxygen species (ROS) are common by-products of normal aerobic cellular metabolism and play important physiological roles in intracellular cell signaling and homeostasis. The human body is equipped with antioxidant systems to regulate the levels of these free radicals (Katerji.M et al., 2019).

Regarding the molecular mechanisms of the *in vivo* toxicity of NPs in general, the oxidative stress plays the most crucial role (Musial J et al., 2020). ROS-generating capability and proinflammatory effects of NPs in the lung (Nel A et al., 2006). ROS generated by mitochondria in cells are normally quickly neutralized by antioxidant substances (Musial J et al., 2020). However, a condition known as oxidative stress (OS) occurs, when ROS overwhelm the body's ability to readily detoxify them.

Excessive amounts of free radicals generated under OS conditions cause oxidative damage to proteins, lipids, and nucleic acids, severely compromising cell health and contributing (Katerji.M et al., 2019) to many types of human chronic diseases, such as cancer, as well as inflammatory, neurodegenerative or cardiovascular diseases (McCord JM, 2000).

The surface of anatase crystals is considered to be more reactive than that of rutile counterparts, as indicated by their ability to generate ROS in aqueous solutions when irradiated with ultraviolet (UV) light (Winkler C et al., 2018). The pathways still unknown as schematized in Figure 5.

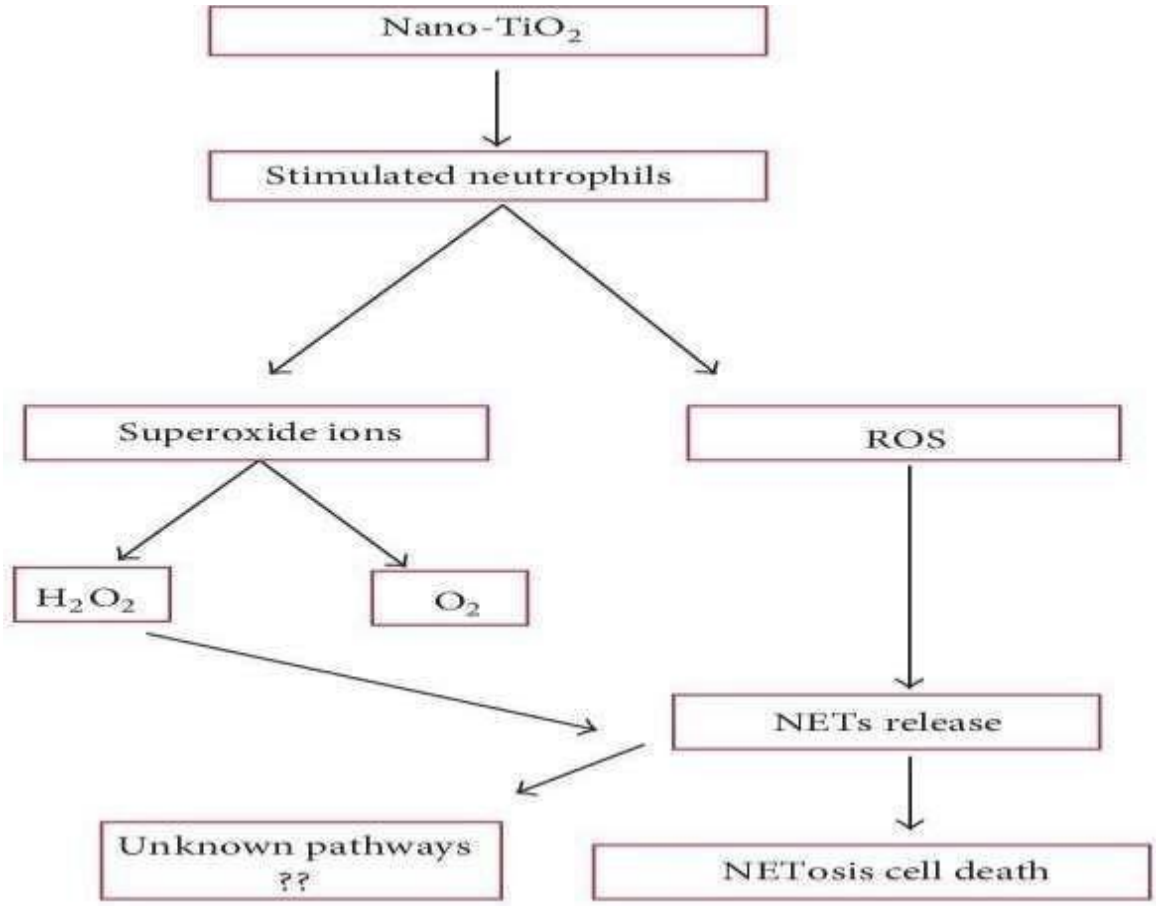

Figure 5: Titanium dioxide nanoparticles -induced NETosis cell death pathway (Shah S. N.A, 2017).

4.Mechanism of toxicity

4.1. Mitochondrial level

TiO_2-NPs cross the cell membrane and accumulate in mitochondria, alter the expression of mitochondrial proteins resulting in depolarization of the mitochondrial membrane, decrease in ATP production, inhibition of cell growth and proliferation, and arrest of the cell cycle (Xia Z et al., 2018).

In addition, TiO_2-NPs could upregulate the expression of a large number of pro-apoptotic substances such as Cytochrome c produced as a result of mitochondrial dysfunction could be released from the mitochondria via this channel into the cytoplasm and irreversibly activated caspase-3, which caused substantial degradation of intracellular DNA (Figure 6).

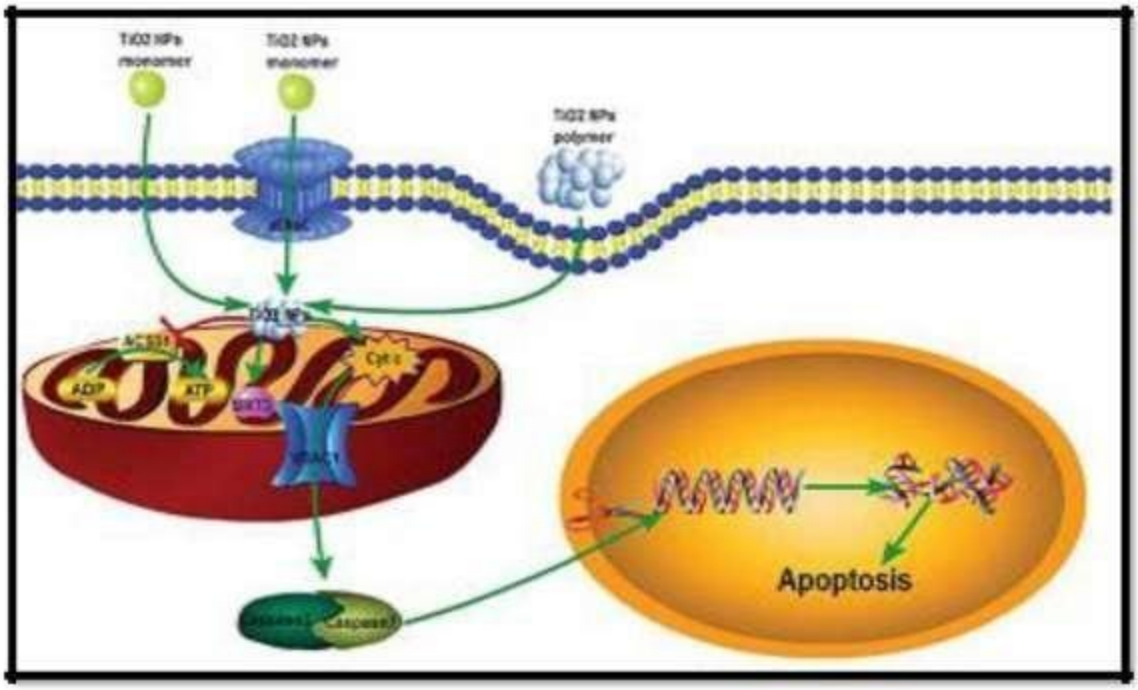

Figure 6: Titanium dioxide nanoparticles induce mitochondria-associated apoptosis in HepG2 cells (Xia Z et al., 2018).

4.2. DNA, peptide and proteins levels

The interaction of free radicals (O_2 - production) with cellular components (nucleus, mitochondria, cytoplasm, etc.) results in the structural modification of cysteine, methionine, histidine, tryptophan and other amino acids. ROS attacks DNA and produces chain breaks, modification of carbohydrate parts and nitrobases by oxidation, nitration, methylation or deamination reactions, finally leading to cell death/apoptosis (Song et al., 2005).

TiO_2-NPs in human liver cells (HepG2 cells) induce oxidative DNA damage and apoptosis in HepG2 cells through mitochondria-mediated pathway. Distribution of these TiO_2-NPs inside cells would therefore enable interactions with biological macromolecules, including lipids, proteins and nucleic acids thereby eliciting toxic responses (Shukla. R.K. et al., 2013).

The mechanism behind mitochondria-mediated apoptosis, p53 levels were measured in HepG2 cells after exposure to TiO2 -NPs. The immunoblot analysis showed a concentration dependent increase in the expression of p53. A concentration-dependent increase in the expression of BAX (pro-apoptotic) and decrease in levels of Bcl-2 (antiapoptotic). It could be

due to increased p53 levels, which result in the modulation in the Bax/Bcl-2 ratio. This leads to the release of cytochrome c, which binds to the apoptotic protease-activating factor (Apaf-1) resulting in the formation of an apoptosome (Shukla. R.K. et al., 2013).

Some studies suggest that caspase-9 and caspase-3 activation leads to cascade of events that trigger cell death in TiO2 -NPs -treated HepG2 cells (Shukla. R.K. et al., 2013).

The next scheme (figure7) illustrates the possible mechanisms of TiO2 -NPs to induce cellular toxicity in HepG2cells according to Shukla et al., 2011.

