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Field: Biological Sciences

Option: Toxicology.

Theme:

Nanoparticles of TiO_2-NPs as stressors of cardiovascular

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بسم الله الرحمن الرحيم

ملخص

ثاني أكسيد التيتانيوم(TiO_2) هو عنصر موجود على نطاق واسع في الطبيعة وذلك لتميزه بالوزن الخفيف والمتانة ومقاومة التآكل، ويستخدم من قبل الإنسان كمضاف غذائي أو محفز أو صبغ.

الهدف من هذه الدراسة هو تحديد سمية جزيئات ثاني أكسيد التيتانيوم(TiO_2) النانوية والإجهاد التأكسدي على نظام القلب لفئران ويستار التي هي مناسبة لدراسة علم السموم لتقييم سمية المواد الغريبة بسهولة.

أجريت الدراسة لمدة 90 يوم على 21 جرذا مقسما إلى 3 مجموعات(7فئران في كل منها)(1) مجموعة تحكم،(2)عولجت بثاني أكسيد التيتانيوم(2.5ml/kg),(3)مجموعة عولجت بثاني أكسيد تيتانيوم (1,25ml/kg).

تظهر نتائجنا أن وجود الجسيمات الثانوية ثاني أكسيد التيتانيوم (TiO_2) يسبب اضطرابا في النمو وأظهرت الدراسات البيوكيميائية اضطراب في المكونات الرئيسية:نقص في البروتينات،والكربوهيدات، وزيادة في الدهون.

كذلك تثير المعايير البيولوجية إلى انخفاض في كمية**MDA** و**GSH**وأيضا نشاط **GPx**عند الجرذان المعالجة بثاني أكسيد التيتانيوم بالنسبة للجرذان الشاهدة.

الكلمات المفتاحية: ثاني أكسيد التيتانيوم، سمية، جسيمات نانوية،MDA, GSH, GPx

Abstract

Titanium dioxide (TiO_2) is an element widely found in nature due to its lightness, durability and resistance to corrosion. It is used by man as a food additive, catalyst or colorant.

The aim of this study was to determine the toxicity of titanium dioxide nanoparticles (TiO_2) and oxidative stress on the cardiovascular system of Wistar rats, which are suitable for toxicological studies to easily assess the toxicity of foreign substances.

The study was conducted over 90 days on 21 rats divided into 3 groups (7 rats each) (1) a control group, (2) treated with titanium dioxide (ml/kg2.5), (3) a group treated with titanium dioxide (ml/kg).kg1.25).

Our results show that the presence of titanium dioxide nanoparticles (Tio2) causes a disturbance in growth, and biochemical studies showed a disturbance in the main components: a deficiency in proteins, carbohydrates, and an increase in fats.

Biological parameters also led to a decrease in MDA and GSH levels, as well as GPx activity, in titanium dioxide-treated rats compared to control rats.

Keywords: titanium dioxide, toxicity, nanoparticles, MDA, GSH, GPx

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Above all **ELHAMDOLILLAH** the Almighty who guides us throughout our lives, who gives us incredible strength, courage and patience to get through all the difficult moments and has enabled this work to see the light of day despite all the obstacles I once thought insurmountable...

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We warmly thank our colleagues and my friends.

Finally, we thank the members of our families for their unfailing support throughout these enriching years.

AYA; ALIMA; SAFOUANE

Dedication

I dedicate this work to.....

To my dearest mother ***BOURAS ZAHIRA***

You are for me the symbol of goodness par excellence, the source of tenderness and the example of devotion who never ceased to encourage me and pray for me.

Your prayers and blessings have been a great help to me in my studies.

No dedication could be eloquent enough to express what you deserve for all the sacrifices you never stopped giving me since my birth, during my childhood and even into adulthood.

You've kept me on the right path in life and continued my studies, may God keep you for me.

To my dear father ***MOUHAMED TAHER***

The man in my life, the one who always sacrificed to see me succeed, may God keep you for me.

To my dear sisters ***NOUR ELHOUDA*** *and* ***ALIMA***

To my dear brother ***ABD ERRAHIM***

To my husband, ***ANIS KECHROUD,*** *may God keep him for me.*

To dear friends KHAWLA, DOUAA, SAFOUANE and SAIF

I also dedicate this work to my family and everyone I know, wishing them all happiness and good health.

AYA

Dedication

After my sincere thanks first to God, who always surrounds us with his care and success, and who has given us the courage and will to complete this work,

I dedicate this work to my father, ***HALFAYA BELGASEM****, may God have mercy on him, and may he rest in peace. I am indebted to him for the support, trust and love that surrounded me with him.*

*To my mother "****KHOUALDIA DALILA****", to the sun that never sets, to my support and my only destination, may God keep you for me.*

To my brothers and sisters ***SOUHA, ABIR, AYA, TAREK, BILAL, AMIR.***

To my dear friends ***KHAWLA, DOUAA, ZAINAB, SAFOUANE, SAIF.***

And I don't forget to dedicate this work to my refuge after my family, ***DJEBBARI SOUFIANE****, may God bless you for me.*

I humbly dedicate this work to all those who have room in my heart for everyone I knew and who inspired me to be patient and strong.

ALIMA

Dedication

The locomotive of my research has gone through many obstacles and difficulties, and despite this, I have tried to overcome all these obstacles and difficulties with great steadfastness, thanks to God Almighty, and thanks to you also, my dear father.

To the one above it, and on it I rely, to the giving heart, my dear and dear mother, and to those on whom I depend in every big and small...

(My dear brothers, Marwan, Haifa, Ziyad, Wail)

And all my friends, especially ***(Saif BLOCK, Mouatez MKT, Zidane LAMIZ, Ninos***

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Who have always been for me as support and support so that I can complete the research.

I cannot forget my honorable teachers who had a great credit and the first role in supporting me and clarifying many important and valuable information for me.

Today, I dedicate my graduation research to you, and I hope that God will prolong your life and bless you always with good things.

SAFOUANE

List of tables

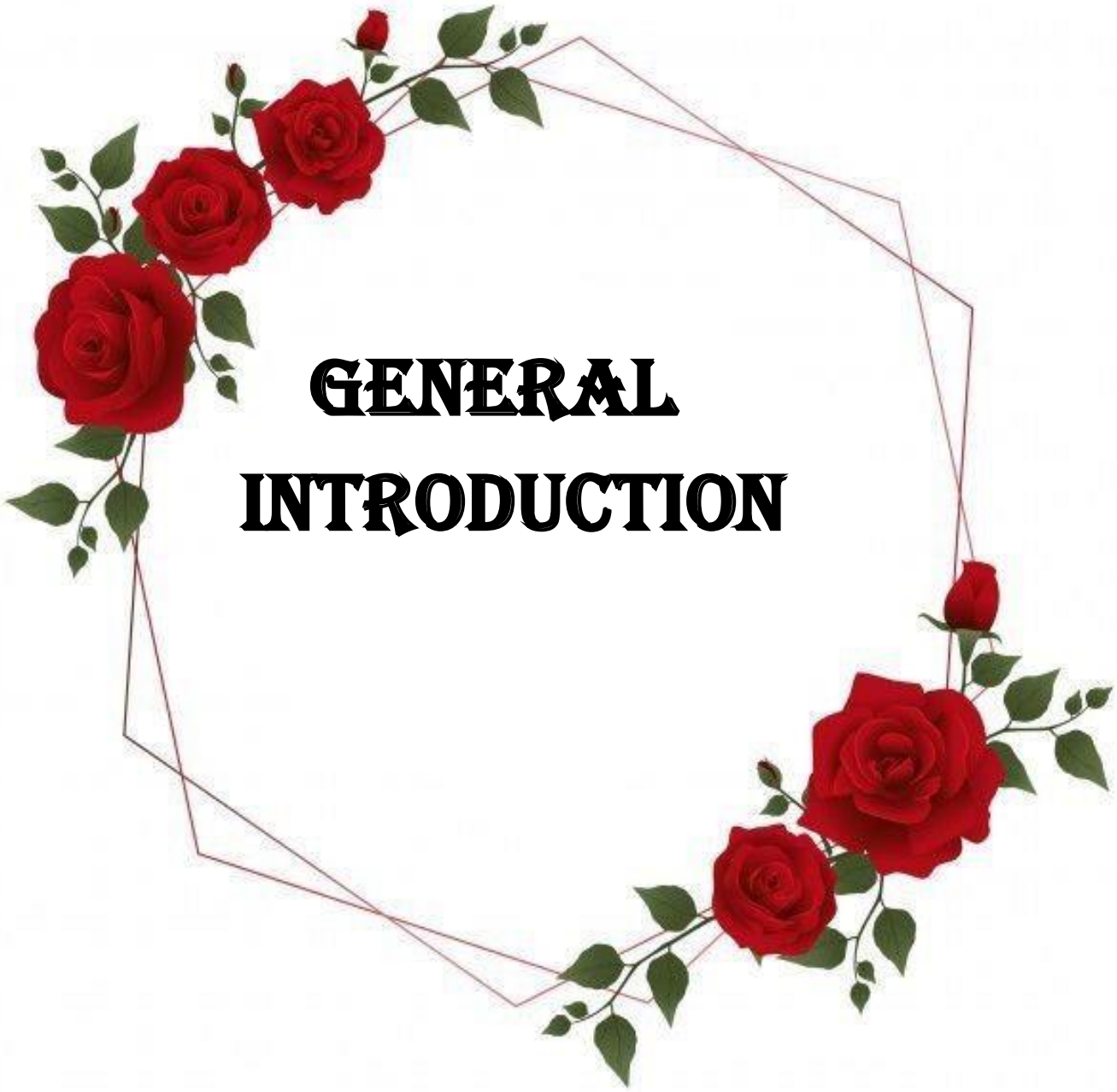
List of Figures

List of abbreviations

Abbreviation	Designation
ANOVA	Analyses of Variance
ASAT	Aspartate aminotransferase
BBC	Bright blue Coomassie
CaTiO3	calcium titanate
CaTiSiO5	even titanite
CDNB	1-chloro, 2.4-dinitrobenzene
DNA	deoxyribonucleic acid
DTNB	5-mercapto-2-nitrobenzoic acid
EDTA	Ethylene-Diamine-Tetraacetic Acid
ERO	Reactive oxygen species
FeTiO3	
GPx	Glutamine peroxidase
GST	Activity of glutathione -S- transferase
GSSG	Oxygenated glutathion
HDL	Low-densitylipoprotéine
H2O2	Hydrogen peroxyde
LDL	Low-densitylipoprotéine.
MDA	Malone-dialdehyde.
MDH	Malate by malate dehydrogenase
SWCNT	single wall carbon nanotube
NacL	sodium chlorite.
NADH	Reduced by nicotinamide-adenine dinucleotide-phosphate
NPM	Producing materials at the Nano-scale
NPs	Nanoparticles.
Mg	Milligramme
PRC	Relative heart weight
Zn	Zinc
Pb	Lead
Hg	Mercury

Cd	Cadmium
POPs	organic pollutants
µmol	Micromoles
TBA	Thiobarbituric acid
TBS	Three-buffered salice.
TCA	Trichloracetic acid
G	Gramme.
TIO2	Titanium dioxide
UFP	ultrafine atmospheric particle
H	Hours
EOA	Active oxygen species
DO	optical density
AVC	cerebrovascular accident

TABLE OF CONTENTS



GENERAL INTRODUCTION

General introduction

Since a number of years ago, there has been a growing interest in nanotechnology research, which focuses on the so-called nanoparticles (NPs), which are defined as ultrafine particles with at least one dimension that is between one and one hundred nanometers **(Baretli, 2015)**.

Their focus is on the intrinsic properties of nanoparticles, particularly when Low dimension gives them physic-chemical properties due to their small size Due to their small size, nanoparticles can be found inside of cell's vacuoles. Likewise, have the ability to hit cellular targets and produce unpleasant effects **(Bettini, 2014).**

We'll be paying closer attention to the titanium dioxide nanoparticles, which are found in the earth's crust in three different crystal forms (anatase, rutile, and brookite). They're primarily used as white food additives and food colorings **(Bettini, 2014).**

Due to their unique properties, titanium dioxide nanoparticles have significant technological promise. They can be produced using a variety of procedures (solvothermal, sol-gel, hydrothermal, etc.). One of the most widely used processes today is sol-gel. Simpler and more effective in terms of quality nanoparticle production methodsof powder, a heterogeneous reaction is set off by the hydrothermal process, requiring the Presence of water-soluble solvents operating at high pressures and temperatures to facilitate dissolution and the re-crystallization of materials that are essentially insoluble in normal conditions **(Baratli, 2015).**

The topic of nanotechnology is a hot topic in today's scientific news, and the case of titan dioxide is particularly fascinating and compelling because its properties and risks are currently being tested. Further questions about Ecological and sustainable development are becoming more and more popular, and titanium dioxide has become an initially appear to be private property **(Site 1).**

Since the year 2000, research on the toxicity of TiO_2 nanoparticles has been advancing rapidly. The number of papers published on the topic each year is increasing, surpassing 550 references in 2014 for a total of 2 975 articles since 1996.The main mechanisms of toxicity associated with inflammation and oxidative stress Additionally, to geno-toxicity **(Boland, 2014).**

This research aims to comprehend and assess the potential toxicity of titan dioxide (TiO_2) nanoparticles on the cardiovascular system while using instruments perfectly suited to toxicological studies to quickly assess the toxicity of xenobiotic.

PART

BIBLIOGRAPHIC

Chapter 01: Nanoparticles of TiO_2

I. General information on heavy metals

1. Definition

Heavy metals are natural metallic elements with densities greater than 5 g/cm3.

Mercury, lead, cadmium, copper, arsenic, nickel, zinc, cobalt, manganese, and other heavy metals are frequently found in trace amounts in the environment **(Arriss, 2008).** Metals are the elements of the periodic table that form cations in solution from a chemical standpoint.

The term "heavy metals" refers to natural metallic elements, metals, or in some cases metalloids (about 65 elements) with a high density greater than 5 g.cm3 **(Adriano, 2001).** From a biological standpoint, we divide metals into two types based on their physiological and toxic effects: essential metals and toxic metals **(Adriano, 2001; Gasmi et *al*., 2016).**

2. Physicochemical property

Adsorption at the solute/solid interface is a physical or chemical phenomenon in which molecules in liquid or gaseous effluent attach to the surface of a solid **(Adriano, 2001).** The adsorption separation process is one of the most important technologies today; it is widely used for depollution and purification in a wide range of fields, including the petroleum, petrochemical, and chemical industries, as well as environmental and pharmaceutical applications. Adsorption is classified into two types:

a. Chemical adsorption (or chemisorption)

One or more covalent or ionic chemical bonds are formed between the adsorbed and the adsorbent **(Adriano, 2001).** Only molecules directly linked to the solid are affected by this type of adsorption, which is generally irreversible and results in a modification of the adsorbed molecules **(Adriano, 2001).**

The majority of chemical phenomena are acid-base reactions (proton transfer), oxidation-reduction reactions (electron transfer), or complication **(Adriano, 2001)**.

b. Physical adsorption

- Physical adsorption takes place at low temperatures.
- Molecules adsorb on one or more layers (multilayers) with adsorption heats that are frequently less than 20 kcal/mol.
- Electrostatic forces ensure interactions between molecules of the solute (adsorbed) and on the surface of the solid (adsorbent).
- Physisorption is a fast, reversible process that does not alter the adsorbedmolecules**(Adriano, 2001).**

3. Classification of heavy metals

3.1. Essential metals

These are trace elements that are required for many cellular processes but are found in very low concentrations in biological tissues. When the concentration exceeds a certain threshold, some can become toxic.

This is true for copper (Cu), zinc (Zn), and iron (Fe).Zinc (Zn), for example, is a trace element that is involved in many enzymatic reactions (dehydrogenases, proteinase, and peptidase) and plays an important role in the metabolism of proteins, carbohydrates, and lipids at a concentration of one mill-molar **(Kabata, 2001)**.

