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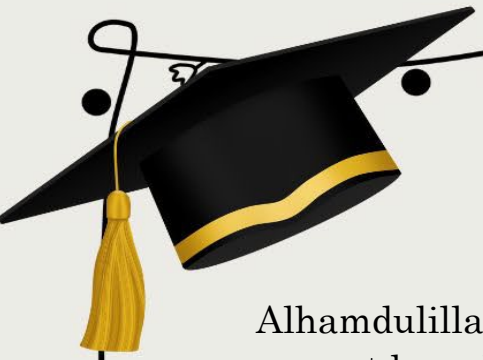
Entitled :

Hepatic alteration in rats exposed to pesticide and potential therapeutic effects of medicinal plants extract

Before the jury :

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Dedication

All praise is to Allah. This work is a blessing from Him and the fruit of years of diligent effort. It also represents the culmination of the unwavering support and inspiration I have received from individuals throughout my academic journey. With immense pride and gratitude, I dedicate this research to my beloved ones, who were instrumental in its success.

To my dearest parents, Ben Hamza Y. and Ben Hamza K., whose constant support and sacrifices made this journey possible—your love and encouragement have been my greatest strength.

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To my pillars and companions,my Giant family—my joy, my strength, and my greatest supporters—my wonderful hopes and the ones to whom I am eternally grateful. You have always encouraged me, and continue to do so, to become a courageous woman unafraid of stumbling. This achievement is as much yours as it is mine, and I am endlessly thankful to walk this journey—and all of life—by your side.

To the companions of the Quran who have profoundly enriched my life with their kindness, optimism, and uplifting prayers—I dedicate this work as a token of gratitude for your love.B.Abla,B.Insaf,K.Isra.

May Allah preserve you for me and bless you all with health, well-being, and happiness.

Ben hamza . A



Dedication

To those who lived life for me, to those who planted dreams in my heart and watered them with prayers, to those who were the light of my path and my shade when the days weighed me down, to my mother and father, the meaning of life and its beauty.

to my firm ribs and the safety of my days, to those who strengthened my arm and were springs from which I quench my thirst, to the best and purest of my days, to the joy of my eyes,

Dhia, Islam, and Loudjain .

to everyone who was a help and support on this path,

Nour, Ikram, Raouia, Imen,Ilhem and Wafaa.

to those who showered me with their feelings and advice,

to you, my family, to loyal friends and companions of the years,

to those who face hardships and crises, and especially my loyal companion in this stage,

Aya.

I dedicate to you this achievement and the fruit of my success that I have always wished for. Today

I have completed and perfected its first fruits, by His grace, glory be to Him

B.Insaf

Abstract

Neonicotinoids, such as acetamiprid (ACE), are highly water-soluble neurotoxic insecticides known for their rapid absorption and bioaccumulation in plant and animal tissues, raising concerns about their potential hepatotoxic effects. This study investigated the impact of ACE exposure on physiological, biochemical, and oxidative stress parameters in Albino-Wistar rats, while also evaluating the protective role of *Juniperus phoenicea* (JNP) extract. 28 rats were divided into four groups of seven rats each: control(receive drinking water and standard food), JNP-only (receive drinking water and a specific dose of a decoction prepared from 200mg/kg of *juniperus* via oral gavage), ACE-only (receive 20 mg/kg of acetamiprid) and a combined ACE+JNP group(administered orally with a dose of acetamiprid at 20mg/kg followed by the administration of 200mg/kg of *juniperus* decoction4 hours later)with assessments conducted over 30 days on body weight, liver weight, biochemical markers, and oxidative stress indicators.

Results demonstrated that ACE induced significant hepatotoxicity (increase in ALT,AST,Triglycerides,Cholesterol,CAT,decrease in Albumin,GST,GSH) whereas JNP alone had no adverse effects. Notably, co-administration of JNP with ACE mitigated ACE-induced toxicity, suggesting that *Juniperus phoenicea* extract possesses hepatoprotective and antioxidative properties capable of counteracting pesticide damage.

These findings highlight the potential of *Juniperus phoenicea* as a natural therapeutic agent for detoxification, warranting further research into its mechanistic pathways and clinical applications in mitigating pesticide-related toxicity.

Keywords: Acetamiprid toxicity, Hepatotoxicity, *Juniperus phoniecea*, physiological parameters, biochemical parameters, stress parameters.

Résumé

Les néonicotinoïdes, tels que l'acétamipride (ACE), sont des insecticides neurotoxiques hautement solubles dans l'eau, connus pour leur absorption rapide et leur bioaccumulation dans les tissus végétaux et animaux, soulevant des inquiétudes quant à leurs effets hépatotoxiques potentiels. Cette étude a examiné l'impact de l'exposition à l'ACE sur les paramètres physiologiques, biochimiques et de stress oxydatif chez des rats Albino-Wistar, tout en évaluant le rôle protecteur de l'extrait de *Juniperus phoenicea* (JNP). Vingt-huit rats ont été répartis en quatre groupes de sept rats chacun : un groupe témoin (recevant de l'eau potable et une alimentation standard), un groupe JNP seul (recevant de l'eau potable et une dose spécifique de décoction préparée à partir de 200 mg/kg de *Juniperus* par gavage oral), un groupe ACE seul (recevant 20 mg/kg d'acétamipride) et un groupe combiné ACE+JNP (recevant oralement une dose d'acétamipride à 20 mg/kg suivie de l'administration de 200 mg/kg de décoction de *Juniperus* 4 heures plus tard). Les évaluations ont été menées sur 30 jours, portant sur le poids corporel, le poids du foie, les marqueurs biochimiques et les indicateurs de stress oxydatif.

Les résultats ont démontré que l'ACE induisait une hépatotoxicité significative (augmentation des ALT, AST, triglycérides, cholestérol, CAT ; diminution de l'albumine, GST, GSH), tandis que le JNP seul n'avait aucun effet indésirable. Fait notable, la co-administration de JNP avec l'ACE a atténué la toxicité induite par l'ACE, suggérant que l'extrait de *Juniperus phoenicea* possède des propriétés hépatoprotectrices et antioxydantes capables de contrecarrer les dommages causés par les pesticides.

Ces résultats soulignent le potentiel de *Juniperus phoenicea* en tant qu'agent thérapeutique naturel pour la détoxification, justifiant des recherches supplémentaires sur ses mécanismes d'action et ses applications cliniques dans l'atténuation de la toxicité liée aux pesticides.

Mots-clés: Toxicité du L'acétamiprid Hépatotoxicité, *Juniperus phoniecea* paramètres physiologiques, paramètres biochimiques, paramètres de stress.

الملخص

تعتبر النيونوتينويدات، مثل الأسيتامبريد(ACE) ، مبيدات حشرية سامة للأعصاب وقابلة للذوبان بشكل كبير في الماء، وتتميز بامتصاصها السريع وتراكمها الحيوي في الأنسجة النباتية والحيوانية، مما يثير القلق بشأن تأثيراتها السامة المحتملة على الكبد. درست هذه الدراسة تأثير التعرض للأسيتامبريد على المعايير الفسيولوجية والكيميائية الحيوية وإجهاد التاكسدي في الجرذان من نوعAlbino-wistar ، مع تقييم الدور الوقائي لاستخراج *العرعر الفينيقي* .(JNP)تم تقسيم 28جرذ إلى أربعة مجموعات تضم كل منها سبعة جرذان :مجموعة ضابطة(تلقت ماء شرب وغذاءً قياسيًا)مجموعة JNP فقط (تلقت ماء شرب وجرعة محددة من مغلي محضر من 200 ملغ/كغ من نبات العرعر عن طريق التلقيح الفموي) مجموعة ACE فقط (تلقت 20 ملغ/كغ من الأسيتاميبريد) ومجموعة ACE+JNP المشتركة (تلقت جرعة فموية من الأسيتاميبريد بمقدار 20 ملغ/كغ تلتها بعد أربع ساعات جرعة من مغلي العرعر بتركيز 200 ملغ/كغ)حيث تم إجراء التقييمات لمدة 30 يومًا المتعلقة بالوزن الجسدي، ووزن الكبد، والعلامات الكيميائية الحيوية، ومؤشرات إجهادالتاكسدي.

أظهرت النتائج أن الأسيتاميبريد تسبب في سمية كبدية واضحة (زيادة فيALT ، AST، الدهون الثلاثية، الكوليسترول، وإنزيمCAT ؛ ونقص في الألبومين، GST، و(GSH، بينما لم يُظهر مستخلص العرعر وحده أي تأثيرات ضارة. ومن الجدير بالذكر أن الإعطاء المشترك لمستخلص العرعر مع الأسيتاميبريد أدى إلى التخفيف من السمية الناجمة عن الأخير، مما يشير إلى أن مستخلص *Juniperus phoenicea*يمتلك خصائص واقية للكبد ومضادة للأكسدة قادرة على مواجهة الأضرار التي تسببها المبيدات الحشرية.

تبرز هذه النتائج الإمكانيات العلاجية الطبيعية لـ *جونيبيروس فونيكيا* كعامل مضاد للتسمم، مما يستدعي المزيد من الأبحاث لفهم آليات عمله وتطبيقاته السريرية في تخفيف السمية المرتبطة بالمبيدات.

الكلمات المفتاحية: سمية الأستامبريد، التسمم الكبدي، *Juniperus phoenicea*، المعايير الفسيولوجية، المعايير الكيميائية الحيوية، معايير الإجهاد.

