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Neurobehavioral impacts of Aluminum oxide nanoparticles on Wistar Rats.

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بسم الله

ملخص

الباركنسون مرض مزمن يصيب الجهاز العصبي و يتلف الذاكرة ، مصدره العديد من العوامل البيئية و الجينية حيث يشارك الإجهاد التاكسي في آليات موت الخلايا العصبية و هذه من أكثر الحالات شيوعا. اهتممنا في هذه الدراسة بتقييم السمية العصبية للألمنيوم المؤكسد في 30 جرذا من السلالة النقية مقسمة إلى ثلاثة مجموعات في كل مجوعة (10 جرذان): المجموعة الأولى التي لوحظت' المجموعة الثانية عولجت ب (5 مغ/كغ/يوم) من الألمنيوم المؤكسد' والمجموعة الثالثة عولجت بجرعة (50مغ/كف/يوم) لمدة 40 يوما.

تنقسم تجربتنا إلى ثلاث مجالات للدراسة: الدراسة السلوكية' ودارسة معلمات العصارة الخلوية للدماغ الكلي' وأخيرا تقييم مستويات الناقل العصبي. حيث أظهرت نتائج السمية العصبية بواسطة جسيمات الألمنيوم المؤكسدة تغيرات في السلوك النفسي للفئران والتي تجلت في حالات القلق و اضطراب الذاكرة والتعلم. من خلال زيادة الإجهاد' وتعطل النوافل العصبية' وتنكس خلايا الدماغ. كشفت الدراسة البيوكيميائية عن اختلال في الجزيئات الرئيسية كالبروتينات والكربوهيدرات والدهون وهدا في نفس وقت تثبيط نمو الجسم (اضطراب الوزن الكلي).

على مستوى أخر كشفت مراقبة المؤشرات الحيوية للإجهاد عن تأثيرات مهمة والتي تتجلى من خلال زيادة نشاط الغليتاتيون س ترونسفيراز. كما أظهرنا أيضا تحريض الإجهاد التاكسي الذي يؤكده زيادة مستوى المانول دي الدهيد. بالإضافة إلى تقييم الناقلات العصبية للاستيلكولينستراز حيث لوحظ فيها انخفاض لدى الفئران المعالجة بجزيئات الألمنيوم المؤكسدة.

الكلمات المفتاحية: الجسيمات النانوية ، أكسيد الألومنيوم ، جرذان من سلالة ويستار، السلوك العصبي و التعرف الاجتماعي

Abstract

Oxidative stress is involved in the mechanisms of cell death in neurodegenerative diseases. Alzheimer's and Parkinson's disease are the most common of these conditions. In these cases, the markers of oxidative stress are unnatural. In this study we were interested in the evaluation of the neurotoxicity of aluminium oxide nanoparticles in 30 rats of the Wistar strain divided into three groups in each of them (10 rats); the first group is a control, the second group treated with (5mg/kg/day) of aluminum oxide nanoparticles and the third group was treated with (50 mg/kg/day) during 40 days. Our experimentation is divided into three areas of study: behavioral study, study of cytosolic parameters of the overall brain and finally the evaluation of neurotransmitter levels. Our results show neurotoxicity by oxidized aluminum nanoparticles. We also demonstrated changes in the psychic behavior of rats which manifested themselves in cases of anxiety, disturbance of memory and learning. Through increased stress, disruption of neurotransmitters and degeneration of brain cells. The biochemical study revealed a disruption of the main macromolecules namely; proteins, carbohydrates, lipids and this at the same time as an inhibition of the body growth (disturbance of the total weight). On another level, monitoring stress biomarkers has revealed significant effects for this molecule, which is manifested by an increase in Gluthation-S-Transferase (GST) activity. We have also demonstrated the induction of oxidative stress confirmed by an increase in the level of ManoldiAldehyde (MDA). In addition, evaluation of acetylcholinesterase neurotransmitters (AChE) shows a decrease in rats exposed to aluminum oxide nanoparticles.

Keywords: Nanoparticles, Aluminium oxide, wistar rats, Neurobehavioural, social recognition.

Résumé

Le stress oxydant est impliqué dans les mécanismes de mort cellulaire lors des maladies neurodégénératives. La maladie d'Alzheimer, la maladie de Parkinson sont les plus fréquentes de ces affections. Dans ces cas, les marqueurs du stress oxydant sont anormaux. Dans cette étude nous nous sommes intéressés à l'évaluation de la neurotoxicité d'aluminium oxydé sur 30 rats de la souche Wistar réparties en trois groupes dans chacun d'eux (10 rats) ; le 1er groupe a observé, le 2ème groupe traité avec (5mg/kg/jour) d'aluminium oxidé et le 3ème groupe à été traité avec une dose de (50 mg/kg/jour) pendant 40 jours.

Notre expérimentation est divisée en trois axes d'études : étude comportementales, étude des paramètres cytosoliques du cerveau globale et en fin l'évaluation de taux des neurotransmetteurs. Nos résultats montrent une neurotoxicité par les nanoparticules d'Aluminium oxydé Nous avons également mis en évidence des changements du comportement psychique des rats qui se sont manifestés par des cas d'anxiété, de perturbation de la mémoire et de l'apprentissage. à travers l'augmentation de stress, la perturbation des neurotransmetteurs et la dégénérescence des cellules cérébrales. L'étude biochimique a mis en évidence une perturbation des principales macromolécules à savoir; protéines, glucides, lipides et ce parallèlement à une inhibition de la croissance corporelle (perturbation du poids total). Sur un autre plan le suivi des biomarqueurs de stress a révélé d'importants effets pour cette molécule, qui ce manifestée par une augmentation d'activité de Gluthation-S-Transférase (GST). Nous avons également mis en évidence l'induction d'un stress oxydatif confirmé par une augmentation du taux de ManoldiAldéhyde (MDA). En outre l'évaluation de neurotransmetteurs d'acétylcholinestérase (AChE) montre une diminution chez les rats exposés aux nanoparticules d'aluminium oxidé.

Mots-clés: Nanoparticules, Aluminium oxydé, rats wistar. Neurocomportementaux, reconnaissance sociale.

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In the Name of Allah, the Most Merciful, the Most

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I hold strongly to dedicate this modest work to my dear father, a unique being, a father of whom I would be very proud

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Halaimia Amira

List of abbreviations

NPs	Nanoparticles
Al_2O_3	Aluminum oxide
AD	Alzheimer's Disease
PD	Parkinson's Disease
HD	Huntington's Disease
NS	Nervous System
CNS	Central Nervous System
PNS	Perepheral Nervous System
IPCC	Intergovernmental Panel on climate change
Wm^{-2}	Watt per square meter
H_2SO_4	Sulfuric acid
Nm	Newton-meter
DNA	Deoxyribonucleic acid
TiO_2	Titanium Dioxide Pigment
ROS	Reactive oxygen species
Fe_2O_3	Ferric oxide
TNF	Tumor necrosis factor
LDH	Lactate dehydrogenase
Cr	Chrome

Zn	Zinc
PZC	Point of zero
XPS	X ray photoelectron spectroscopy
FDA	Food and Drug Admenistrartion
NIOSH	National Institue of Occupational Safety and Health
GST	Glutathione S-transferase
MDA	Malondialdehyde
EDTA	Ethylene Diamine Tetra Acetic Acid
TCA	Trichloroacetic acid
DTNB	5, 5’-dithiobis (2-nitrobenzoic acid or Ellman's reagent
BBC	Brilliant blue of coomassie

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Summary

Summary

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INTRODUCTION

Introduction

Aluminum is a very interesting material that we used in a huge variety of products including cons, foils, kitchen utensils, aeroplane parts and in various medical applications. This is because of its advantages such as its thermal conductivity, chemical, and physical properties, and it is not toxic **(Shirai et *al*., 2010)**. However, this molecule can be exposed to chemicals or biological reactions which can transform it into a very toxic oxidized molecule.

The oxidized aluminum nanoparticles released in all ecosystems, can cause serious impacts on human health and on the environment. The toxicity of NPs is largely determined by their physical and chemical characteristics, such as their small size which allows them to penetrate through epithelial and endothelial barriers into the lymph and blood to be carried by the bloodstream and lymph stream to different organs and tissues, including the brain, heart, liver, kidneys, spleen, bone marrow, and nervous system **(Dukhin et Labib, 2013 ; Oberdorster et *al*., 2005)**,which can manifest in several diseases such as neurodegenerative disease, which are caused by the progressive death of neurons in different regions of the nervous system. The progressive loss of nerve cells is what gives rise to the neurological and neuropsychological signs and symptoms characteristic of each of these disorders.

With few exceptions, the causes of neurodegenerative diseases are essentially unknown, and even when they have been identified, the mechanisms by which they initiate the disease remain, and we still do not know with certainty how mutant huntingtin provokes the disease.

One of the most ferocious debates surrounding the etiology of neurodegenerative disorders concerns the relative roles of genetic and environmental factors in the initiation of these diseases **(Zedborski et *al*., 2003).**

On the basis of these data, we hypothesized that aluminum oxide nanoparticles induced neurotoxicity.

This study is dividing into two parts; bibliographic part which include generalities on the nervous system, nanoparticles and their effects, and informations about aluminum oxide, the second part which is the practical part contains our experiment, their results, their interpretations, and discussion. The objective of our work is the study of the toxic effects of aluminum oxide at the level of central nervous system in Wistar rats, and identifies changes in biochemical and enzymatic parameters of the brain, even the neurobehavioral impacts of this molecule on rats.

BIBLIOGRAPHIC PART

Bibliographic Part

Chapter I: Generalities.

1. Generalities on neurotoxicology

Neurotoxicology is defined as the science that deals with the adverse effects of naturally occurring and synthetic chemical agents on the structure or function of the nervous system. In this context a neurotoxin is a naturally occurring or synthetic chemical agent that can cause a functional or structural change in the nervous system **(Harris et Blain, 2004).** Nanoparticles induce neurotoxicity following inhalation, oral administration, intravenous administration, or injection **(Yuqing et *al.*, 2020).** Neurotoxicity refers to the direct or indirect effect of chemicals that disrupt the nervous system of humans or animals **(Spencer et Pamela, 2014).** Neurotoxicity can result from exposure to substances used in chemotherapy, radiation treatment, drug therapies, and organ transplants, as well as exposure to heavy metals such as lead and mercury, certain foods and food additives, pesticides, industrial and/or cleaning solvents, cosmetics, and some naturally occurring substances **(Koroshetz et *al.*, 2019*).*** Some of the effects of neurotoxicity may appear immediately, while others can take months or years to manifest. He depends on various different factors such as the characteristics of the neurotoxin, the dose a person has been exposed to, ability to metabolism and excrete the toxin, the ability of affected mechanism and structures to recover and vulnerable a cellular target. Some of the symptoms of neurotoxicity include: Paralysis or weakness in the limbs, Altered sensation, tingling and numbness in the limbs, Headache, Vision loss, Loss of memory and cognitive function, Uncontrollable obsessive and/or impulsive behavior, Behavioral problems, Sexual dysfunction, Depression, Loss of circulation, Imbalance and Flu-like symptoms **(Robertson, 2019*).***

1.1. Brain

In our nervous system, the command center is the brain. This three-pound organ (approximately 1.4kg) is made up of billions of cells called neurons, the junctions between them known as synapses **(Goumidi et Fettah, 2020).** The brain controls many of the body's functions including sensation, thought, movement, awareness, and memory. The surface of the brain is known as the cerebral cortex. The surface of the cortex appears bumpy thanks to the grooves and folds of the tissue. Each groove is known as sulks, while each bump is known as a gyros (**Nielsen et *al.*, 2013).**

1.2. Brain histology

The nervous system **(NS)** is the set of systems that bring the body into a state of balance.It is subdivided into two systems: central **(CNS)** and peripheral **(PNS).**

- **CNS:** consists of the brain and spinal cord. It is referred to as "central" because it combines information from the entire body and coordinates activity across the whole organism. The brain is protected by the skull (the cranial cavity) and the spinal cord travels from the back of the brain, down the center of the spine, stopping in the lumbar region of the lower back. The brain and spinal cord are both housed within a protective triple-layered membrane called the meanings. The central nervous system has been thoroughly studied by anatomists and physiologists, but it still holds many secrets; it controls our thoughts, movements, emotions, and desires. It also controls our breathing, heart rate, the release of some hormones, body temperature, and much more. The retina, optic nerve, olfactory nerves, and olfactory epithelium are sometimes considered to be part of the CNS alongside the brain and spinal cord. This is because they connect directly with brain tissue without intermediate nerve fibers **(Newman, 2017).**
- **PNS:** This is the line of communication between the SNC and the organization, and is located at the end of SNC. It is formed mainly from the nerves (cranial and spinal) originating from the brain and the spinal cord which comprises two typesof pathways **(Marieb, 2005).**

1. 3. The components of the human brain and their functions

The human brain is composed of 3 parts: Cerebrum, Cerebellum and Brainstem **(Figure 02):**

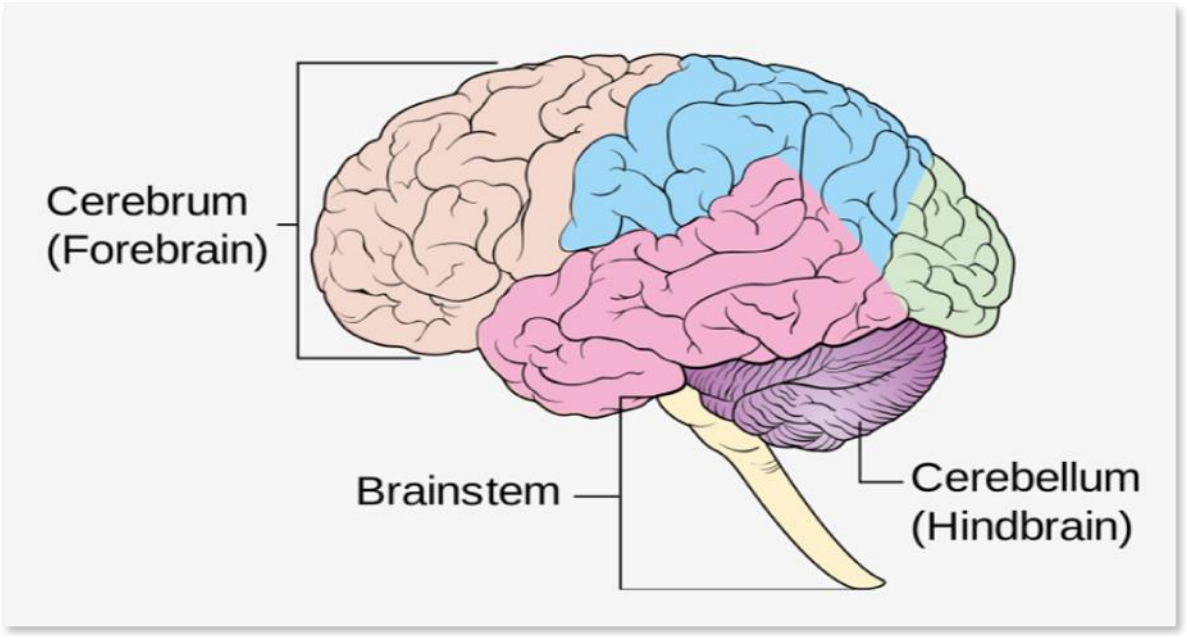


Figure 01: Brain components **(Goumidi et Fettah, 2020).**

1.3.1 Cerebrum: Is the largest part of the brain and is composed of right and left hemispheres. It emotions, learning and fine control of movement (**Hines et *al.*, 2018).** Both sides of the brain work together performs higher functions like interpreting touch, vision and hearing, as well as speech, reasoning, to produce various functions **(Herculano et Hum, 2009).** The cerebral hemispheres have distinct fissures, which divide the brain into lobes. Each hemisphere has 4 lobes: **frontal, temporal, parietal,** and **occipital**. Each lobe may be divided, once again, into areas that serve very specific functions. It's important to understand that each lobe of the brain does not function alone. There are very complex relationships between the lobes of the brain and between the right and left hemispheres (table02) (**Hines et *al.*, 2018).**

Table 01: The location and function of different lobes of the brain. **(Guberman et *al.*, 1986)**

Lobes	Location and function
Frontal lobe	It is located at the front of the brain, this region is involved in the voluntary control of skeletal muscles; the personality ; intellectual processes, verbal communication and decision making
Parietal lobe	Located behind the frontal lobe, it plays an important role in somesthesia, this is to say the perception and integration of different sensory modalities (vision, touch, hearing); but also in the perception of space and in attention. Lesions affecting this area can cause a personality change showing psychotic tendencies.
Temporal lobe	Localized on the side of the brain; it includes the Broke area responsible for the language and the Warnock area which represents an auditory area, these two areas are connected thanks to an arched beam.
Occipital lobe	Located at the back of the brain, the latter represents the visual center; it allows the orientation and processing of visual analysis performed in the primary visual cortex.

The diencephalon: The diencephalon is an important autonomous region of the forebrain, almost entirely covered by the cerebral hemispheres; it comprises the following structures (Table 02):

Table 02: The location and functions of the structures of the diencephalon **(Saalmann et *al.*, 2011).**

Structure	Location and function
The thalamus	The thalamus is an even organ located just below the ventricle lateral, it represents about 80% of the diencephalon. It is the relay of all sensory impulses, except those of smell, towards the cerebral cortex.
Hypothalamus	The hypothalamus has several nuclei interconnected to other regions of the brain, most of the functions it performs are linked to the regulation of visceral activities including cardiovascular regulation, regulation of body temperature, water and electrolyte balance, gastrointestinal activities and hunger, sleep and wakefulness, sexual response, emotions and control of endocrine functions by stimulation of the adenohypophysis.
Epythalamus	It is a pineal gland that secretes melatonin, which could play a role in the onset of puberty.
Pituitary (hypophysis)	The pituitary gland is an endocrine gland that includes a region anterior, the adenohypophysis (antehypophysis), and a region posterior, the neurohypophysis.

1.3.2. Cerebellum

The cerebellum is a major structure of the hindbrain that is located near the brainstem. This part of the brain is responsible for coordinating voluntary movements. It is also responsible for a number of functions including motor skills such as balance, coordination, and posture. The cerebellum makes up just 10% of the total volume of the brain, yet it contains an estimated 50%to 80% of the brain's neurons **(Cherry, 2020).** What the cerebellum does to sensor motor and vestibular control, it also does to cognition, emotion and autonomic function. This hypothesis is based on the theories of dysmetria of thought and the universal cerebellar transform, which hold that the cerebellum maintains behavior around a homeostatic

baseline, automatically, without conscious awareness, informed by implicit learning, and performed according to context **(Schmahmann, 2018).**The increasing appreciation of the role of the cerebellum in motor and non-motor functions is crucial to understanding the outcomes of acquired cerebellar injury and developmental lesions in high-risk fetal and neonatal populations, children with cerebellar damage (e.g. posterior fossa tumors), and neurodevelopmental disorders (e.g. autism). We review available data regarding the relationship between the topography of cerebellar injury or abnormality and functional outcomes. We report emerging structure function relationships with specific symptoms: cerebellar regions that interconnect with sensorimotor cortices are associated with motor impairments when damaged; disruption to posterolateral cerebellar regions that form circuits with association cortices impact long-term cognitive outcomes; and midline posterior vernal damage is associated with behavioral dysregulation and an autism-like phenotype. We also explore the impact of age and the potential role for critical periods on cerebellar structure and child function. These findings suggest that the cerebellum plays a critical role in motor, cognitive, and social behavioral development, possibly via modulatory effects on the developing cerebral cortex **(Stoodley et Limperopoulos, 2016).**

1.3.3. Brainstem

Brainstem as a connection between brain and spinal cord has a critical role to transfer information from the central nervous system (CNS) to the peripheral nervous system (PNS). Brainstem is divided into three regions: the midbrain, the pons, and the medulla (from rostral to caudal). Contains main motor and sensory nerves and facilitates communication between the cerebrum, cerebellum, and spinal cord **(Eskandari et *al.*, 2020).**The brainstem is known to be a major center involved in the integration of sensory information for motor output; however, limitations of functional imaging in humans have impaired our knowledge about the individual roles of sub-nuclei within the brainstem. Thus, the bulk of our knowledge surrounding the function of the brainstem is based on anatomical and behavioral studies in non-human primates, cats, and rodents, despite studies demonstrating differences in the organization of visuomotor processing between mammals **(Winston et *al.*, 2021).**

1.3.4 The Spinal Cord

The spinal cord consists of the axons of major sensory and motor pathways (white matter) and a central region of gray matter (nuclei). The gray matter is composed of the cell bodies of

motor neurons and of interneurons which compose the circuitry for lower level behavioral integration such as reflexes. Traditionally, the spinal cord has been thought to serve only "lower" or simpler functions, while complex or "higher" functions have been attributed to the cerebrum, especially the cerebral cortex **(Thompson et *al.*, 1980).**

2. Cells of the nervous system

The cells of the nervous system are subdivided into two classes gliocytes and neurons.

