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Medicinal Plant Modulates Renal Toxicity And Oxidative Stress Induced By Heavy Metals in Rats

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Abstract

This study focuses on the heavy metal lead nitrate and highlighting its true toxicity on the activity of kidney functions and the extent of the response of the extract of *Eucalyptus Camaldulensis* leaves and *Ziziphus Spina-Christi* leaves as an antioxidant.

This study was conducted on 42 female rats of the female of albino Wistar nature. These rats were divided into 6 groups, each group containing 7 rats, one of which was a control, and the remaining five groups were treated by gavage, where the lead nitrate group was treated with a dose of 50 mg. mg/kg, and two groups were treated with an extract of *Eucalyptus Camaldulensis* and *Ziziphus spina-Christi's* leaves at a dose of 1 ml/day, and two groups were treated with a combination of lead nitrate and an extract of *Eucalyptus Camaldulensis* and *Ziziphus Spina-Christi's* leaves, respectively, for 21 days.

Our results first show that exposure to lead nitrate causes slow growth of the body, an increase in the relative weight of the kidneys, and a disturbance in the biochemical metabolism, mainly characterized by hyperglycemia, a significant increase in the levels of urea, uric acid and creatinine. There is a significant increase in GST and a decrease in GSH in the kidneys.

Secondly, the addition of the leaf extract of *Eucalyptus Camaldulensis* and *Ziziphus Spina-Christi* shows us effective results in reducing the oxidative stress resulting from lead nitrate on the kidneys, and specifically the leaf extract of *Ziziphus Spina-Christi*, as no noticeable difference was recorded compared to the control group.

These results demonstrate the antioxidant effectiveness and ability of these medicinal plants to eliminate oxidative stress resulting from lead nitrate.

Key words: Lead nitrate, toxicity, oxidative stress, *Eucalyptus camaldulensis, Ziziphus spina-christi*, antioxidants.

Résumé

Cette étude se concentre sur le nitrate de plomb, métal lourd, et met en évidence sa véritable toxicité sur l'activité des fonctions rénales et l'étendue de la réponse de l'extrait de feuilles d'*Eucalyptus Camaldulensis* et de feuilles de *Ziziphus Spina-Christi* comme antioxydant.

Cette étude a été menée sur 42 rats femelles de nature Wistar albinos. Ces rats ont été répartis en 6 groupes, chaque groupe contenant 7 rats dont un témoin, et les cinq groupes restants ont été traités par gavage. le groupe contenant du nitrate de plomb. A été traité avec une dose de 50 mg/kg, et deux groupes ont été traités avec un extrait de feuilles d'*Eucalyptus Camaldulensis* et de feuilles de *Ziziphus spina-Christi* à la dose de 1 ml/jour, et deux groupes ont été traités avec une association de nitrate de plomb, et un extrait des feuilles *d'Eucalyptus Camaldulensis* et de *Zizyphus Spina-Christi*, respectivement, pendant 21 jours.

Nos résultats montrent d'abord que l'exposition au nitrate de plomb provoque un ralentissement de la croissance de l'organisme, une augmentation du poids des reins par rapport à l'organisme, et une perturbation du métabolisme biochimique, caractérisée principalement par une hyperglycémie, une augmentation significative des taux d'urée, l'acide urique et la créatinine. Il y a une augmentation significative de la GST et une diminution du GSH dans les reins.

Deuxièmement, l'ajout de l'extrait aqueux des feuilles d'*Eucalyptus* et de *Zizyphus spina-christi* nous fournit des résultats efficaces dans la réduction de la toxicité du nitrate de plomb, sur les reins, en particulier l'extrait des feuilles de *Zizyphus spina-christi*, là où aucune différence significative n'a été enregistrée par rapport à contrôles.

Ces résultats attestent de l'efficacité de *l'Eucalyptus Camaldulensis* et du *Ziziphus Spina-Christi* en tant qu'antioxydants, substances préventives et thérapeutiques contre le stress oxydatif causé par le nitrate de plomb.

Mots clés : Nitrate de plomb, toxicité, stress oxydatif, *Eucalyptus camaldulensis*, *Ziziphus spina-christi*, antioxydants.

ملخَّص

تركز هذه الدراسة حول المعدن الثقيل نيترات الرصاص وإبراز سميته الحقيقية على نشاط وظائف الكليتين ومدى استجابة مستخلص أوراق الأوكالبتوس كامالدولينسيس والزيزوفيس سبينا كريستي كمضاد للأكسدة.

حيث أجريت هذه الدراسة على 42 جرذا من جنس أنثى من طبيعة ألبينو ويستار، حيث قسمت هذه الجرذان إلى 6 مجموعات كل مجموعة تحتوي على 7 جرذان، إحداهما شاهدة، والخمس المجموعات المتبقية تم علاجها عن طريق التزقيم، حيث تم علاج مجموعة بنيترات الرصاص بجرعة 50 ملغ/كلغ، ومجموعتين تم علاجهما بمستخلص أوراق الاوكالبتوس كامالدولينسيس، والزيزوفيس سبينا كريستي بجرعة 1 مل/اليوم، ومجموعتين تم علاجهما بالاشتراك بين نيترات الرصاص ومستخلص اوراق الاوكالبتوس كامالدولينسيس والزيزوفيس سبينا كريستي على التوالي لمدة 21 يوما .

تظهر نتائجنا أولا التعرض لنيترات الرصاص يسبب نموا بطيئا للجسم، وزيادة في وزن الكلى إلى الجسم، واضطرابا في التمثيل الغذائي، الكيميائيّ الحيوي، يتميز بشكل أساسي بفرط سكر الدم، الارتفاع الكبير في نسب اليوريا، حمض اليوريك والكرياتينين. هناك زيادة كبيرة في GST ونقصان في GSH في الكلى .

أيضاً، تتيح لنا إضافة المستخلص المائي لأوراق الأوكالبتوس والسدر نتائج فعالة في خفض من سمية نيترات الرصاص على الكليتين وعلى وجه التحديد مستخلص أوراق الزيزوفيس سبينا كريستي، حيث لم يتم تسجيل فرق كبير مقارنة بالشواهد.

تشهد هذه النتائج فعالية الاوكالبتوس كامالدولينسيس والزيزيفوس سبينا كريستي كمواد مضادة للأكسدة، ووقائية وعلاجية ضد الإجهاد التأكسدي الناتج عن نيترات الرصاص.

الكلمات المفتاحية: نيترات الرصاص، السمية، الإجهاد التأكسدي، الاوكالبتوس كامالدولينسيس، الزيزيفوس سبينا كريستي، مضادات الأكسدة.

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List of abbreviations

Pb	Lead
PbO	lead oxide
HNO3	Nitric acid
GI	Gastrointestinal
ALAS	Aminolaevulinic acid synthetase
ALAD	Aminolaevulinic acid dehydrate
ROS	Reactive oxygen species
DNA	Deoxyribonucleic acid
MDA	Malondialdehyde
ALA	Aminolaevulinic acid
Hg	Mercury
Cd	Cadium
SH	Sulfhydryl
SOD	Superoxide dismutase
CAT	Catalase
GPX	Glutathione peroxidase
G6PD	Glucose-6 phosphate dehydrogenase
MAPK	Mitogen-activated protein kinases
PKA	Protein kinase A
Pb (NO3)2	Lead nitrate
I. P. A	Pasteur Institute of Algiers
Euc	*Eucalyptus camaldulensis*
Zsc	*Ziziphus spina-christi*
GSH	Reduced Glutathione
GST	Glutathione S-Transferase
CDNB	1-chloro, 2-4-dinitrobenzene
OD	Optical density
DTNB	5,5-dithio-bis-2-nitrobenzoic acid
EDTA	Ethylene Diamine Tetra Acetic Acid

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Introduction

Introduction

Lead Nitrate is an environmental and industrial pollutant that accumulates in almost all body tissues such as the liver, lungs, bones, kidneys, reproductive organs, and immune system **(Sudjarwo *et al.,* 2017).** Lead (Pb) is a heavy metal with no specific biological functions, so it is highly toxic even at small concentrations **(Rendón *et al.,* 2017).** Infants and young children are particularly at risk, as low levels of exposure contribute to behavioral problems, learning difficulties, and lower IQ. Long-term exposure in adults can lead to anemia, high blood pressure, and significant brain and kidney damage. Pregnant women are at risk of miscarriage due to exposure to high levels of lead, while chronic exposure can lead to male infertility **(Sani and Amanabo, 2021).**

Given the relationship between lead exposure and oxidative stress, attention has been focused on compounds with antioxidant properties to combat lead-induced toxicity **(Dewanjee *et al.,* 2013).** Medicinal plants have a rich history in treating a wide range of ailments and diseases wing to their therapeutic advantages **(Ghareeb *et al*., 2018).**

Eucalyptus Camaldulensis is a member of the Myrtaceae family and has gained recognition as a reservoir of bioactive compounds used in the treatment of various diseases in recent times **(Dkhil *et al.,* 2023)**. It is employed to alleviate sore throats and combat bacterial infections affecting the respiratory and urinary systems. Its leaf-derived essential oils, rich in eucalyptol (1,8-cineole), are utilized for lung ailments. Additionally, methanol extracts have shown antimicrobial efficacy **(Yepola and Adeniyi, 2008).** In addition, polyphenols in *Eucalyptus* leaves have demonstrated various biological activities, including antioxidant, antitumor, and antibacterial properties **(Fathi *et al*., 2019).**

Moreover*, the Ziziphus Spina-Christi* plant from the Rhamnaceous family holds significant medical value recognized by ancient civilizations. It has been historically utilized to alleviate diarrhea, colon cramps, and rheumatism. The plant's tannin content contributes to its antibacterial properties. Additionally, the leaves are employed in treating headaches, bone pain, abscesses, and superficial wounds, and they can act as an analgesic **(Nabih Ads *et al.,* 2017)**. Studies exploring its phytochemical composition have unveiled numerous significant biological compounds within this plant **(Nabih Ads *et al*., 2017)**. In addition to *Ziziphus spina-christi* containing various biologically active ingredients such as alkaloids, sterols like β-sitosterol, flavonoids, triterpenoids, sapogenins, and saponins, these compounds can potentially serve as antibacterial, antioxidant, antimicrobial, and anti-inflammatory agents **(Hussein, 2019).**

Based on this study: Can *Eucalyptus camaldulensis* and *Ziziphus spina-Christi* plants reduce or eliminate kidney toxicity caused by lead-in rate?

The objectives of this study highlight:

Determine the extent to which *Eucalyptus Camaldulensis* and *Ziziphus spina-Christi* can save kidney tissue from the oxidative stress induced by lead poisoning by evaluating kidney function parameters, redox balance.