3.2. Toxic metals

Have a polluting nature and are toxic to living organisms even at low concentrations. They have no known cell-beneficial effect. This is true for lead (Pb), mercury (Hg), and cadmium (Cd) **(Behanzin, 2014).**

4. Origin of heavy metals

4.1. Natural Springs

Heavy metals occur naturally in rocks and are released during alteration to form the geochemical background **(Bourrelier and Berthelin, 1998)**.Volcanic activity, continental weathering, and forest fires are all important natural sources.Volcanoes can contribute in the form of voluminous emissions caused by explosive activity, or continuous low-volume emissions caused by geothermal activity and magma degassing **(Afnor, 1988)**.

4.2. Anthropogenic sources

Metals derived from anthropogenic inputs are present in highly reactive chemical forms, posing far greater risks than metals derived from natural sources, which are typically immobilized in relatively inert forms **(Weiss, 1999)**.

4.3 Heavy Metal Toxicity

By serving as a trap, sediments lower the concentration of contaminants in surface water **(Wang, 1987)**, making them important witnesses to both recent and historic surface water contamination. A prime location for the buildup of many contaminants is sediment (non-polar organic pollutants, metals, radionuclides, organic matter, etc.). Few of these contaminants are in dissolved form, but metals and non-polar (hydrophobic) organic pollutants have a high tendency to bind to sedimenting particles **(Wang, 1987)**. Metals are not decomposed, and many organic pollutants (POPs) are very poorly degradable, thus they linger in the ecosystem **(Wang, 1987)**.

II. Nanoparticles

1. Definition

Nanotechnology combines all the knowledge necessary to monitor, control, create, alter, and shape matter down to dimensions close to a Nano-scale, they offer new tools that are very helpful for comprehending biological processes and creating novel therapeutic and diagnostic techniques through their biomedical applications for research in vitro and in vivo on animals **(Florin, 2008).** A particle that has a diameter less than 100 nm in at least one spatial direction is referred to as a nanoparticle. Because of this, nanoparticles have very large exchange surfaces (specific surface), several hundred square meters per gram of particle.If nanoparticles come into touch with living things, their unique physical and chemical characteristics, which are yet mostly unknown, may cause their own biological impacts. This is due to the nanoparticles' relatively broad exchange surface.

When compared to larger particles of the same mass, nanoparticles' surface reactivity, which rises as particle size falls, predicts that they will exhibit more biological activity.
This is advantageous and may be applied in treatment methods (drug carriers for their ability to cross biological barriers).Toxic effects linked to their capacity to cause oxidative stress and distribute in the body, on the other hand, can result from this **(Lanone, 2010)**.

2. Nanobiology and nanotoxicology

2.1. Nanobiology

One of the research subfields of Nano-science, called Nano-biology, brings together chemists, biologists, medical professionals, and pharmacists. Due to the impossibility of providing a thorough overview of the various nanoparticle (NP) kinds and uses, only the most important NPs utilized in in vitro medicine, in vivo and in vitro cell imaging, and their current state of clinical development will be covered. Because they may be created and sculpted to act as carriers of (therapeutic) chemicals, these NPs are extremely versatile. Diagnostic imaging probes, simultaneous use of both (theranostic), or both **(Chuto, 2010)**.

2.2. Nano-toxicology

The criteria of traditional toxicology do not appear to apply to a new profession that seeks to carefully examine the risks associated with nanotechnologies.
The evaluation of NPs' toxicity begins with the physico-chemical characterisation of such particles.It is true that changes in size, speciation, and phase can alter their pharmacokinetics and affect their toxicity **(Greco, 2015)**.

3. Areas of use

They have magnetic properties, mechanical resistance, chemical reactivity, and thermal conductivity due to their Nano-metric size, high surface-to-volume ratio, chemical complexity, nature, and expansion of the mineralogical faces. These characteristics enable a wide range of applications in a wide range of fields. Nanotechnology is used in the following industries: textiles, cosmetics, food, medical imaging, drug delivery, environment, electronics, chemistry, and construction **(Greco, 2015)**.

4. Mechanism of action of nanoparticles

NPs often enter cells through the well-known endocytosis process, where they eventually end up in vesicles with a membrane around them in the cell compartment. As a result, the proteins and other biological substances that the NPs have adsorbed and are conveying allow them to enter the cell "masked."

This entrance sets off a chain of chemical events that can lead to cell death and toxicity or to adaptive responses such pro-inflammatory reactions, antioxidant enzyme activation, repair procedures, and changes to cell cycle regulation and proliferation. These occurrences are either directly or indirectly linked to the internalization of NPs, their cellular persistence, their capacity to release free radicals, and their ability to cause oxidative stress.

The formation of free radicals, whether directly or indirectly, in response to the size, chemical makeup, and surface reactivity of many produced NPs is, in fact, a common reaction **(Nel, 2009).** Oxidative stress results when it is not managed by the cell's defense mechanisms, enzymes, or tiny antioxidant molecules. A stepwise reaction would start at the level of free radical generation. When the production of free radicals increases, antioxidant protection is triggered to a modest extent, which then triggers an inflammatory response. Extremely severe cell damage may result in necrosis or apoptosis **(marano, 2011)**.

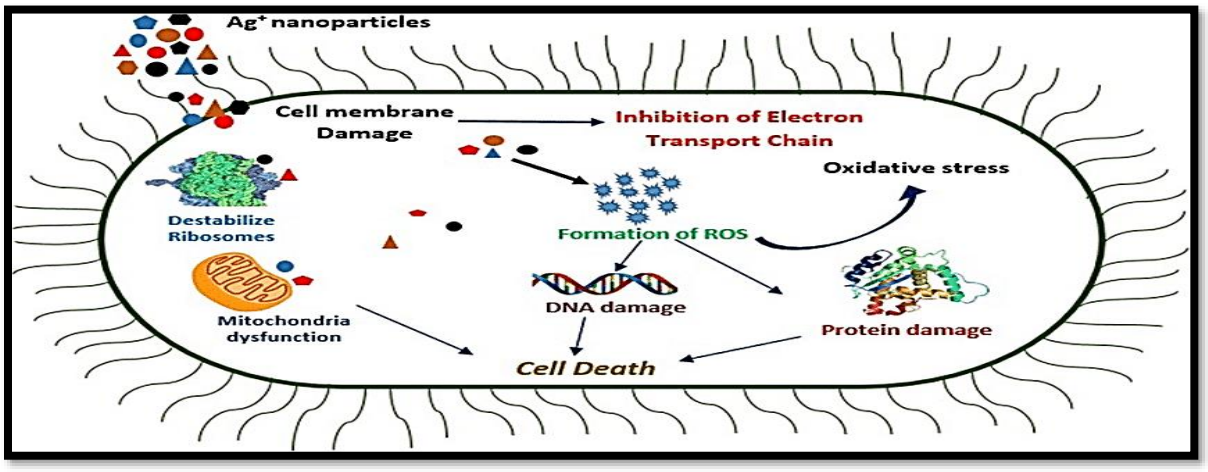


Figure01: mechanisms of action of manufactured nanoparticles (NPs) **(Marano, 2011).**

In particular, metallic NPs can effect on the transcription of multiple genes by activating signaling pathways and transcription factors, producing extracellular and/or intracellular free radicals, and entering the cell by endocytosis. Antioxidant enzymes and pro-inflammatory proteins may be produced as a result. Damage to DNA can result from oxidative stress.

5. The diffèrent types of nanoparticles

5.1.Natural nanoparticles

The environment contains a significant amount of naturally occurring nanoparticles (dust emitted by combustion or by volcanoes, produced by erosion). Imogolite and allophane, two naturally occurring nanoparticles with spherical (allophanes) or tubes-like (imogolites) shapes but uncertain structures, are among them (allophanes). As a result of the weathering of glasses and volcanic ash, imogolites and allophanes are short-range structured aluminosilicate **(Lanone, 2010).**

5.2. Man-made nanoparticles

This group includes "ultrafine" atmospheric particles (UFP). Analysis of the makeup of air particles and their biological impacts has made human exposure to UFPs into a significant public health issue in recent years. UFPs from the burning of fossil fuels can contain more than 80% fine (less than 1 mm) and ultrafine soot in metropolitan areas.

These particles, which are released mostly by diesel and gasoline automobiles as well as district heating systems, were rapidly suspected to be the root cause of the cardiorespiratory morbidity and death seen in epidemiological research on the immediate impacts of pollution atmospheric **(Rachid et *al*., 2008; Lanone, 2010).**

They would mostly trigger inflammatory reactions that could exacerbate lung diseases like asthma. Atherosclerosis and cardiovascular diseases might be brought on by them, and they would also have a prothrombotic effec **(Lanone, 2010)** .

5.3.Intentionally man-made nanoparticles (or engineered nanoparticles)

The creation of engineered nanomaterial's, including engineered nanoparticles, has expanded as a result of the recent growth of nanotechnology, which unifies all the approaches targeted at designing, describing, and producing materials at the Nano-scale (NPM). Due to the novel characteristics of the materials created in this manner, nanotechnologies have seen particularly substantial growth in recent years on a global basis, where we see increasing investment from more and more countries **(Lanone, 2010).**

PUFs and NPMs differ from one another in that the former typically have a wide size distribution and a complicated chemical makeup while the latter frequently have a narrow

distribution and a defined chemical makeup. In a very simplified form, there are carbon NPMs and metallic NPMs (such as titanium and zinc dioxide).Carbon nanotubes are significant among these. Carbon nanotubes can be divided into two primary categories: single wall carbon nanotubes (SWCNT), which are constructed from a single graphene sheet, and multi-wall carbon nanotubes (MWCNT), which are constructed from many sheets that are stacked within one another like Russian dolls. **(Lanone, 2010)**

The potential application of carbon nanotubes in medicine is intriguing in addition to their industrial applications. They could be utilized, for instance, to distribute and transport medications inside of cells. Carbon nanotubes are extremely attractive, but there are concerns about their potential toxicity, long-term side effects, and biodegradability. These issues make up a well-developed subject of study **(Lanone, 2010)**.

III. Titanium dioxide

1. General information on titanium dioxide

The chemical formula for titanium dioxide, often known as titanium **(IV)** oxide, is TiO_2. **(Site 2)**.It can be found naturally as the mineral species rutile, brookite, and anatase in three different crystalline forms **(Herve, 2007).** In addition to being used as a food additive, titanium dioxide is also utilized as a white pigment in paintings, plastics, and paper. **(Bezrodna, 2000)** It demonstrates particular characteristics peculiar to the nanometric scale. It is marketed either in its original form or, more frequently, in modified forms after the particle's surface has been treated. The physic-chemical properties and biological impacts of titanium dioxide are anticipated to change as a result of these treatments **(Site 03)**.

Figure 02: TIO2 Powder **(Site 03)**.

2. Origin of titanium dioxide

The ninth most abundant element in the earth's crust after hydrogen and magnesium is titanium.The majority of the titanium there is found in oxidized form in mixed mineral deposits such as ilmenite ($FeTiO_3$), perovskite ($CaTiO_3$), or even titanite ($CaTiSiO_5$) **(Sugimoto, 2003)**.

The following types of pure TiO_2 are also found in nature. By employing chlorides in an extraction procedure, titanium can be produced. It is possible to produce titanium tetrachloride (TiCl4) using this method, which was improved by the French chemist Henri Sainte-Claire Deville, which maythen be reduced using the "Kroll procedure" to produce pure solid titanium **(Sugimoto, 2003)**.

By treating various ores with sulfuric acid, titanyl-sulphate can also be produced, which can be used to extract TiO_2 ($TiOSO_4$). The result of this compound's hydrolysis and dehydration is TiO_2. **(Combres, 1997).**

3. Structure of TiO_2

Table 01. The 3 crystalline forms of TiO_2

The Anatase phase	The Brookite phase	The rutile phase
This type of natural titanium oxide is less prevalent.“Hauy” made the discovery of anatase in 1801. Its colors range widely, from nearly colorless to brown and greenish. The Anatase unit cell also has tetragonal symmetry **(Goudjil, 2013)**. Anatase is a metastable form with more or less slow kinetics that tends to grow towards the more compact structure of rutile. Each titanium atom is at the heart of an octahedron, similar to rutile. Unlike the temperatures required for the creation of rutile and Brookite, this structure is stable at lower temperatures**(Kertesz, 1991)**	Because Brookite is a metastable phase, it is difficult to prepare pure in the laboratory, but it can be found as a secondary phase with anatase and rutile. Levy discovered it in “Snowen” (England) in 1825. Brookite is a member of the orthorhombic crystal system (**Goudjil, 2013).** Brookite, another metastable form intermediate between anatase and rutile, has received little attention due to its narrow domain of stability **(Kertesz, 1991).**	Rutile is a mineral that is 90% to 95% titanium dioxide. “Wener” discovered it in Spain in 1803. At high temperatures, red rutile is the most stable type of titanium oxide. It can be found in both magmatic and metamorphic rocks **(Goudjil, 2013).** This phase's unit cell has tetragonal symmetry. Six oxygen atoms surround each titanium atom, forming an octahedron. These octahedral are arranged in parallel chains to the lattice axis. Rutile is the most stable structure of titanium dioxide at high temperatures **(Kertesz, 1991).**

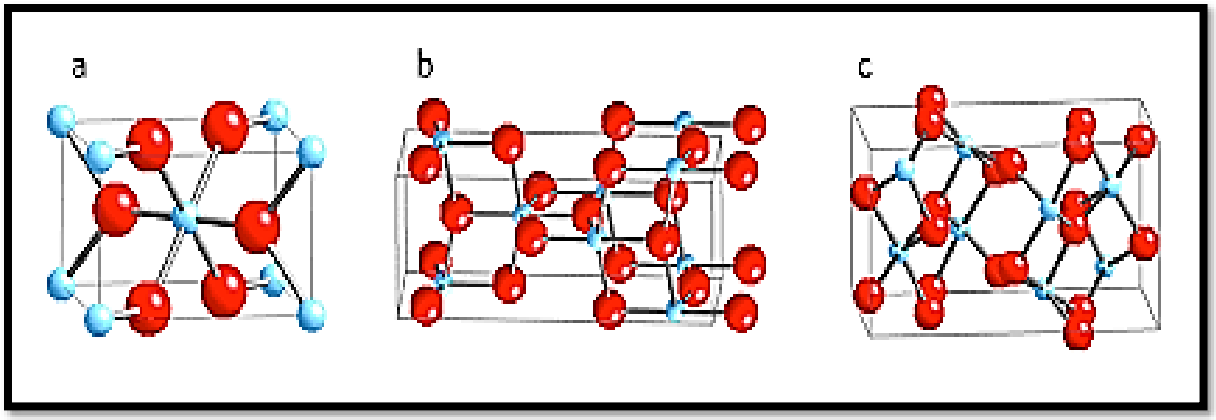


Figure03: Structure of TiO2 **(Kertesz, 1991).**

4. Physical properties

Titanium dioxide is a white solid that is refractile and heat stable. It is sold as a powder or as a liquid dispersion. It has no odor, is insoluble in water, ethanol, and other organic solvents, and absorbs ultraviolet rays to varying degrees depending on particle size. The rutile form is denser and has a higher thermodynamic stability than the anatase form **(Rouabhi et *al.*, 2008; Jargot, 2013)**.

