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Dietary effect of medicinal plants against heavy metal hepatotoxicity in wistar rats

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Abstract

Lead, a long-lasting pollutant, has been used by humans for ages, but its production skyrocketed during the industrial revolution, spreading it widely in the environment. Our study delved into the repercussions of lead exposure on hepatotoxicity, focusing on a range of physiological, biochemical, and stress parameters in rats. While examining the protective effects of *Eucalyptus camaldulensis* and *Ziziphus spina-christi* extracts against lead nitrate-induced toxicity in Albino-wistar rats. The latter were assigned to six groups: control, *Eucalyptus camaldulensis* extract (EU), *Ziziphus spina-christi* extract (ZSC), lead nitrate (Pb), and combinations of lead nitrate with each extract (Pb+EU, Pb+ZSC). Over 21 days, body weight, liver weight, blood parameters, biochemical markers, and oxidative stress indicators were measured.

Lead nitrate exposure was found to be toxic, affecting various physiological and biochemical parameters. The EU and ZSC extracts alone did not significantly alter these parameters. However, when combined with lead nitrate, both extracts demonstrated a protective effect, reducing the negative impact of lead exposure. This suggests that these plant extracts have hepatoprotective and antioxidative properties that may counteract lead toxicity.

The study indicates that *Eucalyptus camaldulensis* and *Ziziphus spina-christi* extracts could serve as natural therapeutic agents for mitigating heavy metal toxicity. Their potential to improve liver function and reduce oxidative stress warrants further investigation into their mechanisms and possible clinical applications in detoxification strategies.

Keywords: Lead toxicity, Hepatotoxicity, *Eucalyptus camaldulensis*, *Ziziphus spina-christi*, physiological parameters, biochemical parameters, stress parameters.

Résumé

Le plomb, un polluant durable, est utilisé par l'homme depuis des lustres, mais sa production a explosé pendant la révolution industrielle, le répandant largement dans l'environnement. Notre étude a exploré les répercussions de l'exposition au plomb sur l'hépatotoxicité, en se concentrant sur une gamme de paramètres physiologiques, biochimiques et de stress chez le rat. Tout en examinant les effets protecteurs des extraits *d'Eucalyptus camaldulensis* et de *Ziziphus spina-christi* contre la toxicité induite par le nitrate de plomb chez les rats Albino-wistar. Ces derniers ont été répartis en six groupes : contrôle, extrait *d'Eucalyptus camaldulensis* (EU), extrait de *Ziziphus spina-christi* (ZSC), nitrate de plomb (Pb) et combinaisons de nitrate de plomb avec chaque extrait (Pb+EU, Pb+ZSC). Pendant 21 jours, le poids corporel, le poids du foie, les paramètres sanguins, les marqueurs biochimiques et les indicateurs de stress oxydatif ont été mesurés.

L'exposition au nitrate de plomb s'est révélée toxique, affectant divers paramètres physiologiques et biochimiques. Les extraits d'EU et de ZSC seuls n'ont pas significativement modifié ces paramètres. Cependant, lorsqu'ils sont combinés avec le nitrate de plomb, les deux extraits ont démontré un effet protecteur, réduisant l'impact négatif de l'exposition au plomb. Cela suggère que ces extraits de plantes possèdent des propriétés hépatoprotectrices et antioxydantes pouvant contrer la toxicité du plomb.

L'étude indique que les extraits d'*Eucalyptus camaldulensis* et de *Ziziphus spina-christi* pourraient servir d'agents thérapeutiques naturels pour atténuer la toxicité des métaux lourds. Leur potentiel à améliorer la fonction hépatique et à réduire le stress oxydatif mérite une enquête plus approfondie sur leurs mécanismes et leurs éventuelles applications cliniques dans les stratégies de détoxification.

Mots-clés: Toxicité du plomb, Hépatotoxicité, *Eucalyptus camaldulensis*, *Ziziphus spina-christi*, paramètres physiologiques, paramètres biochimiques, paramètres de stress.

الملخص

يستخدم الرصاص، وهو ملوث طويل الأمد، من قبل البشر على مر العصور، لكن إنتاجه ارتفع بشكل كبير خلال الثورة الصناعية، وانتشر على نطاق واسع في البيئة. بحثت دراستنا في تداعيات التعرض للرصاص على السمية الكبدية، مع التركيز على مجموعة من المعايير الفسيولوجية والكيميائية الحيوية ومعايير الإجهاد في الفئران. أثناء فحص التأثيرات الوقائية للكليتوس *Eucalyptus camaldulensis* ومستخلصات السدر *Ziziphus spina-christi* ضد السمية الناتجة عن نترات الرصاص في فئران Albino-wistar. تم تعيين هذه الأخيرة إلى ست مجموعات: التحكم، ومستخلص الكليتوس الأوكالبتوس كامالدولينسيس (EU)، ومستخلص السدر زيزيفوس سبينا كريستي (ZSC)، ونترات الرصاص (Pb)، ومزيج من نترات الرصاص مع كل مستخلص (Pb + EU، Pb + ZSC). على مدار 21 يومًا، تم قياس وزن الجسم ووزن الكبد ومعلمات الدم والعلامات الكيميائية الحيوية ومؤشرات الإجهاد التأكسدي.

تم العثور على أن التعرض لنترات الرصاص سام، مما يؤثر على مختلف المعايير الفسيولوجية والكيميائية الحيوية. لم تؤثر مستخلصات EU و ZSC وحدها بشكل كبير على هذه المعايير. ومع ذلك، عندما تم دمجهما مع نترات الرصاص، أظهرت المستخلصات تأثيرًا وقائيًا، مما يقلل من التأثير السلبي للتعرض للرصاص. وهذا يشير إلى أن هذه المستخلصات النباتية لديها خصائص واقية للكبد ومضادة للأكسدة قد تعاكس سمية الرصاص.

تشير الدراسة إلى أن مستخلصات الكليتوس *Eucalyptus camaldulensis* و السدر *Ziziphus spina-christi* يمكن أن تكون عوامل علاجية طبيعية للتخفيف من سمية المعادن الثقيلة. يستحق قدرتها على تحسين وظيفة الكبد وتقليل الإجهاد التأكسدي مزيدًا من البحث في آلياتها وتطبيقاتها السريرية المحتملة في استراتيجيات إزالة السموم.

الكلمات المفتاحية: سمية الرصاص، سمية الكبد، الكليتوس، *Eucalyptus camaldulensis*، السدر، *Ziziphus, spina-christi*، المعايير الفسيولوجية، المعايير الكيميائية الحيوية، معايير الإجهاد.

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List of Abbreviations

ALAD	Aminolevulinic Acid Dehydratase
ALP	Alkaline Phosphatase
ALT	Alanine Amino Transferase
ANOVA	Analysis of Variance
AST	Aspartate Amino Transferase
BSA	Bovine Serum Albumin
CBC	Complete Blood Count
DO	Optical density
DTNB	5,5'-Dithio-bis-2-nitrobenzoic Acid
EDTA	Ethylene Diamine Tetra Acetic Acid
EUC	*Eucalyptus camaldulensis*
GSH	Glutathione
GST	Glutathione S-Transferase
H&E	Hematoxylin-Eosin
Hb	Hemoglobin
HCT	Hematocrit
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
LY	Lymphocytes
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MO	Monocytes
NE	Neutrophils
PAL	Alkaline Phosphatase
PLT	Platelets
Pb	Lead
Pb(NO3)2	Lead nitrate
RBC	Red Blood Cells
ROS	Reactive Oxygen Species
SEM	Standard Error of the Mean

WHO	World Health Organization
WBC	White Blood Cells
ZSC	*Ziziphus spina-christi*

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Introduction

Pollution is one of the greatest concerns of the population in our modern era. Industrial development, urban planning, and technologies have helped humanity and simplified daily life; however, they have caused the deterioration of the ecosystems on our planet and threaten living organisms, including human health **(Payne and Livesy, 2010).**

Among the major environmental contaminants, heavy metals pose serious ecological problems due to their ubiquitous nature, high persistence, and high toxicity **(Chouahda *et al.*, 2013)**. Lead is one of the oldest metals used by humans and has been exploited for millennia, but its use increased with the industrial revolution.

Lead (Pb) is a heavy metal present in the Earth's crust and all compartments of the biosphere **(Elhamalawy, 2018).** Pb enters the body either through food or beverages, particularly water **(Owoeye and Onwuka, 2016).** It is absorbed and conjugated in the liver and partially excreted in urine **(Shukla *et al.*, 2018).** It is known for its oxidative dangers in various tissues by causing an imbalance in the generation and movement of reactive oxygen species **(El-Masry *et al.*, 2016).**

The oxidative stress imposed on human health by environmental oxidants can be reduced by increased consumption of antioxidants. The chemical synthesis of antioxidants with anti-radical properties has become a primary research focus in academic and industrial research settings **(Attri *et al.*, 2017).**

Ziziphus spina-christi and *Eucalyptus Camaldulensis* both exhibit notable antioxidant properties, making them effective in combating oxidative stress. The latter occurs when there's an imbalance between free radicals and antioxidants in the body, leading to cellular damage and contributing to various diseases.

Eucalyptus Camaldulensis species is a genus of over 800 species of trees and shrubs, predominantly native to Australia. Their essential oils contain significant amounts of eucalyptol and other antioxidant compounds. These plants are renowned for their rapid growth, and distinctive aromatic leaves **(Batish *et al.*, 2008)**. Also, these elements help in scavenging free radicals and mitigating oxidative stress, offering potential therapeutic benefits in reducing inflammation and supporting overall health. Additionally, *Ziziphus spina-christi*, commonly known as Christ's Thorn, is a resilient tree native to the Middle East and parts of Africa **(Hussein, 2019).** It is prized for its edible fruits, medicinal properties, and use in traditional medicine. The plant is known for its potential health benefits, including anti-inflammatory, and antioxidant effects **(Hossain *et al.*, 2013).**

Furthermore, both *Ziziphus spina-christi* and *Eucalyptus Camaldulensis* are significant for their medicinal uses and natural therapeutic potential, particularly in traditional and herbal medicine practices. The antioxidant properties of both plants make them valuable in natural therapies aimed at managing and reducing oxidative stress, thereby promoting better health and preventing diseases **(Ghalem *et al.*, 2014 ; Kintz 2012).** The objective of our study is to determine the dietary effect of medicinal plants against heavy metals hepatotoxicity in rats, the impact on physiological evolution, biochemical and hematological parameters.