BIBLIOGRAPHIC PART

1. LEAD METAL

1.1. Physicochemical properties of lead

Lead is a gray-colored, soft, and ductile metal with a density of 11.34 g/cm³ at 293 K (19.9 °C).It has a melting point of 621.5 °F (327.5 °C) and a boiling point of 3180 °F (1749 °C). Lead is insoluble in water but dissolves slowly in weak acid solutions **(Zhang *et al.,* 2015).**

Lead has four isotopes. As a member of the carbon group in the periodic table, it is considered an inert metal. When heated, lead will oxidize to produce PbO, and it will react with HNO_3 to produce Pb $(NO_3)_2$ **(Zhang *et al.,* 2015).**

1.2. Sources of exposure to lead

Lead exposure is indeed a significant global issue due to activities like lead mining, smelting, and battery manufacturing, which are prevalent in many countries. Lead can enter the body through inhalation, ingestion, or skin absorption. Nearly all inhaled lead is absorbed into the body, while the absorption rate for ingestion varies from 20% to 70%, with children generally absorbing a higher percentage than adults. Lead poisoning often occurs due to the ingestion of food or water contaminated with lead. It can also happen after accidental ingestion of contaminated soil, dust, or lead-based paint. Seawater products may contain lead if they are affected by nearby industrial waters. Additionally, fruits and vegetables can be contaminated by high levels of lead in the soils they were grown in. Soil contamination can occur through particulate accumulation from lead in pipes, lead paint, and residual emissions from leaded gasoline. These various sources contribute to the widespread issue of lead poisoning **(Boldyrev, 2018).**

Inhalation is indeed another significant exposure pathway for lead, particularly impacting smokers and individuals working in lead-related occupations. Cigarette smoke contains various toxic substances, including radioactive lead-210, which can contribute to lead exposure through inhalation **(Boldyrev, 2018).**

1.3. kinetics of lead

1.3.1. Absorption

Lead (Pb) can be absorbed into the body through ingestion, inhalation, or skin contact. The primary routes of exposure are through the gastrointestinal (GI) tract and the respiratory system. When lead is inhaled, its absorption depends on the size of the particles, with estimates suggesting that 30-40% of inhaled lead can reach the bloodstream **(Bhasin *et al.,* 2023).**

Absorption rates through the gastrointestinal (GI) tract vary depending on factors like age and nutritional status. In adults, approximately 10 to 15% of ingested lead is absorbed, while in infants, young children, and pregnant women, absorption rates can increase to around 50% **(Sangeetha and Umamaheswar, 2020).** Factors such as fasting, and deficiencies in calcium, iron, phosphorus, or zinc can increase lead absorption **(Bhasin *et al.*, 2023).**

Inorganic forms of lead from sources like lead paint, water pipes, vinyl products, and tetraethyl lead from leaded gasoline can be absorbed through the skin, it is then transported into the plasma and quickly concentrated into extracellular fluid pools such as sweat and saliva, without significant uptake by erythrocytes **(Sangeetha and Umamaheswar, 2020).**

1.3.2. Distribution

After absorption, lead accumulates in the blood, soft tissue, and bone. Approximately 99% of the absorbed lead is bound in erythrocytes, while the remaining 1% is present in plasma and serum **(Bhasin *et al.*, 2023).** The kinetics of lead transfer from blood to soft tissues are slow, taking approximately 4 to 6 weeks, the kidneys take up the highest percentage of lead, followedby the liver and other soft tissues **(Boskabady *et al.*, 2018).** The half-life of lead varies amongdifferent organs: in blood, it's 35 days, in soft tissues it's around 40 days, and in bones, it rangesfrom 20 to 30 years. Understanding these kinetics and distribution patterns is essential for accurately assessing and managing lead exposure and its associated health risks **(Bhasin *et al.*,2023).**

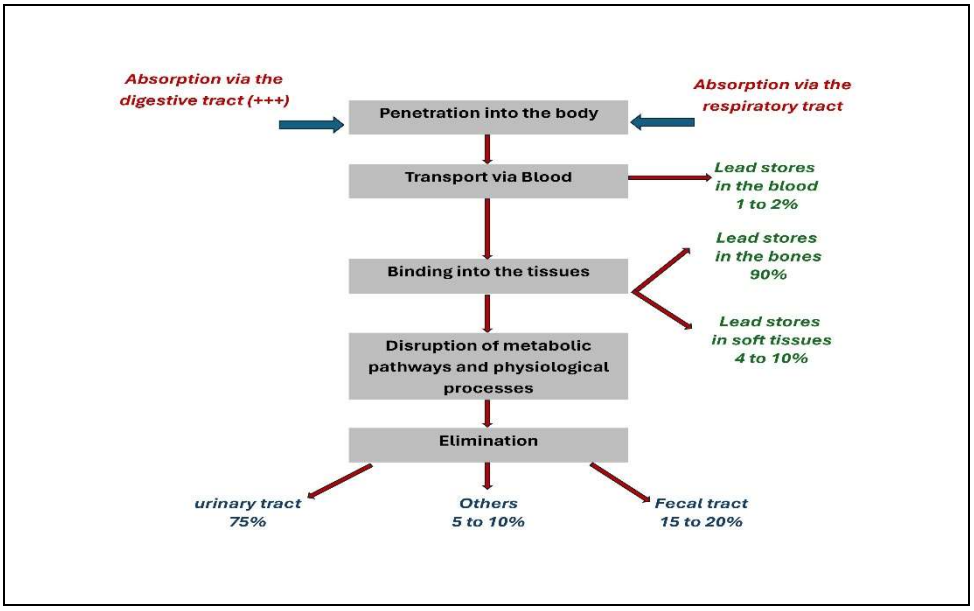


Figure 01. Expresses kinetics in the body **(Duponchel, 2011)**

The distribution of lead in various organs is influenced by the blood flow to the tissues, and lead can cross the blood-brain barrier. Several studies have demonstrated that oral intake of inorganic lead can adversely affect the immune system **(Sangeetha and Umamaheswar, 2020).**

1.3.3. Elimination

Inorganic lead is not metabolized in our body and is excreted unchanged in urine **(Boskabady *et al.,* 2018).** The mechanisms of lead excretion are not fully understood, but it is known to be excreted through various pathways, including secretion into bile, gastric fluid, and saliva Other routes of lead excretion exist including nails and sweat **(Edyta Charkiewicz and Backstrand, 2020).** Overall, lead is excreted slowly and tends to accumulate in the body. Lead can also be excreted in milk, with concentrations of up to 12 µg/L **(Sangeetha and Umamaheswar, 2020).** Children are particularly vulnerable to lead toxicity due to various factors, including their increased hand-to-mouth activity, higher gastrointestinal absorption rates, and the developing nature of their nervous systems **(Sangeetha and Umamaheswar, 2020).**

1.4. Effects of lead on health

1.4.1. Effects of lead on the nervous system

Chronic lead exposure has been found to have significant neurological impacts, especially in children, who are more sensitive to even low levels of exposure. Symptoms include fatigue, motor deficits, cognitive decline, neurodegeneration, emotional disturbances, nausea,headaches, and psychological issues such as depression, anxiety, and irritability **(Tayaba, 2022).**

1.4.2. Effects of lead on Hematological system

Lead induces acute hemolytic anemia by inhibiting the activity of three critical enzymes involved in heme synthesis: ferrochelatase, aminolaevulinic acid synthetase (ALAS), and 6-aminolevulinic acid dehydrase (ALAD) **(Kim *et al.,* 2015).**

1.4.3. Effects of lead on the cardiovascular system

Lead exposure can have detrimental effects on cardiovascular health, including hypertension and vascular damage. Blocked blood vessels due to lead accumulation can precipitate immediate heart attacks, potentially resulting in fatal outcomes **(Debnath *et al.,* 2019).**

1.4.4. Effects of lead on the renal system

Acute lead-induced nephropathy presents functionally as a generalized deficit in tubular transport mechanisms, known as Fanconi syndrome, and morphologically by degenerative changes in tubular epithelium along with the formation of nuclear inclusion bodies containing lead-protein complexes **(Bhattacharjee *et al.,* 2018).** These effects are often reversible with chelation therapy. On the other hand, chronic occupational exposure to lead correlates with chronic renal failure, hypertension, and hyperuricemia. Chronic lead nephropathy represents an irreversible renal disease that progresses over months or years of excessive exposure **(Bhattacharjee *et al.,* 2018).**

1.4.5. Effects of lead on the reproductive system

When blood lead levels exceed 40 µg/dL, lead exposure affects the reproductive system in both males and females. In males, this leads to a reduction in sperm count, changes in sperm volume, and alterations in sperm motility and morphology **(Giulioni *et al.,* 2023).** Toxic levels of lead have more severe impacts on the reproductive system of females. Lead exposure can result in miscarriages, premature births, low birth weight, and developmental issues in childhood **(Wani*et al.,* 2015).**

1.4.6. Effects of lead on the hepatic system

Chronic exposure to lead has indeed been linked to various effects on the liver. Studies have shown that lead toxicity can inhibit cytochrome P450 enzymes, particularly CYP51, which is crucial for cholesterol synthesis. This inhibition can lead to elevated levels of cholesterol. Additionally, lead nitrate has been found to cause the proliferation of liver cells in rats without concurrent liver cell necrosis **(Pervin and Akil Hossain, 2021).**

1.4.7. Effects of lead on bone tissues

In the human body, bones serve as the primary storage site for lead, with two distinct sections: the non-exchangeable pool deep in the bone cortex and the exchangeable pool at the bone surface **(Assi *et al.,* 2016).** The mobilization and storage of lead in bones depends on various factors such as age, pregnancy, lead exposure, race, and gestation. For instance, administeringlead acetate at specific doses and intervals can lead to moderate hyperplasia of hemopoietic tissue, megakaryocyte proliferation, thin trabeculae of calcified cartilage, impaired resorption of osteoclasts, and wider bars of mineralized cartilage projecting further into the metaphyseal marrow cavity compared to normal healthy bones **(Assi *et al.,* 2016).**

1.5. Lead and oxidative stress

When lead exposure occurs, it triggers oxidative stress by disrupting the balance between reactive oxygen species (ROS) generation and removal in tissues and cellular components. This oxidative stress wreaks havoc on cell membranes, DNA, and proteins. Experimental evidence supports that when exposed to lead, the concentration of malondialdehyde (MDA), increased proportionally with the number of double bonds in the fatty acids. Furthermore, lead impacts various membrane-related processes, including enzyme activity, endo- and exocytosis, solute transport across the bilayer, and signal transduction **(Rautray and Swarup, 2011).**

Upon lead exposure, accumulation of 6-aminolevulinic acid (ALA) leads to ROS generation and subsequent oxidative stress. The final oxidation product of ALA, 4,5-dioxovaleric acid, effectively alkylates quinine moieties within nucleosides and isolated DNA. Additionally, increased levels of 8-oxo-7,8-dihydro-2-deoxyguanosine further indicate oxidative DNA damage caused by lead exposure **(Ibrahem *et al.*, 2020).**

The second mechanism underlying lead-induced oxidative stress involves the disruption of cellular antioxidant defense systems. Lead, along with other metals like mercury (Hg) and cadmium (Cd), exhibits a strong affinity for sulfhydryl (SH) groups. When lead interacts with SH groups, it forms fewer stable complexes known as mercaptides. Lead specifically affects antioxidant activities by inhibiting functional SH groups in several enzymes, including ALAD, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glucose-6 phosphate dehydrogenase (G6PD) **(Patra *et al.*, 2011).**