Abbreviations List

ALT	**Alanine Amino Transferase**
ANOVA	**Analysis of Variance**
AST	**Aspartate Amino Transferase**
BSA	**Bovine Serum Albumin**
DO	**Optical density**
DTNB	**5,5'-Dithio-bis-2-nitrobenzoic Acid**
GSH	**Glutathione**
GST	**Glutathione S-Transferase**
CAT	**Catalase**
ROS	**Reactive Oxygen Species**
JNV	***Juniperus phoenicea***
AChE	**acetylcholinesterase**
ACh	**acetylcholine**
SOD	**Superoxide Didmutase**
GPX	**Glutathione Peroxidase**
ACE	**Acetamiprid**
CBC	**Complete Blood Count**
EDTA	**Ethylene Diamine Tetra Acetic Acid**
ALP	**Alkaline Phosphatase**
BCG	**Bromocresol green**
MDH	**Malate dehydrogenase**
TNB	**2-nitro-5-thiobenzoic acid**
SEM	**Standard Error of the Mean**

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Introduction

Introduction

The use of pesticides, dating back to ancient Greece, has significantly boosted agricultural productivity and disease control, supporting food security and public health. However, the surge in global pesticide production, driven by population growth and food demand, raises concerns about their adverse effects on human health and ecosystems. Chronic exposure, even at low levels, is linked to health risks through direct contact or contaminated food. Advances in environmental toxicology highlight pesticides, and emerging contaminants as major toxicological hazards, emphasizing their dual role as essential tools and potential long-term threats to health and the environment **(Bonvallot, 2014).**

Neonicotinoids, particularly acetamiprid, are widely used neurotoxic insecticides with high water solubility, enabling rapid absorption and bioaccumulation in plant and animal tissues. Acetamiprid is metabolized primarily in the liver, with metabolites excreted via urine, making the liver and kidneys key target organs for toxicity. Chronic exposure may induce hepatotoxicity through oxidative stress, generating reactive oxygen species (ROS) that damage DNA, proteins, and lipids, compromising cellular function (**Ghazanfari et al., 2021).**

Medicinal plants have played a pivotal role in traditional healthcare systems since prehistoric times. According to the World Health Organization (WHO), approximately 85% of traditional medicinal practices globally involve the use of plant extracts **(Zouari Bouassida et al., 2018)**. Among these, the genus *Juniperus*, belonging to the Cupressaceae family, is particularly notable. This genus encompasses around of 67 species distributed worldwide and has a long history of utilization in folk medicine. *Juniperus Phoenicea*, an evergreen tree native to North Africa and known as 'Arar' in Algeria, is traditionally used for its medicinal properties, including as a diuretic, stimulant, and treatment for conditions like diarrhea and diabetes. Phytochemical studies have identified diverse bioactive compounds, such as diterpenoids and bioflavonoids, in its leaves and berries. Despite its medicinal significance, research on the antioxidant and broader biological activities of *J. Phoenicea* remains limited, indicating a critical gap in understanding its therapeutic potential **(Boudiba et al., 2021).**

Our study investigates liver damage in rats caused by exposure to acetamiprid, highlighting the biochemical and structural changes that occur. It also explores the potential therapeutic effects of *Juniperus Phoenicea* extracts from medicinal plants as a means to mitigate his damage. Ultimately, the research aims to provide insights into natural remedies that could protect liver health in the context of pesticide exposure.



Introduction

Our work is divided into three chapters:

_Bibliographic research

_Materials and methods

_Results and discussion

_Finally, we conclude our work with a conclusion and perspectives

Bibliographic part

1. Pesticides

1.1. Definition and role of pesticides in agriculture

Pesticides, or phytosanitary/phytopharmaceutical products, are chemical or biological agents derived from Latin *pestis* (plague) and *caedere* (to kill), used to control or eliminate pests threatening agriculture, plant health, and ecosystems. Essential for crop protection and food security, their use requires strict regulation to mitigate environmental and health risks **(Gaouar, 2017).**

1.2. Classification of pesticides

Pesticides are classified into distinct categories based on the specific living organisms they target

-Herbicides: They destroy herbaceous or woody plants, or limit their growth (such as anti-germinatives and desiccants) (**Boukrouet et al., 2018).**

-**Fungicides**: These are products that target only parasitic fungi affecting crops, excluding parasites that affect animals and humans **(Boukrouet et al., 2018).**

-Insecticides: are chemical or biological agents employed to protect plants from insect infestations. Their reproductive capabilities **(Aloui, 2020).**

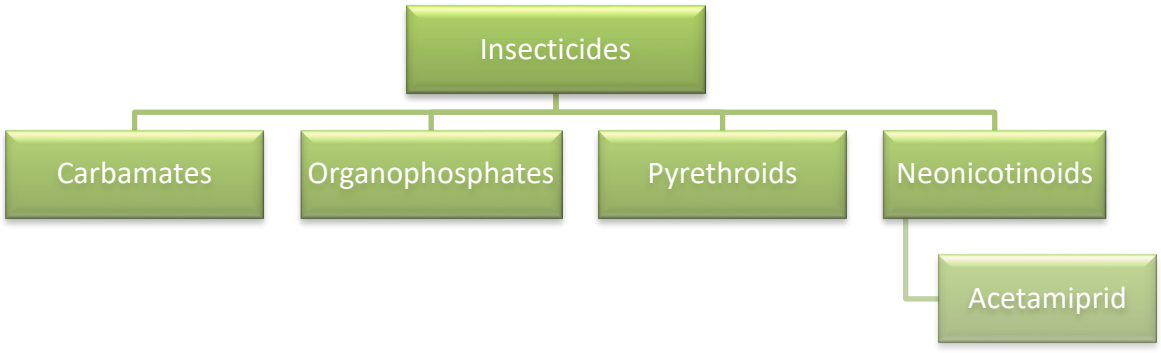


Figure1.Classification of insecticides (Gasmi, 2018; Sparks et al., 2021) .

1.3. Environmental exposure through (air, soil, water)

Human pesticide exposure occurs directly through occupational, agricultural, and household activities, and indirectly via contaminated air, water, soil, and food. Toxicity depends on exposure routes (dermal, ingestion, inhalation) and levels, determining associated health risks **(Tudi et al., 2022).**

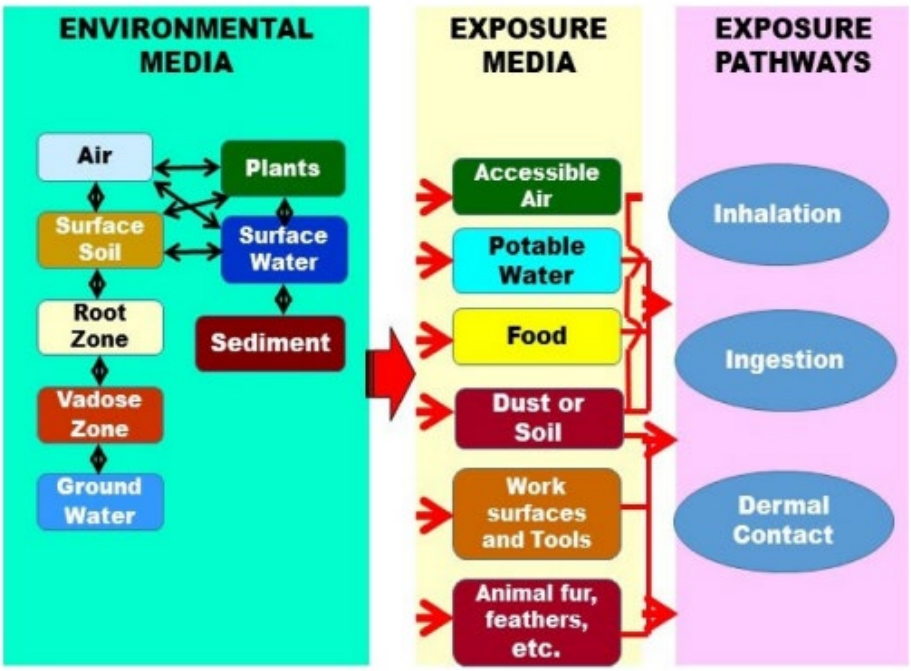


Figure 2.Routes of exposure to pesticides (Tudi et al., 2022).

1.4. General Overview of Acetamiprid Toxicity

Acetamiprid, a neonicotinoid insecticide, exhibits multifaceted toxicological effects across various organ systems, mediated through distinct mechanisms. Neurotoxicity arises from the chronic induction of cytochrome P450 enzymes and irreversible inhibition of acetylcholinesterase (AChE), leading to acetylcholine (ACh) accumulation, sustained cholinergic activity, and disruption of neurotransmission. Developmental exposure reduces neuronal cell populations, induces apoptosis, and alters cell cycle-related gene expression, resulting in neurobehavioral impairments**(EFSA, 2016 ; Gasmi , 2019).**

Reproductive toxicity is linked to oxidative stress in testicular tissue **(Zhang et al., 2011)**. while immunotoxicity manifests as dose-dependent suppression of lymphocyte proliferation and impaired macrophage function **(Shakthi et al., 2015).**

Hematotoxicity is evidenced by reduced hemoglobin levels and altered erythrocyte indices **(Toghan et al., 2022),** and nephrotoxicity is characterized by renal congestion, tubular degeneration, and reduced glomerular filtration rate **(Gathwan., 2016).**

Hepatotoxicity involves elevated serum AST and ALT levels, oxidative stress-mediated lipid peroxidation, thiol group oxidation, and depletion of antioxidant enzymes (e.g., SOD, catalase, GPx), leading to hepatic cell death. These findings underscore the systemic toxicity of acetamiprid, highlighting its potential to induce oxidative stress, cellular damage, and functional impairments across multiple organ systems **(Zhang et al., 2011; Ghazanfari et al., 2021).**

2. General information on *Juniperus phoenicea*

2.1. Medicinal plants and herbal medicine

Medicinal plants, containing bioactive compounds with therapeutic properties, have been used since ancient times, evidenced by historical records and traditional practices. Their therapeutic potential was identified through empirical experimentation, leading to the use of various plant parts for medicinal purposes. Many plant-derived compounds, validated by both traditional use and modern research, are now integral to contemporary pharmacotherapy **(Sofowara, 2010).**

2.2. Geographic repair

The *Cupressaceae* family, comprising ten genera, has a cosmopolitan distribution with most species concentrated in the Northern Hemisphere. Its spatial distribution is influenced by ecological factors (climate, soil properties) and anthropogenic activities, including disturbances and exploitation. *Juniperus phoenicea,* a widely distributed species occupying ~450,000 hectares globally, is primarily found in North Africa (Algeria: 290,000 ha, Tunisia: 80,000 ha, Morocco: 152,000 ha) and Mediterranean regions of Southern Europe, Southern Africa, and Asia. In Algeria, it is a rare, endangered species concentrated in the Kabylie region, Djurjura Mountains, High Plateaus, and Saharan Atlas, with populations in Oran, Algiers, and Constantine **(Bouyahyaoui, 2017).**

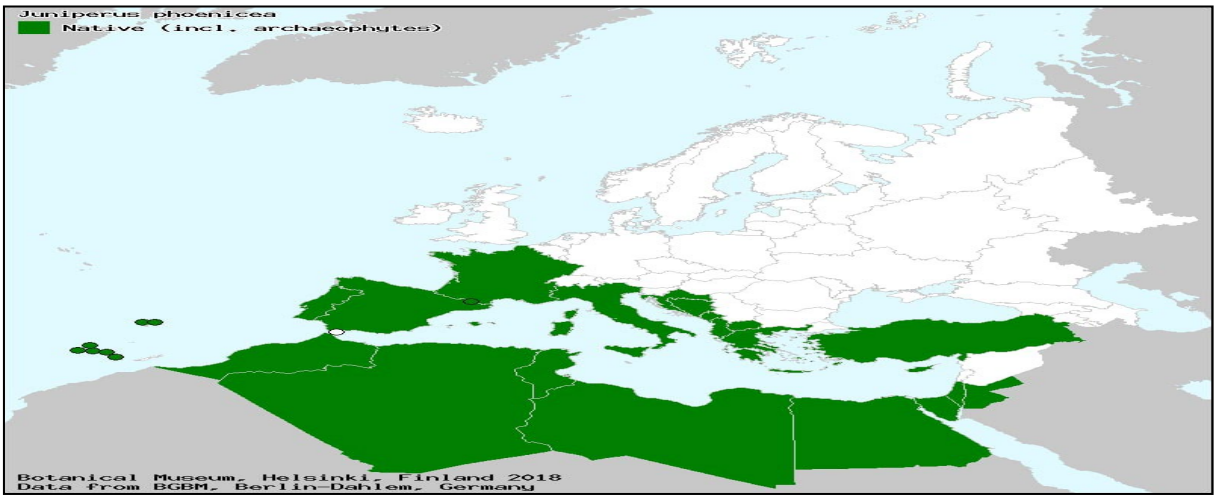


Figure 3.Distribution of *Juniperus phonicea* in Europe and the Mediterrannean region(Malta Wild Plants.,2018).