Our work is divided into three chapters:

- Bibliographic research
- Materials and methods
- Results and discussion

Finally, we conclude our work with a conclusion.

PART ONE. THEORETICAL SECTION

1. GENERALITIES ON LEAD

1.1. Physiochemical properties

Lead, from the Latin *plombum*, is a soft, gray metal with no characteristic taste or odor, typically found in small quantities in the Earth's crust. It belongs to group IVb of the periodic table of elements **(Pourrut, 2008).**

Additionally lead is a moderately reactive metal that dissolves slowly in water and most cold acids, but reacts more rapidly with hot acids. It does not easily react with oxygen in the air and does not burn. There are four naturally occurring isotopes of lead **(Djebbar, 2014)**.

Table 01. The Physiochemical properties of lead nitrate **(Ait hamadouche, 2010).**

atomic number	82
Boiling point	1740 °C
Melting point	327 °C
Density	11,35
Atomic mass (g.mol-1)	207,2
Valency	0, +2, +4
Electronegativity	1,8
Volumic mass	11,34 g.cm-3 to 20 °C
Atomic radius	0,154 nm
Ionic radius	0,132 nm (+2), 0,84 nm (+4)
Pictograms	

1.2. Sources of exposure and contamination

Natural sources of lead rarely result in high concentrations of lead in the environment. Lead poisoning is mainly due to human activities. This can include occupational exposure in industries such as metallurgy, chemicals, cement, or battery manufacturing. Environmental exposure can also occur through dust and paint or plaster flakes, automobile pollution from leaded gasoline, living near artisanal or industrial sites that emit lead into the air, consuming food contaminated with lead, or using lead water pipes **(Shaimi *et al*., 2014).**

1.2.2. Lead in water

The problem of metals contaminating drinking water continues to persist in urban environments, as various factors such as point source contamination, industrial activities, deteriorating pipe infrastructure, and other sources contribute to the elevated levels of these metals beyond the recommended health guidelines **(Triantafyllidou and Edwards, 2012).** Lead contamination in drinking water is often caused by the use of lead service pipes, fixtures, or solder in infrastructure **(Del Toral *et al.*, 2013).**

1.2.3. Lead in food

An international survey conducted by the WHO (World Health Organization) found high lead concentrations in certain foods such as spices and herbs, packaged beverages, shellfish, canned goods, fish, cereals, fruits, and vegetables. Meat products are less contaminated **(Galal-Gorchev, 1993).**

1.2.4. Lead in paints

The presence of white lead (basic lead carbonate) in paints was very significant in concentration (up to 50%) before the discovery of the harmful effects on children from inhaling and absorbing lead-laden dust **(Squinazi, 1994).**

1.3. Lead toxicokinetics

Understanding lead metabolism is crucial for comprehending certain aspects of intoxication, particularly the differences between children and adults, or between pregnant women and fetuses. It's important to assess the various phases of this metabolism, from absorption to elimination, including the transfer mechanisms in different tissues and the storage in certain organs, to derive insights regarding risk assessment and biological monitoring of exposed individuals. Ninety percent of particles smaller than 1µm inhaled through the respiratory tract are retained by the respiratory alveoli, and 3% to 50% of the retained lead passes into the bloodstream **(Missoun, 2012)**.

1.3.1 Absorption

The lead absorption pathways are pulmonary, digestive, and secondarily cutaneous **(Bismuth *et al.*, 2002).** Lead (Pb) is primarily absorbed through the digestive tract. It enters the body through ingesting contaminated food and water or inhaling substances containing lead **(Nemsadze *et al.*, 2009 ; Tamayo y Ortiz *et al.*, 2016).** After absorption, blood lead concentrations reach equilibrium approximately 3 months post-exposure. The majority of lead is stored in the liver.

1.3.1.1. Pulmonary absorption:

Children also absorb more lead through their respiratory tract than adults. Modeling of respiratory tract deposition of inhaled lead depends on particle size. Particles with a mean aerodynamic diameter >5 μm are cleared and swallowed by mucociliary mechanisms **(Nordberg *et al.*, 2014).**

1.3.1.2. Digestive absorption:

The fraction of ingested lead absorbed by the gastrointestinal tract depends on various factors, including age and the physiological characteristics of lead in the ingested medium (which varies at different pH levels). It is also influenced by dietary factors; diets deficient in iron or calcium facilitate the digestive absorption of lead, which occurs through an active transport mechanism and competes with these elements. Digestive absorption is also increased by fasting **(Oleko *et al.*, 2020).**

1.3.1.3. Dermal absorption:

Inorganic lead can be absorbed after exposure through inhalation, ingestion, and dermal contact, but the latter route is much less effective than the first two, so dermal absorption is negligible. Dermal exposure is only notable for organic lead derivatives. Absorption of lead through this route is influenced by contact surface area, concentration, solubility, and exposure duration. Animal studies have shown that organic lead is absorbed through the skin because it is liposoluble **(Abadin *et al.*, 2020).**

1.3.2. Blood transport:

After pulmonary or digestive absorption, lead enters the bloodstream where it distributes into red blood cells in a non-diffusible form. In plasma, lead is partly bound to proteins. The transport of lead into red blood cells occurs 90% via a passive mechanism, both for entry and exit. The entry rate is directly proportional to the amount of lead. Its accumulation system in red blood cells is saturable **(Bismuth *et al.*, 2002).**

1.3.3. Distribution

Lead diffuses and distributes in the body into several sectors, there are many compartments:

- a blood compartment with a half-life of 35 days (95% of lead is bound to red blood cells)
- a compartment consisting of soft tissues with an identical half-life, Soft tissues contain 5 to 10% of the internal dose: the majority of biologically active lead. Lead in soft tissues is responsible for most of the toxic effects.

- Hard tissues, including bones, contain the remaining lead with much longer half-lives, contributing to the body's total lead burden over time. Bone compartments (rapidly exchangeable lead in trabecular bone, slowly exchangeable lead, and very slowly exchangeable lead in the cortical part) with much longer half-lives, ranging from 5 to 20 years. The bone lead stock, unlike tissue, increases with age in an individual exposed to a normal environment: at 20 years old, bone represents 78% of the body's content, at 80 years old, it represents 96%. The slowly and very slowly exchangeable bone lead is extremely difficult to chelate **(Goullé *et al.*, 2012).**

1.3.4. Elimination

Lead is primarily eliminated in urine (75%). Fifteen to twenty percent is excreted in feces. It is also excreted in milk, saliva, sweat, nails, and hair **(Miquel, 2001).**

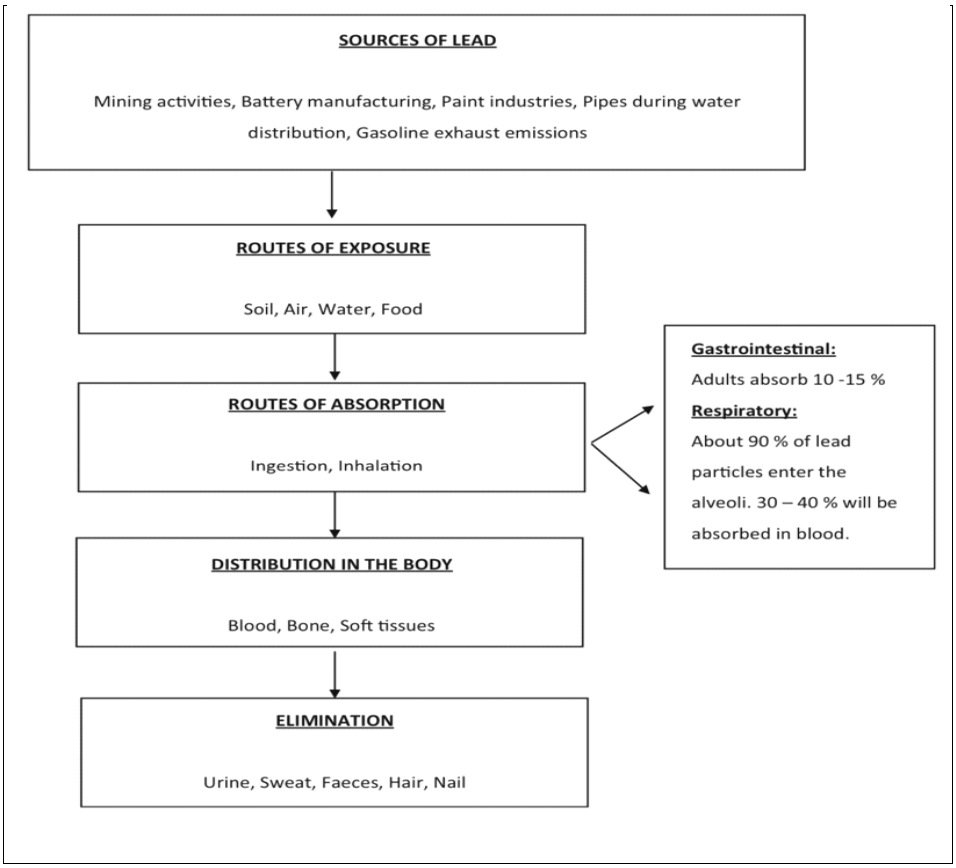


Figure 01. Sources and pathways of lead exposure **(Flora *et al.*, 2006; Shukla *et al.*, 2018)**.