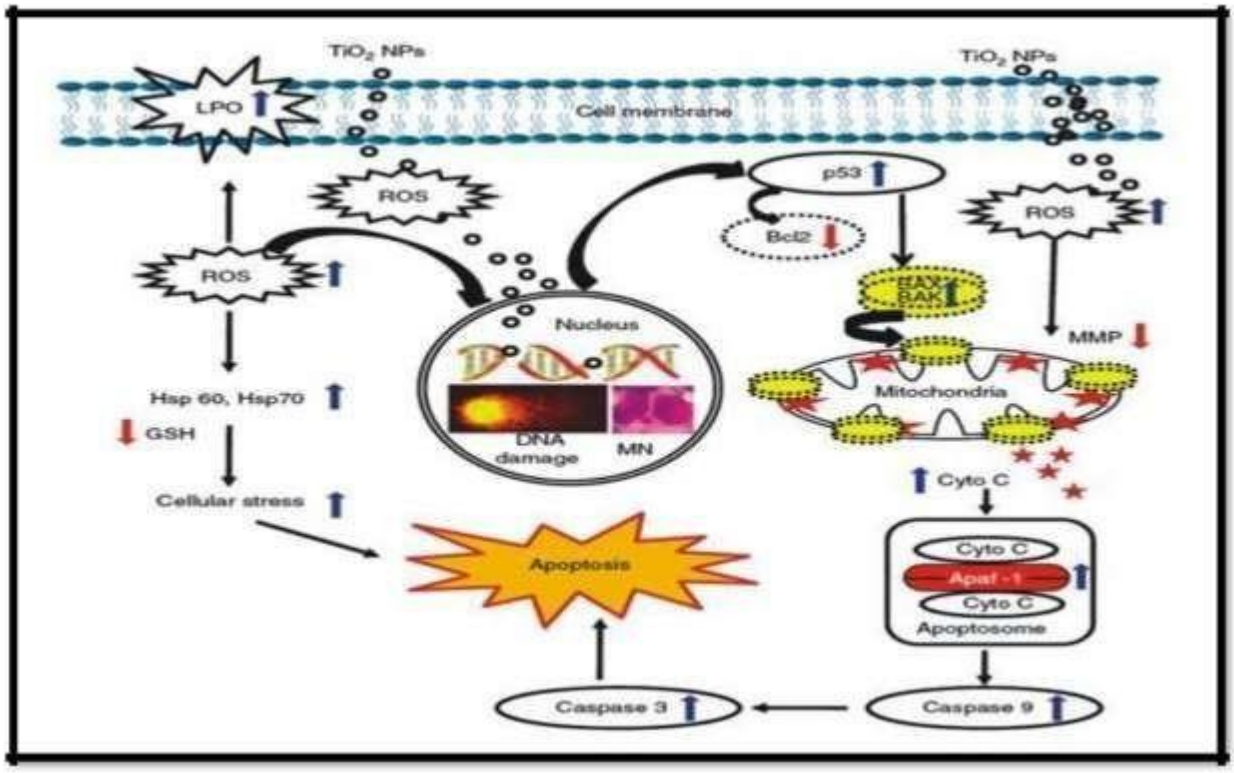

Figure 7: A schematic showing possible mechanisms of titanium dioxide nanoparticles induced cellular toxicity in HepG2cells (Shukla et al., 2011).

5.Evaluation methods of Oxidative stress and Toxicity

5.1.Evaluation method of oxidative stress

Biomarkers of Oxidative stress can be exploited as important tools in the assessment of disease status in humans. Identification of OS biomarkers in clinical samples of cancer

patients and defining their roles in carcinogenesis hold great promise in promoting the development of targeted therapeutic approaches and diagnostic strategies assessing disease status (Katerji M et al., 2019).

Critical evaluation and adaptation of proposed methodologies available in the literature should be undertaken, different methods and approaches used for the evaluation of oxidative stress:

- ✓ Direct Measurement of Reactive Oxygen Species the key molecules responsible for the deleterious effects of oxidative stress.

- ✓ Assessment of Oxidative Damage to bimolecular (DNA, lipids, and proteins), by following protein damage, DNA damage and lipid damage caused by ROS levels.

- ✓ Assessment of Antioxidant Status (enzymatic antioxidant activities, nonenzymatic antioxidant levels, or total antioxidant capacity) the disturbance of this prooxidant and antioxidant balance can be a result of increased free radical production, antioxidant enzyme inactivation, or excessive antioxidant consumption. (Katerji M et al, 2019).

5.2.Evaluation methods of toxicity

The toxicity of TiO_2-NPs test was usually determined following the OECD Test Guidelines and the previously studies which have been don by the other investigators with some modification. Generally, it is done as follow:

- ✓ Biochemical analysis activity levels of AST, ALT, LDH and ALP (Shahsavani et al., 2010).

- ✓ The haematological indices of the mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH) and mean cell volume (MCV) were computed usingthe total RBC count, Hb hemoglobin concentration and Hct (Lee et al., 1999).

- ✓ Organ Coefficient organs including heart, liver, spleen, lung, kidney, brain. (Nie P et al., 2021).

- ✓ Histopathological Examination of Liver; the liver was assessed for histopathological changes (Nie P et al., 2021).

- ✓ Levels of Gene Expression extract the total RNA of the liver (Nie P et al., 2021).

Chapter III:
TiO_2-NPs Effects on health

1. Hematological, cardiovascular and neurological effects

TiO_2 -NPs are able to accumulate in the spleen in a dose-dependent manner inducing histopathological damage. TiO_2 -NPs s induced increased ROS and MDA in the spleen (Li N et al., 2010; Wang J et al., 2011) and decreased levels of SOD antioxidant activity in plasma after intratracheal instillation in rats (Liang G et al., 2009). Moreover, Nemmar A et al., 2008 detected a reduced number of platelets *in vivo* due to a possible aggregation confirming the results obtained by the same authors *in vitro*.

In the cardiovascular system, several experiments in vivo have shown myocardial damage, oxidative stress, inflammation and atherosclerosis in mice exposed to TiO_2 -NPs (Liu, H et al., 2009). Daily gastrointestinal administration of TiO_2 -NPs at 0, 2, 10, 50 mg/kg in rats for up to three months resulted in cardiac dysfunction and inflammatory response (Chen, Z; 2015). TiO_2 -NPs are able to induce high LDH, creatine kinase (CK), alpha-hydroxybutyrate dehydrogenase (HBDH), and aspartate aminotransferase (AST) activities used as markers of myocardial lesions, irrespective of the form of TiO_2 anatase, or the route of exposure (Liu, H et al., 2009).

Once the TiO_2 -NPs are translocated into the CNS, they may accumulate in the brain regions. For their slow elimination rates and the Ti contents would gradually increase with repeated exposure. This would induce pathologic changes, such as inflammation, immunological response, edema, cell injury, cell necrosis, which would lead to CNS dysfunctions, including neurodegenerative diseases and psychiatric disorders (Song et al., 2015).

Studies in experimental animals seen that titanium dioxide can accumulate in the brain and increase oxidative stress (Gültekin F et al., 2019). Studies conducted by on mice (exposed to titanium dioxide at doses (2.5 mg, 5 mg, and 10 mg/kg intranasally for 90 days) shows: In the first study, accumulation in the brain and an increase in oxidative stress were caused by the activation of the proteins signaling pathway (Ze Y et al., 2013). In the second study, accumulation in the brain, increased oxidative stress, increase in all glial cells and tissue necrosis (Ze Y, Hu R et al., 2014). In the last study, Tio_2 accumulates in the hippocampus and causes lesions, excessive proliferation of all glial cells, changes in gene expression of proteins and proteins involved in signaling pathways, neuroinflammation and spatial memory disturbances (Ze Y, Sheng, et al., 2014).

2. Hepatic, renal and respiratory effects

Studies demonstrated TiO_2 -NPs hepatotoxicity effects, in terms of increased ALT levels and ALT/AST ratio both after a single oral administration or repeated intraperitoneal treatments in mice (Liu H et al., 2009). Several studies also detected signs of hepatic damage in terms of histopathological changes and hepatocytes ultrastructural alterations that could lead to impaired liver function (Wu J et al., 2009).

The inflammatory reaction in response to NP insult was also confirmed by significant increase of both mRNA and protein expression levels of several inflammatory cytokines and mediators in liver of treated mice (Ma L et al., 2009; Cui Y et al., 2011).

Several studies report alterations, although opposite in some cases, of renal functional parameters, such as increased (Tang M et al., 2010) and reduced (Wang XG et al., 2009) blood urea nitrogen (BUN), increased and decreased creatinine (Cr), and reduced uric acid (UA) levels (Liu H et al., 2009; Zhao JF et al., 2010).

TiO_2 -NPs inducing kidney damage was demonstrated by increased ROS generation, enhanced lipid peroxidation, as well as decreased SOD, catalase, ascorbate peroxidase, andglutathione peroxidase (GSH Px) antioxidant activities (Liang G et al., 2009; Zhao JF et al., 2010).

Regarding the TiO_2 form, different studies have demonstrated the pulmonary toxicity of acute exposure to anatase in terms of increased bronchoalveolar lavage (BAL) inflammatory parameters, lung tissue structural damage, and inflammatory infiltration although it is modest in both acute and subacute exposure (Ivo I et al., 2012).

3. Genetic, mutagenic, carcinogenic effects

This *in vivo* study shows that the investigated mixture of anatase and rutile nano-sized titanium dioxide administrated orally is distributed to different tissues blood, bone marrow, liver and even the embryo, where it can induce genotoxicity at high exposure levels. The mechanism behind the observed genotoxicity may be due to a secondary response following inflammation and oxidative stress (Gitte S et al., 2014).

The International Agency for Research on Cancer (IARC) stated that the exposure to titanium dioxide is not directly associated with an increased cancer risk. Nevertheless, after assessment of the data derived from animal model studies, the IARC decided that there exists sufficient evidence to claim carcinogenicity of titanium dioxide to animals (IARC, 2010).

TiO_2 -NP-induced generation of ROS and alterations in cell signal transduction pathways which led to cancer at high doses (Shi et al., 2013).

4. Effects on the endocrine system and reproduction

Rats were exposed to anatase at 1 to 2 $mg\,kg^{-1}$ body weight daily by oral gavage for 5 days. Accumulation of Titanium in the spleen tissue and ovaries was documented. In addition, alteration of the thyroid function was observed in male rats, whereas testosterone levels increased in males and decreased in females. The final conclusion was that after exposure to oral dose levels relevant to human exposure, the target tissues for TiO_2 toxicity are active endocrine tissues (Jovanovic B, 2015).

In mice treated with intraperitoneal injections of TiO_2 -NPs demonstrated reductions in sperm density and motility and an increase in sperm abnormality and germ cell apoptosis (Guo et al0, 2009).

Experimental studies

Chapter I :
Material and Methods

1. TiO_2-NPs characteristics

Titanium dioxide nanoparticles (TiO_2-NPs, particle size in 15.2 nm and anatase form in crytal structure) were pprepared by Dr Dehdouh. H from Industrial Technologies Research Center (CRTI), Algeria.

TiO_2-NPs dispersed in sodium chloride 0.9 %, that suspension was sonicated for 10 min before the treatment (Vasantharaja et al.,2015).

2. Preparation of TiO_2 solution and TiO_2-NPs

The TiO_2 solution is obtained by carrying out the following steps (Dehdouh H, 2019):

- ✓ The precursor used for the preparation of the deposition solution is an organometallic alkoxide, namely tetrabutyl-orthotitanate Ti $(C_4H_9O)_4$. The solvent used is butanol (C_4H_9OH). Acetic acid ($C_2H_4O_2$) is used as a catalyst. Adding distilled water helps control the polymerization reactions. The solution obtained is transparent, yellowish in colour and slightly viscous.

- ✓ The hydrolysis is carried out by the humidity of the air in the room, at ambient temperature under continuous magnetic stirring.

Figure (08) summarizes the different steps for preparing the TiO_2 solution.