2.3. Description of *Juniperus phoenicea*

This species, a branched tree in the Sabina section, reaches up to 8 meters in height with a short trunk (up to 2 m circumference) and thick reddish-brown to grayish bark. It flowers in winter, fruits by late summer, producing glossy, 7–10 mm diameter fruits that transition from green to reddish-brown. It has a deep root system, slender rounded branches, naked buds, and cylindrical twigs **(Akrout, 2004).**



Figure 4. Leaves and fruits of *J. phoenicea* (personal photo)

2.4. The taxonomy of the *Juniperus phoenicea*

Table 01:The botanical classification of *Juniperus phoenicea* **(Small et al., 2001).**

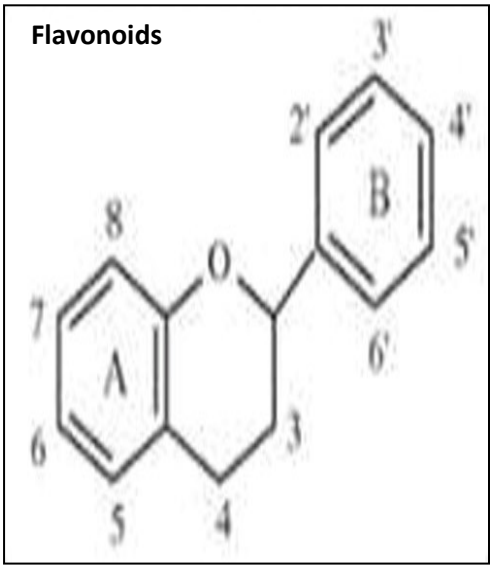
Reign	Plantae
Under reign	Tracheobionta
Branch	Spermatophytes
Under Branch	Gymnospermes
Class	Pinopsida
Order	Pinales
Family	*Cupressaceae*
Gender	*Juniperus* L
Species	*Juniperus phoenicea*

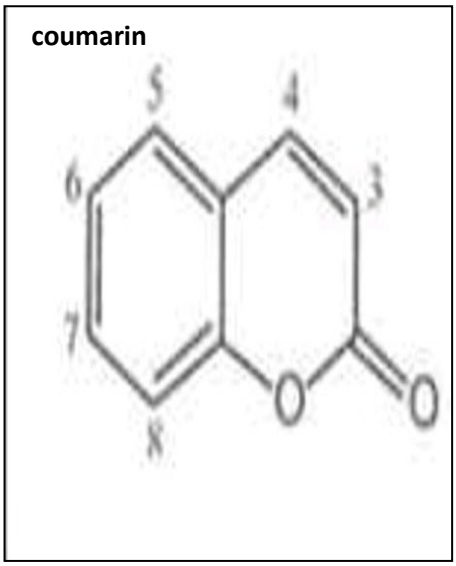
2.5. Use of *Juniperus phoenicea*

This species is a valuable medicinal plant, with foliated branches yielding vegetable tar effective for treating eczema and respiratory conditions like asthma and bronchitis, as well as alleviating cephalalgia, vertigo, and arthritis. Its leaves, prepared as a decoction, are used to manage diabetes, diarrhea, and rheumatism, while powdered dried fruits promote healing of cutaneous ulcerations and abscesses **(Abdelli, 2017; Mansouri et al., 2010)**.

2.6. Chemical constituent and bioactive compounds

Phytochemical investigations on *Juniperus phoenicea* have demonstrated that both aqueous and ethanol extracts contain a diverse array of bioactive compounds, including carbohydrates, proteins, phenols, tannins, flavonoids, alkaloids, coumarins, anthocyanins, saponins, and glycosides. These phytochemicals play critical roles in various biological processes, such as energy provision, structural support, and cellular signaling. Additionally, they exhibit significant pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial, and neuroactive effects **(Elsherif et al., 2024).** Saponins, for instance, are known to contribute to cholesterol reduction, immune system modulation, and inhibition of tumor growth. Glycosides, on the other hand, display a wide range of therapeutic potentials, such as cardioprotective, antidiabetic, and anticancer activities. These findings underscore the medicinal and biological significance of *J. phoenicea* as a source of phytochemicals with multifaceted health benefits **(Elsherif et al., 2024).**





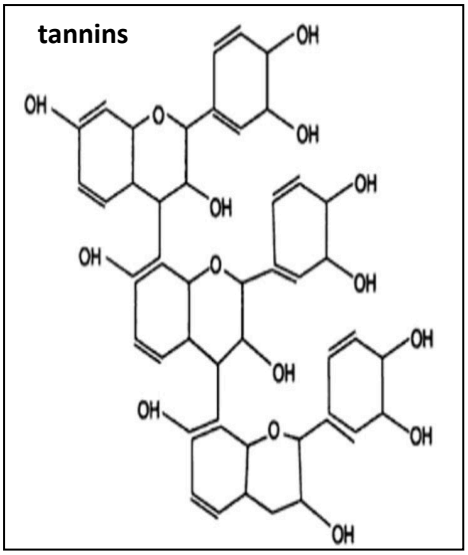


Figure 5 . chemical structure of constituent of *juniperus phoenicea* (Macheix et al., 2005); (Bruneton, 2009); (Guillaume, 1999).

Experimental Part

Materials and Methods

3. Materials and Methods

Our study was conducted in the Biochemistry Laboratory of the Department of Applied Biology at the Faculty of Exact Sciences and Natural and Life Sciences, University of Tebessa, with the objective of evaluating the hepatotoxic effects of the neonicotinoid pesticide acetamiprid in Wistar rats and investigating the potential protective role of *Juniperus phoenicea* extracts in mitigating pesticide-induced oxidative damage. To assess acetamiprid toxicity and the therapeutic efficacy of *Juniperus phoenicea*, we implemented standardized protocols to analyze key biochemical parameters and oxidative stress markers, including glutathione (GSH), catalase (CAT), and glutathione S-transferase (GST)…..

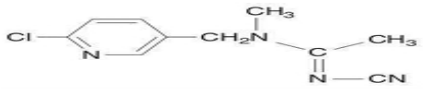
3.1. Chemical and biological materials

3.1.1. Definition of acetamiprid and uses

Acetamiprid is a synthetic insecticide classified within the neonicotinoid class of chemicals. Its molecular structure, represented by the formula $C_{10}H_{11}ClN_4$, demonstrates close structural resemblance to other neonicotinoid compounds, including imidacloprid, a widely used insecticide introduced to the market in 1992.This compound is widely employed in agricultural practices, particularly in the cultivation of citrus fruits, vineyards, cotton, and ornamental plants **(Mikiko et al., 2012)**.

3.1.2. Properties of acetamiprid

Table 02: Main characteristics of acetamiprid **(Testud,2014).**

Structure	
Chemical Name	(E)-N-[(6-Chloro-3-pyridinyl)methyl]-N'-cyano-N-methylethanimine
Properties	White powder with low water solubility and non-volatile
Physicochemical Properties	Vapor pressure < 1 µPa at 25°C
Persistence	Soil half-life between 1 and 8 days
Maximum Residue Levels	0.01 to 5 mg/kg depending on the type of crop
ADI	70 µg/kg/day for humans
LD50	195 mg/kg orally, >2000 mg/kg dermally in rats
Target Organs	Central Nervous System (CNS), **Liver**

3.1.3. Preparation of the solution

We employed a commercially available systemic insecticide, marketed under the trade name Rustilan, containing **acetamiprid 20%** as the active ingredient. The compound was dissolved in mineral water to achieve a concentration of **20 mg/kg** and administered orally via gavage (*per os*) to female rats. This experimental approach ensured precise dosing for evaluating the systemic effects of acetamiprid under controlled conditions **(Shamsi et al., 2021)**.

