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Theme

Characterization of locally extracted red and green clay and study of its effect against toxicity and oxidative stress caused by lead in rats.

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Dédicaces

Une dédicace particulière et sincère à notre enseignant **Dr DEROUICHE SAMIR**

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Abstract

The objective of this work is to study the effects of red and green Clay on changes in biochemical, haematological, oxidative stress and histopathological parameters in rats acutely exposed to lead acetate. The experimental study carried out in the laboratory on 25 male Wistar rats which divided into five group of five rats in each, the first group served as control, the second group is contaminated with lead, the third group is contaminated with lead and treated with red clay, the fourth group is contaminated with lead and treated with green clay. Some biochemical, haematological, oxidative stress parameters were analysis. From the analysis of our results, we observe a considerable change in body weight and an increase in the relative weight of liver, kidney and heart in rats contaminated with lead compared to control. The results also showed notable changes in the biochemical parameters, by a decrease in the serum glucose concentration ($P>0.05$), uric acid calcium level, iron ($p <0.001$) and serum creatinine concentration ($p<0.01$). While urea was significantly increased. However, serum transaminase enzymes (TGP, TGO) activities were increased in rats contaminated with lead in comparison with control. The results obtained revealed also a haematological toxicity and oxidative stress in rats contaminated with lead and a decrease in Hb, HCT, MCV, WBC levels and in tissular GSH concentration and an increase in PLT, RBC, granulocytes, tissular MDA, GST and SOD levels compared to control. Also, the results clearly showed alterations in the structures of the tissues studied in comparison with the controls, with hemorrhage, inflammation with hepatic and testis necrosis. Treatment with red and green clay partially ameliorates biochemical, hematological and oxidative stress parameters, with protection and regeneration of the tissue against free radical attacks caused by lead. In conclusion, this study shows that treatment with red and green clay induces a beneficial effect against lead toxicity at molecular and tissue level.

Key words: Lead, toxicity, clay, oxidative stress, Wistar rats.

الملخص

الهدف من هذا العمل هو دراسة تأثير الطين الأحمر والأخضر على تغيرات في بعض المعايير البيوكيميائية، معايير الدم والاجهاد التأكسدي والأنسجة المرضية في الجرذان المعرضة لخلات الرصاص بصفة حادة. الدراسة التجريبية أجريت في المختبر على 25 من ذكور فأر ويستار مقسمة إلى خمس مجموعات من خمسة فئران في كل منها، المجموعة الأولى كانت شاهدة، المجموعة الثانية ملوثة بالرصاص، المجموعة الثالثة ملوثة بالرصاص ومعالجة بالطين الأحمر والمجموعة الرابعة ملوثة بالرصاص ومعالجة بالطين الاخضر. تم تحليل بعض المعايير للإجهاد التأكسدي والبيوكيميائية ومعايير الدم. النتائج المتحصل عليها تظهر تغيرًا كبيرًا في وزن الجسم وزيادة في الوزن النسبي للكبد والكلى والقلب في الفئران الملوثة بالرصاص مقارنة بالشاهدة. كما أظهرت النتائج تغيرات ملحوظة في المعايير البيوكيميائية، من خلال انخفاض تركيز الجلوكوز في الدم ($P>0.05$)، ومستوى الكالسيوم في حمض البوليك، والحديد($p <0.001$) ، وتركيز الكرياتينين في الدم .($p<0.01$) بينما تم زيادة اليوريا بشكل ملحوظ. ومع ذلك، فقد زادت أنشطة إنزيمات ناقلات الامين في المصل TGP و TGOفي الفئران الملوثة بالرصاص مقارنةً بمجموعة الشواهد. أظهرت النتائج أيضًا وجود اضطراب في معايير الدم الاجهاد التأكسدي في الفئران الملوثة بالرصاص وانخفاض في مستويات الهيموغلوبين، MCV،HCT ، WBC وفي تركيز الغلوتاثيون في الأنسجة وزيادة في مستويات الصفائح الدموية وكريات الدم الحمراء والخلايا الحبيبية وبيروكسيد الدهون النسيجي ونشاط كل من GST وSOD مقارنة بالفئران الشاهدة. كما أظهرت النتائج بوضوح تغيرات في بنية الأنسجة المدروسة مقارنة الشواهد من حيث وجود نزيف الاوعية والتهابات مع تنخر نسيج الكبد والخصية. النتائج اثبتت ان العلاج بالطين الأحمر والأخضر يخفف جزئيًا من عوامل الإجهاد التأكسدي ومن المعايير البيوكيميائية ومعايير الدم، مع حماية وتجديد الأنسجة ضد هجمات الجذور الحرة التي يسببها الرصاص. في الختام، تظهر هذه الدراسة أن العلاج بالطين الأحمر والأخضر له تأثير مفيد جدا ضد سمية الرصاص على المستوى الجزيئي والنسيجي .

الكلمات المفتاحية: الرصاص، السمية، الطين الأحمر، الطين الأخضر، الإجهاد التأكسدي، فئران ويستار.

Abbreviation list

° **OH**: the hydroxyl radical.

8-OHG: 8-hydroxy-guanine.

ALA: Aminolevulinic acid synthase.

ALAD: Delta-aminolevulinic acid dehydratase.

BHT: Buthydrxytoluene.

Ca^{2+}: calcium.

CAT: Catalase.

CDNB: GSH,1–chloro-2,4–dinitrobenzene.

Cu / Zn-SOD: Superoxide dismutase with copper and zinc ions.

DL: Decilitre.

DNA: deoxyribonucleic acid

DTNB: 5,5dithiodis-2-nitrobenzoique

E: Enzyme.

EDTA: ethylenediaminetetraacetate.

F: Unit focuses.

G: unit of gram.

GC: Greene clay.

GOT /ASAT: Glutamic Oxaloacetate Transaminase/ Aspartate aminotransferase.

GPx: Glutathione peroxidase.

GSH: Glutathione.

GST: Glutathione S-transferase.

H+: hydrogene.

H_2O: Dihydrogen monoxide.

H2O2: hydrogen peroxide.

HCL: hydrochloric Acid.

HCT: haematocrit.

HGB: haemoglobin.

HIF-1: a central regulator of hypoxia.

HNE: 4-Hydroxynonenal.

HNO3 :Nitric Acid.

HPGPx : Plasma glutathione peroxidase and membrane glutathione peroxidase.

IR: Infrared light.

L: unit liter.

L˙ : Lipid radical

LH: Luteinizing hormone.

LOO °: The peroxyl radical.

LOOH: Hydroperoxide.

MCV: The mean blood volume.

MDA: Determination of malondialdehyde.

MET: Methionine.

MG: Unit of Milligram.

Ml: Unit millilitre.

N MOLE: Nanomole.

NaCl: Tris Sodium Chloride.

NADHoxidase: 1,4-dihydronicotinamide adenine dinucleotide (NADH) oxidase.

NBT: Nitro blue tetrazolium chloride.

NF-kappaB: Nuclear factor kappa B.

NF-κB: Nuclear factor-kappa B.

NO: Nitric oxide.

O_2 : Oxygen.

O2•− : Superoxide.

UI : International unit.

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Introduction

Introduction

Lead is a naturally occurring heavy metal (Belli, N and *al*; 2010). nonessential, inorganic and is primarily absorbed by the respiratory system and the digestive tract (Saad S and *al*; 2011). extremely toxic, widely distributed in the environment and exposure to this element is still a major public health problem (Belli, N and *al*; 2010). It can accumulate in the body and disrupt the body, especially the system nervous system, blood, gastrointestinal tract, cardiovascular system and kidneys (Pourrut B; 2008). This toxicity is explained by the formation of reactive oxygen species (ROS) (Boubali Z; 2017). which causes an imbalance between the pro-oxidant and antioxidant systems (Zerargui F; 2015). This imbalance potentially leads to structural damage and functional (Bensakhria A; 2018). at the level of the organism. All of this translates that the lead toxist causes oxidative stress (Saad S and *al*; 2011). Cells under oxidative stress exhibit various dysfunctions due to damage have various dysfunctions due to damage caused by ROS to lipids, proteins and DNA. the toxicity associated with this metal could be due to oxidative tissue damage (Uttara B and *al*; 2007). There may be an independent source of oxidative damage related to the direct effect of lead on membrane lipids (Pourrut B; 2008). Considering that lead toxicity is currently one of the world's serious problem, there is still no specific, reliable and safe treatment. Several metals used to inhibit the effects of lead (Klauder, DS; 1975). among this metals iron which is the main constituent of clay (Nevila J; 2003). the latter is a sedimentary rock (Erwan-Nicolas P; 2011). resulting from natural erosion (Tucker, 2001). extremely rich in mineral salts (Nevila J; 2003). Silica, aluminum, zinc, magnesium, calcium, iron, copper, potassium and sodium (Kishk FM; 1975). its mineral wealth and trace elements make it a tool in health (Le Ray Anne-Marie; 2016). In our region, Pregnant Women use clay as a treatment against heartburn by regulating gastric acidity and also used as a face mask. In light of these data, the present study was carried out to investigate the two following Complementary aspects:

The first part: based on the characterization of both type of clay

The third part: based on the in-vivo study for evaluation of the protective effect of red and green clays against toxicity and oxidative stress induced by lead in rat.

First part

Bibliographic synthesis

Chapter I

The lead

1/-Definition:

Lead is a chemical element that exists in nature (Derouiche S and *al*; 2018). is included in heavy metals designates for chemists high atomic number metals (Jeannot and *al*; 2001) .it has the atomic number is 82 (Bozdağ and *al*; 2019). It is recognized as one of the most toxic and harmful heavy metals, even in low quantities (Derouiche S and *al*; 2018). and is an environmental pollutant (Derouiche S and *al*; 2019). It is a widely used metal since the ancient period (Plante and *al*;;1998), and it is used in many industrial applications (Mehmet Bozdağ and *al*; 2019). Lead is a multi-target toxicant, capable of causing different alterations during exposure (Garouri M and al and *al*; 2015). its persistence in the body thus presents a great risk for human health (Gorbela F; 2002). Lead can be combined to form inorganic and organic molecules (Benedetti and *al*; 1998). but, organic lead can be more toxic than inorganic lead because the body absorbs it more easily (CSEM; 2010).

2/ - The physico-chemical properties of lead:

Lead has atomic number Z = 82 (Mehmet Bozdağ and al ;2019), and the atomic mass is 207.21g, for its melting point is 327 ° C and their boiling point is 1720 ° C (Sposito and *al*; 1982), it is a dense metal (d = 11.34 at 20 ° C) and its specific heat capacity at 20 ° C is 0.125J / g, and presented by resistivity is 20.65μΩ / cm (Cezard;1992, Chantal;2000).

3/ - Metabolism:

3-1/- Absorption:

The lead mainly enters the body through three routes through the digestive and pulmonary routes and also through the skin (OPPTBP; 2015).

3-1-1 /- Digestive route:

Is the main route of contamination (intoxication plomb Sites) It can be direct by ingestion of food (contaminated water or food) or by contact of soiled hands with the mouth (Kaminsky P and *al*;1993). The percentage of lead resorbed by the digestive route is 10% in adults; it is 50% in young children ,diets enriched with these minerals decrease its absorption (Rman I and *al*;1978),

and the iron deficit is associated with a greater absorption for lead and diets low in protein or high in fat which support increased lead absorption (Taylor A; 1986).

3-1-2 /- Respiratory route:

The respiratory tract is the second possible route of contamination (OPPTBP;2015). lead vapors, oxides or pulverulent salts, very fine dust or fumes or lead dust found in the air (Lauwerys;1992), it is also to blame for the lead fixed on the particles suspended in the air: only the very fine particles can penetrate into the pulmonary alveoli, the larger ones are rejected, or raised by the mucociliary carpet and swallowed (then borrowing the digestive tract) (WHO;1980).

3-1-3/- Cutaneous use:

Lead can also enter the body following skin lesions (ATSDR; 1988). on the other hand The transcutaneous passage of inorganic lead derivatives is very low compared to organic lead (liposolubility) (Alexander and *al*; 2013).

3-2/- Distribution:

Blood lead represents only 1 to 2% of the quantity present in the body (Bailly C and *al*; 2001). the half-life of lead in the blood can be as short as 20-40 days (WHO;2001). , for distribution Lead absorbed by the digestive tract passes into the bloodstream (Mortureux and *al*; 2013). where it is distributed between red blood cells (90%) and plasma (less than 10%) probably due to its affinity for thiol groups (Bailly C and *al*; 2001). therefore the compartment with very rapid exchanges: plasma proteins, and the second compartment with rapid exchanges: soft tissues (kidneys, brain, spleen, liver, bone marrow, but also red blood cells , etc.) The last compartment with intermediate exchanges: muscles, trabecular bone (Kaminsky P and *al*; 1993).

3-3/-Elimination:

Lead excretion is mainly urinary (> 75%) (Laperche V and *al*; 004). and it not absorbed by the gastrointestinal tract is eliminated by faeces faecal (15-20%) (Garnier R; 2005). Lead can also be eliminated through saliva, sweat, hair and nails. Negligible under normal conditions,

exposure to heat can lead to sweat excretion in humans greater than urinary elimination (Asayama and *al*; 1975), (Piechalak and *al*; 2008). elimination life is greatly increased in the event of renal failure (WHO; 1995)**.** Lead is found in the urine from the daily ingestion of at least 1 mg of dd lead acetate, essentially in free ionized form when blood lead levels are within normal limits (Kehoe;1987).

4/- Main toxic effects of lead on human health:

4-1/- Acute toxicology:

Occur by inhalation or absorption in accidental situations. These effects generally appear for blood lead levels of between 1000 and 2000 µg / l, but can occur in certain subjects at much lower levels of between 400 and 600 µg / l, (Malcom; 1970, Abed and *al*; 1973). in children for intoxications leading to blood lead levels that can vary from 900 to 8000 µg / l (Al Khayay and *al*;1997), Their symptom:

- Digestive disorders are among the earliest symptoms. They result in the appearance of severe colic associated with abdominal pain and cramps (Awad and *al*; 1986).
- Anorexia from vomiting in intermittent phases.
- Renal failure the appearance of tubular lesions characterized by oliguria, albuminuria, glycosuria and hyperphosphaturia (Cramer and *al*; 1974, Bennett; 1985).
- Lesions to the central nervous system (headache, agitation, delirium, hallucinations) are clinically manifested by convulsive encephalopathy and coma which can lead to death (Kehoe;1961).
- Severe neurological or psychomotor (psychomotor delay, epilepsy,blindness and hemiparesis).
- Effects on hepatic metabolism (Swarup and *al*; 1991).

4- 2/- Chronic toxicology:

The two routes of exposure to lead by ingestion, and inhalation for symptoms of poisoning are:

- Neurological effects; The first signs of central neurological damage are headache, asthenia, sleep disturbances (insomnia, nightmares), difficulty concentrating, irritability, decreased libido and depressive thoughts.
- Encephalopathy. And Cardiovascular Effects.
- Peripheral neuropathy. And Abdominal syndrome.
- Renal and hepatic effects.
- Haematological effects., Carcinogenicity. Signs of impregnation.
- Metabolic and endocrine effects. And Effect on reproduction (Robert; 2015).

Chapter II

Oxidative stress

1/-Definition of oxidative stress:

This imbalance comes either from an exaggerated production of oxidizing agents (free radicals and ROS), or from an alteration of the defense mechanisms. This imbalance potentially leads to structural and functional damage (Bensakhria A;2018). The term ROS refers to several types of oxygen-reactive metabolites such as free radicals and other non-radicals such as hydrogen peroxide (H2O2) (Lavoie M;2012).

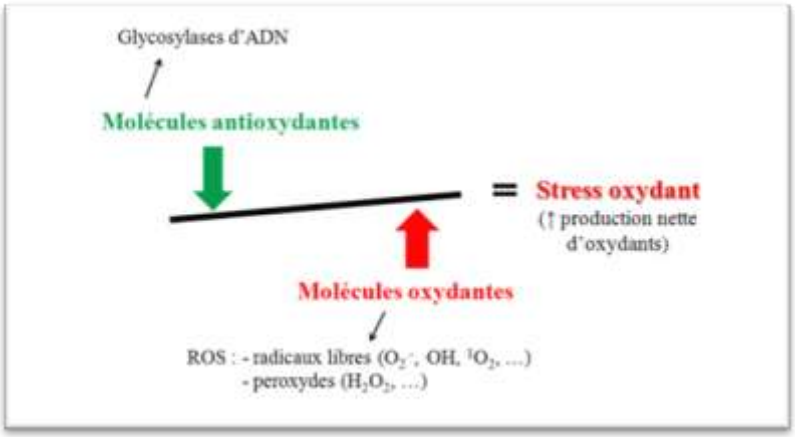


Figure01: Components of the balance between anti- and pro-oxidant molecules.
GPx: glutathione peroxidase, GSH: reduced glutathione, H_2O_2: hydrogen peroxide, O_2.-: superoxide anion, OH: hydroxyl radical, $1O_2$: singlet oxygen, SOD: superoxide dismutase (Clémentine P; 2013).

2/-Free radicals:

2-1/- Definition of Free radicals:

Free radicals (R") are molecules or atoms which have one or more unpaired electrons on their outer shell (Tessier, F; 1995). (chemical species) (Carange J;2010). The magnetic field created by the rotation of this electron, or spin, is 2not compensated by the reverse rotation of a paired electron. This property makes free radicals capable of reacting with various molecules, including cellular compounds: lipids, proteins and nucleic acids, in particular during chain reactions, the best-known example of which is that of the peroxidation of lipids. (Boubali Z;2017). These are products permanence by the organism (Eddhima M Z; 2019). from oxygen in the cell (Jennifer B; 2014). in particular at the level of the mitochondria, in the respiratory chain (Barouki R; 2006).

These are unstable molecules, this compound can react with the most stable molecules for pair its electron (SADOUD M;). their very short lifespan (of the order of a millionth or ten-millionth of a second) (Tessier F;1995). Among the different classes of free radicals, the reactive oxygen species (ROS) are the most abundant radicals. This class of free radicals groups together radicals which derive from oxygen by reductions to one electron, such as the superoxide anion ($0^{2.-}$), the hydroxyl radical ($OH^{.}$), the peroxyl radical ($RO^{.2}$), the alkoxyl radical ($RO^{.}$) and perhydroxyl radical (H^{02}) (Carange J;2010), ROS are reactive and very toxic substances (Munro D; 2014). In oxidative stress phenomena, the free radicals involved have a common characteristic property, that of having a single electron on an oxygen atom ". This gives them the name of reactive oxygen species (ERO or ROS) or nitrogen (ERA or RNS) (Zerargui F; 2015).

2-2/-Nature and source of reactive oxygen species:

2-2-1/-Endogenous source:

- ***ROS of mitochondrial origin:***

The production of ROS in mammalian cells is essentially of enzymatic origin: the membrane NAD (P) H oxidase and the mitochondrial enzymatic complex of the respiratory chain are the main sources (Zerargui F; 2015). this production takes place endogenously and mainly comes from the metabolism of mitochondrial, peroxisome and endoplasmic reticulum. (Boundjah O;2014). during normal metabolism, the tetravalent reduction of oxygen O_2 to water H_2O (Migdal, C; 2011).The leakage of electrons at the level of complexes I and III of the mitochondrial respiratory chain causes a reaction with molecular O_2, which leads to the formation of the superoxide anion ($O_2^{\circ -}$), which will then be transformed into less reactive radicals such as H_2O_2 (Haissaguerre M;2015). by superoxide dismutases (SOD) present in the inter-membrane space of the mitochondria (Bounedjah O;2014). The latter reacts with iron, in Fe^{2+} form to form HO^{-}. And ferric iron (Fe^{3+}): this is the Fenton reaction. Then the ferric iron is reduced to ferrous iron by $O_2^{\cdot -}$. All of these reactions form the Haber Weiss reaction (Poisson C; 2013). These mitochondrial ROSs could be involved in the oxidation of LDL. Mediators of inflammation. In addition, in the body, oxygen is reduced by 95% in the mitochondria enzymatically to a non-toxic molecule like H_2O (Sadoud M).

