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Theme

Mitigation of heavy metal neurotoxicity by medicinal plant extract in rats

Presented by

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Dedication

الحَمْدَ للهِ حَبًا وَ شَكْرًا وإمْتَنانًا علىِ البَدءِ والخَتام

بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيم

{ وَآخِرُ دَعْوَاهُمْ أَنِ الْحَمْدُ لِلَّهِ رَبِّ الْعَالَمِينَ }

صَدَقَ اللهُ العظيمُ

Praise be to God, who has brought me to this day where I stand on the threshold of my graduation, reaping the fruits of my labor and raising my hat with pride.

With all my love, I dedicate the fruits of my success and graduation to:

The one who adorned my name with the most beautiful titles, my father, Tabeche **Abd ElAziz**, may God have mercy on him.

The one whom God placed paradise under her feet and who made adversity easier for me through her prayers, my dear mother, **Alleg Nadjah.**

My dear brothers, each by his name: **Habiba, Nour El-kodes, Abd Errazzak, Montaceur Billah.**

All my family, led by my uncle **Alleg AbdEl Bassit**, thank you very much for your support and encouragement.

All my colleagues and friends, especially my loyal companion on this journey, **Sofia.** May God protect you and grant you more success.

Kenza

Dedication

Today is my graduation day...

I look at my diploma and raise my hat in appreciation, I say goodbye to my best years and start another path, thank God, my day has come, my family is happy, my mother is happy with my diploma and my father appreciates my words full of praise, today I say goodbye to my friends and companions in those years and the day I waited for has come. Here I am today, saying goodbye to my friends and companions in those years and the day I waited for so long has come. Goodbye, years that were my effort and my tiredness, goodbye, years that carried my ambition, the year my name was called a graduate.

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Sofia

Abstract

Lead (Pb) is a metal that has been used by humans for millennia. Its production increased significantly during the industrial revolution, causing intense release and massive accumulation of this indestructible metal in the environment. Numerous studies have elucidated the involvement of lead in neurotoxicity in humans. In our study, we aim to evaluate the potential of two plants, *Ziziphus spina christi* and *Eucalyptus camaldulensis*, as therapeutic alternatives against lead-induced toxicity, a wid espread heavy metal in Algeria and globally, which is likely to cause neurological alterations.

42 female Albino Wistar rats were divided into 6 groups of 7 rats each. The animals under went oral lead treatment at a dose of 50 mg/kg/day and/or extracts of the two plants *Ziziphus spina christi* and *Eucalyptus camaldulensis* at a dose of 1ml/kg/day by force-feeding for 21 days.

The analysis of the obtained results showed that lead causes harmful effects in the organism, manifested by hyperglycemia and weight loss. Neurobehavioral tests reveal an anxiogenic effect, appetite disturbance, and deterioration of exploratory and locomotor activity, associated with the onset of cerebral oxidative stress, indicated by an increase in glutathione S-transferase (GST) activities and a significant decrease in glutathione (GSH) levels. Moreover, the administration of the two plants *Ziziphus spina christi* and *Eucalyptus camaldulensis* improved all the parameters measured in this study. Our study showed that the aqueous extract of *Ziziphus spina christi*was more beneficial than that of *Eucalyptus camaldulensis*.

Keywords: Lead, *Ziziphus spina christi*, *Eucalyptus camaldulensis*, neurotoxicity, behavior, anxiety.

Résumé

Le plomb (Pb) est un métal utilisé par les humains depuis des millénaires. Sa production a considérablement augmenté pendant la révolution industrielle, entraînant une libération intense et une accumulation massive de ce métal indestructible dans l'environnement. De nombreuses études ont mis en évidence l'implication du plomb dans la neurotoxicité chez l'homme. Dans notre étude, nous visons à évaluer le potentiel de deux plantes, *Ziziphus spina christi* et *Eucalyptus camaldulensis*, en tant qu'alternatives thérapeutiques contre la toxicité induite par le plomb, un métal lourd répandu en Algérie et dans le monde, susceptible de provoquer des altérations neurologiques.

42 rats femelles Albino Wistar ont été répartis en 6 groupes de 7 rats chacun. Les animaux ont subi un traitement oral au plomb à une dose de 50 mg/kg/jour et/ou des extraits des deux plantes *Ziziphus spina christi* et *Eucalyptus camaldulensis* à une dose de 1ml/kg/jour par gavage pendant 21 jours.

L'analyse des résultats obtenus a montré que le plomb provoque des effets nocifs dans l'organisme, se manifestant par une hyperglycémie et une perte de poids. Les tests neurocomportementaux révèlent un effet anxiogène, des perturbations de l'appétit et une détérioration de l'activité exploratoire et locomotrice, associés au début du stress oxydatif cérébral, indiqué par une augmentation de l’activitée anzymatique de glutathion S-transférase (GST) et une diminution significative des niveaux de glutathion (GSH). De plus, l'administration des deux plantes *Ziziphus spina christi* et *Eucalyptus camaldulensis* a amélioré tous les paramètres mesurés dans cette étude. Notre étude a montré que l'extrait aqueux de *Ziziphus spina christi* était plus bénéfique que celui *d'Eucalyptus camaldulensis*.

Mots-clés : Plomb, *Ziziphus spina christi*, *Eucalyptus camaldulensis*, neurotoxicité, comportement, anxiété.

ملخص

الرصاص (Pb) هو معدن استخدمه البشر منذ آلاف السنين. وقد زاد إنتاجه بشكل كبير خلال الثورة الصناعية، مما تسبب في إطلاق مكثف وتراكم هائل لهذا المعدن غير القابل للتدمير في البيئة. وقد أوضحت دراسات عديدة تورط الرصاص في السمية العصبية البشرية. و قد نهدف في دراستنا إلى تقدير قيمة نوعان من النبات*Eucalyptus camaldulensis* و *Ziziphus spina christ* كبدائل علاجية ضد السمية الناجمة عن الرصاص، وهو معدن ثقيل منتشر على نطاق واسع في الجزائر والعالم كله، و من المرجح أن يتسبب في حدوث تغيرات عصبية.

تم تقسيم 42 أنثى من فئران Albino Wistar إلى 6 مجموعات تضم كل منها 7 فئران. وقد عولجت الحيوانات بالرصاص بجرعة 50mg/kg يوميا أو بمستخلص مائي لـ *Ziziphus spina christi*أو *Eucalyptus* camaldulensisبجرعة 1ml/kg عن طريق الفم لمدة 21 يومًا.

وأظهر تحليل النتائج التي تم التوصل إليها أن الرصاص يسبب تأثيرات ضارة في الجسم تؤدي إلى ارتفاع نسبة السكر في الدم وانخفاض في الوزن. تكشف الاختبارات السلوكية العصبية عن تأثير مسبب للقلق، واضطراب في الشهية، وتدهور في النشاط الاستكشافي والحركي، مرتبطا بإثارة الإجهاد التأكسدي الدماغي الذي يشير إلى الزيادة في نشاط الجلوتاثيون- S-ترانسفيراز (GST) وانخفاض كبير في نسبة الجلوتاثيون (GSH) من جهة أخرى، أدى إستعمال *Ziziphus spina christi*و*Eucalyptus camaldulensis* إلى تحسين جميع المعلومات المقاسة في هذه الدراسة. أظهرت دراستنا أن المستخلص المائي لنبات*Ziziphus spina* christi كان أكثر فائدة من مستخلص نبات *Eucalyptus camaldulensis.*

الكلمات المفتاحية: الرصاص، *Ziziphus spina-christi*، *Eucalyptus camaldulensis*، السمية العصبية، السلوك، القلق.

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Abbreviation list

BBC	Coomassie Brilliant blue.
BSA	Bovine serum albumin.
CDNB	1-chloro,2.4-dinitrobenzene.
CNS	Central nerveus system.
DTNB	5,5'-dithio-bis-2-nitrobenzoic acid.
EDTA	Ethylene Diamine Tetra Acetic Acid.
EPM	Elevated plus maze.
EUC	*Eucalyptus camaldulensis*.
GSH	Glutathion réduit.
GST	Glutathione S-Transferase.
HE	Hematein-Eosin.
OD	Optical density.
OP	Open Field.
Pb	Plomb (Lead).
Pb (NO3)2	Lead nitrate
PNS	Peripheral nerveus system.
ROS	Reactive oxygen species.
SSA	Sulfosalicylic acid.
ZSC	*Ziziphus spina christi*.

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Introduction

Introduction

Lead (Pb) is a metal that people have used for millennia. Production rose drastically during the Industrial Revolution, resulting in intensive release and vast buildup of this indestructible metal in the environment (**Seddik *et al.*, 2011**).

Lead poisoning is one of the oldest and most extensively researched occupational and environmental dangers (**Seddik *et al.*, 2011**). Many residents of modern cities are persistently exposed to environmental lead levels, which while lowered in the best instances, could be a health risk, primarily affecting the neurological system (**Garza *et al.*, 2006**). Lead is a poison with a polytropic mechanism of action, is manifested by neuropsychiatric effects such as delayed reaction times, headache, and decreased motor nerve conduction, and may be linked to the development of stomach colic, pain paroxysms, and acute encephalopathies (**Slota *et al.*, 2022**). which manifests as distinct toxic effects on hematological organs, and effects on the gastrointestinal tract, cardiovascular, and immunological systems; it damages the liver and kidneys, affects metabolic systems, and increases reactive oxygen species (**Abdel Moneim *et al.*, 2011; Osowski *et al.*, 2023**). There are no known safe blood lead concentrations; even blood lead concentrations as low as 5 µg/dL, previously assumed to be safe, can cause lower intellect, behavioral difficulties, and learning impairments in children (**Offor *et al.*, 2017**). Understanding the precise mechanisms of etiopathogenesis, when a xenobiotic (such as heavy metal) enters the human body, it initiates a series of molecular reactions, and the toxicant's potential harm to cell metabolism is influenced by the organism's overall health (**Slota *et al.*, 2022**).

Researchers have conducted some studies to treat lead poisoning through medical and other popular treatments. because there is a growing interest in the natural antioxidants found in medicinal and dietary plants, as they are considered potential candidates for preventing oxidative damage (**YE and Shams El-Deen Fakher Eldeen, 2021**). We have researched and experimented with *Eucalyptus camaldulensis* and *Ziziphus spina Christi* using Wister rats.

Studies on *Ziziphus spina-christi* shown that this plant contains a large number of phytochemical molecules such as polyphenols and tannins, these compounds have been found to exhibit significant biological activities, including anti-free radical, antimicrobial, antiparasitic, anti-inflammatory, anti-obesity, and anticancer properties (**Elhaj, 2024**). additionally, *Eucalyptus camaldulensis* has been traditionally used to treat various common ailments. especially those related to the respiratory tract and burns. It's commonly employed as a natural antioxidant in food additives (**Musa *et al.*, 2019**). It has been experimentally shown that this plant exhibits extensive therapeutic properties, acting as both an antimicrobial and antidiabetic agent (**Nwaogu *et al.*, 2021**). *Eucalyptus camaldulensis* is utilized in the treatment of other ailments such as diarrhea, male sterility, cough, cold, cancer, ulcers, inflammation, and several others (**Nwaogu *et al.*, 2021**).

