People's Democratic Republic of Algeria
Ministry of Higher Education and Scientific Research
Ministère de l'Enseignement Supérieur et de la Recherche Scientifique
University of Larbi Tebessi –Tebessa-
Faculty of Exact Sciences and Natural and Life Sciences
Department of Applied Biology
Field of Natural and Life Sciences

End-of-study dissertation

Presented for the purpose of obtaining the Master's degree

In : Biological Sciences
Option : Applied Biochemistry

Presented by :

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Entitled :

study of the potential protective effects of medicinal plants extract on kidney function impairment caused by insecticide exposure in rats

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Date of the graduation thesis defense: 02/ 06 / 2025

Acknowledgements

We begin this work by praising and thanking God, who has given us the strength, determination, and will to move forward and reach this stage. We hope for acceptance and success in what we have accomplished.

Because any achievement is not built by individual effort alone, but is shaped by the collaboration of minds and hearts, we extend our highest expressions of thanks and gratitude to all those who helped us and shared the road with us with an opinion, support, or a kind word that spreads hope.

We would also like to thank the two members of the discussion committee, **Guedri Kamilia** and . **Hamiri Manal**, for graciously accepting the evaluation of this work and contributing to its enrichment with their support and appreciation.

All appreciation and gratitude goes to our generous supervisor,**Messaadia Amira** , whose prophetic supervision, valuable guidance, continuous support, and patience throughout the completion of this work have been invaluable. Her expertise and profound observations have been instrumental in the development of this research.

To the members of the laboratory team of the Department of Applied Biology, especially **Nardjesse** ,**Manel** and **Raouia**, we express our deepest thanks for their fruitful cooperation and continuous support that facilitated our steps.

We would also like to thank **misse limami maroua** and **Messaoud el montasser billeh** , **Triki Okba** and **Fethallah Haithem** for their help and support, which had a great impact on overcoming many of the challenges we faced.

Finally, we salute all those who believed in us, pushed us with a word, or encouraged us with a moment. Thanks to them, we were able to continue the journey and reach this important milestone in our scientific journey

Dedication

To God, from Him is success and to Him is favor. We did not take the beginnings except by His facilitation, we did not reach the ends except by His favor, and we did not achieve the ends except by His grace, who inspired me with patience and supported me in every step, so praise be to Him as is due to the majesty of His face and the greatness of His authority.

And to myself I dedicate this achievement to you, because you were patient, you persevered, you endured everything in silence, because you did not give up, and because you believed in your dream until the end...I am proud of you.

To my late father, **Cherfi Ahmed**, I dedicate this work to you, in gratitude for your kindness, in honor of your pure soul, and to make this work a running charity for your soul, may God accept it as a light in your grave.

To my mother **Sellat Fadjara**, whose prayers were the secret of my success, and who taught me patience and giving, I dedicate you the fruit of my efforts and the toil of my years.

To my brother **Hassen** , you are my support after God, you who are the back that does not break, and the hand that holds me when I am weak, you have my gratitude for your support, patience, and encouragement in all my steps.

To **Abir,** thank you for your support, and your sincere love which is priceless

To **Elline**, **ouatin** and **Taline**, the flowers of my heart, your innocent eyes sow hope in my days.

To all those who shared my moments of fatigue and stood by my side in all circumstances, thank you for your pure and priceless hearts.Djamila, Tounes, Amel.

To my partner **Hadjira**, who shared the dream with me step by step, this work is the fruit of our joint effort.

Sana



Dedication

To God, the merciful and gracious, you are the one who lit my path when the roads were dark, and who gave me your strength when my steps were weak. In every letter I wrote and every accomplishment I achieved, you were my helper. Praise be to you until you are satisfied, and thanks be to you as long as there is a heartbeat that reminds you of your blessings.

To my mother **AHMANE nedjma** , you are the home of love, the haven of tenderness, and a pulse that does not fade. Every success in my life is an echo of your prayers, and every tear of joy in my eyes todaywas once a hope in your eyes. You are the beginning, the blessing, and the most beautiful stories.

To my father **AHMANE salem**, you are my pillar and my hope, your silence carries the most sincere prayers, and your gaze gives me the certainty that I am on the right path. From you, I learned that dreams are only made with determination, and that persistence is the first door to success. Every achievement today is an extension of the strength you instilled in my soul.

To all my siblings, especially my brother **Ala eddin** and my sister **Hanane**, you are the heartbeat of my heart when it faded, and the warmth of cold days. Your laughter was a light in the darkness, and your presence was an indescribable support. I owe you immeasurable gratitude.

To the one who was my support, my twin, my other half, my constant presence and pulse despite the silence. In you I found safety, and from you I learned that love is a prayer in the unseen and an unasked-for embrace. Every moment of awareness was by the grace of your heart, and every achievement is your fingerprint.