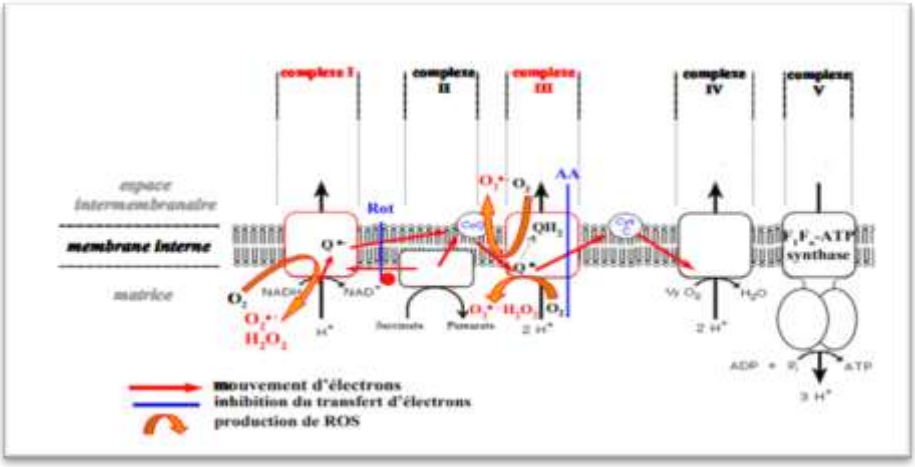


Figure02: Respiratory chain ROS production sites. Two O_2 • - production sites are recognized: complex I and complex III. The use of rotenone (Rot) and antimycin A (AA) made it possible to localize the production of ROS at the level of these complexes and to demonstrate the reverse flow of electrons going up from complex II to complex I.(Julien P;2012).

2-2-2/- <u>Other sources of endogenous ROS and exogenous source:</u>

Free radicals are molecules formed by our body or produced under the effect of external factors, there are several factors that contribute to the production of free radicals which are listed in the following table:

Table 01: Main sources of RL (endogenous and exogenous) (Halliwell., 2006; Durackova., 2008; Rees and al., 2008).

Endogenous sources	Exogenous sources
hemodialysis	toxic environmental
phagocyte	ionizing radiation
atherogenesis	UV radiation
intensively exercised	electric fields
inflammation	prooxidant xenobiotics
lipo-oxygenase	pro inflammatory cytokines
ischemia-reperusion state	smoking
peroxisome	chemotherapy
reactions of transition ions	ozone

2-3/- Targets of free radicals:

Free radicals are permanently produced in small quantities, such as tissue mediators or residues of energy or defense reactions, in the organism The balance between the positive and negative effects of free radicals is particularly fragile (Pincemail; 2003). and this physiological production is perfectly controlled by defense systems, which are moreover adaptive in relation to the level of radicals present (Favier; 2003). Regulatory systems are made up of proteins, enzymes, small antioxidant molecules and essential trace elements. for the activity of enzymes. (Gutteridge;1992, Curtin and al; 2002). An imbalance of the antioxidant balance in the production of ROS constitutes oxidative stress. Oxidative stress damages biological molecules (lipids, proteins, DNA) and cause pathologies (Gutteridge; 1992, Curtin and al; 2002).

2-3-1/- Effects on lipids:

Lipids The first preferred targets of radical attack are lipids and especially their polyunsaturated fatty acids (Bahi A; 2015). which are characterized by one or more elements of chemical structure (- CH = CH - CH2– CH = CH–) are very abundant in nature (Hennebelle M; 2012). The position of one or more methylene groups between two double bonds makes them particularly sensitive to oxidation by metals, oxygenated free radicals (Zaidane E;1993). which lead to the formation of lipid peroxides (Tratner;2003, Delattre; 2005).

Autoxidation is a direct reaction of molecular oxygen with organic compounds (Cillard J and *al* ;2006), among the organic compounds of unsaturated fatty acids introduces a radical chain reaction process which is divided into three stages: the initiation, propagation and termination. (Zaidane E ;1993). :

- ✓ The initiation :

Lipid peroxidation by hydroxyl, alkoxyl, peroxyl, singlet oxygen or peroxynitrite radicals (Zaidane E;1993). attack on reactive species especially the hydroxyl radical (° OH) (Bahi A ;2015) fatty acid located on a carbon placed between two double bonds (weaker hydrogen carbon bond), (Cillard J and al ;2006), which after addition with molecular oxygen gives the peroxyl radical (LOO °) (Delattre and al; 2005).

✓ The propagation :

The peroxyl radical is lipid reacting with another polyunsaturated fatty acid and forming a hydroperoxide (LOOH) (Bahi A ;2015).

✓ Termination:

After having reached a maximum speed of oxidation (Cillard J ;2006). The radicals formed react with each other to lead to a product which is not a free radical therefore decreases (Eymard S;2003). by glutathione peroxidase and vitamin E intercalated in the lipid bilayer of membranes (Esterbauer and *al*;1992, Beaudeux and *al*;2003, Favier;2003).

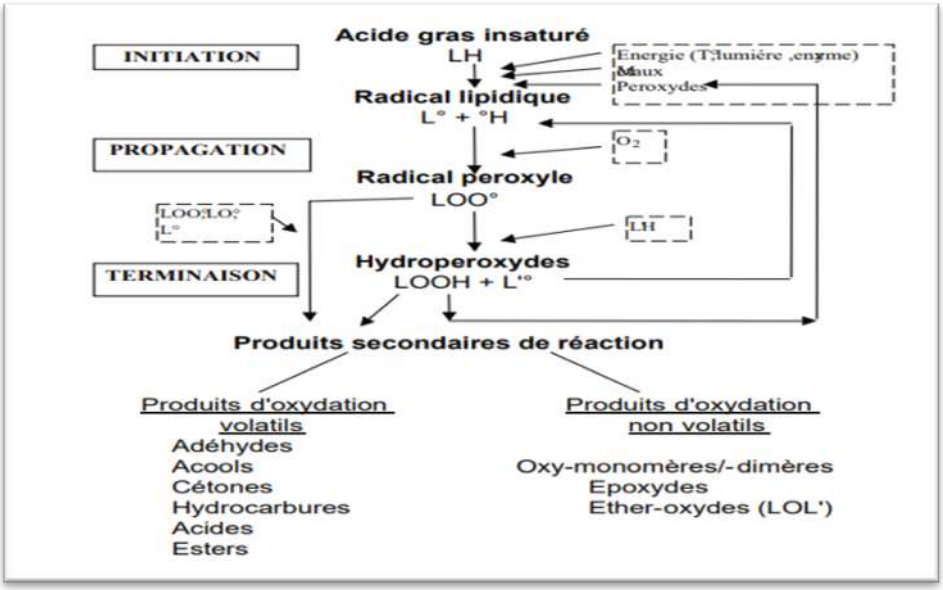


Figure 03: General diagram of lipid oxidation. (Eymard S;2003).

- Polyunsaturated fatty acids, esterified (phospholipids, cholesterol esters, triglycerides) or not (non-esterified fatty acids), are major targets of radical attack (Zaidane E;1993).
- Another important target for radicals is unesterified cholesterol which, upon oxidation, leads to the formation of oxysterols (Michel F and al ; 2008).
- Low density lipoproteins are susceptible to being oxidized by ROS which causes a change in their structure leading to the formation of aldehydes (MDA and HNE) (Nicolosi and al;1999). can cause disease cardiovascular (Michel et al 2008).

- Effects Radicals on membrane phospholipids which modify membrane fluidity (Haleng and *al*;2007). and therefore the functioning of numerous receptors and transporters and signal transduction (Cillard J ;2006).

*2-3-2/- **The effects on proteins:***

Proteins Like lipids, proteins can also be the target of radical or oxidative reactions (Levine;2002). The modifications take place on the polypeptide chain and the nucleophilic or sensitive side chains of amino acids (Zaidane E ;1993). Amino acids basic (arginine, histidine, lysine), sulfur (methionine, cysteine) or aromatic (phenylalanine, tryptophan, tyrosine) are particularly sensitive to oxidation (Durand and al; 2013), is done in particular by the addition of carbonyl groups which can react with amine functions to form imine bonds (-HC = N -) (Durand and al; 2013). cause Protein dysfunctions that can go as far as a complete loss of function or protein aggregation (Magali M ;1998) .

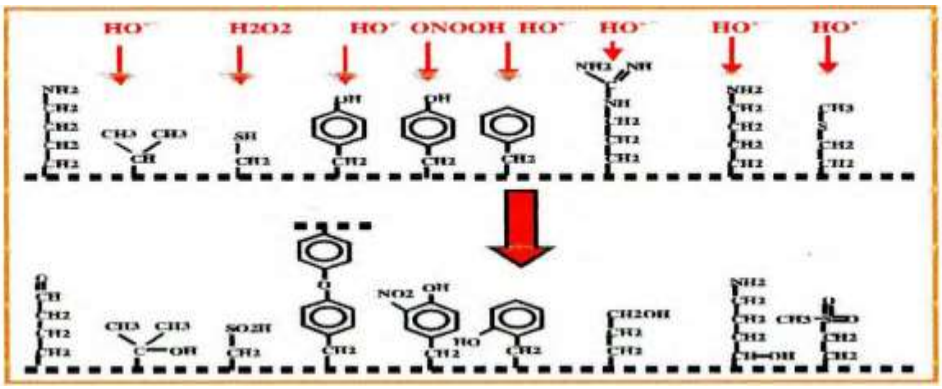


Figure 04: Nature of some side chain modifications of protein amino acids after radical attack (Favier, 2003).

*2-3-3/- **Effects on DNA deoxyribonucleic acid:***

Deoxyribonucleic acid (DNA) is also very sensitive to attack by the OH radical (Pasquier C;1995). Oxidation of DNA Nucleic bases are likely to be directly oxidized by ROS, leading to the formation of 8-oxo-guanine (Levine R.L and *al*;2000). which is normally removed by DNA repair enzymes. If these systems are faulty, 8- OH dG will accumulate within the DNA (Cadet ;1999). therefore this accumulation DNA oxidation reactions creating a large number of DNA damage (Bahi A ;2015). to induce mutations (Fontainel E;2007). which can lead to the development of cancer (Beckman and Ames ;1997).

3/-Physiological defenses against cellular oxidative stress :

1-3/-Antioxidants:

Is a more or less complex chemical species decreasing the oxidative stress within the organism (Camille M and *al*;2011). It is a molecule which is able to neutralize the active forms of oxygen and allows to maintain at the level of the cell and the organism non-cytotoxic levels of free radicals (Delattre J and al;2007). The organism produces free radicals, but it protects against them by antioxidants (Garait P;2006). For each free radical produced by the organism there is an antioxidant capable of neutralizing it (Defraigne J O;2008). They react directly with oxidizing agents and deactivate them. (Haissaguerre M;2015). Antioxidants also come from both endogenous and exogenous sources The body produces its own antioxidants in the form of acids and enzymes: superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) This can be divided into two categories, namely enzymatic antioxidants and non-enzymatic antioxidants. (Delattre J and al; 2007).

1-3-1/-Enzymatic antioxidants :

- ***Superoxide dismutase (SOD) :***

The enzyme superoxide dismutase (SOD) is present in both the dermis and the epidermis (Misra K; 2013). Plays a central role in the defense against ROS (Beyer and *al*;1991). in particular catalyzes the disproportionation of $O_2^{\bullet -}$ to H_2O_2. (Garait B; 2006). SOD exists in three isoforms which are differentiated by their cellular location and by their metal cofactor:

- A cytosolic and nuclear form associated with copper and zinc ions (Cu / Zn-SOD)
- A mitochondrial form associated with manganese (Mn-SOD)
- An extracellular form (EC-SOD) (Haissaguerre M;2015) (J. Haleng and *al*; 2007).

The distribution of these different isoforms varies by tissue (Garait P; 2006). SOD is also known to compete with nitric oxide (NO) for superoxide anion, which inactivates NO to form peroxynitrite. Therefore, by stripping superoxide anions, it promotes the activity of NO (Mamta M; 2013).

❖ ***Catalase :***

It is an enzyme found mainly in peroxisomes, in hepatocytes, erythrocytes and kidney cells (Thomas D; 2016). is also responsible for the elimination of H_2O_2 by transformation into H_2O and O_2 (Garait P; 2006).

It is an essential enzyme for the detoxification of free radicals during stress. It is made up of four polypeptide chains of approximately 500 amino acids (Mamta M; 2013). These constitute the active sites of this enzyme (Benhamdi A; 2014)

The mechanism of hydrogen peroxide disproportionation is as follow:

$$H_2O_2 + Fe\ (III)\ -E \rightarrow \text{——}\ H_2O + O = Fe\ (IV)\ -E$$

$$H_2O_2 + O = Fe\ (IV)\ -E \rightarrow \text{——}\ O_2 + Fe\ (III)\ -E + H_2O$$

(Arbona V and al;2003).

❖ ***Glutathione peroxidase (GPx):***

Are tetrameric enzymes, having a selenium atom in their active sites (Boundjah O; 2014). having the property of being able to catalyze the reduction of hydroxy peroxides (Thomas D; 2016). it acts in synergy with SOD since its role is to accelerate the dismutation of H_2O_2 into H_2O and O_2 (Garait P; 2006). Its main role consists in the elimination of lipid peroxides resulting from the action of oxidative stress on fatty acids polyunsaturated (Haleng and al; 2007). requires the presence of reduced glutathione (GSH) as an electron donor (Haissaguerre M; 2015). It will therefore ensure the intra and extracellular balance of the pro / antioxidant balance (Thomas D; 2016). It is found in extracellular fluids as well as in cells, within the cytosol and mitochondria (Garait P; 2006). Depending on their cellular location, GPx are subdivided into three categories: cytosolic glutathione peroxidase, plasma glutathione peroxidase and membrane glutathione peroxidase (HPGPx) (Boundjah O ;2014).

1-3-2/-Non Enzymatic antioxidants :

Non-enzymatic antioxidants are endogenous constituents or exogenous micronutrients provided by food; we distinguish:

- water-soluble antioxidants like albumin, ascorbic acid (vitamin C) (Singh and al., 2005), glutathione et uric acid (zaidane E; 1993).
- fat-soluble antioxidants: alpha tocopherol (vitamin E), carotenoids, ubiquinone (CoQ10), (Thomas D; 2016).

4/-*Involvement of oxidative stress in lead toxicity in rats:*

Since Lead is a non-essential element for the life of eukaryotic cells, the mechanisms responsible for lead toxicity are multiple and potentially affect all the cells of the body. To this end, we were interested in the oxidative stress generated by lead at the level of different organs (hematopoietic system, liver, kidney and brain) (Kharoubi O; 2009). the free ionized state that lead exerts its toxic effects in the cell according to several mechanisms: interaction with many proteins through their thiol groups and inhibition of the initiation of protein synthesis at the ribosome level; direct or indirect oxidative effect through the accumulation of heme precursors j disruption of calcium homeostasis and interference on many cytoplasmic or membrane cell processes mediated by calcium (Olivier C et al; 1999).

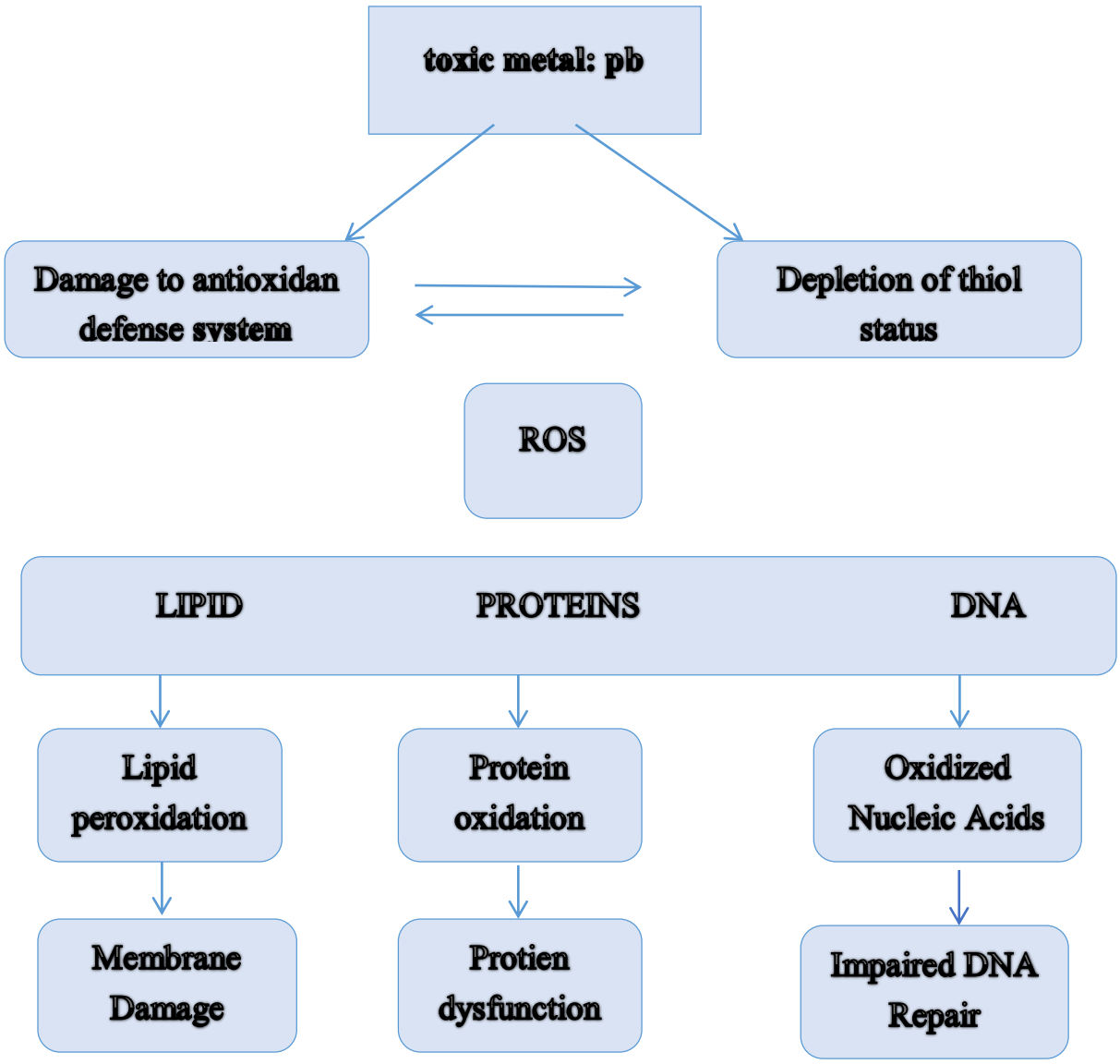


Figure 05: Possible mechanisms for metal-induced oxidative stress.

(Nuran E and al;2001).

Chapter III

the clay

1/-Definition of clay:

Clays Clay is defined as a fatty soil (Ismail K;2012) come from the alteration and degradation of rocks: physical alteration under the effect of temperature variations, and especially chemical alteration in contact with water which allows degradation into very fine particles (Choufa N; 2013) containing a set of fine particles (less than 2 µm in size) with a sheet structure (Ismail K.2012) called clay minerals responsible for its properties (Choufa N; 2013). Its mineral wealth and trace elements make it a tool in health. we can attribute to them, in health, the benefit of a form of Oglio-metallo-therapy which confers some of their properties on clay (Larcher G; 2016) Some "special" clay minerals are components of drugs, both as active and inactive ingredients, when processed to meet regulatory requirements. The special properties of these pharmaceutical grade clay minerals can also be exploited in the development of novel drug delivery systems, designed to deliver therapeutic levels of drug to the site of action and maintain them throughout treatment. (Viseras C and al;2010).