Concerning the interests of these plants, the present study aimed to demonstrate :

- Neurotoxicity of lead ;
- Therapeutic propriétis of the aqueous extract of *Eucalyptus camaldulensis*, and *Ziziphus spina christi*.

Bibliographic part

1. The lead

1.1. Physicochemical properties

Lead (Pb^{2+}) is a heavy metal that is abundant in nature **(Garza *et al.*, 2006)**, it's a silvery-grey metal with a bright luster. It has face-centered cubic crystals and is very soft, making it easy to cast, roll, and extrude. It belongs to Group IV A (Group 14) of the periodic table, lead exhibits valences of +2 and +4. It has four stable isotopes: Pb-204 (1.48%), Pb-206 (23.6%), Pb-207 (22.6%), and Pb-208 (52.3%). Additionally, there are twenty-seven known radioisotopes of lead **(Pradyot, 2002)**.

Table 01. Physical properties of lead **(Pradyot, 2002).**

Atomic number	82
Atomic weight	207.20
Melts (Temperature of fusion)	327.46°C
Vaporizes	1,749°C
Electrical resistivity at 20°C	20.65 microhm-cm
The viscosity of its molten at its melting point	3.2 centipoise
The viscosity at 400°C	2.32 centipoise
Surface tension at 350°C	442 dynes/cm
Density	11.3 g/cm^3
Brinell hardness	4.0
Metallic radius	1.75 Å

1.2. Sources of exposure

Humans are exposed to lead from different environmental sources such as air, soil, food, drinking water, or from occupational and recreational **(Ravi Raja *et al.*, 2008)**, where miners and steelworkers are identified as the primary risk categories **(Slota *et al.*, 2022)**, and also from other sources like folk remedies, moonshine whisky, and gasoline huffing, cosmetics **(Ravi Raja *et al.*, 2008)**, in paints, and artificial jewelry (**Bedi, 2023**), electric batteries, and from cigarette smoking **(Garza *et al.*, 2006)**.

1.3. Kinetics of lead

1.3.1. Absorption

Three ways of exposure to lead are highlighted for humans: inhalation, ingestion, and skin contact **(Ravi Raja *et al.*, 2008).**

a. Respiratory tracts: Inhalation of Pb-containing dust may be regarded as a significant intake channel in a population living in areas with ongoing exposure to the metal processing sector; toxicological kinetics studies revealed that approximately 40% of inhaled Pb is absorbed and deposited in the lungs **(Slota *et al.*, 2022)**.

b. Digestive tracts: A typical human diet is approximately 3 µg of Pb per day, with 1 to 10% effective absorption; children and pregnant mothers typically absorb more. Approximately 95% of the dose ingested is translocated to the bloodstream (**Slota *et al.*, 2022**).

c. Cutaneous tracts: A percentage of lead is absorbed through the skin by exposure to some sources of lead, including industrial jewelry with the highest pink color, as well as in paints, especially yellow paint (**Bedi, 2023**); and also lead acetate present in eye makeup, lipstick, and hair dyes (**Draid *et al.*, 2016**).

1.3.2. Distribution

Lead is difficult to metabolize (**Garza *et al.*, 2006**), the major portion is rapidly absorbed into erythrocytes, but it is also disseminated throughout the soft tissues, lipid-dense tissues (such as the central nervous system) are especially sensitive to organic forms of lead (**Slota *et al.*, 2022**). Lead enters the bloodstream and travels to soft tissues such as the spleen, kidneys, liver, and lungs; absorbed lead deposits are then distributed to bones, teeth, hair, and nails (**Slota *et al.*, 2022**). Lead absorption levels may even rise in the case of occurring nutritional deficits, particularly in the case of calcium, zinc, and iron shortages (**Slota *et al.*, 2022**).

1.3.3. Elimination

Most of the daily Pb consumption is eliminated in the stool following transit through the gastrointestinal tract; erythrocyte turnover of lead occurs within approximately 120 days and the excretion occurs via bile and urine (**Slota *et al.*, 2022**).

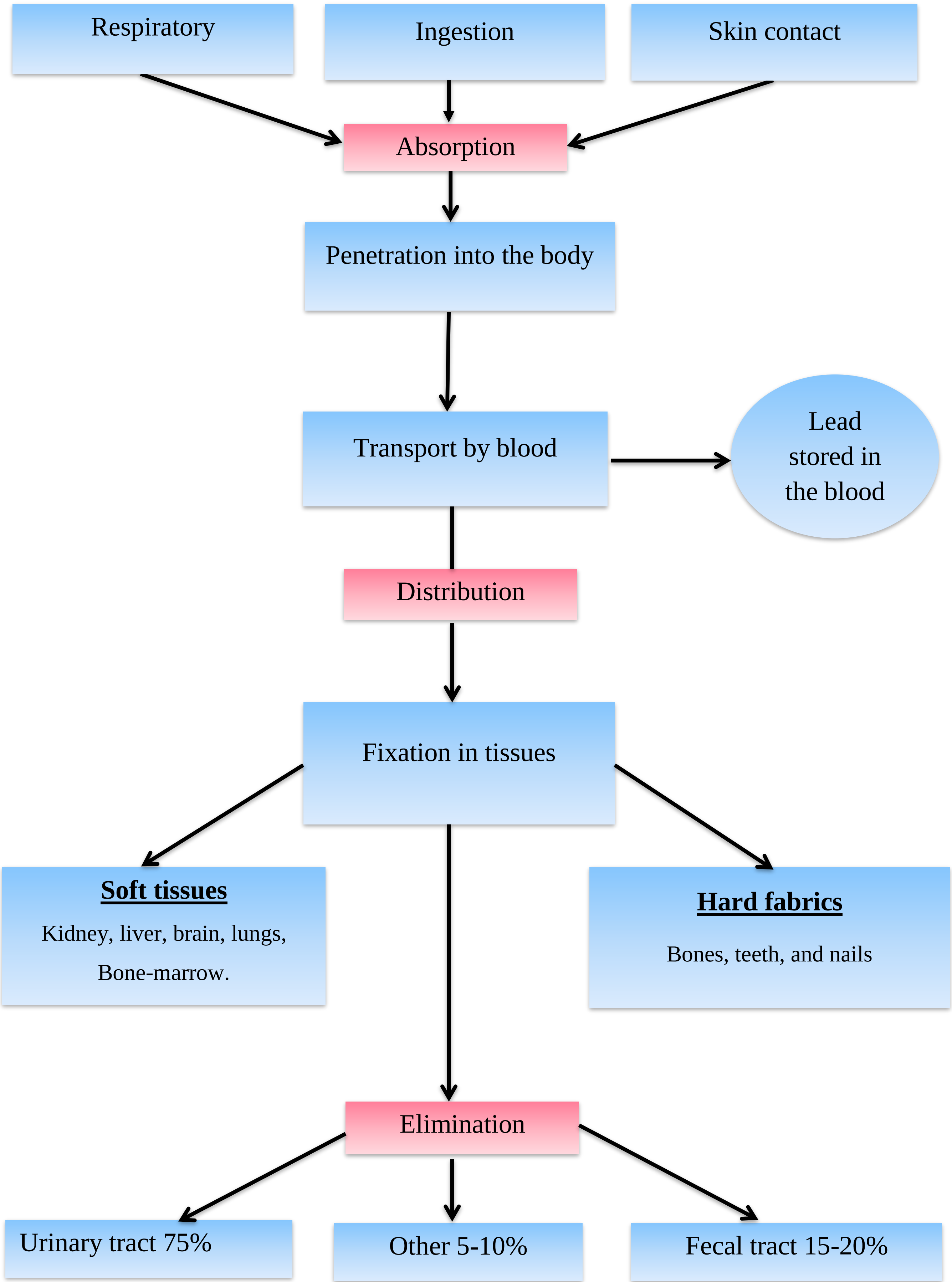


Figure 01. Kinetics of lead in the body **(Slota *et al.*, 2022)**.

1.4. Toxicity of lead and its impact on health

Lead is hazardous to various organs and tissues, including the neurological system. Chronic lead exposure can cause short-term memory impairment, weariness, difficulty with attention and sleep, depression, nausea, abdominal pain, loss of coordination, and anemia (**Slota *et al.*, 2022**). Pb is especially toxic to children, causing potentially permanent learning and behavior disorders (**Quan *et al.*, 2015**). Lead has a toxic effect on organs and tissues, particularly the brain and peripheral nerves; it has an impact on the CNS, PNS, kidney, liver, bone marrow, bone, gastrointestinal tract, blood vessels, reproductive, and endocrine systems **(Al-Khafaf *et al.*, 2021**). Lead has a direct effect on enzymes that contain sulfhydryl groups; in addition to cerebral hemorrhage, edema, and capillary damage, lead suppresses the immune system (**Al-Khafaf *et al.*, 2021**). Is one of several neurotoxins that interfere with and diminish ion calcium communication in nerve processes, and it also affects many distinct areas of the brain (**Al-Khafaf *et al.*, 2021**).

1.5. Lead and oxidative stress

Lead toxicity affects practically every function in the human body (**Osowski *et al.*, 2023**), and significantly lowers the antioxidant system by attaching to sulfhydryl groups and then replacing zinc and copper ions, which serve as key cofactors for antioxidant enzymes. Several studies showed that the pathogenesis of Pb toxicity; due to intracellular lipid peroxidation, could be attributed to the inhibition of the activities of the antioxidant system, by stimulation of free radicals and reactive oxygen species (ROS); as well as, the release of proinflammatory mediators (**El-Boshy *et al.*, 2019**). It was shown that Pb affects neurotransmitter levels, increases ROS, and causes a reduction in mitochondrial potential and death via the release of cytochrome C (**Seddik *et al.*, 2010**).