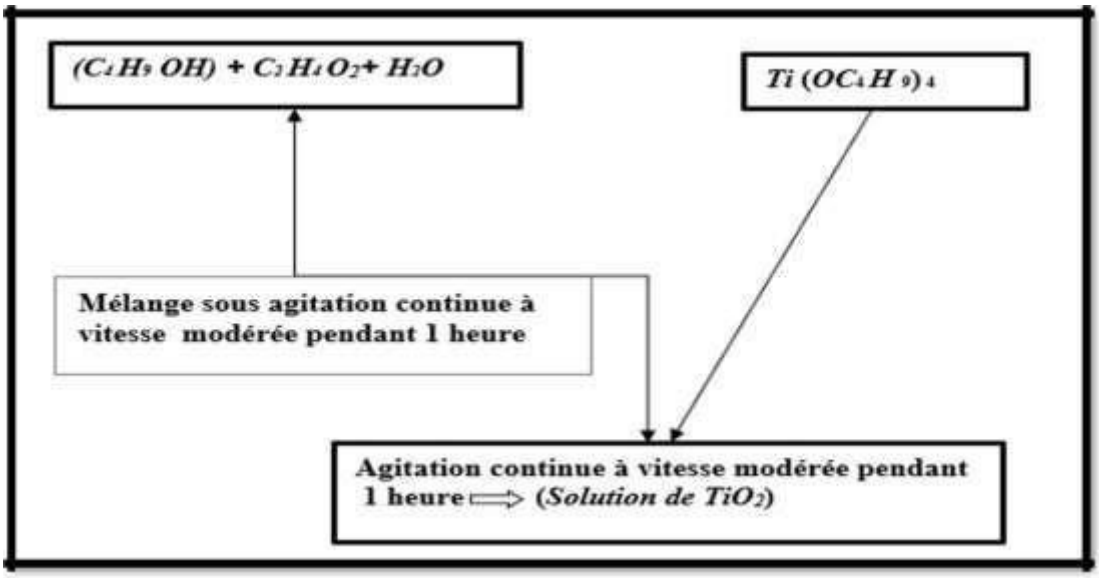

Figure 8: Synoptic diagram of the preparation of the solution of Tio2 (DEHDOUH H, 2019).

The solution remains in a medium away from dust, under atmospheric conditions to evaporate the residual organic elements. After 50 days at an average temperature of 36 ° C, the solution is transformed into xerogel Figure (9. c), then the xerogel obtained is subjected to grinding with a mortar to obtain a fine powder Figure (9. d).

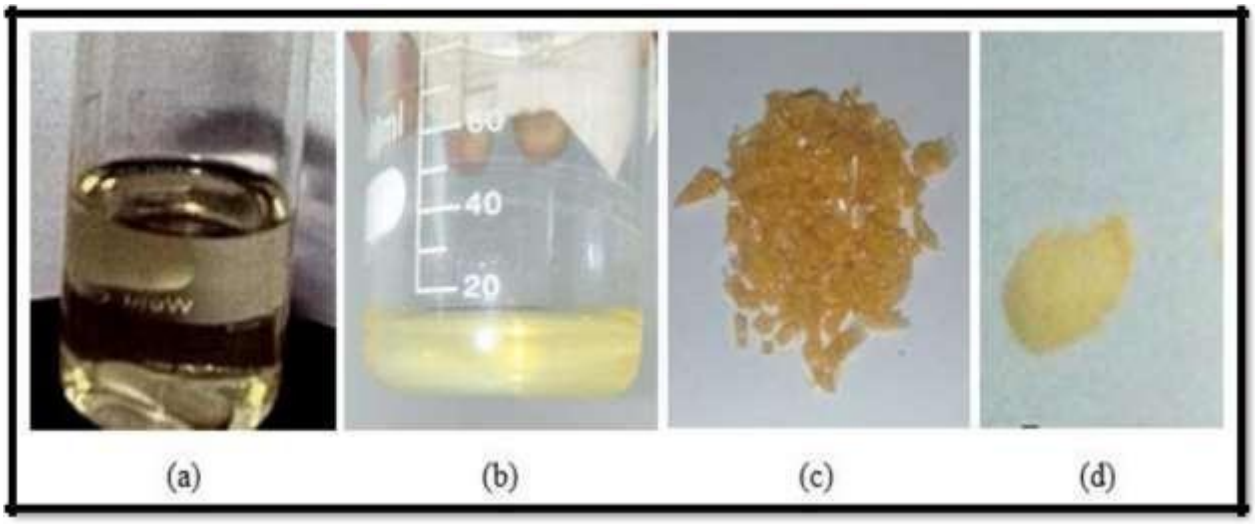

Figure 9: Preparation TiO_2-NPs fromTiO_2 solution**.** (**a**) TiO_2 solution on preparation day, (**b**) TiO_2 solution after 35 days at room temperature, (**c**) TiO_2 xerogel powder after 52 days, (**d**) TiO_2 xerogel powder after grinding**.**

3. Chemicals

The chemicals utilized in the tests were in analytical grade and purchased from Sigma–Aldrich and Roche (see annex 01), they are delivered from Natural and Health Sciences Faculty, Mentouri University and from Pharmaceutical Science Research Center, Constantine, Algeria.

4. Animals

Young adult female Wistar Albino rats (nulliparous and non-pregnant, n = 12, mean age = 25± 5 days) weighing 140 ± 18 g were used in this study. The rats were obtained from the Department of Biology, Mentouri University, Constantine, Algeria (in May, 2021).

They were housed in cages, kept in an air-conditioned room between 22 and 26 ° C and fed standard rat granules with free access to food and water at will. The rats were acclimatized to the laboratory environment for two weeks before the start of the study. The European

Community Directive (86/609 / EEC) and the national rules on animal care have been followed (see annex 02).

5. *In silico* study using the PkCSM ADMET program

Tio2 properties such as absorption, distribution, metabolism, excretion and toxicity (ADMET) profiling of compounds were determined using the PkCSM ADMET descriptors algorithm protocol.

- ✓ The absorption of drugs depends on factors including membrane permeability [indicated by colon cancer cell line (Caco-2)], intestinal absorption, skin permeability levels and P-glycoprotein substrate or inhibitor.
- ✓ The distribution of drugs depends on factors that include the blood–brain barrier (logBB), CNS permeability and the volume of distribution (VDss).
- ✓ Metabolism is predicted based on the CYP models for substrate or inhibition (CYP2D6, CYP3A4, CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4).
- ✓ Excretion is predicted based on the total clearance model and renal OCT2 substrate.
- ✓ Toxicity of drugs is predicted based on AMES toxicity, hERG inhibition,hepatotoxicity and skin sensitization.

6. *In vivo* experimental study

All tests were performed following standard laboratory procedures at the Pharmaceutical Science Research Center, Constantine, Algeria.

6.1. Test of acute toxicity

Limit test was performed at 2000 mg/kg b.w as single dose of TiO_2-NPs administered by gavage. The test was made according to OECD Test Guidelines 425 (OCDE OECD, 2008) as shown in figure (10). Animals (n =3) were kept without food for 3 h before and after getting treatment their doses.

Another group of rats (n =3) was served as a negative control which received 10 mg / kg / b.w of sodium chloride 0.9 % in order to put them in same conditions of the other group. The animal's behaviours and any toxic effect within first 6 hours were closely observed and and mentioned, then at regular intervals for a total period of 14 days.

- ✓ Surviving animal behaviours properties and weight were carefully observed and documented after treatment (30 min, 4 h, 24 h, 7 d and 14 d).

- ✓ Dissection under anesthesia after the 14th day).
- ✓ Blood sampling from the portal vein into dry tubes
- ✓ Serum collecting for biochemical evaluation.
- ✓ Liver harvesting and weighing.
- ✓ Preservation of organs in 10% formalin for histological evaluation

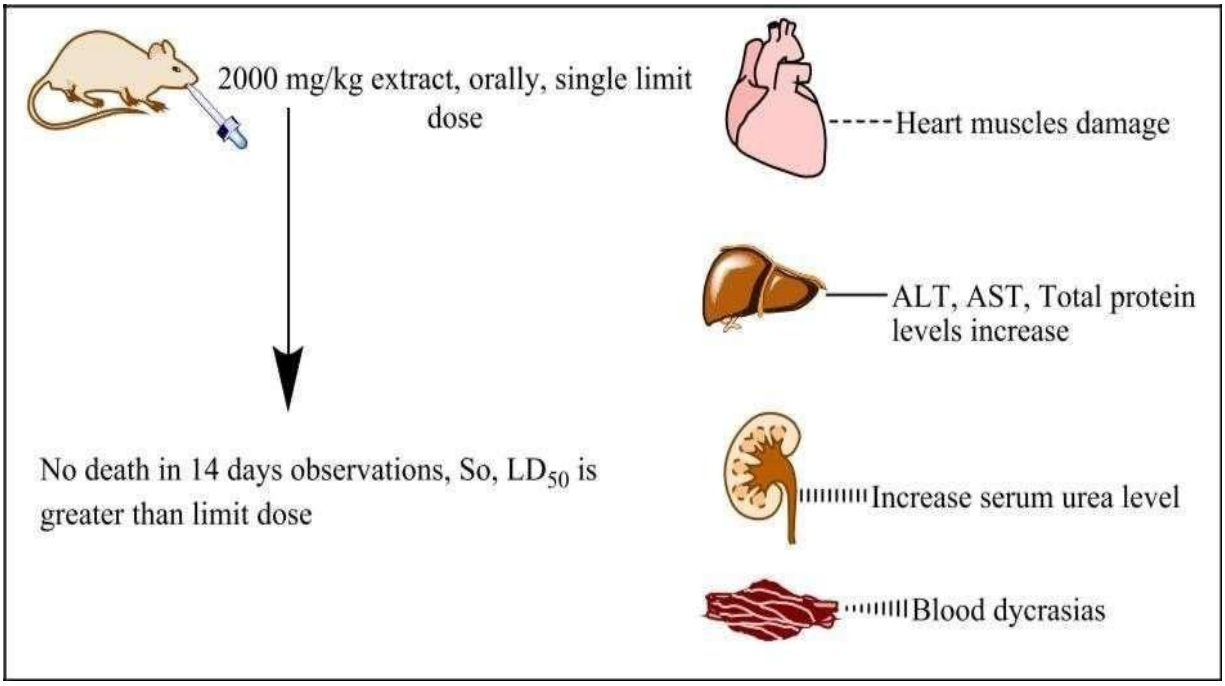

Figure 10: OECD Test Guidelines (425) method to determine the LD_{50} and toxic effects on blood and organs in acute/subacute oral toxicity (Saleem, U et al., 2017).

6.2. Test of sub-acute toxicity

TiO_2 (5 mg / kg b w) was administered daily by gavage for a week. Rats were randomly divided into two groups. Group I (n = 3) was served as vehicle treated control group; animals were received 10 mg / kg / b.w of sodium chloride 0.9 %. While Group II (n = 3), animals were received 5 mg / kg b.w of TiO_2 suspension. The animal's behaviours and any toxic effect within first 6 hours were closely observed and and mentioned, then at regular intervals for a total period of 7 days.

- ✓ Surviving animal behaviours properties and weight were carefully observed and documented daily after treatment.
- ✓ Dissection under anesthesia after the 7th day).
- ✓ Blood sampling from the portal vein into dry tubes.