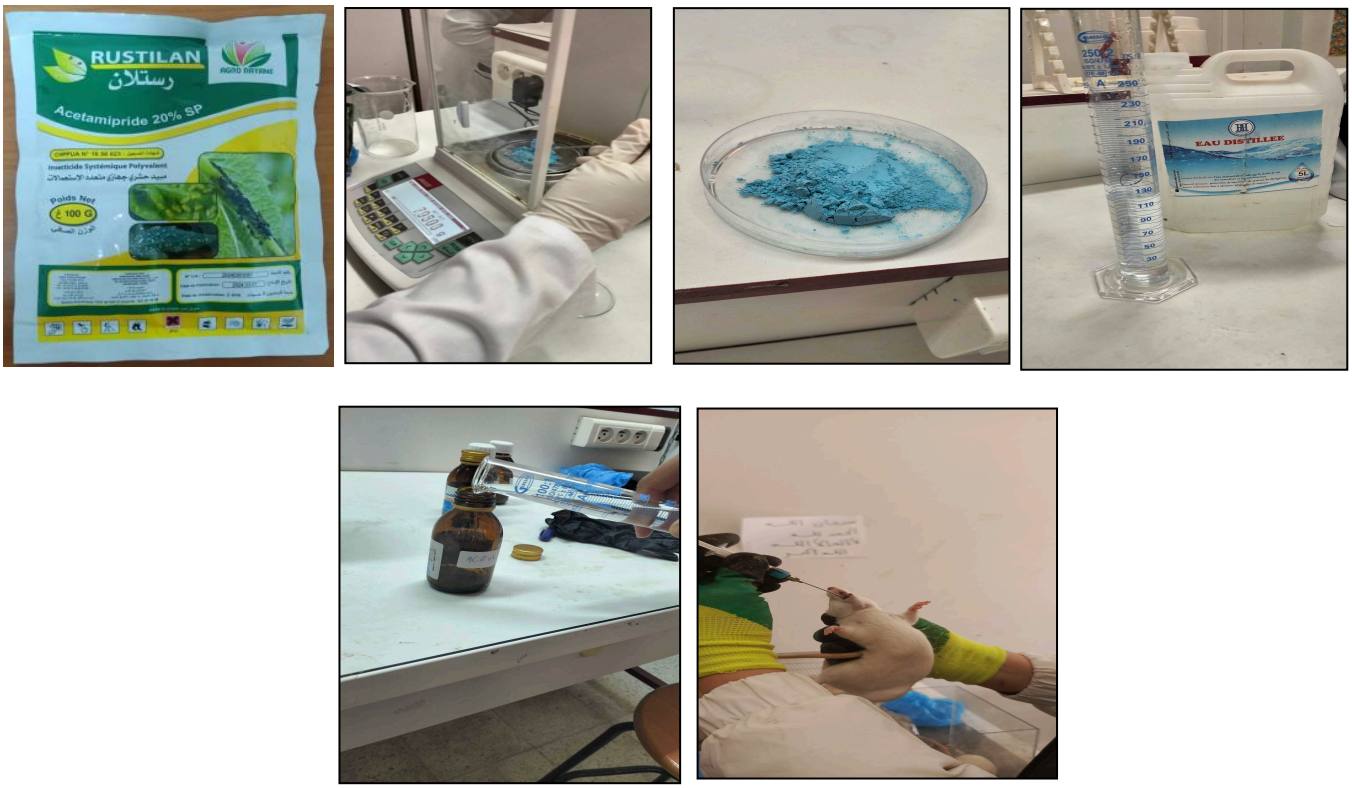


Figure 6. Preparation of acetamiprid solution (personal photo)

3.1.4. Plant materials *:Juniperus phonicea*

To better valorize the biodiversity of plants in Eastern Algeria, our study focuses on *Juniperus phoenicea* a plant species belonging to the *Cupressaceae* family. This selection was informed by an extensive literature review. The criteria for selecting this species are as follows:

- **Prevalence in Algeria**: *Juniperus phoenicea* is widely distributed across Algeria, making it a significant component of the region's flora.

- **Traditional Medicinal Use**: This species has been traditionally utilized in the treatment of various ailments, highlighting its ethnopharmacological importance.
- **Non-Toxicity**: Given its use in culinary preparations and herbal teas, *Juniperus phoenicea* is recognized as a non-toxic plant, ensuring its safety for human consumption.
- **Therapeutic and Pharmaceutical Properties**: The species exhibits notable therapeutic and pharmaceutical potential, warranting further scientific investigation into its bioactive compounds and applications.

3.1.5. Harvesting

The aerial components (stems, foliage, and berries) of *Juniperus* are collected during the month of February in the Safsaf El Ouesra area, located in the Tébessa region in Algeria.



Figure 7. Harvest of *juniperus phoenicea* (personal photo)

3.1.6. Preparation of the decoction

Juniperus phoenicea is decocted in boiling mineral water (100°C), and once cooled, the infusion is filtered and stored in sterile, light-protected vials to preserve its stability and prevent photodegradation. This method ensures the extraction of bioactive compounds while maintaining the solution's integrity for subsequent analytical or therapeutic applications **(Soltani et al., 2018)**.



Figure 8. preparation of the decoction of *Juniperus phoenicea* (personal photo)

3.1.7 Care and treatment of the animals

3.1.7.1. Animals and Housing Conditions

In this study, twenty-eight (28) female Albino Wistar rats (*Rattus norvegicus*), aged five weeks, were obtained from the Pasteur Institute of Algiers (I.P.A.). The animals were bred and maintained in the animal facility of Larbi Tebessi University, Tebessa, The rats were housed in polyethylene cages, each labeled with relevant identifiers, including batch designation, treatment details, and experimental timelines. The cages were lined with sterilized wood shavings as bedding material, which was replaced daily to ensure hygienic conditions.

3.1.7.2. Acclimatization and Environmental Conditions

Prior to experimentation, the rats underwent an acclimatization period of approximately two months within the animal facility. Throughout this period, environmental conditions were strictly controlled, maintaining an ambient temperature of **22 ± 2°C** and a natural **12-hours light/dark cycle** to mimic physiological circadian rhythms.

3.1.7.3. Diet and Hydration

The animals were provided with a standardized nutritionally balanced pellet diet and Fresh drinking water was available at all times to ensure unrestricted access to hydration.

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**

3.2. Methods

3.2.1. Initial Physiological Parameters

At the commencement of the experimental protocol, the rats exhibited an average initial body weight of **200 grams**, confirming uniformity in baseline physiological conditions across the study cohort. Following an acclimatization period, the animals were randomly allocated into four experimental groups of seven rats each.

The subjects received daily treatment administrations over a one-month period. Body weight was monitored at regular intervals to adjust the treatment dosage accordingly.

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

Batch JNP : The rats receive drinking water and a specific dose of a decoction prepared from 200mg/kg of *juniperus* via oral gavage.

Batch ACE : The rats receive 20 mg/kg of acetamiprid via oral gavage.

Batch ACE+JNP: The rats are administered orally with a dose of acetamiprid at 20mg/k followed by the administration of 200mg/kg of *juniperus* decoction 4 hours later



Figure 9. Animal care (personal photo)

3.2.2. Blood sampling

3.2.2.1. Sacrifice

Following a one-month experimental period, the animals are decapitated. Blood samples are promptly collected into two pre-labeled tubes: one containing ethylenediaminetetraacetic acid (EDTA) for complete blood count (CBC) analysis and the other containing heparin as an anticoagulant.

3.2.2.2. Sample Processing

The collected blood samples are immediately centrifuged at 3000 rpm for 15 minutes to separate plasma. The resulting plasma is then aliquoted and stored for subsequent biochemical analyses.

3.2.2. 3.Biochemical Analyses

Plasma samples are assessed for key biochemical parameters, including:

_**Liver function markers:** Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin.

_**Lipid profile:** Triglycerides and total cholesterol.

3.2.3. Liver tissue sampling

A longitudinal abdominal incision was performed to excise the liver. Following dissection, adherent adipose tissue was carefully removed, and the organs were weighed. Subsequently, the liver samples were stored at -20°C in a cryogenic freezer to preserve their biochemical integrity pending further analysis of oxidative stress biomarkers, including reduced glutathione (GSH) and glutathione S-transferase (GST) activity.

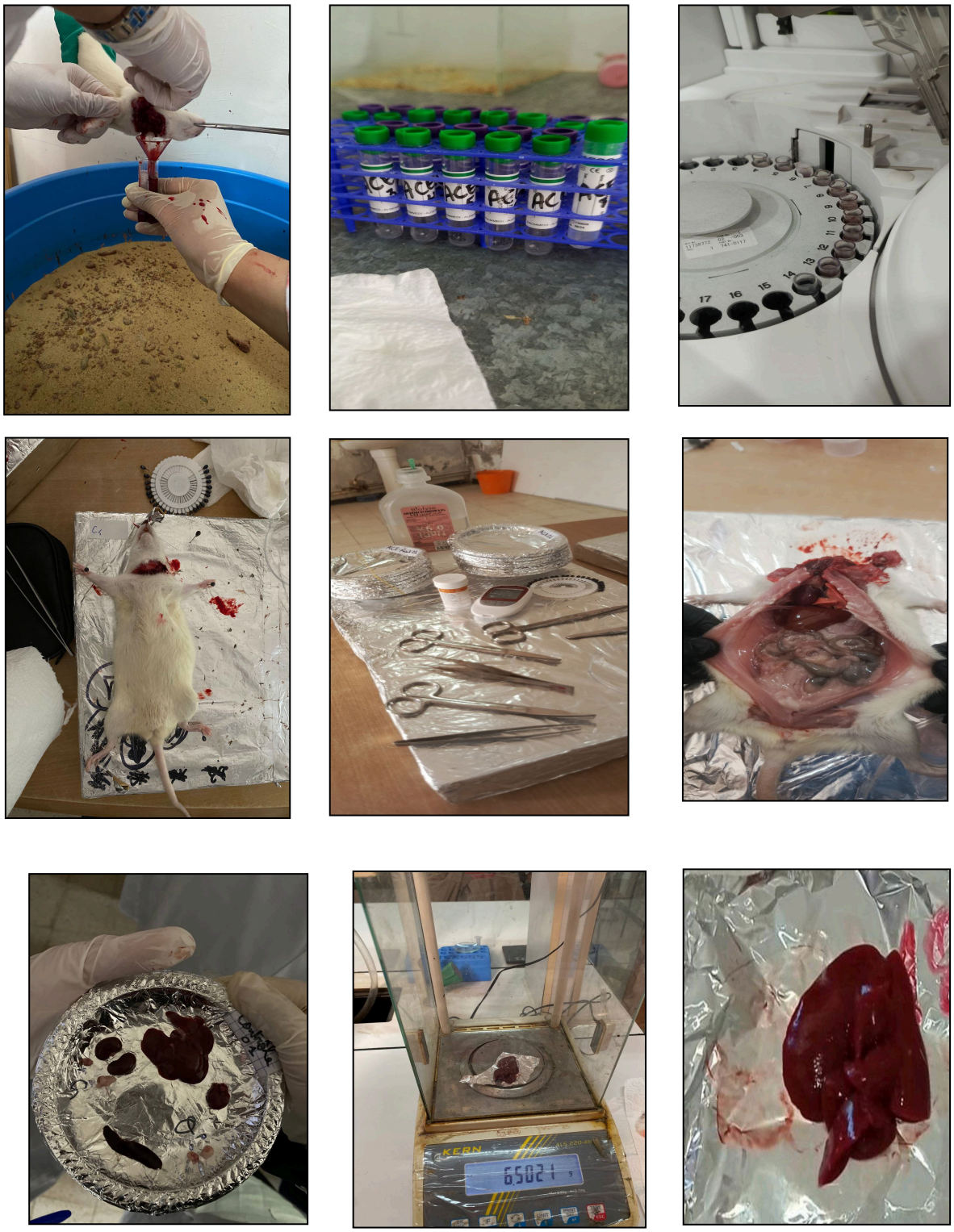


Figure 10. blood sampling and liver tissue sampling (personal photo)