• **Gliocytes:** these are support cells which provide a variety of functions necessary for the proper functioning of the nervous system; there are several types of glial cells:

- Astrocytes: These are the most abundant and voluminous cells of glial origin, they have a star shape with several extensions; a distinction is then made between type I astrocytes, which are in contact with the blood capillaries, and type II astrocytes, which surround the neurons and synaptic slits, thus preventing the dispersion of neurotransmitters by capturing and metabolizing them **(Sun et *al.*, 2012).**

- Oligodendrocytes: smaller than astrocytes and with fewer extensions, they play the role of a support network for neurons but above all ensure the myelination of their axons to isolate them and accelerate nerve impulses **(kierszenbaum, 2006).**

- Ependymocytes: they have a cubic or cylindrical shape, they are often ciliated and form a simple epithelium which lines the central cavities of the brain; they are used to create cerebrospinal fluid and promote its circulation **(Minabe et *al.*, 2015).**

- Microglia: small cells generally mobile and star-shaped, they belong to the group of macrophages thus forming the main defense active immune system of the central nervous system.

• **Neurons (nerve cells**): amitotic cell except for a few specific areas where weak neuronal mitosis takes place, especially in the hippocampus, neurons are blocked in interphase and therefore no longer divide, which makes them cells with extreme longevity, thus explaining the rarity of tumors of neuronal origin **(Silbernagl et *al.*, 2001).**

3. Functioning of the nervous system

3.1 Nerve flow and synaptic integration

The transmission of nerve impulses by nerve cells is due to the properties electrochemical of their plasma membranes. The signal is transmitted from a nerve cell to another by junctions

called synapses **(Purves et *al*., 2004; Brooker et *al*., 2001).** a very particular type of these synapses is the one that connects the neuron to a cell effector such as a muscle fiber, this synapse is then called the neuromuscular junction **(Gerard et *al*., 2002; Ganong et *al*., 2005).** The passage of information through a synapse is provided by chemical molecules called neurotransmitters **(Kolb et *al*., 2002).** At the level synapses, when nerve impulses reach presynaptic endings trigger events that result in the release of a neurotransmitter through the synaptic cleft which modifies the permeability of the postsynaptic membrane **(Marieb, 2005).** Na + channels voltage dependent are responsible for the initiation and propagation of these action potentials along the axon, the sodium channel forms a pore in the membra.ne which is highly selective for Na + ions. In the quiescent state, the channels are closed. When the membrane is depolarized, these channels are open **(figure 02) (Guéguen et *al*., 2005; Imbert, 2006).**

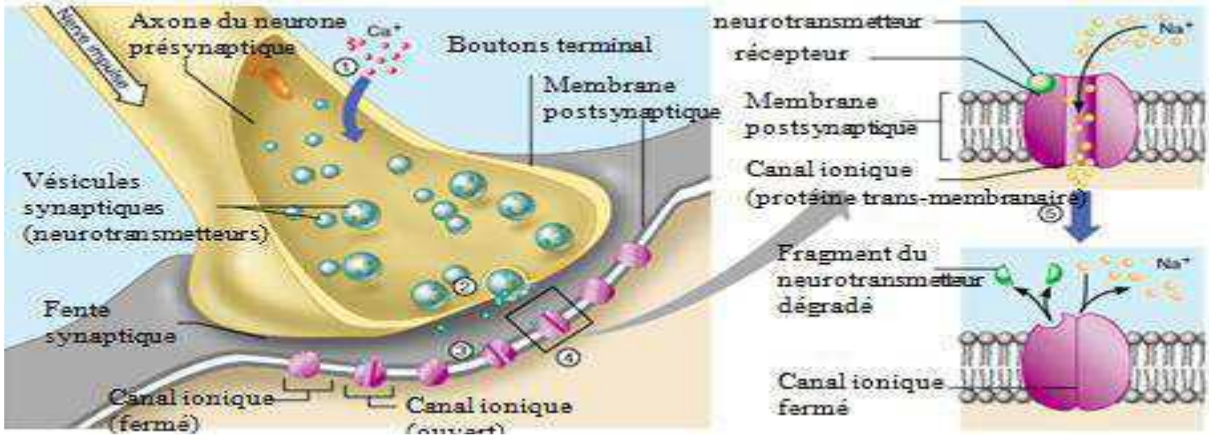


Figure 02: Mode of transmission of nerve signals **(Marieb, 2005)**.

3.2. Function of the brain

Located inside the skull, the brain manages and coordinates most of our functions:

- the internal functions of the body (heartbeat, blood circulation, muscle contractions or digestion),
- the so-called superior functions (thought, emotions, personality, ability to communicate or learn),
- And finally, the five senses, which allow us to relate to the outside world: sight, hearing, touch, smell and taste.

The brain works like a central computer. He constantly receives a wealth of information about what is going on inside and outside the body. This information comes from the spinal

nerves, which run throughout the body, and from the cranial nerves, located inside the skull. Each piece of information is classified, analyzed and processed by nerve cells, which are called: "neurons". These are connected to each other, and are able to manage several pieces of information at the same time. It is what allows us to see, speak, walk and breathe all at the same time and without having to think about it. These billions of neurons are surrounded and nourished by other cells called glial cells, which are ten times more numerous than neurons. They represent 90% of the composition of the brain. There are different types: astrocytes, oligodendrocytes, ependymocytes and microglial cells **(Bensmail et Bouayad, 2012).**

3.3. Neurotransmitters

It's a substance which is released at the end of a nerve fiber by the arrival of a nerve impulse and by diffusing across the synapse or junction effects the transfer of the impulse and to another nerve fiber or muscle fiber or some receptor **(John et Sons, 2001).**They diffuse towards the postsynaptic region to activate their receptors and are then rapidly eliminated **(Guénard, 2001)** either by diffusion outside the synaptic cleft, or degraded by a specific enzyme and reabsorbed by the terminal button **(Guéguen et *al.*, 2005).**

3.3.1. Key neurotransmitters

The first neurotransmitter to be discovered was a small molecule called acetylcholine. It plays a major role in the peripheral nervous system, where it is released by motor neurons and neurons of the autonomic nervous system. It also plays an important role in the central nervous system in maintaining cognitive function. Damage to the cholinergicneurons of the CNS is associated with Alzheimer disease.

- **Glutamate (Glu):** is an excitatory amino acid neurotransmitter in the mammalian central nervous system could play a dual role in mechanisms underlying the maintenance of cellular homeostasis as an excitatory neurotransmitter in the central neurocrine system and an extracellular signal mediator in peripheral autocrine and/or paracrine tissues **(Hinoi et *al.*, 2003).**
- **Acetylcholinesterase (AChE):** Once at the nerve end, the motor impulse causes the calcium channels to open, which triggers a massive entry of calcium ions into the cell. $Ca2^{+}$ promotes AChE secretion in the synaptic cleft **(Guénard, 2001; Kolb et *al.*, 2002).** Each two Ach molecules diffuse on the other side and linked with a cholinergic receptor **(Lodishet *al.*, 2005; Martin et *al.*, 2006**). the latter causing the opening of a

sodium channel, which promotes entry of sodium ions into the muscle fiber, thereby depolarizing the postsynaptic membrane and creating plaque potential **(Lodish et *al*., 2005).** Depending on the number of receptors activated, this potential may exceed a threshold value and thus trigger a muscle action potential which will diffuse to the entire muscle membrane and cause the muscle fiber to contract (fig. 10). Ach molecules are quickly destroyed by the enzyme AchE present at the two-molecule synaptic cleft; acetate and choline **(Martin et *al*., 2006).**

- **Adrenaline:** Adrenaline plays an important role in regulating all organs and the body's reaction to stress, in critical or harmful situations (danger, trauma, infection, cooling). The release of this neurotransmitter then involves a whole series of involuntary nervous reactions, such as the acceleration of rhythms cardiac and respiratory to prepare the body for the effort of fight or flight **(Martin et *al*., 2006; Verkhratsky et Butt, 2007).**
- **Dopamine:** is a neurotransmitter synthesized by certain nerve cells from tyrosine (Bear et al., 2016). She is the inhibitory neurotransmitter of a small group of neurons that affected muscle movement, tissue growth, and muscle function immune system. It is involved in the secretion of growth hormone **(Kazushige et *al*., 2000; Meiser et *al*., 2013; Dias et *al*., 2014).**
- **Gamma aminobutyric acid** is an inhibitory neurotransmitter, the receptor of which is also binds to valium. GABA and valium help relieve anxiety and decrease anxiety. It is the main inhibitory neurotransmitter and plays a critical role in the regulation of neuronal activity (membrane hyperpolarization) in vertebrates and invertebrates **(Buckingham et *al*., 2005; Verkhratsky et Butt, 2007).**
- **Serotonin:** is an inhibitory neurotransmitter would intervene in phenomena such as sleep, consciousness and emotional states **(Wang et *al*., 2007**). The serotonergic system involves 5-HT which is considered to be the most important neurotransmitter involved in anxiety. It has been shown that an anxiolytic acting via the cerebral serotonergic system decreases the level of 5-HT in the brain **(Hoyer et *al*., 2002; Caramaschi et *al*., 2007; Meneses et *al*., 2007).**
- **Morphine:** Natural morphines in the human body are neuropeptides called endorphins and enkephalins **(Martin et *al*., 2006).** Enkephalins are inhibitors and competitors that found in the synapses dealing with the influxes of mood, emotion and pain: by

attaching to postsynaptic receptors instead of effector neurotransmitters, they block the propagation of nerve impulses **(Guéguen et *al*., 2005; Nieuwenhuys et *al*., 2008).**

3.4. Major neuronal pathologies

Alzheimer's disease: The best example of cerebral pathologies is given by the most common neurodegenerative disease: Alzheimer's disease. During this pathology, we observe a diffuse loss of brain neurons. This degeneration, although diffuse, preferentially affects 2 areas: the cerebral cortex and the hippocampus. This neuronal loss is responsible for cerebral atrophy; a decrease in the volume of brain tissue (Figure 03) **(Meziani, 2011).**



Figure 03. Difference between a normal brain and a atrophy brain (Alzheimer's disease) **(Serge, 2008).**

This tissue void is filled by a parallel increase in the fluid volume occupied by cerebrospinal fluid. We thus observe an increase in the size of the cerebral ventricles and cortical grooves (Figure 04) **(Meziani, 2011)**

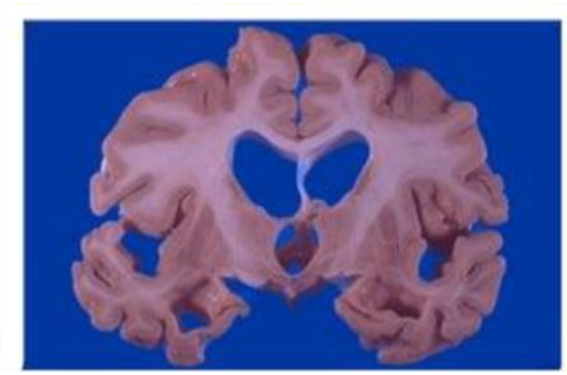


Figure 04. Ventricular dilation Alzheimer's disease **(Serge, 2008).**

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Parkinson's disease: neurodegenerative disease that results from slow and progressive death neurons in an area of the brain that plays an important role in controlling our movements. This is why people with the disease gradually make rigid, jerky gestures and uncontrollable **(Defebvre et Vérin, 2006; Verkhratsky et Butt, 2007).**

- Brain tumor: multiplication in the brain of abnormal cells. The tumor may be benign or malignant **(Peterson et *al.*, 2015**

Chapter II : Nanoparticles

1.Definition of nanoparticles

Nanoparticles are discrete nanometer (10-9 m)-scale assemblies of atoms. Thus, they have dimensions between those characteristic of ions (10-10 m) and those of macroscopic materials. They are interesting because the number of atoms in the particles is small enough, and large enough fraction of them are at, or near surfaces, to significantly modify the particle's electronic, atomic, and magnetic structures, chemical and physical properties, and reactivity relative to the bulk material. Nanoparticle surfaces themselves may be distinctive. Particles may be terminated by atomic planes or clusters that are not common, or not found, at surfaces of the bulk mineral. These and other size-related effects will lead to modified phase stability and changes in reaction kinetics **(Banfield et Zhang, 2001).**

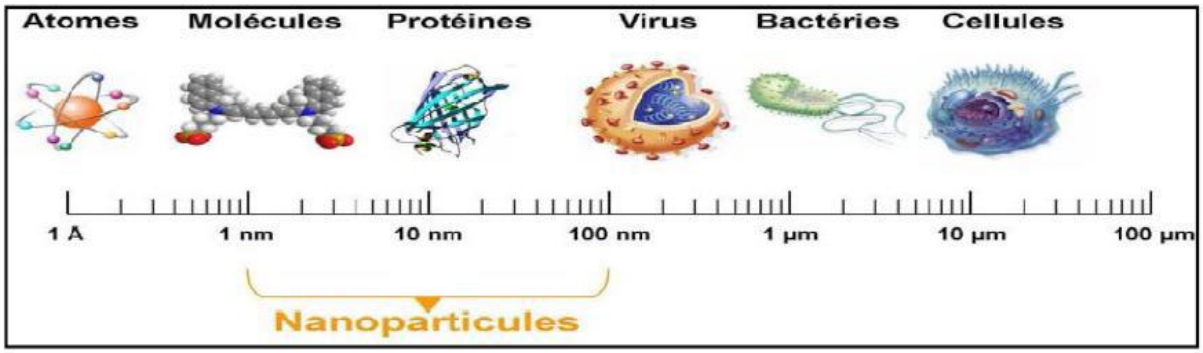


Figure 05: Range of sizes of nanoparticles compared to those of major chemical andbiological structures **(Maafa et Merzouk, 2015).**

2. Characteristics of Nanoparticles that Influence Toxicity

The toxicity of NPs is largely determined by their physical and chemical characteristics, such as their size, shape, specific surface area, surface charge, catalytic activity, and the presence or absence of a shell and active groups on the surface.

The small size of NPs allows them to penetrate through epithelial and endothelial barriers into the lymph and blood to be carried by the bloodstream and lymph stream to different organs and tissues, including the brain, heart, liver, kidneys, spleen, bone marrow, and nervous system **(Dukhin et Labib, 2013; Oberdorster et *al.*, 2005)**, and either be transported into cells by transcytosis mechanisms or simply diffuse into them through the cell membrane. Nanomaterials can also increase access to the blood stream through ingestion

(Holsapple et *al.*, 2005; Hoet et *al.*, 2004). Some nanomaterials can penetrate the skin **(Rasmussen et *al.*, 2006; Shneider et *al.*, 2009)** and even greater microparticles can penetrate skin when it is flexed **(Tinkless et *al.*, 2003)**. Nanoparticles, because of their small size, can extravasate through the endothelium in inflammatory sites, epithelium (e.g., intestinal tract and liver) and tumors or penetrate microcapillaries **(Singh et Lilard, 2009)**. Experiments modeling the toxic effects of NPs on the body have shown that NPs cause thrombosis by enhancing platelet aggregation **(Radomski et *al.*, 2005)**, inflammation of the upper and lower respiratory tracts, neurodegenerative disorders, stroke, myocardial infarction, and other disorders **(Madl et *al.*, 2014; Lucchini et *al.*, 2012; Zhu et *al.*, 2009)**. Note that NPs may enter not only organs, tissues, and cells, but also cell organelles (e.g: mitochondria and nuclei; this may drastically alter cell metabolism and cause DNA lesions, mutations, and cell death) **(Barua et Mitragotri, 2014)**.

Recent studies have shown that contact of lung tissue with NPs about 50 nm in size leads to perforation of the membranes of type I alveolar cells and the resultant entry of the NPs into the cells. This, in turn, causes cell necrosis, as evidenced by the release of lactate dehydrogenase **(Ruenraroengsak et *al.*, 2012)**. There is evidence that NPs penetration increases the cell membrane fluidity **(Wang et *al.*, 2012)**. On the other hand, the formation of reactive oxygen species (ROS) induced by peroxidation of membrane lipids may lead to the loss of membrane flexibility, which, as well as an abnormally high fluidity, inevitably results in cell death.

In addition, the NP cytotoxicity may interfere with cell differentiation and protein synthesis, as well as activate proinflammatory genes and synthesis of inflammatory mediators. It should be specially noted that normal protective mechanisms do not affect NPs; macrophage uptake of large pegylated nanoparticles is more efficient than uptake of small ones, which leads to accumulation of NPs in the body **(Walkey et *al.*, 2012)**. Superparamagnetic iron oxide NPs have been demonstrated to disturb or entirely suppress osteogenic differentiation of stem cells and activate the synthesis of signal molecules, tumor antigens, etc **(Kostura et *al.*, 2004; Chen et *al.*, 2010)**. In addition, interaction of NPs with the cell enhances the expression of the genes responsible for the formation of lysosomes **(Kedziorek et *al.*, 2010),** disturbs their functioning **(Puppi et *al.*, 2011)** and inhibits protein synthesis **(Wang et *al.*, 2017; Poirier et *al.*, 2014)**. A study on the toxic effects of NPs of different compositions on lung epithelial cells and human tumor cell lines has shown that NPs stimulate the synthesis of inflammation mediators, e.g., interleukin 8 **(Choi et *al.*, 2009).**

According to Park, who studied the expression of proinflammatory cytokines in vitro and in vivo, the expressions of interleukin 1 beta (IL-1β) and tumor necrosis factor alpha (TNFα) are enhanced in response to silicon NPs **(Park et Park, 2009).**

Oxidation as well as action of various enzymes on the shell and surface of NPs, results in their degradation and release of free radicals. In addition to the toxic effect of free radicals expressed as oxidation and inactivation of enzymes, mutagenesis, and disturbance of chemical reactions leading to cell death, degradation of NPs leads to alteration or loss of their own functionality (e.g., the loss of the magnetic moment and the changes in the fluorescence spectrum and transport or other functions) **(Lévy et *al*., 2010; Liu et *al*., 2012).**

In summary, the most common mechanisms of NP cytotoxicity are the following:

- NPs may cause oxidation via formation of ROS and other free radicals.
- NPs may damage cell membranes by perforating them.
- NPs damage components of the cytoskeleton, disturbing intracellular transport and cell division.
- NPs disturb transcription and damage DNA, thus accelerating mutagenesis.
- NPs damage mitochondria and disturb their metabolism, which leads to cell energy imbalance.
- NPs interfere with the formation of lysosomes, thereby hampering autophagy and degradation of macromolecules and triggering the apoptosis.
- NPs cause structural changes in membrane proteins and disturb the transport of substances into and out of cells, including intercellular transport.
- NPs activate the synthesis of inflammatory mediators by disturbing the normal mechanisms of cell metabolism, as well as tissue and organ metabolism **(figure 05).**

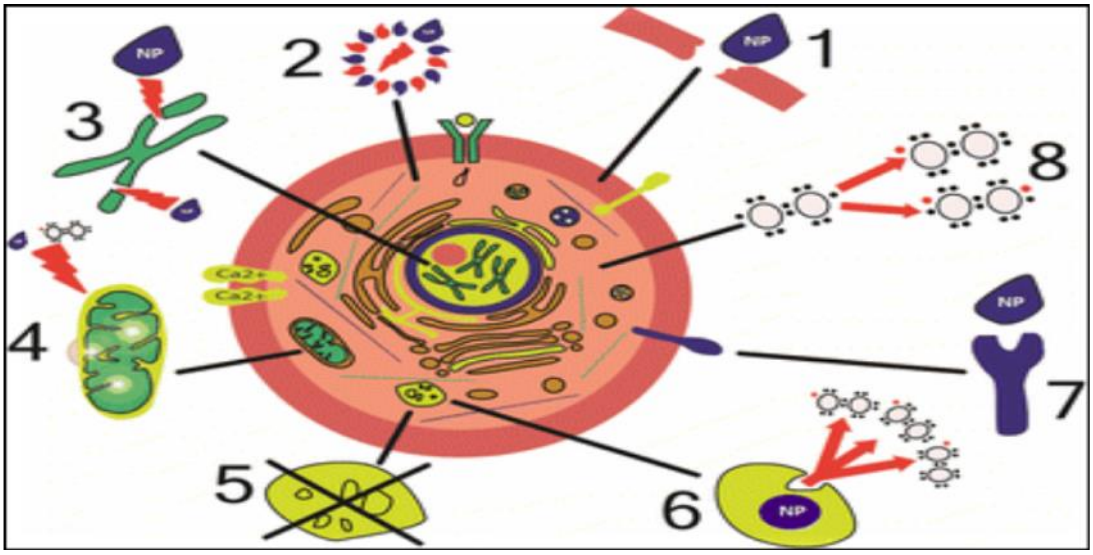


Figure 06. Mechanisms of cell damage by nanoparticles. (1) Physical damage of membranes **(Ruenraroengsak et *al.*, 2012; Zhao et *al.*, 2011; Hu et *al.*, 2011).** (2) Structural changes in cytoskeleton components **(Mao et *al.*, 2015 ; Wu et *al.*, 2010)**. (3) Disturbance of transcription and oxidative damage of DNA **(Soenen et *al.*, 2011; Schmid, 2008).** (4) Damage of mitochondria. (5, 6, 7,) Activation of inflammatory mediators.