- ✓ Serum collecting for biochemical evaluation.
- ✓ Liver harvesting and weighing.
- ✓ Preservation of organs in 10% formalin for histological evaluation.

7. Dosage of biochemical parameters:

The clear serum supernatant of each blood sample was used for analysis of glutamates oxaloacetate transaminases and glutamate pyruvate transaminases (AST and ALT), cholesterol, triglycerides, total bilirubin, urea and creatin. These parameters analysed with Roche Cobas Integre Audit Diagnostics Instrument (Germany), using Roche kits.

8. Histopathological examinations

After dissection of rats, organs were examinated macroscopically and livers were removed, rinsed with normal saline and processed distinctly for histological microscopic interpretations.

All tests were performed following standard laboratory procedures at the laboratory of anatomical and pathological cytology, University Hospital Centre. Constantine. Algeria (Material and Products: see Annex 3). Procedures were followed as described below:

a) Tissue fixation

- ✓ Slide preparation begins with the fixation of your tissue specimen. This is a crucial step in tissue preparation, and its purpose is to prevent tissue autolysis and putrefaction.
- ✓ specimens are fixed in 10% neutral buffered formalin; this will allow most tissues to become adequately fixed within 24 - 48 hours. Formalin containers should be cappedand leak-proof, and labelled correctly.

b) Specimen Transfer to Cassettes

- ✓ After fixation, specimens are trimmed using a scalpel to enablethem to fit into an appropriately labelled tissue cassette.
- ✓ they must not be too thick (ideally, they should be less than 4 mm), the filled tissue cassettes are then stored in formalin until processing begins.

c) Tissue Processing: Processing tissues into thin microscopic sections is usually done using a paraffin block, as follows:

- ✓ Dehydration, which involves immersing your specimen in increasing concentrations of alcohol (75%, 85%, 90%, 95% and 100%) to remove the water and formalin from the tissue.
- ✓ Clearing, in which an organic solvent such as xylene is used to remove the alcohol and allow infiltration with paraffin wax.
- ✓ Embedding, where specimens are infiltrated with the embedding agent – usuallyparaffin wax, this step based on a device called a thermoelectric group (TGE). The tissue becomes surrounded by a large block of molten paraffin wax, creating what is now referred to as the ‑block‖. Once the block solidifies, it provides a support matrix that allows very thin sectioning.

d) Sectioning: The tissue specimen is now ready to be cut into sections that can be placed on a slide:

- ✓ Wax is removed from the surface of the block to expose the tissue.
- ✓ Blocks are chilled on a refrigerated plate or ice tray for 10 minutes before sectioning.
- ✓ A microtome is used to slice extremely thin tissue sections off the block in the form ofa ribbon (3 μm in thickness).

e) Staining

- ✓ Most cells are transparent and appear almost colorless when unstained. Histochemical stains (hematoxylin eosin) are therefore used to provide contrast to tissue sections.

f) Synthesis

- ✓ Following staining, a coverslip is mounted over the tissue specimen on the slide, using optical grade glue, to help protect the specimen.
- ✓ We number the slides according to the samples and classify them in a tray.
- ✓ The slides were observed and the photos were taken using an optical microscope(Leica DM 1000, Germany).

9. statistical Analyis

All studies were performed in each test in triplicate. The data can be obtained on average ± standard deviation of the mean (SDM). The analyses were performed by the SPSS 18(Statistical Package for the Social Sciences) software and evaluated by a T-test. The $p < 0.01$ and $p < 0.05$ values were considered significant.

Chapter II : Results

1. *In silico* study

The prediction of Tio2-NPs properties such as absorption, distribution, metabolism, excretion and toxicity (ADMET) profiling using the PkCSM ADMET program is presented in table (03).

Table 3: Hypotheses of Titanium dioxide nanoparticles properties.

Property	**Model Name**	**Predicted Value**	**Unit**	**Hypotheses**
Absorption	Water solubility	**0.933**	Numeric (log mol/L)	**Soluble in water by 0.933**
Absorption	Caco2 permeability	**1.601**	Numeric (log Papp in 10^{6} cm/s)	**High absorption**
Absorption	Intestinal absorption (human)	**100**	Numeric (% Absorbed)	**Total absorption**
Absorption	Skin Permeability	**-3.22**	Numeric (log Kp)	**Low absorption**
Absorption	P-glycoprotein substrate	**Yes**	Categorical (Yes/No)	**yes**
Absorption	P-glycoprotein I inhibitor	**No**	Categorical (Yes/No)	**No**
Absorption	P-glycoprotein II inhibitor	**No**	Categorical (Yes/No)	**No**
Distribution	VDss (human)	**-0.379**	Numeric (log L/kg)	**Average distribution**
Distribution	Fraction unbound (human)	**0.806**	Numeric (Fu)	**not mention**
Distribution	BBB permeability	**-0.369**	Numeric (log BB)	**Poorly distributed**
Distribution	CNS permeability	**-2.487**	Numeric (log PS)	**average distribution**
Metabolism	CYP2D6 substrate	**No**	Categorical (Yes/No)	**does Not metabolised**
Metabolism	CYP3A4 substrate	**No**	Categorical (Yes/No)	**does Not metabolised**
Metabolism	CYP1A2 inhibitior	**No**	Categorical (Yes/No)	**Not an inhibitor**
Metabolism	CYP2C19 inhibitior	**No**	Categorical (Yes/No)	**Not an inhibitor**
Metabolism	CYP2C9 inhibitior	**No**	Categorical (Yes/No)	**Not an inhibitor**

Metabolism	CYP2D6 inhibitior	**No**	Categorical (Yes/No)	**Not an inhibitor**
Metabolism	CYP3A4 inhibitior	**No**	Categorical (Yes/No)	**Not an inhibitor**
Excretion	Total Clearance	**0.864**	Numeric (log ml/min/kg)	**Excretion by 0.864 ml/min/kg Hepatic and renal Clearance**
Excretion	Renal OCT2 substrate	**No**	Categorical (Yes/No)	**Is not considered as OCT2 substrate**
Toxicity	AMES toxicity	**No**	Categorical (Yes/No)	**AMES negativenot mutant Not a carcinogen**
Toxicity	Max. tolerated dose (human)	**1.271**	Numeric (log mg/kg/day)	**high toxicity**
Toxicity	hERG I inhibitor	**No**	Categorical (Yes/No)	**Not an inhibitor**
Toxicity	hERG II inhibitor	**No**	Categorical (Yes/No)	**Not an inhibitor**
Toxicity	Oral Rat Acute Toxicity (LD50)	**2.264**	Numeric (mol/kg)	**Not mention**
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	**2.071**	Numeric (log mg/kg_bw/day)	**Not mention**
Toxicity	Hepatotoxicity	**No**	Categorical (Yes/No)	**It does not cause liver disorder**
Toxicity	Skin Sensitisation	**No**	Categorical (Yes/No)	**Does not cause skin sensitivity**
Toxicity	*T.Pyriformis* toxicity	**-1.157**	Numeric (log ug/L)	**Toxic**
Toxicity	Minnow toxicity	**2.53**	Numeric (log mM)	**Not toxic**

2. *In vivo* study

2.1.Test of acute toxicity

2.1.1. Animal behaviours properties

After exposing the rats to single dose of TiO_2-NPs, the behaviours of the rats were monitored for 14 days in table (04).

We observe a deterioration in weight compared to the rat control, this was due to the loss of appetite caused by the TiO_2-NPs throughout the examination period.

Respiration and tremor were present in the tested rat during the 30 min only (it was absent in the control rat from the first day until the 14th), the latter were completely absent in the following days until the 14 th.

The skin condition of threats did not change throughout the experiment, no rat was in a coma and the remained alive throughout our study.

Table 04: Animal behaviours properties observed after receiving a single dose of 2000 mg/kg Titanium dioxide nanoparticles.

	30 min		4 h		24 h		48 h		7d		14 d	
Observation	**C**	**TIO_2-NPs 2000mg/kg**	**C**	**TIO_2-NPs 2000mg/kg**	**C**	**TIO_2-NPs 2000mg/kg**	**C**	**TIO_2-NPs 2000mg/kg**	**C**	**TIO_2-NPs 2000mg/kg**	**C**	**TIO2-NPs 2000mg/kg**
Weight	N	N	N	N	N	H	N	H	N	H	N	L
Food Consumption	N	LP	N	LP	N	L	N	L	N	L	N	L
Respiration	A	P	A	A	A	A	A	A	A	A	A	A
Trembling	A	P	A	A	A	A	A	A	A	A	A	A
Skin change	A	A	A	A	A	A	A	A	A	A	A	A
Coma	A	A	A	A	A	A	A	A	A	A	A	A
Alive / dead	V	V	V	V	V	V	V	V	V	V	V	V

C: control; A: absent; N: normal; H: high; P: present; LP: loss appetite.

After an evaluation of the weight of the rats treated with TiO2-NPs (2000mg / Kg) for 14 days, In the first day the animal weight of the tested rats was normal during the first 4 hours, with a very significant increase from the 48 hours until the 7th day, in the following 7 days ,we observe a deterioration in weight compared to the rat control ,this was due to rhe loss of appetite ,difficulty breathinf and tremors were observed for treated rats during 30 min .

2.1.2.Evaluation of body weight of animals

The weight of rats for 14 days was mentioned in table (05).

Table 5: Effect of Titanium dioxide nanoparticles on animal weight after receiving a single dose of 2000 mg/kg. The values are mean ± standard deviation (n = 3). ** p <0.01; ***p <0.001, compared to control group.

Groups	1st Day b. w (mg)	7st Day b. w (mg)	14st Day b. w (mg)
Vehicle control Group	153,33 ± 3,7	163,33 ± 3,4	170 ± 1,5
TiO_2-NPs (2000 mg/kg)	150 ± 1,78**	156,33 ± 1,3*	160,33 ± 1,3*

b. w: body weight.

2.1.2. Evaluation of liver weight of animals

After dissecting animals, livers were separated, weighed and mentioned in the below figure (11).

A significant increase in the liver weight of the treated rats dose 2000mg/kg of TiO_2-NPs for 14 day comparing with the weight liver of control rats.

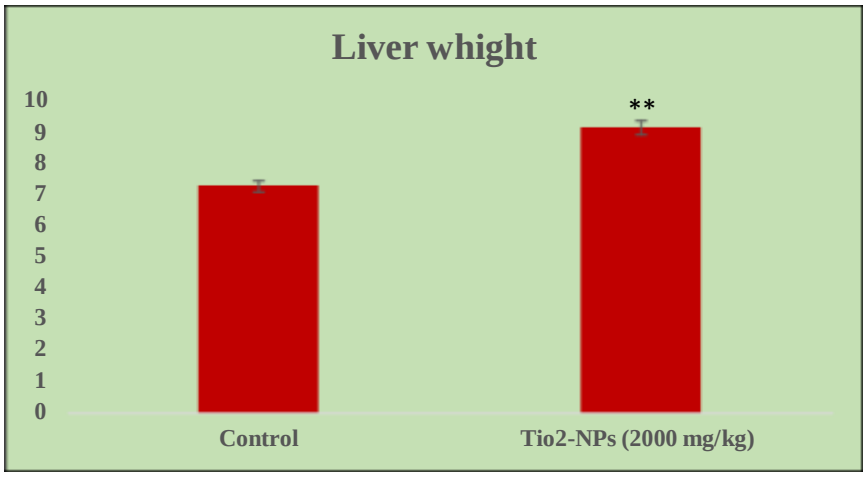

Figure 11: Effect of Titanium dioxide nanoparticles on liver weight of rats after receiving a single dose of 2000 mg/kg. The values are mean ± standard deviation (n = 3). ** p <0.01; ***p <0.001, compared to control group.