5. Chemical properties

Titanium dioxide that is not "ultrafine" is a very inactive material. It is not attacked by hydrochloric or nitric acids, but by hot concentrated sulfuric acid and hydrofluoric acid, which it forms flu-titanic acid with. Concentrated bases can also attack it. Titanium dioxide can be reduced by lithium, magnesium, and zinc **(Jargot, 2013).**

Titanium dioxide nanoparticles have better photo-catalytic properties than larger particles because of their higher specific surface area: they are more likely to generate reactive oxygen species (hydroxyl radicals, singlet oxygen, and superoxide radicals) after exposure to ultraviolet rays and reactions with water or oxygen **(Jargot, 2013).**

6. Use in different areas

Table 02. Summary of TiO_2 applications **(Guitou, 2014).**

Domain	Application	Property
Energy	Solar cells Hydrogen production	Solar Energy Conversion
Medical	Tiles, walls of operating rooms	Antibacterial; Sterilization ; Self-cleaning
Environment	- Wallpaper, paint, coatings, curtains, lampshades -Window, Cement, windows, tiles. -Tunnel walls, lamps tunnels, soundproof	Self-cleaning; Antibacterial Purification o fair Water purification

	walls, -Air purifier, -water disinfection used	

7. Titanium Dioxide Toxicity

TiO_2 nanoparticles form agglomerates after repeated exposures, which can be internalized in cells via yet unknown mechanisms and accumulate in the cytoplasmic territory (vacuoles), making them potentially toxic over longer exposure times. For the double tubular and glomerular targets, a direct correlation between the formation of reactive oxidized species (ROS) and cytotoxic effects has been established. ROS are produced indirectly by metal cations released during nanoparticle dissociation.

On a short period of exposure, these ROS are sufficient to induce oxidative stress and the appearance of cell damage (lipid peroxidation, lysosomal membrane damage), as well as a molecular adaptive response (antioxidant response, cell stress response, and apoptosis: programmed cell death). For TiO_2 nanoparticles, oxidative stress occurs after prolonged and repeated exposure, resulting in cytotoxic effects in vitro.

In vitro nanoparticle impact analysis thus enables a toxicological approach tailored to the identification of molecular and cellular mechanisms. **(Guitou, 2014).**

Chapter 02: rats and cardiovascular system

I. Rats

1. General

The term "laboratory rats" refers to strains or lines of rats that are chosen, bred, and maintained for the purposes of animal experiments in laboratories or, occasionally, for anatomy, anatomy, and dissection classes**(George, 2000)**.

Figure 04: Dissection of a rat showing its main organs**(George**, **2000).**

The Wistar rat is an albino rat that is not inbred. More than half of all laboratory rat strains are descended from the original colony started by physiologist Henry Donaldson, scientific administrator “Milton J. Greenman”, and genetic researcher/embryologist Helen Dean King at the Wistar Institute in 1906 for biological and medical research **(Site 04).**

Because of its metabolic resemblance to humans, the Wistar Rat has become a popular species in toxicology and pharmaco-toxicology. The wistar rat is an opportunistic omnivore that is distinguished by its large head, long ears, and tail length that is always less than its body length (35 to 50 cm with tail, tail length 17 to 23 cm) **(Site 05).** Other characteristics include its relatively docile nature, short gestation period (21 to 23 days), short life span (2 to 3 years), and weights of 150g to 500g with an average of around 300g for females and 250g to 1500g with an**(site 06).**

It has a short gestation period (21 to 23 days), a short life span (2 to 3 years), and is quite docile **(Descat, 2002).**

Figure 05: rat wistar **(George**, **2000).**

2. Classification:

- Phylum : Vertebrates
- Class: Mammals
- Order : Rodents
- Sub-order : Myomorphs
- Family : Muridae
- Sub family: Muridae.

There are four primary categories of strains: inbred strains, outbred strains, first generation hybrids, and mutant strains. Mutant varieties. More than 1400 strains and sub-strains are listed and employed in biomedical research in total **(Descat, 2002)**.

3. Use in research:

The rat is the most often utilized experimental mammal after the experimental mouse, making up roughly 20% of all the mammals employed in research **(Festing, 1979)**.

The rat has been employed in nearly all facets of biological, behavioral, and toxicological research for the previous 80 years. Evidence of study models that have been developed by genetic mutations and selection are provided in item 3 as evidence of their immense value. Toxicology, teratology, experimental oncology, experimental gerontology, cardiovascular research, immunology, dental research ,immunogenic, and experimental parasitology are among the research fields where the rat is frequently used and particularly helpful, according to a recent publication on applications in biomedical research **(Baker, 1980).**

II. Cardiovascular system of rats :

1. Definition of rat cardiovascular system:

The vascular system in rats consists of two parallel networks: the venous network, which

carries blood from the tissues to the heart, and the arterial network, which carries blood from the heart to the tissues, the heart, and the fluid (blood). All body organs are supplied with blood by the cardiovascular system **(Bradley and Calvert, 2009)**.

1.1.Function:

In addition to intercellular communication and moving substances between body parts, it also helps cells communicate with one another and get rid of waste. In order to maintain the body's homeostasis, he delivers to the cells waste as well as oxygen, nutrients, hormones, and many other chemicals. The heart's pumping motion provides force to move blood through the body **(Marieb, 2008).**

2. Cardiovascular anatomy:

2.1.The heart:

The heart is the primary organ of circulation in rats. It is a hollow muscle organ that is very small yet incredibly powerful. His average weight in the 300 g adult rat is 0.3 g **(Bailly, 1990).** an adult's heart beats between 250 and 450 times every four minutes **(Akingbemi, 1994).** It has four chambers—two ventricles and two atria—and is situated between the two lungs. It pumps blood to the arteries.

2.2.Heart anatomy:

2.2.1. Cardiac chamber:

The tricuspid orifice (TRI) connects the right ventricle and atrium, which are connected by the mitral orifice (MI) **(Site 07).**

2.2.2. Heart valves :

Blood circulates one way thanks to four valves. The tricuspid valve on the right and the mitral valve on the left connect each earbud to the appropriate ventricle, while the other two valves are situated between the ventricles and the artery corresponding to Aortic valve and pulmonary valve **(Site 08).**

2.2.3. heart wall:

The wall of the heart consists of three tunics, from outside to inside; pericardium, myocardium and endocardium. The pericardium, which is the outer coat of the wall, is composed of a delicate connective tissue that makes the texture of the outer surface of the heart smooth and slippery. The myocardium is the cardiac muscle tissue; it constitutes most of the mass of the heart and is responsible for the pumping action provided by the heart. The

endocardium is an in layer of connective tissue **(tortora, 2007).**

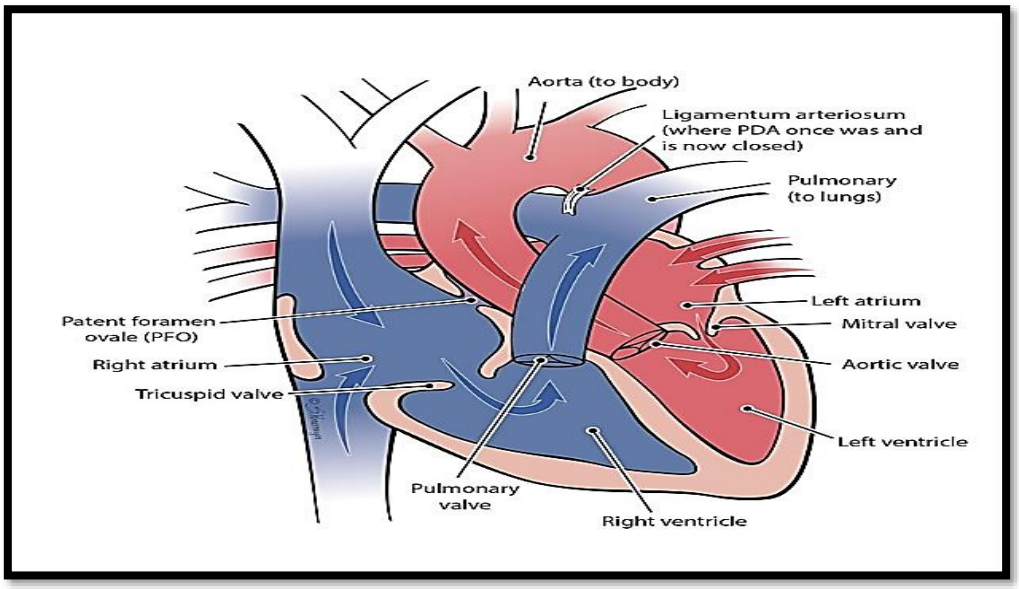


Figure 06: Anatomy of the Heart **(Tortora, 2007)**

3. Heart function:

Ensures blood flow through rhythmic contraction, and in conjunction with the respiratory system, allows for blood oxygenation and carbon dioxide removal. From the peripheral venous network, the right heart pumps oxygen-poor blood into the pulmonary circulation. The peripheral arterial network receives oxygenated blood from the left heart after it passes past the pulmonary filter **(Tortora, 2007).**

4. Cardiovascular physiology:

Deoxygenated blood is pushed from the right ventricle through the pulmonary semi-lemurian valve in the pulmonary trunk, which divides into arteries for the right and left lungs, into the right atrium by the tricuspid valve in the right ventricle of the rat. Blood is oxygenated in the lungs before returning to the left atrium via the pulmonary veins. The blood now travels to the left atrium and enters the left ventricle via the bicuspid valve. Blood is circulated throughout the body by the aorta **(Tortora, 2007).**

Chapter 3: Oxidative stress and cardiovascular diseases.

I. Cardiovascular diseases

Numerous illnesses affecting the heart and circulatory system are categorized as **c**ardiovascular disorders. These illnesses are multifactorial complicated diseases that account for a significant portion of global morbidity and mortality. Additionally, a sizeable amount of healthcare spending is devoted to the corresponding medication therapies.

According to estimates by the World Health Organization, cardiovascular illnesses are the biggest cause of death in the world, responsible for 17.8 million fatalities in 2008, or 30% of all deaths worldwide. With 23.3 million deaths per year, these diseases are predicted to continue being the top cause of mortality worldwide **(Faurie, 2015).**

Cardio-vascular diseases are a collection of issues that affect the heart and blood vessels. They understand **(Dahmouni, 2016).**

- Coronary artery diseases (affecting the blood vessels that feed the heart muscle).
- The cerebral vascular diseases (affecting the blood vessels that supply the brain).
- The peripheral arterial diseases (affecting the blood vessels that supply the arms and hands).
- The rheumatism-related cardiac diseases, which affect the heart's muscle and valves and are caused by an arthritic rheumatism caused by the bacteria streptococcal.
- The malformations cardiaques congenital (malformations of the heart's structure that exist at birth).
- Deep vein thrombosis and pulmonary embolism are conditions in which blood clots in the jambs block blood flow and may go to the heart or kidneys **(Boutahiri, 2011; Bourgou, 2014).**

1. The cerebral vascular accident

The AVC is a localized neurological deficit brought on by damage to one or more cerebral blood vessels. In reality, it occurs when the blood vessels that carry oxygen and nutrients to the brain rupture or become blocked by a blood clot or other particles that damage the affected tissue. A blockage or vessel rupture results in a stoppage of blood flow to one or more areas of the brain. After a short time without oxygen, the nerve cells in the affected area of the brain lose their ability to function and eventually pass away. Additionally, when nerve cells stop working, the body part they control ceases to function, leading to impairments that are

frequently irreversible **(Berkat, 2018).**

1.1.AVC Emulation

AVC dysfunction (Figure 07) results from an oxygen supply shortage in a specific area of the brain. It may be the result of either an emboli (migration of a clot or fat, deposition of debris) or an internal carotid thrombosis (occlusion) of the cerebral artery. In both situations, a portion of the brain is less agitated. There will thereafter be a greater or lesser degree of neurological deficit (paralysis) according to the affected cerebral territory **(Bruins slot, 2008).**

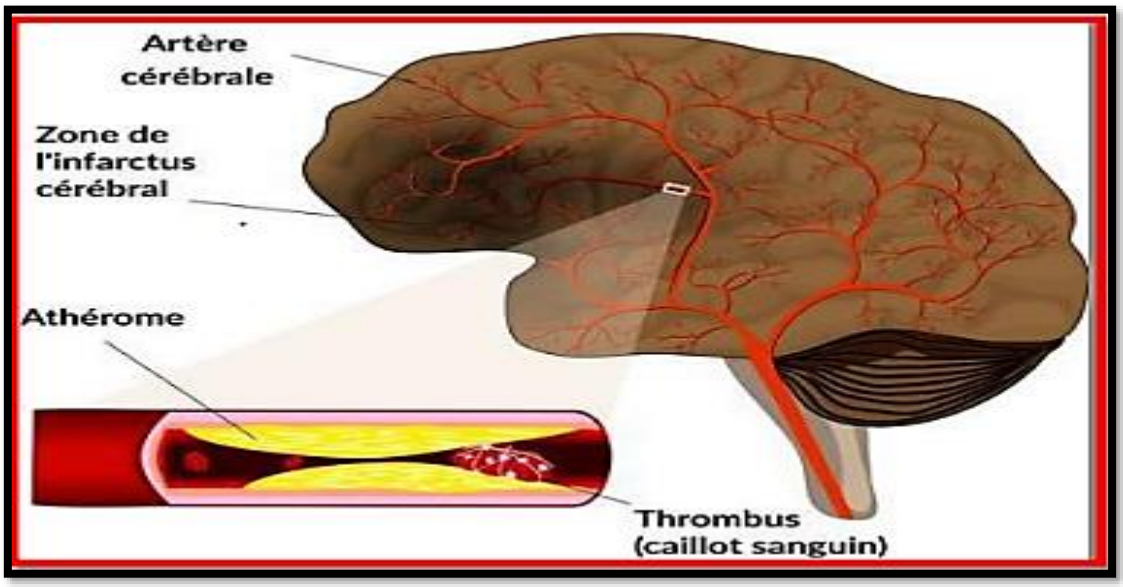


Figure 07: Vascular Cerebral Ischemic Accident (**Bruins, 2008).**

2. Thrombosis

Blood flow slowing down encourages the growth of a clot that completely obstructs the artery. This may result in a chronic illness that causes anoxia and tissue death from necrosis or downstream (brain or myocardial infarction, injury to a body part). Additionally, fibrolipidic plaque fragments or clots (emboli) may separate, migrate, and obstruct smaller arteries away from the primitive foyer, resulting in the formation of an emboli at the level of the brain (hemiplegia), the heart (infarctus), or the lungs (emboli pulmonary) **(Libby, 2002)**.

II. The atherosclerosis

1. Definition

“Felix Marchand” first proposed the word atherosclerosis in 1904 by combining the Greek words atheroma and sclerosis. The term "atheroma" (from the Greek "athere: bouillie") refers to the lipidic portion, and "sclerosis" (from the Greek "scleros: dur") refers to the fibrosis. **(Humphrey, 2002)**.

Since 1958, the OMS has defined atherosclerosis as a factor that influences the internal layer of large and medium-sized arterial walls. She is characterized by the localized deposition of lipids, complex carbohydrate, blood and blood products, fibrous tissue, and calcium deposits. All of this is done in conjunction with a change to the media (arterial vessel intermediate tunic).