2/-General clay composition:

The main components of clays are silicon, aluminum, and other minerals. among these minerals are zinc iron copper calcium sulfur magnesium phosphorus the percentage of these minireax is variable between the types of clay (green and red), the main difference between green and red is the iron percentage (Kishk F and *al*;1976). (Diatta M T; 2006).

3/-Clay minerals:

A summary of the general structure of clay minerals and of the major characteristics of the main families of phyllosilicates is presented in order to provide the necessary context for the understanding of the spectral figures associated with these minerals (Camille T; 2011).

4/-Structure of clay minerals:

Most clay minerals are in leaf shapes (Ismail K;2012). the structure of the sheets is determined by the arrangement of oxygen and hydroxyl, which are much larger in size than the cations, the latter being able to be located in two types of cavities, tetrahedral and octahedral (Boucheta A; 2017).

4-1/-<u>Structural elements of leaflets</u>:

Four main ions form the structure of the sheets: Si^{4+} and Al^{3+} ions, the hydroxyl ion OH-. In addition, depending on the type of clay, other ions are also encountered such as: Fe^{3+}, Ca^{2+}, Mg^{2+}. These ions are in the sheet arranged in a compact structure (Zaidih M.; 2019).

4-2/-<u>Tetrahedral layer</u>:

In the tetrahedral element, the central ion is silica (S^{+4}) which is surrounded by 4 oxygen ions (O^{-2}). Tetrahedra are linked together at their bases by sharing the oxygen union between two tetrahedra to form a tetrahedral layer. The general formula of this set is (Si_2O_5) (Choufa N;2013).

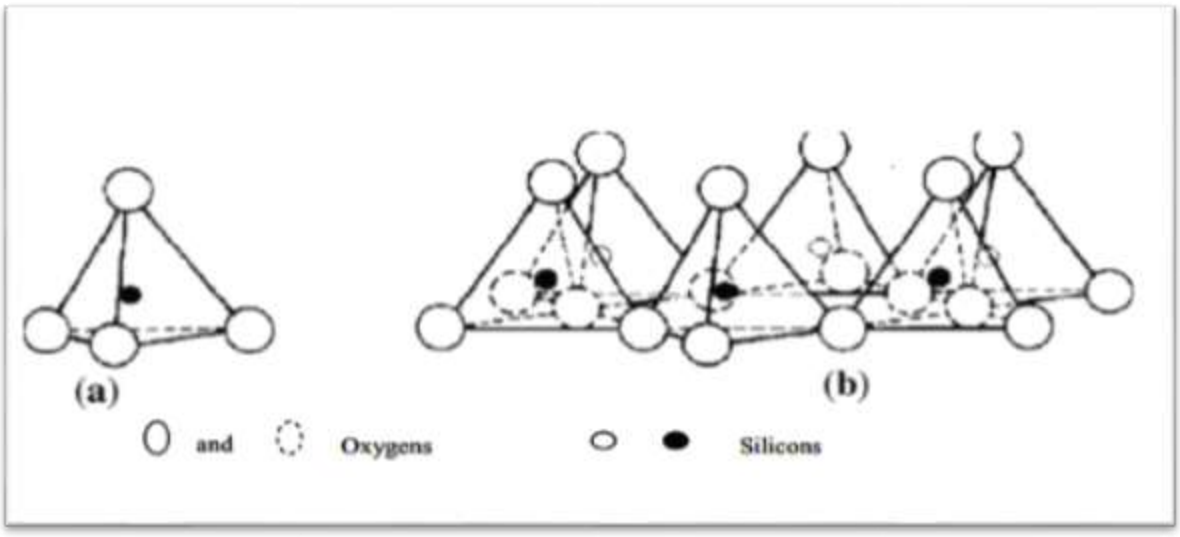


Figure06:

a) silicon core tetrahedral unit.

b) diagram of a tetrahedron layer $[Si_4O_{10}(OH)_2]^{6-}$

(Khenifi A;2010).

4-3-<u>Octahedron layer:</u>

Is formed of metal cations such as Mg^{+2}, Al^{3+}or Fe^{2+} surrounded by six oxygen atoms. The octahedral layer is made up of a sequence of octahedra (Dali Youcef L; 2012).

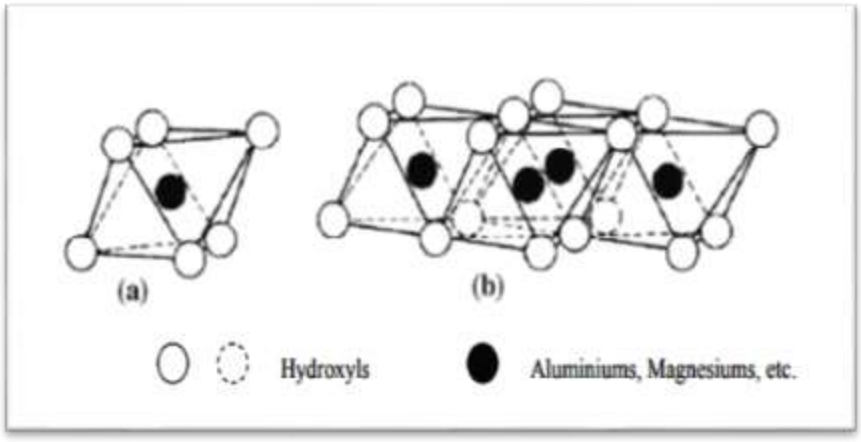


Figure07: a) octahedral unit

b) layer structure based on Brucite $Mg(OH)_2$ octahedron or Gibbsite $Al(OH)_3$

(Khenifi A;2010).

5/-<u>Classification of clay minerals:</u>

According to (AIPEA) the International Association for the Study of Clays, as well as other international authors have classified phyllosilicates according to well-defined criteria:

- Type of sheet combination : T / O or 1 : 1 ; T / O / T or 2 : 1 ; T / O / T / O or 2 : 1 : 1.
- The load of the layer.
- The content of the interfoliar space (cations, water molecules, etc.) (Dali Youcef L; 2012).

There are different classifications of clays: - The most classic is based on the thickness and structure of the sheet (Gomri F; 2010). We can thus distinguish four groups (Jozja N; 2003).

5-1/- <u>Minerals at 7 Å or 1/1 (Te-Oc)</u>, (Kaolinite, Halloysite, Dombasite...):

The sheet consists of a tetrahedral layer and an octahedral layer. It is qualified as T: O or type 1: 1. Its thickness is approximately 7 Å (Khenifi A;2010).

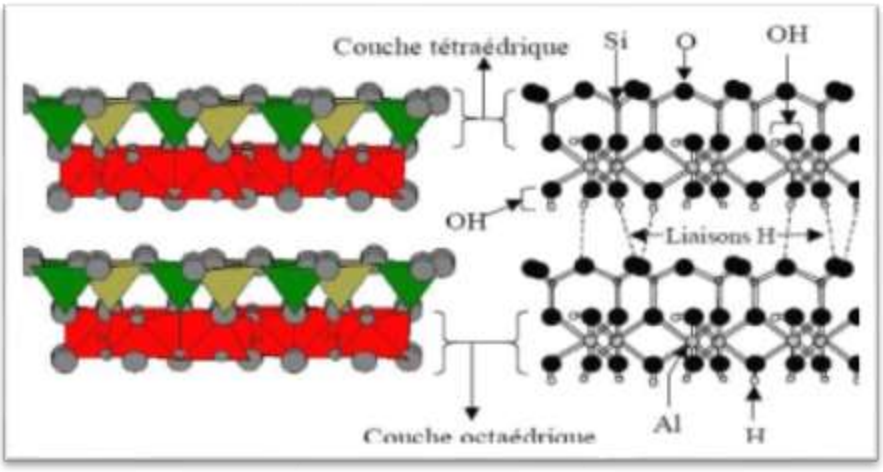


Figure08: Representation of stacks of siliceous tetrahedra and aluminous octahedra of a TO type mineral (Choufa N; 2013).

5-2/- *Minerals at 10 A ° or 2: 1 (T-O-T):*

Their structure is a bit complex than that of the 7 A ° group, this is due to the presence of an octahedral layer between two tetrahedral layers, among these minerals there are smectites and illites (Aoudi M and al;2017).

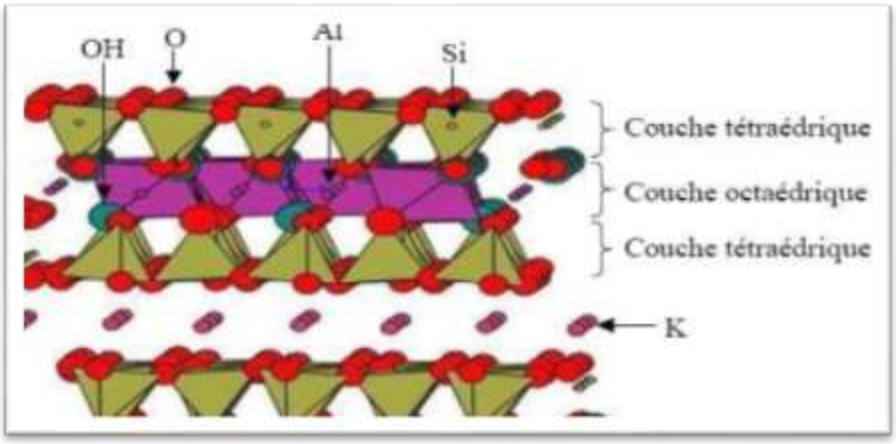


Figure09: Structure of a mineral (Chih-Huang W; 2006).

5-3/-<u>Minerals at 14 A ° or 2/1/1 (Te-Oc-Te-Oc)</u>:

The clay minerals in this group are characterized by a sheet consisting, in addition to the three layers of the 2/1 series, by a fourth octahedral layer which is inserted into the interfoliar space (case of chlorites). These three groups characterize true phyllites, a fourth group is represented by pseudophyllites, or fibrous clays, such as sepiolite and palygorskite (Bernard O; 1975).

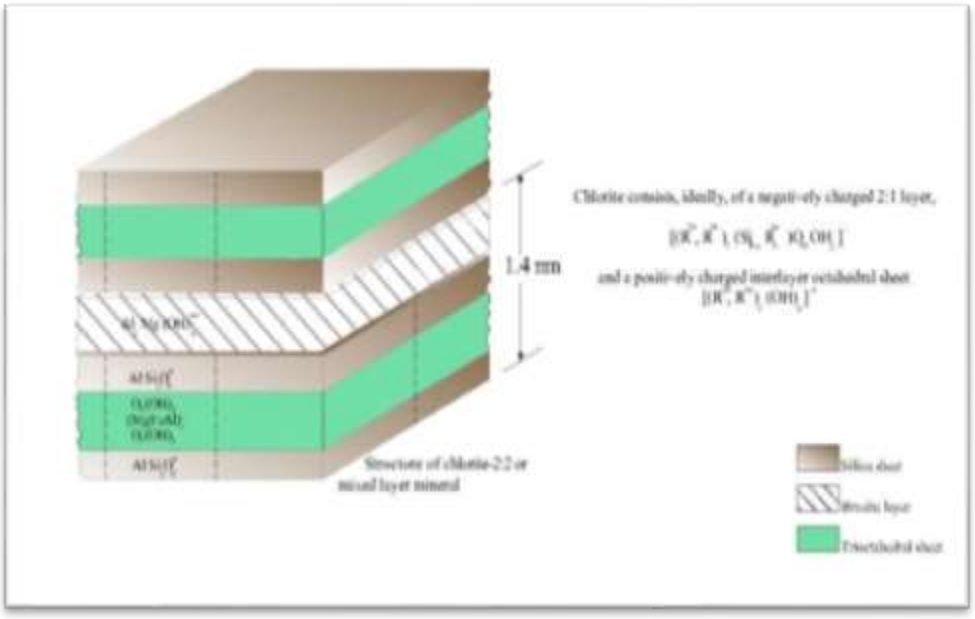


Figure10: Representation of chlorite (F. Arbaoui and al 2014).

6/-<u>Cation exchange capacity:</u>

The ion exchange phenomenon can be invoked for any system made up of at least two immiscible phases. The solid phase, impregnated with liquid, develops positive or negative charges on its surface which are then exactly compensated by charges of opposite sign brought by the ions of the solution. (Emna E;2011).

The ion exchange capacity is mainly related to isomorphic substitutions and edge phenomena that occur following hydrolysis of Si-OH and Al-OH bonds. In this case, the pH plays a very important role. At low pH, H + ions bind more than OH-. A positive charge develops. Under these conditions, clays are therefore characterized by a CEA anionic exchange capacity. At basic pH, the silanol and aluminol functions deprotonate and give rise to the development of a negative charge on the surface (Yassine B;2017).

The clay, impregnated with liquid, develops positive or negative charges on its surface which are then exactly compensated by charges of the opposite sign provided by the ions in the solution. The charges carried by the clay are either localized at the level of ionized functional groups, or delocalized. It is therefore considered that the surface areas where the electrostatic charges are concentrated constitute ion exchange sites. (Sieskind O and Wey R;1957).

7-Biological or clinical effect of clay:

Clays are substances found throughout the earth's crust. Given their ubiquity and particular characteristics, man has used some for a long time. times for their therapeutic effects (López-Galindo, A; 2011). The applications of clays are favored by their colloidal size and crystalline structure. The specific function they have in any formulation depends both on their physical properties (size and shape of particles, specific surface area, texture, color and luminosity) and their chemical characteristics (chemical surface and charge) (C. Viseras and *al*; 2010).Specialists in fields such as geology, mineralogy, geochemistry, pharmacy or medicine should collaborate on the right characterization of the physical and chemical properties of the clays used in the formulations and correlate them with their biological effects (López-Galindo, A; 2011).Clays are also used as adsorbents and protectors in topical and systemic formulations in the treatment of, for example, acne, leg ulcers, inflammatory bowel disease and kidney failure (Alberto L and *al*; 2011).

Second Part

Experimental Part

Chapter I

Materials and Methods

I/-Materials:

I -1/-clay materials:

I-1-1/-Geographical location of the Wilaya Touggourt:

Touggourt is located in the southeast of Algeria, and it is bordered on the north by the states of El-Oued and El-Mughayer, on the east by the wilaya of El-Oued, on the south by the state of Ouargla and on the west by the states of Ouargla and El-Mughir.

Figure11: Geographic location of Touggourt.

I-1- 2/- Studied clay sample area:

Clay was taken from the "BELDAT OMAR " of the Tamassin district of the Wilayat of Touggourt collector as shown in the figure showed that the soil of the Beldat omar region contains 42٪ mineral clays, the rest is silt and sand.

Figure12*:* Clay sampling area.

Figure *13*: career of clay.

I-1-3/- ***History of clay:***

The word clay comes from the Latin Argilla. This same word is derived from the Greek argillos, whose root, argos, means "dazzlingly white". From the 12th to the 16th century, argile was called "Ardille", then this word became "arzille", then "arsille" to finish in "clay " (Larcher G;2016).

I-2/-Animals:

I-2-1/- Animal and husbandry condition:

Our study carried out on twenty male female rats of the Wistar type, coming from the Pasteur Institute of Algiers, aged between eight and ten weeks with a weight of 165.05 ± 4.83 The animals are raised in the animal house of the faculty of nature and life sciences, at Echahid Hamma Lakhdar-El-Oued University. They undergo a period of adaptation to animal house conditions for about 3 weeks at a temperature of 19.600 ± 0.354 C°. The rats are housed in plastic cages and fed a standard diet (table2).

Table02: Mineral composition of standard diet.

Trace elements mg per kg	Quantity (g / Kg)
copper	900
cobalt	70
iron	5000
magnesium	1210
manganese	7290
zinc	6000
sulfur	200
iodine	80
selenium	25
humidity	5%
max mineral matter	45%
salt (sodium chloride)	30%

Table03: Vitamin composition of standard diet.

Vitamin	UI or Mg
Vitamin A	1250000 (UI)
Vitamin D3	300000 (UI)
Vitamin B1	80
Vitamin K3	15
Vitamin B2	80
Vitamin B6	250
Vitamin B12	2
Vitamin PP	5
Vitamin E	3000
Ca^{++} panthotenate	1000
Folic acid	650
Choline	12000

I-2-1/- Treatment of animals:

After the adaptation period, the rats are divided into four groups of five rats each, the rats are treated as follows for 20 days.

Group 1 (T): Healthy rats (control).

Group 2 (Pb): Rats contaminated with lead acetate for 41 days.

Group 3 (Pb + AR): Rats contaminated with lead acetate and treated with red clay for the last 20 days.

Group 4 (Pb + Ar): Rats contaminated with lead acetate and treated with green clay the last 20 days.

The lead acetate $(CH_3COO)_2Pb$ was dissolved in distilled water at a dose (4ppm) The treatment with clays was done at the rate of 5% of the diet.

I-2-3/- Sacrifice and collection of blood and organs :

The rats are anesthetized with chloroform (94%) after 16 hours of fasting and are sacrificed (by decapitation). The blood sample is taken at the time of sacrifice of the rats in EDTA tubes for FNS and biochemical analyzes. Blood sugar is measured by the glucometer for each rat. After, centrifugation the blood at 3000 rpm for 15 minutes. The plasma obtained is stored at a temperature of -20 ° C until the time of biochemical analyzes (urea, uric acid, creatinine, calcemia, TGO, TGP, FER). The liver, kidneys, heart, and testis are carefully removed, rinsed with NaCl (0.9%), then weighed. Organ homogenates are prepared for the determination of oxidative stress parameters (Malondialdehyde (MDA), super oxide dismutase (SOD), gluthaion transferase (GST) and reduced glutathione (GSH). Part of liver and testes are fixed in formalin (10%) in order to carry out the histological study.

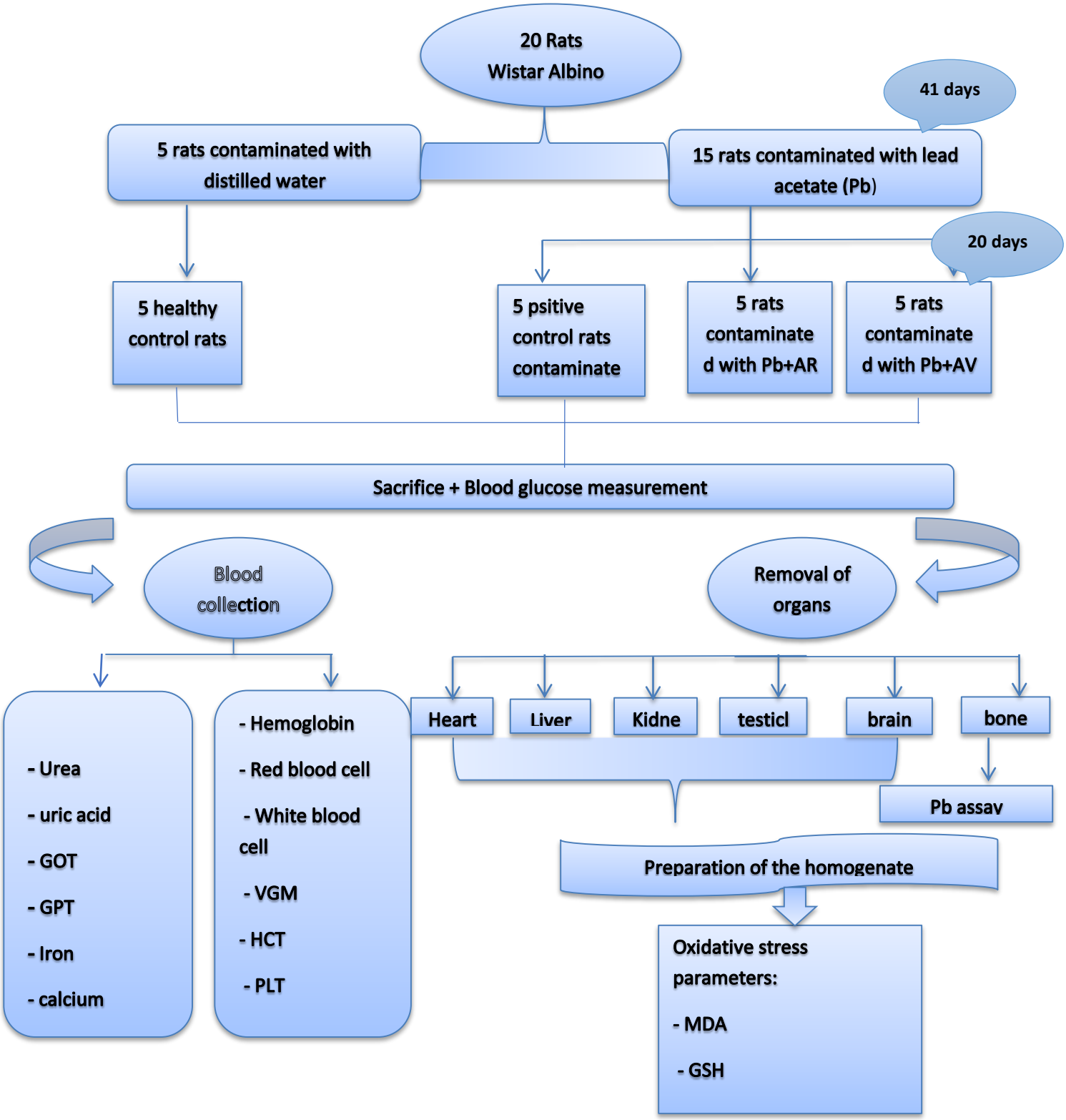


Figure 14: summary diagram of the experimental protocol of the study.