2.1.3. Results of Biochemical parameters

The effect of TiO_2-NPs on liver and kidneys function and also the lipid profile is shown in Table (6).

The result shows changes in biochemical parameters in rat liver serum after TiO_2-NPs dosing 14 days. At a dose of (2000 mg/kg body weight), there were a significant change for all parameters compared with the control group. However, ALT and ALP activities were significantly higher than the control group.

Table 6: Effect of Titanium dioxide nanoparticles on biochemical levels after receiving a single dose of 2000 mg/kg. The values are mean ± standarddeviation (n = 3).

** p <0.01; ***p <0.001, compared to control group.

Analysis	**Vehicle control group**	**TiO_2-NPs (2000** mg/kg)
ALT (U/L)	30,6 ± 1,73	41,6 ± 1,17*
AST (U/L)	65 ± 1,51	76,33 ± 0,78 *
Total bilirubins (mg/L)	5,96 ± 0,37	8,71 ± 0,25 *
Cholesterol (mg/dL)	37 ± 0,89	43,62 ± 2,23 **
Triglycerides (mg/dL)	95,67 ± 2,17	97,55 ± 0,45 **
Urea (g/L)	0,371 ± 0,16	0,43 ± 0,06 *
Creatines (mg/L)	5, 25 ± 0,26	8,02 ± 0,22 *

AST: glutamate oxaloacetate transaminases, ALT: glutamate pyruvate transaminases.

2.2. Test of subacute toxicity

2.2.1. Animal behaviours properties

After exposing rats to TiO_2-NPs (5mg/kg) for 7 days, their behaviours were monitored and mentioned in table (07).

A complete absence of all types of other sign (tremor, pain, skin change and coma) in both rats (control and treated), Both groups control rat and treated rats remain alive throughout the experiment.

Table 7: Animal behaviours properties observed after receiving Titanium dioxide nanoparticles (5 mg/kg/7 days).

	30 min		4 h		24 h		48 h		7 d	
Observation	**C**	**TiO_2-NPs 5** mg/kg	**C**	**TiO_2-NPs 5** mg/kg	**C**	**TiO_2-NPs 5** mg/kg	**C**	**TiO_2-NPs 5**mg/kg	**C**	**TiO_2-NPs 5** mg/kg
Weight	N	N	N	N	H	L	H	H	H	H
Food Consumption	N	L	N	L	N	L	N	N	N	N
Breathing	N	N	N	N	N	N	N	N	N	N
tremors	A	A	A	A	A	A	A	A	A	A
Pain	A	A	A	A	A	A	A	A	A	A
Skin change	A	A	A	A	A	A	A	A	A	A
Coma	A	A	A	A	A	A	A	A	A	A
Living/dead	V	V	V	V	V	V	V	V	V	V

C: control; A: absent; N: normal; H: high; P: present; LP: loss appetite.

After an evaluation of the weight of the rats treated with TiO2-NPs (5mg / Kg) for 7 days, the weight of the rats did not change in the first 4 hours, of the 24 hours the rats tested had a significant deterioration in weight compared to the rat control that there was an increase (at 24 hours), this weight increased again in the rats until the 7th day. Normal weight in the following days compared to the control rat which had a normal weight throughout the duration of this study (7 days), Breathing was completely normal in the treated and control rats. A complet absance of all types of sing.

2.2.2. Evaluation of body weight of animals

The next table (08) notes the occurred changes in the weight that have the animals during a week of daily treatment by TiO2-NPs (5 mg/kg).

Table 8: Effect of Titanium dioxide nanoparticles (5 mg/kg/7 days) on the weight of rats. The values are mean ± standard deviation (n = 3). ** p <0.01; ***p <0.001, compared to control group.

Groups	1st Day b. w (mg)	3st Day b. w (mg)	7st Day b. w (mg)
Vehicle control group	119 ± 3,2	128,66 ± 1,8	138,66 ± 2,8
TiO_2-NPs (5mg/kg/7d)	164,66 ± 4,9	169,33 ± 3,72	171,66 ± 0,51

b. w: body weight.

2.2.3. Evaluation of liver weight of animals

After the dissection, livers were weighed and mentioned in figure (12) below.

An increase in the liver weight of the treated rats with 5mg/kg of TiO_2-NPs for 7 day comparing with the weight liver of control rats.

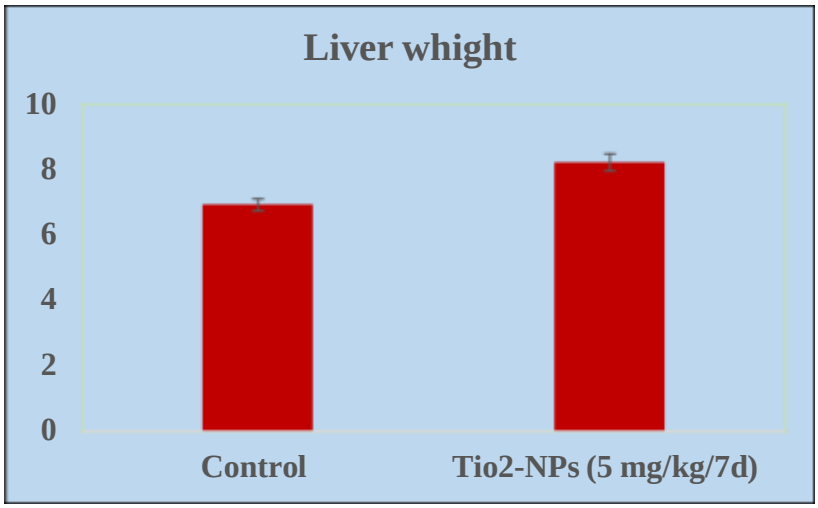

Figure 12: Effect of Titanium dioxide nanoparticles (5 mg/kg/7 days) on the livers weight of rats. The values are mean ± standard deviation (n = 3). ** p <0.01; ***p <0.001, compared to control group.

2.2.4. Results of Biochemical parameters

The effect of TiO_2-NPs biochemical parameters is shown in Table (09).

The result shows changes in biochemical parameters in rat liver serum after TiO_2 nano anatase dosing 7 days. At a dose of (5 mg/kg body weight), there were a significant change for all parameters compared with the control group.

Table9: Effect of Titanium dioxide nanoparticles (5 mg/kg/7 days) on biochemical levels in rats. The values are mean ± standard deviation (n =3). ** p <0.01; *** p <0.001, compared to control group.

Analysis	**Vehicle control group**	**TiO_2-NPs** (**5** mg/kg/7d)
ALT (U/L)	30,5 ± 5,4	47,8 ± 1,81*
AST (U/L)	67,5 ± 3	76,66 ± 2,32 *
Total bilirubins (mg/L)	5,1683 ± 0,44	8,26 ± 0,51 *
Cholesterol (mg/dL)	36,5 ± 2,43	41,5 ± 3,96*
Triglycerides (mg/dL)	95,5 ± 0,77	99,83 ± 0,64*
Urea	0,353 ± 0,05	0,40 ± 0,03**
Creatines (mg/L)	5,7166 ± 0,46	8,89 ± 0,49 *

AST: glutamate oxaloacetate transaminases, ALT: glutamate pyruvate transaminases.

3. Histological study

3.1.Test of acute toxicity

The histological photomicrographs of the hepatic sections of the groups treated by TiO_2-NPs (2000 mg/kg) showed a preserved structure with dilation of the sinusoids (Image 2) compared to the control group (Image 1).

While the histological photomicrographs of kidney sections of TiO_2-NPs (2000 mg/kg)-treated animals showed renal lesions: glomerular congestions, tubular necrosis with a minimal interstitial nephritis (Image 4) compared to the control group (Image 3).

Hepatic sections

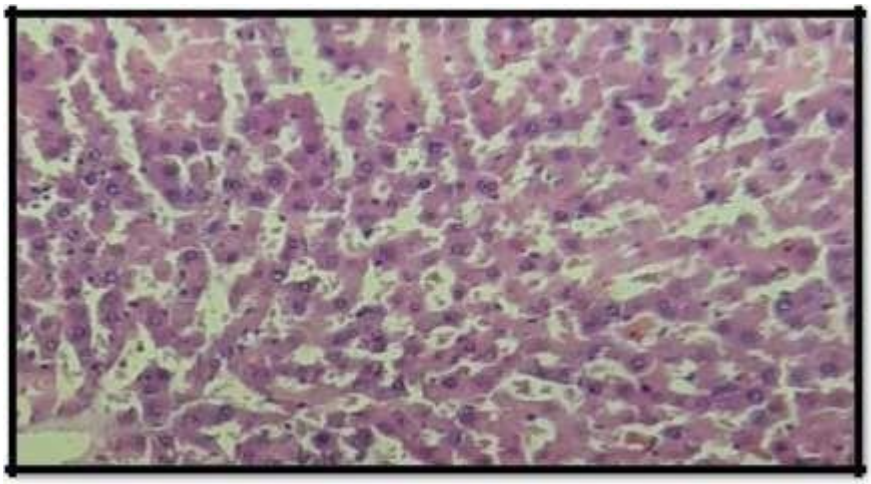

Image 01*:* Histological photomicrography of the liver of the vehicle control group rat (X10).

Preserved architecture with normal appearance.

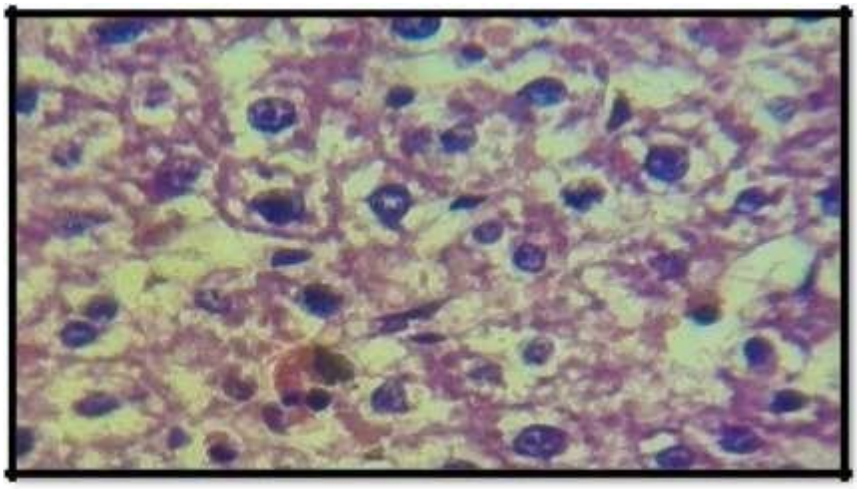

Image 02: Histological photomicrography of the liver of a rat receiving a single dose of 2000 mg/kg (X40).