2. Normal artery structure

Each artery adheres to a common organizational model. Three peaks that go from the interior to the exterior of the parapet are Intima, Media, and Adventice.

a) **The intima:**

At this level, where the tunic is the most internal and fine, is where atherosclerosis develops. She is composed of:

- A specific layer of endothelial cells that rest on a basal membrane to provide an enveloping covering.
- From a virtual a cellular space: the following endothelial zone.
- A limit elastic internal layer that is thick and perforated with fenestration (an opening that allows bidirectional cellular and material transit) separates the intima from the media **(Cohen, 1997)**.

b) **The media:**

This is the middle and thickest tunica, which contains the main component of the artery. It is constrained by both internal and external elastic limiters, and it is made up of:

- A concentrated cluster of lamellar units made primarily of skeletal muscle cells that are encased in an extracellular matrix made of elastin, collagen, and mucopolysaccharide.
- A stretch of elastic; the external limit of elastic separating the middle from the adventure. (**Cohen, 1997)**.

c) The adventitia:

The external tunic, which rests on the external elastic limit, is made up of the following:

- A poorly organized, collagen and elastin-rich tissue containing fibroblasts, adipocytes, and vasa vacuums, a group of small vessels whose function is restricted to the medial exterior.
- A network of non-militated vasomotor that connects the medial skeletal fibers. **(Grignon, 1996).**

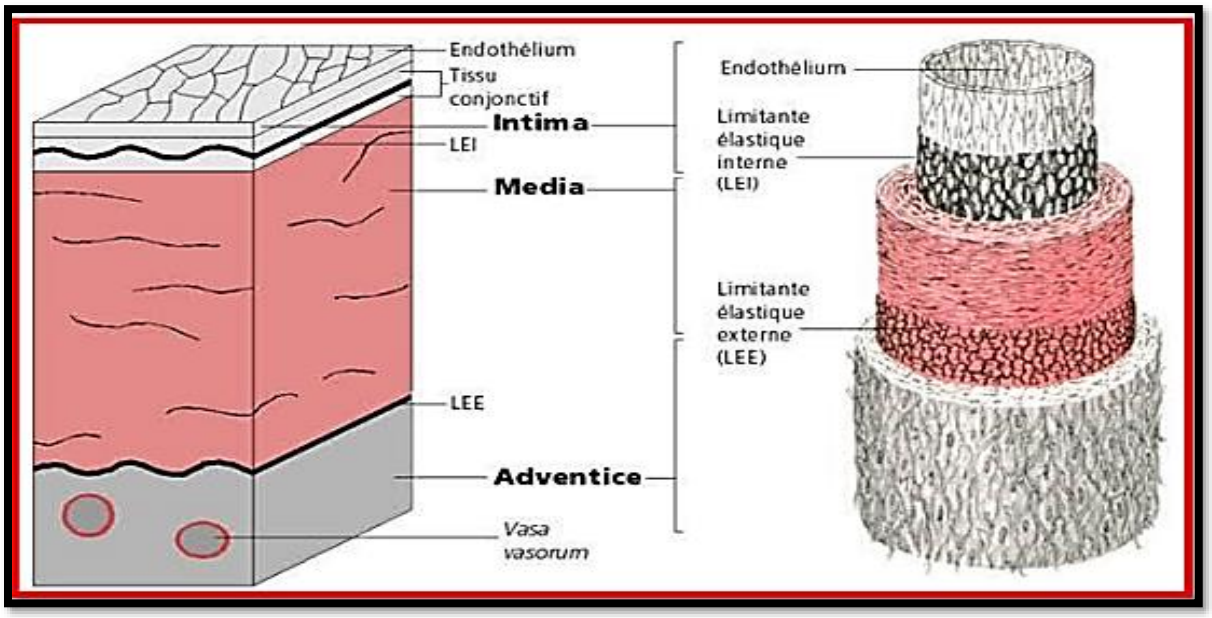


Figure 08: Schematicrepresentation of the arterial pulse **(Stevens and Lowe, 1997).**

3. The Risk Factors for Cardiovascular Diseases:

A condition of a physiological nature (age and sex), a pathological nature (hypertension, diabetes...), or related to a lifestyle habit (smoking, sedentary lifestyle...) can all be considered cardiovascular risk factors **(Houcher, 2012).**

It raises the likelihood that a specific cardiovascular event will occur. Its correlation with pathology must be statistically significant, progressive, and long-lasting **(Berghalout, 2010).**

I.Physiological Factors

a) Age:

One of the most significant factors for cardiovascular risk is age. The clinical signs of atherosclerosis often appear after the fourth decade in men and after the fifth decade in women.

The age at which there is the greatest chance of experiencing a wrongful death accident is fifty-one years for men and sixty-one years for women. Additionally **(Boutahiri, 2011).**Cardiovascular diseases account for one-fourth of all causes of death in people over 75 years old in both sexes **(Minino, 2007)**.

b) **the gender**

Particularly alarming is how much less frequently cardiovascular diseases caused by atherosclerosis affect women than men at middle ages. Nice, whatever the country or region may be. The menopausal woman exhibits an occurrence of similar to the coronary illnesses of the man in that age. This effect is primarily connected to an oestrogen deficiency **(Bella and Khan,**

2016).

Several experimental studies attribute this difference to the beneficial effects of naturally occurring oestrogens in premenopausal women (on endothelial function, labile muscle cells, or angiogenesis) **(Miller and Duckles, 2008).**Appear to be shielded until menopause **(Ayanian, 1991; Goldberg, 1993).** Furthermore, the results of the Framingham study show that the incidence coronary artery diseasesis significantly higher in post-menopausal women than in non-men. Those who are not it.

II. Lifestyle-related factors

a) the tobacco

The use of tobacco is a serious risk factor that can lead to serious consequences. Cardiovascular deaths are the leading cause of death worldwide, accounting for one in ten deaths. Cardiovascular mortality that can be prevented **(Boutahiri, 2011)**. The Cardiovascular Risk of Smoking is correlated with both the frequency and daily amount of consumption.

The main mechanisms of the cardiovascular damage caused by tobacco use are thrombosis and spasm, which are both quickly reversible. The rate of fibrin-genesis, plaque agreeability, and arterial blood pressure are all increased by smoking. Factors that are dependent on the endothelium and encourage thrombosis and spasm. It is also related. To a decrease in HDL cholesterol and an increase in inflammatory markers. Some of these mechanisms aid in development of atherosclerotic plaques. Long-term hypothesis while others are frequently to blame for aigus accidents very prompt **(Boutahiri, 2011)**.

b) Hypertension

The pressure that the blood exerts on the arteries is known as arterial tension (or arterial pressure). This pressure varies depending on the size and elasticity of the arteries, as well as the diameter is reduced, the blood's exerted force to circulate through it must be significant and powerful. As a result, there is increased pressure **(Bourgou, 2014)**.

The two values of arterial hypertension are called systolic and diastolic, respectively. Systolic hypertension refers to the pressure in the arteries at the time when the heart contracts (systole) and expels the blood into the arterial network. Corresponds to the pressure in the arteries at the time the heart expands and fills, between the minimal amounts of blood pressure is two contractions **(Inserm, 2014)**.

The risk of problems like IDM and AVC is increased by HTA and atherosclerosis development, respectively **(Boutahiri, 2011).** Intima and skin lesions are encouraged by a sustained and prolonged increase in pressure.

The formation of the inherited plaque **(Houcher, 2012).** In addition, the increase in by acting to cause the rupture of the plaque, pressure on the vascular parotids problems from aigus being caused by atherosclerosis (AVC).

III. The dyslipidemia

a) Total cholesterol, HDL, and LDL fractions

Plasmatic cholesterol levels, particularly those of LDL cholesterol exhibited a strong correlation that was linear and ongoing with the development of complications. Cardiovascular. A 1% increase in LDL-cholesterol concentration is considered to be significant. Associated with a 2–3% increase in the coronary risk**.** Thisthe associated cardiovascular risk factors significantly alter the association **(Neaton; 1992).**

A too high level of cholesterol will deposit itself on the walls of the arteries, forming fatty plaques that deteriorate over time (arteriosclerosis). These plaques lessen the diameter of the artery, increasing the flow of blood difficult. Cells become closed off when the blood no longer flows at all. Oxygen and were dying. The areas that are most affected include the heart, brain, and limb arteries. When the blood flow is interrupted, the myocardial infarction (heart) is discussed. Either a cerebrovascular accident (cervical) or lower extremity arthritis (**Brian, 2008; Cohen, 2009).**

b) The triglycerides

In addition hypertri-glycemiais linked to an elevated risk of MCV. However, this relationship is weaker than the one with blood cholesterol. Moderate hypertri glyceridemia's are typically accompanied by a group of metabolic changes including a low HDL cholesterol content, an increase in triglycerides, and diabetes, insulin resistance, abdominal obesity, and/or hypertension that are caused by significant risk factors making it challenging to consider hypertriglycemia as an independent factor **(Angelantonio, 2009; Hulley, 1980).**

c) The Diabetes

The primary cause of death in type 1 and type 2 diabetics is cardiovascular disease. Observational studies have shown that the risk of developing type 1 diabetes increases by two to three times the risk of type 2 diabetes. Risk of cardiovascular disease **(Faurie, 2015).** Diabetes increases risk by a factor of 2 to 4.The risk of AVC increases by a factor of 2 to 4 when considering a coronary disease **(Boutahiri, 2011).**

There is currently no established physiological link between diabetes and cardiovascular problems. Not fully understood, but several metabolic, vascular, or other abnormalities the high risk of these problems in the subject may be explained by factors related to coagulation

diabetic:

- The inflammation
- endothelial dysfunction**(Beckman, 2002),**
- The pathogenesis of the oxidative stress (deficiency in antioxidants, glycation products, and glyco-oxydation products) that leads to the oxidation of LDL cholesterol appears to be significant.
- The abnormalities of platelet function and coagulation, which lead to platelet activation and a procoagulant effect.
- The following lipid problems are present: hypertriglycemia, decreased HDL cholesterol, small and dense LDL cholesterol, and an increase in lipoprotein remnants.
- Insulin resistance and hyperinsulinemia **(Boutahiri, 2011).**

PRACTICAL PART

I. Material and methods

1. Materials

1.1.Animals

Adult male rats "*Rattus rattus*" of the Wistar strain from Pasteur Institute of Algiers, Algeria, weighing between 160 and 240g were used for the experiment. The animals were housed in polypropylene cages with soft wood and free access to water and diet every day. They were maintained in the animal house under standard temperature and humidity laboratory conditions **(Figure 09).**

Figure 09: Male rats "*Rattus rattus*" of the Wistar strain **(personal photo).**

1.2.Chemicals

In this work, we used Nano titanium dioxide for the treatment of rats in the form of a two-dose solution for each day.

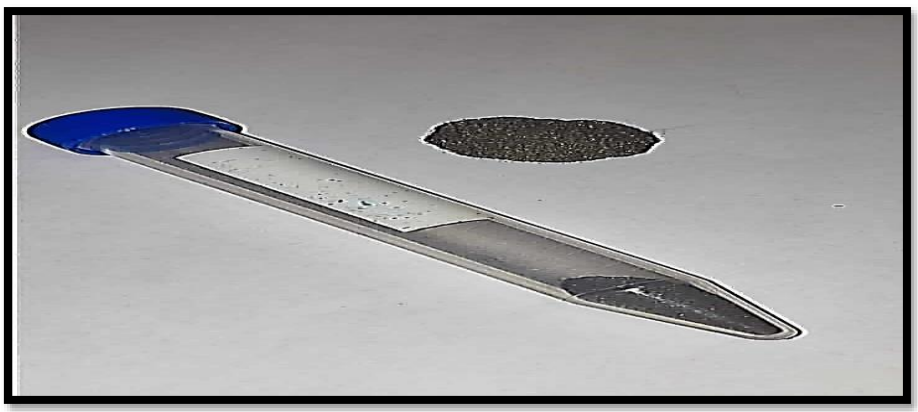


Figure 10: The TiO_2 used in the experiment **(personal photo)**.

2. Methods

2.1.Distribution of rats

The rats were divided into three (03) batches at a rate of seven (07) rats per batch. They were subjected to an adaptation period of 30 days in the animal house of the biology department, Faculty of Sciences, University of Tebessa. The ambient temperature is 23±2°C and a natural photoperiod is 12/12H with a hygrometry of 60%. Rats are reared in polyethylene cages (07 rats for each cage) lined with bedding of wood shavings. The cages were cleaned and the bedding was changed once every two days until the end of the experiment. The animals were fed an energy balanced kibble concentrate **(Figure 11).**

Figure 11: Distribution of rats **(personal photo).**

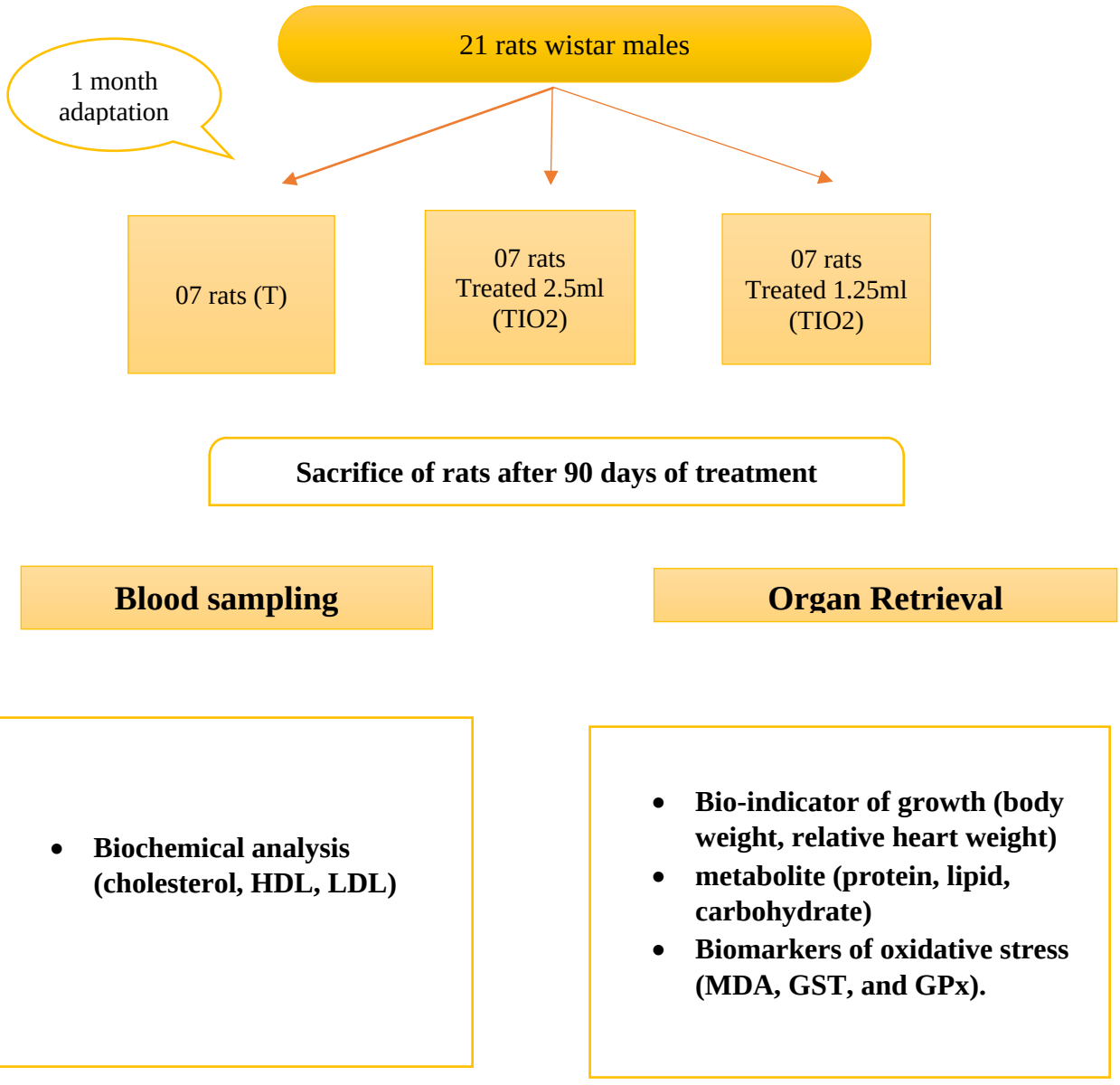


Figure12: Schematic illustration of the experiment.

2.2.Dose selection and preparation of TiO_2

10000 mg nano-TiO_2 was dissolved in 500 ml tap water and administered orally by intragastric gavage using a feeding tube, at consecutive doses of 5000/100, 2150/100 mg/kg body weight/day for 90 days at a dose volume of 2.5, 1.25 ml/kg body weight respectively in batches (LOT 01, LOTS 02) at approximately the same time of day by oral gavage **(Figure 13).**

Figure 13: TiO2 used in the experiment (**personal photo).**

2.3.Treatment of rats

The allocation and treatment of the animals is illustrated as follows:

Lots (T): control lot (T) receiving distilled water by gavage 0.5ml/day for 90days.