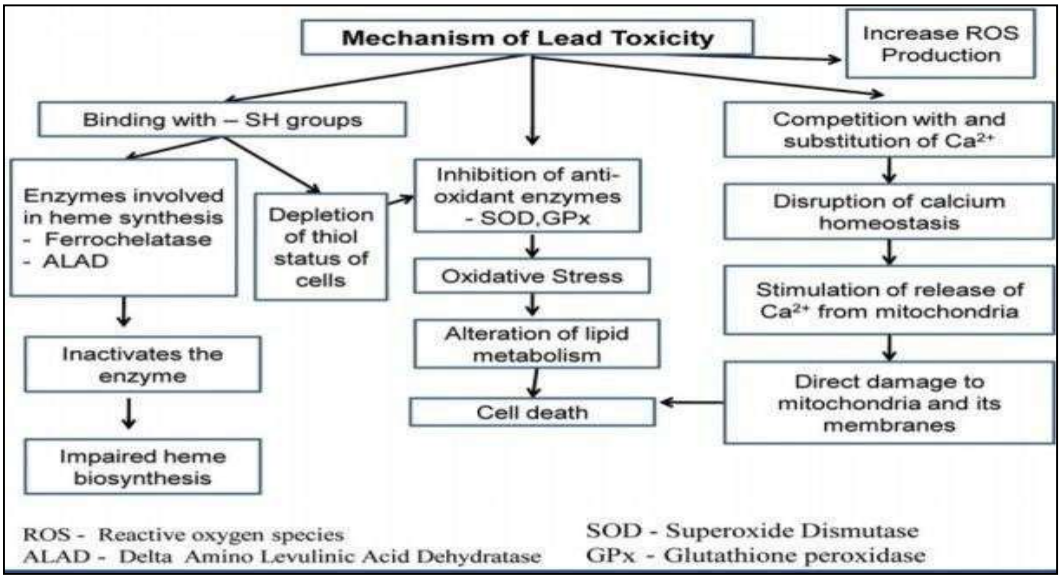


Figure 02. Schematic representation of various mechanisms of lead toxicity **(Sangeetha and Umamaheswari, 2020)**

2. Eucalyptus camaldulensis

2.1. Botanic study

Eucalyptus camaldulensis are trees of the Myrtaceae family that typically grow up to 20 metersin height, occasionally reaching 50 meters, with a trunk diameter ranging from 1 to a maximumof 2 meters. The bark is smooth and can be white, grey, yellow-green, grey-green, or pinkish- grey, shedding in strips or irregular flakes; rough bark covers the lower 1-2 meters of the trunk.Its leaves are grey-blue, alternate, drooping, 8-22 cm long, and 1-2 cm wide, often curved or sickle-shaped, narrowing with a short pointed base. The inflorescence is axillary, solitary, and consists of 7-11 flowers; flower buds are white, globular-rostrate or ovoid-conical, with an operculum that is hemispherical, rostrate, or conical, measuring 4-6 mm x 3-6 mm and endingobtusely. Its fruits are very small capsules located at the tips of thin stalks, about 5-8 mm in size, with 4 valves containing minute seeds **(Al-Snafi, 2017).**

Figure 03. Photograph of tree and leaves of *Eucalyptus camaldulensis* **(Laadel, 2014)**

Table 01. Scientific classification **(Rezzag and Ghelui, 2020)**

Kingdom	Plantae
Phylum	Spermatophytes
subphylum	Angiosperms
Class	Dicotyledons
Subclass	Rosidae
Order	Myrtales
Family	Myrtaceae
Genre	Eucalyptus
Species	*Eucalyptus Camaldulensis*

2.2. Geographic Distribution

Native to Australia and Tasmania, certain species, notably *E. globulus*, have been introduced to Europe, where they have acclimatized very well on the Mediterranean shores and in Portugal,a country in which immense eucalyptus forests have been planted for the production of paper pulp. The tree has also been planted in North Africa, notably in Algeria, Morocco, and Libya. It is also found in Madagascar, Sri Lanka, South Africa, and California **(Rojas Sandoval and Acevedo-Rodríguez, 2019)**.

2.3. Chemical compositions

Eucalyptus camaldulensis leaves are rich in essential oil, comprising 0.1-0.4% of the leaf composition, with 77% of this oil being 1,8-cineole. Other significant constituents include cuminal, phellandrens, aromadendren (or aromadendral), valerylaldehyde, geraniol, cymene, and phellandral, as identified in several research. The leaves also contain about 5-11% tannins.The kino, a resinous substance from the tree, contains 45% kinetannic acid along with kino red, glucoside, catechol, and pyrocatechol. Flavonoids and sterols are detected in both leaves and fruits. The bark is rich in tannins, ranging from 2.5-16%, while the wood and kino contain 3-14% and 46.2-76.7% tannins, respectively. Reported phytoconstituents of the tree encompass essential oils, sterols, alkaloids, glycosides, flavonoids, tannins, and phenols **(Aleksic Sabo and Khezevic, 2019).**

2.4. Therapeutic properties.

E. camaldulensis extract products have the potential to be used as antibacterial and antifungal agents in cosmetic and pharmaceutical products **(Jaradat *et al.,* 2023).** It is used as a food additive to reduce the dependency on synthetic chemicals in food preservation. *E. camaldulensis* flower essential oil inhibited melanogenesis through its antioxidant properties and by down-regulating both mitogen-activated protein kinases (MAPK) and protein kinase A(PKA) signaling pathways **(Huang *et al.,* 2015).**

Besides the beneficial effects, essential oils and plant extracts can exert potentially unfavorable effects as complex mixtures of different compounds. A risk assessment of their hazard is always necessary before utilization **(Aleksic Sabo and Knezevic, 2019).**

For centuries, Eucalyptus leaves and their essential oils, used as a traditional Aboriginal herbal remedy, have been applied in various aspects of daily life due to their antiseptic, anti-inflammatory, and fever-reducing properties. In ancient Aboriginal society, Eucalyptus

camaldulensis plants were utilized in medicinal practices to address gastrointestinal issues like colic, diarrhea, and dysentery, as well as respiratory ailments such as colds, coughs, asthma, laryngalgia, laryngitis, pharyngitis, and sore throat. They were also used to control bleeding, heal wounds, cuts, and bruises, and their decoctions were employed for relieving spasms, muscle aches, joint pains, toothaches, and even as a smoke bath where the patient was surrounded by the smoke from burning leaves, a method used to treat fevers, colds, influenza, and general illnesses. In some regions like Senegal, a preparation of Eucalyptus leaf decoctionwith sugar was administered to alleviate stomach pain **(Aleksic Sabo and Khezevic, 2019).**

3. Ziziphus Spina-Christi

3.1. Botanic study

Ziziphus is a genus within the Rhamnaceous family, encompassing approximately 100 species of deciduous or evergreen trees and shrubs. These plants are found in tropical and subtropical regions globally **(Abdullah Almalki *et al.*, 2018).**

Ziziphus spina-Christi, also known as Christ's thorn jujube, is a resilient spiny shrub that thrivesin hot and dry conditions with a deep taproot. The tree can reach heights of 5-10 m and trunk diameters of up to 45 cm. Its bark is characterized by whitish brown or pale grey coloration with deep fissures. Light brown paired spines, one longer and straight and the other shorter andcurved, adorn its branches. The leaves are simple, alternate, narrowly ovate-lanceolate, rangingfrom 1 to 9 cm in length and 1-3.5 cm in width. They have a smooth upper surface and a denselypubescent lower surface, with three prominent basal veins. *Ziziphus spina-Christi* produces small and greenish-yellow flowers, with a pleasant fragrance, typically arranged in dense clusters within the leaf axils. The fruit of Ziziphus spina-christi is a globose drupe, approximately 1-1.5 cm in diameter, red-brown in color, containing a hard stone surrounded bya sweet, edible pulp **(Saied *et al.*, 2007).**

Figure 04. *Zizphus spina-Christ* tree **(Asparpanah and Haghighat, 2012)**

Table 02. Scientific classification **(Rojas-Sandoval, 2017)**

Kingdom	Plantae
Phylum	Spermatophyta
Subphylum	Angiospermae
Class	Dicotyledonae
Order	Rhamnales
Family	Rhamnaceae
Genus	Ziziphus
Species	*Ziziphus Spina-Christi*

3.2. Geographic Distribution

Ziziphus spina-christi (L) is a tropical evergreen tree originating from Sudan. It thrives in semi-arid tropical regions of sub-Saharan Africa and subtropical zones in the Near and Middle East. Known locally as dum, nabq, sadr, sidr, and tsal, it typically grows below 500 meters above sea level, demonstrating remarkable resilience to heat and drought. This tree flourishes in desert climates with annual rainfall ranging from 50 to 300 mm and shows adaptability to various soil types, although it favors light, silty soils **(Alamin, 2016)**.

3.3. Chemical composition

The literature survey indicates that various compounds such as cyclopeptide and isoquinoline alkaloids, flavonoids, terpenoids, glycosides, betulinic acid, ceanothic acid, saponins, erols, tannins, triterpenes, and cardiac glycosides are found in different amounts in Ziziphus species. Additionally, the main components of Z.spina-christi leaves essential oil include geranyl acetate, methyl hexadecanoate, methyl octadecanoate, farnesyl acetone C, hexadecanol, and ethyl octadecanoate **(Alamin, 2016).**

Zizyphine-F, jubanine-A, amphibine-H, and spinanine-A have been isolated from the stem barkof *Z.spina-Christi*. *Christinin-A* is identified as the major saponin in the leaves while dodecaacetylprodelphinidin B3 has also been isolated from the leaves. Flavonoids such as quercetin 3-xylosyl (1-2) rhamnoside-4-rhamnoside, along with rutin, hyperin, quercetin, apigenin-7-O-glucoside, isovitexin, and quercetin-3-O-glucoside-7O-rhamnoside, have been characterized from Z. spina-christi fruits. Additionally, a flavonoid C-glycoside, 3',5'-di-C-B- D-glucosylphloretin, was identified in *Z. spina-christi* leaves. Furthermore, two new cyclic amino acids, 4-hydroxymethyl-1-methylpyrrolidine-2carboxylic acid and 4-hydroxy-4-

hydroxymethyl-1-methylpyrrolidine-2-carboxylic acid, were characterized from Z. spina- christi seeds **(Asparpanah and Haghighat, 2012).**

3.4. Therapeutic properties

Ziziphus spina-christi has a long history in traditional medicine for addressing various ailments such as digestive issues, weakness, liver problems, obesity, urinary issues, skin infections, lossof appetite, fever, pharyngitis, bronchitis, anemia, insomnia **(Al-Ghamdi *et al.,* 2019).** In Sudan, the fruits of Z. spina-christi are consumed to alleviate diarrhea, malaria, and spasms. Twigs are powdered and applied externally for rheumatism and scorpion stings. Powdered leaves are used in poultices for swelling, while boiled leaves are applied to wounds **(Dogara Abdulrahman *et al.*, 2022).**

Macerated roots serve as anti-purgatives, and bark extract decoctions are used for intestinal spasms. In Egypt, a fruit-based beverage is considered as a sedative. Palestine utilizes leaves and branches for eye inflammation, toothache, and stomachaches **(Alamin, 2016).**

Materials & Methods

4. Materials & Methods

4.1. Materials

4.1.1. Chemical and Biological Materials

4.1.1.1. Preparation of the Lead Nitrate Solution

We used lead nitrate Pb (NO3)2 obtained from the company BIOCHEM Chemopharma. It was dissolved in mineral water at a concentration of 50 mg/kg and administered to rats orally (per os).

Figure 05. Preparation of the lead solution **(personal photos)**

4.1.1.2. Plant Material and Choice of Medicinal Plants

To better valorize the biodiversity of plants in Eastern Algeria, our study focused on *Ziziphus spina-Christi* and *Eucalyptus camaldulensis*, which belong to the families of Rhammanceous and Myrtaceae respectively. A bibliographic study and a simple survey among the local population with knowledge of traditional medicine allowed us to make this choice. Thus, the selection criteria are as follows:

– Abundance of both plants in Algeria.

– Traditional use in the treatment of several diseases.