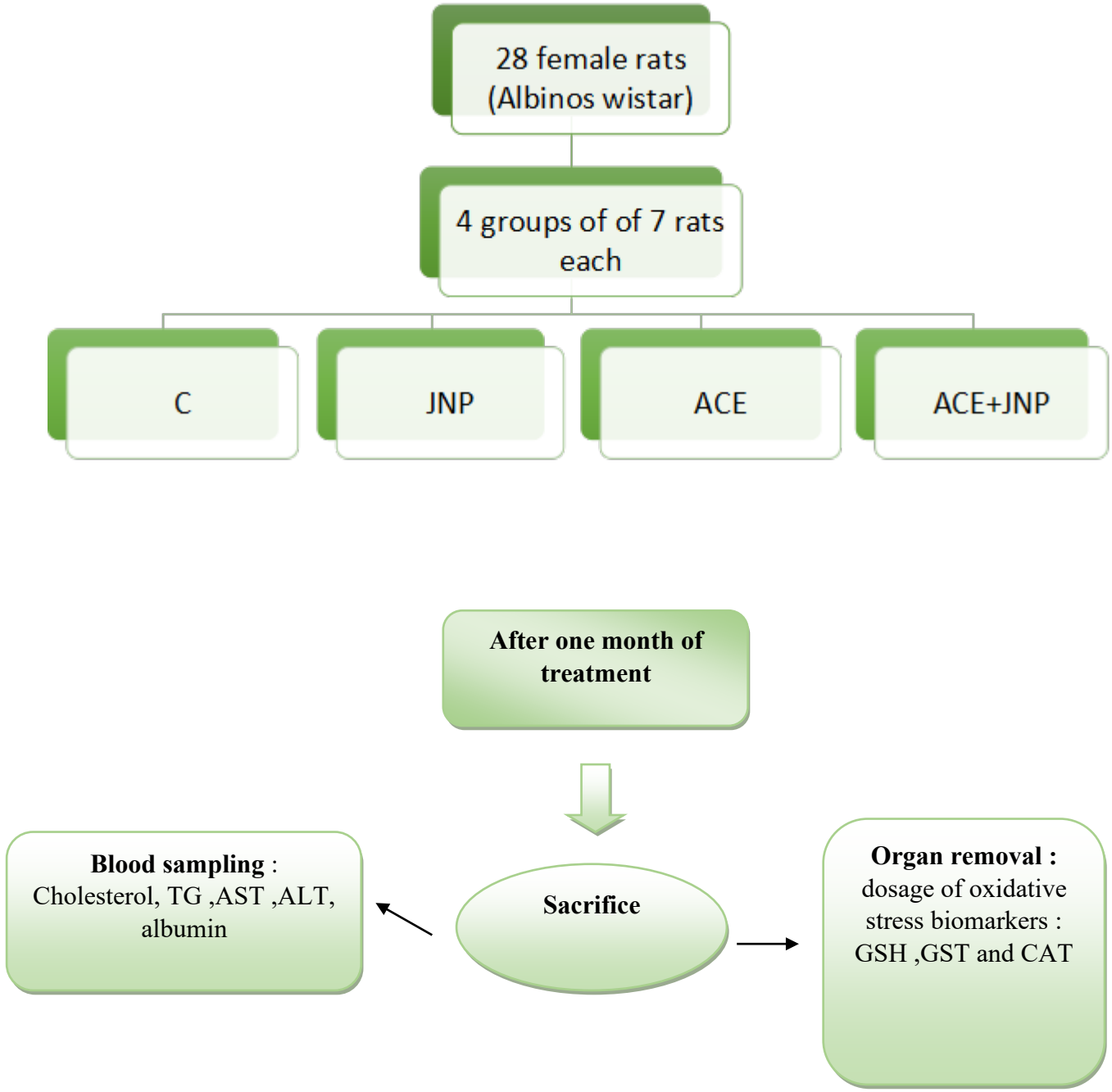


Figure 11. Diagram summarizing the experimental protocol.

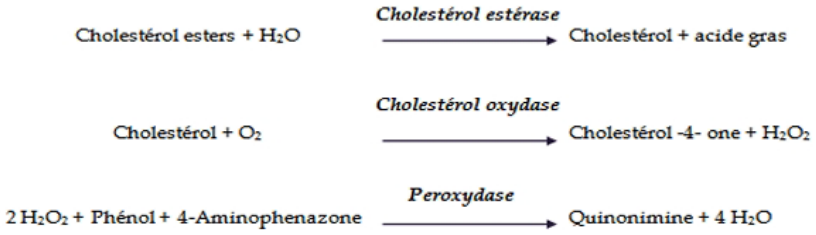
3.2.4 .Albumin Assay

Principle

In a buffered aqueous solution maintained at pH 4.2, bromocresol green (BCG) undergoes a selective binding interaction with albumin, resulting in the formation of a chromogenic complex. The absorbance of this complex exhibits a linear relationship with albumin concentration when measured spectrophotometrically at its λ_max of 630 nm (with an acceptable measurement range of 620-640 nm). This photometric property serves as the basis for quantitative albumin determination in biological specimens **(Doumas et al., 1997).**

3.2.5. Cholesterol Assay

The principle is shown in the following reaction diagram:



The cholesterol assay employs enzymatic reactions (cholesterol esterase and cholesterol oxidase) to hydrolyze cholesterol esters into free cholesterol, which is oxidized to produce hydrogen peroxide. Peroxide then reacts with a chromogen (e.g., 4-aminoantipyrine and phenol) via peroxidase, forming a quinoneimine dye. Spectrophotometric measurement of its absorbance allows precise, sensitive, and specific quantification of total cholesterol in biological samples **(Allain et al., 1974).**

3.2.6. Triglycerides Assay

Principle

The assay is based on an enzymatic colorimetric method in which triglycerides are enzymatically hydrolyzed to produce glycerol and free fatty acids. The glycerol is subsequently oxidized, leading to the generation of a colored chromophore through a series of coupled enzymatic reactions. The intensity of the resulting colorimetric signal, measured spectrophotometrically, is

directly proportional to the concentration of triglycerides in the sample, enabling quantitative analysis **(Parhofer and Laufs, 2019).**

3.2.7. Assay of Aspartate amino transferase AST

Principle

The principle is shown in the following reaction diagram:

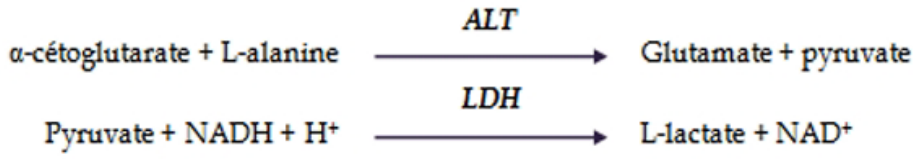


The spectrophotometric assay of Aspartate Aminotransferase (AST) quantifies enzymatic activity by monitoring the transamination of L-aspartate and α-ketoglutarate to yield oxaloacetate and L-glutamate. This reaction is coupled with the oxidation of NADH to NAD^+, catalyzed by malate dehydrogenase (MDH), wherein the decrease in absorbance at 340 nm—proportional to NADH consumption—serves as a direct measure of AST activity. The assay thus provides a kinetic assessment of enzyme function through the stoichiometric relationship between substrate depletion and cofactor conversion **(Rosalki and Rau, 1972)**

3.2.8. Assay Of Alanine amino transferase ALT

Principle

The principle is shown in the following reaction diagram:



The alanine aminotransferase (ALT) assay quantitatively measures enzymatic activity through spectrophotometric analysis by monitoring the transamination reaction between alanine and α-ketoglutarate, which yields pyruvate and glutamate as products. This reaction is coupled with the reduction of nicotinamide adenine dinucleotide (NAD+) to NADH, enabling the kinetic assessment of ALT activity based on absorbance changes at 340 nm **(Bradford, 1976).**

3.2.9. Assay of Glutathione S-Transferase GST enzymatic activity

Principle

The measurement of GST (glutathione S-transferase) activity involves providing the enzyme with a substrate, typically 1-chloro-2,4-dinitrobenzene (CDNB), which readily reacts with various GST isoforms and glutathione (GSH). The conjugation reaction between these two compounds results in the formation of a new molecule, GS-DNB, along with hydrochloric acid (HCl). In this study, the method employed for GST quantification is based on the protocol described by **(Habig et al., 1974)**. This approach involves incubating the GST-containing homogenate with a mixture of GSH and CDNB at 25°C and pH 7. The change in optical density, resulting from the formation of the GSH-CDNB conjugate, is monitored spectrophotometrically at 340 nm for 1-minute intervals over a 5-minute period.

Operating mode

Table 04: Operating mode with reagents used

Reagents used	Blank (µl)	Sample (µl)
Phosphate buffer (0.1M, pH 7)	850	**830**
CDNB (0.0 2M)	50	**50**
GSH (0.1M)	100	**100**
Supernatant	-	**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

3.2.10. Catalase (CAT) Assay

Principle

The method employed was based on **(Aebi et al., 1984),** in which catalase activity was measured spectrophotometrically at 240 nm by monitoring the decrease in optical density resulting from the decomposition of hydrogen peroxide (H_2O_2) at 25°C in the presence of an enzymatic source from the cytosolic fraction. Absorbance readings were initiated after a 15-second delay and recorded over a 60-second measurement period. Given the rapid decline in catalase activity, it was crucial to maintain consistent timing between pipetting and placing the cuvette in the spectrophotometer to ensure reproducibility.

Operating mode

Table 05: Operating mode with reagents used

Reagents used	Blank (µl)	Sample (µl)
Phosphate buffer (0.1M, pH 7)	800	**780**
H2O2 (0.5 M)	200	**200**
Supernatant	-	**20**

Calculation: The activity of catalase is determined using the following equation:

- ✓ **Catalase Activity (µmol H_2O_2/min/mg protein) = (ΔA × 10) / (ε × L × X × Fd)**

Where:

- **ΔA**: Change in absorbance per minute
- **ε**: Extinction coefficient of H_2O_2 = 0.04 $mM^{-1} \cdot cm^{-1}$
- **L**: Path length of the cuvette = 1 cm

- **X**: Protein concentration in mg/mL
- **Fd**: Dilution factor of H_2O_2 in the buffer (0.02)

This equation quantifies the enzyme's ability to decompose hydrogen peroxide (H_2O_2) per minute per milligram of protein.