Lots 1: treated with TiO2 receiving 2.5 ml/kg/day for 90 days.

Lots 2: treated with TiO2 receiving 1.25 ml/kg/day for 90 days.

Figure 14: treatment of rats **(personal photo)**

2.4.Evaluation of the neurotoxicity of TiO_2

After 90 days of exposure of the animals to TiO2, two approaches were applied to evaluate the impact of TiO2 on the neurobehavioral of rats.

2.5.Weight measurement

Weight measurement was performed on the rats every 7 days for the duration of the treatment using an electronic scale.

Figure 15: Measuring the weight of rats **(personal photo).**

2.6.Study of the toxicity of TiO_2

A. Sacrifice and heart extraction

At the end of the TiO_2 administration period, the rats were sacrificed after intracardiac blood sampling and the heart was quickly removed and rinsed in cold wash buffer, then dried by a semi-absorbent paper.

The samples to be stored at -80°C for the determination of biochemical parameters (proteins, lipids, carbohydrates) and oxidative stress such as MDA ,GST and GPx,.

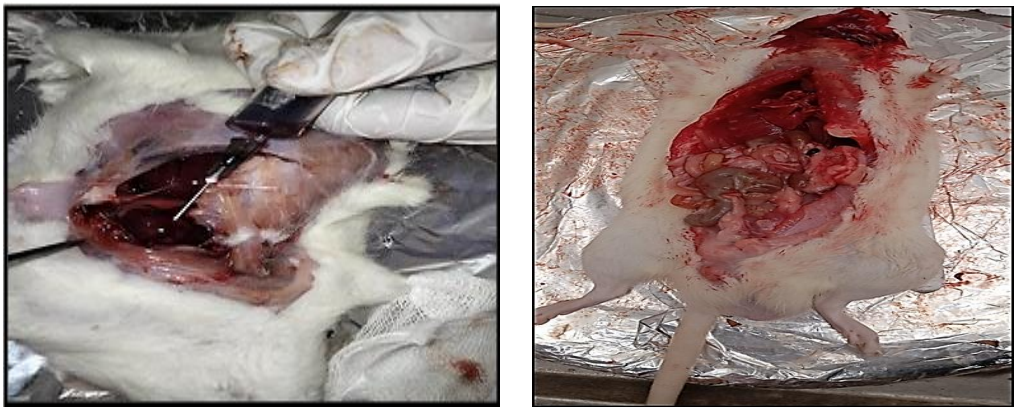


Figure 16: Sacrifice and heart extraction **(personal photo)**

B. Blood collection

After 90 days of experiment, animals were fasted overnight and the blood was obtained by intracardiac sampling and collected into EDTA tube.

C. Estimation of relative heart weight

The relative weight of the hearts extracted from the rats (PRC [g/100g of body weight]) is calculated with respect to the total weight of the rat according to the following formula:

$$\mathbf{PRC\ (g/100g\ de\ PT) = PC/PT \times 100}$$

PC: heart weight (g).**PT:** total rat weight (g). **PRC:** relative heart weight (g).

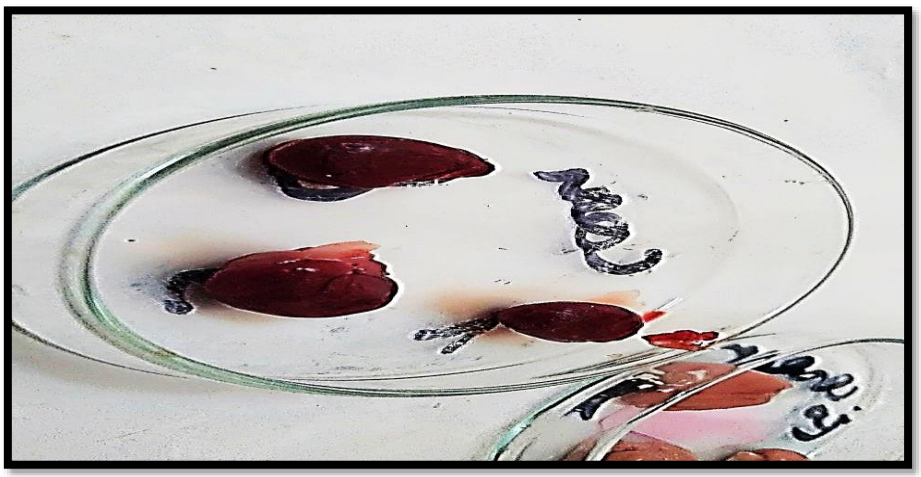


Figure 17: hearts of the rats **(personal photo).**

2.7.Biochemical parameters

The blood numbering, to estimate the figurative elements of the blood (cholesterol, LDL, HDL) this analysis is carried out on the blood preserved in tubes with EDTA or heparin were determined at the end of the experiment, the Counting was carried out at the level of the laboratory ELITE LAB (Tebessa).

- **Measuring Cholesterol**

The assay is done according to the Biomaghreb data sheet **(Fasce, 1982; Richmond, 1973; Trinder, 1969)**. The cholesterol present in the sample forms a colored complex according to the following reactions:

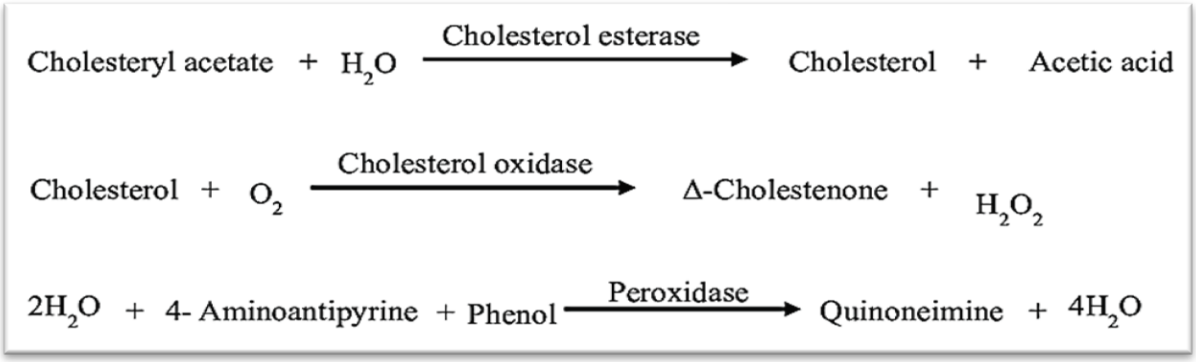


Table 03: Reagents used in the determination of cholesterol.

Reagent 1: **Tampon**	**PIPES pH 6.9** **Phenol**	**90mmol/l** **26mmol/l**
Reagent 2: **Enzyme**	Cholesterol esterase (CHE) Cholesterol oxidase (CHOD) Peroxidase 4-Aminophenazone(4-AP)	300 U/L 300U/L 1250U/L 0.4mmol/l
Etalon	Cholesterol aqueous (Standard)	2b/l

3-Working reagent: dissolve the contents of reagent2 in the bottle of reagent1 and mix lightly.

4-Sample: Plasma

Table 04: Working reagents

	Blanc	**Etalon**	**sample**
Working reagent (ml)	1.0	1.0	1.0
Etalon (µl)	--	--	--
Sample (µl)	--	--	--

- **Operating mode**

Mix, incubate for 5 min at 37°C, or 15-20 min at room temperature. Read absorbance of samples and standard against reagent blank at 505 nm. The final coloration is stable for at least 30 min.

Calculation of the concentration:

$$Cholestérol\ (g/l) = \frac{(A)\ Echantillon}{(A)\ Etalon} \times 2g/l$$

A: Densité optique

- **Dosage of cholesterol (HDL–LDL)**

Principal:

Chylomicrons and very low density lipoproteins (VLDL) and low density lipoproteins (LDL) contained in the sample are precipitated by addition of phosphotungstic acid in the

presence of magnesium ions. The supernatant obtained after centrifugation contains the high density lipoproteins (HDL).

2.8.Evaluation of biochemical parameters

A. Carbohydrate dosing

The method of **(Duchateau, 1959).** is used to determine the amount of carbs. This method uses a stock solution of glucose (1 g/l) as a standard and anthrone as a reagent (150 mg anthrone, 75 ml sulfuric acid, and 25 ml distilled water). The procedure is as follows:

- To an aliquot of 100 µl of supernatant from the different samples, add 4 ml of anthrone reagent
- When the mixture is heated to 80 degrees for 10 minutes, a green hue develops.
- The coloration's intensity was measured at a wavelength of 620 nm.

B. Lipids dosing

The method of **(Goldsworthy, 1972)**. is used to determine the lipid content. This method uses vanillin as the reactant (0, 38 g of vanillin, 195 ml of 85% orthophosphoric acid, and 55 ml of distiller's water) and a mother's lipid solution (2, 5 mg/ml) as the standard. After stirring and adding 1 ml of 98% sulfuric acid, the tubes are heated in a bath at 100°C for 10 minutes; 200 l of each tube are then pre-collected, and 2.5 ml of reactant is added.

After 30 minutes in complete darkness, the absorbance were measured at a wavelength of 530 nm.

C. Protein assessment

The proteins are measured using the Bradford method of (Bradford, 1976), which uses the blue brilliant of coomassie (BBC) as the reactant and (B.S.A.) as the standard. The range of etaloning was produced using a mother solution of B.S.A. (1 mg/ml) and B.B.C. (conservation approximately 21 days at 4°C), which is prepared as follows:

- 100 mg of BBC and 50 ml of ethanol are agitated for two hours.
- 100 ml of orthophosphoric acid is then added, and the mixture is finished off with distillate water to make 1000 ml.
- The dosage of proteins was administered in a fraction aliquot (100 ml).
- The absorbance were measured at a 595 nm wavelength.

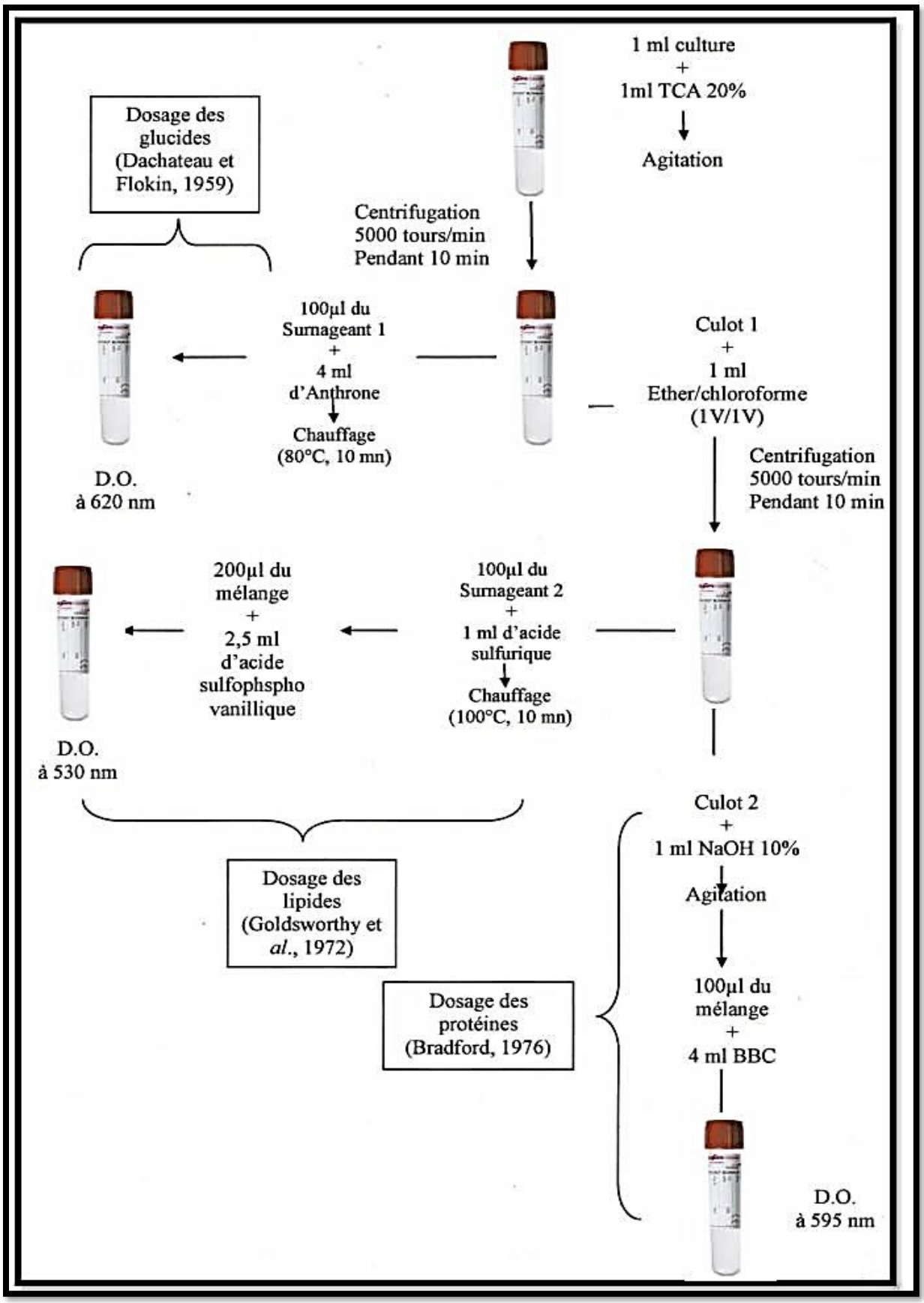


Figure 18: Extraction and dosage of carbohydrates, lipids and proteins according to the method of **(Shibko, 1967)**.

2.9.Evaluation of oxidative stress parameters

A. Malonedialdehyde (MDA)

Malone-dialdehyde (MDA) are determined by the method of **(Buege and Aust, 1984)**. This method is based on the colorimetric measurement of the reaction between thiobarbituric acid (TBA) and Malone-dialdehyde (MDA) in an acidic and hot medium (100°C) giving a red-brown product whose intensity of measured at a wavelength of 530 nm.

➢ Experimental protocol

- Prepare homogenates from 200mg of organ with TP
- (pH 7.4)
- Centrifuge at 3000 rpm for 10 min
- Collect 375 µl of supernatant
- Add 150 µl of TBS buffer (50 mM Tris, 150 mM NaCl pH 7.4)
- Add 375 µl of TCA-BHT solution (TCA 20%, BHT 1%)
- Shake and centrifuge at 1000 rpm for 10 min
- Collect 400 µl of supernatant
- Add 80 µl of 0.6 M HCl
- Add 320 µl of Tris-TBA solution (Tris 26 mM, TBA 120 mM);
- Mix and incubate in a water bath at 80°C for 10 min
- Read Optical density at λ = 530 nm.
- The absorbance is directly proportional to the amount of MDA formed, thus giving an accurate assessment of the peroxide lipids. The concentration of MDA is calculated according to the of Beer-Lambert (DO = E.C.L).

$$[C]\ (\text{nmol/mg protéine}) = \frac{DO.10^6}{\varepsilon.L.X.Fd}$$

-**C**: concentration in nmole/mg of protein.

-**DO**: optical density read at 530 nm.

-***ε***: Molar extinction coefficient of MDA = 1.56.105 M-/cm.U.

-**L**: Length of the cell used (1cm).

-**X**: Protein concentration of the extract (mg/ml).

-**Fd**: Dilution factor (Fd = 0.2083).