3. Classsification of nanoparticles and nanomaterials

Nanoparticles can be classified into various types, according to their size, shape, and material properties.

3.1 Classification of nanoparticles according to their forme of use

NPs can be classified according to their forme of use into difrent types:

- ✓ **Dimension 0 materials:** these materials are in dispersed form, randome or organized, like in colloidal crystals for optics or magnetic fluids.
- ✓ **Dimension 1 materials:** these materials are in the forme of nanofils and nanotube.
- ✓ **Dimension 2 materials:** these materials are in the forme of thin layer, as in deposits of thick coating obtained by plasma spraying or aggregates or electrochemical means.
- ✓ **Dimension 3 materials:** these materials are in the forme of compacts as in nanostructural metals and ceramics **(Taib, 2018).**

3.2. Classification of nanoparticles depending of their source

According to the source of NPs, we classified:

- **The nanoparticles of natural origines**

Biologic: like protenes nanoparticles, for exemple albumine (major molecul of transport in the organism), liposoms (the most mature form of NPs) witch contains amphiphilic compounds, as in phospholipids, glycolipids, and aminolipids of the cell membrane **(Debbage et Jaschke, 2008)**, the DNA of 2,5 nm of demension, some bacteria of 30 nm of demension, and virus between 10 to 60 nm of demension **(Prosie et *al.*, 2008)**.

Mineral or environmental: as in armospheric dust, the fine fraction of desert sand and fumes originating from volcanic activity or forest fires **(Prosie et *al.*, 2008)**.

- **The nanoparticles of human origines**

Manufactured NPs are nanomaterials synthetised for technological applications, such as ultrafine metals and ultrafine particles of titanium oxide **(Boczkowski et Lanone, 2010).**

The bulk nanoparticleslike carbon black**,** smoked silica, oil smoke, the smog, diesel particles **(Boczkowski et Lanone, 2010; Prosie et *al.*, 2008).**

3.3. Classification of nanoparticles according to their nature and function

- Organic nanoparticles

The organic nanoparticules like micells, wich is a spheroidal aggregate formed by the self assembly of a phospholipid bilayer whose polar head is hydrophilic and the tail hydrophobic. Also nanoparticles based on polymers and carbon nanotubes have only two nanometric dimensions, their length can currently be around a centimeter. They can be observed by electron microscopy, Raman spectroscopy, nuclear magnitic resonance spectroscopy or photoluminescence **(Taib, 2018).**

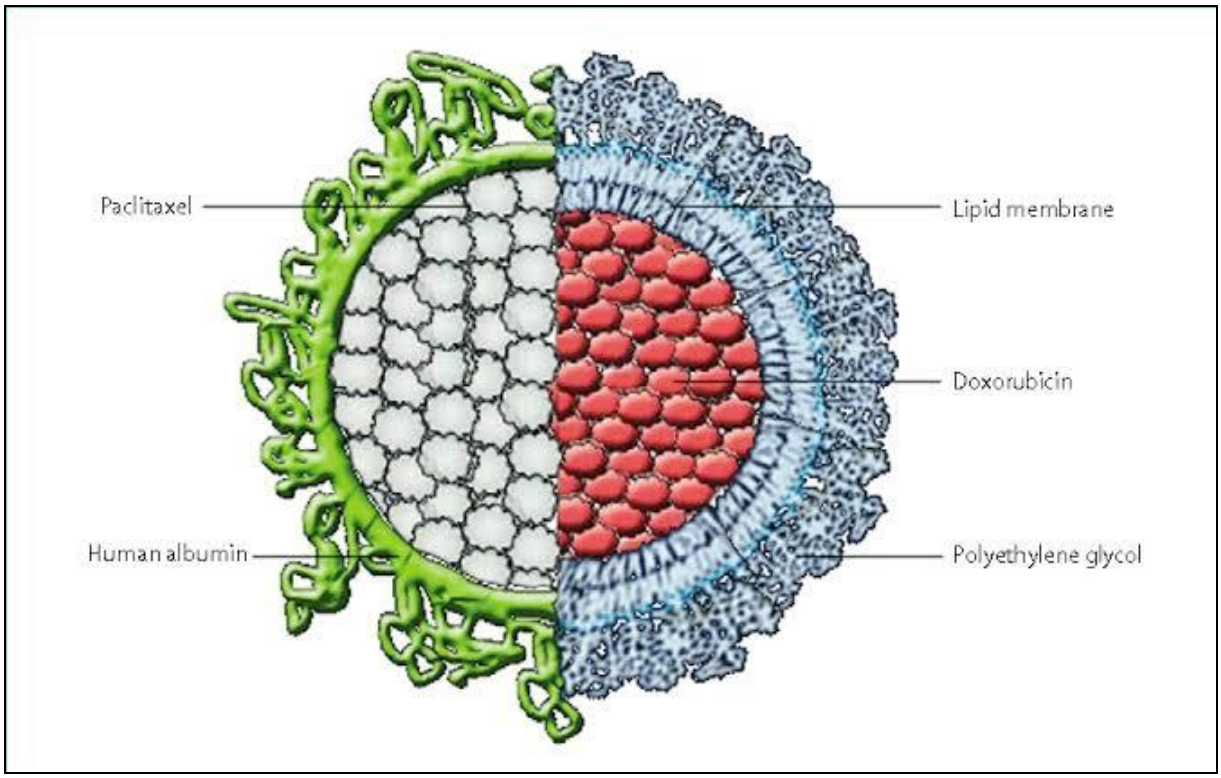


Figure 07. Basic structure of organic nanoparticles **(Yezhelyev et *al*., 2006).**

- Inorganic nanoparticles

They have the same base structure of organic NPs, with a core with fluorescence, optical or magnitic propreties and an organic protective layer on the surface. Among which we can cite photoluminescent dimond nanoparticules, quantum dots, Raman probs, magnitic and supermagnitic iron oxide nanoparticles formed from a magnetically active metal core (mainly iron but also cobalt, nickel) and are used as a contrast agent in magnetic resonance imaging MRI. They are covered with a protective organic layer on which be attached to carrier molecules. They can be encapsulated in micelles **(Chuto et Riffaud, 2010).**

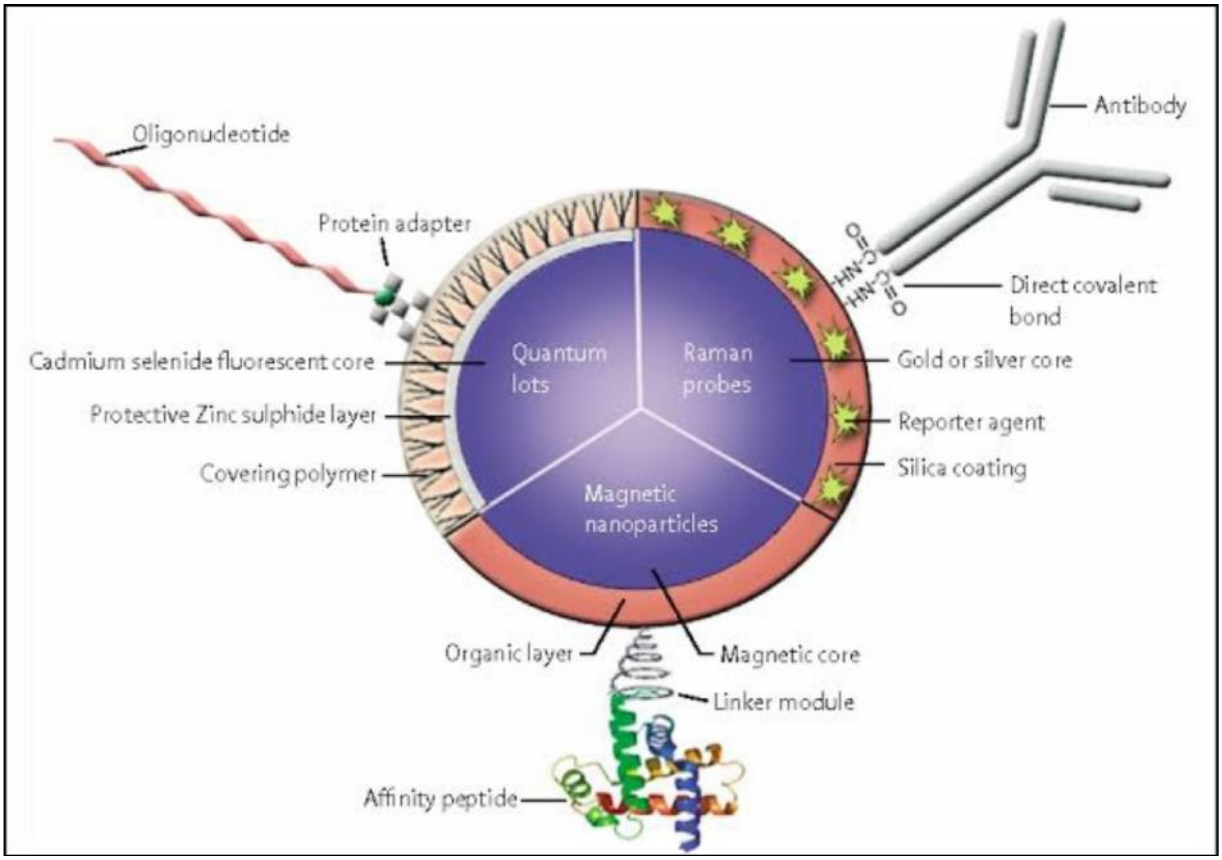


Figure 08. Basic structure of inorganic nanoparticles **(Yezhelyev et *al.*, 2006).**

4.Application of nanoparticles

Nanoparticles have many applications in a wide variety of fields:

- The Field of medicine

Nanoparticles are used in the Victorization of medicaments, for exemple magnitic nanoparticles allow gindance and release of a drug in a specific site **(Sauzedde et *al.*, 1999; Chomoucka et *al.*, 2010).** They are also used in the treatment of cancer **(Jain et *al.*, 2005).** Nanotechnology has enabled the development of nanoscale devices that can be conjugated with several functional molecules simultaneously, including tumor-specific ligands, anticancer drugs, antibodies,. Since these are 100 to 1000 times smaller than cancer cells; they can be easily transferred through leaky blood vessels and interact with tumor-specific proteins both on the surface and inside cells cancerous **(Wang et *al.*, 2008).** They are used in medical resonance imaging magnitic **(Thorek et *al.*, 2006; Sahoo et *al.*, 2007).** They are also used as an agent antimicrobial. They are seen as targeted approaches to reduce damage caused by microorganisms, and more specifically fungi, treating areas **(Ghasemian et *al.*, 2012; Sanjenbam et *al.*, 2014)**.

- The field of cosmetics

The cosmetics industry seeks to improve the properties of products cosmetics such as hold, transparency, shine and optical properties. Several types of applications are developed by the cosmetics industry; filtration ultraviolet radiation, adaptation to the environment by effects photochronic, new visual effects, anti-aging of the skin **(Prosie et *al.*, 2008)**.

- The field of environment

Magnetite iron oxide nanoparticles are used in the purification of the water.

- The field electronic

Zinc oxide nanoparticles are starting materials for electronic products **(Aruojaa et *al.*, 2009)**.

- The field of agriculture

NPs are used in the field of agriculture, such as the use of pesticides of nanometric dementions.**(Grillo et *al.*, 2015)**.

- Others :

The field of chemicals, construction, textiles and food **(Greco et *al.*, 2014)**.

5.Routes of exposure to nanoparticles

The human been is exposed to many nanoparticles, the most frequent route of penetration is the route of inhalation. It's important to know that the site of deposition of nanoparticles in the lungs is depending their dimensions. They can change in the process of production the deposition of dust along the lung tree varies considerably depending on their grain size and their behavior in air **(Prosie et *al.*, 2008).** There are also other routes of exposure; the transcutaneons route **(Sadrieh et *al.*, 2010; Jeanjean et *al.*, 2012),** the blood also represents a portal of penetration of nanoparticles in the organism **(Leonard et *al.*, 2015).**

6. Mechanism of action of nanoparticles

6.1. Cut (contact surface)

The size is a determining element explaining their mechanisms of toxicity. Due to their very small size, these particles offer a very large surface contact area per unit mass. It has been clearly shown that the degree of toxicity is related to these surface properties of these nanoparticles and not to their mass **(Prosi et *al.*, 2008)**. The peculiarity of a nanoparticle lies in the fact that the majority of the atoms which constitute it are found on the surface.

Nanoparticles have very large exchange surfaces (specific surface), of several hundred square meters per gram of particle. This exchange surface very important gives nanoparticles physical and chemical properties that may cause specific biological effects, which still remain largely misunderstood, if they come into contact with the living. The responsiveness of surface of the nanoparticles, which increases as the particle size decreases, suggests that they will have a greater biological activity, at mass comparable, than larger particles **(Lanone et Boczkowski, 2010).**

Multifunctional nanoparticles exceeding 20 to 30 nm are easily taken into cells via endocytosis vesicles, which are typically 40 to 60 nm in diameter **(Debbage et Jaschke, 2008).**

6.2. Biopersistence

The two mechanisms for removing foreign bodies from which the body human disposals are chemical dissolution (for soluble particles) and physical translocation (for insoluble and poorly soluble particles). Over there translocation, the largest particles that normally setter in upper parts of the lungs (Trachea and bronchi) are removed by mechanism of mucociliary lift to the digestive system. The purification system macrophage of the lungs in less efficient for ultrafine particles, probably due to the oxidative potential, which results in a heigher biopersistence high **(Prosie et *al*., 2008).**

7. Nanoparticles impacts

7.1. Impacts on environment

There is to many sources of nanoparticle pollution such as synthesized sources (e.g. herbicides and insecticides from agricultural lands, grease and toxic chemicals from urban runoff, energy production and factories), and natural sources (like crops and forest lands, and eroding stream banks Salt from irrigation practices and volcanos…). NPS pollution generally results from land runoff, precipitation, atmospheric deposition, drainage, seepage or hydrologic modification, plus the consequences of human activities (**Buseck et Adachi, 2007).**

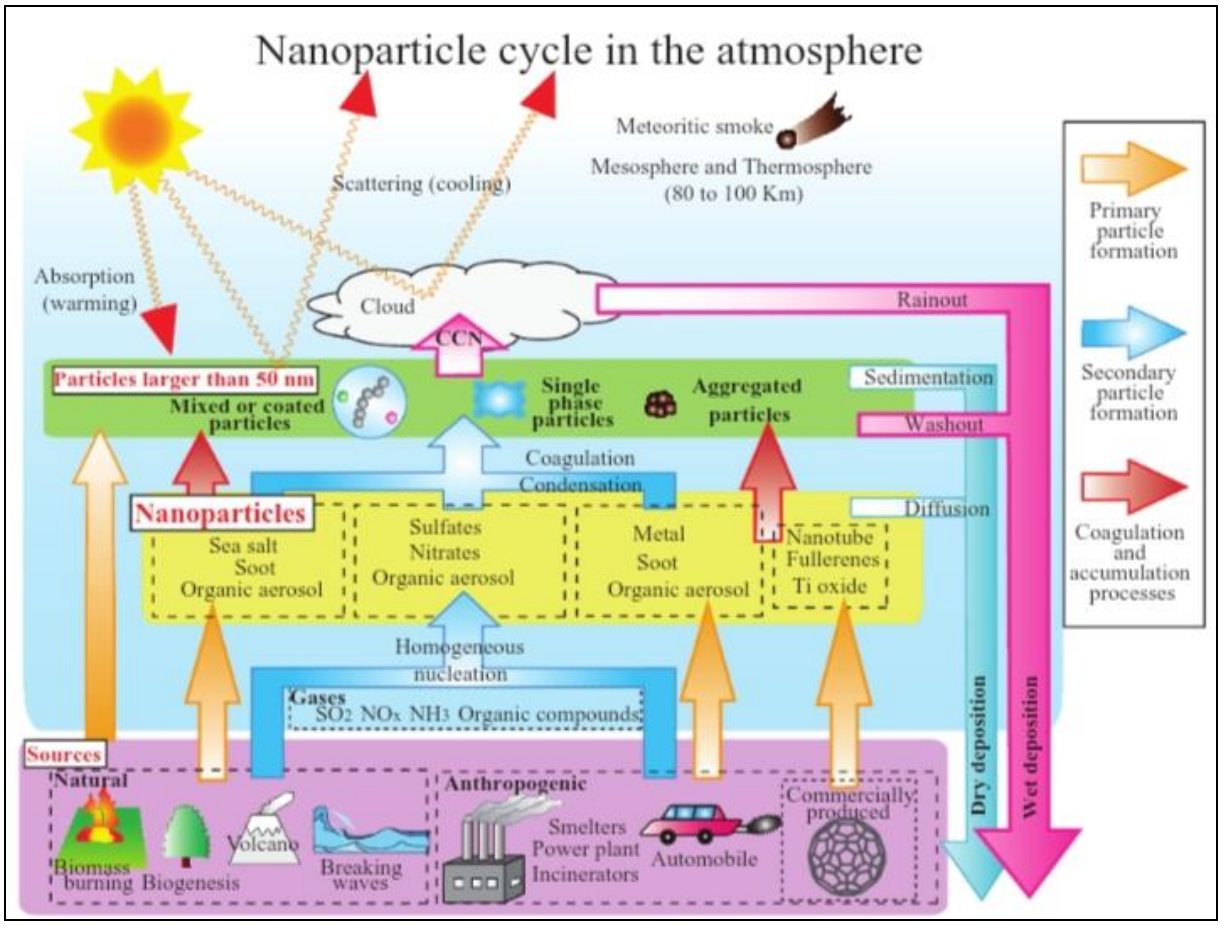


Figure 09. Schematic of the nanoparticles cycle in the atmosphere. (CCN; cloud condensation nuclei**) (Buseck et Adachi, 2007).**

7.1.1. Atmospheric impacts (effects on climate)

Depending on their composition and sizes, aerosol particles influence global climate through both warming and cooling effects.

Sulfate aerosol particles are extremely abundant, and they produce a net cooling effect of -0.4 Wm^{-2} **(Solomon, 2007).** On the other hand, soot, minerals such as hematite, and colored organics (e.g. humic substances) absorb sunlight and cause warming **(Bergstrom et *al*., 2007).**

The direct radioactive effect of the soot, which is the largest contributor to warming among aerosol particles, is 0.34 Wm^{-2} **(Solomon, 2007).** If soot particles are coated with sulfate or organic compounds (internally mixed, in atmospheric jargon), which is common in the ambient atmosphere, their radioactive forcing increases to about 0.6 Wm^{-2}**(Chung et Seinfeld, 2005)**. In such cases, their radiative forcing is the second largest contributor to global warming **(Ramanathan et Carmichael, 2008).**

At times, volcanic emission can overwhelm all other sources. For example, the Pinatubo (Luzon, Philippines) eruption in 1991 injected vast quantities of aerosol particles and SO2, much of which produced H_2SO_4 and ultimately other sulfate particles, into the atmosphere, includes into the stratosphere. The net effect was global cooling of -0.5°C and significant ozone depletion **(Soden et *al*., 2002).**

7.1.2. Effects of nanoparticles on water

The engineered nanoparticles on aquatic ecosystems have significant effects on water quality, and aquatic organisms, such as fishes, plants, and microscopic living organisms in the water. The issues of penetration and accumulation of nanoparticles in the body of aquatic organisms and their toxic effects, biotransformation and migration along food webs are considered. This huge case of nanoparticles pollution will increase the health of all living organisms even the human been**. (Krysanov et *al*., 2010).**

7.1.3. Impacts of nanoparticles on ground

Nanoparticles have a detrimental effect on terrestrial ecosystems (fauna and flora). NPs have an effect on the growth of terrestrial plants. Indeed, Al2O3 nanoparticles are able to inhibit the growth of different plant species **(Yang et Watts, 2005).**

7.2. Impacts on Human health

Toxicity of nanoparticles is of increasing interest because of the rapid development and growth of nanotechnology **(Nel et *al*., 2006; Cameron et Mitchell, 2007)**. To date, interactions of nanoparticles with the human body are not fully understood, although an epidemiological study showed that excess morbidity from fine particles, prominently sulfates, resulted from air pollution episodes in several U.S. cities **(Dockery et *al*., 1993).** Whene nanoparticles are absoebed or ingested, their behavior differs from that of larger particles. They pose a particularly large health risk because they are likely to be more reactive and toxic than larger particles **(Peter et *al*., 2007).**

Nanoparticles maybe are taken into the body through the skin, lung, and gastrointestinal tract. When inhaled, they deposit on all regions of the respiratory tract by diffusion, with particles smaller than 20 nm having the highest deposition efficiency. After nanoparticles deposit in the alveolar region, only 20 % of them are removed, whereas 80 % of particles larger than 500 nm are removed **(Oberdorster et *al*., 2005).** In contrast to larger particles, nanoparticles travel into the circulatory and lymphatic systems. Their toxicity depends on

their surface areas and compositions, and surface atoms and molecules work as catalysts for chemical reactions. The smallest particles have the largest specific surface areas, making nanoparticles especially reactive. Thus, they potentially cause adverse effects such as oxidation stress, pulmonary inflammation, and cardiovascular events like heart attacks and cardiac-rhythm disturbance **(Oberdorster et *al.*, 2005; Nel et *al.*, 2006).**

Chapter III: Aluminum oxide nanoparticles

1. Definition of aluminum oxide nanoparticles

Aluminum oxide is an atmospheric nanoparticle. He's chemical formula Al_2O_3. It is used in various chemical, industrial and commercial applications. It is considered an indirect additive used in food contact substances by the FDA.