– Non-toxicity of the plants, as they are used in the preparation of herbal teas.

4.1.1.3. Harvesting and Identification

The aerial part of the *Eucalyptus* is harvested in the month of February in the region of Ferkane south of Tebessa state and botanical identification was done on an herbarium specimen by Mrs. Guenez Radja, a teacher at the University of Tebessa. The dried Christi's leaves are purchased from the local market originating from Soug Ahras

4.1.1.4. Preparation of Decoctions

10 g of each plant is infused in 100 ml of boiling mineral water (100°C). Once cooled, the decoction is filtered and stored in sterile containers, protected from light.

Figure 06. Preparation of decoctions **(personal photos)**

4.1.2. Animal Care and Treatment

For this study, we received 42 female rats (Albino Wistar) from the Pasteur Institute of Algiers (I.P.A.), aged 05 weeks. The breeding of the rats was carried out at the Larbi Tébessi University animal facility in Tébessa. The animals were housed in polyethylene cages equipped with labels indicating the lot name, the treatment received, and the dates of the experiments. The cages were lined with wood shavings and cleaned daily.

These rats underwent an adaptation period of approximately one month under the conditions of the animal facility: a temperature of (22 ± 2°C) and a natural photoperiod (12/12 hours). They were fed an energetically balanced concentrate in the form of pellets, along with ad libitum access to water. The table 03 summarizes the composition of the diet. The average weight of the rats at the beginning of the experiment was approximately 200 g ± 210 g.

Table 03: Composition of 1kg of nurishment.

Foodstuff	Quantity in g/kg of feed	%
Maize	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 : Group time, the rates to receive potable water and a simple nourishment.

Euc :Recipes for refilling potable water and 1ml with a force-feeding of a decoction prepared on *Eucalyptus* base.

ZSC : The amount of refill of potable water and 1ml of force-feeding of a decoction prepared on the base.

Pb : The recommended rates of forced-feeding are1 mg/kg of nitrate content.

Pb + EU : The rats are eaten in an administration by force-feeding of a dose of nitrate in the blood of 1 mg/kg, After few hours of administration of 1 ml of decoction in the eucalyptus base.

Pb + ZSC : The rats are eaten in an administration by force-feeding of a dose of nitrate in the mixture of 1 mg/kg, After few hours of administration of 1 ml of decoction in the base syrup.

Figure 07. maintenance of the animals **(personal photos).**

4.1.3. Blood Sampling

After a period of 21 days, the animals are sacrificed by decapitation. Blood is immediately collected into labled tubes containing heparin. These tubes are promptly centrifuged at 3000 revolutions per minute for 15 minutes. The recovered plasma will be used for the measurement of the following biochemical parameters: urea, creatinine, uric acid.

Figure 08. Blood sample **(personal photos)**

4.1.4. Renal Sampling

A longitudinal abdominal incision is made to collect kidneys. Once freed from their adipose tissues, the organs are weighed and stored in a freezer at -20°C for the quantification of oxidative stress biomarkers (GSH and GST). The figure 09 summarizes the various steps of the experimental protocol.

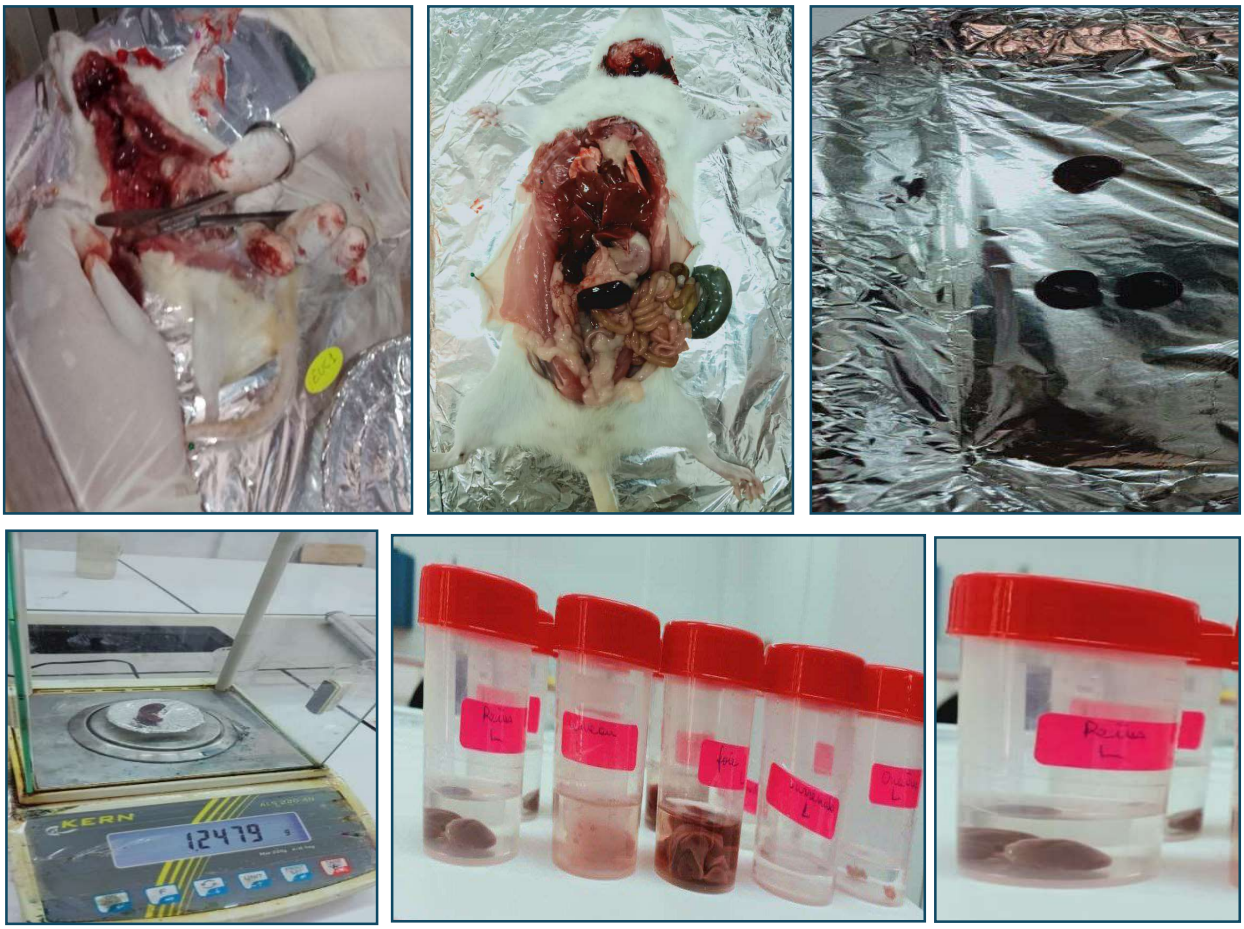


Figure 09. Kidneys removal **(personal photos)**

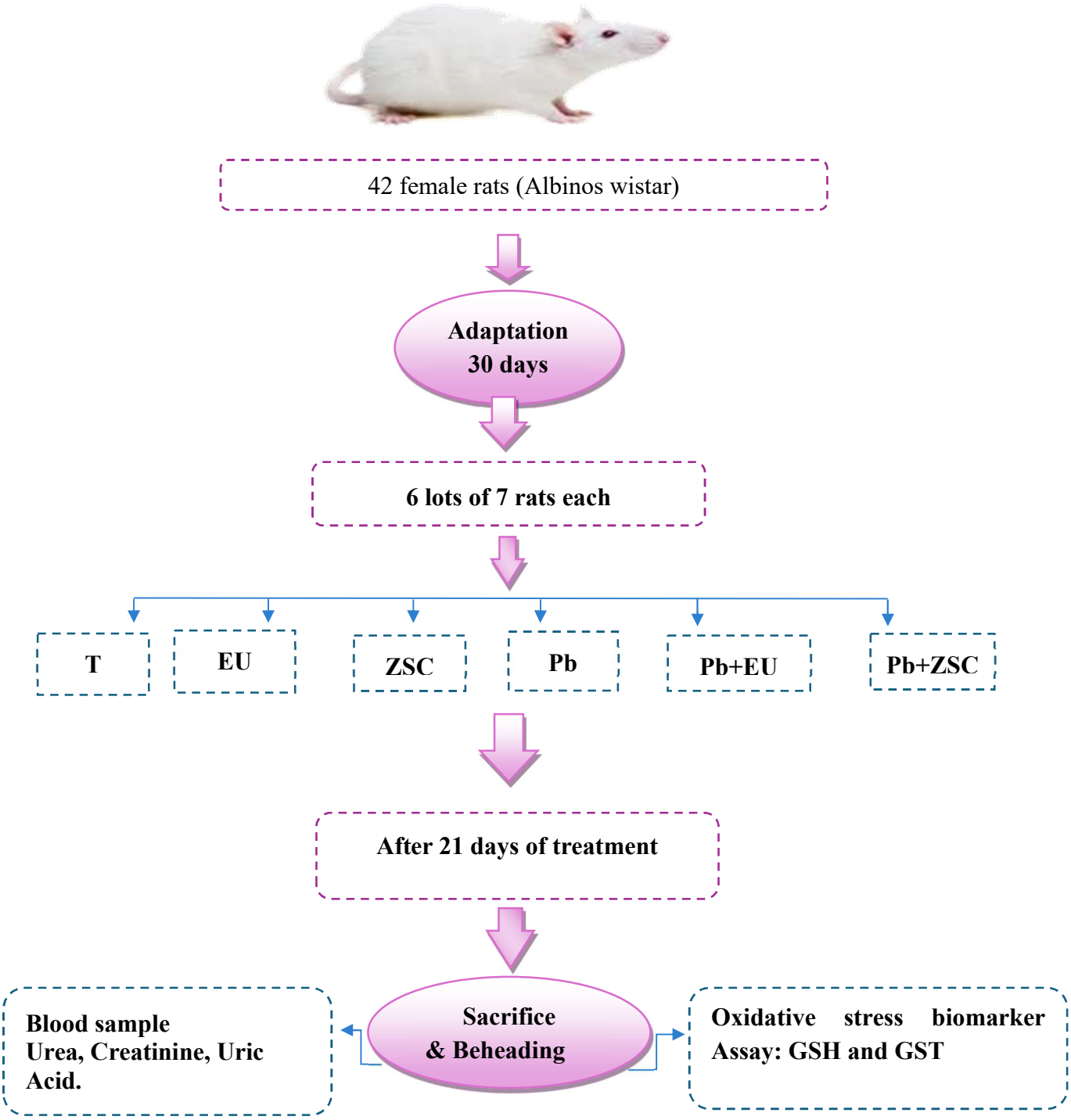


Figure 10. Diagram summarizing the experimental protocol

4.2. Methods

4.2.1. Blood Glucose Measurement

Principle

Blood glucose measurement was performed using a glucometer that utilizes reactive test strips. These test strips are intended for in vitro (external) diagnostic use for blood glucose testing. They are designed to measure glucose in capillary whole blood. The reactive test strip contains glucose oxidase, an enzyme that oxidizes glucose in the blood, producing D-gluconic acid and hydrogen peroxide.

Procedure

1. The glucometer automatically turns on when you insert the One Touch reactive test strip (in the direction of the arrows and until it clicks into place).
2. The symbol of a droplet blinks.
3. Place a drop of blood on the test strip's designated area. The result will be displayed within 5 seconds. Blood glucose levels are given in g/dL.