B. Glutathion S- Transférase (GST) assessment

The measurement of GST activity consists in providing the enzyme with a substrate, usually 1-chloro, 2.4-dinitrobenzene (CDNB), which reacts readily with glutathione under the action of many GST forms. The reaction of these two products results in the formation of a new molecule that absorbs light at 340nm wavelength **(Habig, 1974).**

➢ Experimental protocol

The method used in this study to determine GST is that of (**Habig, 1974)** which consists in to make the GST contained in the homogenate act on a mixture (GSH + CDNB) at a temperature of 37°C and at a pH of 6.5. The variation of the optical density, due to the appearance of the GSH-CDNB complex, is measured for 15 minutes for 1 minute at a wavelength of 340 nm. According to the following steps:

- Samples are homogenized in 1ml TP (0.1 M, pH 6)
- Centrifuge at 1400 rpm for 30min
- Collect the supernatant as an enzyme source
- Remove 200µl of supernatant and add 1.2ml of CDNB (1mM), GSH (5mM) mixture

$$\text{L'activitéde la GST} = \frac{\text{DOéchantillon} - \text{DO blanc}}{\text{9,6mg de protéine}}$$

- The blank containing 200µl of distilled water replacing the amount of supernatant.

-**DO**: sample/min: optical density of the sample per minute.

- **DO**: blank/min: optical density of the blank per minute.

- **9.6**: extinction coefficient of GSH-CDNB expressed in mM-1.cm-1.

C. (GPx) dosage

Using the method described in **(Flohe, 1984)**. the glutathion peroxidase (GPx) enzyme activity was measured. This method relies on the reduction of reduced glutathion (GSH) into reduced peroxide of hydrogen (H2O2), which is then converted into (GSSG) under certain conditions. The following reaction, as determined by GPx,

$$H_2O_2 + GSH \xrightarrow{GPx} GSSG + 2H_2O_2$$

➢ **Experimental protocol**

- Prepare homogenates from 1ml of culture with TP homogenization buffer (pH 7.4)
- Centrifuge at 3000 rpm for 10 min
- Remove 0.2 ml of supernatant
- Add 0.4 ml of GSH (0.1 mM)
- Add 0.2 ml of TBS buffer (Tris 50 mM, NaCl 150 mM, pH 7.4)
- Incubate in a water bath at 25°C, for 5 min
- Add 0.2 ml of H2O2 (1.3 mM) to initiate the reaction, left for 10 min
- Add 1 ml of TCA (1%) to stop the reaction
- Place the mixture in an ice bath for 30 min
- Centrifuge for 10 minutes at 3000 rpm
- Remove 0.48ml of supernatant
- Add 2.2ml of TBS buffer
- Add 0.32ml of DTNB (1mM)
- Mix and after 5 minutes read the optical densities at 412 nm
- The determination of the enzymatic activity of GSH-Px is done using the following formula:

$$GPx(\mu mol.\ mg\ de\ protéine) = \frac{(DOe - DOb).\ 0{,}04}{DOb}$$

-**DO**: sample: Optical density of the sample.

- **DO**: standard: Optical density of the standard.

- **0.04**: Substrate concentration (GSH).

2.10. Statistical analysis

The results are presented as mean plus or minus standard deviation (m±s) and illustrated by tables and histograms. The statistical treatment of the results is exploited by performing a one-factor analysis of variance (ANOVA), the "Tukey" test was used to compare the treated groups with the control group and with the fenthion group. All statistical analyses were

performed using Minitab 17.1 and Excel 13 (Microsoft, Inc.) statistical software. The level of statistical significance was set at $p < 0.05$.

- *Significant difference ($p \leq 0.05$).
- **: Highly significant difference ($p \leq 0.01$).
- ***: Very highly significant difference ($p \leq 0.001$).

RESULTS

II. Results

1. Study of biological parameters

1.1.Effect of TiO_2 on weight change

Our results show a decrease in body weight in the TiO_2 treated batches compared to the control batch **(figure 19).**

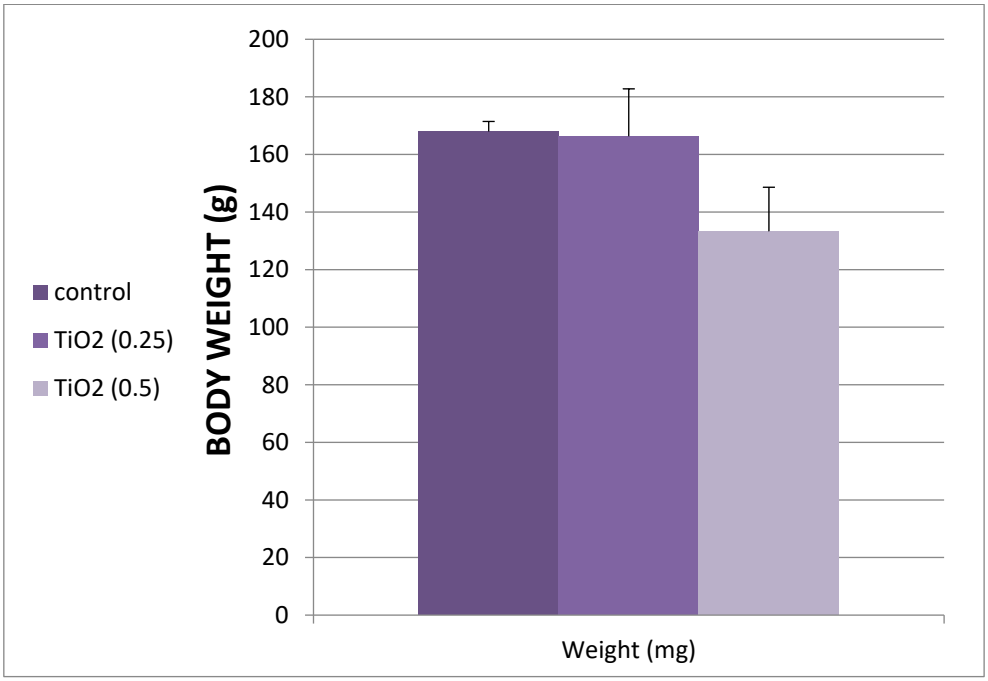


Figure 19: Change in body weight in control and TiO_2 treated rats.

1.2.Effect of TiO_2 on relative heart weights

Figure (20) and **table (05)** show the changes in relative heart weights in control and TiO_2-treated rats. The results obtained show that there was a significant ($p \leq 0.05$).increase in relative heart weight in theTiO_2-treated groups compared with the control group.

Table 05: Change in relative heart weight in control and treated batches.

Relative weight (mg/100mg of live weight)	**control**	**Lots 01**	**Lots 2**
Relative heart weight (mg/100mg)	0.269 ± 0.052	0.268 ± 0.024	0.305 ± 0.018

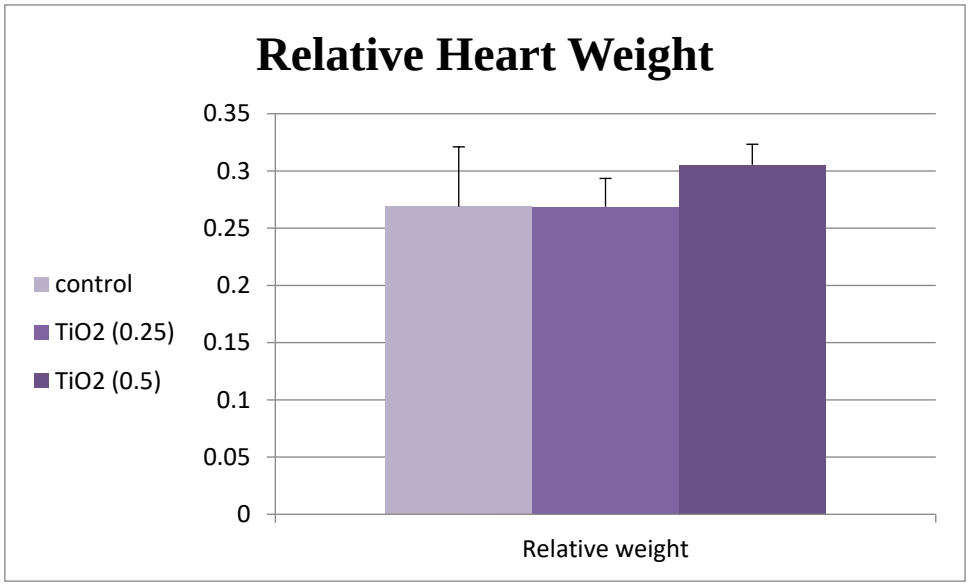


Figure 20: Change in relative heart weight in control and treated batches.

2. Study of biochemical parameters

2.1.Effect of TiO_2 on plasma cholesterol, HDL and LDL concentration

According to the results obtained, a highly significant increase ($p \leq 0.01$) in plasma cholesterol concentration was observed in the TiO_2-treated batches compared to the control batch.

Concerning the HDL level, we recorded a significant decrease ($p \leq 0.05$) in the TIO2-treated batch compared to the control.

As for the LDL level, we recorded a significant increase ($p \leq 0.05$) in the TIO2-treated batches compared to the control batch. **(Table 06).**

Table06: Variation of cholesterol, HDL and LDL in control and treated batches.

	Control	L 1	L 2
Cholesterol	**0.573 ± 0.175**	**1.426± 0.794**	**1.113 ± 0.473**
LDL	**0.190± 0.010**	**0.796 ± 0.921**	**0.526 ± 0.562**
HDL	**0.433 ± 0.035**	**0.426 ± 0.116**	**0.400 ± 0.088**

3. Effect of TiO2 on biochemical parameters in the heart of rats

3.1.Effect on the level of carbohydrates

The variation of carbohydrate/rate in the heart in control and treated rats are represented in **(Figure 21).**

The results obtained show a decrease in carbohydrate with a significant difference ($p \leq 0.05$)

in [TiO_2]-treated rats compared with the control.

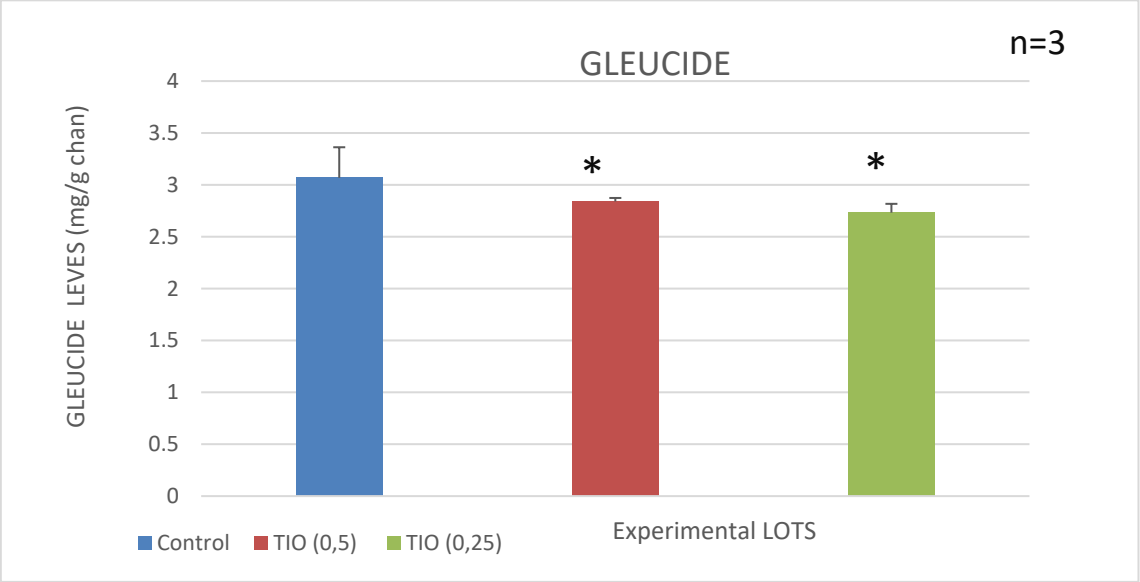


Figure 21: evaluation of carbohydrate activity.

3.2.Effect on lipid levels

The variation in lipid levels in the heart of control and treated rats is shown in (**figure22).** The results obtained show an increase in lipid with a significant difference ($p \leq 0.05$) in rats treated with [TiO_2] in (L1), compared with the control; and an increase with a highly significant difference ($p \leq 0.01$) in treated rats in (L2) compared with the control.

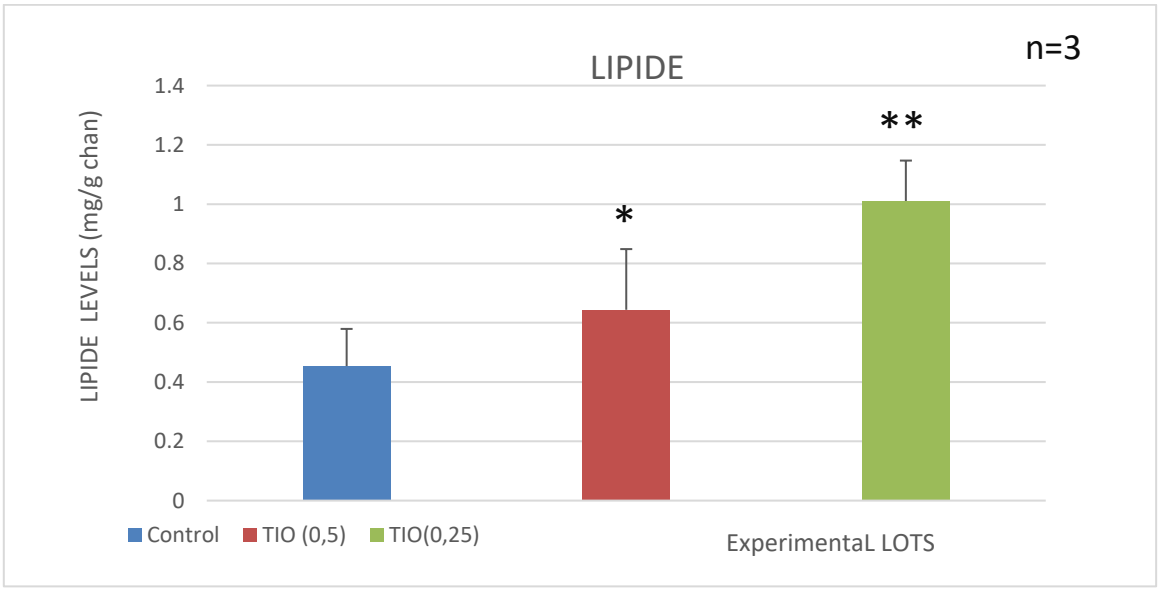


Figure 22: lipid activity evaluation.

3.3.Effect on the protein rate

The variation in protein levels in the heart of control and treated rats is shown in **(figure 23).** The results obtained show a decrease in protein with a significant difference ($p \leq 0.05$) in rats treated with [TiO_2], compared with the control.

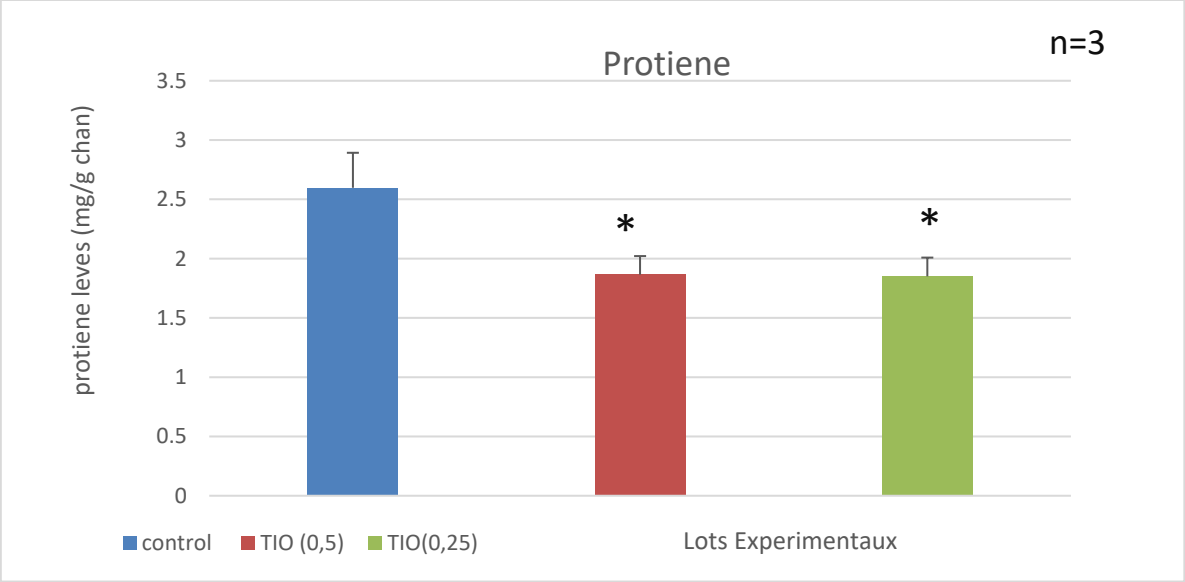


Figure 23: evaluation of protein level activity.