I-2-4/-Reagents and products used:

Hydrogen Chloride (HCl), Chloroform, Tris Sodium Chloride (NaCl), Nitric Acid (HNO3), Salicylic Acid, Trichloroacetic Acid (TCA), Thiobarbituric Acid (TBA), Buthydrxytoluene (BHT), Phosphate Buffer (KH2PO4), 5,5dithiodis-2-nitrobenzoique (DTNB), GSH,1–chloro-2,4–dinitrobenzene (CDNB), EDTA, Riboflavin, ethanol, metheonine (MET), Nitro blue tetrazolium chloride (NBT).

II/-Methods:

II-1/- Sample preparation:

We cleaned the clay and then crushed it with a mortar.

Figure15: Read clay (RC).

Figure16: Green clay (GC).

II-2/- Characterization of clay:

The characterization of red and green clay were identified by Ultraviolet-visible spectroscopy (UV-VIS), Fourier transform infrared spectroscopy (FTIR) and X-ray powder diffraction (XRD) analysis. The analysis were done by direct reading through JENWEY, Thermo Scientific iS5, PROTO AXRD Benchtop and Thermo Scientific Apreo S apparatus respectively.

II-3/- Toxicity test:

The test was performed using healthy albino rats of Wistar. The animals were divided into two groups for each one of them tree rats, which administered clay (50ppm) orally. Animals were observed after dosing at least once during the first 30 min, periodically during the first 24 h with special attention given during the first 4 h, and daily thereafter for a total of 14 consecutive days (Derouiche et al., 2017).

II-4/-Hematological parameters analysis:

Hematologic parameters are determined by Coulter's method using the Medonic-type hematology auto-analyzer specific for FNS (blood count formula).

II-5/- Biochemical parameters analysis:

The levels of urea, uric acid, triglycerides, cholesterol, TGO and TGP activity in the plasma were determined using the commercial kit from BIOLABO France (ref: urea-92032, uric acid-80351, Triglyceride-80019, cholesterol-90206, TGO-80025, TGP-80027).

II-6/-Oxidative stress parameters:

One gram of tissue (liver, brain, testis, kidneys and heart) from each rat from the different groups studied was used. After grinding and homogenization of the tissues in TBS (50 mM Tris, 150 mM NaCl, pH 7.4), the cell suspension was centrifuged (3000 holes / min, 15 min), then the supernatant (homogenate) obtained is obtained. stored at -20 ° C while awaiting the determination of glycogen and oxidative stress parameters.

II-6-1/- Determination of malondialdehyde (MDA) level:

MDA was measured according to the method described by (SASTRE and *al* ;2000). In brief, Pipette 100 µl of sample, 400 µl of TBA reagent into the glass and screw test tubes and seal. Heat the mixture in the Marie bath at 100 ° C for 15 minutes. Then cool in a cold-water bath for 30 minutes leaving the tubes open to allow evacuation of the gases formed during the reaction. Centrifuge at 3000 rpm for 5 minutes and read the absorbance of the supernatant at 532 nm using a spectrophotometer. TBARS concentration was determined using the MDA molecular extinction

coefficient (å = 1.53 105 M-1. Cm-1). The results were expressed in µmol / mg proteins (Yagi; 1976).

II-6-2/- <u>Determination of reduced glutathione (GSH) level:</u>

GSH concentration was performed with the method described by Ellman. based on the development of a yellow color when DTNB is added to compounds containing sulfhydryl groups. In brief, 0.8 mL of tissue homogenate was added to 0.2 mL of 0.25% sulphosalylic acid and tubes were centrifuged at 2500 g for 15 min. Supernatant (0.5 mL) was mixed with 0.025 mL of 0.01 M DTNB and 1 mL TBS (pH 7.4). Finally, absorbance at 412 nm was recorded. Total GSH content was expressed as nmol GSH/mg proteins (Weckbercker and Cory., 1988).

II-6-3/-<u>Glutathione-S-transferase (GST)Activity assay:</u>

GST activity was measured spectrophotometrically by the method of (Habig and *al*;1974), based on the formation kinetics of a complex between a GST substrate: 1-chloro-2-4-dinitrobenzene (CDNB) and GSH. 50 µl of CDNB (0.02M) was mixed with 850µl and 830 µl of phosphate buffer in blank and test tube respectively, then 100 µl of GSH (0.1M) was added to mixture, 20µl of homogenate was puted test tubes. results are measured each1 min during 5 min. The complex formed can be visualized by increasing the optical density at a 340 nm. The GST activity was expressed as nmol CDNB /min/mg prot:

$$\mathbf{GSTs(nM\ /min/\ mg\ of\ pro)} = \frac{\boldsymbol{DO\ sample/min} - \mathbf{DO\ blanc/min}}{\mathbf{9.6} \times \boldsymbol{mg\ of\ pro}}$$

II-6-4/- <u>Determination of Super Oxide Dismutase (SOD) activity:</u>

- ***<u>Principal:</u>***

The assay method of SOD activity using the NBT by the superoxide anion ($O_2^{\cdot-}$), is used as a basis for detecting of presence of SOD by measuring the spectrophotometrically absorbance at 560 nm (Beauchamp & Fridovich;1971).

- ***Procedure:***

Collect in tubes	Blank	Sample
EDTA-Met (0.1mM, 13mM)	1000µL	1000µL
Phosphate buffer (50Mm)	892,2µL	892,2µL
Sample	-	50
Phosphate buffer (50Mm)	1000µL	950µl
NBT (75µM)	85,2µL	85,2µL
Riboflavin (2µM)	22,6µL	22,6µL

- ***Expression of results:***

Inhibition percentage of NBT reduction by SOD

$$\text{SOD} = \frac{OD\ blanc - \text{OD sample}}{OD\ blanc} \times 100$$

II - 7/- Histopathological study of liver and testis tissue:

After the rats were sacrificed, the tissues of liver and testicular were removed and immersed in a fixative (solution formaldehyde) during the preparation of the slices. Graduated series of ethanol, cleaned with toluene %36Whish dehydrated ascending immersed in paraffin and stained hematoxylin and eosin. Histopathological evaluation was performed under an optical microscope.

II-8/- Statistical analysis:

Our statistical study is carried out by the Minitab software using (Student t test) to compere means among our different experimental groups; Differences were considered statically significant at $p<0.05$.

Chapter II

Results & Discussion

I /-Results:

I -1/-Characterization of clay:

I -1-1/-UV–Vis and infrared spectrum:

UV–Vis absorption and infrared spectrum of clay is shown in Figure 17 and 18 The presence of several band indicates the richness of red and green clay in several components with minor differences between the two types.

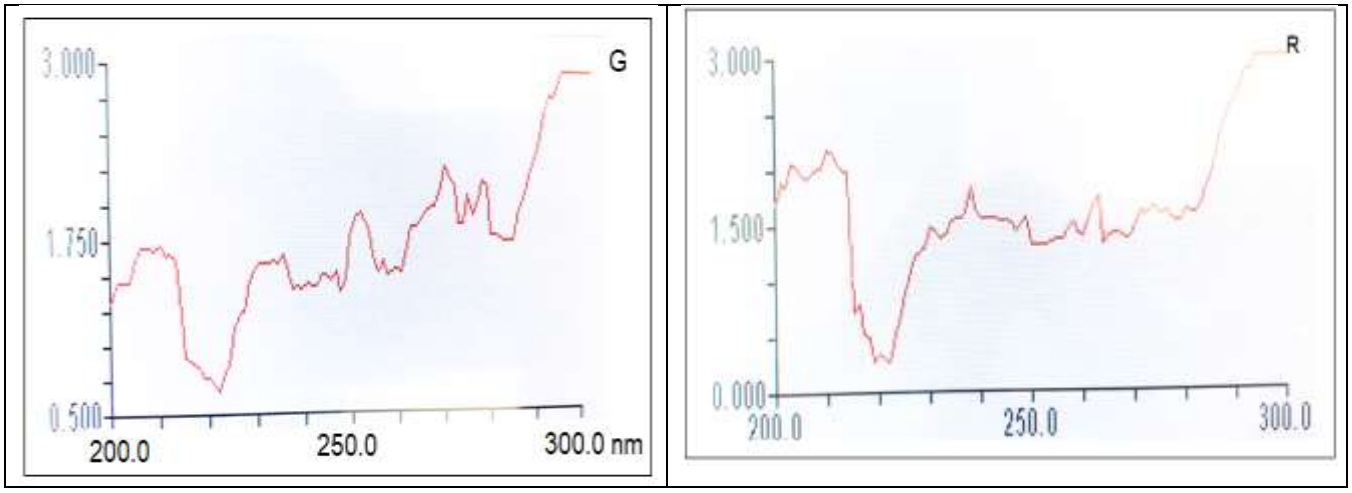


Figure17: UV-Vis spectrum of red clay (R) and green clay (G).

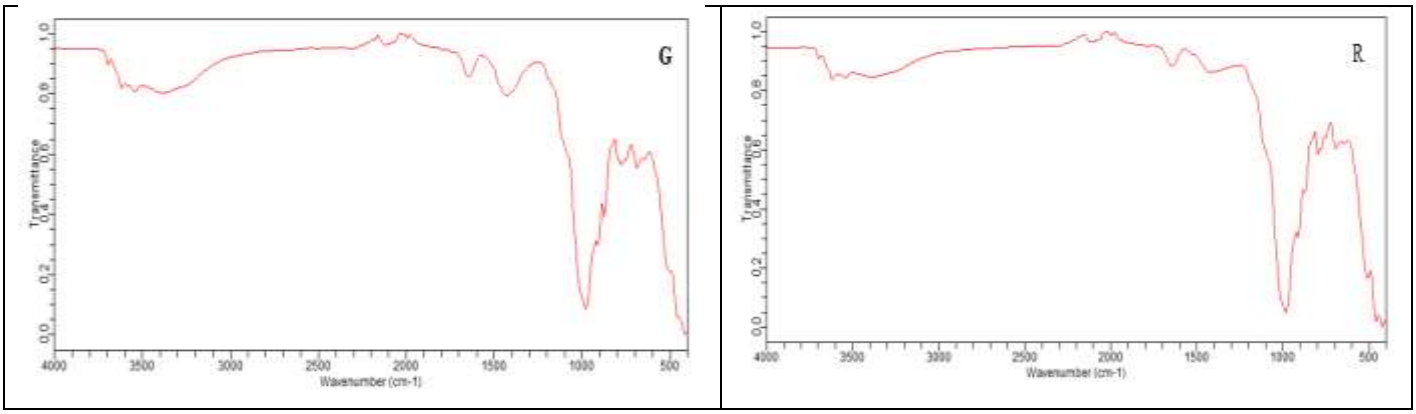


Figure18: Infrared spectrum of red clay (R) and green clay (G).

I -1-2/-XRD and light microscope analysis:

The results of XRD and light microscope analysis are presented in figure 19 and figure 20 which show the different elements present in the two types of clay with clearly different crystalline shape between the two types.

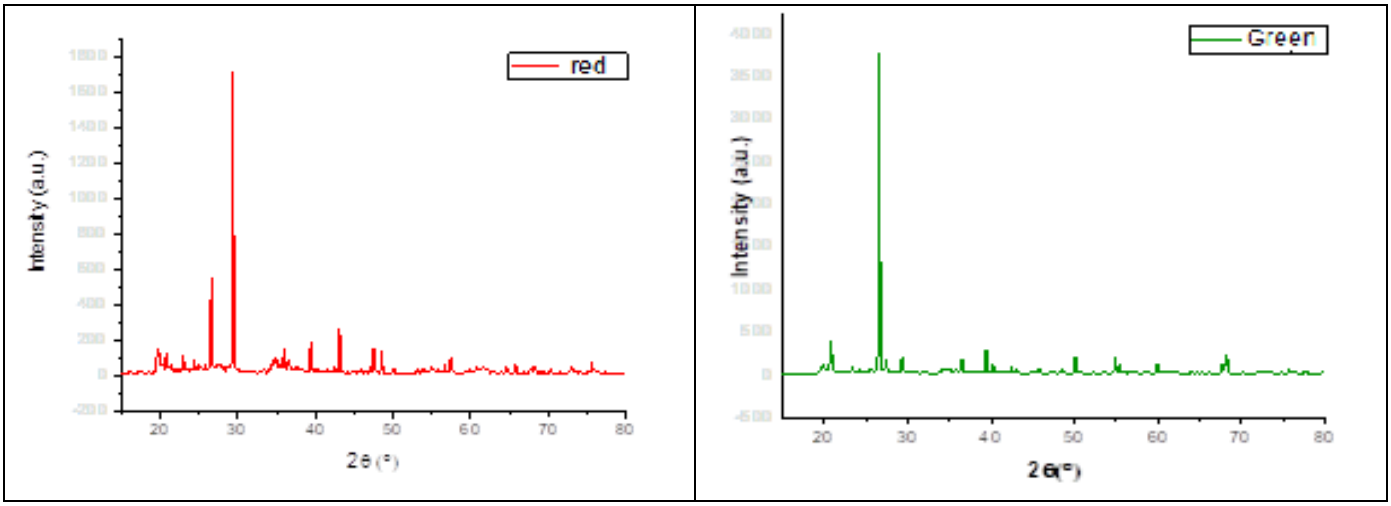
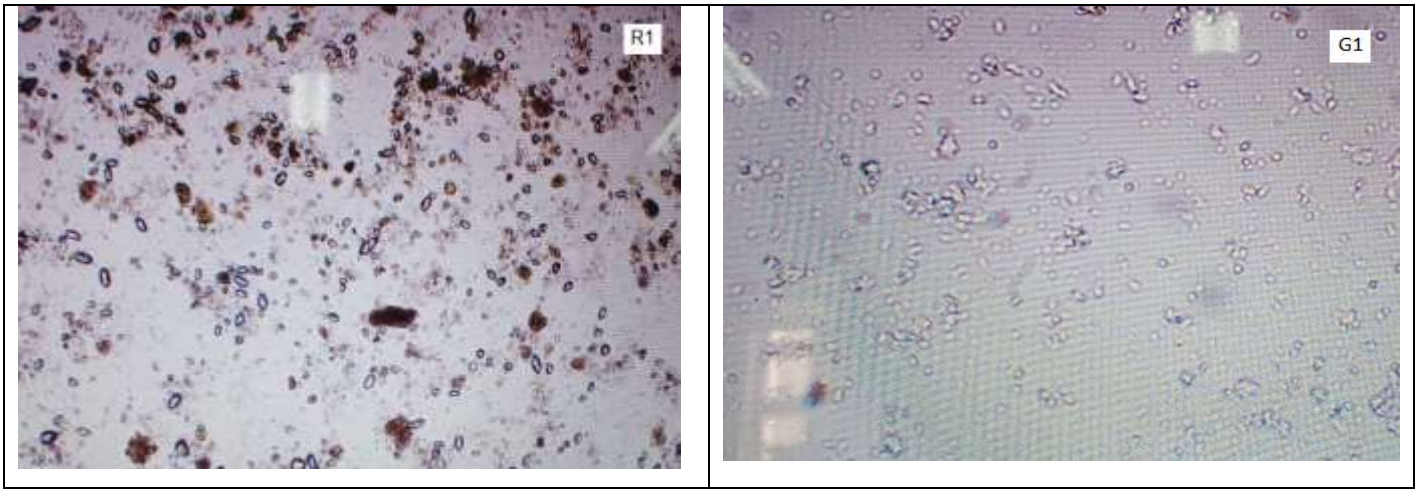
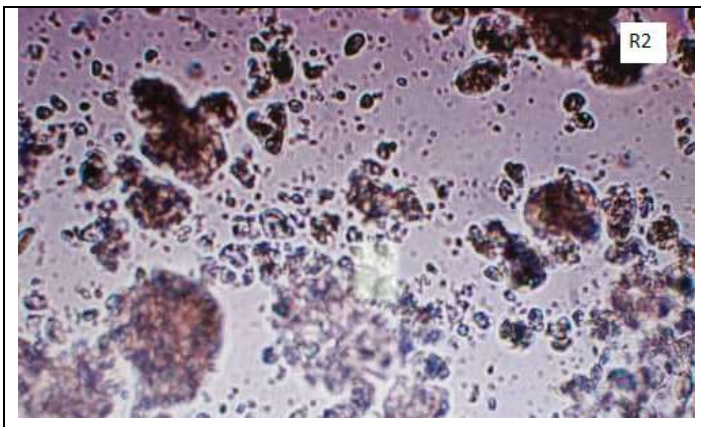


Figure19: XRD spectrum of red and green clay.





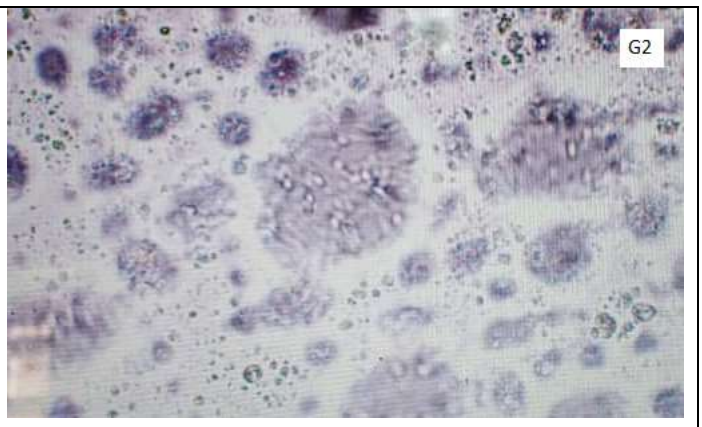


Figure 20: Light microscope analysis of red (R1x10, R2x40) and green clay (G1x10, R2x40).

I -2/- <u>Acute toxicity essays of clay:</u>

Physiological parameters of the rats were determined during the experimental period and showed that treatment with the clay caused no symptoms or complications and also no adverse effects in the rats during the treatment period (Table5):

Table04: Acute toxicity test of clay on physiological parameters in Wister albino rats.