Aluminum oxide is a white oderless crystalline powder.Water insoluble. Properties vary according to the methode of preparation; different methods give different crystalline modifications. The variety formed at very high temperature is quite inert chemically.

2. Physical and chemical properties

Table 03. Chemical and physical properties of aluminum oxide (Al_2O_3). **(PubChem)**

Property name	Property value	Reference
Color	White	NIOSH.2016
Oder	Non	NIOSH.2016
Solubility	-Insoluble in water -Difficult solubility in mineral acids and strong alkalin.	NIOSH.2016
Density	4 g/cm^3	NIOSH.2016
Molecular weight	101.961 g/mol	Computed by PubChem 2.1.(PubChem release.2019.06.18).
Topological Polar surface Area	3 $A^{\circ 2}$	Computed by Cactvs 3.4.6.11.(PubChem release.2019.06.18)
Formal charge	0	Computed by PubChem

Covalently bonded unit count	5	Computed by PubChem
Boiling point	-5396 F˚ at 760 mmHg -2977 C˚	NIOSH.2016
Melting point	-3623 F˚ -2030 C˚	NIOSH.2016
Vapor pressure	-0 mmHg -1mmHg at 2158 C˚	-NIOSH.2016 -LEWIS (ed) Sax's Dangerous Properties of industrial materials.Mth Edition Wiley-interscience.Wiley and Sons , Inc Hoboken.2004.P136
Biological half-life	-4.5 h in dogs -8h in urine	- Ellenhorn and Barceloux. Medical Toxicology - Diagnosis and Treatment of Human Poisoning. New York, NY: Elsevier Science Publishing Co., Inc. 1988., p. 1009 - Friberg, Nordberg, Kessler, and Vouk. (eds). Handbook of the Toxicology of Metals. 2nd ed. Vols I, II.: Amsterdam: Elsevier Science Publishers B.V., 1986., p. 10

3. Toxicokinetics of Al2 O3 NPs

3.1. Absorbation, distribution, elimination

- Absorption

In a healthy adult only approximately 15µg of the average daily dietary aluminium intake of 3-5mg is absorbed **(Winship, 1992).** The intestinal absorption of aluminium and its oxide is enhanced by citrate (which is found frequently in effervescent drug formulations) and reduced by silica. Since aluminium oxide is insoluble it is poorly absorbed following inhalation.

- Distribution

Since aluminium oxide is insoluble some will be retained in the lung following inhalation. More than 90 percent of that which is systematically absorbed is bound to transferrin which does not cross the blood-brain barrier readily. The remaining ten per cent is associated with low molecular weight complexes, such as citrate, which can accumulate in brain tissue. Systematically absorbed aluminium is stored mainly in bone (up to 40 per cent) and liver.

- Excretion

Aluminium is excreted predominantly via the kidneys and therefore will accumulate in patients with renal failure **(Alfrey, 1980).** Following long-term occupational inhalation, aluminium oxide exposed workers with normal renal function may also accumulate aluminium. In two such cases the total body aluminium half-life was estimated as three years **(Elinder et *al*., 1991).**

3.2. Metabolism

Aluminium oxide ingestion is rare and does not lead to significant toxicological problems; most exposures are via inhalation. No features following acute inhalation have been reported.

4. Use of aliminum oxide

We use aluminum oxide in different productions, such as :Production of aluminum, manufacture of abrasives, refractory, ceramics, electrical insulators, catalyst and catalyst supports, paper, spark plugs, crucibles and laboratory wares, adsorbent for gases and water

vapors, chromatographic analysis fluxes, light bulbs artificial gems, heat resistant fibers, food additive (dispersing agent) **(Lewis et *al.*, 2007)**. In hollow-fiber membrane units used, in water desalination, industrial ultrafiltration, hemodialysis**. (John et sons, 1980).**

Aluminum oxide (Al_2O_3) is a bioceramic widely used in various medical applications such as tooth filling and implantation, which is also cost-effective, biocompatible and has good mechanical properties including high corrosion and wear resistance **(Dong et *al.*, 2012).**

5. Aluminum oxide toxicity

There is experimental evidence that aluminum inhibits bone mineralization partly by the deposition of aluminum at the steroid/calcified-bone boundary thereby directly inhibiting calcium influx, and partly by aluminum accumulation in the parathyroid glands with suppression of parathyroid hormone secretion **(Visser et Van, 1985; Berland et *al.*, 1988; Firling et *al.*, 1994).**

Proposed mechanisms of aluminium-induced neurotoxicity include free-radical damage via enhanced lipid peroxidation, impaired glucose metabolism, effects on signal transduction and protein modification and alterations in the axonal transport and phosphorylation state of neurofilaments **(Birchall et Chappell, 1988; Exley et Birchall, 1992; Erasmus et *al.*, 1993; Winship, 1993; Haug et *al.*, 1994; Joshi et *al.*, 1994; Strong, 1994)**. It has also been suggested that low-level aluminium exposure may influence the body distribution of other essential metals with potential adverse metabolic effects **(Rollin et *al.*, 1991).**

PRACTICAL PART

Practical Part

Chapter IV: Material and methods

1. Material

1.1. Biological material

This study was conducted on 30 male rats *Rattus rattus* (2 - 2.5-month-old) of the Wistar strain, from the Pasteur Institute in Algiers. Weighing between (170 and 230 g) was housed in polypropylene cages with woodchip shavings on the floor, and free access to food and water. They were maintained in the animal house under standard laboratory conditions of temperature and humidity.

1.2. Chemical material

In this work, we used aluminium oxide nanoparticles for the treatment of rats in solution form with two doses (5mg/Kg/day and 50 mg/Kg/day).

2. Methods

2.1. Raising rats

In This study we have divided 30 males rat in 3 experimental groups, consisting of 10 rats in each group **GI(C), GII (dose 01: 5 mg/kg/J)** and **GIII (dose 02: 50 mg/kg/J).** They were subjected to a 30 day adaptation period in the pet store in the animal center of the Faculty of Science, University of Tebessa. The ambient temperature is 23 ± 2 ° C and a natural 10/14h photoperiod with a hygrometry of 60%. The rats are raised in polyethylene cages (05 rats for each cage) which are lined with a litter made of wood shavings. The cages are cleaned and the litter is changed once every two days until the end of the experiment, the rats were fed with white bread.

2.2. Distribution and treatment of rats

The distribution and treatment of animals is summarized in Figure 2 and illustrated as following in table:

Table 04: Shows the treatment of rats.

Experimental groups	Treatment	Number of rats	Duration	Dose
Control Group	Force-feeding by Distilled water	10	40 days	0,5ml/day
GI (Dose 01)	Force-feeding of Aluminum oxyde	10	40 days	5mg/kg/day
GII(Dose 02)	Force-feeding of Aluminum oxyde	10	40 days	50mg/kg/day

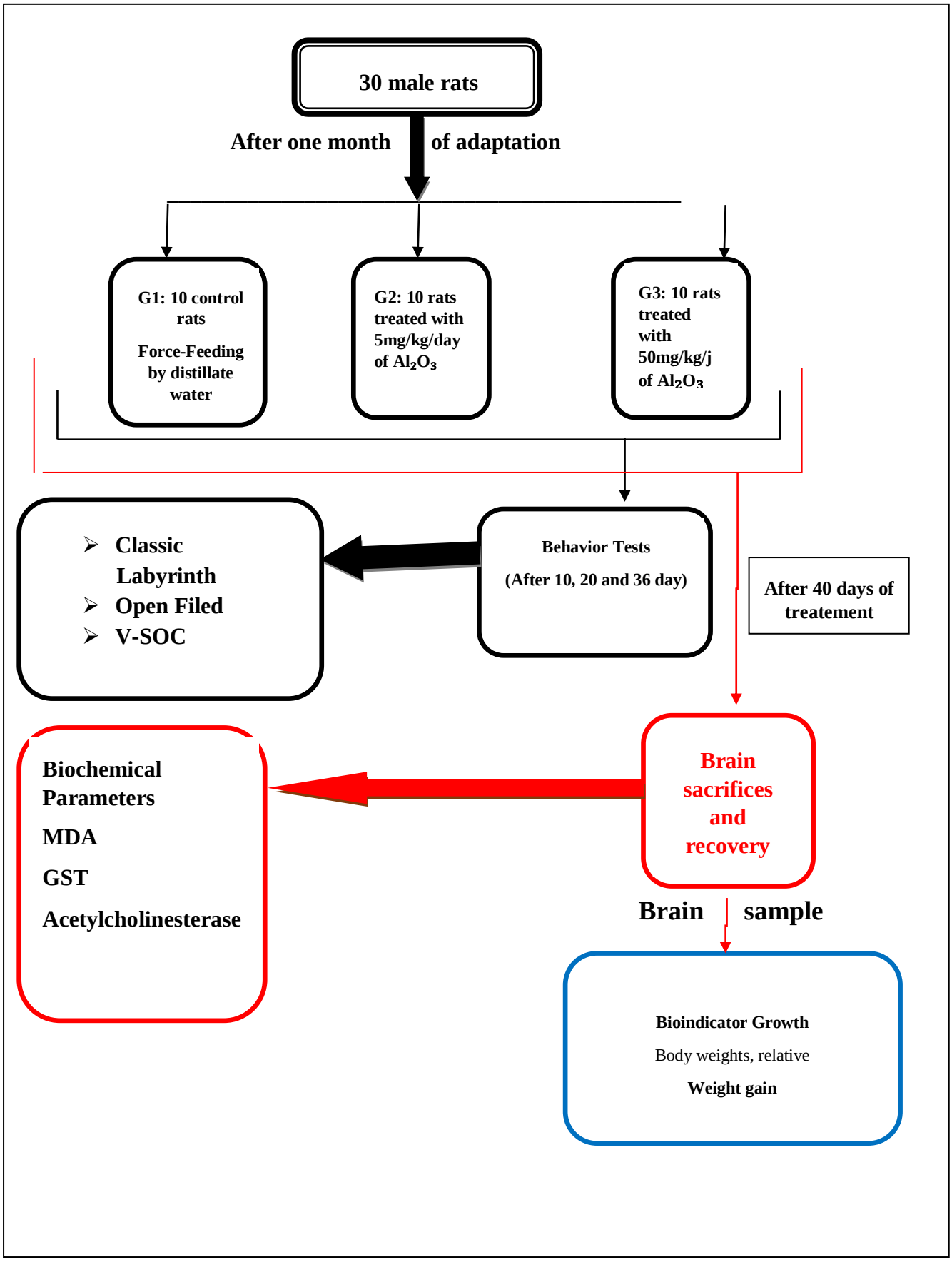


Figure 10. Summary diagram of the experimental protocol.

2.3. Evaluation of neurotoxicity of aluminum oxide nanoparticles

After 40 days of exposure of the rats to oxidized aluminum, two approach methods were applied to assess the impact of this nanoparticle on the neurobehaviour of the rats and also to assess the structural and functional integrity of brain cells.

2.3.1. Weight measurement

The weight measurement is performed on rats very day during the breeding period, either during the adaptation (to evaluate possible changes by the food or the place (external factors) or during treatment (to assess the effects of xenobiotics) using a small (SF-7000g balance brand JAYA).



Figure 11. Wistar rat mesurement.

2.3.2. Neurobehavioral Study

This approach to evaluate the neurotoxic effects induced by exposure to xenobiotics on the overall behavior of the animals, namely memorization, learning and anxiety, to achieve this goal, we have done several tests such as: Classic Labyrinth, Open Filed and L-MAZE.

- **Classic Labyrinth Test**

The Classic Labyrinth Test (CLT) is a simple way to evaluate behaviors in rodents such as learning ability, memory, and anxiety. The CLT uses a square-shaped maze with a starting point and a stopping point. After the animal is trained, the animal is allowed to view and explore the labyrinth freely for 5 min. During this time, all of the animal's vertical and horizontal movements within the labyrinth are recorded. This is a very challenging task

because it requires the animal to remember the quickest path between the starting points and the end. In cases where the labyrinth is designed so that the animal only needs to walk forward, it is quite easy for healthy rats, but for rats exposed to neuro-xenobiotics (drugs, pesticides, nanoparticles) there will be disturbances in their path. The CLT is performed in a square-shaped plastic enclosure (59x 59 x 21 cm) with several labyrinth passages of identical width and height (21 x 40 cm), but with variable length. Usually, this labyrinth is placed on a table 90 cm high. Control rats can quickly move through the labyrinth between the starting point and the ending point. When the animal explores the maze, it increases the time it spends in the maze's passages, which will be considered as aversive or anxiety-provoking for the stressed animal; while the leak behavior will be observed when the animal spends more time in the starting point or corners, which will be associated with a refuge **(Leo et Pamplona, 2014; Gasmi et *al.*, 2017)**. There are many tests similar to this test, but this test is unique because it is concerned with measuring the impact of environmental pollutants on the behavior and psychology of animals, especially rats **(Serchov et *al.*, 2016).** Some advantages of this approach are that this test is inexpensive, and it is quick; the test usually takes about 10 min, and after cleaning the labyrinth with 70% ethanol, it can be used to test the next animal **(Gasmi et *al.*, 2017).**

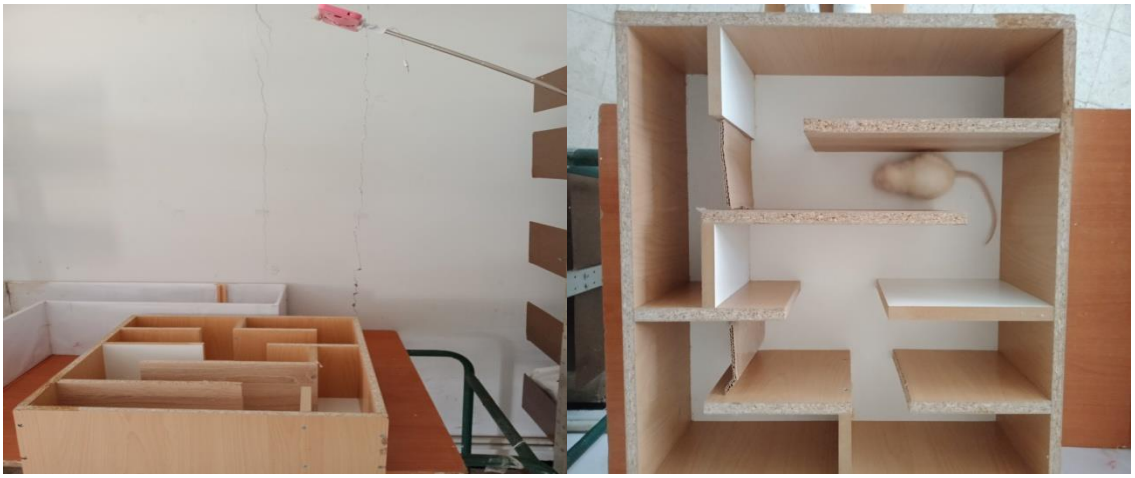


Figure 12. Instruments of Classic Labyrinth.

- **Procedure**

1. The apparatus (labyrinth) is placed in an isolated room away from any external interferences and noises with a low-intensity white light source.

2. Clean both compartments of the apparatus with 30% hydroalcolic **(Serchov et *al.*, 2016).**

3. Turn on the camera and place the first rat in the starting point of labyrinth.

4. We stay as far away as possible from the box and out of sight of the test animal.

5. The rats are allowed to move freely within the labyrinth for 5 min (between the starting point and the stopping).

6. After each trial, all urine and fecal matter are removed, and the maze is cleaned with 30% Hydroalcolic.

7. The recorded videos are analyzed manually with the aid of a chronometer.

- **Data analysis**

The data were visualized using Excel 2007 to represent these results in the form of graphs and histograms. Statistical analysis was performed using the Minitab® 17.1 software.

- **Open field test**

- **Principle**

The open-field test is used to assess locomotor and exploratory activity as well as the emotional state of the animal.

- **Experimental apparatus**

The device consists of square plexiglass enclosure 50 cm per side and 37 cm high. It is divided into 2 parts of the same area: 1 central part and 1 peripheral part. The central part serves as a starting point for the animals for each test.



Figure 13. Open-field experimental set-up.

Practical Part

- **Procedure**

The test begins after the establishment of the animal at the center device and movements are filmed for 5 minutes. The device is cleaned with a hydroalcoholic solution of 10% and dried after the passage of each animal.

- **Variables measured**

The variables measured are the distance crossed, the number of adjustments made, the number of passages in the central box, the time spent in the central box, time spent in moving and time in the peripheral part.

- **V-SOC Maze test**

We applied this test with the aim of evaluating the neurobehavioral and social effects and preference for the social novelty of wistar rats after the exposure of aluminium oxide nanoparticles.

Studying social behavior in rat models empowers the understanding of the neurobiolgical Mechanisms involved, are affected in nouropsychiatric disorders, allowing the evaluation.

Social behaviors are important in numerous species to establish the networks and relationships that define social communities **(Berry et Bronson, 1992**).Among those behaviore, sociability is defined as the tendency to, seek out social interaction **(Caldwell, 2012).**Some neuropsychiatic disorders, such as autism spectrum disorders(ASD) display a maeked alteration in sociability combined with ather features **(American, Psychitic Assocaition, 2013).**This characteristic highlights meed for experimental behavioral setting in animal models to address the research on such complex mlti-factted desorders **(Caldwell, 2012)** .Mus musulus is a social specie showing reciprocal social interaction, sexual and parenting behavoirs territorial scent marking and aggressive behaviores **(Ricceri et *al.*,2007 ;Silverman et *al.*, 2010)** .The wide repertoire of mouse behaviors makes this specie suitable for modeling human disorders characterized by disruption in social recognition and social behavior **(Crawley, 2004 ;Yang et *al.*, 2007).**Such rodent models wodels warrant the evaluation of potential therapeutic approaches for treatment **(Moy et *al.*, 2004)** .Commonly mic conserve a charactristic pattem of social behaviore ,initiating social contact and approach when exposed to an unfamiar conspecific **(Moy et *al.*, 2004).**

Practical Part

Several paradims have been described to measare social behavior in mouse modles **(Silverman et *al*., 2010).**Among those ,the most common is the crawley's sociability test also called three-chamber apparatus **(Chadman et *al*., 2008 ;Mc Farlan et *al* ., 2008 ;Moy et *al*., 2008)**.Which allaws to study two different parameters :sociability and prefernce for social novelty .Sociability is defined as the tendency of the expermental mouse to spend more time exploring an unfamiliar mouse than exploring an object or an empty chamber Insted preference.

For social novelty is defined as the propensity to spend more time exploring a new unfamiliar mouse than the now-familair mouse that was explored in the sociability phase **(Moy et *al*., 2004 ;Yang et *al*., 2011).**Due to the length of the different phase in the Crawly's sociability test ,the procedure is time-consuming and experimental variability is sometimes troublesome.

We have adapted a V-maze,previously used successfully to evaluate novel object – recognition memory **(Puighermanal et *al*., 2009 ;Busquets-Garcia et *al*., 2011 et 2013 ;Navarro-Romero et *al*., 2019)**,to assess social behavior .We found that the using this new maze (Vsoc maze) provides an advantageous approach in time ,while maintaining accuracy, to reveal particular social phenotypes in tow mouse lines frequently used in biomedical research.