1.4. Toxicity of lead and its impact on health

Lead toxicity can have severe effects on health, including:

1.4.1. The hepatic system

Lead induces significant hepatotoxicity and causes changes in cholesterol metabolism, hepatocyte proliferation, leading to hepatic hyperplasia or hypertrophy **(Dini *et al.*, 1999).** Hepatic cytolysis is a sign of acute lead poisoning, observed after massive contamination (> 1500µg/l) **(Niamane *et al.*, 2002).**

1.4.2. The hematopoietic system

Lead alters the properties of numerous cytosolic and membrane proteins by reversibly binding with thiol groups (-SH) or by displacing other metals. Specifically, in the biosynthesis of heme, it inhibits enzymes, particularly those in the heme biosynthesis pathway such as aminolevulinic acid dehydratase (ALAD) and ferrochelatase. Consequently, it decreases the lifespan of erythrocytes and alters iron metabolism by reducing binding capacity, causing maturation disorders of reticulocytes responsible for the presence of basophilic stippling of erythrocytes **(Saka *et al.*, 2011).**

1.4.3. The cardiovascular system

Permanent hypertension can develop in workers chronically exposed to high levels of lead or after several episodes of acute poisoning. This hypertension is linked to the metal's effect on vascular smooth muscle tissue **(Hajem *et al.*, 1990).**

1.4.4. The immune system

The effects of lead on the immune system are subtle and do not typically manifest in infectious symptoms. However, in cases of acute or chronic poisoning, the consequences are reflected in decreased levels of serum immunoglobulins (IgG, IgA, IgM), as well as a reduction in the percentage and absolute value of T cells and helper T cells. There is also a significant decrease in the adhesion and chemotaxis of macrophages and neutrophils **(Başaran and Ündeğer, 2000).**

1.4.5. The reproductive system

High lead exposure is associated with a risk of infertility or malformation, as it can affect the male reproductive system by impairing spermatogenesis and reducing testosterone levels **(Missoun, 2012)**. 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. Carcinogenic effect of lead

At high doses, lead can cause gastric, broncho-pulmonary, and urinary tract cancers. The International Agency for Research on Cancer has classified lead as a Group 2B substance, meaning it's possibly carcinogenic to humans **(Hervé-Bazin and Hoet, 2004).**

1.5. Lead and oxidative stress

Lead significantly reduces the performance of antioxidant systems by binding to sulfhydryl groups in antioxidant systems and subsequently displacing zinc and copper ions, which are important cofactors for antioxidant enzymes **(Flora *et al.*, 2007).** In the liver, lead binds to glutathione, and its conjugates accumulate in hepatorenal tissues, leading to oxidative stress, lipid peroxidation, and subsequent oxidative tissue damage **(Kumar *et al.*, 2016).**
Furthermore, some studies have shown that the pathogenesis of lead poisoning due to intracellular lipid peroxidation is due to the inhibition of antioxidant system activity through stimulation of free radicals and reactive oxygen species (ROS) **(Lakshmi *et al.*, 2013).**

2. GENERALITIES ON EUCALYPTUS

With over 800 species, the genus Eucalyptus is one of the most varied groups of flowering plants in the world **(Raho and Benali, 2014).** *Eucalyptus camaldulensis* is a tropical and subtropical plant that is a member of the Myrtaceae family. Also referred to as river redgum, it is a fast-growing plant that may flourish in a variety of climatic and edaphic circumstances **(Ahmad *et al.*, 2024).**

2.1. Botanic Study

Over 19 million hectares are covered by eucalyptus species plantations, which are among the most economically significant plantation plants globally **(Degefu *et al.*, 2023).** *Eucalyptus camaldulensis* grows to 20 m tall, at times to 50 m, with a trunk length of 1 (max. 2) m; in open formations, it has a large, spreading crown with a short, thick bole; in plantations, it has an erect, lightly branched crown with a clear bole of 20 m; the bark is smooth, white, grey, yellow-green, or pinkish-grey, and it sheds in irregular flakes or strips; the first 1-2 m of the trunk is occupied by rough bark. Grey-blue, alternating, drooping leaves that are 8–22 cm long and 1-2 cm wide. They are frequently sickle-shaped or curved, tapering, and short-pointed at the base. An axillary, single inflorescence with seven to eleven flowers; white, globular-rostrate or ovoid-conical flower buds; a hemispherical, rostrate or conical operculum measuring 4-6 x 3-6 mm and obtuse. Fruit tiny capsules with four valves, measuring 5-8 mm, at the end of thin stalks that hold tiny seeds **(Al-Snafi, 2017).**

Figure 02. *Eucalyptus camaldulensis* **(personal picture)**

2.2. Documented speice distribution

Known as river red gum, it is native to Australia. The world's countries with the highest growth rates of eucalyptus include Pakistan, India, Algeria, East Africa, Sudan, Malaysia, Philippines, and Ethiopia **(Ahmad *et al.*, 2024).**

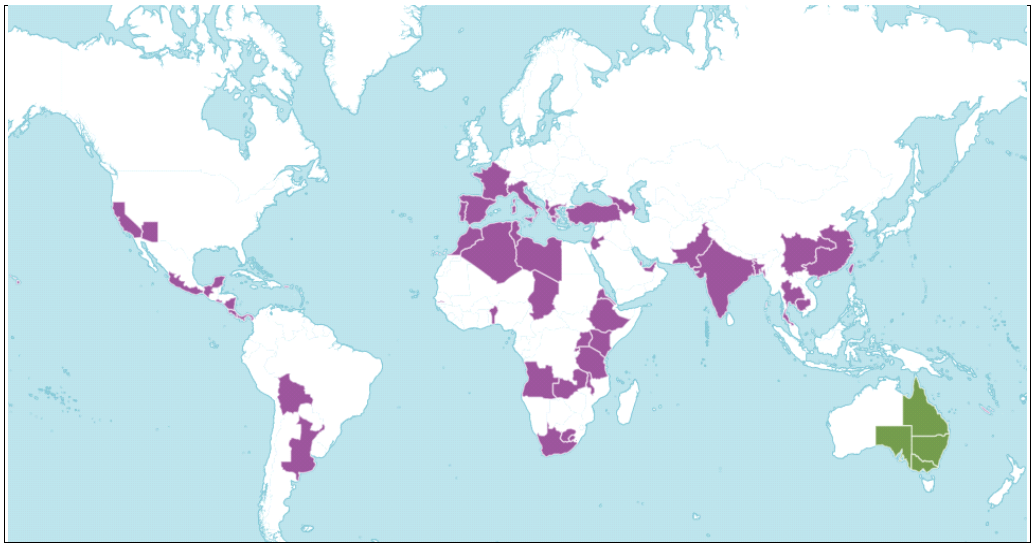


Figure 03. Geographic Distrubution of *Eucalyptus camaldulensis* **(Web).**

2.3. Chemical composition

The plant exhibits a diverse array of chemical components, prominently featuring terpenoids, alkaloids, flavonoids, tannins, saponins, and glucosides, alongside phenolic acids. Notably, fresh leaves primarily contain 1,8-cineole as the predominant compound **(Sànchez-Lordeo *et al.*, 2024).** also known as eucalyptol, is a cyclic ether and a monoterpenoid. Its chemical formula is C10H18O **(PubChem, 2022).**

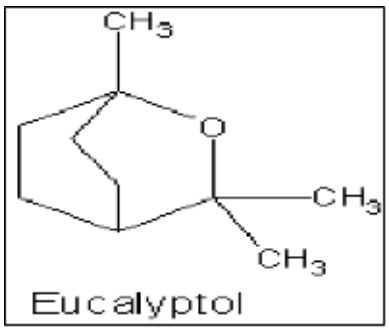


Figure 04. Structure of 1,8-cineole **(Rassaeifar *et al.*, 2013).**

2.4. Therapeutical properties

Leaves of *Eucalyptus camaldulensis* have been widely utilized in traditional medicine to cure a variety of illnesses **(Ghasemian *et al.*, 2019).** It is frequently used as a natural food addition for antioxidants. Experimental evidence indicates that this plant exhibits diverse therapeutic properties, including antimicrobial and antidiabetic effects **(Nwaogu *et al.*, 2021).**

3. GENERALITIES ON *ZIZIPHUS SPINA-CHRISTI*

Ziziphus spina-christi belongs to the family (Rhamnaceae) and is an armed shrub or evergreen tree called Christ's thorn having common Arabic names such as Sidr and Nabeq**.**

Figure 05. *Ziziphus spina-christi* **(personal photos)**

3.1. Botanic study

Ziziphus spina-christi thrives within specific parameters. It typically flourishes in altitudes ranging from sea level to 2,000 meters, favoring regions with mean annual temperatures spanning from 19 to 28 degrees Celsius. Moreover, its ideal habitat encompasses areas receiving annual rainfall between 100 to 500 millimeters. When it comes to soil preferences, *Ziziphus spina-christi* exhibits adaptability, thriving in alluvial plains with deep soils, as well as clayey terrains where water accessibility is ensured, and even saline soils. Understanding these biophysical limits is essential for cultivating and preserving diverse ecosystems **(Hussein, 2019).**

Table 02. Taxonomical classification of *Ziziphus spina-christi* within the plant kingdom. (Orwa *et al.*, 2009)

Kingdom	Plantae
Phylum	Angiosperms
Class	Eudicots
Orde	Rosales
Family	Rhamnaceae
Genus	Ziziphus
Species	spina-christi

3.2. Geografic distribution

Ziziphus spina-christi, originating across a wide expanse of Africa from Mauritania to the Red Sea, thrives in arid conditions, enduring high temperatures and minimal rainfall. It typically grows along the peripheries of water bodies like ponds and rivers where groundwater is accessible, exhibiting resilience to waterlogging for a limited duration and enduring prolonged dry seasons. With its ability to form dense thickets, it demonstrates a robust capacity for colonization **(Orwa *et al.*, 2009).**