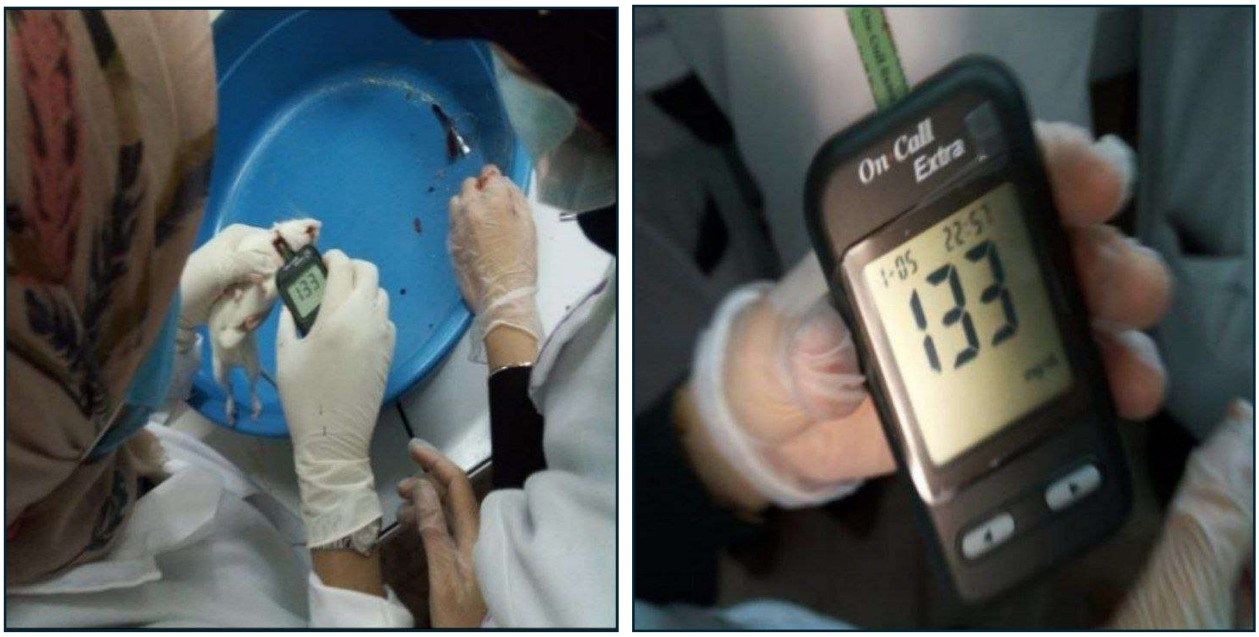


Figure 11. blood glucose measurement **(personal photos)**

4.2.2. Urea Measurement

Principle

This method is based on the specific action of urease, which hydrolyzes urea into ammonium ions and carbonate. The ammonium ions then react with chlorine and salicylate to form a blue-green colored complex. The intensity of coloration, proportional to the amount of urea in the specimen, is measured at 600 nm (570–610) **(Searcy, 1967).**

4.2.3. Uric Acid Measurement

Principle

Uricase acts on uric acid to produce allantoin, carbon dioxide, and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide reacts with a chromogen (dichloro-hydroxybenzene sulfonate and amino-antipyrine) to form a red-colored quinonimine complex. The absorbance at 505 nm is proportional to the amount of uric acid in the specimen **(Fossati, 1980).**

4.2.4. Creatinine Measurement

Principle

Colorimetric reaction of creatinine with picric acid in an alkaline medium, with kinetic development measured at 490 nm (490-510) **(Fabiny, 1971; Labbé, 1996).**

4.2.5. Measurement of Glutathione S-Transferase (GST) Enzymatic Activity

Principle

The measurement of GST activity involves providing the enzyme with a substrate, typically 1-chloro-2,4-dinitrobenzene (CDNB), which readily reacts with various forms of GST and glutathione. The conjugation reaction between these two compounds results in the formation of a new molecule that absorbs light at 340 nm 1. **(Habig *et al.,* 1974).**

Preparation of the Homogenate

100 mg tissue + 1ml tampon phosphate →→→→ cold milling→→→→→→ homogenate .

Note: keep homogenates cold until homogenization is complete.

Centrifugation of the homogenate to 9000 tr/min for 15min →→→→→ recovery of the supernatant for the determination of protein GST and mg.

Procedure

used reagents	white (µl)	Sample (µl)
Tampon phosphate (0.1M, pH 6.5)	830	830
CDNB (0.0 2M)	50	50
GSH (0.1M)	100	100
supernatant/distilled water	20	20

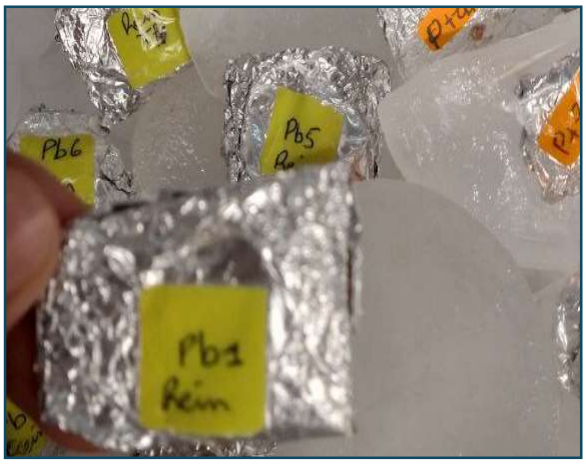
Calculation: the GST concentration is obtained by the following formula.

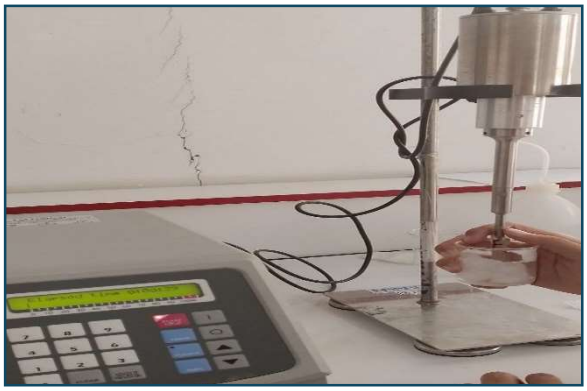
$$\mathbf{GST\ (nmol\ gst/min/mg\ protein)} = \frac{\mathbf{(OD\ SAMPLE/MIN - OD\ blanc/min)}}{\mathbf{9.6\ \times mg\ protein}}$$

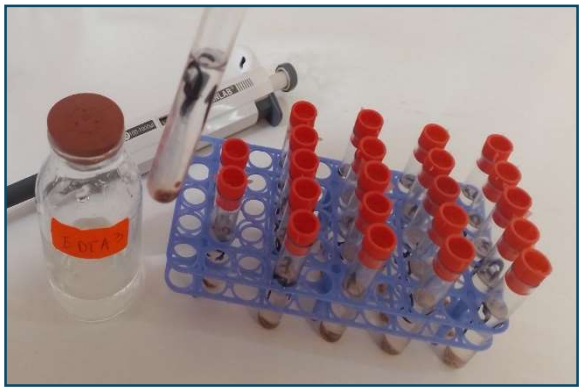
OD sampl / min : Optical density of the sample per minute

OD white / min : Optical density of white per minute

9.6 : GSH-CDNB extinction coefficient expressed in mM-1.Cm-1







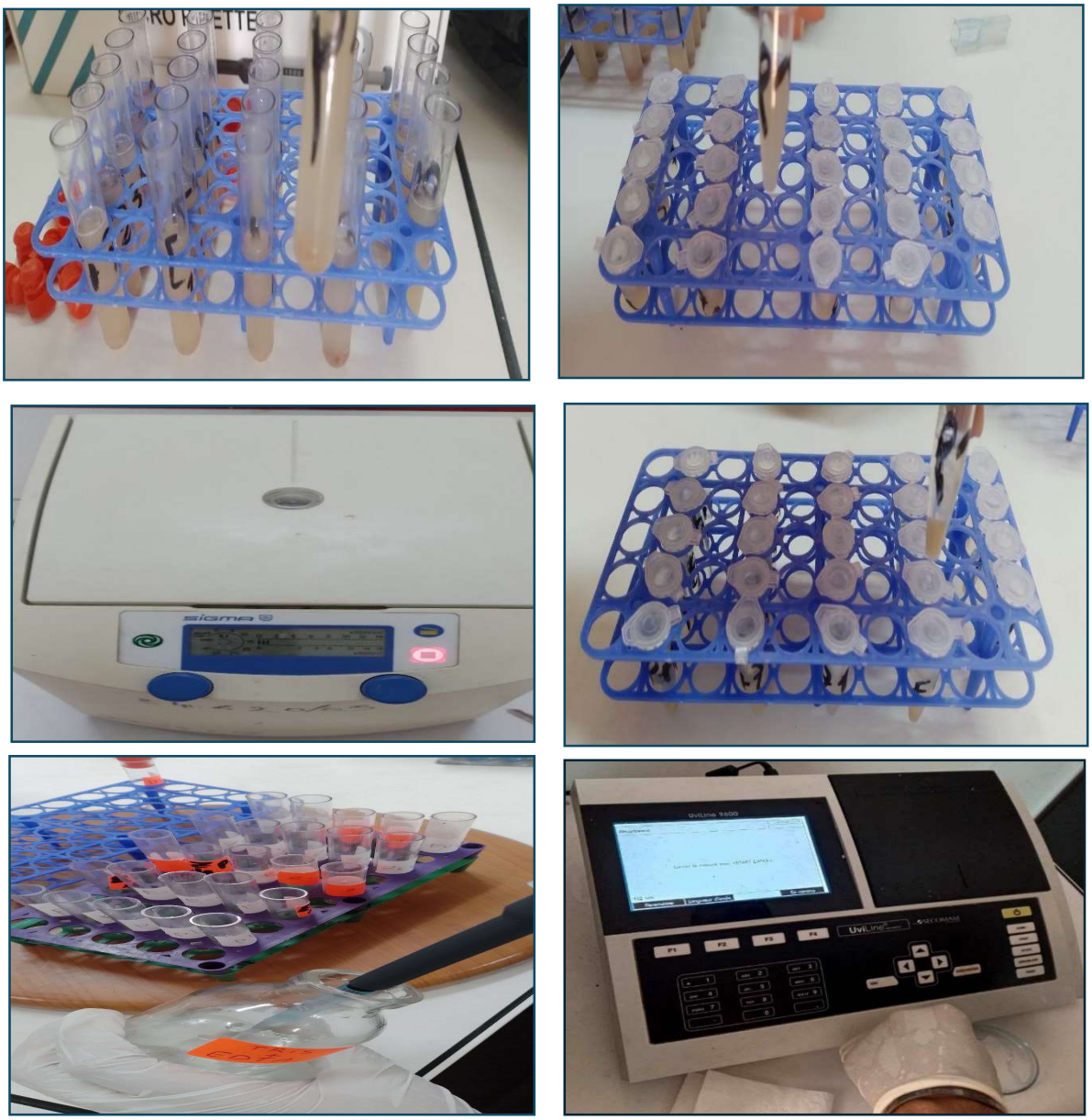


Figure 12. GST dosage photo gallery **(personal photos)**

4.2.6. Reduced Glutathione (GSH) Assay

Principle: The measurement of glutathione was carried out using the method described by **(Wekbeker and Cory 1988).** The principle of this assay is based on the optical absorbance measurement of 2-nitro-5-mercapturic acid. This compound results from the reduction of 5,5'-dithio-bis-2-nitrobenzoic acid (Ellman's reagent, DTNB) by the thiol (-SH) groups of glutathione. To achieve this, homogenate deproteinization is essential to retain only the specific thiol groups of glutathione.