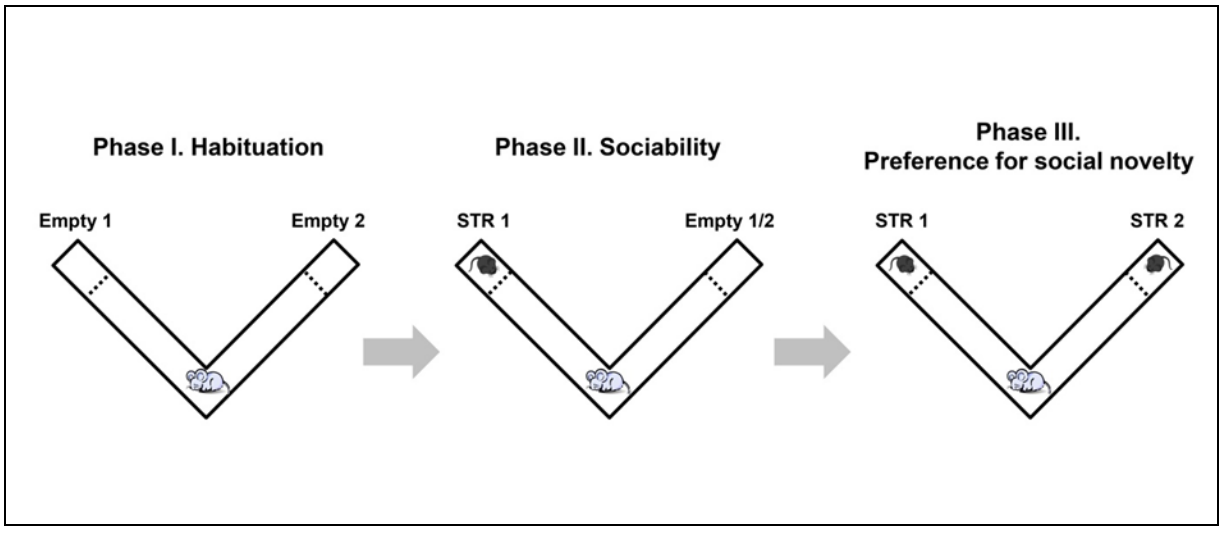


Figure14: The phase of maze Vsoc test **(Moy et*al*,.2004)**

Practical Part

Instruments

- Tissue paper
- Experimental mice
- 30% ethanol (v/v)
- Video Camera phone (OPPO A5S)

Labyrinth of V-soc we used a modified version of the V-shaped maze **(Busquets-Garcia et *al*., 2011)** V-soc Figure. It consists of a drawing structure: the maze wall (height 25cm), made of wood (6 mm wide) and the maze cover, and made of transparent glass. The lanes in the V-soc maze are (90 cm long and 25 cm wide) internal procedures in the passages when the cover was inserted into the V-soc maze, and rooms were used to allocate strange mice events, the design was intentionally simple to enhance the exploratory activity of the experimental.

Rats under analysis, and to facilitate animal handling cleaning between sessions **(Martinez et *al*., 2019)**. We applied this test during the treatment period after 10, 20 and 39 day.

2.3.3. Study of the cellular and molecular integrity of neurocytes

A. Sacrifice and brain extraction

At the end of the xenobiotic administration period (Aluminium oxide) of 40 days, the animals were sacrificed by decapitation; the brains were carefully excised and immediately rinsed in physiological solution, then dried at low temperature (4 ° C) with a semi-absorbent paper. The brains are weighed and then stored at -80 ° C until analysis for the determination of following parameters: **(Carbohydrate, Proteins, Lipids, MDA, GST and AChE).**

B. Estimation of the relative weight of the brain

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

RWB (g/100g of TW) = WB/TW x 100

RWB: Relative weight of Brains (g). TW: Total weight of rat (g). WB: Weight of Brain.

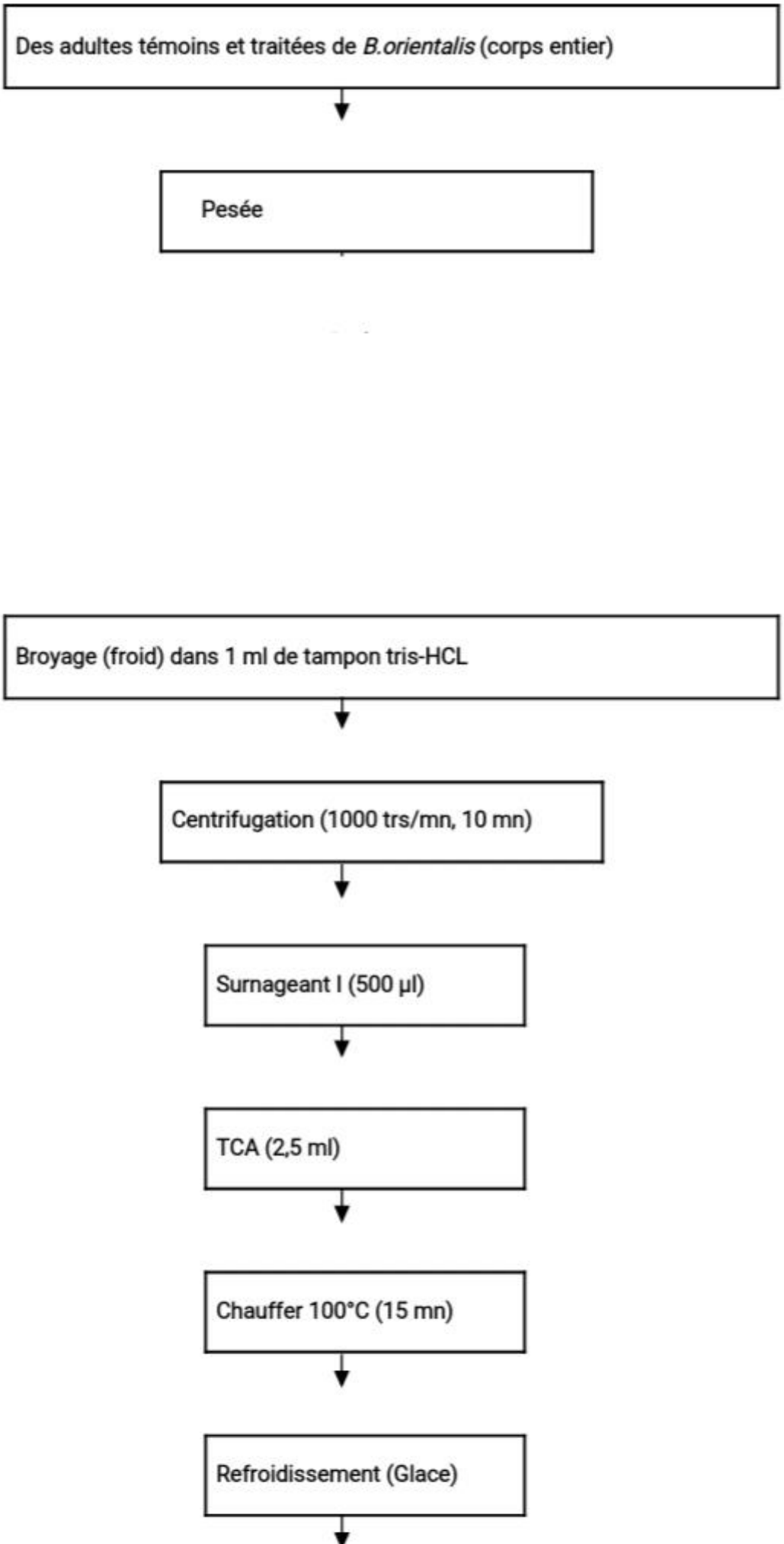
C. Preparation of cytosolic samples

One gram of nervous tissue was homogenized in 2 ml of phosphate buffered saline solution (PBS; pH 7.4). Then the homogenates were centrifuged at 3000 rpm for 15 min at 4 °C and the resulting supernatant was used for the determination of the level of **MDA** and the enzymatic activity of **GST.**

D. Evaluation of oxidative stress parameters

- **Manoldialdehyd assay (MDA)**

The **MDA** assay is carried out according to the method of **(Esterbauer et *al.*, 1992**). The principle of this assay is based on the condensation of MDA in an acidic medium and hot with thiobarbituric acid, to form a pink pigment. A quantity of 375µl of supernatant is taken in a dry tube, to which is added a volume of 150µl of the TBS solution (50mM tris, NaCl (150mM; pH7.4) and 375µl of the TCA-BHT solution (20% TCA, BHT 1%), the mixture is Vortexed and centrifuged at 1000 rpm for 10 min. A volume of 400 µl is taken from the supernatant to which 80 µl of 0.6M HCL and 320 µl of the tris-TBA solution (26 mM tris, TBA120 mM) are added. Finally, the mixture is vortexed and is then incubated in a water bath at 80 ° C. for 10 minutes. The reading of the optical density of the samples is measured by spectrophotometry at 530 nm.


Des adultes témoins et traitées de *B.orientalis* (corps entier)
Pesée
Broyage (froid) dans 1 ml de tampon tris-HCL
Centrifugation (1000 trs/mn, 10 mn)
Surnageant I (500 µl)
TCA (2,5 ml)
Chauffer 100°C (15 mn)
Refroidissement (Glace)

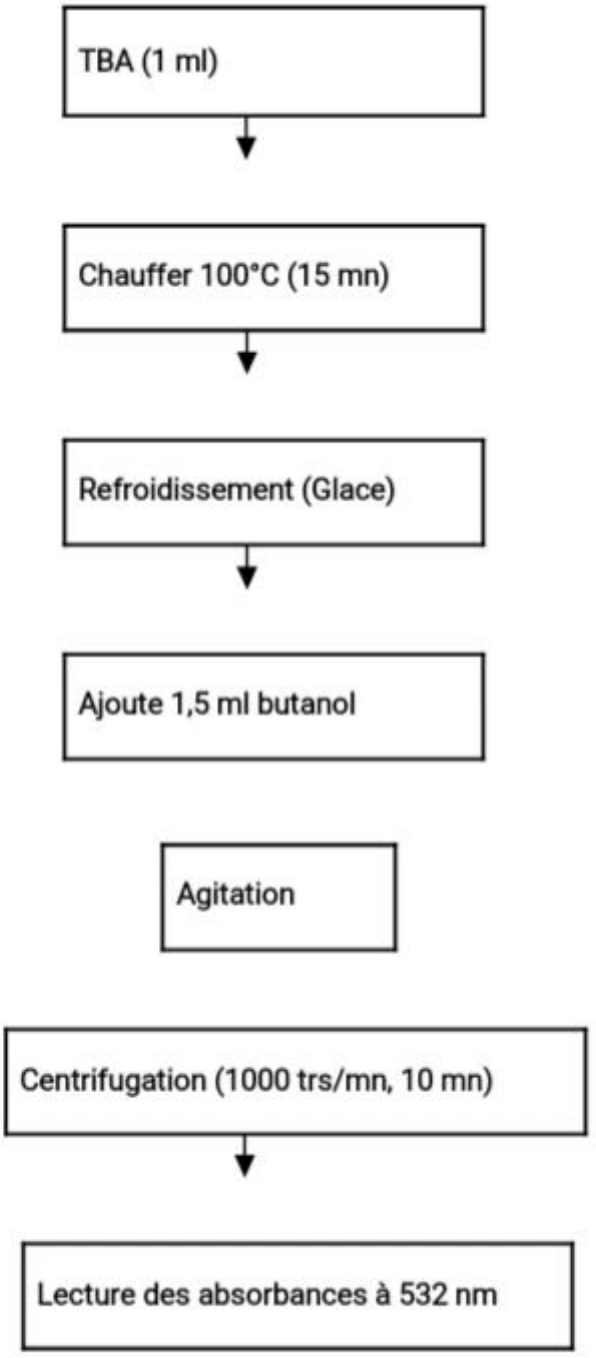


Figure 15. Extraction and dosage of malondialdehyde. **(Drapper et Hdley, 1990).**

Practical Part

- **Assaying of the activity of glutathione S-transferase (GST)**

The measurement of glutathione S-transferase (GST) activity is determined according to the method of **(Habig et *al*., 1974),** It is based on the conjugation reaction between GST and a substrate, CDNB (1-Chloro2,4-dinitrobenzene) in a cofactor glutathione (GST), the conjugation leads to the formation of a molecule novelle; 1-S-Glutathionyl 2-4Di nitrobenzene to measure GST activity.

The value of the optical density measured is directly proportional to the amount of conjugate formed, itself related to the intensity of the GST activity. The samples are homogenized in 1ml of phosphate buffer (0.1M, pH6). The homogenate is centrifuged at 14,000 rpm for 30 min and the recovered supernatant will serve as a source of enzymes.

The assay consists in reacting 200 µl of the supernatant with 1.2 ml of the CDNB (1 mM), GSH (5 mM) mixture [20.26 mg CDNB, 153.65 mg GSH, 1 ml of ethanol, 100 ml of phosphate buffer (0.1M, PH 6)]. The absorbance reading is taken for one minute and every 15sec at a wavelength of 340 nm against a blank containing 200 µl of distilled water replacing the amount of supernatant.

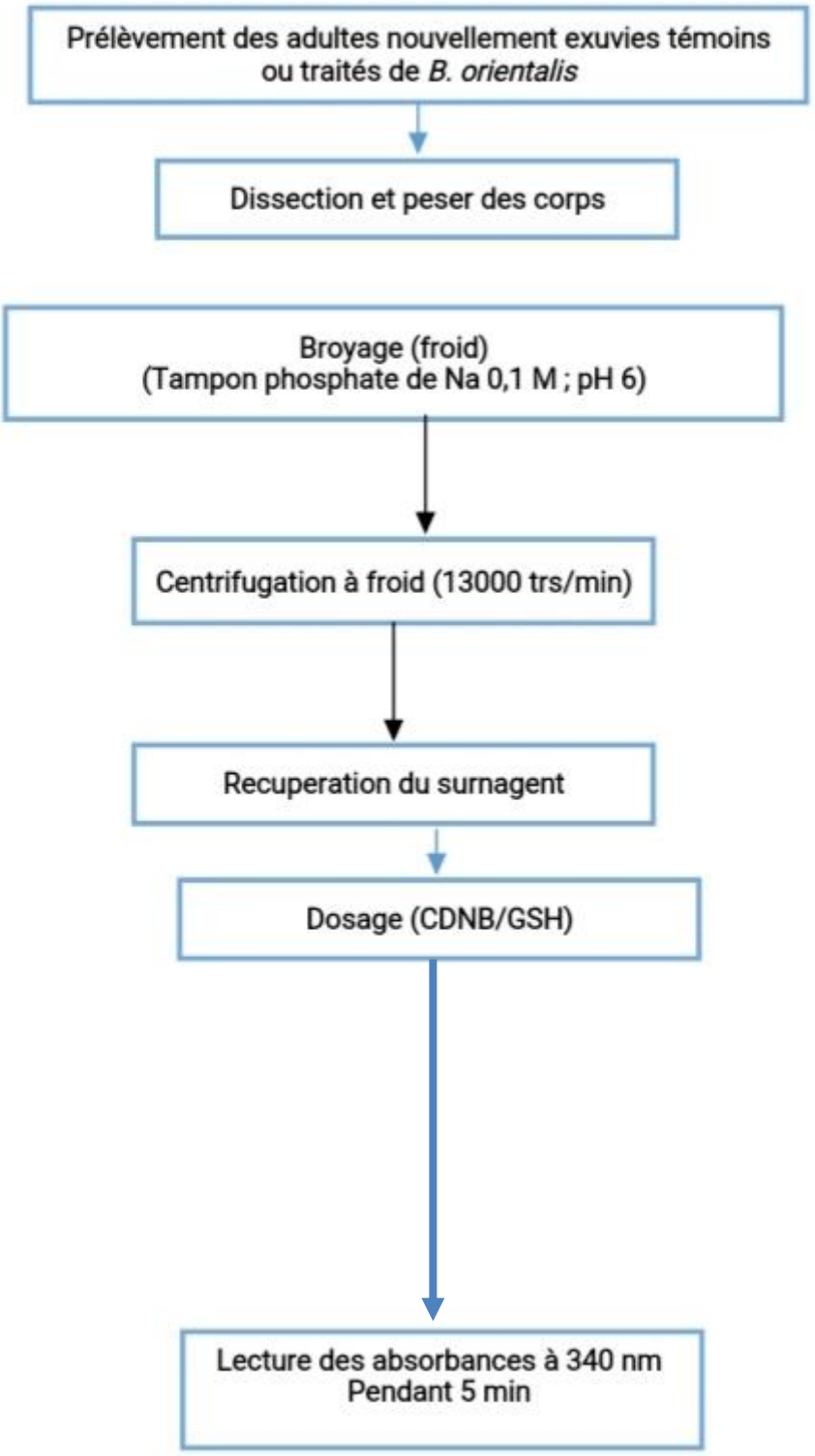


Figure 16. Extraction and dosage of glutathion-S-transfrase **(Habig et *al.*, 1974)**

E. Neurotransmitter estimation

Practical Part

Acetylcholinesterase assay (AChE)

The most common method of determining acetylcholinesterase (AChE) **(Ellman et al., 1961)** consists of providing the enzyme with a substrate, acetylthiocholine, the hydrolysis of which releases thiocholine and acetic acid. The samples are homogenized in 1ml of detergent solution (38.03mg ethylene glycol tris-β-aminoethyl ether NN N 'N, 1ml triton X 100%, 5.845g NaCl, 80ml 10mM tris buffer) using a homogenizer with ultrasound then centrifuged at 5000 rpm for 5 minutes. The supernatant is used immediately for measurement of AChE activity. The steps of the AChE assay are as follows: 100µl of supernatant are added to 100µl of DTNB (0.1M, pH 8) (39.6mg of DTNB, 15mg CO3Na, in 10ml 0.1M tris, pH 7) and 1ml tris buffer (0.1M, pH 7). After 5 min of rest necessary to exhaust the spontaneous reaction, 100µl of acetylthiocholine substrate (118 mg ACh in 5 ml of distilled water) are added. Optical densities are read at 412nm every 4min for 20min.

F. Evaluation of biochemical parameters

- **Carbohydrate dosage**

The determination of the total soluble carbohydrates was carried out according to the method of **(Dubois, 1956).** The extraction of soluble sugars consists in putting 100µl of homogenate in test tubes then adding 2 ml of 80% ethanol, the whole is left 48h. The assay is done by total evaporation of the alcohol by placing the test tubes in a water bath at 70 ° C. After cooling, the volume of each test tube is made up to 20ml with distilled water, then 1ml of the solution is taken and 1ml of 5% phenol is added, taking care to shake well, then 2ml of acid is added. Concentrated sulfuric acid in tubes previously placed in an ice bath. Finally everything is left to rest for 25min, and then we proceed to theread at 490nm wavelength. The calculation of the actual concentrations is done by the equation deduced from the calibration range prepared from a stock glucose solution.

- **Lipids dosage**

Tissue lipids are evaluated according to the method **(Goldsworthy et *al.*, 1972),** using 200µl of homogenate in 5ml of 20% trichloroacetic acid (TCA), and ground and filtered this mixture; and directly applied centrifugation at 5000rpm for 10min. The pellet is kept in a tube containing 1ml of the Ether / Chlorophorme mixture, and after centrifuging this mixture at 5000t/min for 10min, 100µl of the supernatant is taken, to which 1ml of sulfuric acid is added

and after stirring the tubes in a water bath at 100 ° C for 10min. After cooling, the sample is taken again using a 200 µl micropipetteof the extract to which is added 2.5ml of the 85% sulfophosphovanillin mixture (0.38g vanillin + 195ml orthphosphoric acid + 55ml H2O) and left this mixture 30min "in the dark", the reading at a wavelength of 530nm. The calculation of the actual concentrations is made from the equation deduced from the calibration range carried out from a stock solution prepared using oil of sunflower (Appendices).

- **Protein dosage**

The method used for the determination of proteins is that of **(Bradford, 1976)** which uses the BSA as standard, on the same sample used to measure the lipids, the pellet from the second centrifugation to which 1 ml of NaOH (0.1N) was added and stirred energetically for protein dissolution. Then, we take, by means of a micropipette, a volume of 100µl to which is added 4ml of the BBC reagent (Brilliant Blue of Coumassie) (50mg BBC + 50ml of 85% orthophosphoric acid and make up to 500ml with distilled water). Thus a blue color develops and the samples are passed directly for reading at a wavelength of 595nm. The concentration is calculated by the equation deduced from the calibration range produced from a solution of bovine serum albumin (Appendices).