Preserved architecture with dilation of the sinusoids.

Renal sections

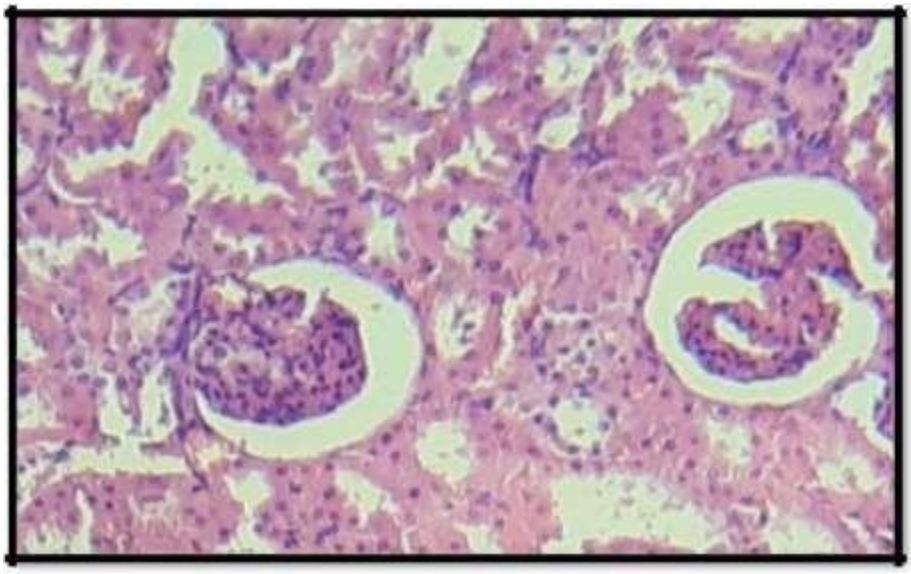

Image 03: Histological photomicrography of kidney of a rat from the vehicle control group (X40).

Preserved architecture.

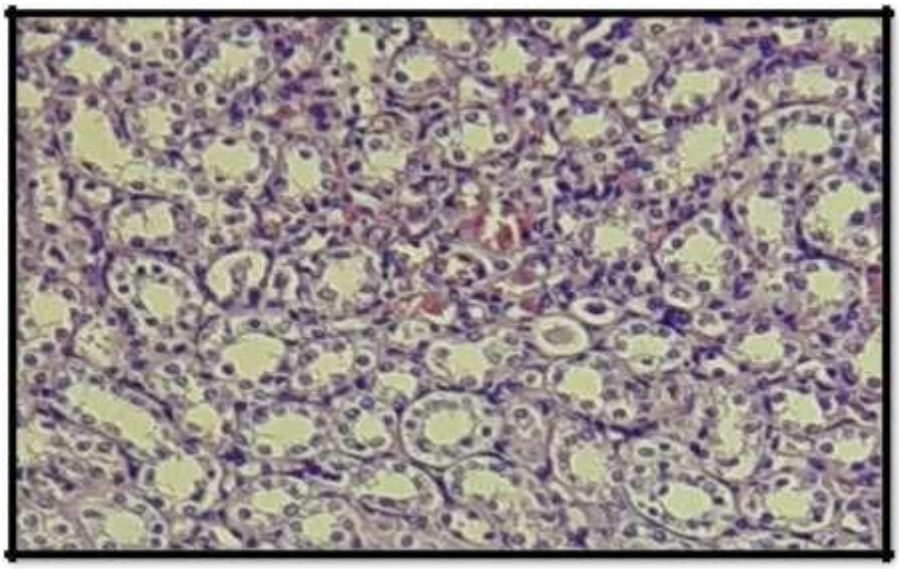

Image 04: Histological photomicrography of the kidney of a rat receiving a single dose of 2000 mg/kg (X10).

Minimal interstitial nephritis with tubular necrosis.

3.2.Test of subacute toxicity

The TiO_2-NPs -treated group at 5 mg/kg/7 days demonstrated a preserved architecture of liver and kidney (Image 6 and 8) in comparing by the control group (Image 5 and 7).

Hepatic sections

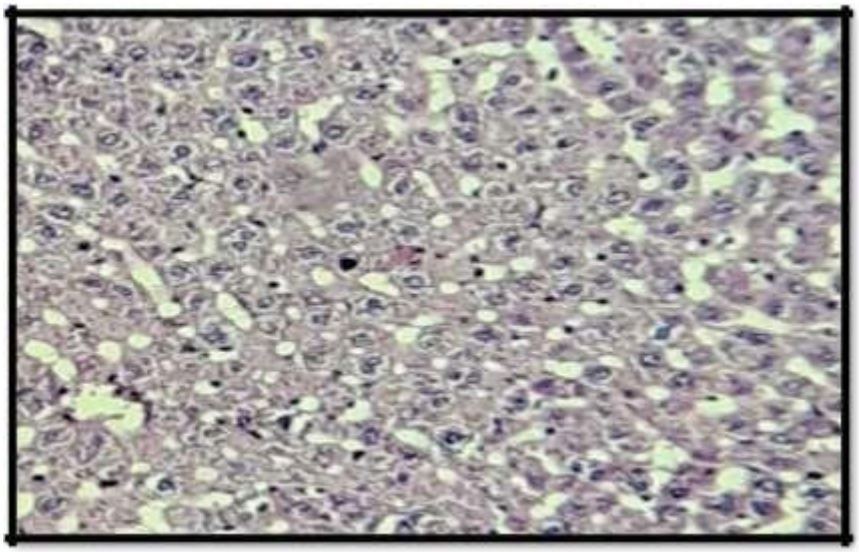

Image 05*:* Histological photomicrography of the liver of a rat from the vehicle control group (X10).
Preserved architecture.

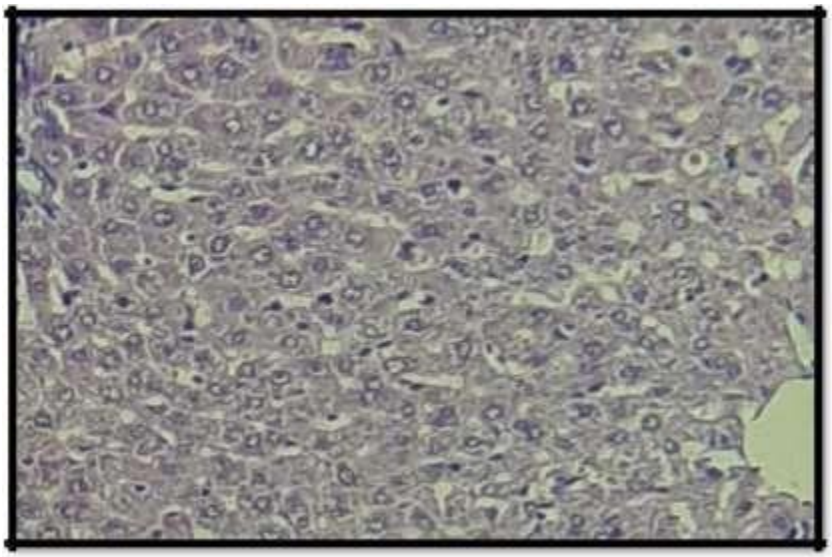

Image 06*:* Histological photomicrography of the liver of a rat receiving Titanium dioxide nanoparticles (5 mg/kg/7 days) (X40).
Preserved architecture (nothing to report).

Renal sections

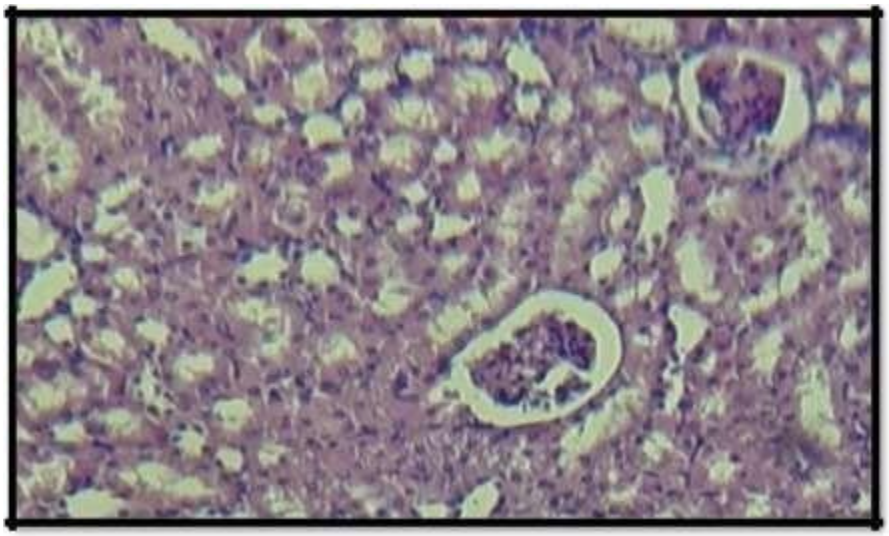

Image 07: Histological photomicrography of the kidney of a rat from the vehicle control group (X40).

Preserved architecture.

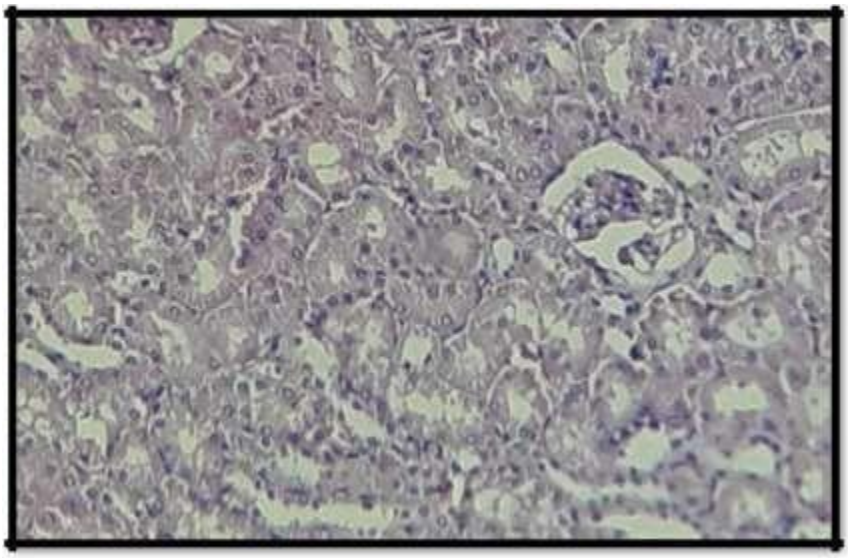

Image 08: Histological photomicrography of the kidney of a rat receiving Titanium dioxide nanoparticles (5 mg/kg/7 days) (X40).