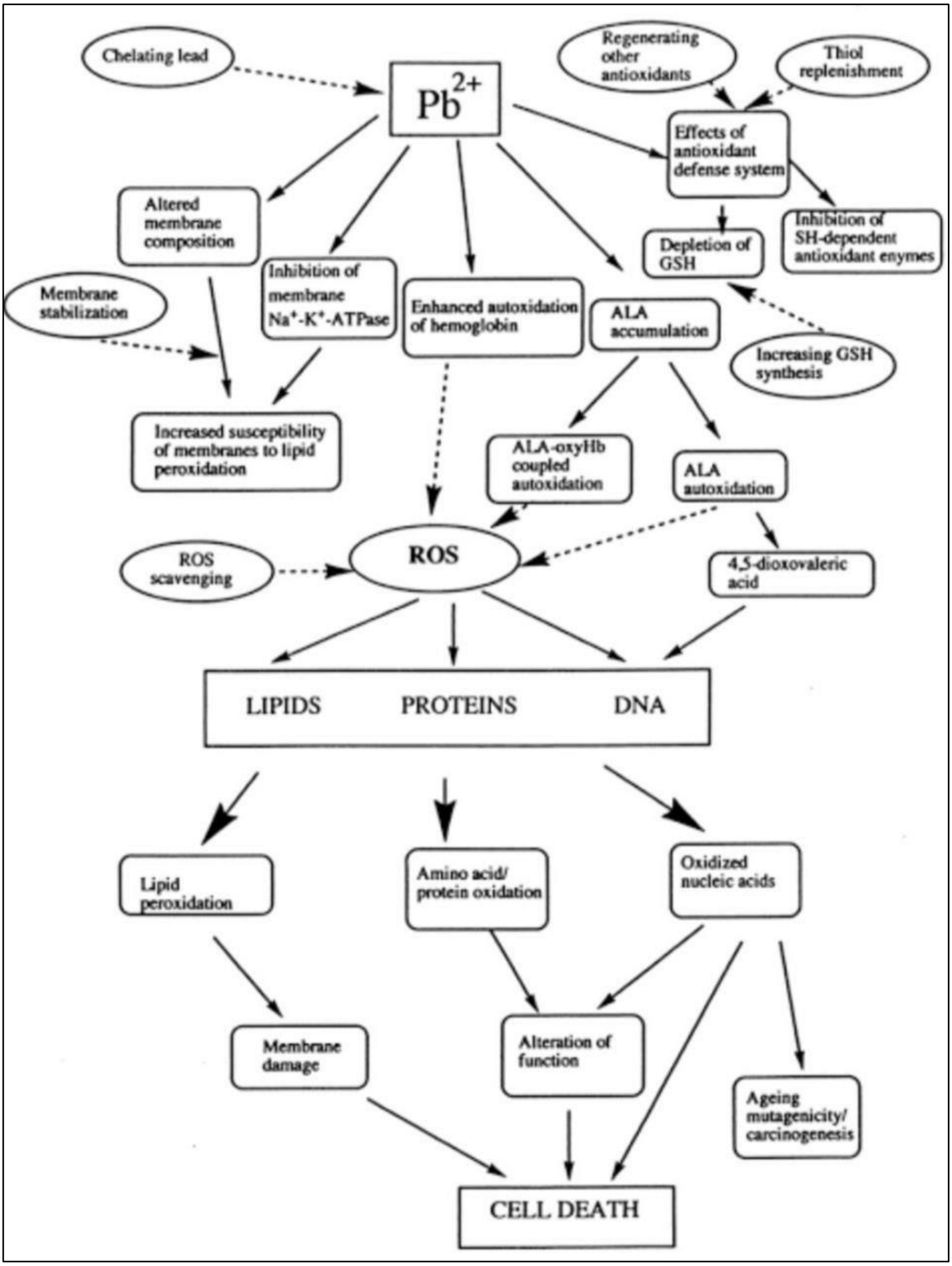


Figure 02. Involvement of lead in the appearance of oxidative stress **(Gurer and Ercal, 2000)**

2. *Ziziphus spina-christi*

2.1. Geographic distribution

The genus Ziziphus is part of the Rhamnaceae family, which comprises approximately 100 species of deciduous or evergreen trees and shrubs found across tropical and subtropical regions worldwide (**Saied *et al.*, 2008**). It also grows extensively in upper Egypt, and Sinai and is widespread in the Mediterranean region, Australia (**El-Beltagy *et al.*, 2019**). *Z. spina-christi* is present in the Sahelian region, spanning from Senegal to Sudan, and across a significant area in North Africa, the Middle East, eastern Afghanistan, and northwestern India (**Saied *et al.*, 2008**).

2.2. Botanical description and taxonomy

Ziziphus spina-christi, commonly referred to as Christ's Thorn Jujube; is a shrub or occasionally a tall tree reaching heights of 20 meters and a diameter of 60 centimeters (**Saied *et al.*, 2008**).

Figure 03. General appearance of one of the *Ziziphus spina-christi* species **(personal photos).**

2.2.1. Vegetative apparatus

The trunk: is twisted and heavily branched, with a light-grey bark that is deeply cracked (**Asgarpanah and Haghighat, 2012**); and it has a diameter of up to 45 cm (**Saied *et al*, 2008**).

a) **The leaves:** are smooth on the upper surface and finely hairy below, with an ovate-lanceolate or ellipsoid shape, acute or obtuse apex, nearly entire margins, and prominent lateral veins (**Asgarpanah and Haghighat, 2012**).

b) **The shoots:** are whitish, flexible, and drooping (**Asgarpanah and Haghighat, 2012**).

2.2.2. Reproductive system

a) **The flowers:** are arranged in cymes (**Asgarpanah and Haghighat, 2012**); emitting a sweet scent and appearing in dense clusters in the leaf axils (**Saied *et al*, 2008**).

b) **The fruits**: are orange-yellow, with a taste resembling a blend of dates and apples; and they are typically consumed fresh or dried (**YE and Shams El-Deen Fakher Eldeen, 2021**).

Figure 04. Different parts of the Ziziphus tree: trunk, leaves, fruits, and flowers **(Web 1)**

2.2.3. Classification of the *Ziziphus spina-christi*

The taxonomy is as follows :

Table 02. Classification of *Ziziphus spina-christi* **(Rojas-Sandoval, 2017).**

Domain	Eukaryota
Kingdom	Plantae
Phylum	Spermatophyta
Subphylum	Angiospermae
Class	Dicotyledonae
Order	Rhamnales
Family	Rhamnaceae
Genus	Ziziphus
Species	*Ziziphus spina-christi*

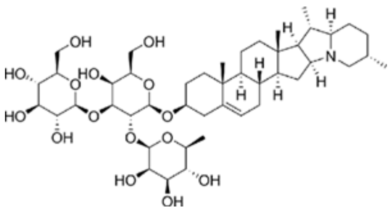
2.3. Chemical compositions

Phytochemical screening studies have revealed that the *Ziziphus spina-christi* plant is abundant in tannins, saponins, lipids, proteins, free sugars, and mucilage. Additionally, it contains cardiac glycosides, which are associated with numerous therapeutic properties (**Elhaj, 2024**). The flesh of *Z. spina-Christi* fruits is high in carbohydrates as well as iron, protein, fats, calcium, thiamine, riboflavin, niacin, and ascorbic acid (**Saied *et al.*, 2008**); additional studies have identified various active compounds in *Ziziphus spina-christi*, including triterpenoid sapogenins, geranyl acetate, sterols, methyl hexadecanoate, peptides, cyclopeptide alkaloids, methyl octadecanoate, and flavonoids (such as rutin and quercetin derivatives) (**El-Beltagy *et al.*, 2019**). The Zizyphus genus is known for its high content of phenolic compounds, particularly flavonoids, and anthraquinones; these compounds are recognized as potent antioxidant molecules (**El-Beltagy *et al.*, 2019**); moreover, two cyclic amino acids, 4-hydroxymethyl-1-methyl pyrrolidine-2-carboxylic acid, and 4-hydroxy-4-hydroxymethyl-1-methyl pyrrolidine-2-carboxylic acid were identified from *Z. spina-Christi* seeds (**Asgarpanah and Haghighat, 2012**). The main compounds are presented in figure **05**.

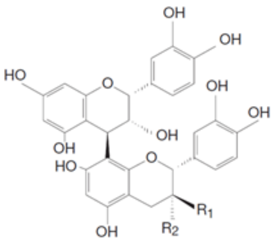
2.4. Therapeutic properties

The genus Ziziphus is renowned for its medicinal properties, acting as a hypoglycemic, hypotensive, anti-inflammatory, antimicrobial, antioxidant, antitumor, and liver-protective agent, as well as an immune system stimulant (**Saied *et al.*, 2008; YE and Shams El-Deen Fakher Eldeen, 2021**). Several pharmacological screening studies have shown that the aqueous extract of *Ziziphus spina Christi* root bark exhibits antinociceptive activity in mice and rats (**Ads *et al.*, 2018**).

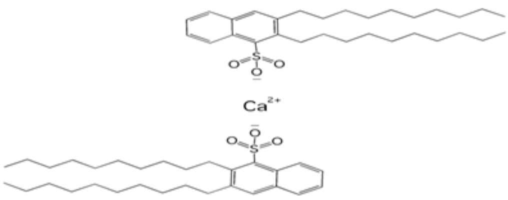
The use of *Ziziphus spina-christi* led to a significant decrease in colonic inflammation (**Almeer *et al.*, 2018**), restoring the equilibrium between oxidants and antioxidants (**AL-Marzooq, 2014**) and effectively regulating the RNAm expression of redox-sensitive transcription factors (**Asgarpanah and Haghighat, 2012**). Wood ash mixed with vinegar is applied topically to treat snake bites (**Asgarpanah and Haghighat, 2012**) while tea made from the fruit is used to alleviate symptoms of measles and to relieve chest pains and respiratory issues. Additionally, Extract from *Z. spina-christi* has demonstrated a protective effect against aflatoxicosis, leaves may potentially serve as a safe sedative (**Asgarpanah and Haghighat, 2012**).



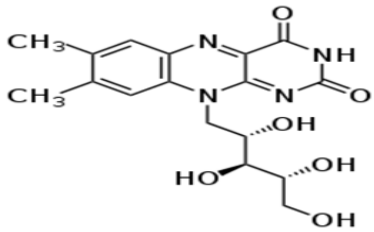
Saponins



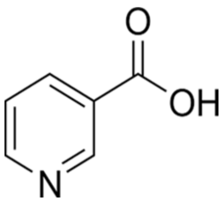
Tannins



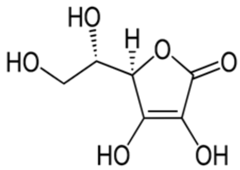
Calcium



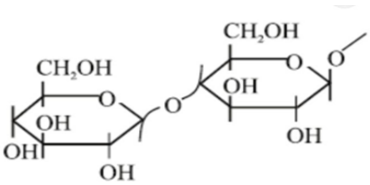
Riboflavin



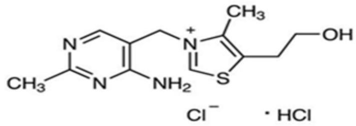
Niacin



Ascorbic acid



Mucilage



Thiamine

Figure 05. Structure of the main components of *Ziziphus spina-christi* **(Web 2-9)**

3. *Eucalyptus camaldulensis*

3.1. Geographic distribution

The genus consists of about 700 species of evergreen trees and shrubs. This native Australian plant has been introduced to Europe and North Africa, where they have become well-adapted to the Mediterranean coastlines (**Mohammed and Faris, 2016**; **Nwaogu *et al.*, 2021**).

3.2. Botanical description and taxonomy

Eucalyptus camaldulensis, commonly referred to as the "river red gum," is a tall evergreen plant belonging to the Myrtaceae family; it typically reaches heights of 20 to 40 meters and features a sturdy trunk and wide-spreading branches; its bark is smooth and sheds periodically, revealing shades of white, yellow, and grey, which eventually become rough at the base (**Musa *et al.*, 2019**).