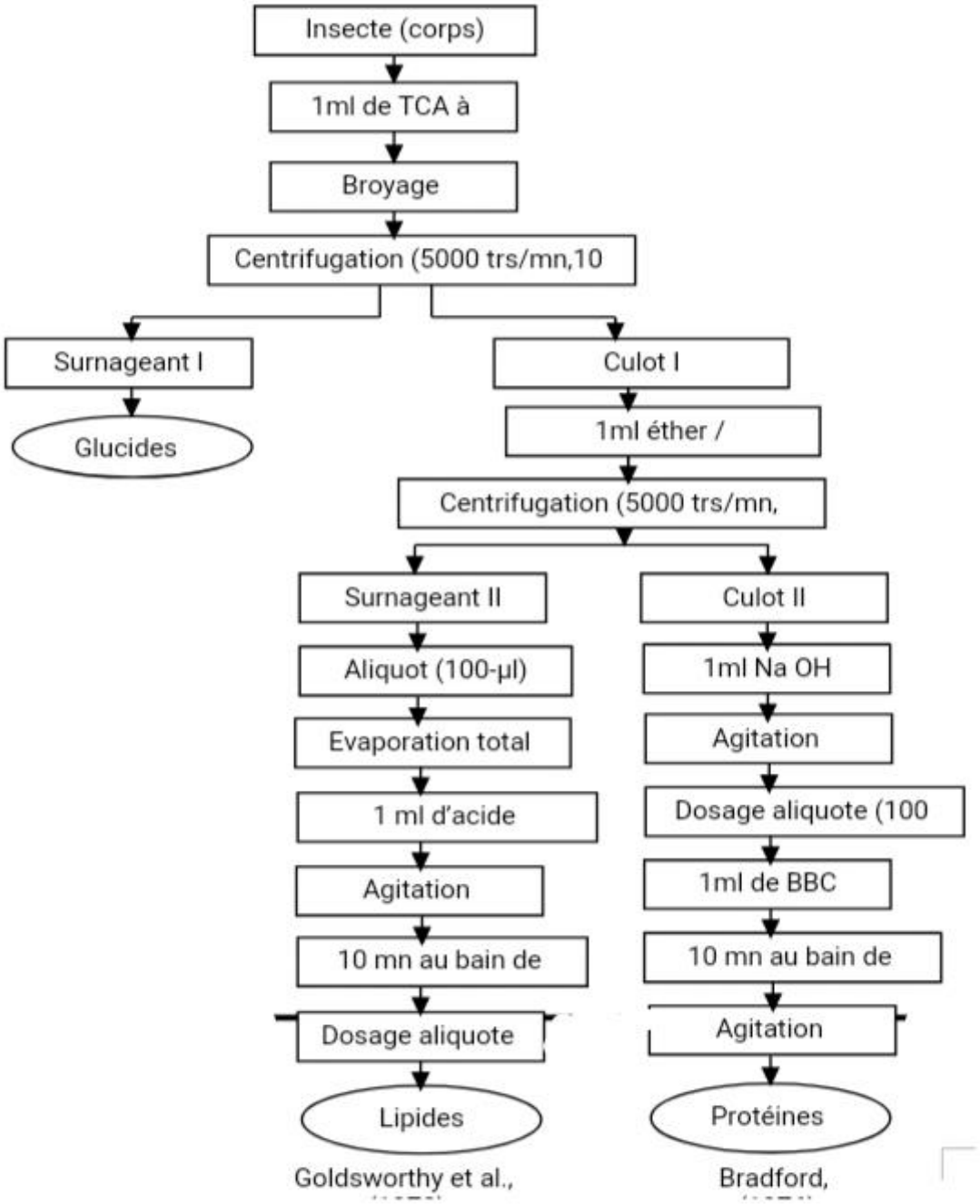


Figure 17. Extraction and dosage of biochemical parameters

2.3.4. Statistical analyzes

The results obtained were expressed by the mean of two repetitions (mean± deviationtype), and to better visualize using the Excel 2013 office to represent these results in the form of graphs and histograms. The statistical analysis was carried out using the Minitab® software 17.1. The significance of the difference between the control lot and the treated lots is

verified using the Dunette test and the “Student” t test, and the comparison result as following:

- P> 0.05 = the difference is not significant;
- (*) 0.05> P> 0.01 = the difference is significant;
- (**) 0.01> P> 0.001 = the difference is highly significant;
- (***) P <0.001 = the difference is very highly significant.

Chapter V: Results

1. Effects of aluminium oxide nanoparticles on overall growth parameters of animals

The results of the evaluation of growth parameters in terms of body weight, gainweight and relative weight during the 40 days of treatment of the different groups of animalsby the aluminium oxide NPs are illustrated by thefigures 16.

1.1. Body weight

The results of the body weight assessment show an increase in body weight in rats treated with D01 (5 mg/kg/day) and D02 (50 mg/kg/day) compared to control group of rats (fig18).

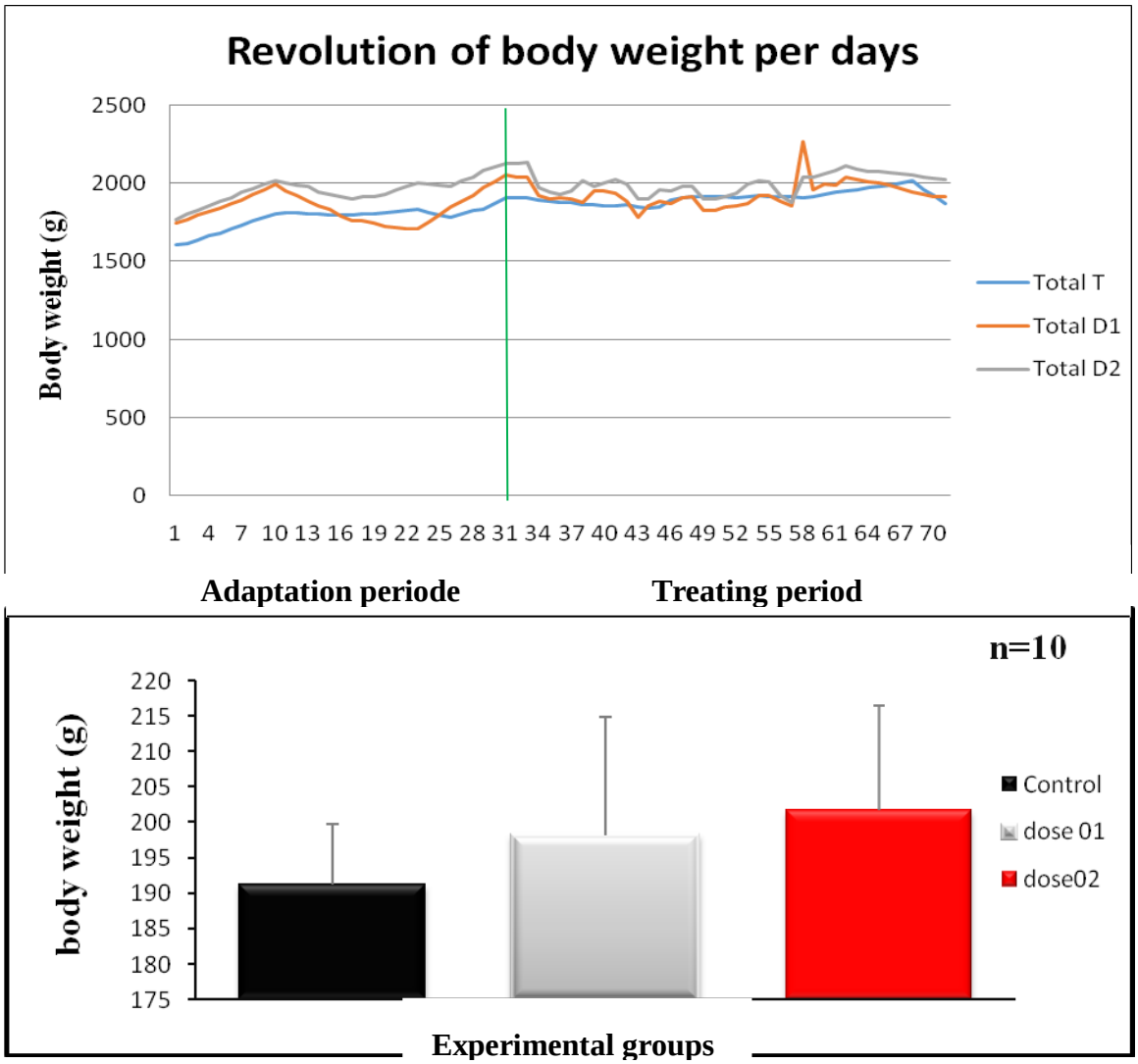


Figure 18. Evolution of body weight (BW) in the different groups treated for 40 Days by aluminium oxide nanoparticle**.**

1.2. Weight gain (WG)

The results of the weight gain assessment (fig 19) show a very hightly significant decrease ($p \leq 0.001$) weight gain in the group treated with 5mg/kg/day of Al_2O_3 compared to the control group. These results also show avery hightly significant decrease ($p \leq 0.01$; $p \leq 0.05$) in WG respectively in rats treated with 50 mg/kg/ day of Al_2O_3 compared to the control group.

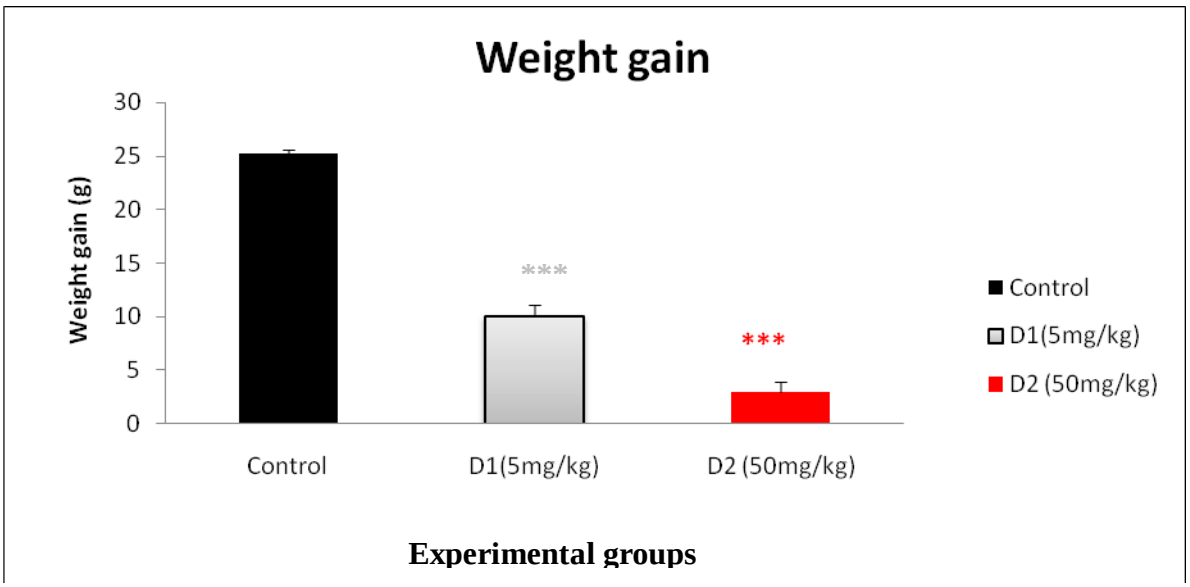


Figure 19. Evolution of body weight gain (WG) in control and treated rats after 40 days of treatment by 5 and 50 mg/kg/day of Al_2O_3.

1.3. Brain Weight (BW)

The results of the brain weight assessment (fig.20) show a hight significant increase weight in the group treated with 5mg/kg/day of Al_2O_3 compared to the control group. These results also show a significant increase in rats treated with 50 mg/kg/day of Al_2O_3 compared to the control group.

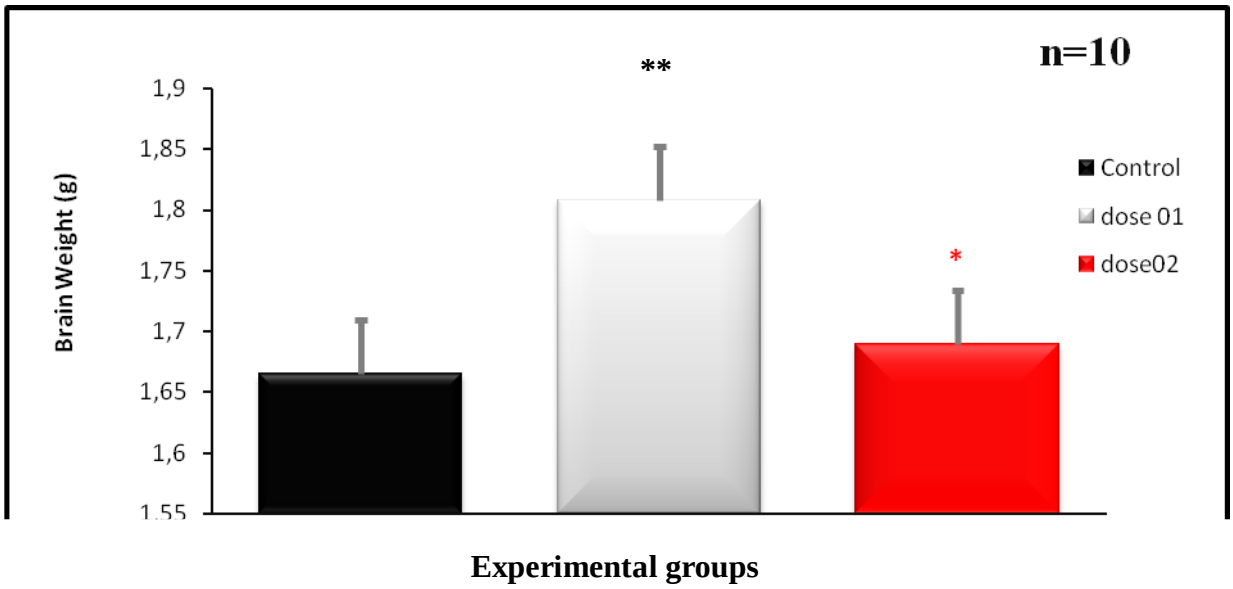


Figure 20. Evolution of brain weight (BW) in the different groups treated for 40 Days by aluminium oxide nanoparticle.

1.4. Relative weight of the brain (RW)

The results of the Relative weight of the brain assessment show a significant increase weight in the group treated with 5mg/kg/day of Al_2O_3 compared to the control group. These results also show a significant decrease weight in rats treated with 50 mg/kg/day of Al_2O_3 compared to the control group.

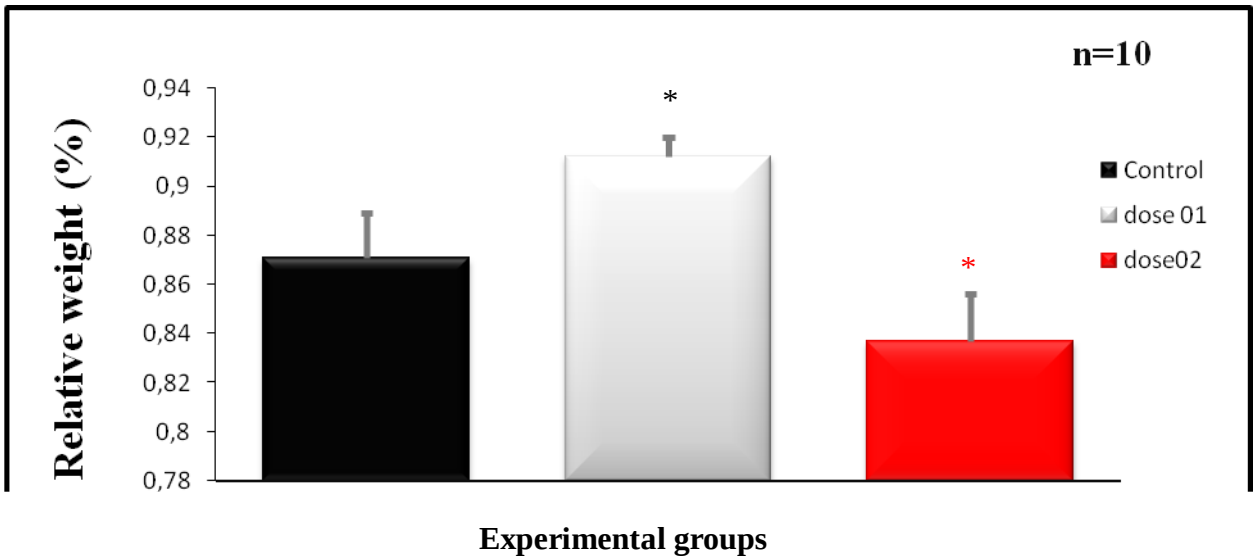


Figure 21. Evolution of the relative weight of the brain (RWB) in rats treated for 40 days with5 and 50 mg/kg/day of Al_2O_3.

2. Effects of Aluminium oxide nanoparticles on biochemical parameters in the total brain

2.1. Carbohydrate

The results of the evaluation of the variation in the level of carbohydrates in the brain are shown in figure 22. Statistical analysis of these results shows that the administration of Al_2O_3NPs causes a very highly significant decrease ($p \leq 0.001$) in both groups which are treated by 5 and 50 mg/kg/day of Al_2O_3NPs compared to the control group.

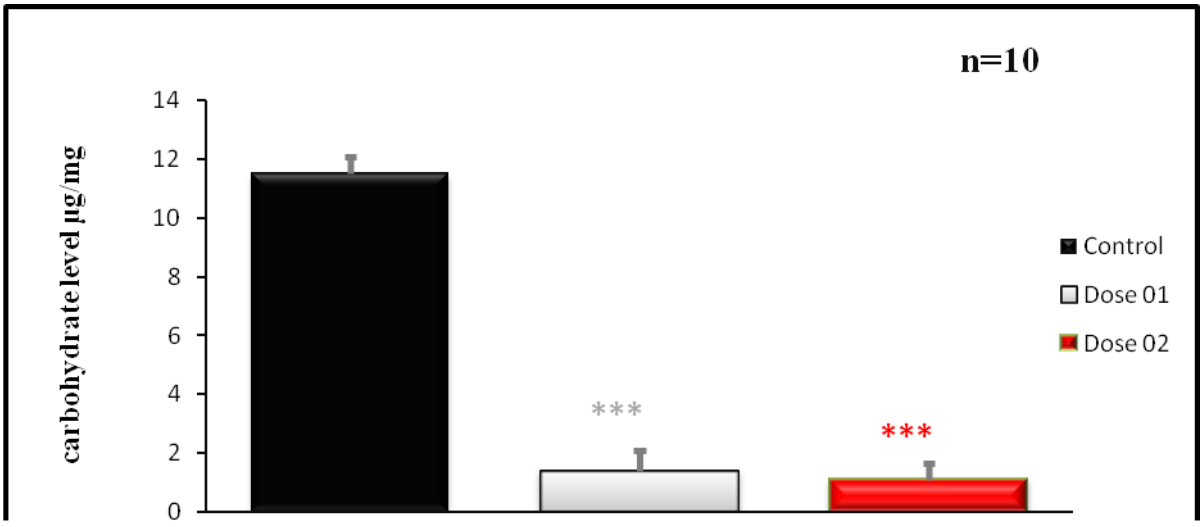


Figure 22. Variation in carbohydrate levels in control and treated rats after 40 days of treatment by 5 and 50mg/kg/day of Al_2O_3.

2.2. Lipids

The results of the evaluation of the variation in the level of lipid in the brain are shown in figure 23. Statistical analysis of these results shows that the administration of Al_2O_3 NPs causes a very highly significant decrease ($p \leq 0.001$) in the group treated by 5 mg/kg/day of Al_2O_3 and also a very highly significant decrease ($p \leq 0.001$) in the group treated by 50 mg/kg/day of Al_2O_3 compared to the control group.

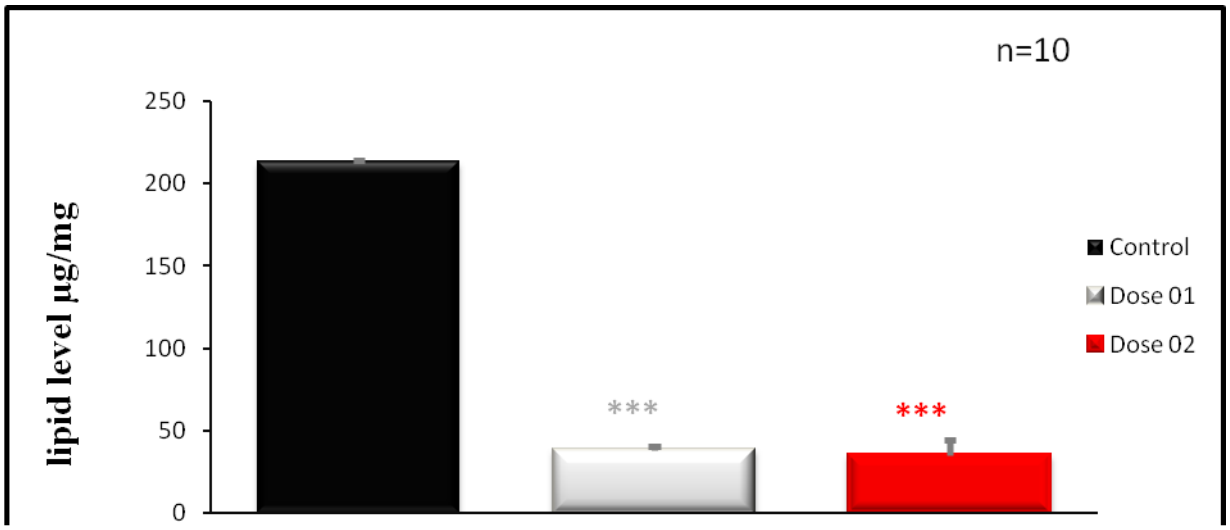


Figure 23. Variation in lipids levels in control and treated rats after 40 days of treatment by 5 and 50mg/kg/day of Al_2O_3.