Some atrophic glomeruli.

Chapter III :

Discussion

According to the database of pubchem site, we can discuss the properties of TiO_2-NPs and its effects on the animals.

Starting with the **ADMET** properties of TiO_2 /TiO_2-NPs and its impurities as shown in table (03). According to the pkCSM program, the Tio_2 molecule at a low rate is soluble in water, after matching the results, it can be said that the TiO_2 -NPs molecule can be distributed in water more than it is soluble.

Caco-2 permeability, intestinal absorption (human), skin permeability, and P-glycoprotein substrate or inhibitor were used to predict the absorption level of the Tio_2 , where the results of the experiment showed that for the pkCSM predictive model, high caco-2 permeability would translate in predicted values>0.90 , So we find TiO_2-NPs has a high permeability at the colon level , For the intestine, the molecule with less than 30% absorption is considered poorly absorbed, so we find that the TiO_2 -NPs molecule has a high absorption of 100% , The TiO_2 is expressed because the skin permeability is constant logKp, the compound is considered to have relatively low skin permeability if it has logKp > -2.5 , so we find that the TiO_2 –NPs molecule has a low absorption (Accumulation of TiO_2-NPs may give undesirable results) , the P-glycoprotein is an ATP-binding cassette transporter ;it functions as a biological barrier by extruding toxins and xenobiotics out of cells ,the model predicts whether the $Ttio_2$ is likely to be a substracte of Pgp or not , so we find that the TiO_2 -NPs molecule considered as substracte of Pgp.

Modulation of **Pgp** mediated transport has significant pharmacokinetic implication for Pgp substractes, which my either be exploited for specific therapeutic advantages or result in contraindication, the predictor will determine if TiO_2 is likely to be a P-glycoprotein I/II inhibitor, so we find that the TiO_2 molecule is not an inhibitor.

The **distribution volume (VDss), Fraction unbound (human), CNS permeability and blood–brain barrier membrane permeability (logBB)** were used to characterize the distribution of TiO_2 ,The distribution volume is a parameter to characterize the distribution of drugs in various tissues in vivo ,VDss is the theoretical volume that the total dose of a drug would need to be uniformly distributed to give the same concentration as in blood plasma ,is considered low if below (log VDss < -0.15) and high if above (log VDss >0.45) , so we find that the TiO_2 molecule is average distribution the brain is protected from exogenous compounds by the blood-brain barrier ,for TiO_2 , a logBB >0.3 considered to readily cross the

BBB ,while with logBB <-1 are poorly distributed to the BBB , so we find that the TiO_2 -NPs molecule is poorly distributed.

Measuring blood brain permeability can difficult with confounding factors, the blood brain permeability-surface area product (logPS), if TiO_2 with a logPS>-2 is considered to penetrate the central nervous system, if logPS <-3 is considered as unable to penetrate the CNS, so we find that the TiO_2 -NPs molecule is average distribution.

The cytochrome P450 are responsible for metabolism of many drugs, the two main isoforms responsible for drug metabolism are 2D6 and 3A4 , the predictor will assess whether the TiO_2 is likely to be metabolised by either P450 , The pkCSM program predicted that TiO_2 could not be metabolized at the liver level, however, the experiment showed that there is an effect of TiO_2 -NPs on the liver tissue, which means that TiO_2-NPs has been penetrated into the organ and reactions occur that lead to unwanted effects.

The PKCSM program also predicted the possibility of Tio_2 being a **cytochrome P450 inhibitor**. The predictions showed that it is not an inhibitor. However, the results of the experiment show that the liver was damaged after exposure to TiO_2 doses, which means that the efficiency of liver enzymes responsible for metabolism was affected in the presence of TiO_2 -NPs. It can be said tentatively that hepatotoxicity occurred. **The drug clearance** is measured by a proportionality constant, and it occurs mainly as a combination of hepatic synthesis and renal clearance. According to the pkCSM system, excretion is predicted by 0.864 ml/min/kg.

Organic cation transporter 2 is a renal uptake transporter that plays an important role in disposition and renal clearance of drug and endogenous compounds, the predictor will assess whether the TiO_2 is likely to be an OCT2 subtract, pkCSM predicted that Tio_2 Is not considered as OCT2 substrate, however, the results show the effect of Tio_2 -NPs on the efficiency of the filtering process.

The pkCSM results also indicate different levels of toxicity that can be expressed by: AMES toxicity, hERG I/II inhibitor, Hepatotoxicity, Skin Sensation. The **Ames** test, it is for the detection of genetic mutations of compounds using bacteria, it predicts whether TiO_2 gives a positive or negative Ames, the pkCSM appears to be a negative Ames test, however, the TiO_2-NPs compound is hypothesized to be a carcinogen, TiO_2 -NP-induced generation of ROS and alterations in cell signal transduction pathways which led to cancer at high doses (Shi et al, 2013).

Drug-induced liver injury is a major safety concern for drug development, it predicts whether the Tio_2 is likely to be associated with disrupted normal function of the liver, the pkCSM system gave a prediction that TiO_2 does not cause any liver damage, however, the results of the experiment showed the effect of the liver with Tio_2 – NPs doses.

Skin sensation is a potential adverse effect ,for dermally applied products ,it predicts whether the TiO_2 is likely to be associated with skin sensation , PkCSM program predicted that Tio_2 does not affect the skin, our study gives another possibility that once TiO_2 -NPs accumulates in the skin (continuous use) it can give unwanted effects, accumulation of TiO_2- NPs may take place in some organs like the lungs, alimentary tract, liver, heart, spleen, kidneys and cardiac muscle (Shi H et al, 2013).

Inhibition of the potassium channels encoded by hERG are the principal causes for the development of acquire long QT syndrome-leading to fatal ventricular arrhythmia, the predictor will determine if the TiO_2 is likely to be a hERG I/II inhibitor, the predictions of pkCSM were similar to the results of the experiment, so that TiO_2 -NPs is not yet considered an inhibitor of hERG I/II.

TiO_2-NPs damage the function of liver and lipid metabolism as showed in the **biochemical analysis**, (AST, ALT, ALP increased) which may be due to by the generation of ROS and disorders of structure of liver as proved in our *in vivo* study.

The result shows changes in biochemical parameters in rat liver serum after TiO_2 nano anatase dosing 14 days. At a dose of (2000 mg/kg body weight), there were a significant change for all parameters compared with the control group. However, ALT and ALP activities were significantly higher than the control group.

There is no obvious difference between the other parameters than the control group, indicating that TiO2 nano anatase in a single dose shows no observed effect on liver function comparing with rat treated with doe of (5mg/kg) which show apparent effect , This study is rather preliminary in terms of safety evaluation of TiO2. Even though no biochemical markers were altered as compared to control animals, TiO2 has not been cleared from liver and spleen within the observation period, indicating that after continuous exposure TiO2 would be accumulating in these organs even at low exposure levels (Fabian et al,2008).

The results showed differences in **the tissue section of the liver** of the rats treated with TiO_2-NPs compared to the rats treated with saline serum, so that the tissue section of the control rats had no changes in their **histological** structure, while the rats treated with a dose of (5 mg/kg) showed some histological changes like some congestion of sinusoids, while the renal sections presented an atrophic glomerulus.

For the results of functional parameter in kidney of rat after TiO_2 nano anatase dosing shows increase in the level of these parameter; increased creatinine level indicating its retention in the kidney as a result of renal insufficiency, as for the increase in the urea, it indicates a defect in the metabolism of purines, an indication of renal insufficiency.

As for the rats treated with a single dose of TiO_2 -NPs (2000 mg/kg) for a week, the tissue changes were more significant compared to the control rats, as it was observed microscopically that the liver tissue of the control rats did not undergo any structural changes, while the rats treated with TiO_2-NPs recorded some changes in liver tissue like dilatation and congestion of sinusoids. Our histological study results demonstrated this renal insufficiency by showing interstitial nephritis with tubular necrosis.

From the previous results, it can be said that TiO_2-NPs had an undesirable effect on liver and kidney functions, which indicates that it has caused oxidative stress at the level of mitochondrial enzymes and its enzymes leading to a defect in its efficiency. In addition, the repeated dose of 5mg/kg is less toxic than a single dose of 2000mg/kg and had a greater effecton hepatocytes and nephrocytes.

Conclusion

Conclusion

Nanotechnology known a large interest and progress offering a considerable adverses in many fields, titanium dioxide nanoparticles are one of most popular material used in wide variety ofproducts, medcine, indestrial, cosmetic product, food product, cleaning .

Titanium dioxide nanoparticles is most used nowadays for it's special and excellent physiochemical properties such as small size, large surface area shape, crystallinity, surface, resistance whitening and photocatalytic, and electrical properties take place in different uses cosmetics product, food, paints, medicine, pharmaceutical ,decomposing organic matters, etc.

Studies has been shown that the extensive use higher doses and concentration for long time of these metal can lead to dangerous consequences to both ecosystem and humans.

Refers to ADMET system of TiO2-NPs it has the ability to pass the cell membrane and accumulate in different organs liver, kidney, lung, heart induce oxidative stress responsible of pathological effects on reproductive, renal, liver, respiratory, endocrine, inflammatory, nervous systems, according to results it can be carcinogen.

In this paper ,we present *in vivo* investigations which we administered orally to rats (Wistar Albinos) with titanium dioxide nanoparticles for 7 days dose 5mg/kg subacute toxicity and for 14 days dose 2000 mg/kg acute toxicity, rats gain weight even though they suffer from anorexia (loss of appetite), and from biochemical analysis we obtained an increase of AST, ALT, ALP which indicate damage to liver, increase of urea acid and creatinine indicate a damage at kidney level, the histological shots shows necrosis at kidney level, and liver cell swelling.

Even though the results we obtained in our experiment we can say that there is no systematic damage caused by the acute and subacute toxicity of TiO2-NPs.

The toxic effect of the titanium dioxide nanoparticles still improving, future studies should focus in there research at the mode of action with the biological system in order to determine a direct solution to protect our health from the risk of these nanoparticles.