3.2.11. Protein assay in a sample

Principle

This method employs Coomassie Blue as a reagent, which reacts under acidic conditions primarily with arginine and, to a lesser extent, with histidine, lysine, and aromatic amino acids. Coomassie Blue binds to proteins through non-covalent interactions, including hydrogen bonding, hydrophobic effects, and ionic interactions. Upon binding, its maximum absorption wavelength shifts from 465 nm (red) to 595 nm (blue), reflecting the formation of a protein-dye complex. This shift serves as the basis for quantifying protein concentrations in spectrophotometric assays **(Bradford.,1976).** .

Operating mod

Pipette 0.1 mL (100 µL) of the sample (supernatant).

- Add 4 mL of Bradford reagent.
- Mix gently and incubate for 5 minutes to allow color stabilization.
- Measure the optical density at 595 nm, using the blank as a reference (4 mL of Bradford reagent + 0.1 mL distilled water).

The optical density measured is plotted against a pre-established calibration curve, and the protein concentration is determined by comparison with a standard curve generated using bovine serum albumin (BSA) (1 mg/mL) as a reference, under identical experimental conditions. This method ensures accurate quantification by correlating absorbance values with known BSA concentrations, following the principle of spectrophotometric analysis in biochemical assays

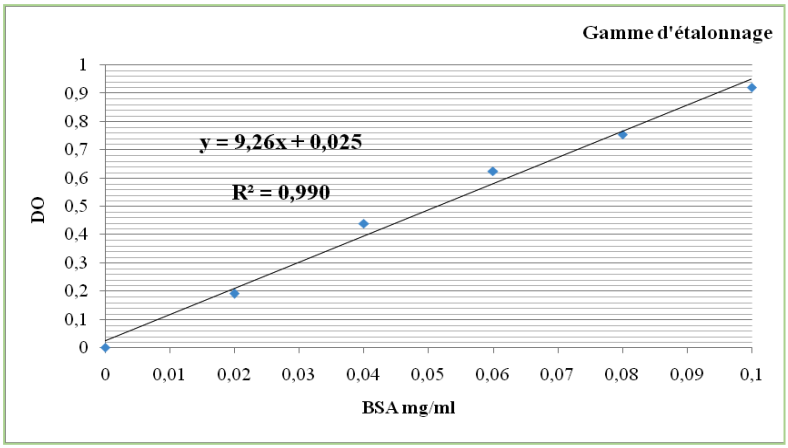


Figure 12 . Bovine serum albumin calibration curve

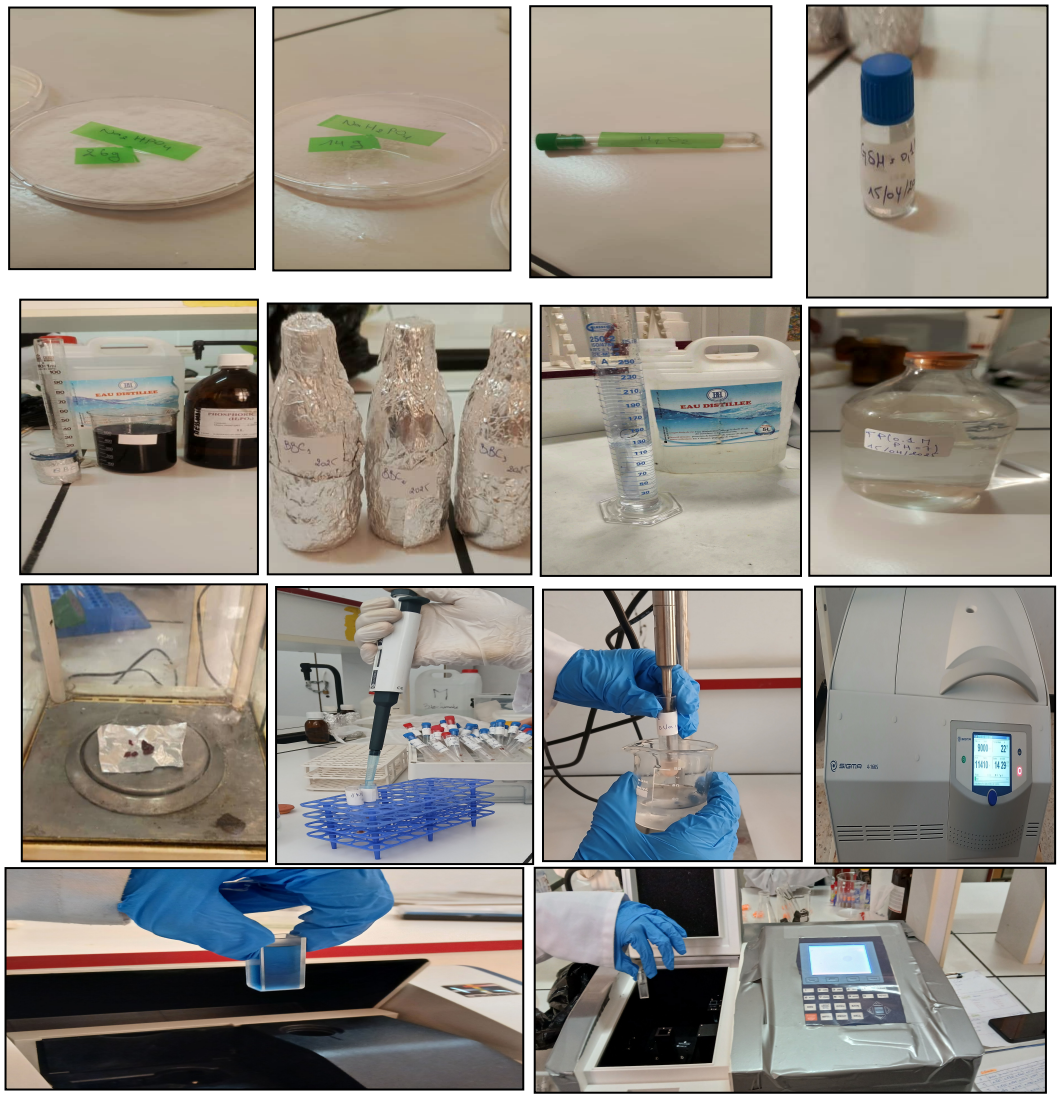


Figure13. photo galleries summarizing the different stages of the dosage of GST/CAT/Protein (personal photo)

3.2.12. GSH Assay

Principle

The glutathione concentration was determined according to the established protocol of **Wekbeker and Cory (1988)**, which relies on the spectrophotometric detection of 2-nitro-5-thiobenzoic acid (TNB) formed via the reduction of 5,5′-dithiobis-2-nitrobenzoic acid (DTNB, Ellman's reagent) by the sulfhydryl (-SH) moieties of glutathione. To ensure assay specificity, the homogenate underwent deproteinization to remove extraneous proteins, thereby isolating thiol groups exclusively attributable to glutathione for accurate quantification **(Dilmi et al., 2018).**

Preparation of the homogenate

In a tube (No. 1), add

- 100 mg of tissue + 4 mL of Ethylene Diamine Tetraacetic Acid (EDTA)
- Cold grinding → Homogenate.

Operating mode

In tube (No. 2), proceed as follows

- Take 0.8 mL of the homogenate.
- Deproteinize by adding 0.2 mL of a 0.25% sulfosalicylic acid (SSA) solution.
- Mix the solution and let it stand for 15 minutes in an ice bath.
- Centrifuge at 1000 rpm for 5 minutes.

In a tube (No. 3), place the following

- Take 0.5 ml of the supernatant.
- Add 1 ml of Tris + EDTA buffer (0.02 M EDTA), pH 9.6.
- Mix and add 0.025 ml of 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) at 0.01 M.
- Let stand for 5 minutes at room temperature for the color to stabilize, as it develops instantly.
- Read the optical densities at 412 nm against the blank.

The glutathione concentration is determined using the following formula:

✓ **[GSH] (nM GSH/mg protein)=(OD×1×1.525)/(13100 ×0.8×0.5×mg protein)**

Where:

- **OD**: Optical density at 412 nm.
- **1**: Total volume of solutions used in deproteinization (0.8 mL homogenate + 0.2 mL SSA).
- **1.525**: Total volume of solutions used in the GSH assay (0.5 mL supernatant + 1 mL Tris-EDTA + 0.025 mL DTNB).
- **13,100**: Extinction coefficient of the thiol group (–SH) at 412 nm.
- **0.5**: Volume of supernatant present in the 1.525 mL total assay volume.
- **0.8**: Volume of homogenate present in the 1 mL total deproteinization volume.



Figure14. Dosage of GSH (personal photo)

3.3. Comparative Statistical Evaluation of Experimental Groups

A statistical analysis was performed to compare the effects of the different treatments among the experimental groups: control (C), *juniper* extract-treated group (JNP), acetamiprid-exposed group (ACE), and the co-treated group (ACE+JNP). Data were expressed as mean ± standard deviation (SD), and one-way ANOVA followed by Tukey's post hoc test was used to determine statistical significance. Differences were considered statistically significant at $p< 0.05$. This analysis revealed significant variations between groups, confirming the toxic effect of acetamiprid and the potential protective role of the *juniper* extract.

Results and Discussion

4. Results and Discussion

4.1. Physiological parameters

4.1.1 Effect of treatments on body weight

The extract of *Juniperus phoenicea* (JNP) demonstrated the most significant positive effect on weight gain, whereas acetamiprid (ACE) exhibited the most pronounced negative impact. However, when co-administered with *Juniperus phoenicea*, the combined treatment (ACE + JNP) showed a modest but notable attenuation of the adverse effects induced by acetamiprid, suggesting a partial protective role of the *Juniperus* extract against ACE-induced toxicity.