2.3. Protein

The results of the evaluation of the variation in the level of protein in the brain are shown in figure 24. Statistical analysis of these results shows that the administration of Al_2O_3 NPs causes a very hightly significant increase ($P \geq 0.001$) in the group treated by 5 mg/kg/day of Al_2O_3 and also a very hightly significant increase ($p \geq 0.001$) in the group treated by 50 mg/kg/day of Al_2O_3 compared to the control group.

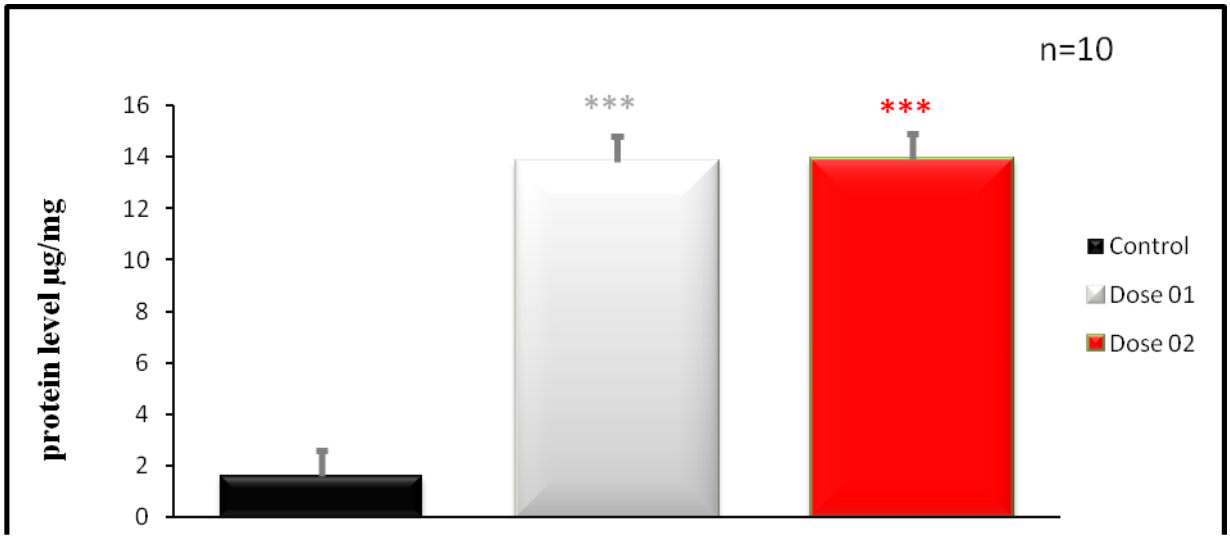


Figure24. Variation in protein levels in control and treated rats after 40 days of treatment by 5 and 50mg/kg/day of Al_2O_3.

3. Effect of of Aluminium oxide nanoparticles on cytosolic redox status in total brain

3.1. Glutathione-s-transferase (GST)

The results of the evaluation of the variation in the level of GST in the brain are shown in figure 25. Statistical analysis of these results shows that the administration of Al_2O_3 NPs causes a very hightly significant increase ($p \leq 0.001$) in the group treated by 5 mg/kg/day of Al_2O_3 and hightly significant increase ($p \leq 0.001$) in the group treated by 50 mg/kg/day of Al_2O_3 compared to the control group.

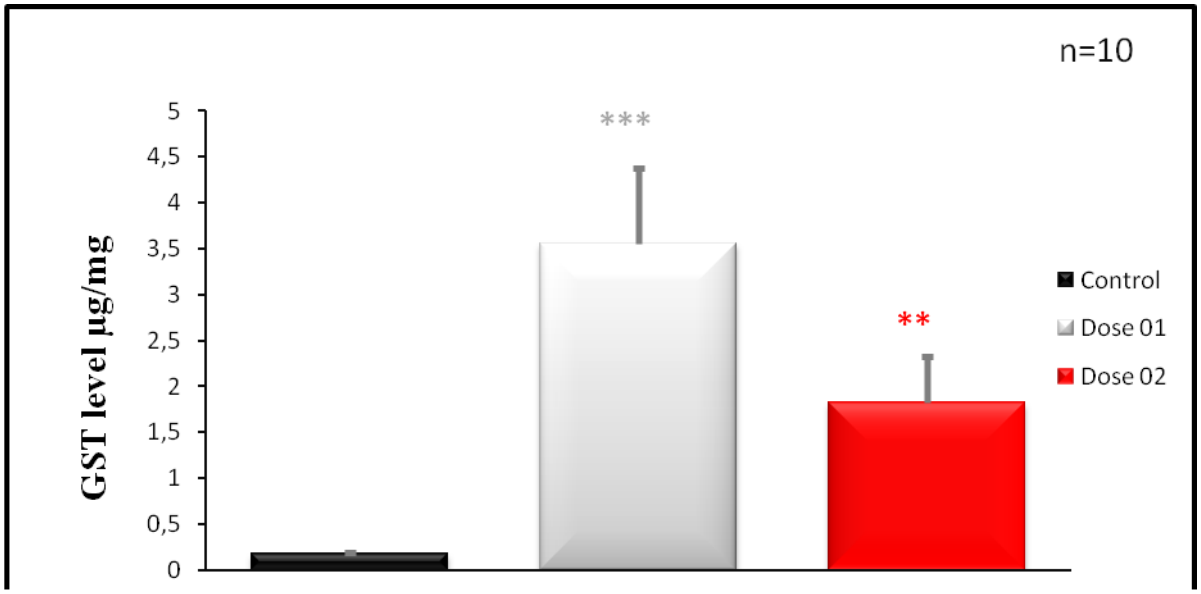


Figure 25. Variation in GST levels in control and treated rats after 40 days of treatment by 5 and 50mg/kg/day of Al_2O_3.

3.2. Malondialdehyde (MDA)

The results of the evaluation of the variation in the level of MDA in the brain are shown in figure 26. Statistical analysis of these results shows that the administration of Al_2O_3 NPs causes a significant increase ($0.05>P>0,01$) of the MDA level in the total brain respectively in the treated with 5 mg/kg/day of Al_2O_3 when compared to the control group anda very highly significant increase($P> 0.001$) in brain malondialdehyde levels in rats treated with 50 mg/kg/day compared to the control.

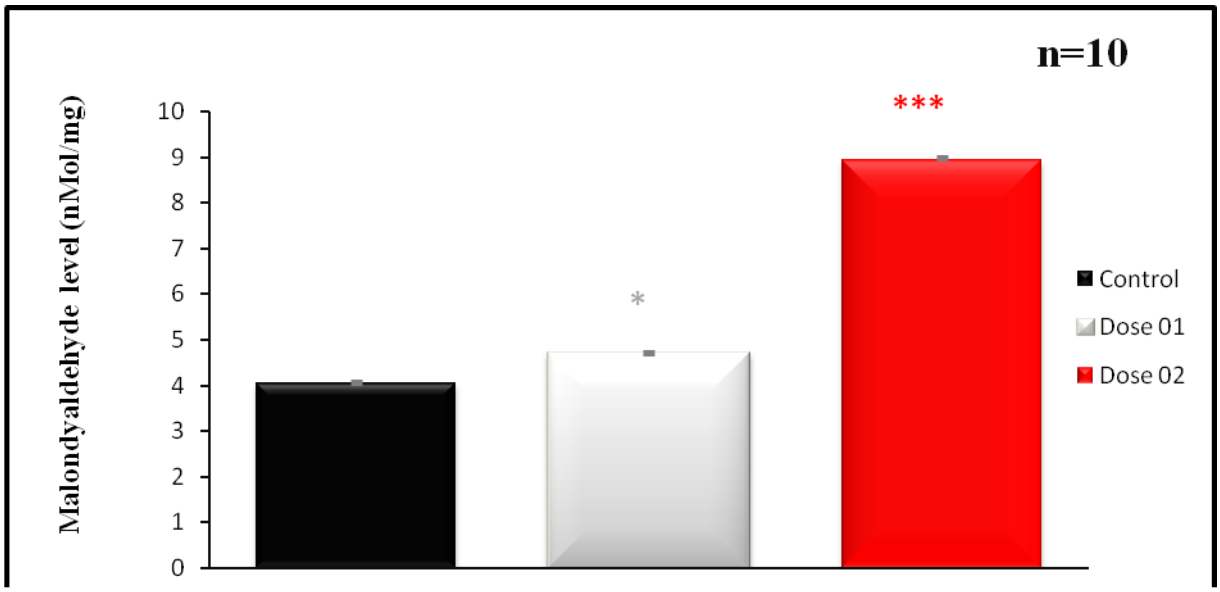


Figure 26. Variation in MDA levels in the brains of control and treated rats after 40 days of treatment by 5 and 50mg/kg/day of Al_2O_3.

4. Effects of Aluminium oxide nanoparticles on neurotransmitters in the total brain

The results obtained during the evaluation of the activity of acetylcholinesterase in the total brain in rats treated with aluminium oxyde nanoparticles in (figure27). Statistical analysis of these results shows that the administration of Al_2O_3 NPs causes no significant increase ($P>0.05$) in the group treated groups by 5mg/kg/day compared to the control, and a significant decrease in the second group treated by 50 mg/kg/day compared with control group.

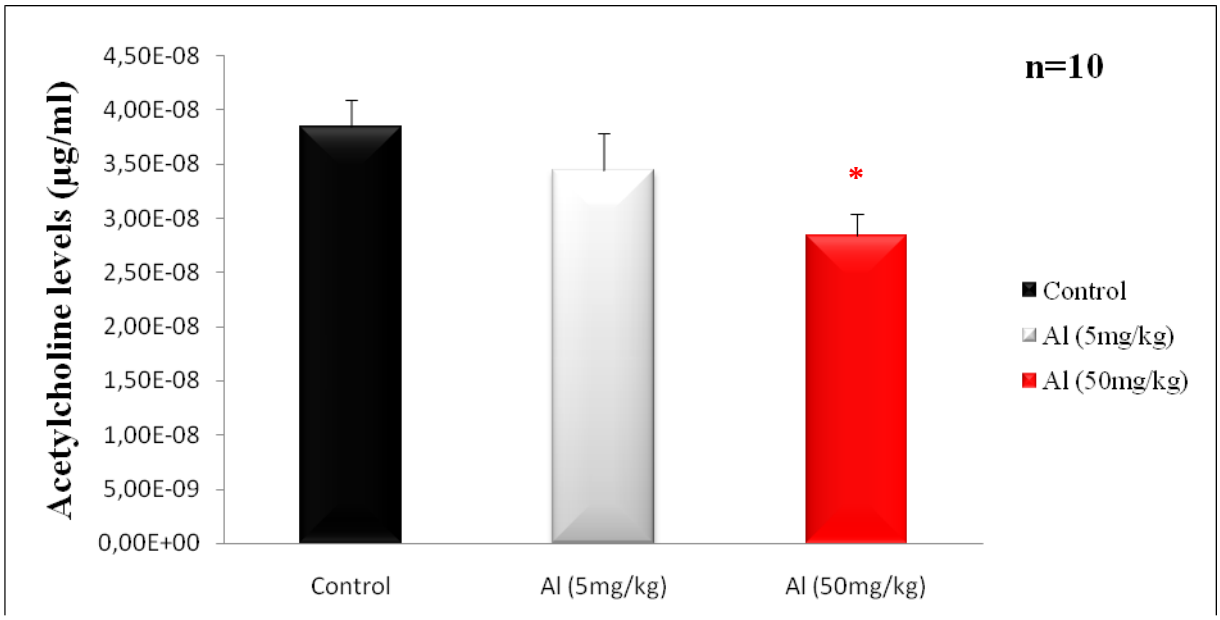


Figure 27. Variation in Acetylcholinesterase levels in control and treated rats after 40 days of treatment by 5 and 50mg/kg/day of Al_2O_3.

5. Effects of Aluminium oxide nanoparticles on neurobehavioural in rats

5.1. Classic Labyrinth Test

The results obtained following the examination of this test on animals are illustrated in figure 28. Statistical analysis of these results showed no significant difference ($p>0.05$) of the arrival time (figure 28) in D1 (5mg/kg/day) and D2 (50mg/kg/day) lots when compared to the controls.

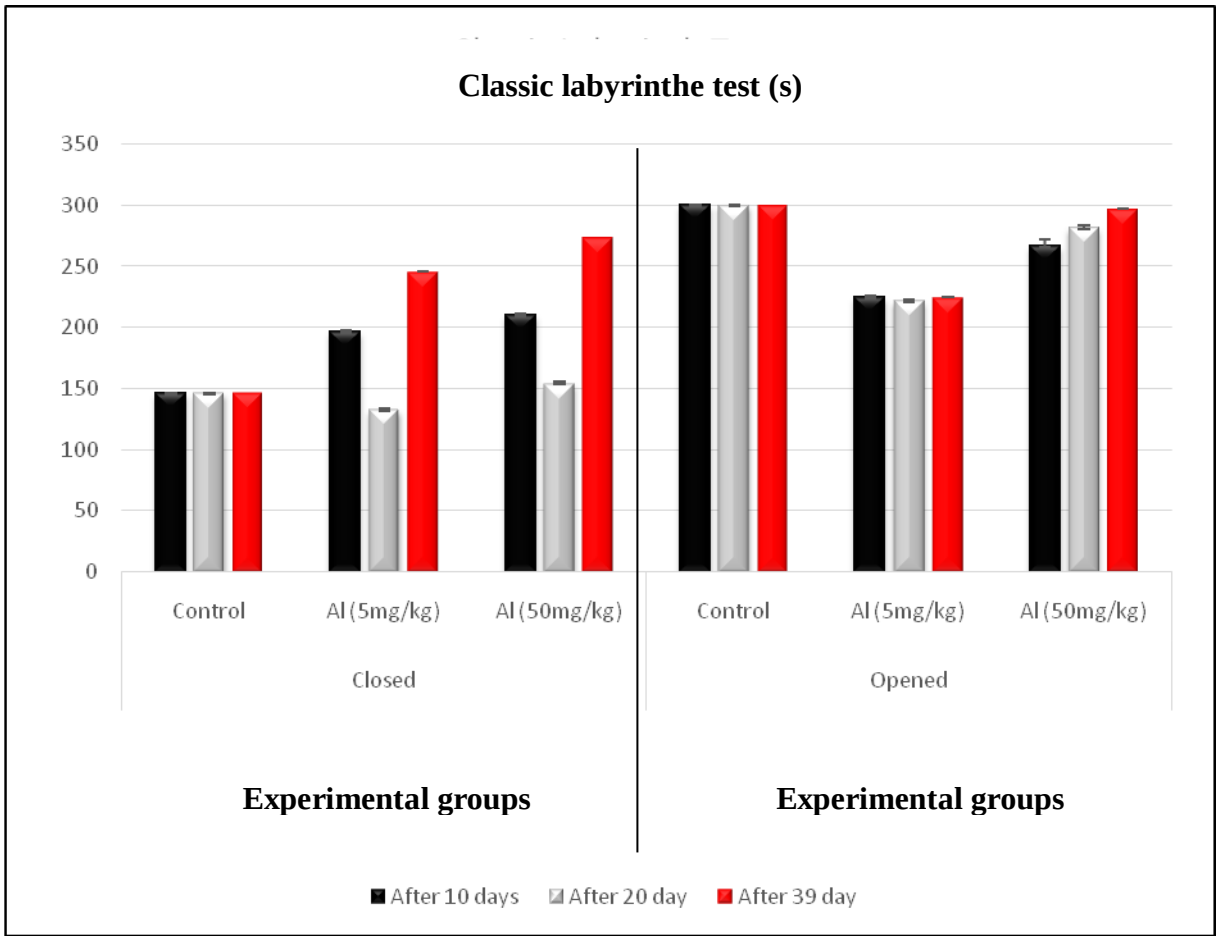


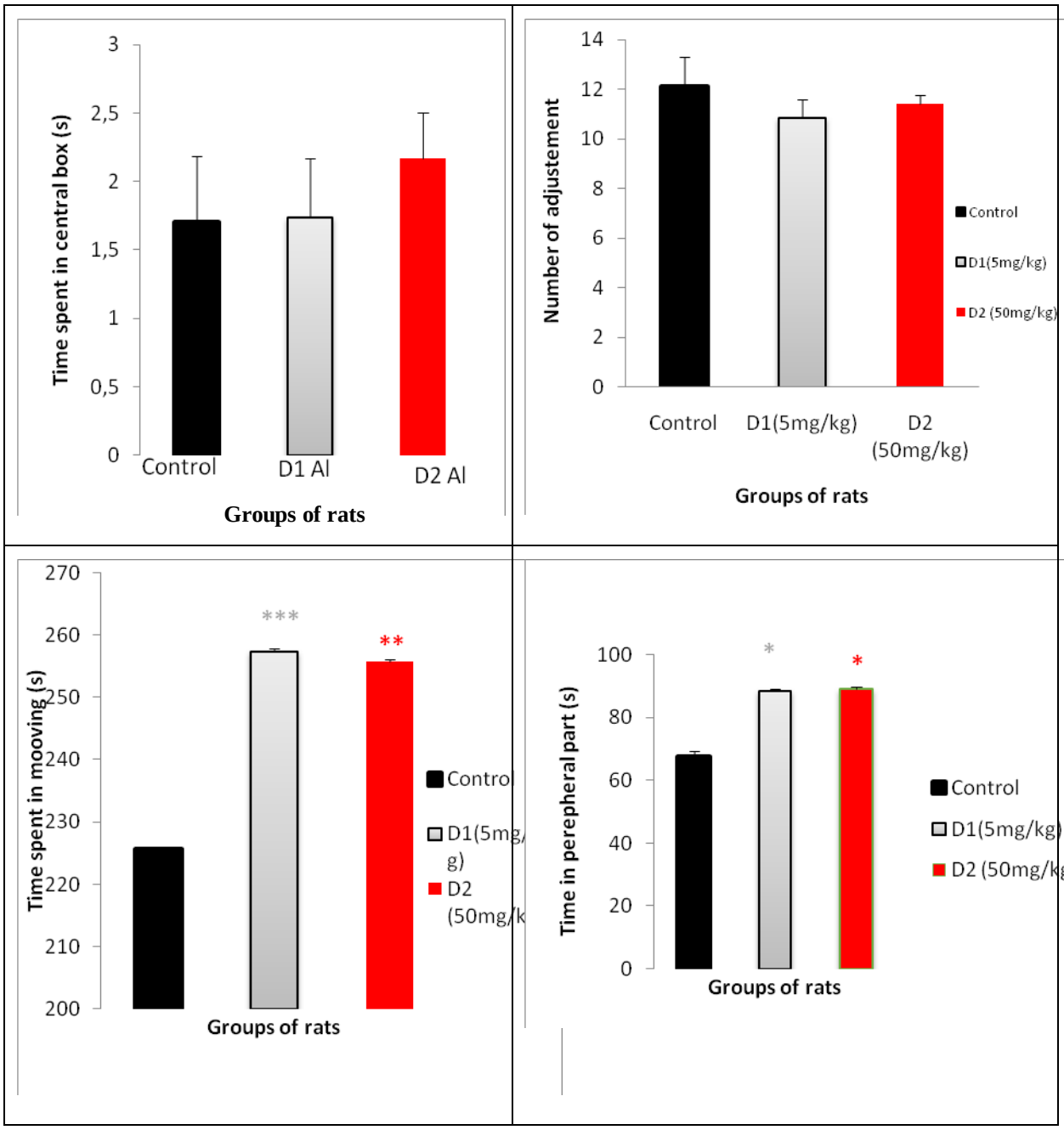
Figure 28. Arrival time in the Classic Labyrinthe in control and all lots treated with Al_2O_3.