Homogenate Preparation: 100 mg of tissue was mixed with 4 ml of a 0.02 M Ethylenediaminetetraacetic acid (EDTA) solution and then cold homogenized using an ultrasonic homogenizer to obtain a homogenate.

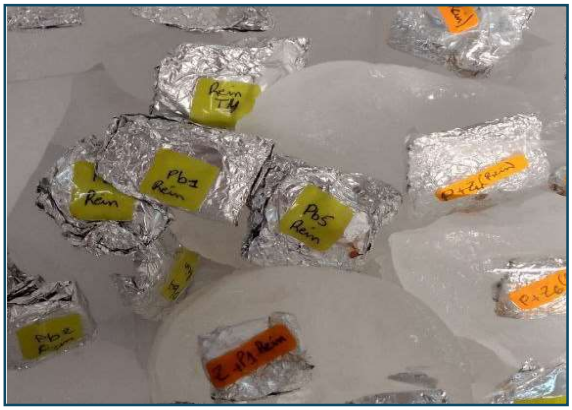
Procedure

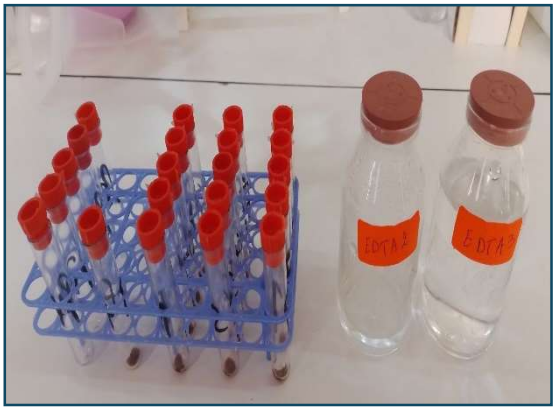
1. Take 0.8 ml of the homogenate.
2. Deproteinize by adding 0.2 ml of a 0.25% sulfosalicylic acid solution.
3. Agitate the mixture and let it sit for 15 minutes in an ice bath.
4. Centrifuge at 1000 rpm for 5 minutes.
5. Collect 0.5 ml of the supernatant.
6. Add 1 ml of Tris + EDTA buffer (0.02 M EDTA), pH 9.6.
7. Mix and add 0.025 ml of 0.01 M DTNB (dissolved in absolute methanol).
8. Allow it to stabilize for 5 minutes at room temperature, during which the color develops instantly. Read the optical densities at 412 nm against the blank.

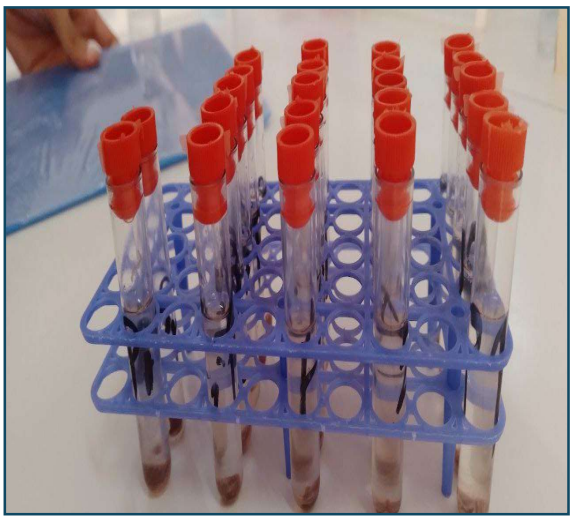
Calculation: The concentration of glutathione is obtained using the following formula.

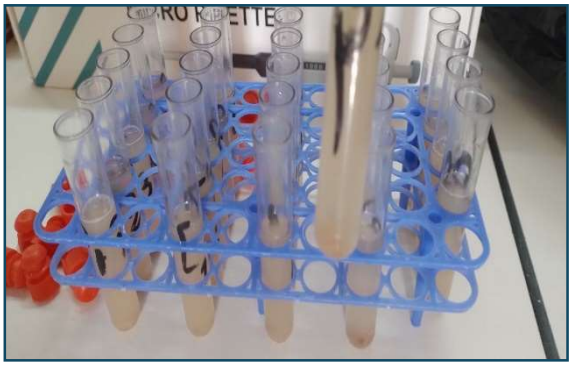
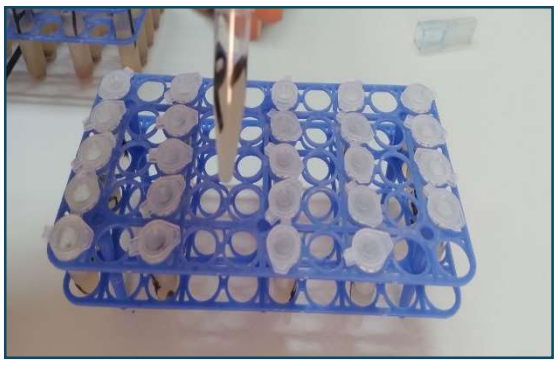
$$[\boldsymbol{GSH}](\boldsymbol{nM\ GSH/mg\ protide}) = (\boldsymbol{OD} \times \mathbf{1} \times \mathbf{1.525})/(\mathbf{13100} \times \mathbf{0.8} \times \mathbf{2.5}) \times \boldsymbol{mg\ proteide}$$

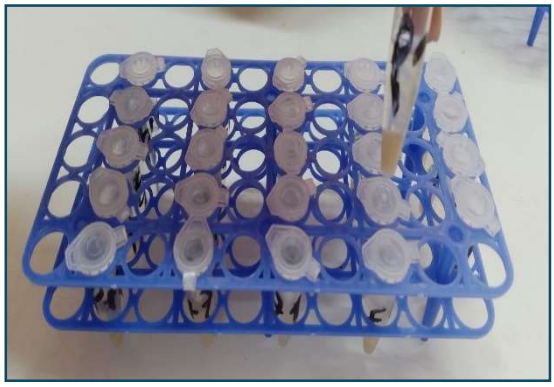
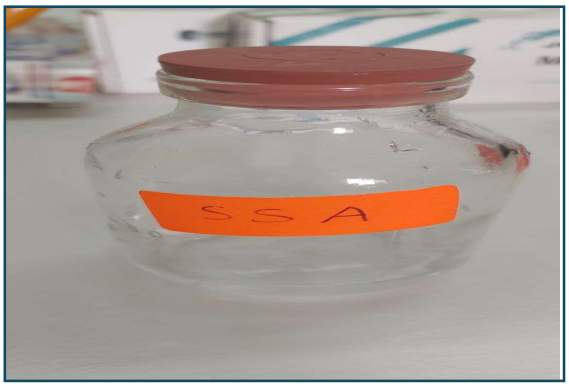
OD	:	optical density at 412nm.
1:	:	Total volume of solutions used in deprotonisation.
1,525	:	Total volume of solutions used in GSH assay.
13100	:	Absorbance coefficient of the group (-SH) à 412nm
0.5	:	Supernatant volume found in 1,525 ml
0.8	:	Volume of the homogenate found in 1ml





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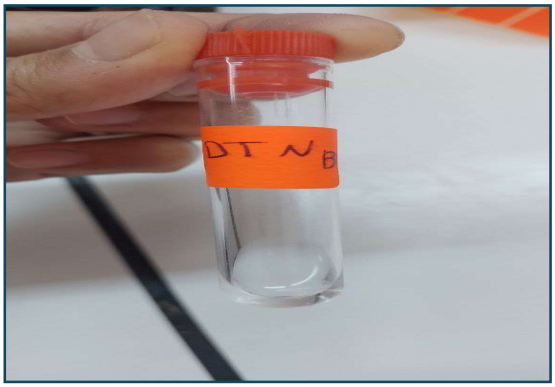

Figure 13. GSH dosage photo gallery **(personal photos).**

4.2.7. Protein Quantification

Principle: The protein concentration is determined using the **Bradford method (1976),** which utilizes Coomassie Blue (G-250) as the reagent. The amino groups (-NH2) of proteins react with a mixture of orthophosphoric acid, ethanol, and Coomassie Blue to form a blue-colored complex. The appearance of this color reflects the degree of ionization in the acidic medium, and its intensity correlates with the protein concentration in the sample.

Procedure

1. Take 0.1 ml of the homogenate.
2. Add 5 ml of the Bradford reagent.
3. Mix thoroughly and allow it to rest for 5 minutes to stabilize the color.
4. Measure the optical density at 595 nm against a blank (no protein).
5. Compare the obtained optical density with a pre-established standard curve.
6. Determine the protein concentration by comparing it to a standard range of bovine serum albumin (BSA) (1 mg/ml) prepared under the same conditions (see Figure 22).

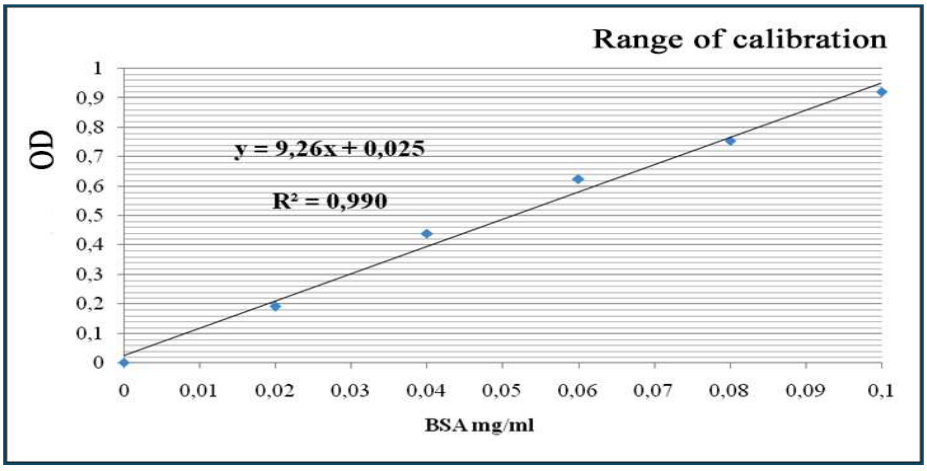

Figure 14. Bovine albumin serum calibration curve

Figure 16. Protein Measurement Photo Gallery **(personal photos).**

Statistical analysis

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

Results
&
discussion

5.1. Physiological parameters

5.1.1. Effect of treatments on body weight

Body mass monitoring of animals during the treatment period indicates a gradual increase in body weight of the control group (+10.44%) and animals receiving aqueous extracts of *Eucalyptus camaldulensis* and *Ziziphus spina-christi* (+16.27%), and (+10.45%) respectively. However, slow growth was noted in rats exposed to lead nitrate (+0.90%).