Table 6 . Effect of aqueous extracts of *Juniperus phoenicea* and/or Acetameprid treatment on body weight gain after 30 days of experience (values represent the mean±SEM of seven rats)

Experimental groups				
Parametrs	**C**	**JNP**	**ACE**	**ACE+JNP**
Initial weight (g)	240.00±20.88	237.43±22.13	243.57±25.05	242.71±22.03
Final weight (g)	250.57±29.1	251.71±23.6	232.42±22.7	241.43±29.4
Gain (%)	**4.40**	**6.02**	**-4.57**	**-0.53**

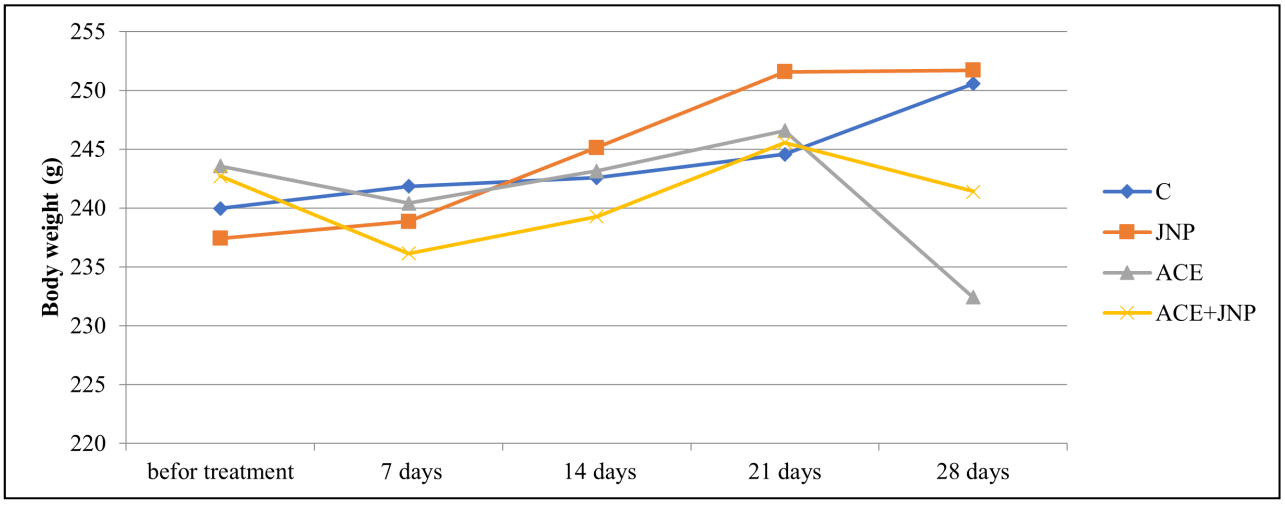


Figure15. Effect of various treatments on body weight over 30days

Research has demonstrated that *Juniperus phoenicea* extract promotes weight gain in mammals, attributed to its high total protein content and bioactive compounds that modulate stress responses, suggesting potential nutritional and therapeutic applications for both humans and animals **(Elsherif et al., 2024)**. Conversely, exposure to neonicotinoid-based insecticides has been shown to induce significant body weight reductions in rats, primarily due to decreased food intake linked to the anxiogenic and anorexigenic effects of these synthetic compounds **(Askri et al., 2022).** These contrasting findings underscore the divergent physiological impacts of natural phytochemicals and synthetic pesticides, emphasizing the beneficial role of plant-derived bioactive substances compared to the adverse metabolic effects associated with pesticide exposure.

4.1.2. Effect of treatment on liver weight

The administration of acetamiprid resulted in a significant increase in liver weight, indicating hepatotropic effects. In contrast, *Juniperus phoenicea* extract alone did not induce any alterations in liver weight. However, when co-administered with acetamiprid, the extract demonstrated a mitigating effect, normalizing liver weights to levels comparable to the control group. This suggests that while *J. phoenicea* extract alone lacked hepatomodulatory activity, it exhibited a protective role against acetamiprid-induced hepatic alterations when used in combination, highlighting its potential as an antagonistic agent against pesticide-induced hepatotoxicity.

Table7: Effect of aqueous extractsof *Juniperus phoniecea* and/or Acetameprid treatment on Liver weights after 30 days of experience (values represent the mean±SEM of seven rats)

	Liver			
Groups	**C**	**JNP**	**ACE**	**ACE+JNP**
Absolute weight (g)	6.237±0.35	6.201±0.58	6.408±0.42*	6.283±0.32
Relative weight (%)	2.403±0.25	2.468±0.29	2.833±0.38*	2.675±0.56

*$P < 0,05$ relative to the control group (C)

The previous research indicate that acetamiprid exhibits significant hepatotoxicity, inducing severe hepatic damage characterized by necrosis, sinusoidal dilation, cellular accumulations, and pronounced inflammatory cell infiltration in the liver parenchyma **(Khovarnagh et Seyedalipour, 2021).** In contrast, various fractions derived from the aerial parts of *Juniperus phoenicea* demonstrated notable hepatoprotective efficacy, as evidenced by the normalization of biochemical parameters and corroborated by histopathological analysis **(Alqasoumi et al., 2013).** This protective effect is likely attributed to the presence of flavonoids and phenolic acids, which exhibit potent antioxidant properties, thereby counteracting hepatotoxic mechanisms. Notably, the therapeutic potential of such plant-derived extracts aligns with their historical use in traditional medicine, underscoring their viability as safe and cost-effective candidates for the

development of natural hepatoprotective agents to address diverse liver disorders. These insights may pave the way for novel pharmacological interventions in hepatotherapy **(Ali et al., 2010)**

4.2. Biochemical parameters

Significant alterations in various biochemical parameters were observed in ACE group in comparison with the control group (incease in ALT,AST,Triglycerides,Cholesterol, decrease in Albumin). Furthermore, the ACE+JNP co-treatment group exhibited significant improvements in hepatic function markers and lipid metabolism profiles, suggesting potential hepatoprotective and ameliorative effects against Acetamiprid-induced metabolic disturbances.

Table 8: Effect of aqueous extracts of *Juniperus phoniecea* and/or Acetameprid treatment on several biochemical parameters after 30 days of experience (values represent the mean±SEM of seven rats)

Parametrs	C	JNP	ACE	ACE+JNP
ALT (UI/L)	36.00±2.65	38.00±4.58	45.33±1.53*	41.33±2.52
AST (UI/L)	130.0±1.00	127.33±1.53	142.33±4.16*	151.66±4.73*
Triglycerides (g/L)	0.716±0.11	0.730±0.07	1.730±0.06**	0.856±0.09
Cholesterol (g/L)	0.350±0.05	0.400±0.06	0.576±0.015*	0.507±0.031*
Albumin (g/L)	43.33±1.53	41.00±1.00	33.00±1.00**	35.67±1.52*
*P< 0,05 **P<0,01 relative to the control group (C)				

According to **Ghazanfari et al. (2021), s**ub-acute exposure to acetamiprid has been shown to induce significant hepatotoxicity, as evidenced by elevated serum levels of hepatic enzymes and severe histological alterations in hepatic tissue, indicating substantial liver injury**.** The liver, an organ, which plays a central role in xenobiotic metabolism, is particularly susceptible to damage from toxic substances **(Gulati et al., 2018).** Serum hepatic enzymes, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), serve as critical biomarkers for evaluating liver integrity and function, with their elevated levels reflecting structural membrane damage and cellular necrosis **(Chakroun et al., 2016)**. ALT, being liver-specific, is a particularly reliable indicator of hepatic injury, while AST, though present in other tissues, also increases significantly in liver damage. The release of these enzymes into the bloodstream following

hepatocellular membrane disruption and necrosis suggests that prolonged oral exposure to acetamiprid accumulates hepatotoxic concentrations, ultimately leading to cell death **(Chakroun et al., 2016)**. These findings align with prior studies demonstrating acetamiprid-induced hepatotoxicity in sub-chronic and chronic exposure scenarios **(Devan et al., 2015).** Damage to the mitochondria in liver cells impairs fatty acid β-oxidation, promoting the accumulation of triglycerides. Acetamiprid may inhibit the conversion of cholesterol into steroid hormones, indirectly affecting lipid metabolism **(Kong et al., 2017).**Albumin is synthesized by the liver. Acetamiprid induces hepatic oxidative stress, which can impair the function of liver cells and diminish albumin production **(Gad EL-Hak et al., 2022).**

In contrast**,** The extracts of *Juniperus phoenicea* exhibit significant hepatoprotective activity, as evidenced by their ability to reduce serum AST and ALT levels, indicating hepatic cell regeneration, while also increasing and albumin levels, which suggests enhanced protein synthesis and liver repair **(Laouar et al., 2017)**. According to **Elsherif et al. (2024)**, phytochemical analysis reveals that these extracts are rich in bioactive compounds, including total phenolic compounds, tannins, flavonoids, carbohydrates, proteins, coumarins, anthocyanins, saponins, and glycosides, all of which contribute to their therapeutic potential. These phytochemicals possess diverse biological functions, such as antioxidant and anti-inflammatory effects, while saponins additionally demonstrate cholesterol-lowering and immunomodulatory properties, further supporting the extract's hepatoprotective and regenerative mechanisms **(Elsherif et al., 2024).**

4.3.Oxidative Stress parameters

4.3.1.Effect of treatments on Glutathione S. transferase And Glutathione And Catalase

Exposure to acetamiprid has been shown to significantly elevate catalase (CAT) activity, an effect that is effectively mitigated by treatment with *Juniperus* extracts. Additionally, acetamiprid exposure induces a reduction in both glutathione levels(GSH) and glutathione S-transferase (GST) activity in hepatic tissue; however, therapeutic intervention with *Juniperus phoenicea* extracts demonstrates a protective role by attenuating these adverse effects. These findings suggest that *Juniperus* extracts possess antioxidative properties capable of counteracting the oxidative stress induced by acetamiprid exposure.