Figure 06. Botanical description of the *Eucalyptus camaldulensis* **(personal photos).**

The classification of the *Eucalyptus camaldulensis* is as follows :

Table 03. Classification of the *Eucalyptus camaldulensis* **(OO and Adeniyi, 2008**; **Hirsch, 2020; Nwaogu *et al.*, 2021).**

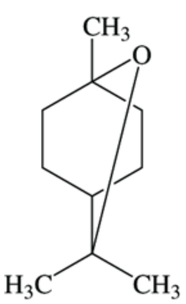
Domain	Eukaryota
Kingdom	Plantae
Phylum	Spermaphytes
Subphylum	Angiosperms
Class	Magnoliopsida
Order	Myrtales
Family	Myrtaceae
Genus	Eucalyptus
Species	***Eucalyptus camaldulensis***

3.3. Chemical compositions

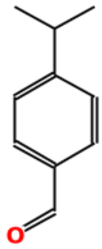
Eucalyptus camaldulensis leaves contain essential oils ranging from 0.1% to 0.4%, with 77% being 1,8-cineole; they also contain notable amounts of cuminal, phellandrene, aromadendren (or aromadendral), valerylaldehyde, geraniol, cymene, and phellandral. Reported phytoconstituents of the tree include essential oils, sterols, alkaloids, glycosides, flavonoids, tannins, and phenols (**Sabo and Knezevic, 2019**). The main compounds are presented in figure **07.**

3.4. Therapeutic properties

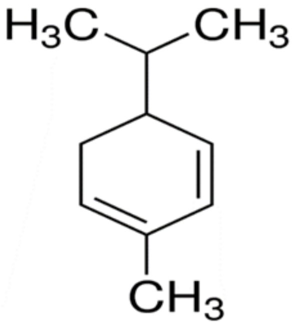
According to traditional medicinal practices and indigenous knowledge, *E.camaldulensis* is employed in treating various ailments including diarrhea, leprosy, male sterility, diabetes, cough, cold, cancer, ulcer, inflammation, and antimicrobial issues. It serves as a remedy for bacterial infections of the respiratory and urinary tracts. The essential oils extracted from its leaves are utilized in treating lung diseases, while the volatile oils act as expectorants (**Nwaogu *et al*, 2021**).



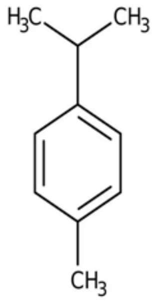
Being 1,8- cineole



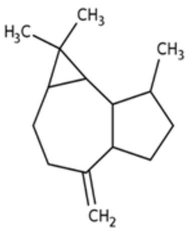
Cuminal



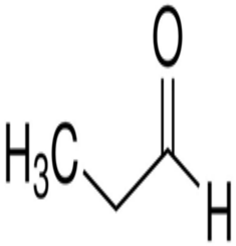
Phellandrene



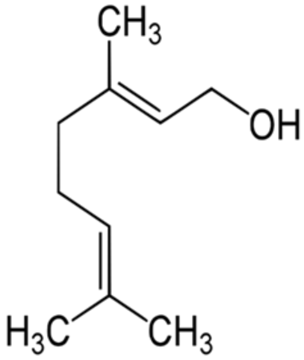
Cymen



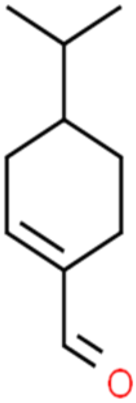
Aromadendren



Valerylaldehyde



Geraniol



Phellandral

Figure 07. Structure of the main components of *Eucalyptus camaldulensis* **(Web 10-17)**

Experimental part

Materials and methods

4. Materials and methods

4.1. Chemical and biological materials

4.1.1. Preparation of the lead nitrate solution

We used lead nitrate (Pb (NO3)2) from the company **Biochem** it was solubilized in mineral water at a concentration of 50 mg/kg and administered to the rats by oral gavage (*per os*).

Figure 08. Preparation of the lead nitrate solution **(personal photos)**.

4.1.2. Plant materials and choice of medicinal plants

To better promote plant biodiversity in eastern Algeria, our study focused on *Ziziphus spina Christi* and *Eucalyptus camaldulensis* belonging to the Rhamnaceae and Myrtaceae families respectively. A bibliographic study and a simple survey of the local population with knowledge of traditional medicine allowed us to make this choice. Thus, the selection criteria are as follow :

- Abundance of both plants in Algeria.
- Traditional use in the treatment of several diseases.
- The non-toxicity of plants, given that they are used in the preparation of herbal tea.

4.1.3. Collection and identification

The aerial part of the *Eucalyptus camaldulensis* is harvested in the month of February in the region of Ferkane south of Tebessa, the botanical identification was made on a herbarium specimen by Ms. Guenez Radja, teacher at the University of Tebessa. Dry sidr leaves are purchased from the local market from Souk-Ahras.

4.1.4. Preparation of decoctions

10 g of each plant is infused in 100 ml of boiling mineral water (100ºC), once cooled the decoction is filtered and stored in sterile bottles protected from light.

Figure 09. Preparation of decoctions **(personal photos)**.

4.1.5. Animal care and treatment

For this study we received 42 female rats (Albino wistar), from the Pasteur institute of Algiers (I.P.A.), age 05 weeks. The breeding of rats was carried out at the animal facility of the University of Larbi Tebessi -Tebessa - The animals were housed in polyethylene cages provided with labels indicating the name of the batch, the treatment undergone and the dates. experiments, the cages are lined with wood shavings bedding which is cleaned daily.

These rats were subjected to an adaptation period of approximately one month to the conditions of the animal facility; at a temperature of (22 ± 20°C) and a natural photoperiod (12/12H). They were fed with an energetically balanced concentrate in the form of: Croquette and water and libitum. The table **04** summarizes the composition of the food. The average weight of the rats at the start of the experiment is approximately 200 g ± 20 g.

Table 04. Composition of 1kg of food.

Food material	Quantity in g/kg of food	%
Corn	620	62
Soy	260	26
Phosphate	16	1.6
Limestone	9	0.9
Cellulose	10	1.0
Minerals	10	1.0
Vitamins	10	1.0

After a period of habituation, the animals were randomly divided into six groups of seven rats each. They underwent different treatments daily for 21 days, we note that the follow-up required weighing the body weight in order to determine the appropriate treatment dose.

C **:** Control group, rats receive drinking water and simple food.

EUC **:** The rats receive drinking water and 1ml by force-feeding of a decoction prepared from Eucalyptus.

ZSC **:** The rats receive drinking water and 1ml by force-feeding of a decoction prepared from Sidr.

Pb **:** Rats receive by force-feeding 50 mg/kg of lead nitrate.

Pb+EUC **:** The rats are administered by force-feeding a dose of lead nitrate at a rate of 50 mg/kg, after few hours the administration of 1 ml of a decoction based on eucalyptus.

Pb+ZSC **:** The rats are administered (per-os) a dose of lead nitrate at a rate of 50 mg/kg, after few hours the administration of 1 ml of a Sidr-based decoction.

Figure 10. Animal care **(personal photos)**.

4.1.6. Organ harvesting

The brain is remove immediately, weighed, and stored in the freezer at -20°C for the determination of oxidative stress biomarkers (GSH and GST). The figure **12** summarizes the different stages of the experimental protocol.

Figure 11. Organ harvesting **(personal photos).**

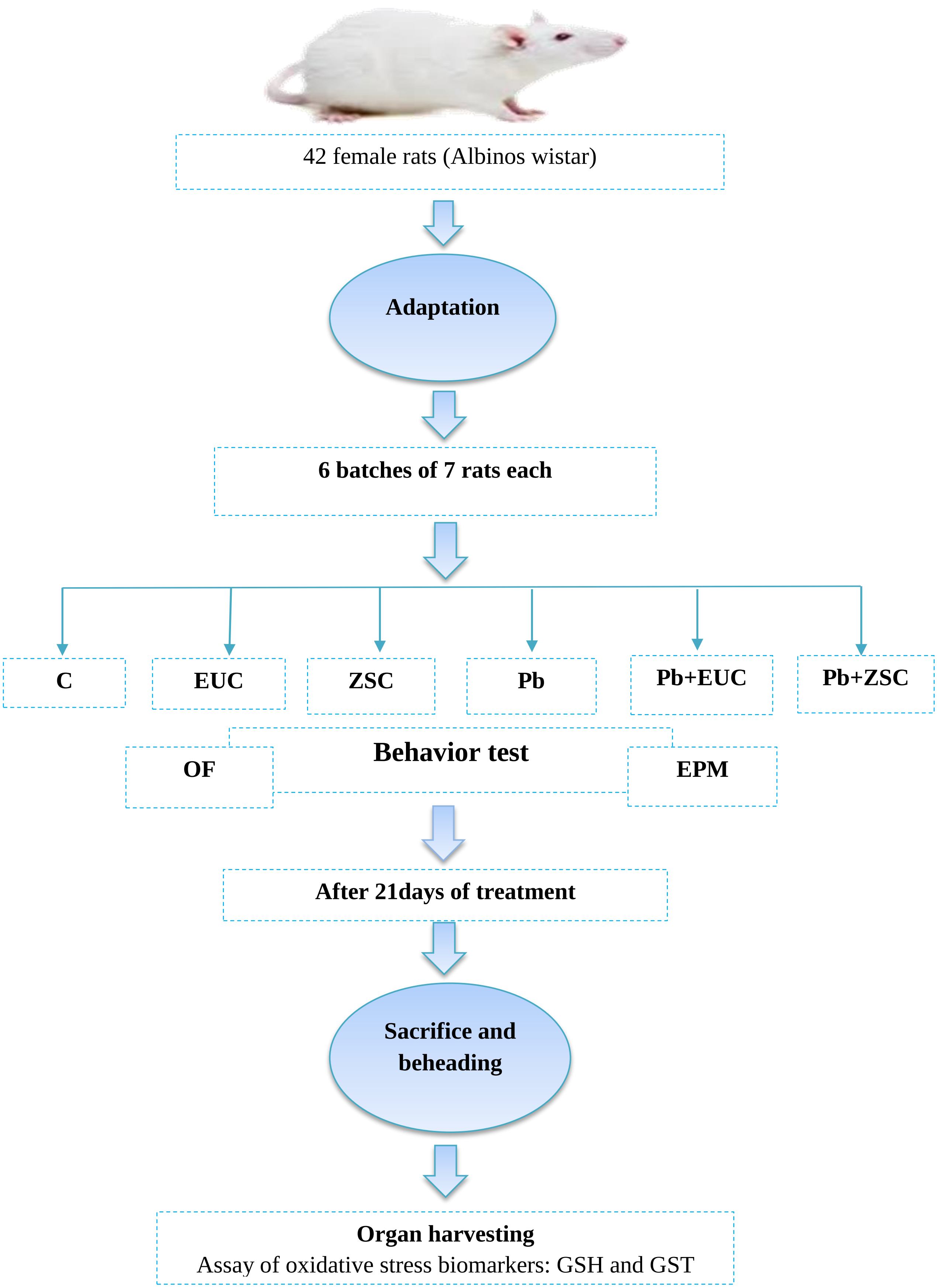


Figure 12. Diagram summarizing the experimental protocol.

4.2. Methods

4.2.1. Behavioral study

The behavioral tests were carried out at the animal facility, Larbi Tebessa University-Tebessa- to evaluate the deleterious effect of lead and the protective effect of *Eucalyptus camaldulensis* and *Ziziphus spina-Christi* on locomotor activity, the state of anxiety and depression. To achieve these objectives, we performed the Elevated Cross Test (EPM) and the Open Field (OF) test.