Parameters	0 h		3 h		24 h		Day- 7		Day-14	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
Dead rats	0	0	0	0	0	0	0	0	0	0
Eyes	N	N	N	N	N	N	N	N	N	N
Sleep	N	N	N	N	N	N	N	N	N	N
Movement	N	N	N	N	N	N	N	N	N	N
Diarrhea	N	N	N	N	N	N	N	N	N	N

N: Normal

I -3/- <u>Growth parameters:</u>

- ✓ ***<u>Study of body growth and relative organ weight:</u>***

From our results shown in Table 5, the body weight of the rats was affected by lead, indeed, we noticed that the lead contamination increased the weight of the rats remarkably compared to the control rats. For the treatment groups that consumed red (Pb + RC) and green (Pb + GC) clay, we also saw a significant increase in weight compared to the control group and the toxic group. In addition, the results obtained show that there is a very highly significant increase in the relative

weight of kidneys ($p<0.001$), liver and heart ($p<0.05$) and testis ($p<0.001$) in the pb group compared to the control. In the groups treated with red and green clay, we find significant improvements ($p<0.05$) in the relative weight of the organs compared to organ relative weight of lead group.

Table 05: Initial and final body weight and relative organ weight in the control group and the experimental groups.

Group		Control (n=5)	Pb (n=5)	Pb+RC (n=5)	Pb+GC (n=5)
Initial weight (g)		150±0.58	168.2±1	158.2±1	172.6±1
Final weight (g)		152.5±0.58	198±1	190.6±1	206.8±1
Relative weight (%)	Liver	2,62 ±0,0105	3,10±0,154*	2,58±0,0183^{*c}	2,79 ±0,0080^{***c}
	Kidney	0,57 ±0,0187	0,73 ±0,0284***	0,61± 0,014^{*c}	0,65 ±0,0087^{***c}
	Heart	0,27±0,004	0,34±0,0189*	0,33±0,0065^{***NS}	0,29 ± 0,0080^{NSC}
	Testis	1,56 ± 0,048	1,23± 0,022***	1,43±0,031^{**c}	1,32±0,012^{***c}

* p <0.05; ** p <0.01; *** p <0.001 comparison with the control lot.
^{a} p <0.05; ^{b} p <0.01; ^{c} p <0.001 comparison with the lead batch.

I-4/- Hematological and biochemical parameters:

I-4-1/-Study of haematological parameters:

The results of our study (table 6) show that lead acetate contamination leads to a very highly significant decrease ($P<0.001$) in hemoglobin (HGB) and hematocrit (HCT) and mean corpuscular volume (MCV) levels, and for white blood cell numbers (WBC) we notice a non-significant decrease ($P>0.05$), on the other hand we notice a very highly significant increase ($p<0.001$) in platelets (PLT) and a non-significant ($P>0.05$) for de red blood cell count (RBC) when comparing to control rats. Green clay treatment improves hemoglobin values ($P>0.01$) in comparison with Pb group, while green clay increase significantly the level of hematocrit (HCT) ($P>0.05$), and mean globular volume (MCV) ($P>0.001$) compared to pb group. With no significant changes to the rest of the hematological markers.

Table 06: Hematological parameters in control and experimental groups.

Group	Control (n=5)	Pb (n=5)	Pb+RC (n=5)	Pb+GC (n=5)
WBC ($\times 10^9$ /L)	7,45±0,24	6,42±0,840 ^{NS}	5,66±0,35^{***NS}	7,225±0,39^{NSNS}
HGB(g/dl)	15,30 ± 0,60	13,56 ± 0,060***	14,620±0,23^{*b}	14,0 ±0,21^{***NS}
RBC($\times 10^{12}$/L)	8,670±0,36	8,77±0,0724^{NS}	9,05 ±0,16^{*NS}	8,62 ±0,09^{NSNS}
HCT (%)	45,38 ±1,82	40,16±0,333***	9,058 ±0,162^{***c}	41,67 ± 0,51^{***a}
MCV (FL)	52,45±0,85	46,98±0,383***	47,90±0,48^{***NS}	48,86 ±0,26^{***c}
PLT($\times 10^9$/L)	758,67±6,93	870,8 ±12,3***	815,3±34,7^{NSNS}	875,3±33,9^{*NS}

* p <0.05; ** p <0.01; *** p <0.001 comparison with the control group.
[a] p <0.05; [b] p <0.01; [c] p <0.001 comparison with the Pb group.

I-4-2/-<u>Study of biochemical and enzymatic parameters:</u>

Our results (table 7) show a non-significant decrease in the serum concentration of glucose and uric acid and a very highly significant (p <0.001) decrease in calcium and iron and a significant (p<0.01) decrease in serum concentration of creatinine and a non-significant increase (P>0.05) in urea and for serum transaminase enzymes (TGP, TGO) we notice a very highly significant increase (P<0.001) in TGO and a very highly significant decrease (P<0.001) of TGP, in Pb rats compared to the control. On the other hand, in the rats treated with red clay, our results show a significant increase in concentration of serum uric acid (P<0.05) and iron (P<0.01), and a significant decrease in serum calcium, urea and GOT levels compared to Pb group. About rats treated with green clay our results show a significant decrease (P<0.001) in concentration of serum calcium, iron and urea and activity of serum GOT and a significant increase (P<0.001) in serum uric acid when compared to rats exposed to lead. But no significant change for the other parameters.

Table 07: Blood glucose and biochemical parameters levels in control and experimental groups.

Group	Group (n=5)	Pb (n=5)	Pb+RC (n=5)	Pb+GC (n=5)
Blood glucose (g/l)	1,55 ±0,10	1,43 ±0,12 ^{NS}	2,07±0,09^{*b}	1,62 ±0,09 $^{NS\ NS}$
Serum calcium (mg/l)	35,00 ±0,89	31,66±0,44***	30,75±0,31^{***a}	9,33 ±0,16^{***c}
Serum iron (mg/l)	0,23±0,05	0,15±0,004***	0,33±0,0491^{NSb}	0,04 ±0,014^{***c}
Serum urea (g/l)	0,46±0,03	0,49±0,013^{NS}	0,28±0,023^{***c}	0,25±0,022^{***c}
Serum creatinine (mg/l)	5,66±0,16	5,20±0,13**	5,800 ±0,13^{NSc}	6,50 ±0,18^{**c}
Serum uric acid (mg/l)	14,00 ±1,26	11,50±1,48^{NS}	14,00±1,15$^{NS\ a}$	19,75 ±1,45^{**c}
Serum GOT (UI/l)	147,5 ±14,5	305,00±4,02***	203,7±10,3^{***c}	201,00 ±9,35^{***c}
Serum GPT (UI/l)	54,50±1,12	45,33±1,74***	61,50±1,12^{***c}	77,33 ±5,34^{**c}

* p <0.05; ** p <0.01; *** p <0.001 comparison with control group.

[a] p <0.05; [b] p <0.01; [c] p <0.001 comparison with the Pb group.

I -5/- Oxidative stress parameters:

I -5-1/-Study of lipid peroxidation (MDA) and reduced glutathione (GSH):

Our result illustrated in the (table 8) show a significant increase in lipid peroxidation (MDA) in kidney, liver and heart and a significant decrease (P<0.001) in reduced glutathione (GSH) in the liver, heart, testes, kidney (p<0.01) and brain (p<0.05) of rats exposure to lead compared to control group. On the other hand, in rats treated with red clay, results show a significant decrease of MDA level in liver (P<0.001), brain (P<0.05) and testis (P<0.001) and a significant increase (P<0.001) in GSH concentration in all tissues studies.

Table 08: MDA and GSH levels in tissues of control and experimental groups.

Parameters		Group (n=5)	Pb (n=5)	Pb+RC (n=5)	Pb+GC (n=5)
GSH (nmol/g tissu)	Liver	24,93 ±6,49	11,37 ±1,54***	58,72±3,00^{***c}	48,57±5,46^{***c}
	Kidney	19,24±2,78	12,04±1,52**	37,407±0,703^{***c}	55,58±7,82^{**c}
	Heart	36,57±2,43	9,189±0,731***	41,15±3,39$^{NS\ c}$	30,85±4,42 $^{NS\ c}$
	Brain	13,89±2,04	9,54±1,59*	13,49±1,84^{NSNS}	23,64±2,71^{**c}
	Testis	21,63±2,62	5,631±0,880***	19,63±1,31 ^{NSC}	24,05±2,98^{NSC}
MDA (nmol/g tissu)	Liver	6,764±0,544	18,76±1,60***	11,98± 1,07 ^{**c}	6,623±0,808$^{NS\ c}$
	Kidney	10,095±0,079	15,192±0,533***	16,040±0,631^{***NS}	10,998±0,880$^{NS\ b}$
	Heart	14,90 ± 1,49	29,09 ± 3,65**	25,47 ± 4,02^{*NS}	11,31±3,18^{NSc}
	Brain	14,90±1,49	15,78±1,33^{NS}	12,60±1,32$^{NS\ a}$	9,25±1,35^{**c}
	Testis	1,9112±0,0920	3,047±0,702^{NS}	1,676±0,161^{NSc}	1,235±0,225^{*c}

* p <0.05; ** p <0.01; *** p <0.001 comparison with the control lot.
^{a} p <0.05; ^{b} p <0.01; ^{c} p <0.001 comparison with the lead batch.

I -5-2/-<u>Study of Superoxide Dismutase (SOD) and Glutathione S Transferase (GST) activities:</u>

Our result illustrated in the (table 9) show a significant increase (P<0.001) of superoxide dismutase (SOD) and Glutathione S Transferase (GST) activities in all tissues studies except that SOD in heart no significant change in Pb group compared to control. On the other hand, in rats treated with red clay, results show a significant amelioration of SOD and GST activities in all tissues study exept SOD in brain and GST in liver and testis compared to Pb group. In adition, in rats treated with green clay, our results show a significat decrease of GST in liver, kidney, heart, brain and testis and a significant decrease of SOD in heart (P>0.05), brain (P>0.01) and testis (P>0.05) compared to Pb group.

Table 09: GST and SOD activities in control and the experimental groups.

Parameters		Group (n=5)	Pb (n=5)	Pb+RC (n=5)	Pb+GC (n=5)
SOD (UI/g tissu)	Liver	16,76±0,27	17,54±0,033***	16,77±0,23^{NSa}	17,37±0,10^{***NS}
	Kidney	15,82±0,23	17,17±0,084***	15,98±0,12^{NSc}	17,01±0,09^{***NS}
	Heart	17,620±0,05	17,58±0,036^{NS}	16,04±0,55^{*a}	17,60±0,01^{NSa}
	Brain	17,37±0,13	17,70±0,023***	17,65±0,02^{***NS}	17,79±0,02^{***b}
	Testis	13,63±1,94	17,84±0,02***	17,11±0,14^{***c}	17,46±0,13^{***a}
GST (nmol GSH/min/g tissu)	Liver	0,41±0,023	3,97± 0,23***	0,37± 0,03^{NSc}	0,40±0,01^{NSc}
	Kidney	0,05±0,0219	0,68± 0,27*	0,10± 0,01^{*c}	0,04±0,009^{NSc}
	Heart	0,023±0,001	0,041±0,001***	0,02± 0,001^{*c}	0,018±0,004^{NSb}
	Brain	0,001±0,0001	0,04± 0,004^{***c}	0,026±0,0005^{***c}	0,008±0,0008^{***c}
	Testis	0,25± 0,02	0,38± 0,01***	0,39±0,013^{**NS}	0,32±0,007^{***c}

* p <0.05; ** p <0.01; *** p <0.001 comparison with the control lot.
[a] p <0.05; [b] p <0.01; [c] p <0.001 comparison with the lead batch.

I- 6/-<u>Histological study:</u>

The testis histological sections of control and different experimental group revealed a significant difference between the rats treated with lead compared to the control rat and the rat treated with green and red clay compared to Pb group. Photomicrograph of the testis tissues of control showing the normal tissue structure with spermatozoon, spermatid, spermatocyte, Sertoli cell and basal lamina of eptilieume spermatogonia and normal nucleus we observe the different stages of spermatogenesis, which takes place in a centripetal fashion, with small spermatogonia on the periphery, larger spermatocytes I and II, with large nuclei sometimes in mitosis, spermatids smaller, located towards the inside of the tubes and, finally, mature sperm, the flagella of which fill almost all of the light from the tubes. (A). In testis cells, treatment with lead for 21 days causes a marked cell lysis (damage to the level of tissue) and causes fairly marked cell necrosis. Necrosis predominantly peripetous, sometimes decreased rate of sperm production and / or degeneration, and abnormalities in the strecture of the walls of the seminiferous tube (deformation of cells) and increase in the void between the cell. (B). For rats treated with green and red clay for 21 of them noticed an improvement in testis tissues levels (C and D).

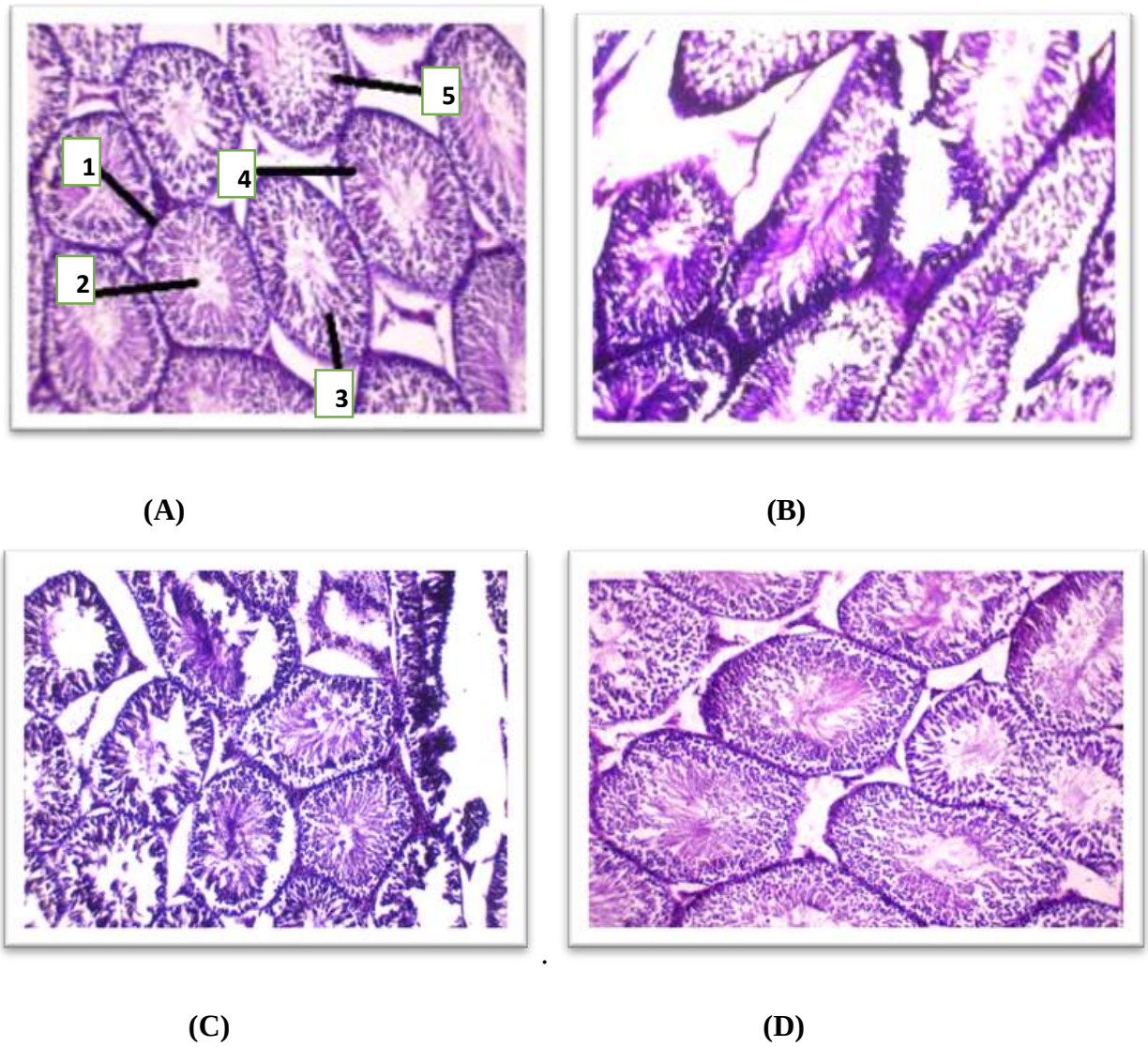


Figure 21: Photomicrograph of histopathological examination of testis sections of control (A), Pb group (B), treated with red clay (C) and treated with green clay (D), coloration with hematoxylin and eosin (x40). (1- basal lamina of eptilieume spermatogonia /2- spermatozoon/ 3- spermatid /4- spermatocyte /5-, Sertoli cell)

The liver histological sections of rats under different experimental conditions reveals significant differences between the rats treated with lead compared with the control rat and the rat

treated with green and red clay. Photomicrograph of the liver tissues of control showing the normal tissue structure with striations and branched appearance and normal nucleus (A1). In liver cells, treatment with lead for 21 days causes a marked cell lysis (damage to the level of tissue) and causes fairly marked cell necrosis. Necrosis predominantly peripetous, sometimes pericentrolobular and usually accompanied by fairly significant sinusoidal inflammation. Scattered vacuolations are also observed as well as macrocytic steatosis (B1). For rats treated with green and red clay for 21 of them noticed an improvement in liver tissue level but the improvement that caused by green clay although red (C1 and D1).

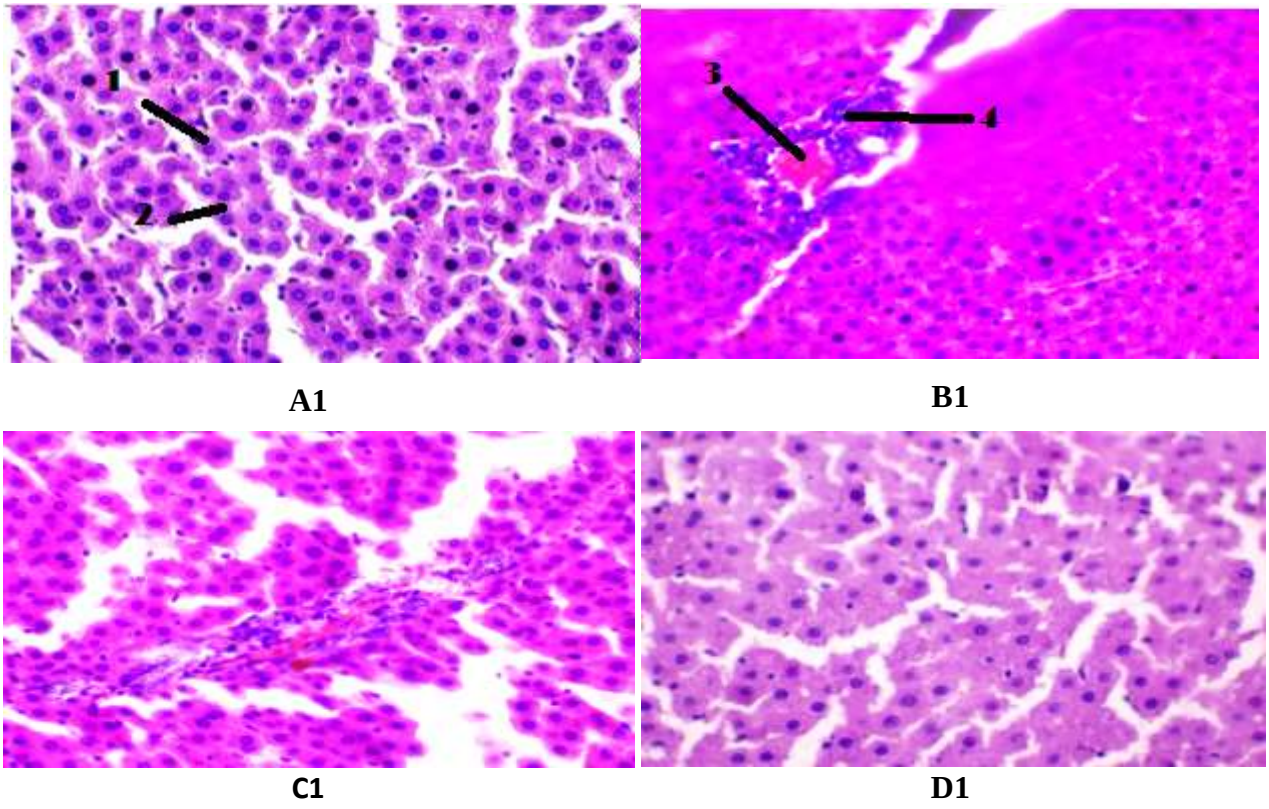


Figure 22: Photomicrograph of histopathological examination of liver sections of control (A1), Pb group (B1), treated with red clay (C1) and treated with green clay (D1), coloration with hematoxylin and eosin (x40);(1 nucleus / 2-hepatic cell / 3- necrosis / 4-hemorrhage).