3.2.1. Documented specie distribution

Native: Chad, Djibouti, Eritrea, Ethiopia, Kenya, Libyan Arab Jamahiriya, Mali, Mauritania, Nigeria, Pakistan, Senegal, Somalia, Tunisia, Turkey, Zimbabwe

Exotic: Algeria, Comoros, Egypt, India, Iran, Iraq, Israel, Jordan, Madagascar, Morocco, Netherlands, Saudi Arabia, Syrian Arab Republic, United Arab Emirates, Zanzibar **(Orwa *et al.*, 2009).**

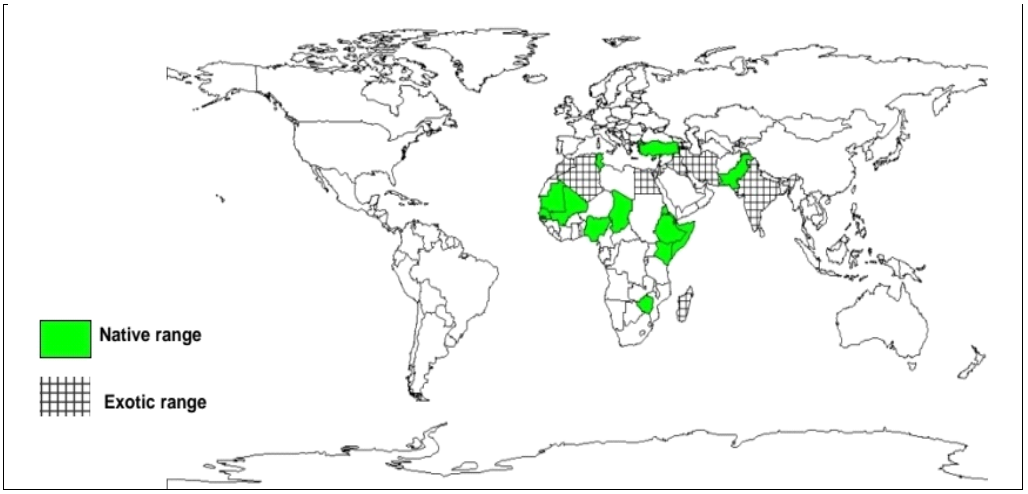


Figure 06. The map above displays the native and exotic regions in which the plant plant can be found **(Orwa *et al.*, 2009).**

3.3. Chemical composition

According to reports, 431 compounds were isolated from the genus *Ziziphus*, with alkaloids and flavonoids identified as the primary compound classes. In *Ziziphus spina-christi*, various components including saponins, fatty acids, and phenolics, alongside alkaloids and flavonoids, have been documented from different parts **(Abdulrahman, 2022).**

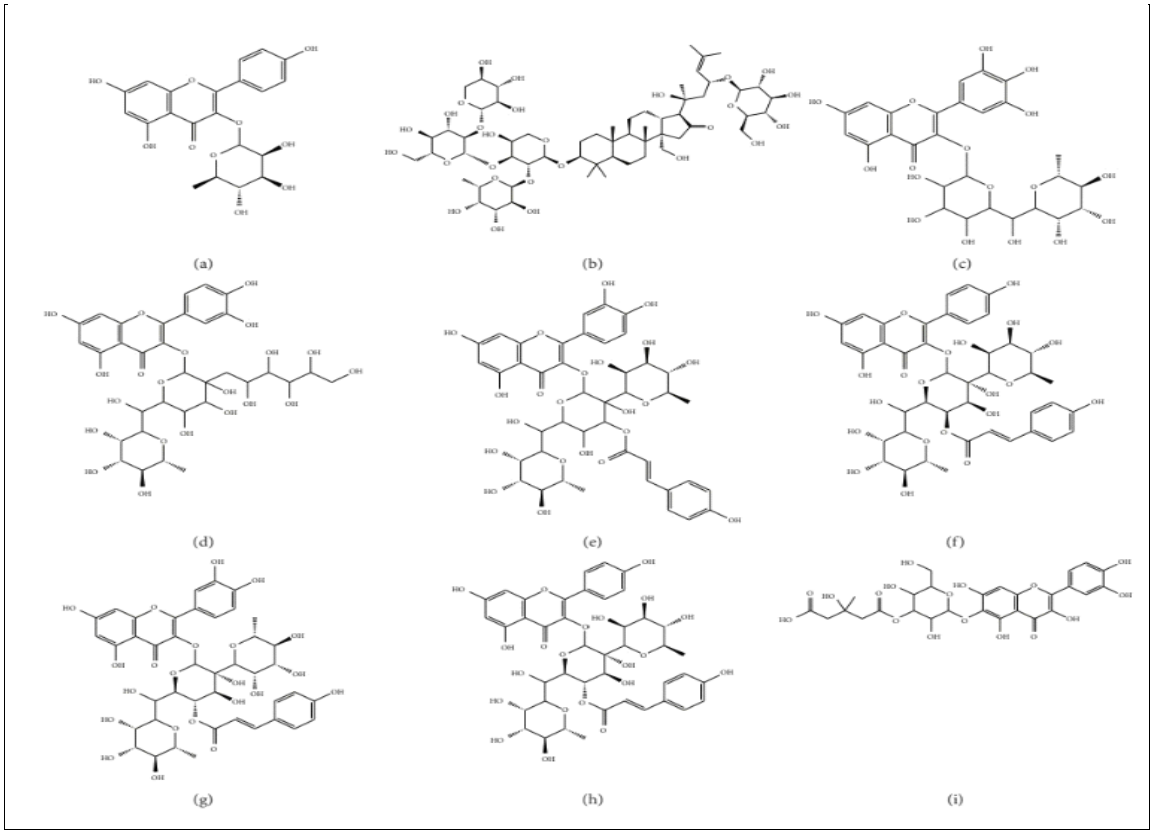


Figure 07. Some of the major compounds from *Ziziphus spina-christi* (a) Kaempferol-3-O-rhamnoside. (b) Jujuboside B1. (c) Myricetin-3-O-(6-rhamnosyl) hexoside. (d) Quercetin-3 O-[(2-hexonyl)-6-rhamnosyl]-hexoside. (e) Quercetin-3-O-p-coumaroyl (2,6-dirhamnosyl)-hexoside. (f) Kaempferol-3-O-(4-O-(p)-coumaroyl)-2-rhamnosyl-[6-rhamnosyl]-galactoside. (g) Quercetin-3-O-(4-O-p-coumaroyl)-2-rhamnosyl-[6-rhamnosyl]-glucoside. (h)Kaempferol-3-O-(4-O-p-coumaroyl)-2-rhamnosyl-[6-rhamnosyl]-glucoside. (i) Quercetin 3-O-[4-carboxy-3-hydroxy-3-methylbutanoyl]-(6)-hexoside **(Abdulrahman, 2022).**

3.4. Therapeutical properties

Ziziphus spina-christi has been utilized in unconventional medical practices to alleviate fever, pain, dandruff, wounds, ulcers, inflammatory issues, asthma, and eye ailments. *Ziziphus spina-christi* has recently been shown to have Antibacterial and antifungal properties, Antinociceptive effects, Antioxidant activity, and Anti-diabetic properties **(Asgarpanah and Haghighat, 2012).**

PART TWO. EXPERIMENTAL SECTION

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 dissolved 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-based solution and oral gavage **(personal photos)**

4.1.2. Plant materials and selection 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 *Rhamnaceae* and *Myrtaceae*, respectively. This selection was informed by a literature review and a simple survey conducted among the local population familiar with traditional medicine. The selection criteria are as follows :

- Wide availability of both plants across Algeria
- Traditional use in treating a variety of illnesses
- Non-toxicity of the plants, ensuring suitability for herbal tea preparation

4.1.3. Harvesting and identification

The aerial part of EU is harvested in February in the southern region of Ferkane – Tebessa. Botanical identification was conducted on a herbarium specimen by Ms. Guenez Radja, a doctor at the University of Tebessa. Dried sidr leaves are purchased from the local market originating from Souq-Ahras.

4.1.4. Preparation of decoctions

10 grams of each plant are steeped in 100 ml of boiling mineral water (at 100°C). After cooling, the decoction is filtered and stored in sterile bottles, shielded from light.

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

4.1.5. Care and treatment of the animals

In this study, we obtained 42 female Albino Wistar rats, aged 5 weeks, from the Pasteur Institute of Algiers (I.P.A.). The rats were bred at the animal facility of Larbi Tebessi University in Tebessa. They were housed in polyethylene cages labeled with batch names, treatment information, and experiment dates. The cages were lined with wood shavings and cleaned daily. During the adaptation period lasting about one month in the animal facility, the rats were kept under controlled conditions: a temperature of (22 ± 2°C) and a natural light/dark cycle (12/12 hours). They were fed a nutritionally balanced diet consisting of pellets, with water available ad libitum. Details of the diet composition are summarized in the table. The rats had an average initial weight of approximately 200g at the start of the experiment.

Table 03. Composition of 1 kg of feed

Feed Composition	Quantities in grams per kilogram (g/kg)	**%**
Corn	620	62
Soybean	260	26
Phosphate	16	1.6
Limestone	9	0.9
Cellulose	10	1.0
Minerals	10	1.0
Vitamins	10	1.0

After an acclimatization period, the animals were randomly divided into six groups of seven rats each. They underwent daily treatments for 21 days. Regular weighing was conducted to determine the appropriate treatment dosage.

Batch C **:** Control group: rats receive plain drinking water and standard food

Batch EU **:** The rats receive plain drinking water and 1 ml of a decoction prepared from Eucalyptus via oral gavage.

Batch ZSC **:** The rats receive plain drinking water and 1 ml of a decoction prepared from Sidr via oral gavage.

Batch Pb **:** The rats receive 50 mg/kg of lead nitrate via oral gavage.

Batch Pb+EU **:** The rats are administered orally with a dose of lead nitrate at 50 mg/kg, followed by the administration of 1 ml of an eucalyptus decoction 6 hours later.