4.2.2. Elevated plus maze (EPM)

It is a test that measures anxiety-like behavior in rodents. The device is a cross labyrinth raised 50 cm from the ground, it is made up of two arms 50 cm long and 10 cm wide and devoid of side walls (open arms) and opposing perpendicularly to two closed arms of the same dimensions. The test lasts 5 minutes during which the rat is placed in front of an open arm and can freely explore the entire slide. Since the rat is afraid of empty spaces, it will naturally take refuge in closed arms and remain confined. Thus the exploration of open arms by the animal indicates a less anxious behavior while the exploration of closed arms by the animal indicates a more anxious state. The variables measured at the end of this test are: the number of entries into the open arms, the number of entries into the closed arms, the time spent in the open arms, the time spent in the closed arms **(Pellow *et al.*, 1985).**

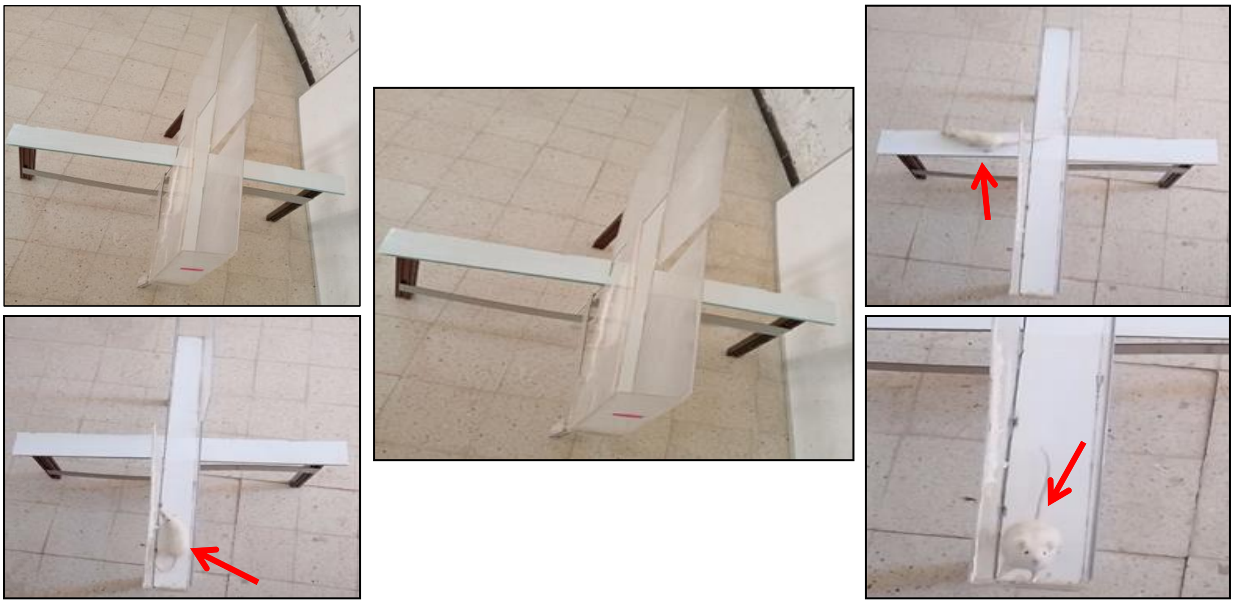


Figure 13. Photograph portraying the study of anxiety **(personal photos).**

4.2.3. Open field test (OF)

This test is widely used in neuroscience, it is designed to evaluate differences in emotional reactivity in rodents, but also used to predict an anxiolytic type activity of a molecule. The device is a square enclosure made of plexiglass measuring 70 cm on each side with side walls 40 cm high. The floor of this device is divided into two parts of the same surface area, a peripheral part and another central one, so each part is in the form of squares each 10 cm wide, this makes it possible to quantify the distances traveled by the rat. In general, an anxious rat exhibits a high degree of avoidance of the central area compared to the periphery, so less anxious behavior will be more pronounced when the rat explores the central area. The test begins by placing the animal in the central area to allow it to explore the device for 5 minutes during which the number of entries into the central part, the immobility time and the total distance traveled are recorded **(Hall, 1934).**

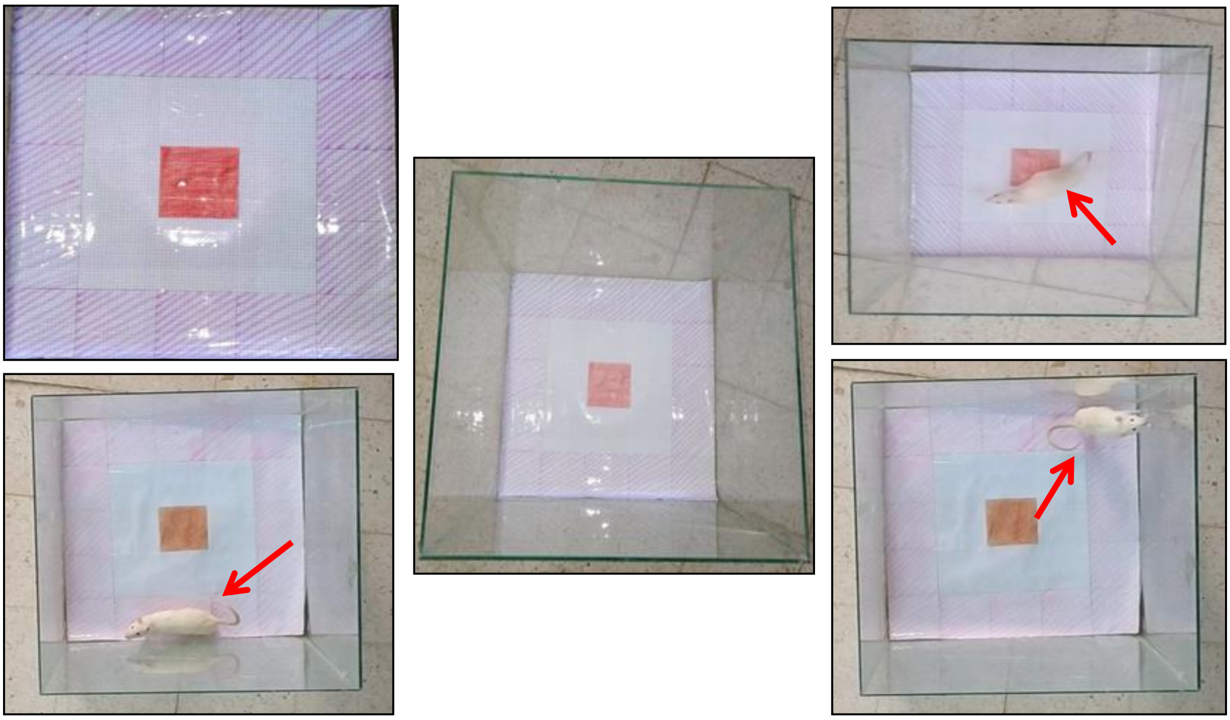


Figure 14. Photograph portraying the study of locomotor activity **(personal photos).**

4.2.4. Blood sugar measurement

Principle

Blood glucose was measured by a glucometer using test strips. These are intended for in vitro (external) diagnostic use for blood glucose testing. They are designed to measure glucose in capillary whole blood.

The test strip contains glucose oxidase, an enzyme that oxidizes glucose in the blood and produces D-gluconic acid and hydrogen peroxide.

Operating mode

- The reader turns on automatically by simply inserting the one touch test strip (in the direction of the arrows and up to the stop).
- The drop symbol flashes.
- Place the drop of blood on the strip application area. The result is displayed in 5 seconds. Blood sugar is given in g/dl.

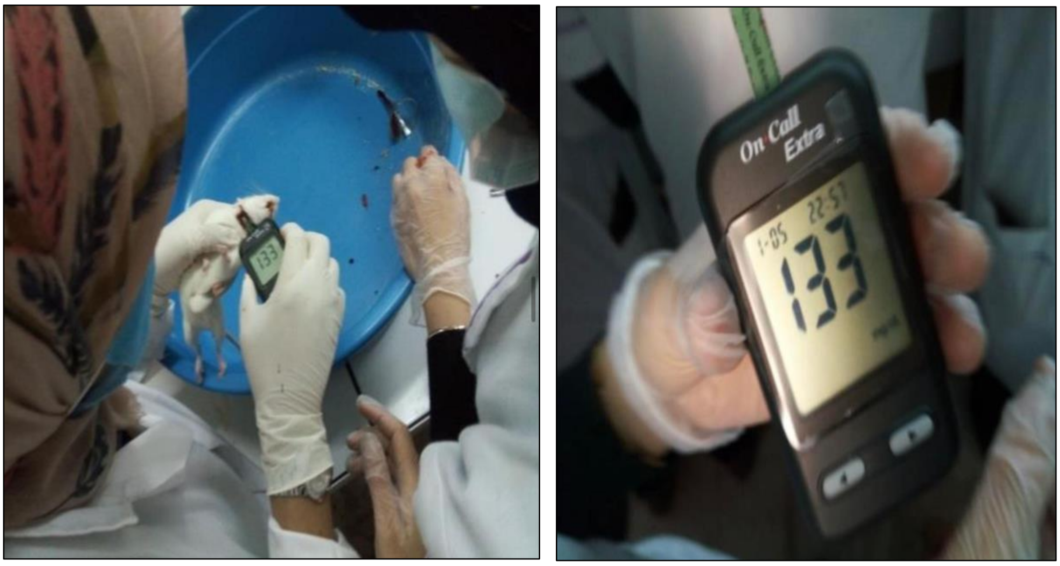


Figure 15. Blood sugar measurement **(personal photos).**

4.2.5. The dosage of the enzymatic activity of Glutathione S-Transferase (GST)

Principle

Measuring GST activity involves providing the enzyme with a substrate, generally 1-chloro,2.4-dinitrobenzene (CDNB), which reacts easily to many forms of GST and glutathione. The conjugation reaction of these two products results in the formation of a new molecule which absorbs light at 340 nm **(Habig *et al.*, 1974).**

Preparation of the homogenate

100 mg tissue + 1 ml Phosphate buffer → → → → cold grinding → → → → homogenate.

Note: keep the homogenates cold until the end of homogenization.

Centrifugation of the homogenate at 9000 tr/min → → → → recovery of the supernatant for the determination of GST and mg of proteins.

Operating mode

Table 05. The dosage of the enzymatic activity of Glutathione S-Transferase (GST).

The reagents used	Blank (µl)	Sample (µl)
Phosphate buffer (0.1M,Ph 6.5)	830	830
CDNB (0.02M)	50	50
GSH (0.1M)	100	100
Supernatant/ distilled water	20	20

Calcula: the GST concentration is obtained by the following formula

$$\textbf{GST (nmol GST/min /mg proteins)} = \frac{\textbf{(OD Sample /min – OD blanc / min)}}{\textbf{9.6} \times \textbf{mg proteins}}$$

OD Sample / min : Optical density of sample per minute.