II/-Discussions:

II-1/- Growth parameters:

In our study the weight of rats exhibiting on lead we noticed an increase in their weight, this result not in agreement with the study (derouiche and *al*;2018).

Regarding the relative weight of the organs, we notice hepatomegaly and nephromgaly in rats exposed to lead. This is explained by nephro- and hepato-toxicity caused by the build-up of lead in these target organs. This increase in relative organ weight may be due to lead-induced necrosis and apoptosis on these organs (Ibrahim and *al*;2011). On the other hand, the reduced testis weight compared to the control group our result is agreed with (Gorbel F and *al*;2002). On the other hand, the treatment of the rats with red and green clay we notice an improvement in the level of the body weight and their organs this improvement refer to clay compositions.

II-2/-Haematological parameters:

Our results show significant changes in haematological parameters in rats exposed to lead acetate. Overall, it appears that the lead effect resulted in and decrease in HB, MCV and HCT the latter mean Lead interferes with heme synthesis by inhibiting delta-aminolevulinic acid dehydratase (ALAD) (Kehili N;2017). by binding to SH groups (thioloprive mechanism) at the same time these fixation causes inactive various enzymatic systems hence the diversity of its effects. At a low but prolonged dose of exposure, they mainly relate to erythropoiesis (anemia) At the hematological level, lead acts at the level of three enzymatic systems of heme biosynthesis (ALA synthetase, ALA dehydrase and ferrochelatase), resulting in particularly an accumulation of ALA (deltaamino levulinic acid) in blood and urine and an increase in erythrocyte protoporphyrins (Landrigan;1989). The hematological effects of lead poisoning include hypochromic microcytic anemia with sideroblastic erythropoiesis, anemia hemolytic and leuco erythroblastic blood smear. The cause of the well-known basophilic stippling and hemolysis is inhibition of pyrimidine 5'nucleotidase while hypochromic microcytic anemia and sideroblastic erythropoiesis are due to inhibition of enzymes involved in heme synthesis. (Ray R R, 2015). For Hct is the ratio of erythrocyte volume to total blood volume, its value may, unlike Hb, reflect all hematological enzymatic inhibitory effects of lead, including the last step of the haematological route and destruction of erythrocytes. Additionally, lead may interfere with heme synthesis in

susceptible individuals, resulting in reduced Hb protein and erythrocyte function, as indicated by reduced Hct in our study and by small erythrocyte size in another. study14 rather than by a quantitative reduction in Hb levels. This suggests that Hct is the best parameter to use in studies of the hematological effects of lead, which is why we have used it here. There was a slightly significant relationship between iron intakes and Hct. This may be relevant for the biosynthetic pathway of erythrocytes and different stages of iron deficiency development. Hct or Hb values are the last parameters to be influenced by altered iron status, 1 representing a long-term consequence of low dietary iron intake. The relationship was negative (and slightly more significant) in the high Pb group, which might lead to the interpretation that dietary iron absorbed by high Pb subjects is not used as effectively as those with low Pb and as a consequence, results in their lower iron status. Therefore, the lower iron status observed in primary rats could be the result of the combined mechanisms of high Pb and low dietary iron intake. Our results confirmed that a high Pb level alters iron utilization in the body, leading to higher incidences of iron deficiency in high Pb subjects (Kim H-S and *al*;2003). on the other hand, the reduction in haematological values could also be attributed to the binding of lead to the RBCs, which increase the fragility of the membranes and therefore the destruction of the RBCs (Rous and *al*;2000). On the other hand, the iron deficiency induced by competitive inhibition lead is a proven cause of anemia (Goyer Ra;1993).Regarding white blood cells and platelets during gestation of rats, a significant decrease in GB is in agreement with the study by(Orji and al; 2016) and a significant increase in PLT this study agrees with (Zahmati's M; 2016). study.

Furthermore, the treatment of rats exposed to lead by red and green clays significantly improved the hematological parameters by their competition among the zinc and iron compositions.

Zinc functions not only as an antioxidant but also as an anti-inflammatory agent and it is essential for the normal functioning of the immune system, infecting both the innate immune system and adaptive response (Marreiro D;2017). Zinc acts as an inhibitor of NADPHoxidase, inducer of metallothionein (effective radical scavenger) and is an integral metal of Cu, Zn-SOD. The main transcription factors regulating the inflammatory responses there are NF-κB and HIF-1a and it is shown that Zn regulates both .NF-kappaB which activates by ROS which in turn activates growth factors, anti-apoptotics molecules causing cell proliferation (cancer),

inflammatory cytokines and adhesion molecules. Reduced prod uction of inflammatory cytokines by zinc, which upregulates a zinc finger protein, A20, which inhibits activation of NF-kB via the TRAF pathway (Jomovaa K;2011).

The influence of lead exposure, iron deficiency or a combination thereof on certain biochemical parameters in the blood, plasma and urine of rats was studied in order to identify specific diagnostic tests for two conditions and to establish a possible interrelation between the two factors. Decreased activity of blood glutathione peroxidase, packed cell volume, the increase in blood delta-aminolevulinic acid dehydratase (ALAD) activity without altering the blood zinc protoporphyrin (ZPP) level appears to be a specific effect of Fe deficiency that could be distinguished from intoxication with lead, a condition characterized by inhibition of blood ALAD activity accompanied by an increase in the blood level of ZPP. Linear regression analysis of the data showed that blood levels of Pb and free plasma cholesterol increase with decreasing plasma Fe levels, excessive Pb concentrations and Fe deficiency have an effect on Hb level and the size of red blood cells (HASHMI NS and *al*;1989).

II-3/-<u>Biochemical markers:</u>

Compared to the control, the results for the blood sugar level show a decrease in the main rats but there is no significance and the results for the serum calcium level show a remarkable decrease in the lead rats. first result is not agree and the second result agrees with those published by (Derouiche S and *al*; 2019). The results concerning the iron level show a decrease in significance in lead rats These results are in agreement with those published by (Andrew crowe;1995). The results concerning the iron level uric acid show a remarkable decrease in lead rats (Derouiche S and *al*; 2018). uric acid is a major end product of purine metabolism, at physiological pH it is mainly ionized in the form of urate, a powerful scavenger of free radicals (Haleng, J and *al*; 2007). Without being an antioxidant in the true sense of the term , uric acid has major antioxidant properties, (Fendri C and *al*; 2006). in the case of rats treated with lead, the level of uric acid decreases on the other hand in the case of rats treated with clay in a noticed the uric acid level almost like the control rat levels.lead causes oxidative stress (Saka S and *al*; 2011). this explains why the decrease in uric acid level has been proposed as one of the best antioxidants in plasma, where it could contribute to 35-60% of the total antioxidant capacity (Massart A; 2011), The antioxidant properties of urate can be appreciated indirectly by the fact that a reaction product of

urate with ROS, is present at high levels during oxidative stress (Haleng, J and *al*; 2007).The clay component capable of reducing oxidative stress, this explains the increase in acid level.

Furthermore, the treatment of rats exposed to lead with red and green clays, their calcium and iron composition, a significant decrease in calcium levels is noted because lead competes with calcium when the calcium channels open; it thus enters the cell, accumulating in the mitochondria. It inhibits the activation of Ca^{++} dependent proteins by interfering with the transmission of the hormonal signal (M. Hauber, J, Sibilia;1999) and the gastrointestinal absorption of Ca + 2, increases the excretion of Ca^{+2} by the kidneys, also Pb^{+2} and Ca^{+2} interact for storage in bone, which leads to the alteration of calcium homeostasis (Derouiche S ;2019). On the other hand, there is a remarkable increase in the iron content because the iron-rich clay.

П-4/- <u>Markers of renal function:</u>

Regarding the effect of lead on renal function, the results of our study show that a non-significant increase in urea and a significant decrease, in the case of poisoning of lead acetate in rats caused a chronic accumulation of lead in the body eventually leads to impaired renal function, urea and creatinine are a waste of the metabolism of amino acids that they eliminated by the kidneys (Faisal.H G Q A L and *al*; 2018), These results are in agreement with the work of (Thylambal and *al*;2004), who showed that kidney cells are no longer able to control the process of urinary excretion, beca use the kidneys are among the most sensitive to lead (Bonsignore and *al*; 1965), when these tests are abnormal, the nephropathy has already reached the irreversible phase which can lead to renal failure (Faisal.H G Q A L and *al*; 2018)

Moreover, the treatment of the rats exposed to lead by the red and green clays significantly improved the renal parameters by their competition among the zinc compositions.

These data suggest that zinc status is associated with a decline in renal function (Damianaki and *al*;2019). Low dietary zinc levels may increase the risk of developing chronic kidney disease in rats with normal kidney function. (Joo Y S and *al*;2020). result does not agree with our result.

II-5/- Liver function markers:

Regarding the results of the effect of lead on hepatic physiology, a significant increase in TGO activity and a significant decrease in TGP were shown in rats exposed to lead. Aspartate Aminotransferase (ASAT or TGO) TGO or glutamate oxaloacetic transaminase is an intracellular transaminase of mitochondrial origin. It is analyzed as part of the hepatic exploration. It is not specific to the liver; it is a tissue enzyme. The liver is an important target for lead, which has a g high affinity for thiol groups of hepatic cell membranes, which leads to hepatic necrosis and the release of TGO in serum (Da Silva and *al*; 2010). and Leakage of transaminases into the bloodstream may be the result of liver dysfunction (Vaglio and *al*;1999, Ulusoy and *al*;2005). For TGP It decreases in our results compared to the results of this study (Da Silva and al.; 2010), because it increases and is not in line with our results.

Moreover, the treatment of the rats exposed to lead by the red and green clays significantly improved the hepatic parameters by their competition among the zinc and magnesium compositions.

Zinc and magnesium treatments improved the activity of TGO and TGP transaminase enzymes in lead contaminated rats. These antioxidant can stabilize the hepatic cell membrane and protect hepatocytes from the toxic effects of lead which can decrease the leakage of enzymes to plasma. (kouadria M and *al*; 2019).

II-6/- Oxidative stress parameter:

The results of the analysis of the effect of lead on markers of oxidative stress show that lead affects these markers by increasing the level of tissue AMDA and the activity of hepatic and renal GST tissue SOD and decreasing the GSH in rats lead poisoning which indicates oxidative stress state which causes by lead. Some of the known biochemical mechanisms of lead toxicity are the effects of components of the antioxidant defense system and lead to oxidative damage by causing a degradation of the prooxidant / antioxidant balance, due to the increased production of ROS, suppression of the activity of antioxidant enzymes, decrease in GSH levels, mitochondrial dysfunction, oxidative DNA damage and ultimately apoptosis are observed (Gurer H and *al* ;2000; Hsu PC and *al*;2002). as one of the main mechanisms of Pb toxicity. Oxidative stress has been suggested as one of the main mechanisms of Pb toxicity (Moreira and *al*;2001). This occurs

when one or many of the unpaired electronic structures of free radicals exceed the capacity of the antioxidant defense mechanisms that provide protection against the damaging effects of free radicals. With the depletion of glutathione and other sulfhydryl groups, adjusting the activity of various antioxidants enzymes that prevent lipid peroxidation play an important role in lead-induced oxidative tissue damage (Flora and *al*;2012) . In the case of exposure lead, superoxide ions, hydroxyl radicals and hydrogen the production of peroxide is accelerated from free radicals (Firuzi and *al*; 2011). Lead to toxic effects on the structure and the function of the cell membrane. The effects on the erythrocyte membrane are very sensitive lead (Carocci and *al*; 2016). Another mechanism of lead-induced oxidative membrane damage is its effect on changes in fatty acid membrane composition. Since there is a correlation between fatty acids chain length and unsaturation and peroxyd action and membrane sensitivity, the increase in arachidonic acid leads to an exacerbation of membrane lipid peroxidation (Ercal and *al*; 2001).

The most common parameters used for the assessment of lead poisoning are the level of GSH and the antioxidant enzyme SOD, also known as the metalloprotein, exhibit an antioxidant effect by detoxifying peroxides (-OOH), hydrogen peroxide (H_2O_2) and simple oxygen ($1O_2$), enzymatically. The CAT converts H_2O_2 into H_2O and O2, respectively. It takes an important role in the deterioration of H_2O_2 (Firuzi and al :2011) GSH-Px requires GSH for peroxide degradation and is used to break down weaker and less stable H2O2. These antioxidant enzymes are potential targets for lead toxicity. The other is SOD and there are two subtypes, MnSOD and CuSOD. There is a close relationship between SOD and low copper levels in the blood. Exposure to lead results in a decrease in copper level and therefore indirectly decreases SOD activity (Croft; 1998, Carocci and *al*;2016). One of the effects of lead exposure is the metabolism of glutathione. Glutathione is a tripeptide comprising three amino acids: glutamic acid, cysteine and glycocoll It exists in reduced form (commonly known as GSH) and in oxidized form (GSSG), these two forms balancing each other containing a - group - Reactive SH having power. The direct interaction of -SH groups with ROS acts as a non-enzymatic antioxidant or is has an effect for enzymatic detoxification reactions as a cofactor / coenzyme for ROS. There is a carboxylic acid group, an amino group, a sulfhydryl group, and two peptide bonds to which metals are attached. Lead binds to the -SH group, which decreases the level of GSH, and a low level of GSH directly or indirectly affects antioxidant activity (Ercal and *al*; 2000, Hsu PC and *al*;2002, Patrick 2006a, b) As a result, GSH plays an important role in the protection of cell or tissue life against oxidative

stress / lipid peroxidation. The inactivation of these antioxidant enzymes by lead (SOD, CAT and GSH-Px) is also linked to the substitution of zinc, an important cofactor. Besides lipid peroxidation, it induces oxidation of Hb which causes hemolysis of erythrocytes. This is attached to inhibition of ALAD and leads to increased concentration of ALA substrate in urine / blood High level of ALA results in formation of hydrogen peroxide and superoxide radicals which interact with hydroxyl radicals and oxy-Hb. The mechanism makes the cell highly sensitive to oxidative stress and causes cell death. Only / divalent cations such as Ca^{+2}, Mg^{+2}, Fe^{+2} (Assi and *al*; 2016, Singh and *al*;2018).

This pro-oxidant action was also defined by an increase in the level of MDA in the group treated with lead our result is in agreement with (Aouacheri O and *al*;2020).

We observed an increase in the level of GST activity in the lead-treated group While the antioxidant enzyme GST is known to provide protection against oxidative stress, the increase in this enzyme on exposure to lead may be due to depletion of tissue thiol fraction status. These enzymes are important for maintaining a critical balance in the redox state of glutathione. Results of current work clearly demonstrate the generation of oxidative stress to be an important mediator after exposure to lead (Saxena G and *al*;2004).

Also, during the gestation period of rats, treatment with red and green clay. Their composition is silicon, magnesium, aluminium, calcium, sulfur, iron, phosphorus, zinc and copper in noticed an improvement in oxidative stress parameters (a significant increase in GSH level and a significant decrease in SOD, GST, MDA levels).

Iron (Fe), copper (Cu) and zinc (Zn) are microelements essential for the proper functioning of living organisms. These elements participate in many processes, including cell metabolism and antioxidant and anti-inflammatory defenses, and also influence enzyme activity, regulate gene expression and participate in protein synthesis (Grzeszczak K and *al*;2020).

In our study we use red clay rich in iron and green clay poor in iron, iron having an indirect action for the oxidative stress induced by lead. In cases where there is a considerable amount of iron will decrease the absorption of lead then decrease the toxic effects of this metal (oxidative stress) our result against this study (David.S. K ;1975).

Zinc is an essential trace element for living organisms More than 300 enzymes require Zn for their activity acts as a cofactor of important enzymes which contribute to the proper functioning of the antioxidant defense system (Patra R. C and *al* ;2011). In addition, this mineral protects cells against oxidative damage because it acts in the stabilization of membranes, inhibits the enzyme nicotinamide adenine dinucleotide phosphate oxidase (NADPH-oxidase), a pro-oxidant enzyme, and induces the synthesis of metallothionein. Metallothionein is involved in the reduction of hydroxyl radicals (OH) and in the sequestration of reactive oxygen species produced under stress conditions (Marreiro D N and *al*; 2017). Zinc deficiency has been associated with increased levels oxidative damage, including increased oxidation of lipids, proteins and DNA (Prasad;2009). The latter against our study.

Copper is a co-factor of many enzymes among this enzyme is superoxide dismutase (Cu / Zn-SOD 1 and 3) or SOD and ceruloplasmin, these enzymes key in protecting against free radicals They also inhibit lipid peroxidation and superoxide anion scavenger. Superoxide dismutase (SOD) responsible for the dismutation of superoxide anions into oxygen and hydrogen peroxide. It is therefore one of the defense systems against free radicals, which can cause serious tissue damage and degenerative processes (Lamer A-C; 2014).

Copper performs the catalytic functions of the enzyme, while zinc only enters its conformation. SOD could be stimulated initially to try to counteract the production of free radicals, then its excessive use could lead to a decrease in its activity (Blouin; 2008).

Magnesium is a mineral salt which has a very important role in many intracellular enzymatic reactions. It also participates in the neuromuscular transmission of nerve impulses. It is often considered to be the natural “stress reliever”. (Fabienne R; 1993).

Having a protective effect is in part due to the inhibition of the secretion of stress hormones (Jennifer R ;2017). In the state of stress causes the secretion of substances called "stress hormones". These are essentially adrenaline and cortisol. Adrenaline increases lipolysis and therefore the level of circulating fatty acids which capture plasma magnesium, thereby reducing cell penetration. Cortisol decreases the intestinal absorption of magnesium and increases its urinary excretion, further reducing intracellular intake. (Montaigne Medical Academy ;2013).

During chronic stress, the hypothalamic-pituitary adrenal axis plays an essential role. Faced with a stressful element, there is first the synthesis of CRH which will bind at the level of the anterior pituitary gland. This will cause the production of ACTH which in turn will cause the release of glucocorticoids, especially cortisol, in the adrenal cortex. There is a negative feedback phenomenon in the hypothalamus and pituitary gland. Long-term presence of cortisol is harmful to the body The availability of magnesium helps decrease the chemical messengers of stress by modulating the hypothalamic-pituitary adrenal axis and reducing adrenaline and cortisol released into the bloodstream. The adequate level of magnesium therefore makes it possible to better manage stressful situations and reduce their impact. However, it is also important to take charge of the cause of this stress (Jennifer R;1992).

Calcium is a mineral salt whose main function is the mineralization of bone in the form of salts of calcium phosphates. Discover the role of this vitamin, its recommended nutritional intakes, the risks of deficiencies or overdose as well as its medical applications (Fabienne R;1993). The present study shows that the doubling of dietary calcium with $Ca_3 (PO_4)_2$ limits the absorption of lead in rats. This was shown both by the oral absorption of 203Pb and by the decrease in the body burden of stable lead assessed by enzyme activities and tissue lead content. Retention of intraperitoneal 203Pb was unaffected by calcium dietary supplementation, suggesting decreased absorption of lead by calcium orthophosphate was mediated from the gastrointestinal tract. The similar decrease in oral absorption of 203Pb caused by other calcium salts suggests that the decrease in lead absorption is associated only with calcium supplementation and not with the high phosphorus contained in the diet. (PETER A and *al*; 1977).