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Annexes

Annex 1

Products

Table I: Products used

TiO_2 powder	
Chloroform	
Distilled water	
salty serum	
Formol	
alcohol	
KCL	

Equipment

Table II: The devices used

Balance	
Centrifuge	
Vortex	
Magnetic agitator	
Rotary homogenizer	

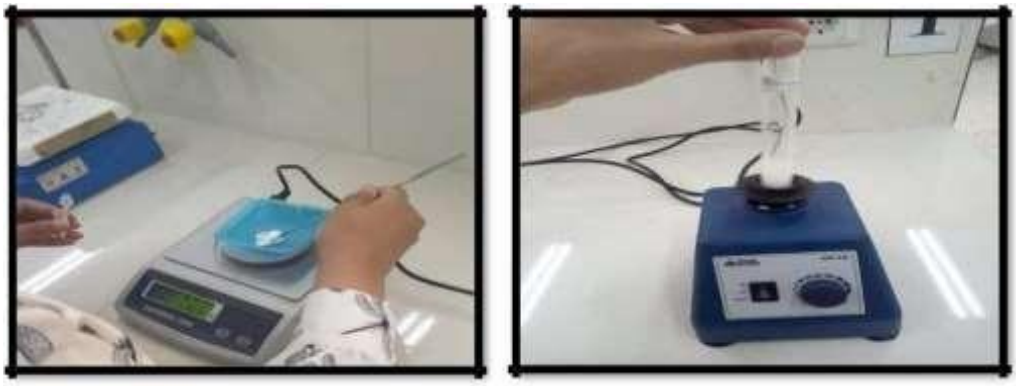
Table III: Other materials

Blood tubes (Dry tube)	
Medical syringes (5/10ml)	 
Dissection box	
Flacon	 
Tubes	 
laboratory rack	
laboratory spatula	
Aluminum /filter paper/ cotton	  
Petri dish	

Pipette/ cylinder/pastuer	  
Force-feeding probe	
Beaker	

Annex 2

Methods



The process of weighing tio2-NPs powder in proportion to each dose **(original photo)**

Force feeding technique **(original photo)**

Stages of the dissection process **(original photo)**

Blood centrifugation process (3000 rpm/15 min) **(original phot**

Biochemical samples preparation process **(original photo)**

Annex 3

Histopathological examinations

Material

- ✓ Dehydration machine
- ✓ Cassettes
- ✓ The scissors
- ✓ Knives
- ✓ The scalpel
- ✓ The clamps
- ✓ The tape measure
- ✓ Slide and coverslip
- ✓ Refrigerated plate
- ✓ Coverslip
- ✓ Slides
- ✓ Tray

Devices

- ✓ Microtome
- ✓ Optical microscope
- ✓ Thermoelectric group (TGE)

Products

- ✓ Alcohol
- ✓ Paraffin
- ✓ Xylene
- ✓ Formalin
- ✓ Blotting paper
- ✓ Hematoxylin eosin
- ✓ Optical grade glue

Abstract

Abstract

Titanium dioxide nanoparticles (TiO_2-NPs) have a varied range of applications in several fields. Over the past decade research, TiO_2-NPs have been focused on the potential toxic effects of these beneficial materials.

This study was designed to evaluate *in silico* and *in vivo* the susceptibility of adult female rats to oral toxicity induced by titanium dioxide nanoparticles (TiO_2-NPs). In the current study, *in silico* study was done using pkCSM program to predict the ADMET (Absorbance, Distribution, Metabolism, Excretion) properties of Tio_2 and compered them with that fond for the Tio2-NPS. While in our *in vivo* study, we investigated the effects of acute and subacute exposure rats to TiO_2-NPs on their sensitive behaviours, biochemical parameters, and histology of liver and kidney. In the acute toxicity test, animals were exposed to TiO_2-NPs (2000 mg/kg body weight) as a single oral dose and controlled 14 days after getting treatment. While in the sub-acute toxicity test, animals received by gavage a repeated dose of TiO_2-NPS during 7days (5 mg/kg b. w).

The results demonstrated by the pkCSM program showed that the TiO_2-NPs can be distributed in solvents but does not decompose, its accumulation leads to undesirable effects on the level of the liver and kidneys, as well as affects the metabolic enzymes. The fewer elevated AST/ALT enzyme activity, lipidic profile and renal toxicity indicators. However, histological examination showed some changes in liver architecture (lobular congestion and dilatation) and also in kidneys (necrotic and atrophic glomerulus) which were more confused in acute toxicity test more than that in the sub-acute toxicity. Moreover, our results provide strong evidence that the oral treatment of TiO_2-NPs increased the accumulation of titanium in the liver and kidneys. The results suggest that TiO_2-NPs could alter the neurobehavioral performance of adult Wistar rats and promotes alterations in hepatic and renal tissues.

In our study on TiO_2-NPs toxicological effects were associated to the repeated dose of 5 mg/kg b. w/ days. Compared to the control groups or to the animals treated by 2000 mg/kg b.w as a single dose but we improve that its LD50 is greater than 2000 mg/kg b. w. as for the TiO_2.

Key words: Titanium Dioxide Nanoparticles, Acute Toxicity, Sub-acute Toxicity, Biochemical parameters, Histological examination.

Résumé

Les nanoparticules de dioxyde de titane (TiO_2-NPs) ont une gammevarié d'applications dans plusieurs domaines. Au cours de la dernière décennie, les recherches sur les TiO_2-NP se sont concentrées sur les effets toxiques potentiels de ces matériaux bénéfiques.

Cette étude a été conçue pour évaluer *in silico* et *in vivo* la sensibilité de rats femelles adultes à la toxicité orale induite par les nanoparticules de dioxyde de titane (TiO_2-NPs). Dans la présente étude, une étude in silico a été réalisée à l'aide du programme pkCSM pour prédire les propriétés ADMET (Absorbance, Distribution, Métabolisme, Excrétion) de TiO_2 et les comparer avec celles du Tio_2-NPS. Dans notre étude *in vivo*, nous avons étudié les effets d'une exposition aiguë et subaiguë de rats au TiO_2-NPs sur leurs comportements sensibles, leurs paramètres biochimiques et l'histologie du foie et des reins. Dans le test de toxicité aiguë, les animaux ont été exposés au TiO_2-NPs (2000 mg/kg de poids corporel) en une dose orale unique et contrôlée 14 jours après avoir reçu le traitement. Pendant le test de toxicité subaiguë, les animaux ont reçu par gavage une dose répétée de TiO_2-NPS pendant 7 jours (5 mg/kg p.c.).

Les résultats démontrés par le programme pkCSM ont montré que le TiO_2-NPs peut être distribué dans les solvants mais ne se décompose pas ,son accumulation entraîne des effets indésirables au niveau du foie et des reins, ainsi qu'affecte les enzymes métaboliques. L'activité enzymatique AST/ALT, le profil lipidique et les indicateurs de toxicité rénale les plus faibles. Cependant, l'examen histologique a montré quelques modifications de l'architecture hépatique (congestion et dilatation lobulaires) ainsi que des reins (glomérule nécrotique et atrophique) qui étaient plus confus dans le test de toxicité aiguë que dans la toxicité subaiguë. De plus, nos résultats fournissent des preuves solides que le traitement oral de TiO_2-NPs a augmenté l'accumulation de titane dans le foie et les reins. Les résultats suggèrent que les NP de TiO_2 pourraient altérerles performances neurocomportementales des rats Wistar adultes et favoriser des altérations des tissus hépatiques et rénaux.

Dans notre étude sur les TiO_2-NPs, effet toxicologique systémique important a été associé à la dose répétée de 5 mg/kg p.c /jours. Comparé aux groupes témoins ou aux animaux traités par 2000 mg/kg p.c. en dose unique mais nous améliorons que sa DL50 est supérieure à 2000 mg/kg p.c comme pour le TiO_2.

Mots clés : Nanoparticules de dioxyde de titane, Toxicité aiguë, Toxicité sub aiguë, Paramètres biochimiques, Examen histologique.

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الملخص

الجسيمات النانوية لثاني أكسيد التيتانيوم (TiO2-NPs) لها مجموعة متنوعة من التطبيقات في العديد من المجالات. على مدار أبحاث العقد الماضي ، ركزت TiO2-NPs على التأثيرات السامة المحتملة لهذه المواد المفيدة.

تم تصميم هذه الدراسة لتقييم قابلية إناث الفئران البالغة للتسمم الفموي الناجم عن جزيئات ثاني أكسيد التيتانيوم النانوية-TiO2) (NPsفي السيليكو وفي الجسم الحي. في الدراسة الحالية ، أجريت دراسة السيليكو باستخدام برنامج pkCSM للتنبؤ بخصائص ADMET(الامتصاص والتوزيع والتمثيل الغذائي والإفراز) لـ Tio2 وتم تصنيفها مع هذا المولع بـ .Tio2-NPS أثناء دراستنا في الجسم الحي ، قمنا بالتحقيق في آثار تعرض الفئران الحادة وتحت الحاد لـ TiO2-NPs على سلوكياتهم الحساسة ، والمعايير الكيميائية الحيوية ، وأنسجة الكبد والكلى. في اختبار السمية الحادة ، تعرضت الحيوانات لـ TiO2-NPs (2000 مجم / كجم من وزن الجسم) كجرعة فموية واحدة وتم التحكم فيها بعد 14 يومًا من تلقي العلاج. بينما في اختبار السمية تحت الحادة ، تلقت الحيوانات بالتزقيم جرعة متكررة من TiO2-NPS خلال 7 أيام (5 مجم / كجم من وزن الجسم).

أظهرت النتائج التي أظهرها برنامج pkCSM أن TiO2-NPs يمكنها التوع في المذيبات لكن لا يتم تحللها بشكل كلي ، يؤدي تراكمها إلى تأثيرات غير مرغوب فيها على مستوى الكبد والكلى ، وكذلك يؤثر على الإنزيمات الأيضية. انخفاض نشاط إنزيم / AST ALTالمرتفع والمظهر الدهني ومؤشرات السمية الكلوية. ومع ذلك ، أظهر الفحص النسيجي بعض التغييرات في بنية الكبد (احتقان الفصيصات والتوسع) وكذلك في الكلى (الكبيبة النخرية والضامرة) والتي كانت أكثر تشويشًا في اختبار السمية الحادة أكثر من السمية تحت الحاد. علاوة على ذلك ، تقدم نتائجنا دليلًا قويًا على أن العلاج الفموي لـ TiO2-NPs زاد من تراكم التيتانيوم في الكبد والكلى. تشير النتائج إلى أن TiO2 NPs يمكن أن يغير الأداء السلوكي العصبي لفئران ويستار البالغة ويعزز التغيرات في الأنسجة الكبدية والكلوية.

في دراستنا على TiO2-NPs وجدنا تأثيرات سمية نظامية كبيرة بالجرعة المتكررة البالغة 5 مجم / كجم ب. ث / أيام. يتم مقارنتها مع مجموعات الشاهدة أو الحيوانات المعالجة بمقدار 2000 مجم / كجم من وزن الجسم كجرعة وحيدة ولكننا نؤكد أن الجرعة المميتة LD50 أكبر من (2000 مجم / كجم من وزن الجسم). بالنسبة لـ.TiO2

الكلمات المفتاحية: الجسيمات النانوية لثاني أكسيد التيتانيوم ، السمية الحادة ، السمية شبه الحادة ، المعايير البيوكيميائية ، الفحص النسيجي

Presented by : Khenafra Kaouther , Redjouah Fatima Zahra , Gasmi Amel

In silico and *in vivo* study of the Susceptibility of adult rats to the oral toxicity of titanium dioxide nanoparticles

Abstract

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Evaluation jury :

Jury president:	**Lalaoui .K**	Prof -University Freres Mentouri.
Reporter:	**Bekhouche. K**	Dr – Pharmaceutical Science Research Center
Examiner:	**Boubekri .N**	Dr –University Freres Mentouri .
Examiner:	**Mouri .F**	Dr -University Freres Mentouri.

Defense date : 12/09/2021