OD blank / min : Optical density of blank per minute.

9.6 : Extinction coefficient of GSH-CDNB expressed in $mm^{-1}.cm^{-1}$.

Figure 16. GST assay **(personal photos)**

4.2.6. The dosage of glutathione reduces GSH

Principle

The glutathione assay was carried out according to the method of **Wekbeker** and **Cory (1988)**. The principle of this assay is based on measuring the optical absorbance of 2-nitro-5-mercapturic acid. The latter results from the reduction of 5,5'-dithio-bis-2-nitrobenzoic acid (Ellman's reagent, DTNB) by the (-SH) groups of glutathione. For this, deproteinization of the homogenate is essential in order to keep only the thiol groups specific to glutathione.

Preparation of the homogenate: 100 mg of tissue were placed in the presence of 4 ml of a 0.02 M solution of Ethylene Diamine Tetra Acetic Acid (EDTA), then were ground cold using an ultrasonic homogenizer to obtain a homogenate.

Operating mode

- Take 0.8 ml of the homogenate.
- Deproteinize by adding 0.2 ml of a 0.25% sulfosalicylic acid solution.
- Shake the mixture and leave for 15 minutes in an ice bath.
- Centrifuge at 1000 rpm for 5 min.
- Take 0.5 ml of the supernatant.
- Add 1 ml of Tris+EDTA buffer (0.02 M EDTA), pH 9.6.
- Mix and add 0.025 ml of 0.01M DTNB (dissolved in absolute methanol).
- Leave for 5 min at room temperature for color stabilization which develops instantly. Read optical densities at 412 nm against blank.

Calcul : the concentration of glutathione is obtained by the following formula.

[GSH] (nM GSH /mg protein) = (OD × 1 × 1,525) / (13100 × 0,8 × 0,5 × mg protein)

OD **:** Optical density at 412 nm.

1 **:** Total volume of solutions used in deprotonization.

1,525 **:** Total volume of solutions used in GSH assay.

13100 **:** Absorption coeficient du groupement.

0,5 **:** Volume of supernatant found in 1.525 ml.

0,8 **:** Volume of the omogenate found in 1 ml.

Figure 17. GSH assay **(personal photos).**

4.2.7. Protein assay

Principle: The protein concentration is determined according to the method of Bradford (1976) which uses Coomassie blue (G 250) as reagent. The amine groups (-NH2) of proteins react with a reagent based on orthophosphoric acid, ethanol and Coomassie blue to form a blue complex.

The appearance of this color reflects the degree of ionization of the acidic environment and the intensity establishes the concentration of proteins in the sample.

Operating mode

- Take 0.1 ml of the homogenate.
- Add 5 ml of Bradford reagent.
- Shake and leave for 5 min for color stabilization.
- Read the optical density at 595 nm, against blank.
- The optical density obtained is reported on a previously drawn calibration curve.
- The protein concentration is determined by comparison with a standard range of bovine serum albumin (BSA) (1 mg/ml) carried out under the same conditions Figure 22.

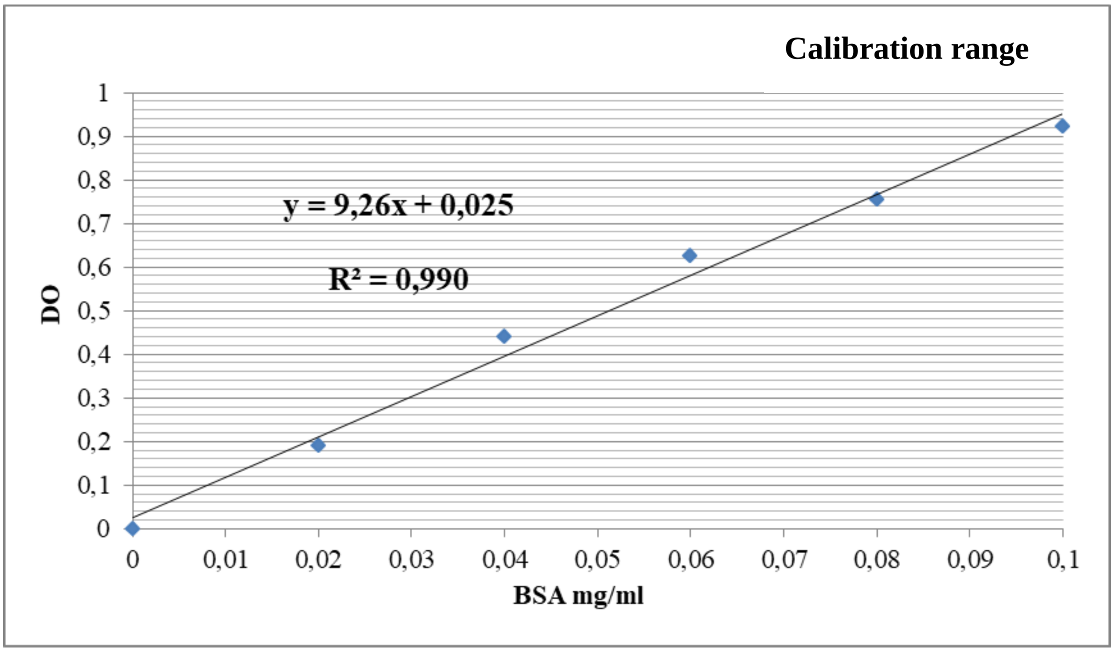


Figure 18. Bovine serum albumin calibration cuve.

Figure 19. Preparation of Bradford reagent **(personal photos).**

Figure 20. Protein assay **(personal photos).**

4.3. statistical analysis of results

The data from various groups were presented as mean±SEM. Statistical analysis was conducted using one-way analysis of variance (ANOVA) with multiple comparaison. A significance level of $P<0,05$ was approved as statistically significant.

Results and discussion

5. Results and discussion

5.1. Physiological parameters

5.1.1. Effect of treatments on body weight

Monitoring the body mass of the animals during the treatment period indicates a progressive increase in body weight in the control groups (+10.44%) and those treated with the *Eucalyptus camaldulensis* extract (+16.27%) or the *Ziziphis spina christi* extract (+10.45%). However, there is a slow increase in weight in rats treated with lead only (+0.90%) and by a combination of Eucalyptus and lead (+1.31%) as well as a combination of *Ziziphis spina christi* and lead (+3.35%) compared to the control group.

The food intake of the animals did not change throughout the treatment period in the control group and the groups treated with *Eucalyptus camaldulensis* and *Ziziphis spina christi*. Unlike the groups exposed to lead only or in combination with aqueous extract of both plants, we notice a reduction in the food intake and the appetite of the animals compared to the control group.

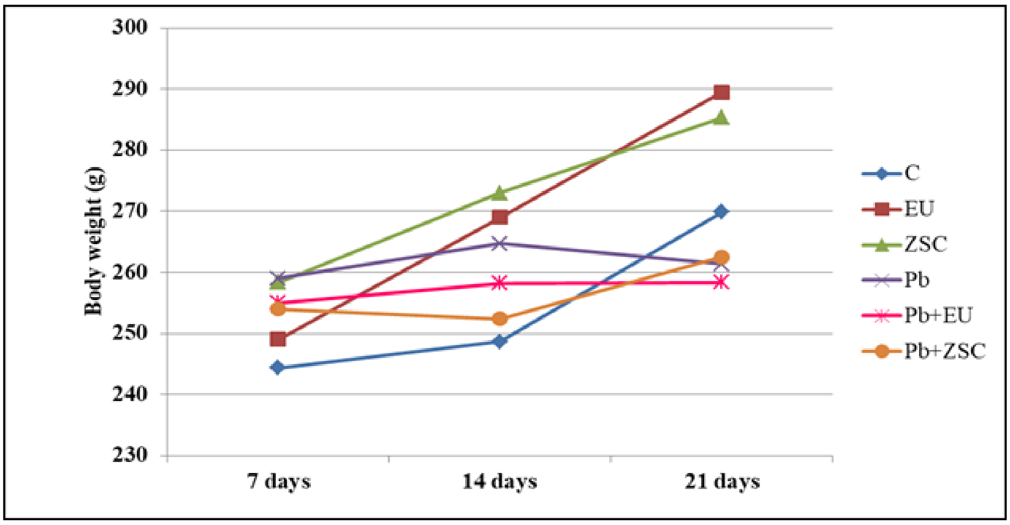


Figure 21. Effect of aqueous extracts of *Eucalyptus camaldulensis*, *Ziziphus spina-christi* leaves and/or lead nitrate treatment on body weight after 21 days.

5.1.2. Effect of treatment on organs weight

According to the results obtained in table **07**, we notice a significant increase in absolute brain weight in the groups subjected to lead alone or with a combination of Eucalyptus and lead ($P< 0.05$). Unlike, no significative increase is observed in the other groups. The results reveal no significant change in relative brain weight in all groups compared to the control.

Table 06. Effect of aqueous extracts of *Eucalyptus camaldulensis*, *Ziziphus spina-christi* leaves and/or lead nitrate treatment on body weight gain after 21 days of experience **(values represent the mean±SEM of seven rats).**

	Experimental groups					
Parameters	**C**	**EU**	**ZSC**	**Pb**	**Pb+EU**	**Pb+ZSC**
Initial weight (g)	244,33±11,4	249,00±12,1	258,33±14,1	259,00±18,7	255,00±12,9	254,00±9,94
Final weight (g)	269,83±18,6	289,50±17,8	285,33±12,4	261,33±18,8	258,33±21,6	262,50±13,4
Gain (%)	**10,44**	**16,27**	**10,45**	**0,90**	**1,31**	**3,35**

Table 07. Effect of aqueous extracts of *Eucalyptus camaldulensis*, *Ziziphus spina-christi* leaves and/or lead nitrate treatment on brain weight after 21 days of experience **(values represent the mean±SEM of seven rats).**

	Experimental groups					
Parameters	**C**	**EU**	**ZSC**	**Pb**	**Pb+EU**	**Pb+ZSC**
Absolute brain weight (g)	1,443±0,41	1,484±0,30	1,676±0,29	1,760±0,25*	1,710±0,12*	1,435±0,34
Relative brain weight (%)	0,586±0,17	0,570±0,12	0,628±0,08	0,700±0,16	0,754±0,10	0,652±0,23

***(P< 0.05) :** Significant difference compared to control.

Lead is one of the most toxic materials encountered in everyday life and is capable of causing numerous acute and chronic illnesses. It affects every organ and system in the body **(Seddik *et al*., 2010)**. Lead is a powerful toxic agent that primarily affects the nervous system, with brain tissue being particularly vulnerable to lead injury **(Al-Khafaf *et al*., 2021).**

In the present study, we reported that subchronic lead exposure for 21 days was associated with a decrease in body weight gains compared to control rats. This is due to the negative effect of lead, which induces growth depression and reduces food consumption by interacting with appetite-depressant receptors in the gastrointestinal tract. Our results support the findings of **Seddik *et al*. (2010)**, who investigated lead toxicity at a concentration of 500 mg/l for 90 days. Another study by **Sabbar *et a*l. (2012)** revealed that lead exposure leads to disorders in intestinal absorption, consequently causing body weight loss.