Batch Pb+ZSC **:** The rats undergo oral gavage with a dose of lead nitrate at 50 mg/kg, followed by the administration of 1 ml of a Sidr decoction6 hours later

4.1.6. Blood sampling

After a 21-day period, the animals are euthanized by decapitation. Blood is promptly collected into two labeled tubes: one with EDTA for complete blood count (CBC), and the other with HEPARIN. These tubes are immediately centrifuged at 3000 rpm for 15 minutes. The resulting plasma will be used to analyze various biochemical parameters including AST, ALT, ALP, albumin, total bilirubin, triglycerides, and total cholesterol.

4.1.7. Liver tissue sampling

A longitudinal abdominal incision is made to extract the liver. Once cleared of adipose tissue, the organs are weighed and stored at -20°C in a freezer for oxidative stress biomarker analysis (GSH and GST).

Figure 10. Animal care, blood sampling and liver tissue sampling **(personal photos)**

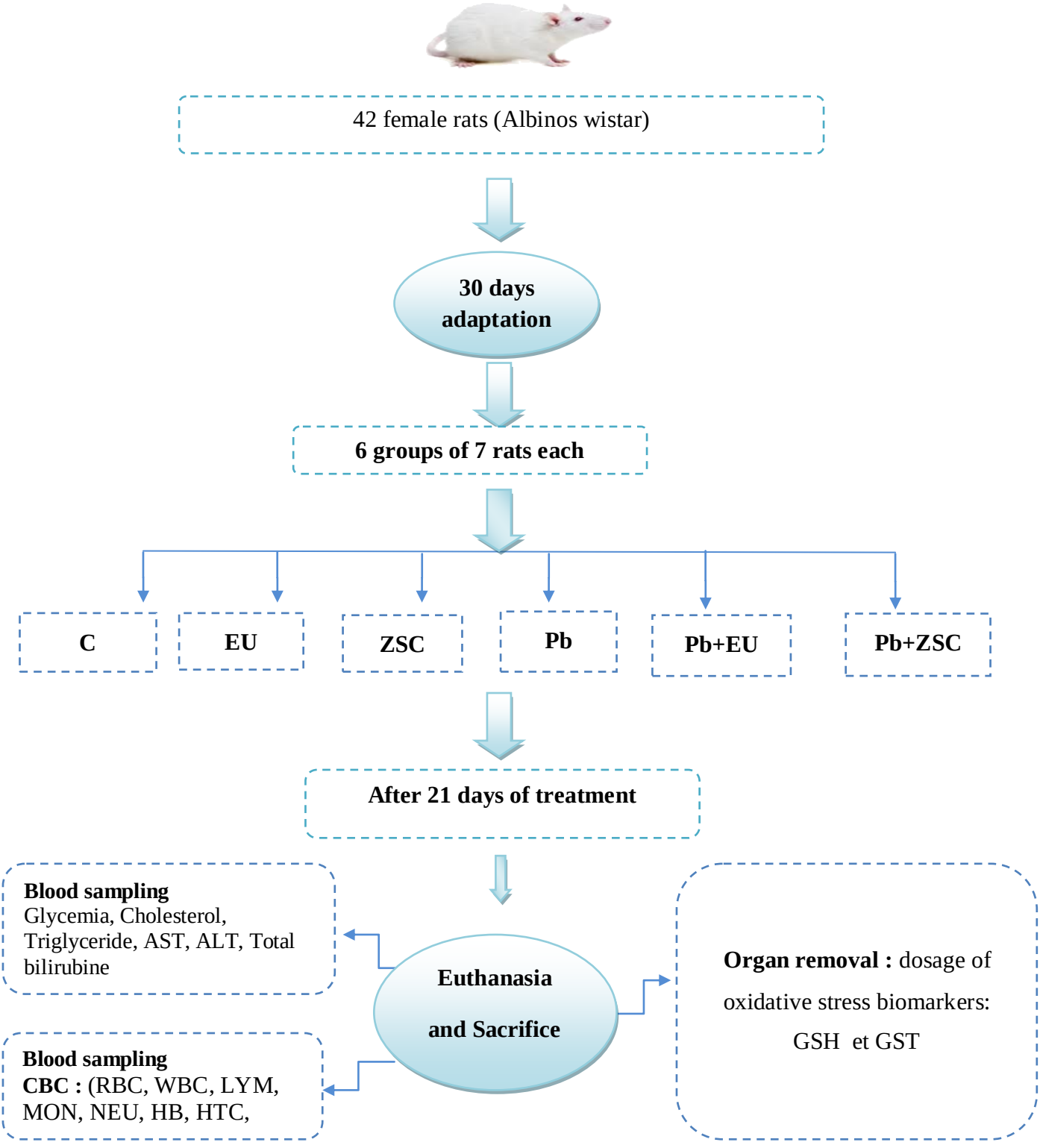


Figure 11. Diagram summarizing the experimental protocol.

4.2. Methods

4.2.1. Blood glucose measurement

Principle

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

Operating mode

- The reader automatically turns on upon inserting the OneTouch reactive strip (in the direction of the arrows until it stops).
- The symbol of a drop blinks.
- Place a drop of blood on the application area of the strip.
- The result displays within 5 seconds. Blood glucose levels are shown in g/dL.

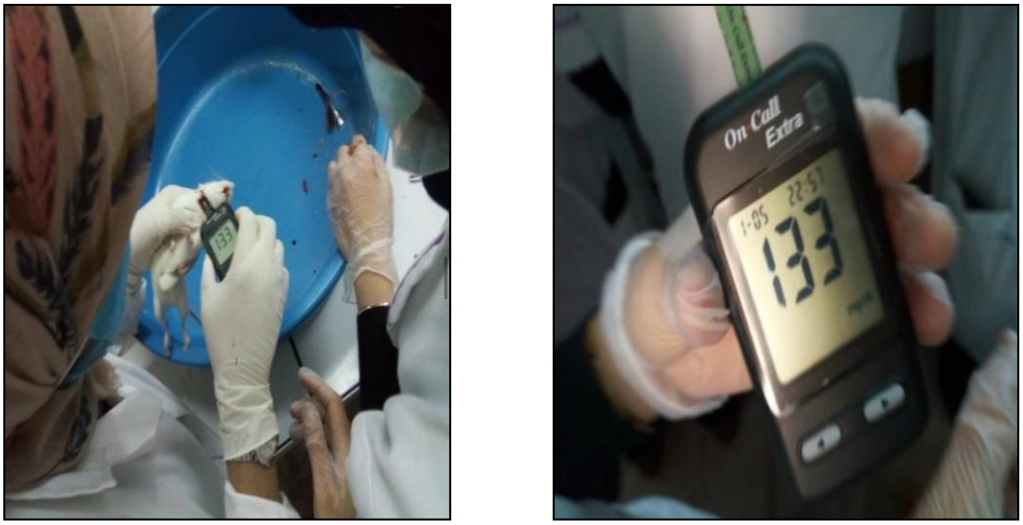


Figure 12. Blood glucose measurment **(personal photos)**

4.2.2. Hematological parameters assay

The complete blood count (CBC) was performed using the ERMA INC full automatic blood cell counter model (PCE-210N). A tube of whole blood with EDTA as the anticoagulant is placed into the analyzer, and the CBC analysis begins. Results are automatically displayed on the screen and then printed out. The parameters measured include: red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), hematocrit (HCT), platelets (PLT), lymphocytes (LY), monocytes (MO), neutrophils (NE), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC).

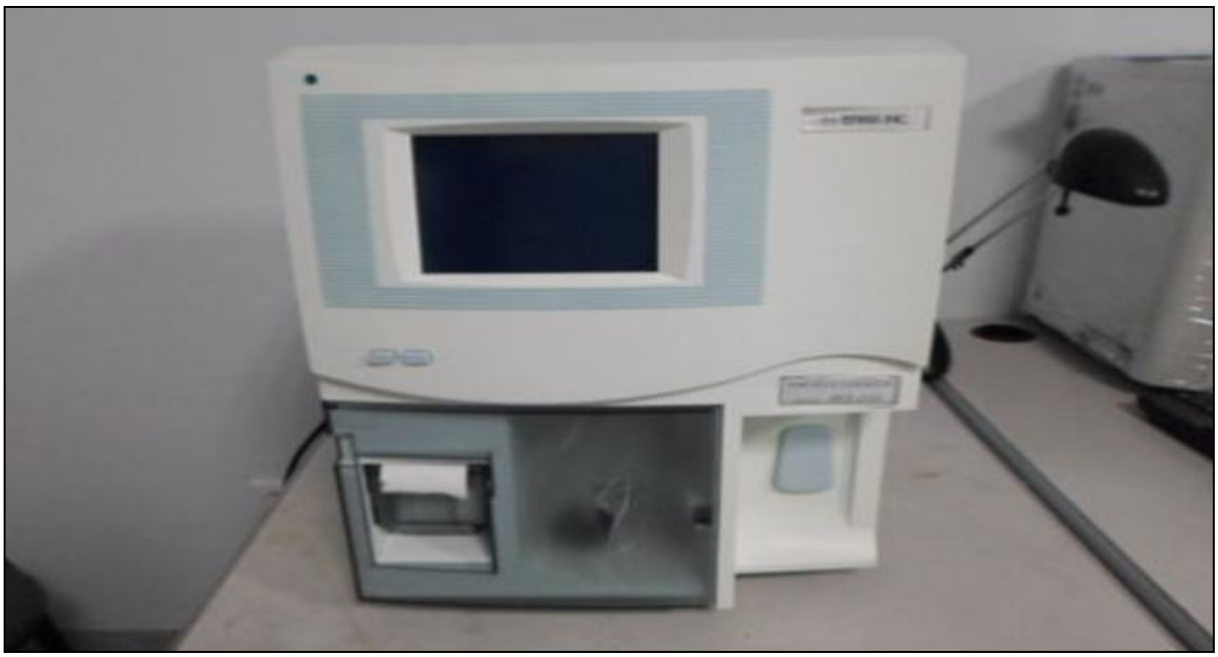


Figure 13. Analyzer PCE-210N for determining the complete blood count (CBC)