The results also show no notable physiological growth changes in rats treated with a combination of lead nitrate and *Eucalyptus camaldulensis* leaf extract or *Ziziphus spina-christi* (Pb + EU and Pb + ZSc) compared to the control group. Dietary intake remained unchanged, and the animals maintained their natural appetite, preserving their weight

Table 04. 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

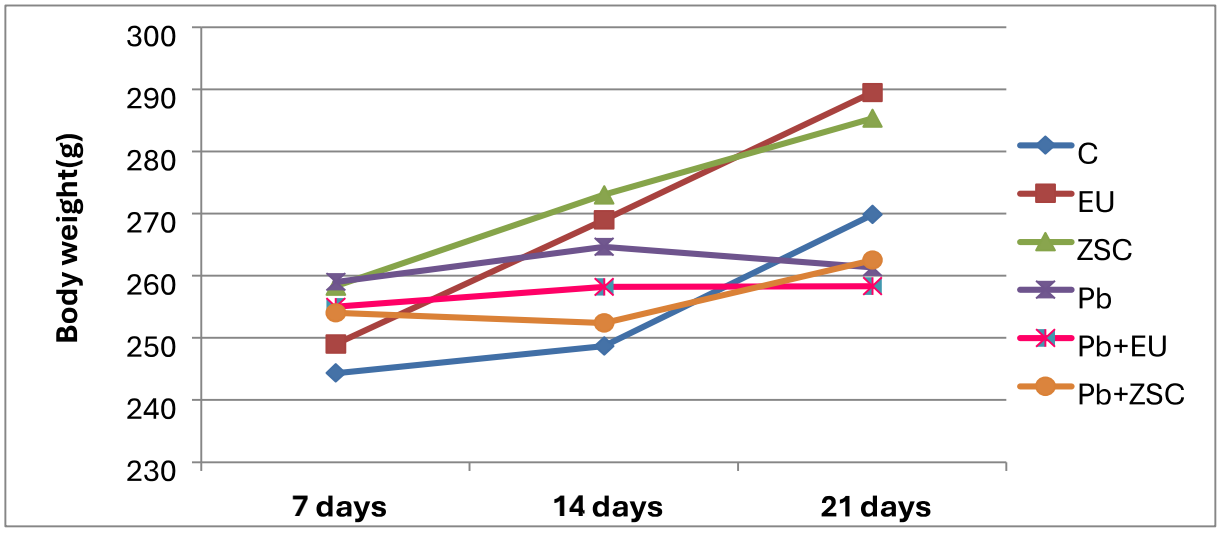

Figure 17. A graph representing changes in body weight in terms of days

As shown in the current study conducted on rats exposed to lead nitrate at a dose of 50 ml/kg, there was a very slight and unnoticeable increase in body weight, as the percentage of increase was estimated at (0.90%) compared to the control group, whose rate was estimated at (10.44%). We find that the body weight did not undergo significant changes, which indicates that rats lost their appetite in the presence of daily food consumption. This suggests that lead nitrate leads to a loss of appetite in animals and disrupts their daily nutritional factor, and this is what previous studies have proven **(Ramah *et al.,* 2015)**. It has been proven that the decrease or decline in weight growth compared to the control group is the result of the effect of lead nitrate due to the poor selection of some essential trace elements.

Our study showed that there was a slight improvement in body weight in rats treated with *Eucalyptus camaldulensis* leaf extract combined with lead nitrate. *Eucalyptus camaldulensis* showed effectiveness in restoring appetite compared to the group that was treated with lead only. Studies have shown that *Eucalyptus camaldulensis* extract treatment improved the health of mice infected with *P. chabaudi* by reducing both weight loss and anemia relative to non-infected controls **(Dkhil *et al.,* 2021; Aljawdah *et al.,* 2022).**

Moreover, our results showed that when aqueous extracts of *Ziziphus Spina-Christi* leaves were added to rats exposed to lead liters at a dose of 50 mg/kg, there was a significant increase in body weight. This can be attributed to the ability of *Ziziphus Spina-Christi* leaf extract to stimulate appetite and enhance gastric secretion. This facilitates the process of digestion and absorption of food. Similar results were obtained by **Ali et al. (2019)** who found that supplementation of goats with above 50% level of dried ZSC leaves in their diet significantly increased feed intake and body weight gain by improving the feed conversion efficiency of goats. Parallel with the finding of **Abalaka et al (2011)** who reported that regular supplementation of Ziziphus fruit extract allows for regular assimilation of all body nutrients that maintain the growth rate.

5.1.2. Effect of treatment on organ weight

Statistical studies in Table 05 showed changes in absolute and relative kidney weight. There was a notable increase in these parameters in animals exposed to lead nitrate only and treated with *Eucalyptus Camaldulensis* leaf extract compared to the control group. However, a slightly insignificant difference was observed in the other groups (EU, ZSC, and Pb + ZSC) compared to the control group.

Table 05. Effect of aqueous extracts of Eucalyptus Camaldulensis, Ziziphus Spina-Christi leaves and/or lead nitrate treatment on organ weight after 21 days of experience (values represent the mean ± SEM of seven rats)

Groups	C	EU	ZSC	Pb	Pb+EU	Pb+ZSC
Absolute weight (g)	0,968±0,30	1,191±0,23	1,248±0,11	1,528±0,29*	1,432±0,33*	1,216±0,41
Relative weight (%)	0,404±0,12	0,458±0,07	0,486±0,07	0,594±0,05*	0,622±0,10*	0,530±0,14
***P< 0,05 relative to the control group (C)**						

The kidney is more severely affected by lead toxicity than are other tissues because it is the fundamental route for elimination, and thus the main organ providing protection against lead poisoning **(Ghorbel *et al.,* 2001)**. Statistical analysis showed that in lead-treated rats the weight of the kidney, and its organo-smatic index, significantly increased compared with the control group. This increase could be due to a lead-induced retention of liquid and hypertrophy in the kidney **(Bornet *et al.,* 2003).**

Our results show that the aqueous extract of Eucalyptus leaves improves the relative and absolute weight of the kidneys at values that remain statistically significant compared to the control group, this is perhaps explained by the low dose of our decoction. According to **Uadia et al. (2024),** after a treatment of diabetic animals with an *E. camaldulensis* leaf-based diet for fourteen days, kidneys had normal tubular, vascular, and glomerular architecture. This established that *E. camaldulensis* leaf-based diet could reverse kidney damage.

Furthermore, no significant changes are mentioned in rats treated with a combination of lead nitrate and leave aqueous extract of *Ziziphus spina-christi* compared to the control group. A previous study suggests that *Ziziphus spina-christi* leaf extract has a protective effect against multiple-organ impairment, this may be attributed to the antioxidant, anti-inflammatory, and anti-apoptotic activities of this plant **(Dkhil *et al.,* 2018).**

5.2. Biochemical profile

The force-feeding of lead nitrate to rats at a dose of 50 mg/kg led to disturbances in chemical metabolic representation characterized by a notable increase ($P < 0.001$) in uric acid and urea levels. Additionally, minor fluctuations in blood glucose and creatinine levels were observed.

However, the addition of *Eucalyptus Camaldulensis* and *Ziziphus spina-christi* in the form of aqueous extracts restored all measured biochemical parameters in our study. Despite a slight decrease in blood glucose levels, a substantial increase in uric acid and urea was recorded compared to the control group.

Table 06. Effect of aqueous extracts of *Eucalyptus Camaldulensis*, *Ziziphus Spina-Christi* leaves, and/or lead nitrate treatment on several biochemical parameters after 21 days of experience (values represent the mean ± SEM of seven rats).

Experimental groups						
Parameters	C	EU	ZSC	Pb	Pb+EU	Pb+ZSC
Glycemia (g/L)	1.388±0.08	1.393±0.066	1.362±0.118	1.411±0.085	1.310±0.186	1.285±0.065 *
Creatinine (mg/dl)	3,396±0,27	3,492±0,12	3,468±0,21	3,672±0,12	3,658±0,259	3,546±0,38
Uric acid (mg/dl)	24,41±2,74	27,27±3,60	20,65±2,96 *	34,28±1,89 ***	29,75±2,75 *	29,20±2,47 *
Urea (g/L)	0,185±0,04	0,213±0,04	0,196±0,04	0,527±0,06 ***	0,417±0,05 ***	0,422±0,03 ***
***P< 0,05 ***P<0,001 relative to the control group (C)**						

Earlier research has demonstrated that rats treated with lead nitrate at a dose of 50 mg/kg exhibit a notable elevation in blood sugar levels. This increase is directly linked to the detrimental impact of lead nitrate on the pancreas, specifically affecting insulin secretion within the islets of Langerhans **(Saka, 2011)**. Additional research has indicated that elevated blood sugar levels result from lead nitrate toxicity, either due to the inhibition of insulin production within the islets of Langerhans or impaired glucose utilization by target cells **(Karami-Mohajeri and Abdollahi, 2011)**. This has been proven by our current study.

However, *E. camaldulensis* leaf administrated alone or in combination with lead normalized blood sugar levels. According to **Uadia et al. 2024** results, who suggest that this plant stimulated

the regeneration of both alpha and beta cells in the pancreas, indicated by increased insulin and decreased amylase levels.

We also found that the utilization of *Ziziphus spina-christi* considerably decreases the level of glycemia in poisoning lead rats. The results of our study agreed with those of **Abdel-Zaher et al. (2005); Avizeh et al. (2010); and Michel et al. (2011)** who noted that treatment with ZSC extract reduced blood glucose in experimental animals. **Al-Awar (2019)** demonstrated a significant reduction in blood glucose, insulin, pyruvate, and lactate in diabetic rats treated with ZSC seeds embryo extract. In addition, previous studies have demonstrated that the aqueous extract of *Ziziphus spina-christi* leaves can lower effectively blood sugar levels by exhibiting antioxidant and anti-diabetic effects in rats **(Benamar and Baghdad, 2014; Khaleel *et al.,* 2020; Al-Sayari and Wahab, 2021)**. *Ziziphus spina-christi* is rich in antioxidants such as saponin, polyphenols glycosides, flavonoids, and terpenoids which may inhibit oxidative stress and reduce the accumulation of lipids around cells, thereby facilitating insulin binding to cellular receptors and glucose entry into cells.

The renal system plays a vital role in maintaining the hydroelectrolytic homeostasis of the body and in purifying bodily fluids. Indeed, the cells in our body produce waste that must be eliminated to prevent it from becoming toxic (metabolic waste, urea, uric acid, excess ions). Additionally, xenobiotics, pesticides, and environmental toxins that enter our body are also largely eliminated by the kidneys. Urine production by the kidneys facilitates the elimination of these compounds **(Rossary, 2021).** Urea, uric acid, and creatinine are metabolic waste products that must be excreted by the kidneys. As a result, they serve as the most sensitive biochemical markers used in diagnosing renal damage **(Gökçe Apaydın *et al.,* 2016).**

Based on our current results, we recorded a significant increase in creatinine levels, in addition to an increase in uric acid and urea in rats treated with lead nitrate at a dose of 50 mg/kg. This is due to the kidneys losing their normal functions, as confirmed by **(Gökçe Apaydin *et al.,* 2016).** It was reported that high levels of urea in the blood are linked to increased protein hydrolysis in mammals and/or the conversion of ammonia to urea, which occurs due to increased protein synthesis of the enzyme arginase involved in the production of urea. In addition, the elevation in plasma concentrations of urea, uric acid, and creatinine observed in the current trial may be indicative of kidney dysfunction, as indicated by pathological changes and oxidative stress. The researchers also highlighted the strong relationship between high urea and creatinine levels and poor kidney function.