II-7/- <u>Histological analysis:</u>

lead toxicity causes quite marked cell lysis (damage to hepatic and testicular tissue). Lead can cause an increase in the production of reactive oxygen species (ROS) such as the hydroxyl radical (HO.). The superoxide radical (0^{2-}) or hydrogen peroxide (H_2O_2). Increased generation of ROS can overwhelm cells' intrinsic antioxidant defenses and lead to a condition known as “oxidative stress” (Clementine P; 2014). Cells under oxidative stress exhibit various dysfunctions due to damage caused by them. ROS to lipids, proteins and DNA. the toxicity associated with this metal could be due to oxidative tissue damage (Lavoie M-E; 2012).

. There can be two independent sources of oxidative damage; the first is the pro-oxidant effect of δ-ALA and the second is linked to the direct effect of lead on membrane lipids (Nuran E and *al*; 2001).

Phosphate is an intracellular anion and its levels in tissues can be altered during cell membrane damage. The increase in phosphate levels in this study may be due to cell membrane damage due to lead exposure (Khan D and *al*;2008). Necrosis predominantly periportal, sometimes pericentrolobular and most often accompanied fairly significant sinusoidal inflammation. Scattered vacuolations are also observed as well as macrocytic steatosis. These histological alterations testify to the toxic effect of lead on the liver and also confirm the biochemical results and the stress which they induce.la treatment with red and green clay improved with negative conceconce which causes by lead.

Conclusion

Conclusion

Our work was carried out for the objective of evaluating the toxic effects of lead in rats of the Wistar strain and to study the effectiveness of metallotherapy (red and green clay) against this toxicity. In light of the results obtained, we can conclude that:

- The qualitative and quantitative analysis shows that red and green clay are rocks rich in minerals, giving them a positive effect against several pathologies.
- The physiological study showed that the treatment with red and green clay increases the body weight of the rats and improves the relative weight of the organs of the liver, kidneys, heart and testes, which clearly shows the absence of the toxic side effect on these two target organs.
- The treatment with red and green clay induces a significant restoration of certain hematological and biochemical parameters, which shows the protective effect of these therapeutic types against metabolic and physiological alterations in several biological systems in relation to the parameters studied.
- Treatment with red and green clay the state of oxidative stress induced by exposure to lead by limiting radical phenomena and repairing oxidative damage by reducing lipid peroxidation at the hepatic, renal and testicular level and in the brain and cardiac; and by improving the mobility of the antioxidant defense in the studied organs, which also shows in another way the protective effect of these two plants against the pathologies associated with oxidative stress including inflammation, cancer… .etc.
- The tissue analysis carried out at the level of the liver and the testes made it possible to show that our red and green clay causes a remarkable protection and regeneration of hepatic and testicular tissue with preferential dominance of green clay.
- In the light of this study, we have recorded that the use of red and green clay has no disruptive effect on the organs studies which allows us to confirm the beneficial effect of these two plants on the state of user health.

Perspective:

Our results are remarkable for us because they open up experimental perspectives in the future which should allow us to clearly identify:

- Studies the therapeutic effect of red and green clay against acute and chronic diseases, in particular nervous diseases.
- Study the effect of red and green clay on genomic DNA.

- The molecules involved in these therapeutic effects.
- Carry out a study of the mechanisms of action at the molecular and cellular level of the types of treatments studied.

Reference

1. ABED D, BOLLINELLI R, BROUSSY G, CARLES P, SORBARA R. Encephalopathie
2. AL KHAYAT A, MENON NS, ALIDINA MR. Acute lead encephalopathy in early infancy- clinical presentation and outcome. Ann Trop Pediatr. 1997; **17** (1): 39-44.
3. Alberto L and al ;2011 ;Chapitre 13 - Utilisations pharmaceutiques et cosmétiques des argiles fibreuses ; developments in Palygorskite-Sepiolite Research; 299-324.
4. Alexander, J., Benford, D., Boobis, A., Ceccatelli, S., Cravedi, J-P., Di Domenico, A., Doerge, D., Dogliotti, E., Edler, L., Farmer, P., Filipic, M., Fink-Gremmels, J., Fürst, P., Guerin, T., Knutsen, H. K., Machala, M., Mutti, A., Schlatter, J. et Leeuwen, R. (2013). Scientific Opinion on Lead in Food. European Food Safety Authority (EFSA), European Food Safety Authority journal, 8(4), 1-151.
5. *Aouacheri O; Saka S ;2020; Cytoprotective Effects of Zingiber officinale Against the Oxidative Stress Induced by Lead Acetate Toxicity in Rats; Phytothérapie, Publication ahead of print*
6. Aoudi M et al ;2017 ; Caractéristiques environnementales de l'argile et des minéraux à base d'argile ; Taylor Fracis Online ;139.
7. Arbona, V., Flors, V., Jacas, J., García-Agustín, P., & Gómez-Cadenas, A. (2003). Enzymatic and Non-enzymatic Antioxidant Responses of Carrizo citrange, a Salt-Sensitive Citrus Rootstock, to Different Levels of Salinity. Plant and Cell Physiology, 44(4), 388–394.
8. ASSAYAMA M, OGAWA T, MORIMOTO T. Excretion of heavy metals in sweat. *J Aichi Med Uni Assoc*. 1975; **3** (4): 230-235.
9. *Assi, M. A., Hezmee, M. N. M., Haron, A. W., Sabri, M. Y., & Rajion, M. A. (2016). The detrimental effects of lead on human and animal health. Veterinary World, 9(6), 660–671*
10. ATSDR. 1988. The nature and extent of lead poisonning in children in the United States: areport to congress-Agency For Toxic Substances and Disease Regisry Public H ealth Service- U S Departement of Health and Human Services. Public Health Service. Atlanta. Georgia. P150-155.
11. ATSDR. 1988. The nature and extent of lead poisonning in children in the United States: areport to congress-Agency For Toxic Substances and Disease Regisry Public H ealth Service- U S Departement of Health and Human Services. Public Health Service. Atlanta. Georgia. P150-155.
12. AWAD EL KARIM MA, HAMED AS, ELHAIMI YA, OSMAN Y, EL KARIM MA.
13. *BAHI A; 2015; L'effet protecteur des antioxydants naturels dans l'intoxication du mercure chez le rat Albinos Wistar (Aspects biochimiques, immunologiques et histologiques); Mém doctorat ;1-188.*
14. Barouki R ; 2006 ; Stress oxydant et vieillissement ; MEDECINE/SCIENCES ; 22 : 266-72.
15. Beauchamp, C., and Fridovich, I. (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry. 44(1): 276-287.
16. Benhamdi A ; 2014 ; Etude des enzymes de stress oxydatif chez Hedysarum pallidum Desf. Et Lygeum spartum L. en réponse à la pollution du sol par l'antimoine ; Mém doctorat ; UC1 ; 146.
17. BENNETT WM. Lead nephropathy. Kidney Intern. 1985; 28: 212-220.

18. Bensakhria A ;2018 ; Toxicologie Générale ; RecgercheGate ;70-86
19. Bernard O ;1975 ; modification mecanique et structurales de trois argiles pures consolidees electro-chimiquement ; Mèm dotorat; UDSETDL; 159.
20. *BLOUIN Eric et VIGNON Eric Arthrose et Cuivre. Réflexions Rhumatologiques, 2008 Février, Tome 12, n°106, 2-7.*
21. *Bonsignore, D., Calissanon, P., et Cartasegna, C. (1965). Un senylice metado per la determinazione della α-amino-levulinicodeidratasi nel sangue: comportamento dell'ertzima nell'intossicazione saturmina. Med Lavoro. 56: 199–205.*
22. Boubali Z ; 2017 ; Biomarqeurs du stress oxidatif; Mém doctorat; UMVR; 137
23. Boundjah O ;2014 ; Mécanismes d'assemblage des granules de stress dans des conditions de stress oxydatif et osmotique ; Mém doctora ; UEVE GAO ;197.
24. *Bozdağ, M., & Eraslan, G. (2019). The effect of diosmin against lead exposure in rats‡. Naunyn-Schmiedeberg's Archives of Pharmacology.*
25. *BURDIN L; 2014; OLIGOTHERAPIE ET PERSONNE AGEE: INTERET DU CHROME, DU SELENIUM, DU ZINC ET DU CUIVRE?; Mém doctorat ; 1-142.*
26. C. Fendri C ; Mechri M ; Khiari G ; Othman A ; Kerkeni A; Gaha L;2006; Implication du stress oxydant dans la physiopathologie de la schizophrénie : revue de la littérature; EM cosulte; 32-N°2; 244-252 .
27. C. Viseras et al; 2010; Current challenges in clay minerals for drug delivery; Applied Clay Science.
28. *Cadet J, Delatour T, Douki T, Gasparutto D, Pouget JP, Ravanat JL, Sanvaigo. (1999). Hydroxyl radicals and DNA base domage. Mutat. 424, 9-21.*
29. Camille M and al ;2011 ; Espèces réactives de l'oxygène et stress oxydant ; médecine/sciences ; 27 : 405-12
30. Camille T ; 2011 ; Caractérisation et quantification des minéraux argileux dans les sols expansifs par spectroscopie infrarouge aux échelles du laboratoire et du terrain ; Mém doctorat ; UT3 Paul sabatier; 230.
31. Carange J ; 2010 ; RÔLE ANTIOXYDANT ET ANTI-APOPTOTIQUE DES BRASSINOSTÉROïDES, UNE NOUVELLE STRATÉGIE DE NEUROPROTECTION ; Mém doctorat ; UDQ ; 138.
32. *Carocci, A., Catalano, A., Lauria, G., Sinicropi, M. S., & Genchi, G. (2015). Lead Toxicity, Antioxidant Defense and Environment. Reviews of Environmental Contamination and Toxicology, 45–67.*
33. *Catherine ROUSSEL, Marielle SCHMITT ;2001 ; Dépistage du saturnisme infantile autour des sources industrielles de plomb ; tome2 ; MINISTÈRE DE L'EMPLOIET DE LA SOLIDARITÉ*
34. Cezard C. 1992. Toxicologie du plomb chez l'homme, Lavoisier. Paris. P 123-131.
35. Chantal B. 2002. Toxicologie clinique. Ed. Flammarion Médecine Science. Paris. P 638.
36. Chih-Huang, W., Yi-Fong, P. (2006). Adsorption of a cationic dye (méthylène blue) onto spent activated clay, Journal of Hazardous Materials 144 ,355–362.
37. Choufa N ; 2013 ; Epuration des eaux usées : l'élimination des micropolluants dans les eaux usées par un matériau argileux ; Mémoire De Magister ; Université Mohammed

Chérif Messaadia Souk Ahras; Faculté Des Sciences Et De Technologie Département Des Sciences De La Matière; 124.

38. Choufa N ; 2013 ; l'élimination des micropolluants dans les eaux usées par un matériau argileux ; Mém magister ; UBMA ; 110.
39. *Christian BAILLY, Christophe DECLERCQ, Bruno FABRES, Nadine FRERY,*
40. *Cillard, J., & Cillard, P. (2006). Mécanismes de la peroxydation lipidique et des anti-oxydations. Oléagineux, Corps Gras, Lipides, 13(1), 24–29.*
41. Clémentine P ; 2013 ; Rôle du stress oxydant au niveau hépatique et rénal dans la toxicité de l'uranium après exposition chronique ; Mém doctorat ; UPS ; 416)
42. *Croft KD (1998) The chemistry and biological effects of flavonoids and phenolic acids. Ann N Y Acad Sci 854:435–442*
43. Crowe, A., & Morgan, E. H. (1996). *Interactions between tissue uptake of lead and iron in normal and iron-deficient rats during development. Biological Trace Element Research, 52(3), 249–261.*
44. *Curtin J F, Donovan M, Cotter TG. (2002). Regulation and measurement of oxidative stress in apoptosis. J of Imm Methods. 265, 49-72.*
45. *Da Silva A S. Wolkmer P. Costa M M. Tonin A A. Eilers T L. Gressler L T. Otto M A. Zanette R A. Santurio J M. Lopes S T A et Monteiro S G. 2010. Biochemical changes in cats infected with Trypanosoma evansi. Vet. Parasitol. Vol 171. P 48-52.*
46. Dali Youcef L ; 2012 ; perification et caracterisation de l'attapulgite Algereienne application a l'adsorption de bleu de methylene ; Mèm magister ; UO ;120.
47. *Damianaki, K., Lourenco, J. M., Braconnier, P., Ghobril, J.-P., Devuyst, O., Burnier, M., … Pruijm, M. (2019). Renal handling of zinc in chronic kidney disease patients and the role of circulating zinc levels in renal function decline. Nephrology Dialysis Transplantation.*
48. DEFRAIGNE J O ;2008 ; STRESS OXYDANT ET ANTIOXYDANTS : mythes et réalités ; Rev Med Liège 2008 ; 63 ; 10-19.
49. *Delattre J., Beaudeux J.L. et Bonnefont-Rousselot D. (2005). Radicaux libres et stress oxydant: aspects biologiques et pathologiques; Paris: Lavoisier; 3ème édition ;1-548*
50. *Derouiche S; Zeghib K; Gharbi S; Khelef Y ;2018; Ameliorative effect of Aristolochia longa and Aquilariamalaccensison lead acetate inducednephrotoxicityin female rats; International Journal of Biological and Agricultral Reasearch (IJBAR) ;1-13.*
51. *Derouiche S; Zeghib K ;2019; Evaluation of the Chelating Efficacy of Aquilaria malaccensis and Aristolochia longa Against Biochemical Alterations Induced by Lead Bioaccumulation in Rats; Pharmaceutical and Biosciences Journal; Vol. 7(2), 11-15, 2019; 1-5.*
52. DIATTA M T ; 2006 ; Matières premières argileuses du Sénégal : caractéristiques et applications aux produits céramiques de grande diffusion ; Mém doctorat,UL; 167.
53. *DRASS Rhône-AlpesCIRE Rhône-Alpes Auvergne.*
54. Durackova Z., Djrolo F., Houngbe H., Avode G., Attoulou V., Addra B., Kodjoh N Avimadj M., 2008. Oxidants, Antioxidants and Oxidative stress. Mitochondrial medicine Gvozdjakova A (ed). P :19-43.

55. *Durand, D., Damon, M., & Gobert, M. (2013). Le stress oxydant chez les animaux de rente: principes généraux. Cahiers de Nutrition et de Diététique, 48(5), 218–224.*
56. *eckman K and Ames B.N., 1997. Oxidative decay of DNA. J Biol Chem, 272: 19633-19636.*
57. Eddhima M Z ; 2019 ; les radicaux libres : effet, mecanisme et approches therapeutiques; Mém doctorat; UMVR; 228.
58. *Eddhima; Z ;1993; Les radicaux libres: effets, mecanismes et approches therapeutique ; Mém doctorat ;1-228.*
59. Effects of exposure to lead among lead-acid battery factory workers in Sudan. *Arch Environ Health*. 1986; 41 (4): 261-265.
60. Emna E ;2011 ; Réactivité de surface d'argiles naturelles Etude de l'adsorption de colorants anioniques ; Mèm docorat ; UDS ; 210.
61. *Esterbauer H, Gebicki J, Puhl H, Jurgens G. (1992). The role of lipid peroxidation and antioxidants in oxidative modification of LDL. Free Rad Biol Med. 13, 341 - 349.*
62. *EYMARD S; Mise en évidence et suivi de l'oxydation des lipides au cours de la conservation et de la transformation du chinchard (Trachurus trachurus): choix des procédés; Mém doctorat ;1-217.*
63. F. Arbaoui and M. N. Boucherit, J; 2014; Appl. Clay Sci. 91–92, 6.
64. *Faisal.H.G.Q.AL-Amier, Hind.S.A, Salah .M.M ;2018 ; Effect of Lead Exposure on Some Biochemical Parameter of Battery Factory and Benzene Fuel Stations Workers ; Department of Biology, College of Science, Baghdad University,Baghdad,Iraq. Biotechnology Research Center, AL-Nahrain Unversity,Baghadad, Iraq ; Vol. 18, No.2 ;1-6 .*
65. *Favier A. (2003). Intérêt conceptuel et expérimental dans la compréhension des mécanismes des maladies et potentiel thérapeutique. L'act Chim. 108 - 115.*
66. *Favier, A. (2003). Le stress oxydant: intérêt conceptuel et expérimental dans la compréhension des mécanismes des maladies et potentiel thérapeutique. L'actualité chimiqu, 108-115.*
67. *Firuzi, O., Miri, R., Tavakkoli, M., & Saso, L. (2011). Antioxidant Therapy: Current Status and Future Prospects. Current Medicinal Chemistry, 18(25), 3871–3888.*
68. *Flora, G., Gupta, D., & Tiwari, A. (2012). Toxicity of lead: a review with recent updates. Interdisciplinary Toxicology, 5(2)*
69. *FONTAINE E ;2007; RADICAUX LIBRES ET VIEILLISSEMENT; Université Joseph Fourier, Laboratoire de Bioénergétique Fondamentale et Appliquée, 38041 Grenoble; vol42, 2, 2007. ;110-115.*
70. *Fourier J; 1993; La supplementation des aliments par des mineraux interet nutritionnel; Mém doctorat; 1-131.*
71. Garait P ; 2006 ; LE STRESS OXYDANT INDUIT PAR VOIE METABOLIQUE (REGIMES ALIMENTAIRES) OU PAR VOIE GAZEUSE (HYPEROXIE) ET EFFET DE LA GLISODIN®. Biologie cellulaire. Université Joseph-Fourier - Grenoble I, Français.
72. *Gargouri M; Ben amarad I; Ksourib R; Zeghaid K; El fekia A; Magnec C; Hakimd ;2015; IMPACT DE DEUX PLANTES HALOPHYTES SUR*

L'HEPATOTOXICITE INDUITE PAR LE PLOMB CHEZ LE RAT MALE ADULTE; Etude Expérimentale; N°21 / 22; 72 – 80.