4. Effect of TiO2 on stress parameters in the heart of rats

4.1.Malone-dialdehyde (MDA) levels

The variation in MDA levels in the heart of control and treated rats is shown in **(figure 24)**. The results obtained show a decrease in MDA with a highly significant difference ($p \leq 0.01$) in rats treated with [TiO_2], compared with the control

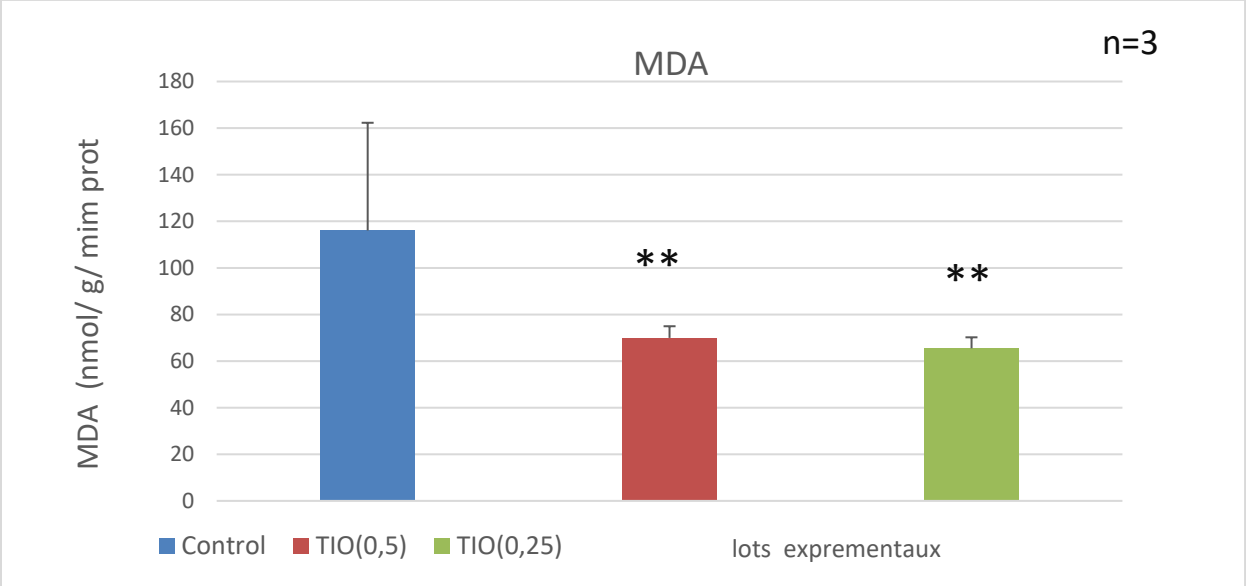


Figure 24: Evaluation of MDA activity.

4.2.Activity of glutathione -S- transferase (GST)

Variations in GST activity in the heart of control and treated rats are shown in **(figure 25).** The results obtained show a reduction in GST with a very highly significant difference ($p \leq 0.001$) in rats treated with [TiO_2] in (L1) and (L2), compared with the control.

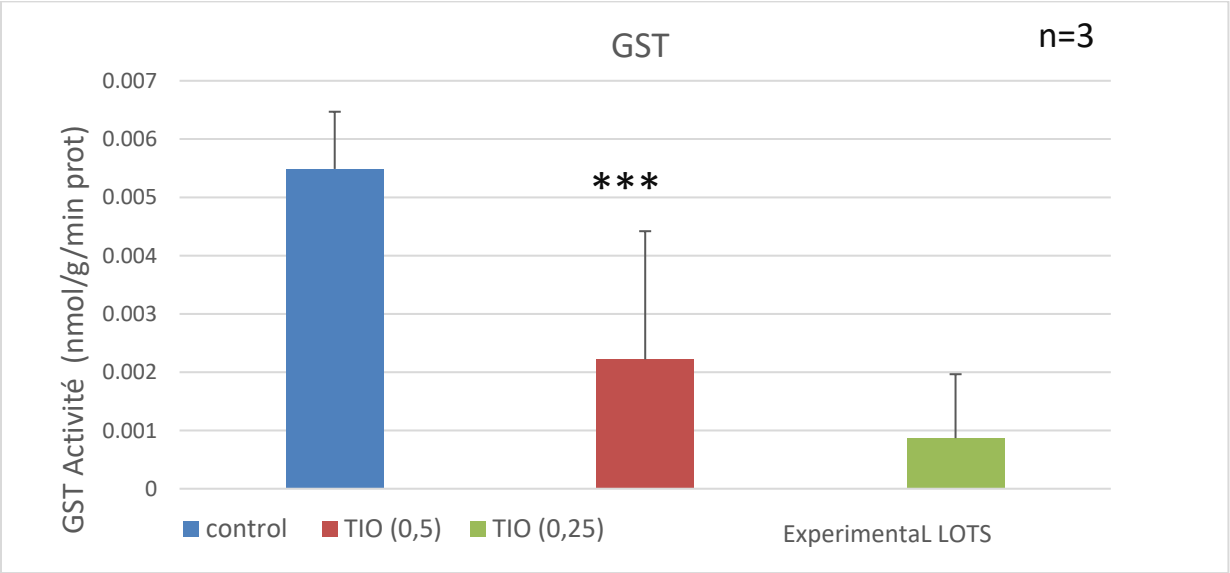


Figure 25: Evaluation of GST uygggugky0 `luactivity.

4.3.Activity of GPx

The variation in GPx activity in the heart of control and treated rats is shown in **(figure 26).** The results obtained show a decrease in GPx with a very highly significant difference (p≤

0.01) in rats treated with [TiO_2] in (L1) and (L2), compared with the control.

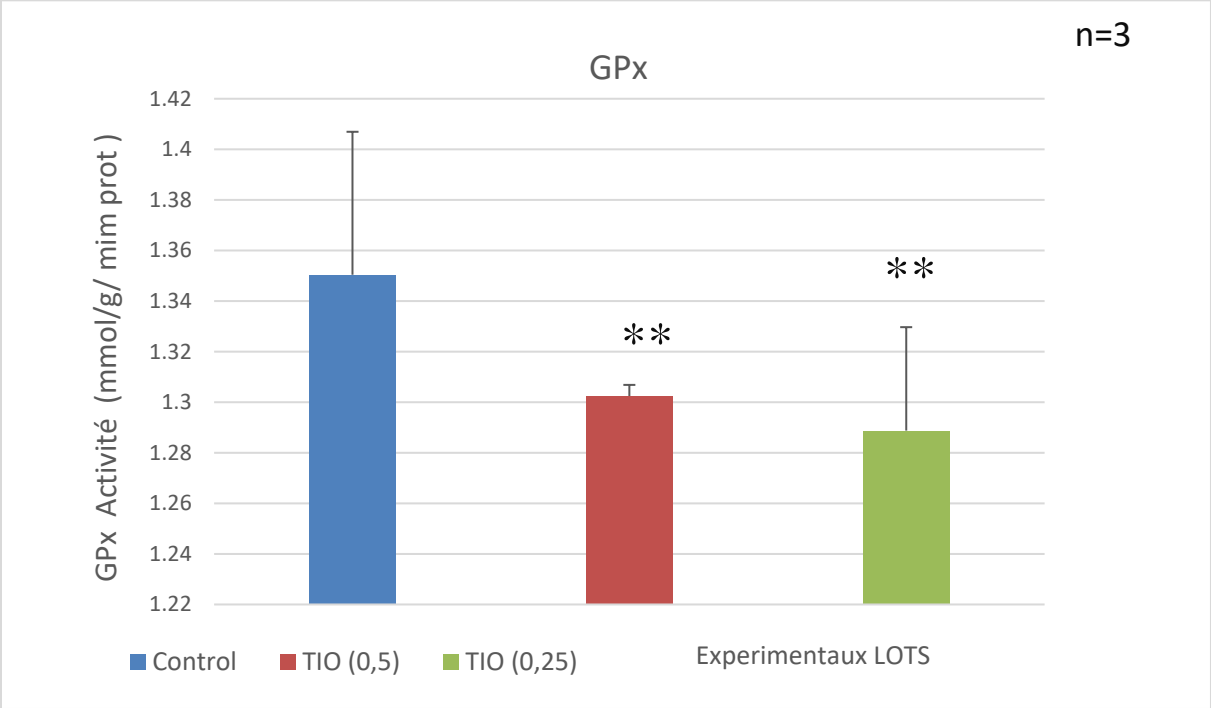


Figure 26: Evaluation of GPx activity.

DISCUSSION

III. Discussion

The size of nanoparticles, which ranges from 1 to 100 nanometers, can be dangerous to one's health. Because they are smaller than human cells, NPs pose a threat to the body as a whole. They may also have a negative effect on the environment because, due to their size, they are not filtered out by water or air and spread out into the natural environment **(Pompella, 2003).**

The very specific chemical and physical properties of the nanoparticles' size and shape facilitate their use in biological and medical applications. The material quickly spreads throughout the entire body. After injection, the substance circulates throughout all tissues and organs **(Berry, 2004)**. Before using nanoparticles as drug delivery systems, it is important to assess their environmental impact, biocompatibility, and potential toxicity to both humans and animals. Due to their smaller size, these particles have a higher surface area and are more reactive, which is a key factor in their toxic effects **(Carlson, 2008)**. TiO_2 nanoparticles are used extensively in several industries, including the industrial sector. Pigments, solar panels **(Mital, 2011)**.

The TiO_2 treatment resulted in changes to the metabolic parameters and the oxidative stress. According to a study, the cytotoxicity of dioxide of titanium nanoparticles is caused by the creation of oxidative stress, which leads to the death of cells **(Naqvi, 2010).**

The production of oxygen-active species (EOA) and the body's anti-oxidant defenses are out of balance during oxidative stress, favoring the first **(Haleng, 2007).**

In this work, we set ourselves the primary objective of demonstrating the potential toxicity of Titanium Dioxide nanoparticles as a cardiovascular stressor on Wistar rats as a biological model.

The results of our study showed that oral treatment of Wistar rats with titanium dioxide nanoparticles resulted in cardiovascular toxicity and oxidative stress.

1. Parameters for overall animal growth

The results of the weight parameter evaluation suggest that TiO_2 administration causes a significant reduction in body growth in the different groups of treated rats. The reduction in body weight may be the result of the anorexic phenomenon that animals may undergo with the time of exposure to xenobiotic and the state of stress in which they live during the period of this exposure**(Viviana,2015; Chakroun, 2016)**. These results are in line with the work of

(Monir, 2015). who reported a reduction in food consumption in male rats occurring at sub-chronic toxicity.

2. Effect of TIO_2 on plasma HDL and LDL cholesterol concentration

Our results reveal a significant increase in LDL and Cholesterol levels and decrease of HDL in treated rats compared to controls, signifying metabolic distress and a direct effect on blood circulation.

These results are in line with those of **(El-Hussainy, 2016)**. who found an increase in cardiovascular parameters (LDL, HDLand Cholesterol) in rats treated with the same xenobiotic.

Taken together, these results confirm the toxic effects of TIO_2 nanoparticles on the cardiovascular system of rats, a sign that the use of this type of technology should be monitored.

3. Biochemical parameters (Carbohydrates, Lipids and Proteins)

- **Carbohydrates**

A decrease in carbohydrate levels in the group treated with titanium dioxide nanoparticles can be observed.

These results are in line with those of **(Armand, 2016; Chen, 2018)**. having highlighted a negative disruption of glucose metabolism following exposure to TiO_2 nanoparticles.

This can be explained by the direct use of glycogen for energy regeneration or following hypoxia. Carbohydrates are the primary and immediate sources of energy, and under stress conditions carbohydrate reserves are depleted to meet increased energy demands.

- **Lipids**

Treatment of Wistar rats with the nanoparticles studied resulted in this increase in lipid levels compared with controls.

These results are not the same as those of **(Husain, 2013)**. who showed in mice a disruption of lipid metabolism following intratracheal instillation of low doses of TiO2 NPs. According to **(Aurousseau, 2002)**. metal nanoparticles can be the source of toxic free oxygen radicals via lipid degradation "lipid peroxidation induction"; thus inducing a loss of lipid molecules.

- **Protein**

Our results indicate a small decrease in protein levels in the group treated with titanium dioxide nanoparticles.

These results differ from those of **(Grara, 2016).** who demonstrated a similar dose-dependent increase in liver protein concentration in the presence of metallic dusts.

These results differ from those of **(Nzengue, 2008)**. the increase in total protein levels can be explained by the triggering of the detoxification process by a regulatory system consisting of enzymes, proteins and antioxidant molecules.

4. Oxidative stress parameters (MDA, GST and GPx)

- **MDA**

The adverse biological effect of nanoparticles is an unavoidable scientific problem because of their small size and high surface activity. In this review, we focus on TiO2 to clarify the toxicological effect and cytotoxic response in order to explore the mechanisms of NP toxicity.

MDA or plasma Malondialdehyde is a marker of lipid peroxidation. It is considered one of the end products of polyunsaturated fatty acid oxidation.

Malondialdehyde is a chemical compound with the formula CH2 (CHO) 2, naturally present in tissues, where it is a manifestation of oxidative stress. It is derived in particular from the action of reactive oxygen derivatives on polyunsaturated fatty acids.

Our results revealed that a decrease in MDA levels in TiO_2-treated rats; reflects that **(Aliabadi, 2016).** who suggested that exposure to TiO_2 resulted in increased MDA content, according to **(Smolinski, 1999).** the production of malondialdehyde (MDA) as an index to assess cell membrane damage by lipid peroxidation.

Our results showed that exposure to TiO_2 decreased the level of (MDA) in the heart, a result that differed from the study by **(Li, 2005)**.