4.2.3. Albumin Assay

Principle

In a buffered solution at pH 4.2, bromocresol green combines with albumin to form a colored complex. The absorbance of this complex, measured at 630 nm (620-640 nm), is proportional to the albumin concentration in the specimen **(Doumas *et al.*, 1997).**

4.2.4. Assay of Alkaline phosphatases ALP

Principle

Colorimetric determination of alkaline phosphatase activity according to the following reaction scheme:

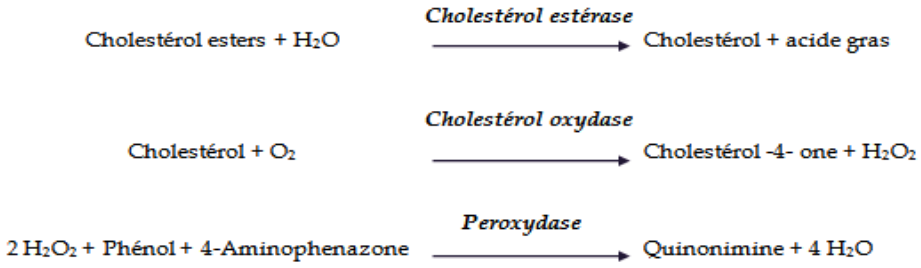
Alkaline phosphatase + Phenylphosphate → Phenol + Phosphate.

The phenol released by the substrate hydrolysis then forms, in the presence of amino-4-antipyrine and potassium ferricyanide, a red complex whose absorbance measured at 510 nm is directly proportional to the ALP activity in the specimen **(Kind *et al.*, 1954 ; Belfield *et al.*, 1971).**

4.2.5. Cholesterol Assay

Principle

The principle is shown in the following reaction diagram:



The cholesterol assay relies on enzymatic reactions to produce a colored product, proportionate to cholesterol concentration **(Allain *et al.*, 1974).**

4.2.6. Triglycerides Assay

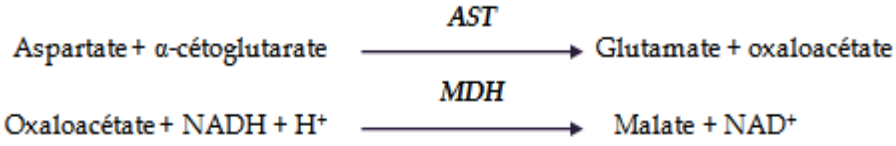
Principle

The assay principle involves enzymatic colorimetric reactions producing a color complex, its intensity correlating with triglyceride concentration **(Parhofer and Laufs, 2019).**

4.2.7. Assay of Aspartate amino transferase AST

Principle

The principle is shown in the following reaction diagram:

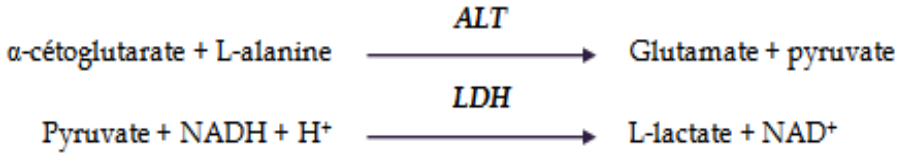


The assay of Aspartate Amino Transferase (AST) involves spectrophotometric measurement of its enzymatic activity, typically assessing the conversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate, with the concomitant reduction of NAD+ to NADH **(Rosalki and Rau, 1972).**

4.2.8. Assay Of Alanine amino transferase ALT

Principle

The cholesterol assay relies on enzymatic reactions to produce a colored product, proportionate to cholesterol concentration **(Allain *et al*., 1974).**



The ALT assay quantifies enzymatic activity spectrophotometrically, measuring the conversion of alanine and α-ketoglutarate to pyruvate and glutamate, with NAD+ reduction **(Bradford, 1976).**

4.2.9. Assay of Glutathione S-Transferase GST enzymatic activity

Principle

To measure GST activity, the enzyme is supplied with a substrate, usually 1-chloro,2.4-dinitrobenzene (CDNB), which reacts readily with the many forms of GST and glutathione. The conjugation reaction of these two products results in the formation of a new molecule that absorbs light at 340 nm **(Habig *et al.*, 1974).**

Homogenate preparation

100 mg tissue + 1ml TBS →→→→ cold grinding →→→→→ homogenate

Note: keep homogenates cold until homogenization is complete.

Centrifugation of the homogenate at 9000 rpm for 15min →→→→→ recovery of the supernatant for GST and mg protein determination

Operating mode

Table 04. Operating mode with reagents used

Reagents used	**Blank (µl)**	**Sample (µl)**
Phosphate buffer (0.1M, pH 6.5)	830	830
CDNB (0.0 2M)	50	50
GSH (0.1M)	100	100
Supernatant/distilled water	20	20

Calculation: GST concentration is obtained by the following formula

$$\text{GST (nmol GST / min / mg protéine)} = \frac{\text{(DO échantillon / min – DO blanc / min)}}{\text{9.6 x mg protéine}}$$

DO Sample / min : Sample optical density per minute

DO blanc / min : Optical density of the blank tube per minute

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

Figure 14. Assay of Glutathione S-Transferase GST enzymatic activity preparation **(personal photos)**

4.2.10. GSH reduced glutathione assay

Principle

Glutathione was determined using the method of Weckbecker 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-nitrobenzoïic acid (Ellman's reagent, DTNB) by glutathione (-SH) groups. To achieve this, the homogenate must be deproteinized to retain only the thiol groups specific to glutathione **(Dilmi *et al.*, 2018).**

Homogenate preparation

100 mg of tissue were placed in the presence of 4 ml of 0.02 M Ethylene Diamine Tetra Acetic Acid (EDTA) solution, then cold-ground 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% solution of sulfosalicylic acid.
- Shake the mixture and leave for 15 minutes in an ice bath.
- Centrifuge at 1000 rpm for 5 minutes.
- Take 0.5 ml of the supernatant.
- Add 1 ml of Tris buffer + EDTA (0.02 M EDTA), pH 9.6.
- Mix and add 0.025 ml of 0.01 M DTNB (dissolved in absolute methanol).
- Leave for 5 minutes at room temperature for color stabilization, which develops instantly. Read the optical densities at 412 nm against the blank.

Calculation: concentration of Glutathione is obtained by the following formula

$$[GSH]\ (nM\ GSH/mg\ protide) = (DO \times 1 \times 1{,}525) / (13100 \times 0{,}8 \times 0{,}5 \times mg\ protide)$$

DO : Optical density at 412nm

1 : : Total volume of solutions used in deprotonation

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

13100 : Absorbance coefficient of the (-SH) group at 412nm

0.5 : Volume of supernatant found in 1.525 ml

0.8 : Volume of homogenate found in 1ml



Figure 15. GSH reduced glutathione assay preparation **(personal photos)**

4.2.11. Protein assay

Principle

The protein concentration is determined using the Bradford method, which utilizes Coomassie Blue (G 250) as the reagent. The amine groups (-NH2) of proteins react with a reagent containing 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 the intensity establishes the protein concentration in the sample**.**

Operating Mode

- Take 0.1 ml of the homogenate.
- Add 5 ml of the Bradford reagent.
- Mix and let it stand for 5 minutes to stabilize the color.
- Read the optical density at 595 nm against the blank.
- The obtained optical density is compared against a previously constructed standard curve.
- The protein concentration is determined by comparing it to a standard range of bovine serum albumin (BSA) (1 mg/ml) prepared under the same conditions (see figure)

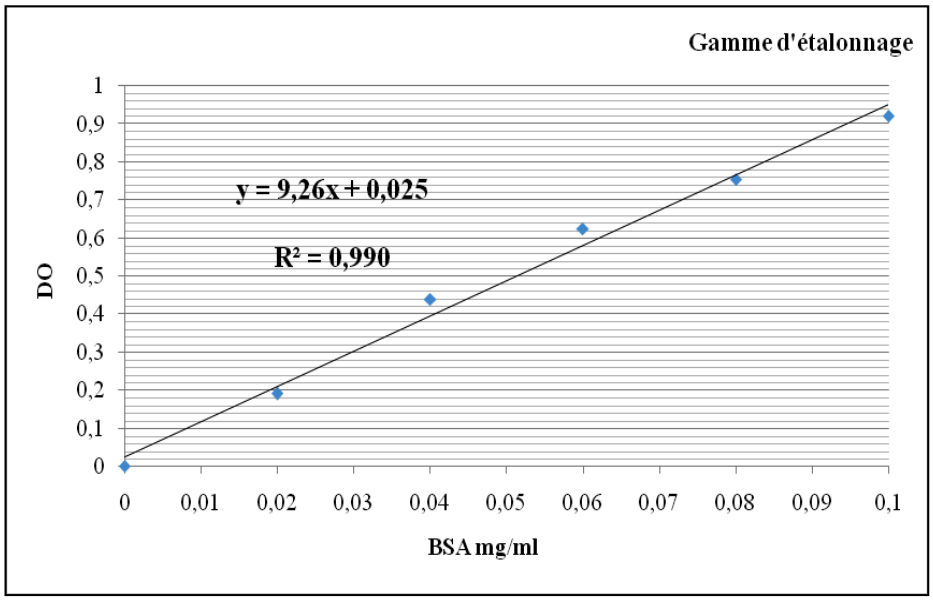


Figure 16. Bovine serum albumin calibration curve

Figure 17. Protein assay preparation **(personal photos)**

4.3. Statistical analysis

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.