Our study showed that *Eucalyptus camaldulensis* leaf extract improved creatinine concentration very effectively, while there was a slight improvement in both urea and uric acid induced by lead nitrate. This has been proven by previous studies **(Duskaev *et al.*, 2020).**

The observed modulatory nature of the aqueous extract on levels of kidney biomarkers may be due to the antioxidant properties of the plant, perhaps originating from its active phytochemicals' components like Vitamins A, C, and E and flavonoids ho act as primary antioxidant or free radical scavengers that fight against oxidative damage. Hence, *E. camaldulensis* extracts containing appreciable amounts of these phytochemicals might show beneficial activity via any of these mechanisms **(Anigboro *et al.*, 2020).**

Besides, Ziziphus supplementation plays a notable role in maintaining biochemical parameters in their near-normal values in comparison with control rats. Our results are concurrent with those obtained by **(Khaleel *et al.*, 2021).** They show that oral administration of the ethanolic leaf extract of *Z. spina-christi* is relatively safe and did not have deleterious effects on the kidney and the serum biochemical parameters.

5.3. Influence of treatments on oxidative stress parameters

5.3.1 S-Transferase enzyme activity

The results mentioned in Figure 18 indicate a notable increase in renal enzymatic activity of GST in rats exposed to lead nitrates, compared to the control group. However, we observe a noticeable improvement when *Eucalyptus camaldulensis* and *Ziziphus Spina-Christi* are added in the form of aqueous extracts.

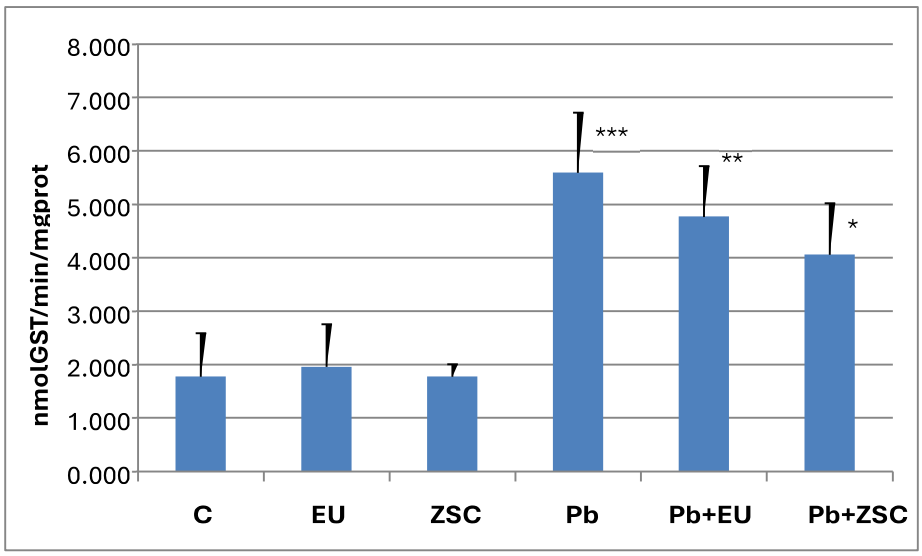

Figure 18. The variation in the enzymatic activity of glutathione S-transferase (µmol/min/mg/prot) in the kidneys of both control rats and those treated with (EU, ZSC, Pb, Pb + EU, and Pb + ZSC) for 21 days.

Oxidative stress is an abnormal condition in which our body's tissues are exposed to an increased influx of free radicals and can no longer capture or eliminate them effectively **(Agrawal et Sharma, 2010).** Lead excretion from organisms and lead's ability to be distributed and accumulated in the kidneys allows for direct toxic effects. Pb has been shown to induce kidney damage through several mechanisms, including general oxidative stress induction, essential cation interaction, inflammation, and induction of glomerular and tubular cell apoptosis. Some of its additional mechanisms are changes in renal gangliosides (plasma membrane lipids that play a role in the control of glomerular filtration), changes in renal vascular tone, and alterations in the renin-angiotensin-aldosterone hormonal system **(Vukelić *et al.,* 2023).**

The antioxidant enzymes, such as glutathione S-Transferase (GST), are regarded as the primary cellular defense against oxidative damage. The GST enzyme family plays a role in detoxification processes. Additionally, a crucial function of GST in response to oxidative stress is its ability to conjugate GSH with the products of lipid peroxidation **(Manawadi et Kaliwal, 2010).**

Our results recorded a significant increase in the antioxidant enzyme glutathione S-Transferase in the kidneys of lead nitrate-treated rats, which we find to be consistent with the findings of **Saka et al. (2011)** who showed an increased GST activity in lead acetate-treated rats. This enzyme participates in the conjugation reactions of electro toxic compounds resulting from the metabolism of lead acetate with GSH. These reactions lead to the formation of non-toxic mercapturic acids, which are subsequently excreted in the urine.

In our study, the administration of aqueous leaf extracts of both Eucalyptus and Ziziphus has shown promoting effects on glutathione S transferase activity in the kidneys of animals exposed to lead poisoning for 21 days, with a much better result for Ziziphus extract. Previous studies have demonstrated an amelioration of glutathione S-transferase activity in rats that received extracts of *Ziziphus spina-christi* leaves. This improvement is likely due to the presence of saponin, glucosides, and flavonoids, which act as antioxidants **(Agata *et al.,* 2009).** These compounds enhance the coupling with lead nitrate and mitigate its toxic effects. The extracts also elevate glutathione levels, attributed to the antioxidant properties of the flavonoids and moracin found in the leaves **(Othman *et al.,* 2009).** According to **Uadia et al. (2024),** *E. camaldulensis* reduced oxidative damage by mopping up free radicals, ameliorating the activities of antioxidant enzymes and thereby preventing lipid peroxidation.

5.3.2 Glutathione reduces GSH

The castration of rats using lead nitrates at a rate of 50 mg/kg for 21 days led to a notable decrease ($P < 0.01$) in renal glutathione (GSH) levels compared to the control group. However, when *Eucalyptus camaldulensis* leaf extract was added, we observed a noticeable improvement in renal GSH levels. Additionally, the *Ziziphus Spina-Christi* leaf extract restored renal GSH levels to their natural state, with no notable change compared to the control group.

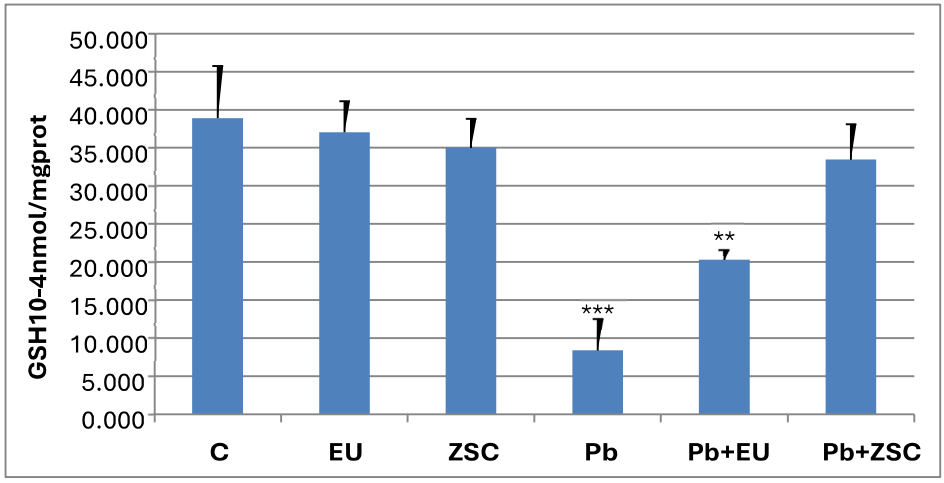

Figure 19. The variation in reduced glutathione levels (in nanomoles per milligram of protein) in the kidneys of both control rats and those treated with EU, ZSC, Pb, Pb+EU, and Pb+ZSC for 21 days.

Glutathione (GSH) serves as an excellent marker of oxidative stress. Previous research has shown that a significant decrease in glutathione (GSH) concentration, along with an increase in antioxidant enzyme activity, is due to the accumulation of peroxides **(Haleng *et al.*, 2007)**. This is what our results indicate, as they showed a significant decrease in the GSH value in the group treated with lead nitrate compared to the control group.

Because oxidative stress plays an essential role in the pathogenesis of lead poisoning, natural extracts, which may have antioxidative properties, may be useful for the treatment of this injury. Treatments with *Eucalyptus camaldulensis* significantly improved GSH activity and reduced toxicity. Previous analyses revealed the improvement of antioxidant status induced by *Eucalyptus camaldulensis* supplementation **(Marchant and Tawes, 2011; Li *et al.*, 2020).**

In the present study, we also evaluate the antioxidant potential effects of the leaf extract of Ziziphus in rats subjected to 50mg/kg/day of lead nitrate. ZSC extract inhibited Pb-induced

oxidative stress by increasing the content of GSH, restoring the activity of GST, and modulating the biochemical biomarkers in the kidneys. Thus, treatment with ZSC may have protected the rats against oxidative stress-induced organ damage by preserving cellular integrity; these protective effects may be due to the potent antioxidant component present in this plant **(Aliomrani *et al.,* 2016; Dkhil *et al.,* 2018; Almeer *et al.,* 2018).** Several studies have demonstrated the antioxidant activity of *Ziziphus spina-Christi*, its attenuated aflatoxin B1-induced oxidative damage by inhibiting lipid peroxidation and NO production and increasing the activity of SOD and GPx, in rats **(Abdel-Wahhab *et al.,* 2007)** Moreover, the methanolic extract of ZSC restored the levels of GSH and total antioxidant capacity in the colons of azoxymethane-treated rats **(Guizani *et al.,* 2013).** Additionally, the methanolic extract of ZSC decreased the activity of MPO, inhibited the level of lipid peroxidation, and elevated the activities of SOD and CAT, as well as the content of GSH, in rats intoxicated with carbon tetrachloride **(Amin and Mahmoud-Ghoneim, 2009).**

Conclusion
&
Perspective

Conclusion & Perspective

Lead is considered a metal that is dangerous to human health and the environment, as it causes serious consequences to the body, whether acute, immediate, or chronic in the long term. In this study, we wanted to investigate the damage and effect of lead nitrate on the kidneys, and work to reduce this damage through medicinal plants. In this context, our research focused on the effects of *Eucalyptus camaldulensis* and *Ziziphus spina-christi*. It is among the most widely used medicinal plants in herbal medicine and has therapeutic properties.

In this study, the effect of aqueous extract of *Eucalyptus camaldulensis* and *Ziziphus spina-christi* leaves on the biochemical parameters of antioxidants in the kidneys of Wistar albino rats was demonstrated.

Our results tried to show that the administration of lead nitrate by oral route at the rate of 50 ml/kg during 21 days, seems he cause a notable increase of renal obsolete and relative weight, uric acid and urea. GST activity in kidneys and depletion in GSH levels.

Eucalyptus camaldulensis and *Ziziphus spina-christi* leaves extract improves, reduces and reduces lead nitrate toxicity.

Specifically, the extract of *Ziziphus Spina-Christi* leaves played a major role in reducing the toxicity of lead nitrate, by returning the renal GST ratio to its normal value.

The observed potential therapeutic effect of these extract a on kidney parameters could be attributed to their biochemical contributes.

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

- Deepening our findings through a laboratory study to reveal the mode of action of *Eucalyptus camaldulensis* and *Ziziphus spina-christi* oils.
- Conduct a genotoxic study to look for DNA damage caused by lead.
- Study the basic composition of extracts to identify the molecules responsible for the therapeutic effects.
- Conducting a histological study to determine the extent to which cells are affected by lead nitrate, and the extent of their response to the leaf extract of *Eucalyptus Camaldulensis* and *ziziphus Spina-Christi.*

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