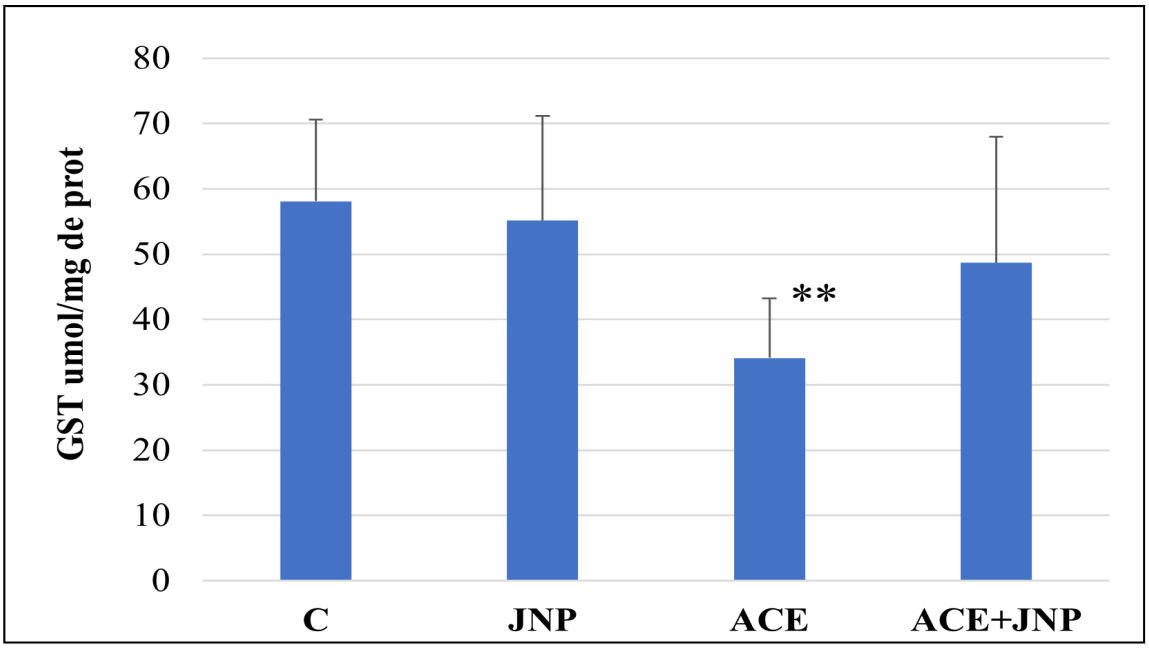


Figure16. Effect of aqueous extracts of *Juniperus phoniecea* and/or Acetameprid treatment on Glutathione S. transferase (GST) activityin the liver after 30 days of exposure (values represent the mean±SEM of seven rats)

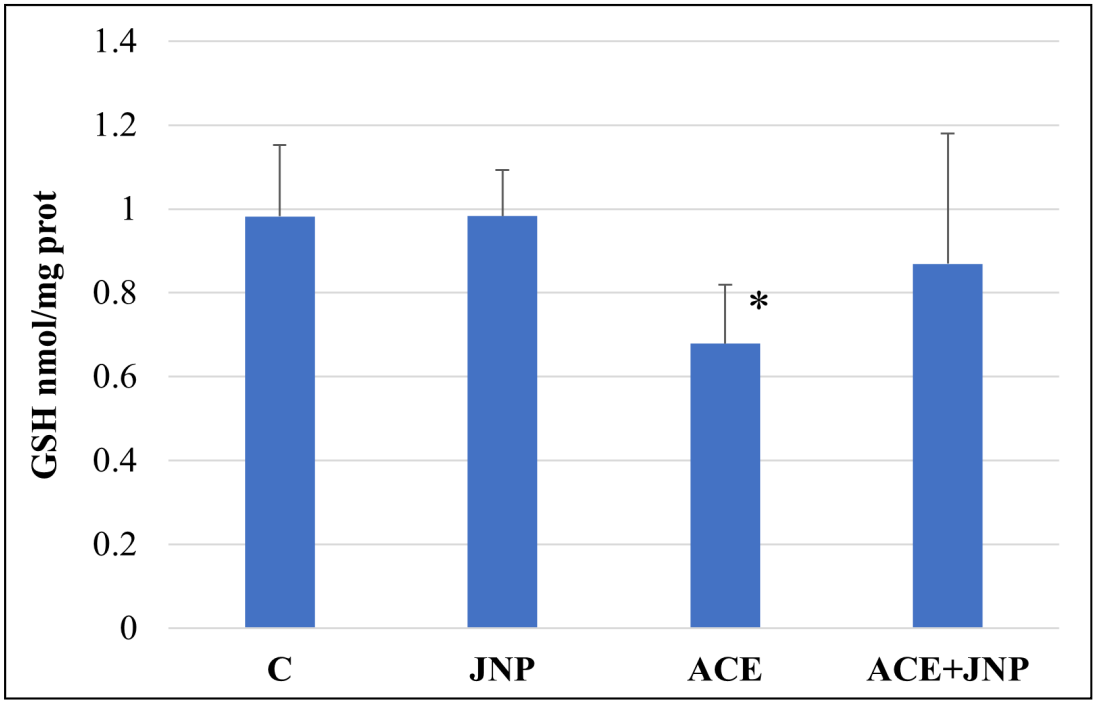


Figure17. Effect of aqueous extracts of *Juniperus phoniecea* and/or Acetameprid treatment on reduced Glutathione level (GSH)in the liver after 30 days of exposure (values represent the mean±SEM of seven rats)

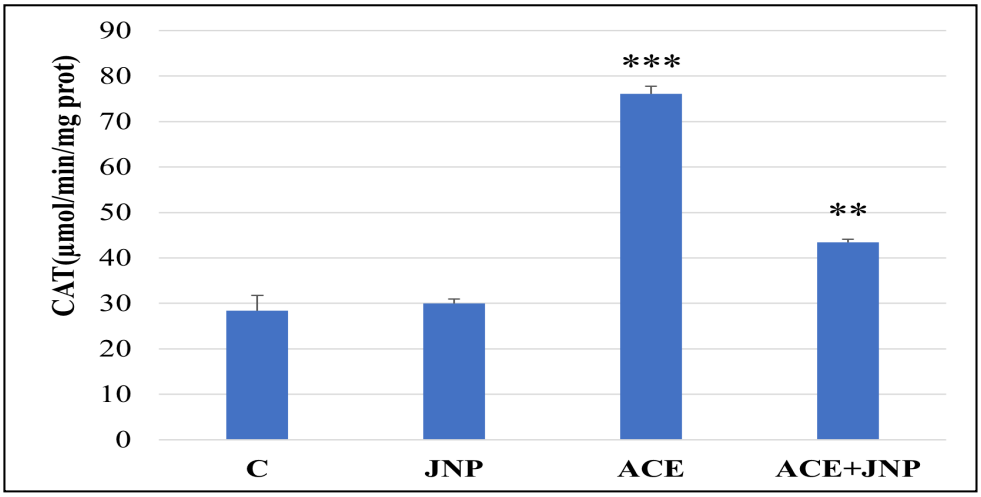


Figure18. Effect of aqueous extracts of *Juniperus phoniecea* and/or Acetameprid treatment on Catalase activity (CAT)in the liver after 30 days of exposure (values represent the mean±SEM of seven rats)

Oxidative stress is defined by an imbalance between increased levels of reactive oxygen species (ROS) and a low activity of antioxidant mechanisms. An increased oxidative stress can induce damage to the cellular structure and potentially destroy tissues **(Preiser, 2012).**

Several studies have demonstrated that acetamiprid exposure alters antioxidant defense mechanisms in rats, particularly affecting the activity of key enzymes such as glutathione S-transferase (GST) and catalase (CAT), as well as glutathione (GSH) levels. Research by **Chakroun et al. (2016)**, reported increased GST and CAT activities in the liver of rats after exposor to 1/5 of LD50 of acetamiprid dose .suggesting an adaptive response to oxidative stress. Similarly, **Khovarnagh et al. (2021),** observed a significant reduction in hepatic GSH levels alongside elevated GST activity in acetamiprid-exposed rats, indicating oxidative perturbation. Furthermore**,(Noaishi & Abd alhafez, 2016)** highlighted that acetamiprid induces oxidative stress .As evidenced by increased CAT activity, which may reflect a compensatory mechanism to counteract reactive oxygen species (ROS). These findings collectively underscore the dual role of acetamiprid in both promoting oxidative stress .The administration of *Juniperus phoenicea* extract significantly elevated glutathione S-transferase (GST) activity, suggesting a restoration of the disrupted oxidant-prooxidant balance in experimental rats by modulating antioxidant enzyme levels **(Sotelo et al., 2002)**. This effect may be attributed to the upregulation of GST gene transcription, as phase II enzyme genes are highly inducible by antioxidant compounds. Additionally, *J. phoenicea* treatment increased hepatic glutathione (GSH) levels, likely through de novo synthesis or regeneration, thereby enhancing cellular defense against free radicals and peroxides **(Attia et al., 2012; Klibet et al., 2016; Laouar et al., 2017).** The extract also promoted catalase (CAT) and GSH activities, indicating a potential mechanism by which *J. phoenicea* reinforces cellular antioxidant defenses, possibly by stimulating the expression and activity of antioxidant enzymes during oxidative stress **(Bouassida et al., 2018)**.

Conclusion and Perspectives

In conclusion, the findings of this study provide compelling evidence that *Juniperus phoenicea (J. phoenicea)* extracts exert a protective role against the physiological, biochemical, and oxidative stress-related disturbances caused by exposure to acetamiprid, a widely used neonicotinoid pesticide. The administration of *J. phoenicea* extract not only counteracted the acetamiprid-induced reduction in body weight but also revealed significant hepatoprotective effects, as demonstrated by improved liver histopathology and normalization of hepatic enzyme activity. Furthermore, the co-treatment group (ACE+JNP) exhibited notable improvements in lipid profiles, antioxidant status, and liver function markers compared to the group exposed to acetamiprid alone.

These findings underscore the therapeutic potential of *J. phoenicea* as a natural phytotherapeutic agent capable of mitigating pesticide-induced toxicity, particularly in relation to liver health and metabolic disturbances. The bioactive compounds present in the extract, such as flavonoids and phenolic acids, likely contribute to its antioxidant and anti-inflammatory effects. This study not only adds to the growing body of evidence supporting the medicinal value of plant-based interventions but also highlights the urgent need for developing alternative strategies to safeguard human and environmental health in the face of rising pesticide exposure.

Future research should aim to further isolate and characterize the active constituents of *J. phoenicea*, explore their mechanisms of action at the molecular level, and evaluate their safety and efficacy in long-term and clinical settings. Ultimately, the integration of such phytotherapeutic agents into public health strategies may offer a sustainable and effective approach to combat the adverse effects associated with chemical pollutants like acetamiprid.

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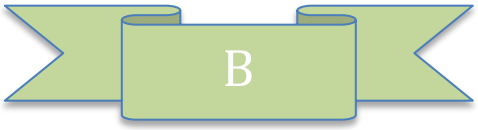
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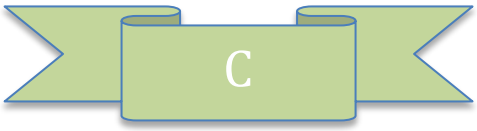
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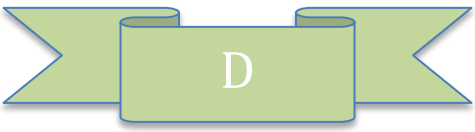
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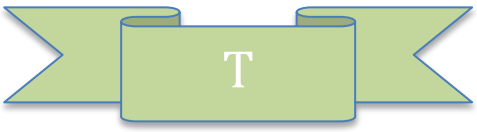
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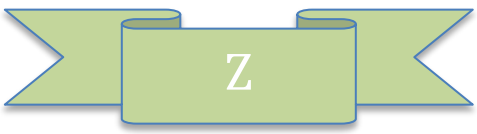
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Webographic

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