Our results showed that the mean body weights of lead-treated rats were significantly lower than those of the control group. However, co-supplementation of *Ziziphus spina-christi* leaves aqueous extract to lead-treated rats successfully restored body weight to near-normal levels. These findings are in agreement with previous studies by **El-Beltagy *et al*. (2019)**. The data concerning the modulatory role of Ziziphus leaves aqueous extract in maintaining body weight align with the findings of **YE and Shams El-Deen Fakher Eldeen, (2021)**, who reported that regular supplementation of Ziziphus leaves extract allows for the regular assimilation of nutrients that maintain growth rates.

We also observed an increase in body weight in rats treated with a combination of lead and Eucalyptus. Contrary to our results, **Fathi *et al*. (2019)** reported that dietary Eucalyptus had no significant effect on live body weight and daily weight gain in rats. This discrepancy may be due to the different content of our Eucalyptus sample.

Our results show a significant increase in absolute brain weight in rats treated with lead, which may be due to the deposition and accumulation of lead in the brain. According to studies by **Seddik *et al*. (2010)**, lead is capable of crossing the blood-brain barrier and acts primarily in the hippocampus.

5.2. Effect of treatment on the assessment of blood sugar levels

According to our results in table **08** no significant difference in blood sugar levels was observed in several groups in comparison with the control. However, rats exposed to a combination of lead and Sidr shown a significant decrease (P< 0.05) in glycemia, compared to the control group.

Table 08. Effect of aqueous extracts of *Eucalyptus camaldulensis*, *Ziziphus spina-christi* leaves and/or lead nitrate treatment on glycemia after 21 days of experience **(values represent the mean±SEM of seven rats).**

	Experimental groups					
	C	**EU**	**ZSC**	**Pb**	**Pb+EU**	**Pb+ZSC**
Glycemia (g/L)	1.388±0.082	1.393±0.066	1.362±0.118	1.411±0.085	1.310±0.186	1.285±0.065*

***(P< 0.05) :** Significant difference compared to control.

Lead is one of the most toxic heavy metals of great public health significance. Exposure to low-level heavy metals such as lead can significantly contribute to the causation of chronic diseases, including diabetes **(Offor *et al*., 2017).**

Our study showed that lead nitrate elevated blood glucose levels insignificantly, which aligns with the results observed by **Draid *et al*. (2016)**. They suggested that the elevation in blood glucose levels may be due to an increase in glucose transport from the tissues to the blood or decreased removal of glucose from the blood to the tissues. According to **Saka *et al*. (2011)**, there was a significant increase in serum glucose concentration in rats treated with lead acetate. This is directly linked to the harmful effects of lead acetate on the pancreas, specifically on the excretion of insulin by the islets of Langerhans.

We noticed a decrease in blood sugar levels in the groups treated with lead and Ziziphus spina-christi (ZSC) leaves, which are known for their ability to adjust blood sugar levels **(Al-Ghamdi and Shahat, 2017)**. These findings are similar to those of **Mohammed *et al*. (2023)**; **Othman *et al*. (2009)**; and **Asgarpanah and Haghighat, (2012)** who demonstrated the anti-hyperglycemic effect of ZSC. This effect improves peripheral insulin sensitivity and stimulates cellular glucose uptake in muscles and tissues. They showed that ZSC increases insulin secretion in the blood, which may be due to saponins and polyphenols, and also controls hyperglycemia by reducing the absorption of meal-derived glucose.

The results of the groups treated with the combination of lead and Eucalyptus showed normal blood sugar levels. This suggests that Eucalyptus leaves have antidiabetic properties due to their chemical components that help adjust blood sugar levels **(Musa *et al*., 2019).**

5.3. Effect of treatment on oxidative stress parameters

5.3.1. Reduced glutathione (GSH)

Gavage of rats with lead 50 mg/kg/day or with a combination of lead and Eucalyptus for 21 days led to a collapse of reduced glutathione resulting in a very highly significant decrease (P< 0.001) in the GSH level at the brain. However, the addition of *Ziziphis spina christi* with a combination of lead restored the GSH level to almost normal. No significant change was recorded compared to the control group.

5.3.2. Glutathione S-Transferase (GST)

According to the results obtained, we observe a very highly significant increase (P< 0.001) in the level of GST in the brains of the rats receiving lead compared to the control rats. In the presence of Sidr the GST decreases considerably to reach values close to those of the control rats. However, we still note a significant increase (P<0.05) in the combination group (pb+Euc) compared to the control group.

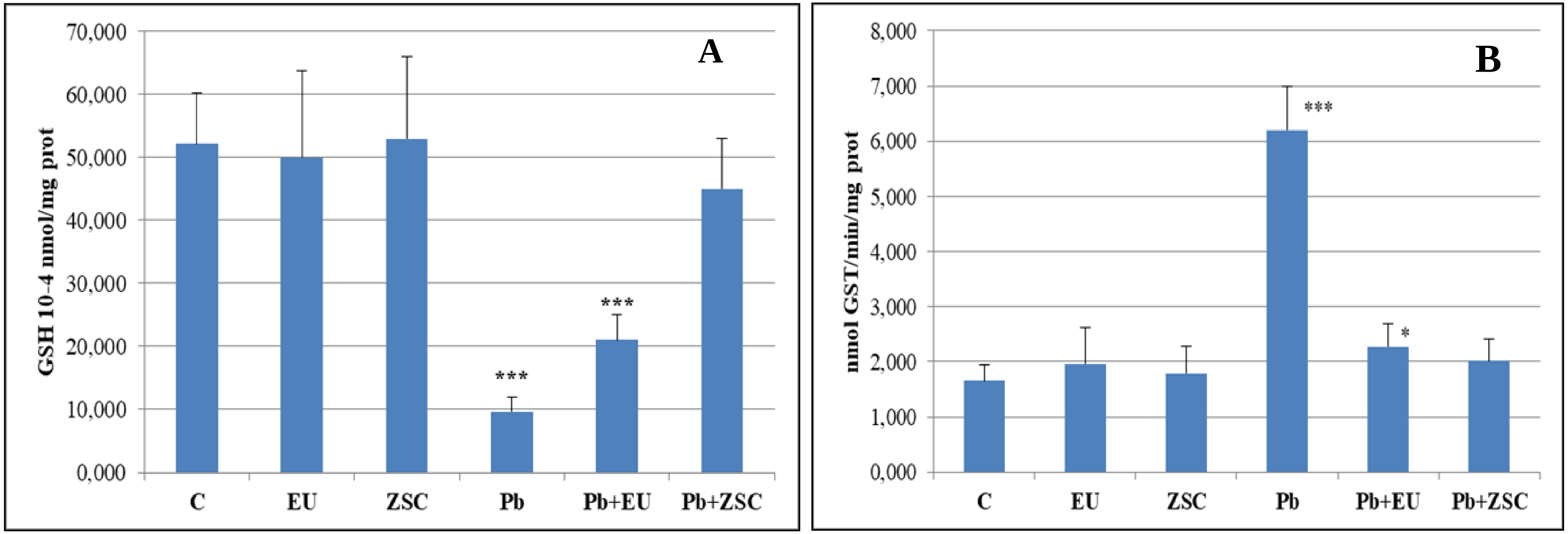


Figure 22. Effect of *Eucalyptus camaldulensis*, *Ziziphus spina-christi* leaves and/or lead treatment on oxidative stress biomarkers after 21 days of experience. **A**. Reduced glutathione (GSH) levels **B**. glutathione S-transferase (GST) activities * $P \leq 0.05$; *** $P \leq 0.001$ significant difference from the control.

Oxidative stress is defined as an obvious imbalance between antioxidants and the production of free radicals. The origins of oxidative stress are multiple, resulting from the formation of reactive oxygen species (ROS) within the body. A free radical is a chemical species with an unpaired electron in its outer shell. These unstable and toxic derivatives of oxygen can damage DNA, proteins, and lipids. There is ample evidence that lead effectively alters cellular signaling and is considered a major mediator of inflammation and neuropathological effects resulting from oxidative stress **(Seddik *et al*., 2011).**

The results obtained from the group exposed to lead only showed a clear reduction in glutathione (GSH) levels in the brain. Our findings are consistent with those of **Sharma *et al*. (2014)**, which demonstrated that the decrease in GSH levels results from the utilization of glutathione in the detoxification process against lead. Similar results were also found by **Seddik *et al*. (2010)**; **Abdel Moneim *et al*. (2011)**; and **El-Masry *et al*. (2011)**, who showed that GSH depletion is induced due to increased cytotoxicity of hydrogen peroxide (H2O2) in endothelial cells, resulting from the inhibition of GSH reductase, thereby preventing GSH from remaining in its reduced state. Lead can stimulate lipid peroxidation by affecting the activity of antioxidants, enzymes, and GSH concentration.

When treating lead-poisoned rats with *Ziziphus spina-christi*, we noticed that GSH levels returned to normal. This is because Sidr is rich in tannin compounds that can reduce ROS production by accelerating the conversion of H2O2 into H2O and preventing the accumulation of H2O2 **(El-Beltagy *et al*., 2019)**. According to **YE and Shams El-Deen Fakher Eldeen, (2021)**, ZSC has protective activity against lead and neurodegeneration through its ability to scavenge free radicals generated by lead.

Additionally, in rats treated with a combination of lead and Eucalyptus, we observed a slight increase in GSH levels. This is in line with the results of **Mohammed and Faris, (2016)**, which showed positive results and a significant decrease in GSH levels in experiments on rats exposed to acetaminophen (2.5 mg/kg) as a single dose, then treated after 2 hours with Eucalyptus (150 mg/kg) for 42 days. Our results may be explained by the short duration of the treatment or the concentration of the Eucalyptus extract used.

The GST enzyme is one of the most important factors indicating the level of oxidative stress and antioxidants in cells **(Seddik *et al*., 2011).**

We noticed an increase in GST levels in the group treated with lead only, which can be explained by the elevated activity of GST. This enzyme participates in the conjugation reactions of electrophilic toxic compounds derived from acetate metabolism of lead with GSH to form non-toxic mercapturic acids excreted in urine **(Saka *et al*., 2011).**

Consumption of Sidr leaf extract reduced the level of GST in the brains of rats, making it similar to the control group, which indicates its antioxidant activity. This may be due to the high content of antioxidant compounds such as saponins, tannins, and flavonoids. ZSC leaves were able to increase the internal activities of antioxidant enzymes as they conjugated with harmful free radicals, reducing their size and toxic properties **(Othman *et al*., 2009).**

Biochemical estimations revealed the antioxidant and anti-inflammatory effects of Eucalyptus leaves by decreasing the GST level in the brains of animals. Phytoconstituents present in Eucalyptus leaves, such as 1–8 cineole, β-pinene, myrcene, terpinolene, carvacrol, aromadendrene, phallandrene, and cymene, have been reported to have antioxidant and anti-inflammatory effects in various preclinical studies **(Yadav *et al*., 2019).**

5.4. Effect of treatment on rats behavior

5.4.1. Elevated plusmaze (EPM)

The results of rats show a significant increase in the time spent in closed arms in the groups treated with lead alone and with a combination of lead and Eucalyptus. A significant decrease in the time spent in the open arms compared to the control groups. However, the group treated with the combination of lead and Sidr modulated the exposure time in both arms.