5. Results and Discussion

5.1. Physiological parameters

5.1.1. Effect of treatments on body weight

The *Eucalyptus camaldulensis* extract (EU) was found to have the most positive impact on weight gain, while lead nitrate (Pb) had the most negative effect. When combined with lead, both the Pb+EU and Pb+ZSC treatments showed a slight improvement in mitigating lead's harmful effects. Interestingly, the combination including *Ziziphus spina-christi* (Pb+ZSC) was more effective in this regard compared to *Eucalyptus camaldulensis* (Pb+EU).

Table 05. 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**

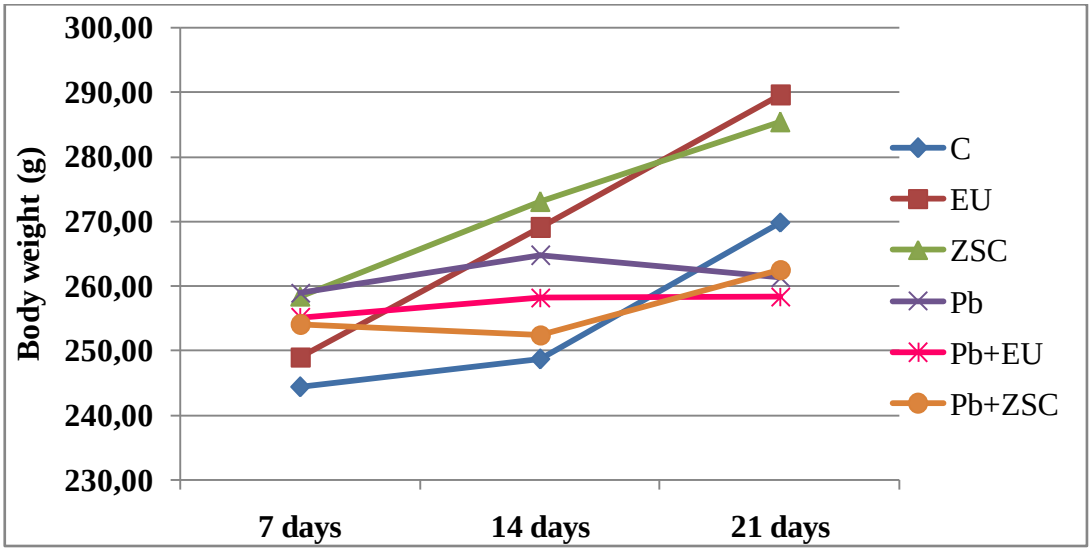


Figure 18. Effect of various treatments on body weight over 21 days

Studies have suggested that certain constituents of *Eucalyptus camaldulensis* extract may have a positive effect on weight gain, potentially enhancing appetite or improving nutrient absorption (**Silva *et al.*, 2018**). Conversely, lead exposure, particularly lead nitrate, consistently correlates with impaired growth and reduced weight gain in both animal and human studies due to its interference with nutrient absorption, metabolism, and hormonal regulation (**Patrick, 2006**). To counteract the adverse effects of lead toxicity, including its impact on weight gain, researchers have explored the antioxidant and hepatoprotective properties of various plant extracts, our research study focuses on *Eucalyptus camaldulensis* and *Ziziphus spina-christi*. These extracts, by reducing oxidative stress and enhancing liver function, offer potential mitigation strategies (**Amin and Mahmoud-Ghoniem, 2009**). Comparatively, the combination of lead nitrate with *Ziziphus spina-christi* extract (Pb+ZSC) demonstrates greater efficacy in mitigating lead's negative impact on weight gain than the combination with *Eucalyptus camaldulensis* extract (Pb+EU). This heightened effectiveness is attributed to the specific pharmacological properties of *Ziziphus spina-christi*, which appear particularly adept at counteracting lead toxicity **(Amin and Mahmoud-Ghoniem, 2009)**.

5.1.2. Effect of treatment on liver weight

Lead nitrate had a notable impact, significantly increasing liver weight. Interestingly, when administered alone, the extracts of *Eucalyptus camaldulensis* and *Ziziphus spina-christi* did not induce any changes in liver weight. However, when combined with lead nitrate, both extracts showed a mitigating effect, bringing liver weights closer to control levels. This suggests that while the individual extracts did not affect liver weight, they were effective in counteracting the effects of lead nitrate when used in combination with it.

Table 06. Effect of aqueous extracts *of Eucalyptus camaldulensis, Ziziphus spina-christi* leaves and/or lead nitrate treatment on liver 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 weight (g)	5,41±0,79	5,74±1,22	5,99±0,59	6,38±1,36*	5,59±0,66	5,64±0,93
Relative weight (%)	2,22±0,23	2,20±0,40	2,34±0,38	2,48±0,37	2,44±0,10	2,46±0,27
*P< 0,05 relative to the control group (C)						

Results show that lead nitrate induce liver damage, leading to hepatotoxicity characterized by alterations in liver function and morphology, including hepatocyte hypertrophy and damage (**Liu *et al.*, 2008**). In contrast, *Eucalyptus camaldulensis*, has been investigated for its potential hepatoprotective properties. Its bioactive compounds, such as flavonoids and phenolic compounds, exhibit antioxidant and anti-inflammatory activities, suggesting a protective effect against liver damage **(Aljawdah *et al.*, 2022).** Similarly, *Ziziphus spina-christi*, has been traditionally utilized for its medicinal properties. Studies have demonstrated its hepatoprotective effects, attributed to its antioxidant, anti-inflammatory, and anti-fibrotic properties, which could mitigate liver damage **(Yossef *et al.*, 2011).**

5.2. Blood parameters

Lead nitrate caused significant changes in blood parameters. When used alone, the extracts from *Eucalyptus camaldulensis* and *Ziziphus spina-christi* had little to no effect on these parameters. However, when combined with lead nitrate, these extracts partially reduced the negative effects of lead, with *Eucalyptus camaldulensis* showing slightly better results in certain parameters than *Ziziphus spina-christi*.

Table 07. Effect of aqueous extracts *of Eucalyptus camaldulensis, Ziziphus spina-christi* leaves and/or lead nitrate treatment on blood 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**
WBC 10^3/µl	8,29±1,50	9,17±1,42	10,35±1,13*	16,58±3,37**	14,04±2,10**	14,13±3,47**
Lym 10^3/µl	6,74±1,35	6,91±1,59	6,89±2,47	12,74±3,55***	9,81±1,85**	10,16±2,02**
Neu 10^3/µl	0,977±0,25	1,121±0,07	1,155±0,34	1,687±0,13**	1,577±0,16**	1,515±0,32*
Eosin 10^3/µl	0,141±0,02	0,135±0,01	0,170±0,06	0,293±0,06**	0,208±0,04*	0,226±0,07*
Baso 10^3/µl	0,656±0,09	0,921±0,09*	0,635±0,12	1,548±0,25***	1,442±0,13***	0,835±0,22
Mon 10^3/µl	0,083±0,02	0,085±0,01	0,095±0,03	0,390±0,06***	0,111±0,03	0,253±0,09**
RBC 10^6/µl	9,290±0,50	9,222±0,63	9,115±0,32	8,822±0,71	8,893±0,37	8,847±0,72
Hb g/dl	15,333±0,30	15,183±0,75	14,780±1,51	13,800±0,58**	13,950±0,88**	13,933±0,52**
HTC %	47,20±1,15	47,13±2,58	45,68±4,40	43,45±1,61**	44,58±2,43*	43,58±1,75**
MCV fl	53,30±1,72	55,17±1,51	54,08±1,11	47,97±1,10***	50,10±0,88**	49,48±3,14**
MCHC g/dl	32,150±0,25	32,183±0,17	32,150±0,49	31,550±0,39*	31,183±0,37*	31,200±1,20*
PLT 10^6/µl	1006±123	1044±162	1041±199	1155±123	1089±145	1106±102
*P< 0,05 **P<0,01 ***P<0,001 relative to the control group (C)						

Lead exposure has been associated with immunotoxic effects, leading to alterations in white blood cell counts and their differentials. An increase in WBC count, along with changes in lymphocytes, neutrophils, eosinophils, basophils, and monocytes, suggests an inflammatory or immune response to lead toxicity (**Patrick, 2006**). Conversely, Exposure to lead can disrupt both heme synthesis and erythropoiesis. This interference predominantly manifests through the inhibition of enzymes crucially involved in the heme biosynthesis pathway, notably including aminolevulinic acid dehydratase (ALAD) and ferrochelatase **(Saka *et al.*, 2011).** such as aminolevulinic acid dehydratase (ALAD) and ferrochelatase., resulting in a slight decrease RBC count, and significantly decreasing hemoglobin levels, hematocrit, mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC)

(**Flora *et al.*, 2007**). These changes indicate possible anemia or impaired red blood cell production due to lead-induced toxicity. Platelet count slight elevation in response to lead exposure suggests a potential reaction to inflammation or tissue damage **(Ji *et al.*, 2021).** Platelets are involved in hemostasis and can increase in response to various stressors, including inflammatory processes. While studies have shown that *Eucalyptus camaldulensis* and *Ziziphus spina-christi* possess various pharmacological properties, including anti-inflammatory and immunomodulatory effects, their direct impact on blood parameters may be limited when administered alone **(Aljawdah *et al.*, 2022 ; Yossef *et al.*, 2011).**

5.3. Biochemical parameters

Significant changes were observed in various biochemical parameters compared to the control group. The combination treatments (Pb+EU and Pb+ZSC) showed promising reductions in liver enzyme levels against lead alone, indicating potential protective effects. Additionally, the Pb+EU and Pb+ZSC groups displayed improvements in markers of liver function and lipid metabolism