5.2. Open Field Test

The results of the Open Field test showed in (Figure 29):

- There is a significant increase ($p \leq 0.05$) in the movement of rats treated with aluminum oxide in the intermediate zone compared to the controls.
- The total number of movements; (C) Distance crossed; (D) Movement in the peripheral zone; (E) Movement in the central zone, showing a significant decrease in the parameters cited in rats treated with the aluminum oxide compared to control rats.

Practical Part



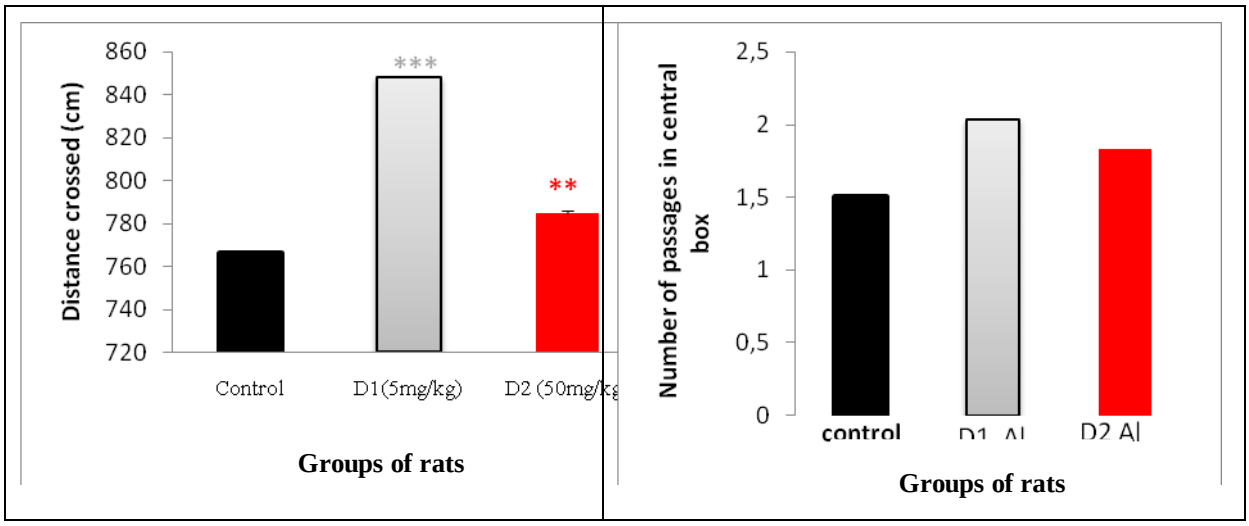


Figure 29. Variation of the parameters of Open field test in groups of rats treated with Aluminum oxide.

5.3. Maze V-soc test

The following figures show the results of the Maze V-soc test, where:

- Figure (30-A) shows the movement of rats at two empty maze after treatment with Al_2O_3 compared to controls Habituation phase.

- The figure (30.B) represents phase II sociability the results obtained from this test show a very significant decrease in same in first strange and very significant increase in empty 2 in rats treated with Al_2O_3compared to controls.

- The figure (30-C) preference and social novelty is a very height significant increase in stranger 1, and a very significant decrease in stranger 2 in rats treated with aluminum oxide compared to control rats.

Practical Part

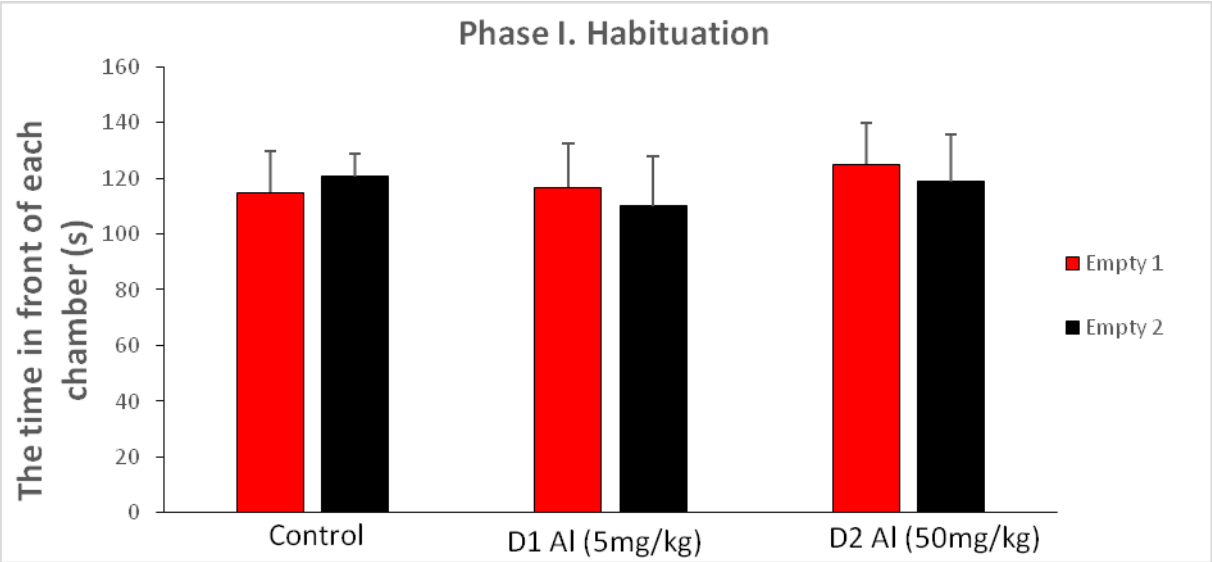


Figure 30-A. Variation in phase I data from the maze Vsoc test in groups of rats treated with Aluminum oxide. . (n = 04, p = 0.000).

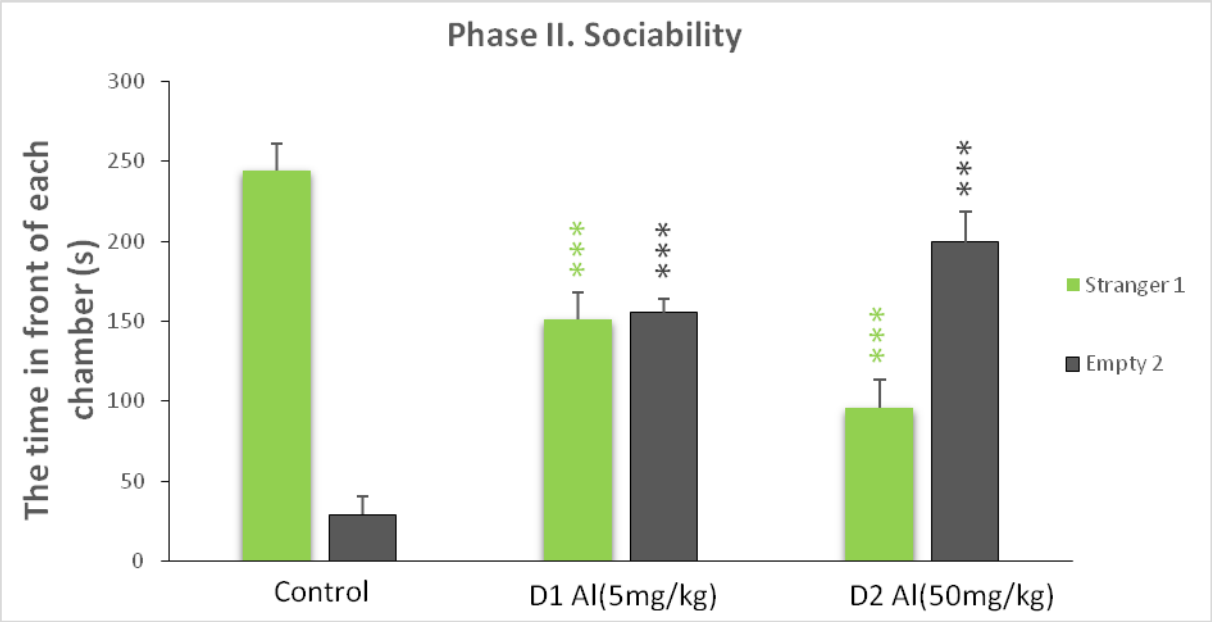


Figure 30-B. Variation in phase II data (sociability) of the maze Vsoc test in groups of rats treated with Aluminum oxide. . (n = 04, p = 0.000).

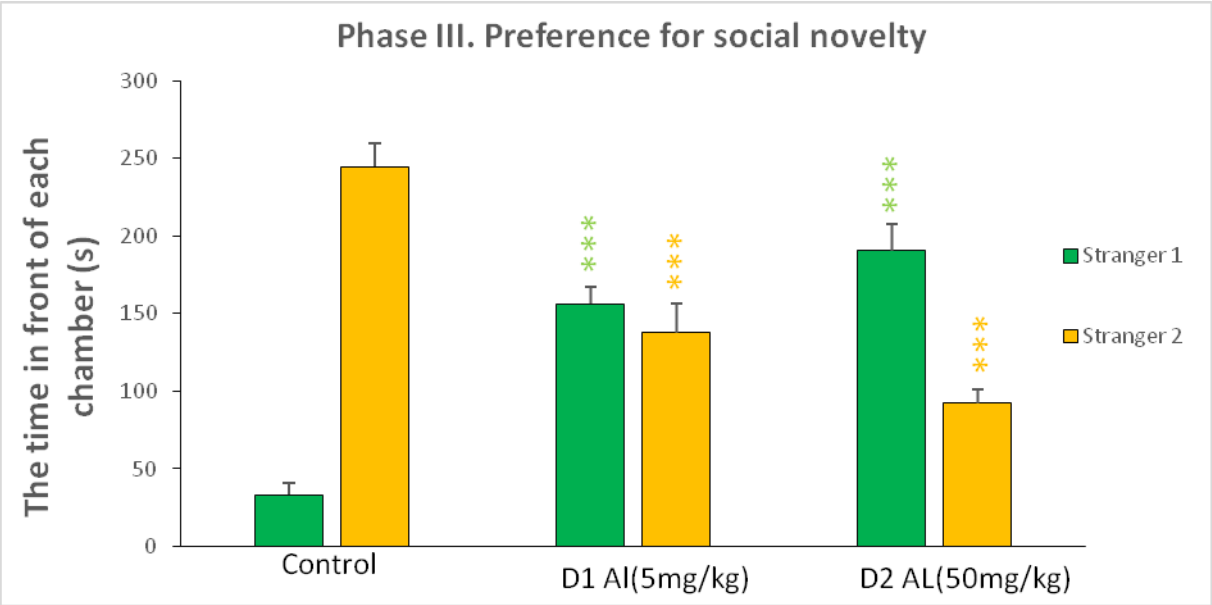


Figure 30. Variation in phase III data (preference for social novelty) of the maze V-soc test in groups of rats treated with aluminum oxide. (N = 04, p = 0.000).

Chapter VI: Discusion

Little is known about the uptake, biodistribution, and biological responses of nanoparticles (NPs) and their toxicity in developing animals such as Wistar rats.

Nanoparticles, when ingested into the body, can travel to different regions and different organs of the body due to their small size. They can cross the small intestine and then find their way into the blood, brain, lung, heart, kidney, spleen, liver, intestine and stomach **(Hillyer et Albrecht, 2001).**

Oxidative stress is caused by exposure to reactive oxygen intermediates, such as superoxide anion (O_2^{*-}), hydrogen peroxide (H_20_2), and hydroxyl radical (HO*), which can damage proteins, nucleic acids, and cell membranes. Increasing evidence suggests that the cumulative damage caused by reactive oxygen species contributes to numerous diseases **(Storz et Imlayt, 1999)**.

The Ultrafine particlescause brain dysfunction. The purpose of this study was to examine the effects of Al_2O_3-NPs on spatial learning, memory, oxidative, biochemical and neurotransmittersresponse by subacuteorale route at two doses of 5 and 50 mg/kg/day in rats. The spatial learning capacities of rats were evaluated in the following tests: Classic Labyrinth and Open Filed; and the social knowning capacities of Wistar rats were evaluated by V-soc test. The assesement of parameters were evaluated in carbohydrate, lipids, proteins, GST, MDA and Acetyl choline.

1. Neuropsychiatric effects of oxidized aluminum

There is increasing speculation that Alzheimer's disease may be linkedaetiologically to the accumulation of aluminium in the brain but this remains a highly contentious issue **(Ebrahim, 1989; Petit, 1989; Murray et *al*, 1991; Crapper, 1994; Munoz, 1994).**

Animal studies have demonstrated the ability of aluminium to inducethe formation of neurofibrillary tangles **(Klatzo et *al*, 1965)**, impair the learning ability of rats, and increase brain acetylcholinesteraseactivity in a similar way to that seen in Alzheimer's disease **(Bilkei-Gorzó, 1993).**

Other workers have shown elevated aluminium concentrations in braintissue from patients with Alzheimer's disease **(Crapper et *al*, 1973)** and laser microprobe studies have demonstrated aluminium accumulationin the neurofibrillary tangles of these patients **(Good et**

***al*, 1992).** He found that although there was no increased incidence of neurological diagnoses in miners exposed between 1944 and 1979 to a mixture of powdered aluminium and aluminium oxide, exposedworkers performed significantly less well on cognitive testing thanunexposed controls; the likelihood of impairment increased withduration of exposure.However, the intraperitoneal injection of aluminum oxide nanoparticles at a dose of 50 mg/ kg/day for 40 days causes the rat to increase in the specific activity of this enzyme, thereby inducing alteration of the nervous system **(AbouDonia et *al*., 2008).**

1.1. Effect on memory and learning capacities

Several uncontrolled studies **(Sjögren et *al*., 1990; White et*al*., 1992; Hänninen et *al*., 1994)** have reported subtle memory defects in aluminium workers **(Hänninen et *al*., 1994).** Demonstrated a negative association between short-term memory loss, learning and attention.

Our study shows the same results including The results of the Open-field (OF) test, classic labyrinth test (CL), and L Maze test which showed behavioral changes such as anxiety increase, nervousness, fear, short-term memory loss, and inability to think or learning. these due to the gradual increase in ROS of brain cells.

1.2. Effects of oxidized aluminum on overall growth parameters

Other studies have also shown that Treatment with Al_2O_3 recorded a significant decrease in body weight gain and feed intake group compared with healthy control group. In this respect **(Domingo, 1987)** found a significant decrease in body weight gain associated with a decrease in food consumption at 260 mg of aluminium per kg of body weight per day.The physiologic basis for this outcome is unclear, but it was reported that animals exposed to highdoses of aluminium in drinking water consumed less food.

1.3. Effects of oxidized aluminum on biochemical parameters

Aluminium has the potential to be toxic for both humans and animals. It was included in the priority list of hazardous substances identified by the Agency for Toxic substances and Disease Registry **(ATSDR, 2007).**

The assessment of harmful effects of aluminium is realized by measuring biochemical parameters **(Kowalczyk, 2004).**

1.4. Effects of Al_2O_3on metabolites

Practical Part

When the intensity of free radical release increases abnormally, cellular components can be affected such as carbohydrates **(Jaeschke, 1995),** lipids andproteins **(Halliwell et Chirico, 1993; Radi et *al.*, 1991)**.

- **Effects on carbohydrates level**

Treatment with aluminum oxide nanoparticles at a dose of 5mg/kg/day and a dose of 50 mg/kg/day for 40 days in Wistar rats is capable of modifying the general level of brains carbohydrates in comparison with the control, these changes reflect a general disturbance of metabolites in the brain with tisular degradation that influence on energic products.

Our results show that we have a very highly significant decrease ($p \leq 0.001$) in both groups which are treated by 5 and 50 mg/kg/day of Al_2O_3 NPs compared to the control group, which may be due to chemical stress caused by the test compound. The biochemical reaction caused by the stress needs more energy, which comes from degradation of carbohydrates; that's explain trhe decrease of the carbohydrate level

- **Effects on lipids**

The dose-dependent decrease in lipid levels after exposure of rats to aluminum oxide nanoparticles may be due to chemical stress caused by the test compound. Indeed, results reported that the harmful effect of chemical compounds could be attributed to the increased formation of malondialdehyde (MDA); the result of the activation of lipid peroxidation (degradation of polyunsaturated fatty acids) by frees radicals **(Dixit et *al.*, 2001; Alpha Jalloh et *al.*, 2009**). or may be due to reduced lipid synthesis or lipase activity involved in lipid oxidation. According to **(Kemajl Bislimi et *al.*, 2015)** the decrease in lipid levels in treated snails is the consequence of oxygenated free radicals which are the cause of lipid degradation through β-oxidation.Our expirment confirm these studies, thats because we have found the same Lipids decrease in the two groupes of rats (5mg/kg/day) and (50mg/kg/day) compared with the group of control.

- **Effects on protein**

In our work we have shown a very hight increase in the protein level in rats treated with aluminum oxide nanoparticles in the two groupes of rats(dose 1 and dose 2) compared to the control group.

The increase in the level of proteins in a dose-dependent manner means that the acetamiprid can be explained by the synthesis of enzymes and peptides defending against the homeostatic imbalance of oxidative stress; by inducing the synthesis of stress proteins in relation to the phenomenon of bio activation / biotransformation on the one hand and on the other hand by lipid peroxidation generated by free radicals **(Anadn et *al.*, 1991; Benbouzib, 2012; Rouabhi et *al.*, 2015)** .In addition, during stress, animals synthesize stress proteins (resistance proteins) where they cana ct with thiol groups (-SH). Bain proteins and reduces metabolism of these substances, thus promoting storage for the second pathway of metabolism.

1.5. Effects of aluminum oxideonthe rate of biomarkers of oxidative stress

- **GST activity**

Our results are shown that, the exposure of rats to aluminum oxide (5 and 50mg/kg/day) induces a very highly significant increase in GST activity. The increase in GST activity is highly correlated with the decrease in the level of GSH, and a form of defense which translates the detoxification of xenobiotics in the body to allow its elimination, and a physiological response to compensate for the alterations which are due to free radicals and which suggests that the xenobiotic conjugated by GSH is catalyzed by GST for reduce its toxic effect, these results are confirmed by studies of **(Ognjanovićet et *al.*, 2008)**.

The response of GST activity depends on several factors such as the type of xenobiotic, the concentration, the exposure time and the species **(Oruç andÜner, 2000).**

- **Effect of aluminum oxide on the level of malondialdehyde (MDA)**

Malondialdehyde is considered to be a major biomarker of oxidative stress in general and of lipid peroxidation in particular **(Grana et *al.*, 2012)**. It is the result of the attack of polyunsaturated lipids by ERO under certain stress conditions, in particular with organic contaminants and inorganic (metalic nanoparticles)**.**It is a major oxidant produced from peroxidized polyunsaturated fatty acid **(Zhang et *al.*, 2004)**.

Our study shows the same decrease in MDA levels in the two groups of rats (5 and 50 mg/kg/day) treated with aluminum oxide, compared with the control group.

1.6. Effects of aluminum oxideon neurotransmitters

Little is known about the impact of nanoparticles on their biodistribution, and biological responses in the brain and their toxicity on neurotransmitters.

Aluminum oxide nanoparticles altered neurotransmitter/metabolite concentrations in juvenile rats' brain tissues. These data suggest that orally administered Al_2O_3 NP interferes with the brain biochemistry **(Mortensen et *al*., 2020).**

CONCLUSION

CONCLUSION

The objective of the present study was to assess the subacute neurotoxicity in Wistar rats of aluminium oxide nanoparticles. The light of the results obtained, we can conclude that:

- The force-feeding of Al_2O_3 NPs respectively at a dose of 5 mg/kg/d and 50 mg/kg/d of adult male rats induced behavioral disturbances of the rats (Open field test, Labyrinth test, V-Soc test).

- Also exposure to Al_2O_3 NPs for 40 days alters carbohydrate metabolism, lipid and protein, accompanied by a remarkable weight disturbance (weight, relative weight and weight gain).

- Oxidized aluminum also causes alterations in the balance of oxidative stress which results a disruption of MDA and GST activity in the total brain.

- Evaluation of neurotransmitters parameters such as Acetyl choline esterase also shows a very clear disturbance after the sub-acute administration of Al_2O_3 NPs.

Knowing that the massive use of oxidized aluminum nanoparticles in several areas (cosmetics, medicines, electronics, aerospace, chemical engineering, food contact materials, catalysts, ceramics and rubber), we must therefore seek of course the details of this molecule to avoid all forms of intoxication or health problems induced has neurodegenerative diseases linked with this molecule. Of this effect, this thesis work can be completed by more developed studies and as perspectives we propose from:

- To determine the effects of final metabolites of oxidized aluminum on the body after chronic exposure under the same experimental conditions and also under different conditions.
- Try to use a medicinal plant as a specific antidote against the different types of poisoning by this molecule (hepato, neuro. nephro and pneumotoxicity).
- To develop the goal of this study by other competency tests.
- To develop the goal of this study by assaying other biomarkers.

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