Regarding the number of entries into the arms, Rats treated with lead only and by the combination of lead and Eucalyptus showed a significant decrease in the number of entries into the open arms and closed arms. Also a significant decrease in the number of entries into the closed arms in the groupe treated by *Ziziphus spina-christi* only compared to the control group. However, the group treated with the combination of lead and Sidr recorded almost balance in the numbers of entries in both arms.

For the recovery number,our results show a significant decrease in rats treated with the lead, and by combination of lead and Eucalyptus compared to the control groups. But when Sidr was added with lead, the recovery number returned to almost normal.

Table 09. Variation in parameters linked to the EPM raised cross test in control rats and those subjected to different treatments for 21 day

Settings	Units	C	EU	ZSC	Pb	Pb+EU	Pb+ZSC
TSC	**sec**	53,83±7,03	44,16±11,7	41,33±12,50	10,67±5,92***	11,67±4,68***	34,83±4,88**
TSCA	**sec**	226,40±16,5	218,38±19,5	235,09±13,1	295,69±7,33***	264,95±15,2**	204,59±19,0
TSCA/P	**sec**	100,83±8,16	101,17±9,15	114,66±12,0*	50,83±11,1***	24,66±9,33***	60,16±29,3**
TSCA/D	**sec**	125,57±13,7	117,14±12,3	120,42±17,1	244,85±15,4***	240,29±8,08***	144,42±11,4*
TSOA	**sec**	39,52±9,07	39,57±12,6	31,88±10,1	14,28±5,88***	10,71±2,5***	35,04±12,2
TSOA/P	**sec**	32,86±6,57	32,57±8,48	27,71±6,18	14,28±6,90***	10,71±3,64***	31,71±5,91
TSOA/D	**sec**	6,67±1,51	7,00±3,35	4,17±1,22	0,17±0,41***	0,50±0,84***	3,33±2,58*
N adj		10,67±4,37	6,83±1,94	10,33±3,56	3,50±2,17**	5,67±3,50*	9,83±4,83
NEOA		5,83±3,06	4,50±2,26	4,33±2,88	1,50±1,22**	1,83±1,17*	2,83±1,6
NECA		6,67±2,25	4,17±1,60	3,67±2,50*	1,67±1,63**	2,50±1,38**	4,00±3,22

***(P< 0.05) :** Significant difference compared to control.

****(P< 0.001) :** Highly significant difference compared to control.

*****(P< 0.001) :** Very highly significant difference compared to control.

TSC	Time spent in the center	**TSOA/P**	Time spent in the open arms : proximit part
TSCA	Time spent in the closed arms	**TSOA/D**	Time spent in the open arms : distal part
TSCA/P	Time spent in the closed arms : proximit part	**N adj**	Number of adjustments
TSCA/D	Time spent in the closed arms : distal part	**NEOA**	Number of entries into open arms
TSOA	Time spent in the open arms	**NECA**	Number of entries into closed arms

The Elevated Plus Maze (EPM) test was used to study exploratory behavior and state anxiety in rats. This experiment assesses the degree to which animals fear open spaces and their desire to escape to enclosed areas, where closed arms represent safety while open arms represent exploratory value **(Seddik *et al*., 2010)**. Naturally, an anxious animal tends to prefer closed and dark spaces. Based on this principle, behavioral anxiety is measured by the extent to which an animal avoids the open spaces of the maze. Spending a short time in the open arms is considered an indicator of anxiety.

Using the EPM, we demonstrated that rats treated with 50 mg/kg of lead spent less time in the open arms compared to control rats, with a reduced number of entries into both the open and closed arms. Our data suggest that rats with chronic exposure to lead have less exploratory activity and a high state of anxiety. These findings are in agreement with other studies that support the role of lead intake in behavioral changes in animals **(Seddik *et al*., 2010)**. Our results are similar to those of **Sansar *et al*. (2011)**, who concluded that lead exposure negatively affects nerve cells, leading to disturbances in animal behavior and increased anxiety.

When treating lead-exposed animals with *Ziziphus spina-christi* leaves, we observed an increase in exploratory activity and a reduction in anxiety, as indicated by increased time spent in the open arms. Sidr leaves are rich in compounds that produce natural antioxidants, which can defend against oxidative damage by reducing or inhibiting free radicals and reactive oxygen species. These compounds work to calm the nerve centers responsible for controlling and alleviating oxidative stress **(Al-Ghamdi and Shahat, 2017; Asgarpanah and Haghighat, 2012).**

Additionally, treatment with Eucalyptus leaves slightly increased the exploratory activity of the animals, according to the results of **Nwaogu *et al*. (2021)**. Eucalyptus leaves play a role in the elimination of free radicals and the inhibition of lipid peroxidation thanks to their content of phenolic compounds, tannins, steroids, resins, and glycosides. Tannins are compounds that participate in one of the reaction steps to delay oxidative stress, thereby preventing programmed cell death and tissue damage.

5.4.2. Open field test (OP)

After the results obtained in the table **10**, we note that is a significant increase in the immobility time in the groups treated with lead alone and with a combination of lead and Eucalyptus or with a combination of lead and Sidr, compared to the control group.

Additionally, there was a significant decrease in the number of adjustments in the groups treated with the lead alone and with a combination of lead and Eucalyptus.

Also, rats treated with lead alone recorded a significant increase of the time in the peripheral part. a significant decreased of the time in the central part in the groups treated with lead alone and a combination of lead and Eucalyptus or a combination of lead and Sidr compared to the control group.

However, rats treated with Eucalyptus or Sidr recorded an improvement in all parameters evaluated in this test.

Table 10. Variation in parameters linked to the open field test, in control rats and those subjected to different treatments for 21 days.

Settings	Units	C	EU	ZSC	Pb	Pb+EU	Pb+ZSC
TDT	**cm**	888,00±168	845,00±152	863,00±192	905,00±98,5	773,00±170	805,00±282
TI	**sec**	167,16±15,4	184,66±29,2	194,7±27,8	218,0±24,7**	228,0±28,7**	207,5±26,8*
TPP	**sec**	56,33±16,5	59,33±12,9	47,30±11,3	73,50±4,09*	43,50±5,47	44,83±6,71
TCP	**sec**	73,41±17,8	53,66±14,0	68,33±16,0	9,33±3,50***	29,83±31,5**	44,17±10,5**
N adj	/	13,00±2,83	12,57±2,44	10,71±2,06	9,43±3,46*	4,86±2,61***	10,43±2,23

***(P< 0.05) :** Significant difference compared to control.

****(P< 0.001) :** Highly significant difference compared to control.

*****(P< 0.001) :** Very highly significant difference compared to control.

TDT	Total distance traveled
TI	Time of immobility
TPP	Time in the peripheral part
TCP	Time in the central part
N adj	Number of adjustments

The open field (OF) test is used to measure motor skills, anxiety levels, and emotional responses. Anxious rats tend to spend more time in the corners and periphery of the apparatus. A decrease in the distance traveled in the open field reveals a decrease in locomotor activity, and a reduction in the number of adjustments indicates a deterioration in exploratory activity. This suggests that lead can impair the locomotor and exploratory abilities of rats **(Seddik *et al*., 2010).**

In the OF test, we observed a lack of exploratory activity in the lead-treated groups compared to the control groups, along with an increase in anxiety. This is supported by the research of **Benammi *et al*. (2014)** and **Sabbar *et al*. (2012)**, which indicates that changes in motor behaviors are associated with a high level of anxious behavior, evidenced by a decrease in time spent in the central region and an increase in time spent in the peripheral region. Thus, our findings support the idea that lead exposure profoundly affects cognitive and motor functions. Cumulative exposure to lead has been shown to increase the risk of developing Parkinson's disease, which is associated with motor symptoms. Many studies, including the research of **Al-Khafaf *et al*. (2021)**, have suggested that lead exposure reduces the percentage of neurotransmitters and is linked to a decrease in calcium transmitters, thereby interfering with synapse formation.

The activity of lead-treated rats was significantly improved by the aqueous extract of *Ziziphus spina-christi* and Eucalyptus, as shown by the increased time spent in the central part of the OF and the number of adjustments. These plants have powerful medicinal properties, such as anti-inflammatory and antioxidant effects, and contain a wide variety of natural compounds such as phenolic acids, flavonoids, tannins, α-pinene, α-terpineol, terpinen-4-ol, limonene, and germacrene-D **(El-Beltagy *et al*., 2019; Allanto *et al*., 2022).**

Conclusion and perspective

Lead presents a major risk to human health. Numerous epidemiological studies suggest a correlation between lead exposure and the development of neurodegenerative diseases.

In this context, our project aimed to evaluate the neurotoxicity of lead in rats and demonstrate its impact on health. We focused on studying the therapeutic effects of *Ziziphus spina-christi* and *Eucalyptus camaldulensis*, aromatic and medicinal plants rich in secondary metabolites with therapeutic and pharmacological properties.

To complete our research, we measured body weight, blood sugar levels, markers of oxidative stress, and neurobehavioral parameters in control and rats treated by lead (50mg/kg/day) and/or the two plants (*Ziziphus spina-christi* and *Eucalyptus camaldulensis* at 1 ml/kg/day) for a period of 21 days.

Our initial results showed:

- Slow body growth and poor weight gain.
- Increased relative brain weights.
- Hyperglycemia.

Neurobehavioral tests revealed anxiogenic effects and deterioration in exploratory and locomotor activity. On the oxidative level, we observed an increase in glutathione S-transferase (GST) and a decrease in reduced glutathione (GSH) levels in the brain.

However, the addition of *Ziziphus spina-christi* and *Eucalyptus camaldulensis* (1 ml per day) improved all the measured parameters. This improvement demonstrates the antioxidant, neuroprotective, and anxiolytic propriéties of these medicinal plants against the disruptive and oxidative effects of lead.

It would be wise to supplement this research with an in-depth study focusing on:

- How these substances affect the body to resist lead poisoning and what is the active substance present in these plants that works to reduce the effects of lead-induced toxicity on the body.
- Histological sectioning to further investigate the plants effect on mitigating lead induced toxicity on organs tissue.
- Investigate the synergistic interaction of both plants on lead induced toxicity.

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FACULTY OF EXACT SCIENCES, NATURAL AND LIFE

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Spécialité :......Biochimie Appliquée

Année universitaire 2023/2024

Formulaire de levée de réserves après soutenance d'un Mémoire de Master

Données d'identification du candidats (es) :

Nom et prénom du candidat :

......................Tabeche Kenza / Guenez Sofia......

Intitulé du Sujet :

.....Mitigation of heavy metal neurotoxicity by.........

..............medicinal plant extract in rats...........

Données d'identification du membre de jury :

Nom et prénom :

....① Messaadia Amira : Encadrante / ② Guedrie Kamilia : presidente

③ Hamiri Manel : Examinatrice

Grade :

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Tébessa le : ..01/07/2024........

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Guedri Kamilia.

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