- **GST**

GST plays an important role in protection against oxidative stress **(Hu, 2019).** and is the major enzyme for the detoxification of a wide variety of xenobiotic **(Fujioka, 2007).**

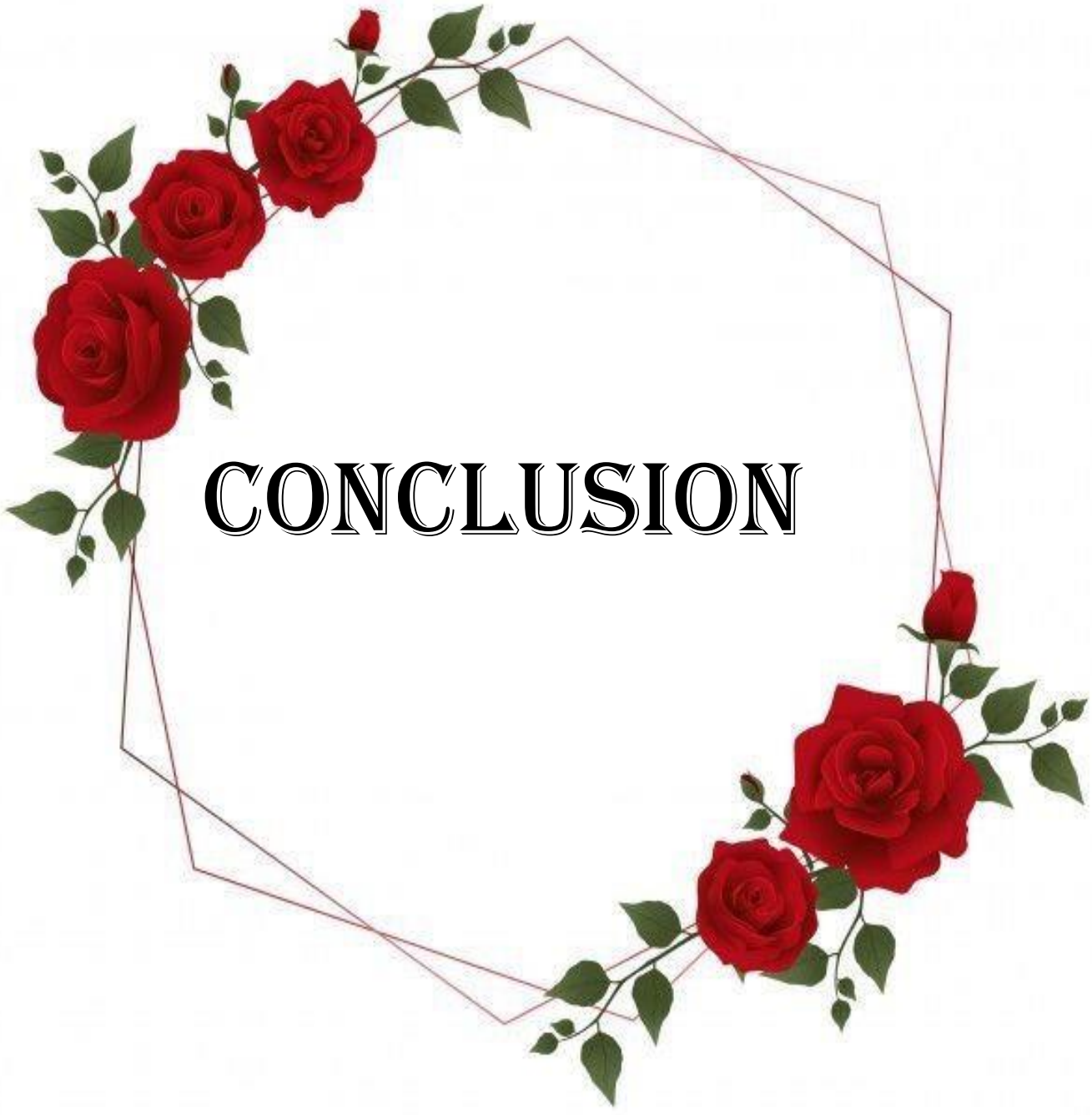
According to our results, we observed a highly significant decrease in the enzymatic activity of cardiac GST, in contrast to **(Meksem et *al.*, 2007; Lai, 2011)**. This decrease in

GST activity is associated with lipid peroxidation, which is confirmed by the very low level of MDA **(Clasen, 2018).**

- **GPx**

Glutathione peroxidase (GPx) is a cytosolic enzyme **(Fanucchi, 2014).** It constitutes the second line of defense against hydro peroxides (**Nedjoud et *al*., 2009**; **Engelking, 2015)**, in fact GPx catalyzes the reduction of hydrogen peroxide via GSH. This reaction is important for eliminating low concentrations of hydrogen peroxide that can damage the cell **(Beutler, 1972).**

Our results show a highly significant decrease in cardiac GPx enzymatic activity in treated rats, in agreement with (**Cui, 2017).**



CONCLUSION

IV. Conclusion

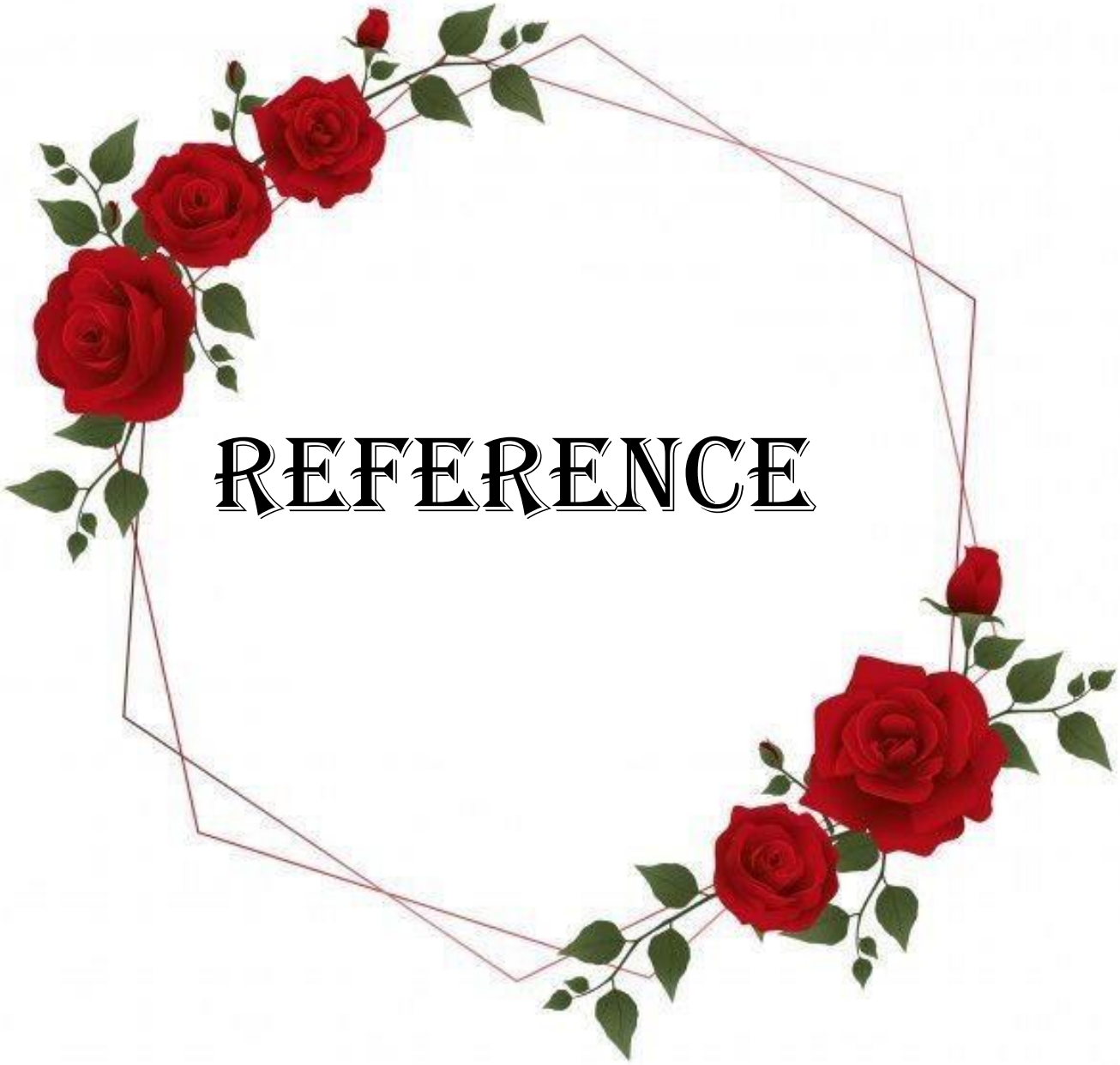
The nanoparticles used in this study are TiO_2 titanium dioxide NPs, which lead to disruption of growth and oxidative stress parameters that differ depending on the dose of administration, and the effect on the cardiovascular system.

We studied the effect of titanium dioxide TiO_2 on some biochemical parameters of the heart such as proteins, carbohydrates and lipids; also on oxidative stress parameters (MDA, GST and GPx).

We investigated the effect of titanium dioxide TiO_2 in 3 groups of Wistar rats. After performing the necessary assays, we found that there was a disturbance in the parameters evaluated as follows:

- A significant decrease in body weight.
- A significant decrease in relative heart weight.
- A significant increase in cholesterol and LDL and a significant decrease in HDL.
- A significant decrease in carbohydrate levels.
- A highly significant increase in lipid levels.
- A significant decrease in protein levels.
- A significant decrease in MDA levels.
- A highly significant decrease in GST levels.
- A highly significant decrease in GPx activity.

So, titanium dioxide TiO_2 NPs clearly change these parameters, and provoke the cardiovascular system.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ANNEXES

1. **Materials used in the various stages of the study**

- **Large equipment and appliance**
  - Centrifuge
  - Analytical balance
  - Precision balance
  - Oven
  - PH meter.
  - Magnetic stirrer
  - Dissecting equipment.
  - Vortex shaker
  - Spectrophotometer
  - Sigma centrifuge.
  - Refrigerator.
  - Water bath
- **Small equipment**
  - Mortar + pestle (manual grinder).
  - Spatula.
  - Baromagnet.
  - Micropipettes.
  - Racks.
  - Test tubes.
  - Glass and plastic dry tubes.
  - Eppondorf tubes for sigma centrifuges.
  - Spectrophotometer cuvettes (plastic and quartz).
  - Aluminum foil.
  - Wattman paper.
  - Beakers.
  - Funnels.
  - Graduated test tubes.
  - Volumetric flasks.
  - Glass flasks.

2. **Calibration curves for metabolite assay**

➢ **Calibration curve for carbohydrate determination**

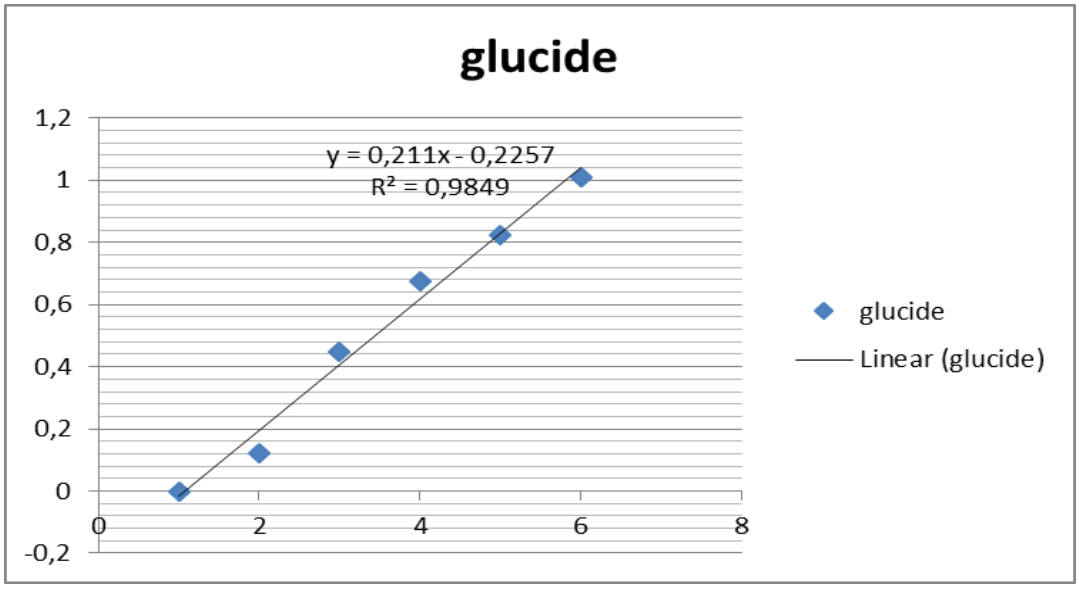

Figure L: Calibration curve for carbohydrate determination

➢ **Calibration curve for lipid determination**

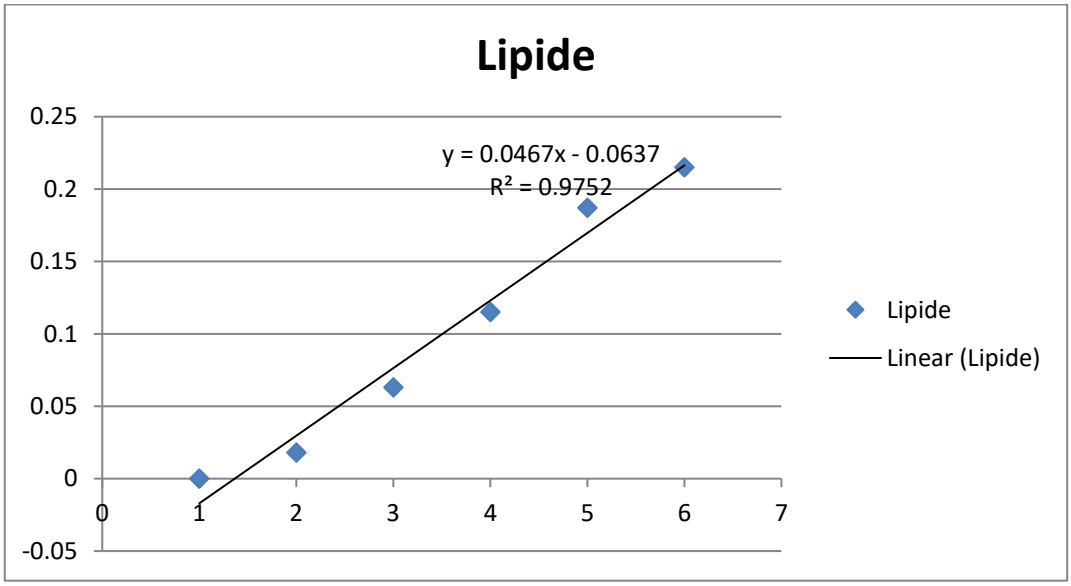

Figure M: Calibration curve for lipid determination

➢ **Calibration curve for protein determination**

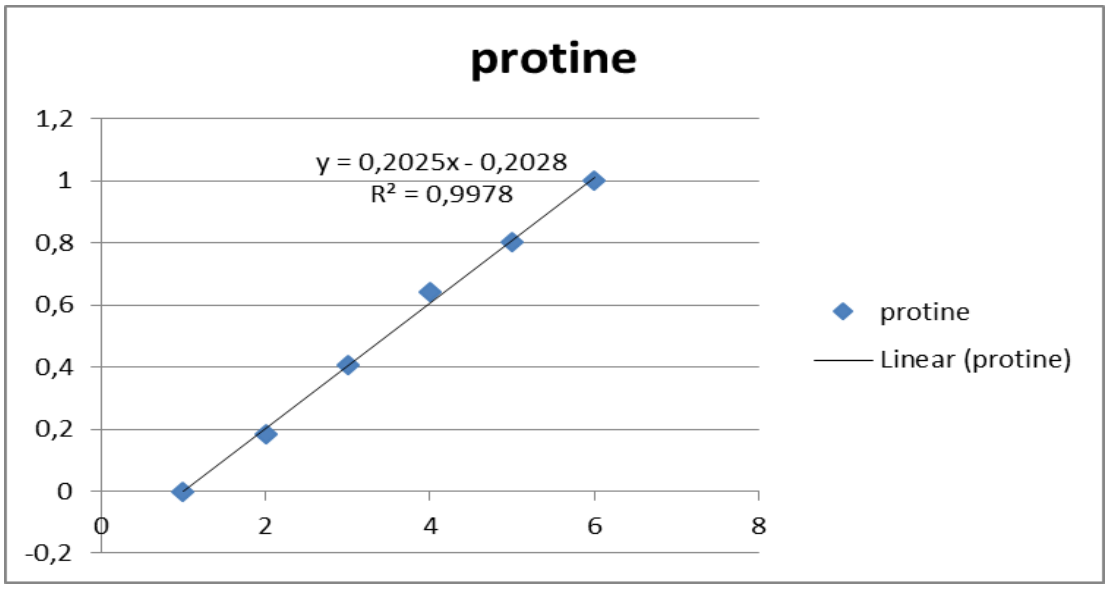

Figure N: Calibration curve for protein determination