Table 08. 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±0.082	1.393±0.066	1.362±0.118	1.411±0.085	1.310±0.186	1.285±0.065*
ALT (UI/L)	26,66±1,23	23,46±1,03**	26,72±5,14	37,70±3,73**	31,90±5,21	28,66±6,42
AST (UI/L)	147,81±8,81	146,42±7,62	148,6±11,8	166,0±11,2*	151,8±17,8	155,9±23,0
PAL (UI/L)	71,0±14,6	91,8±15,3	97,20±7,22*	121,11±4,67**	102,4±21,9*	114,2±18,9**
T. bilirubin (mg/L)	1,556±0,20	1,592±0,17	1,598±0,12	1,836±0,27	1,844±0,34	1,662±0,33
Albumin (g/L)	39,68±2,63	38,90±0,91	36,49±4,29	33,31±2,42**	34,77±2,20**	33,44±5,41*
Triglycerides (g/L)	0,790±0,16	0,813±0,29	0,700±0,22	0,952±0,21*	0,885±0,25*	0,848±0,24
Cholesterol (g/L)	0,657±0,10	0,678±0,05	0,666±0,06	0,778±0,11	0,705±0,06	0,737±0,10
$^{*}P< 0,05$ $^{**}P<0,01$ $^{***}P<0,001$ relative to the control group (C)						

The data demonstrate increased ALT levels, which is indicative of liver stress or damage, and lead exposure has been associated with hepatotoxicity, leading to elevated ALT levels (**Flora *et al.*, 2007**). Similarly, increased AST levels suggest liver damage, as AST is another enzyme released into the bloodstream when liver cells are damaged (**Bernal and Wendon, 2013**). The increased level of these enzymes might be due to leakage from the tissue as a result of damage caused by lead. This observation is similar to the report of (**Al-Megrin *et al.*, 2019 ; Alhusaini *et al.*, 2019**). They reported that elevated serum levels of ALT and AST can be linked to damage of the membrane due to oxidative stress induced by lead (**Ilesanmi *et al.*, 2022**). Elevated PAL levels can indicate liver or bone issues, and lead exposure has been linked to increased PAL levels, reflecting liver dysfunction (**Burtis and Ashwood, 1999**). Increased bilirubin levels suggest liver dysfunction or impaired bilirubin

metabolism, which can occur due to lead exposure disrupting hepatic function (**Schuppan and Afdhal, 2008**). Decreased albumin levels, synthesized in the liver, can indicate liver or kidney issues, and their reduction is associated with liver dysfunction **(Giacobbe *et al.*, 2018).** Several studies have also reported associations between lead exposure and alterations in lipid metabolism, including increased triglyceride levels. As mentioned earlier, studies like **(Zhang *et al.*, 2022 ; Wang *et al.*, 2015).** have found positive associations between blood lead levels and triglycerides. The observed protective effects of *Eucalyptus camaldulensis* and *Ziziphus spina-christi* extracts against lead nitrate-induced biochemical changes suggest their potential in ameliorating liver damage and restoring liver function **(Flora *et al.*, 2007).** The hepatoprotective effects of these extracts may be attributed to their ability to scavenge free radicals, inhibit inflammatory pathways, and enhance antioxidant defenses in the liver **(Bencheikh *et al.*, 2019).**

5.4. Stress parameters

5.4.1. Effect of treatments on Glutathione S-Transferases And Glutathione

Lead exposure significantly increases glutathione S-transferases activity, yet treatment with *Eucalyptus camaldulensis* (EU) and *Ziziphus spina-christi* (ZSC) extracts mitigates this effect. ZSC demonstrates slightly more consistent results compared to EU. Additionally, lead exposure reduces glutathione levels in the liver; however, both EU and ZSC treatments help alleviate this effect. Interestingly, EU exhibits slightly less variability in its protective effect compared to ZSC.

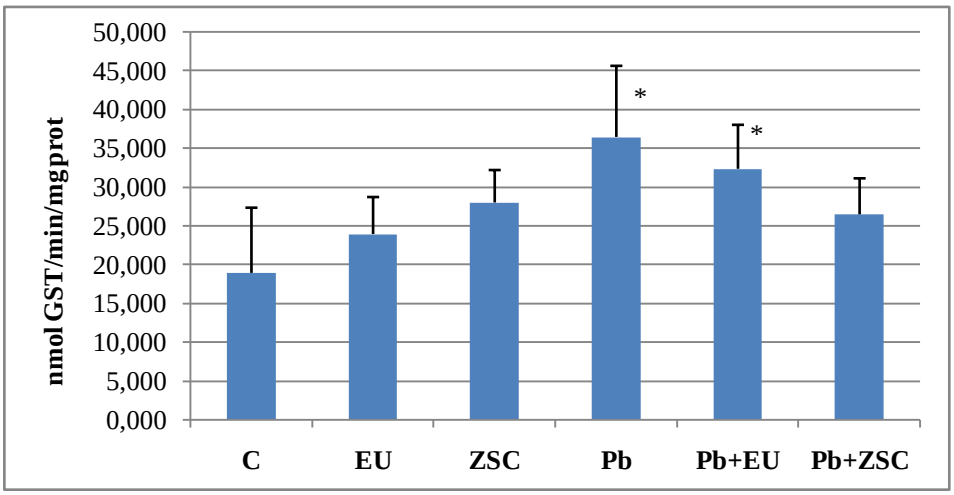


Figure 19. GST activity on various treatments

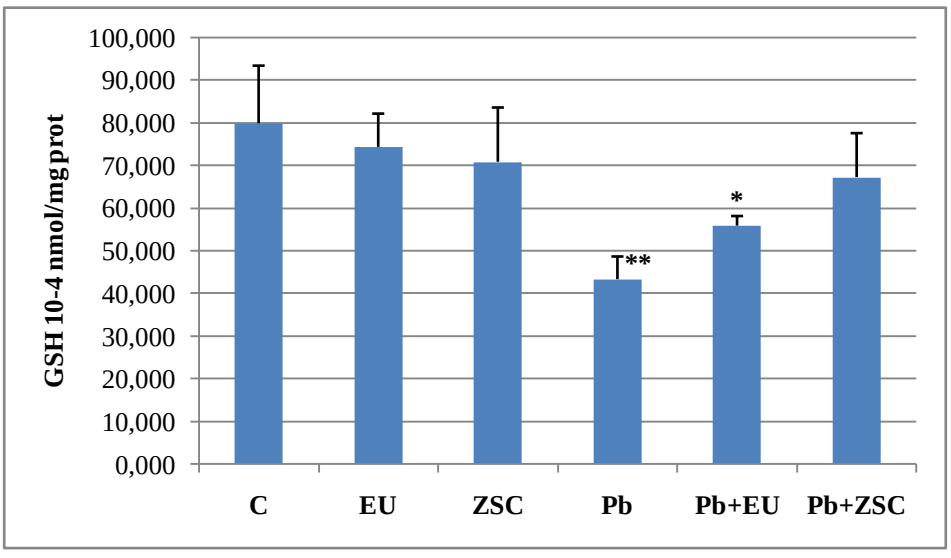


Figure 20. GSH levels on various treatments

Lead exposure induces oxidative stress and disrupts cellular antioxidant defenses, prompting the body to upregulate enzymes like glutathione S-transferases (GSTs) **(Vàzquez-Medina *et al.*, 2007).** GSTs play a crucial role in detoxification by conjugating glutathione with electrophilic compounds, including lead. The observed mitigation of lead-induced GST increase by *Eucalyptus camaldulensis* (EU) and *Ziziphus spina-christi* (ZSC) extracts suggests their potential in attenuating the oxidative stress and cellular damage caused by lead exposure **(Bencheikh *et al.*, 2019).** This effect could be attributed to their antioxidant properties and ability to modulate detoxification pathways. While both EU and ZSC extracts show some efficacy in mitigating the increase in GSTs induced by lead exposure, ZSC appears to demonstrate slightly more consistent results. This difference could be due to variations in the composition and potency of bioactive compounds present in the extracts **(Sharma *et al.*, 2021).** Additionally, lead exposure induces oxidative stress by generating reactive oxygen species (ROS) and depleting antioxidant reserves, including glutathione (GSH) (**Patrick, 2006**). GSH plays a crucial role in scavenging ROS and maintaining cellular redox balance. The observed mitigation of lead-induced glutathione depletion by EU and ZSC extracts suggests their potential in restoring cellular redox balance and protecting against oxidative damage **(Bencheikh *et al.*, 2019).** This effect could be attributed to their ability to enhance GSH synthesis or prevent its depletion. While both EU and ZSC extracts show efficacy in mitigating lead-induced glutathione depletion, EU appears to exhibit slightly less variability in its protective effect compared to ZSC. This difference could be due to variations in the composition and potency of bioactive compounds present in the extracts **(Sharma *et al.*, 2021).**

Conclusion and perspective

In conclusion, the findings of this research underscore the significant impact of various treatments, particularly *Eucalyptus camaldulensis* and *Ziziphus spina-christi* extracts, on mitigating the adverse effects of lead exposure on physiological, blood, biochemical, and stress parameters. The positive effect of *Eucalyptus camaldulensis* extract on weight gain, coupled with its hepatoprotective properties, highlights its potential as a therapeutic agent against lead-induced toxicity. Furthermore, the combination treatments, Pb+EU and Pb+ZSC, showed promising results in alleviating lead's adverse effects on liver function and lipid metabolism. Notably, *Ziziphus spina-christi* extract demonstrated marked efficacy in mitigating lead-induced changes in blood parameters and oxidative stress. This indicates its strong potential in ameliorating the detrimental effects of lead exposure. Specifically, *Ziziphus spina-christi* was effective in restoring normalcy in red and white blood cell counts, hemoglobin levels, and other critical hematological parameters that are typically disrupted by lead toxicity. Additionally, its antioxidant properties played a crucial role in reducing oxidative stress markers, thereby protecting cellular integrity and function. These findings contribute to the growing body of evidence supporting the use of plant extracts as natural remedies for lead toxicity. They underscore the importance of *Ziziphus spina-christi*, not just as a complementary treatment, but as a significant standalone intervention against lead-induced physiological and biochemical disruptions. The study advocates for further research into the mechanisms by which *Ziziphus spina-christi* exerts its protective effects, as well as its potential applications in clinical settings. This could pave the way for developing effective, natural intervention strategies to combat lead toxicity, enhancing public health outcomes.

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