73. Garnier, R. (2005). Toxicité du plomb et de ses dérivés. EMC - Toxicologie-Pathologie, 2(2), 67–88
74. *Gerli, E. M., & Ruiz, J. (1990). Libro del Arcipreste (Tambien llamado "Libro de buen amor"). Hispania, 73(2), 413.*
75. Gomri F ;2010 ; Synthèses et caractérisations d'argiles cationiques et anioniques organophiles. Elimination des chlorophénols; Mèm magister; UFAS (ALGERIE); 234.
76. *Gorbela F; Boujelbenea M; Makni-Ayadib F; GuermazicF; Croutee F; Soleilhavoupe JP; El Fekia A;* 2002; *Exploration des effets cytotoxiques du plomb sur la fonction sexuelle endocrine et exocrine chez le rat pubère mâle et femelle. Mise en évidence d'une action apoptotique; 2002 Académie des sciences / Éditions scientifiques et médicales Elsevier SAS. Tous droits réservés; C. R. Biologies 325 (2002) 927–940.*
77. *Goyer, R. A. (1993). Lead toxicity: current concerns. Environmental Health Perspectives, 100, 177–187.*
78. *Grzeszczak, K., Kwiatkowski, S., & Kosik-Bogacka, D. (2020). The Role of Fe, Zn, and Cu in Pregnancy. Biomolecules, 10(8), 1176.*
79. *Gurer, H., & Ercal, N. (2000). Can antioxidants be beneficial in the treatment of lead poisoning? Free Radical Biology and Medicine, 29(10), 927–945.*
80. *Gutteridge J. (1992). Invited review free radicals in disease processes: a compilation of cause and consequence. Free Rad Res Comm. 19, 598-620.*
81. Habig, W.H., Pabst, M.J., & Jakoby, W.B., (1974). Glutathione S-transferase. The first enzymatic step in mercapturic acid formation. Journal of Biological Chemistry, 249, 7130-7139.
82. Haissaguerre M ;2015 ; Etude de l'intraction entre lesROS et la voie mTORC1dans la regulation de la balance energeique; Mém doctorat ; UB; 157.
83. Haleng, J., Pincemail, J., Defraigne, J.O., Charlier, C., Chapelle, J.P. (2007). Le stress oxydant. Revue médicale de Liège 62 : 628-638
84. *Haleng, J., Pincemail, J., Defraignem J.O. et Chapelle, J.P. (2007). Le stress oxydant. Revue Médicale de Liège, 62(10): 628-638.*
85. Halliwell B., 2006. Phagocyte-derived reactive species: salvation or suicide. TRENDS in biochemical scienes. Vol 31. n°9: 509-15.
86. *Hashmi, N. S., Kachru, D. N., & Tandon, S. K. (1989). Interrelationship between iron deficiency and lead intoxication (part 1). Biological Trace Element Research, 22(3), 287–297*
87. *HENNEBELLE M; 2012; Acides gras polyinsaturés n-3 (AGPI n-3) et prévention des dommages cérébraux induits par un stress chronique ;1-416.*
88. *Hsu PC, Guo YL (2002) Antioxidant nutrients and lead toxicity. Toxicology 180:33–44*
89. Identification et caractérisation des propriétés structurales et fonctionnelles de l'albumine glyquée ; Mém doctorat ; UR ; 196.
90. IPCS. Environmental health criteria 165. Inorganic lead. Geneva : WHO ; 1995 300p
91. *Isabelle GIRARD, Martine LEDRANS, Patrick PEIGNIER, Michel ROUGE,*

92. Ismail KHAY,Etude physico-chimique des interfaces chaux/chanvre/argile : Impact sur la rhéologie des mortiers et sur les propriétés mécaniques, thermiques et hydriques du matériau composite, Thèse Pour Obtenir Le Grade De Docteur, Université De Limoges Faculté des Sciences et Techniques, 2012.
93. J. Haleng and al; 2007; Le stress oxidant; Rev Med Liege;62 ,10, 628-638.
94. J.-L. Beaudeux and al ; 2006 ; Sources cellulaires des espèces réactives de l'oxygène et de l'azote ; Em-conculte ;64 ; N°6 ; 373-381.
95. J.M. Haguenoer; C. Declercq ;2004; Les explorations complémentaires selon le seuil volume 16, no spéc., pp. 51-61.
96. Jeannot R., Lemière B., Chiron S., Augustin F.et Darmendrail D. (2001). Guide méthodologique pour l'analyse des sols pollués, Ed:BRGM p 19. Juste C., 1988. Appréciation de la mobilité et de la biodisponibilité des éléments en traces du sol. Sci. Sol 26 (2) 103-112.
97. Jennifer B ; 2014 ; Stress oxydant et pathologie diabétique à l'île de La Réunion
98. Jomova, K., & Valko, M. (2011). Advances in metal-induced oxidative stress and human disease. Toxicology, 283(2-3), 65–87.
99. Joo, Y. S., Kim, H. W., Lee, S., Nam, K. H., Yun, H.-R., Jhee, J. H., … Park, J. T. (2020). Dietary zinc intake and incident chronic kidney disease. Clinical Nutrition.
100. Jozja N ; 2003 ; etude de materaux argilaux albanais : Caracterisation multi-échelled'une bentonite magnesienne.Impact de l'interaction avec le nitrate de plomb sur la perméabilite ; Mém doctorat; UO;294.
101. KAMINSKY P; KLEIN M; DUC M ;1993; Physiopathologie de I'intoxication par le plomb inorganique; MISE AU POINT; RevMed Interne 1993; 14: 163-170.
102. Kehili, N., Saka, S., & Aouacheri, O. (2017). L'effet phytoprotecteur de la nigelle (Nigella sativa) contre la toxicité induite par le cadmium chez les rats. Phytothérapie.
103. KEHOE R A. The metabolism of lead in health and disease. J. Roy. *Inst Public Heulth Hyg*. 1961; 24: 81-96.
104. KEHOE RA. Studies of lead administration and elimination in adult volunteers under natural and experimentally induced conditions over extended periods of time. *Food Chem Toxicol*. 1987; **25** (6): 421-493.
105. Khan, D., Qayyum, S., Saleem, S., & Khan, F. (2008). Lead-induced oxidative stress adversely affects health of the occupational workers. Toxicology and Industrial Health, 24(9), 611–618.
106. Kharoubi O ; 2009 ; Effet prophylactique de l'extrait aqueux d'Absinthium Artémisia L. sur le pouvoir pro oxydant du plomb chez le rat Wistar en croissance ; Mém doctorat ; UO1 ;169.
107. Kharoubi O ; 2009 ; Effet prophylactique de l'extrait aqueux d'Absinthium Artémisia L. sur le pouvoir pro oxydant du plomb chez le rat Wistar en croissance ; Mém doctorat ; UO1 ;169.
108. Khenifi A ;2010 ; elaboration de materiaux a base d'argiles, caracterisation, et application a l'elimination des polluants organiques ; Mém docorat;USTOMB; 228.

109. Khenifi A ;2010 ; elaboration de materiaux basz d'argile, caracterisation, et application a l'elimination des polluants organiques ; Mèm doctorat ; UNIVERSITE DES SCIENCES ET DE LA TECHNOLOGIE D'ORAN MOHAMED BOUDIAF ;229.

110. Kim, H.-S., Lee, S.-S., Hwangbo, Y., Ahn, K.-D., & Lee, B.-K. (2003). Cross-sectional study of blood lead effects on iron status in Korean lead workers. Nutrition, 19(7-8), 571–576.

111. Kishk, F. M., El-Attar, H. A., Hassan, M. N., & El-Sheemy, H. (1976). Mineralogical and chemical composition of the clay fraction of some Nile aluvial soils in Egypt. Chemical Geology, 17, 295–305.

112. Kouadria M, Djemli S, Tahraoui A ;2019; THE PROTECTIVE EFFECT OF ZINC AND MAGNESIUM AGAINST SUBCHRONIC CADMIUM TOXICITY IN WISTAR RATS (BIOCHEMICAL AND NEUROBEHAVIORAL EFFECTS); Asian Journal of Pharmaceutical and Clinical Research; Vol 12, Issue 5, 2019 ;1-9

113. Landrigan, P. J. (1989). *Toxicity of lead at low dose. Occupational and Environmental Medicine, 46(9), 593–596.*

114. Larcher G ; 2016 ; L'ARGILE, SON UTILISATION A L'OFFICINE ; Mém.doctorat; HE ; 127.

115. Larcher G ; 2016 ; L'ARGILE, SON UTILISATION A L'OFFICINE ; Mém.doctorat; HE ; 127

116. Lauwerys R. 1992. Toxicologie industrielle et intoxication professionnelles, 3éme Ed. Masson. Paris. P 123-131.

117. Lavoie M ;2012 ; Inflammation, stress oxydant, profil métabolique : influence des apports alimentaires et de la dépense énergétique ; Mém doctorat; UM;326.

118. *Levine R.L., Garland D., Oliver C.N., Amici A., Climent I., Lenz A.G., Ahn B.W., Shaltiel S., Mc Cord JM., 2000. The evolution of free radicals and oxidative stress. Am J Med. 108: 652-659*

119. Levine RL. (2002). Carbonyl modified proteins in cellular regulation; aging and disease. Free Radic Biol Med. 32, 790-796.

120. López-Galindo, A., Viseras, C., Aguzzi, C., & Cerezo, P. (2011). Pharmaceutical and Cosmetic Uses of Fibrous Clays. Developments in Palygorskite-Sepiolite Research, 299–324.

121. M. Hauber, J. Sibilia ;1999 ; Les complications ostéoarticulaires liées au plomb : un problème méconnu ; M ISE AU POINT ; n° 257 - décembre 199 ,27-31.

122. M. ZAIDI H; 2019; Etude du mécanisme de fixation du potassium dans les sols : Application à une smectite bionique Na-Ca; Mém doctorat; UAHIBM; 124.

123. M.J. Kassim, A. Pizzi; 2009;Characterization and performance of Rhizophora apiculata mangrove polyflavonoid tannins in the adsorption of copper (II) and lead (II), Industrial Crops and Products», 30 152–161.

124. MALCOLM D. The effects of lead on the kidney. *Trans Soc Occup Med*. 1970; **2** (2): 50- 53.

125. Mamta, Misra, K., Dhillon, G. S., Brar, S. K., & Verma, M. (2013). Antioxidants. Biotransformation of Waste Biomass into High Value Biochemicals, 117–138.

126. Mamta, Misra, K., Dhillon, G. S., Brar, S. K., & Verma, M. (2013). Antioxidants. Biotransformation of Waste Biomass into High Value Biochemicals, 117–138.

127. Marreiro, D., Cruz, K., Morais, J., Beserra, J., Severo, J., & de Oliveira, A. (2017). Zinc and Oxidative Stress: Current Mechanisms. Antioxidants, 6(2), 24.

128. Massart A; 2011; Supplémentation en oméga 3 et antioxydant et stress oxydant au cours d'un entrainement de judo. Mém doctorat; UO; 192.

129. Melle. Choufa Nassima Epuration des eaux usées: l'élimination des micropolluants dans les eaux usées par un matériau argileux Mémoire de magister a l'université Mohahammed messaadia souk ahras 2012-2013.

130. Meredith, P. A., Moore, M. R., & Goldberg, A. (1977). The effect of calcium on lead absorption in rats. Biochemical Journal, 166(3), 531–537.

131. Meschy, F. (2010). Nutrition minérale des ruminants, Éditions Quæ c/o Inra, RD 10, 78026 Versailles Cedex © Éditions Quæ, 2010. 292 .

132. Michel1 F ; Bonnefont-Rousselot D; Mas E ; Drai J; Thérond P ; 2008 ;*Biomarqueurs de la peroxydation lipidique : aspects analytiques ; revue générale ; vol. 66, no 6 ;1-16.*

133. Migdal, C., & Serres, M. (2011). Espèces réactives de l'oxygène et stress oxydant. Médecine/sciences, 27(4), 405–412.

134. Migdal, C., & Serres, M. (2011). Espèces réactives de l'oxygène et stress oxydant. Médecine/sciences, 27(4), 405–412.

135. MONGENS M ;1998 ; ORIGINE ET CONSÉQUENCES DU STRESS OXYDANT ; ; Mém doctorat ;1-121.

136. Monteiro, H. P., Abdalla, D. S., Arcuri, A. S., & Bechara, E. J. (1985). Oxygen toxicity related to exposure to lead. Clinical Chemistry, 31(10), 1673–1676.

137. Mortureux, M. (2013). Expositions au plomb: effets sur la santé associés à des plombémies inférieures à 100 µg/ Janvier. Agence nationale de sécurité sanitaire alimentation, environnement, travail. (Ed.) Vol. (9).

138. Munro D; 2014; production des espèces réactives de l'oxygène et composition lipldique des membranes mitochondriale comme determinants de la longevité de la cap a cié d'adaptation au froid chez les bivalves veneroides; Mém doctorat; UDM;266.

139. Nicolosi R.J., Lawton C.W and wilson T.A., 1999. Vitamin E reduced plasma LDL-C, LDL oxidation, and early aortic atheroscleroris compared with black tea in hypercholesterolemic

140. Nuran Ercal, B. S. P., Hande Gurer-Orhan, B. S. P., & Nukhet Aykin-Burns, B. S. P. (2001). Toxic Metals and Oxidative Stress Part I: Mechanisms Involved in Me-tal induced Oxidative Damage. Current Topics in Medicinal Chemistry, 1(6), 529–539

141. Nuran Ercal, B. S. P., Hande Gurer-Orhan, B. S. P., & Nukhet Aykin-Burns, B. S. P. (2001). Toxic Metals and Oxidative Stress Part I: Mechanisms Involved in Me-tal induced Oxidative Damage. Current Topics in Medicinal Chemistry, 1(6), 529–539.

142. Nuran Ercal; Hande Gurer-Orhan and Nukhet Aykin-Burns; 2001; Toxic Metals and Oxidative Stress Part I: Mechanisms Involved in Metal- induced Oxidative Damage; Current Topics in Medicinal Chemistry ; 1, 529-539.

143. Olivier Chanel, Catherine Dollfus, Jean-Marie Haguenoer, Philippe Hartemann, Guy Huel, et al.. Plomb dans l'environnement : quels risques pour la santé ?. [Rapport de recherche] Institut national de la santé et de la recherche médicale(INSERM). 1999, 451 .
144. Olivier Chanel, Catherine Dollfus, Jean-Marie Haguenoer, Philippe Hartemann, Guy Huel, et al.. Plomb dans l'environnement : quels risques pour la santé ?. [Rapport de recherche] Institut national de la santé et de la recherche médicale(INSERM). 1999, 451.
145. OMS. 1980. Exposition aux métaux lourds. limites recommandées d'exposition professionnelle à visée sanitaire. Genève. Série de rapports techniques. P647-132.
146. OMS. 1980. Exposition aux métaux lourds. limites recommandées d'exposition professionnelle à visée sanitaire. Genève. Série de rapports techniques. P647-132.
147. OPPTBP. (2015). Traitement des peintures au plomb. Vol. (978). O.P.P.B.T.P (Ed.).
148. Pasquier, C. (1995). Stress oxydatif et inflammation. Revue francaise des laboratoires,. 27,: 87-92.
149. Patra, R. C., Rautray, A. K., & Swarup, D. (2011). Oxidative Stress in Lead and Cadmium Toxicity and Its Amelioration. Veterinary Medicine International, 2011,
150. Patrick L (2006a) Lead Toxicity, A review of the literature. Part I: Exposure, evaluation, and treatment. Altern Med Rev 11:2–22
151. Patrick L (2006b) Lead toxicity part II: the role of free radical damage and the use of antioxidants in the pathology and treatment of lead toxicity. Altern Med Rev 11:114–127
152. **PIECHALAK A, MALECKA A, BARAŁKIEWICZ D, TOMASZEWSKA B**. lead uptake, toxicity and accumulation in Phaseolus vulgaris.Biologia *Plantarum*. 2008; **52** (3): 565-568.
153. Pincemail J, Meurisse M, Limet R, Defraigne JO. (1999). L'évaluation du stress oxydatif d'un individu : une réalité pour le médecin. Vaiss Cœur Poumons. 4(5), 359-370.
154. Plant R ; Benedetti, J.L ;1998. Définition d'une maladie à déclaration obligatoire ou d'une intoxication et d'une exposition significative : le plomb. Direction des risques biologiques, environnementaux et occupationnels. 62p.
155. Poisson C; 2013; Rôle du stress oxydant au niveau hépatique et rénal dans la toxicité de l'uranium après exposition chronique; Mém doctrat; UPS; 416.
156. Prasad, A. S. (2009). Zinc: role in immunity, oxidative stress and chronic inflammation. Current Opinion in Clinical Nutrition and Metabolic Care, 12(6), 646–652.
157. Ray, R. R. (2015). Haemotoxic Effect of Lead: A Review. Proceedings of the Zoological Society, 69(2), 161–172.
158. Rees M.D., Kennett E.C., Whitelock J.M and Davies M.J., 2008. Oxidative damage to extracellular matrix and its role in human pathologies. Free radical biology & Medicine 44:1973-20010.
159. Rman I, Morrison IN. The influence of high dietary calcium and phosphate on lead uptake and release. Environ Res 1978 ; 17 : 60-7.
160. Robert G. 2015. Le plomb et le saturnisme. 4 éme Edition .Paris. P33.
161. Romain CORIAT M; 2012; APPLICATIONS D'UNE MODULATION PHARMACOLOGIQUE DES DERIVES DES FORMES REACTIVES DE L

OXYGENE POUR UNE OPTIMISATION THERAPEUTIQUE DES PATIENTS TRAITES PAR CHIMIOTHERAPIE; Mém doctorat; UP5RD; 208.

162. ROTH J; 1992 ; Le magnésium, minéral incontournable ? Enquête auprès des patients de l'officine ; Mém doctorat ; 1-118.

163. SADOUD M; 2010; Stress oxydatif; Maitre-assistant A, département NSA, Faculté SNV; 9.

164. Saka S; Aouacher O; Bahi A; 2011; L'effet du stress oxydant induit par l'acétate de plomb sur le système enzymatique du glutathion chez les rats; ResearchGate; 23(3); 139-145.

165. saturnine mortelle. A propos d'une observation anatomoclinique. *Arch Mal Prof*. 1973; 34

166. Saxena, G., & Flora, S. J. S. (2004). Lead-induced oxidative stress and hematological alterations and their response to combined administration of calcium disodium EDTA with a thiol chelator in rats. Journal of Biochemical and Molecular Toxicology, 18(4), 221–233.

167. Scientific Opinion on Lead in Food. EFSA Journal, 8(4), 1570. (2010).

168. Sieskind O et Wey R;1957; Sur la variation de la capacité d'échange de cations base de la montmorillonite - H en fonction du pH; PARCOURIR LES COLLECTIONS

169. Sivaprasad, T. R., Malarkodi, S. P., & Varalakshmi, P. (2004). Therapeutic efficacy of lipoic acid in combination with dimercaptosuccinic acid against lead-induced renal tubular defects and on isolated brush-border enzyme activities. Chemico-Biological Interactions, 147(3), 259–271.

170. SPOSITO G, LUND LJ, CHANG AC**.** Trace metal chemistry in arid-zone field soils amended with sewage sludge: I. Fractionation of Ni, Cu, Zn, Cd and Pb in solid phases. *Soil Science Society of America Journal*. 1982; 46: 260-264.

171. Swarup ,D., &Maitis,K.(1991) . Changes in some biochimical constituents in blood and cerebrospinal fluid of lead intoxicated calves. Indian journal of Animal Sciences 61(9): 942-945.

172. Taylor A. Metabolism and toxicology of lead. Rev Environ Health 1986 ; 6 : 1 - 83.

173. Tessier, F., & Marconnet, P. (1995). Radicaux libres, systèmes antioxydants et exercice. Science & Sports, 10(1), 1–13.

174. Tessier, F., & Marconnet, P. (1995). Radicaux libres, systèmes antioxydants et exercice. Science & Sports, 10(1), 1–13.

175. Thomas DESMIER; 2016; LES ANTIOXYDANTS DE NOS JOURS : DEFINITION ET APPLICATIONS; Mém doctorat; UL; 88.

176. Ulusoy,U., & Simsek,S .(2005). Lead removal by polyacrylamide-bentonite and zeolite composites: effect of phytic acid immobilization. J Hazard Mater 127:163-171.

177. Uttara, B., Singh, A., Zamboni, P., & Mahajan, R. (2009). *Oxidative Stress and Neurodegenerative Diseases: A Review of Upstream and Downstream Antioxidant Therapeutic Options. Current Neuropharmacology, 7(1), 65–74.*

178. V. Laperche, M.C. Dictor, B. Clozel-Leloup, P. Baranger; 2004, Guide metodologique du plomb applique a la gestion des sites et des sols polles; BRGM; 138.

179. Vaglio, A ., &Landriscina, c. (1999). Changes in liver enzyme activity in the teleost Sparusaurata in response to cadmium intoxication. Ecotoxicol EnvironSaf 43:111-116

180. Weckbercker, G ., & Cory, J.G. (1988). Ribonucleotide reductase activity and growth of glutathione-depleted mouse leukemia L1210 cells in vitro. Cancer Letters. . 40(3): 257264**.**

181. Who. (2001). Air Quality Guidelines (Second Edition). Chapter 6.7: Lead. Regional Office for Europe, Denmark.288.

182. Yagi,K. (1976). Simple fluorimetric essay for lipoperoxide in blood plasma. Biochem. Med. (15) : 212-216

183. Yassine B;2017; Caractérisation physico-chimique des argiles marocaines : application à l'adsorption de l'arsenic et des colorants cationiques en solution aqueuse; Mèm doctorat ;UAE ; 171.

184. Zerargui F; 2015; Activité antioxydante des extraits de racines Tamus communis L. et caractérisation des substances bioactives ; Mém doctorat; UFAS;169.

185. Zerargui F; 2015; Activité antioxydante des extraits de racines Tamus communis L. et caractérisation des substances bioactives ; Mém doctorat; UFAS;169.