To my dear friend **ilham**, sister of my soul and companion of my path, you were my mirror when my features were lost, and the voice of tranquility amidst the noise of life.

To **the grandchildren** of my family, you are the flower of life and the joyof the soul, you are the future we hope for, your laughter fills the heart with hope, and your innocence reminds me that all the fatigue of life is gone.

To my partner **Sanaa**, who shared the dream with me, and together we built days of hope and beautiful fatigue. This work is the fruit of our tired hearts.

Hadjira



Abstract

Acetamiprid is a neonicotinoid insecticide known for its rapid efficacy in controlling insect populations. However, it poses a risk in terms of residue accumulation in agricultural products, raising concern about its potential effects on human health, including nephrotoxicity. In our studies, we aimed to evaluate the value and relevance of *Juniperus phoenicea* as a therapeutic alternative against acetamiprid-induced toxicity. To minimize its risk to human health.

twenty-eight female Albino Wistar rats were divided into 4 groups of 7 rats each. The animals were treated with acetamiprid at a dose of 20 mg/kg per day or an aqueous extract of *Juniperus phoeniceas* at a dose of 200 mg/kg orally for 30 days.

The analysis of the results showed that acetamiprid causes adverse effects in the body leading to decreased nutritional capacity, loss of appetite, and decreased relative weight in rats and increased relative weight of the kidneys. Relative change in the biochemical parameter (decreased in albumin,eGFR and increased in uric acid, urea, and creatinine)and significant changes in antioxidant levels in the kidneys where we observed a decrease in glutathione S-transferase (GST), a decrease in glutathione (GSH), and an increase in catalase (CAT) activity

Nonetheless, the addition of *Juniperus phoenicea* improved the observed parameters. This improvement demonstrates the antioxidant, anti-inflammatory, and therapeutic characteristics of these medicinal plants in mitigating the disruptive and oxidative effects of acetamiprid.

Key words: Acetamiprid, *juniperus phoenicea*, nephrotoxicity, biochemical parameter, antioxidant.

Resume

L'acétamipride est un insecticide néonicotinoïde connu pour son efficacité rapide dans le contrôle des populations d'insectes. Cependant, il présente un risque en termes d'accumulation de résidus dans les produits agricoles, ce qui soulève des inquiétudes quant à ses effets potentiels sur la santé humaine, notamment en termes de néphrotoxicité. Dans nos études, nous avons cherché à évaluer la valeur et la pertinence de *Juniperus phoenicea* en tant qu'alternative thérapeutique contre la toxicité induite par l'acétamipride. Pour minimiser le risque pour la santé humaine.

Vingt-huit rats albinos Wistar femelles ont été divisés en 4 groupes de 7 rats chacun. Les animaux ont été traités avec de l'acétamipride à une dose de 20 mg/kg par jour ou avec un extrait aqueux de *Juniperus phoenicea* à une dose de 200 gmg/k par voie orale pendant 30 jours.

L'analyse des résultats a montré que l'acétamipride provoque des effets indésirables dans l'organisme, entraînant une diminution de la capacité nutritionnelle, une perte d'appétit, une diminution du poids relatif chez les rats et une augmentation du poids relatif des reins. Un changement relatif dans les paramètres biochimiques a été observé (diminution de l'albumine, du DFG estimé \[eGFR], et augmentation de l'acide urique, de l'urée et de la créatinine), ainsi que des changements significatifs dans les niveaux d'antioxydants au niveau des reins, avec une diminution de la glutathion S-transférase (GST), une diminution du glutathion (GSH), et une augmentation de l'activité de la catalase (CAT).

Néanmoins, l'ajout de *Juniperus phoenicea* amélioré les paramètres observés. Cette amélioration démontre les caractéristiques antioxydantes, anti-inflammatoires et thérapeutiques de cette plante médicinale dans l’atténuation des effets perturbateurs et oxydatifs de l’acétamipride.

Mots-clés : Acétamipride, *juniperus phoenicea,* nephrotoxicité, paramètres biochimiques, antioxydantes.

ملخص

يُعد الأسيتاميبريد مبيدًا حشريًا من مجموعة النيونيكوتينويد، ويُعرف بفعاليته السريعة في مكافحة تجمعات الحشرات. ومع ذلك، فإنه يُشكل خطرًا من حيث تراكم الرواسب في المنتجات الزراعية، مما يثير القلق بشأن تأثيراته المحتملة على صحة (Juniperus phoenicea) الإنسان، بما في ذلك سُميته الكلوية. في هذه الدراسة، سعينا إلى تقييم فعالية نبات العرعر الفينيقي كبديل علاجي للحد من السمية الناتجة عن الأسيتاميبريد، بهدف تقليل مخاطره على الصحة البشري

تم تقسيم ثمانية وعشرين فأرًا من إناث فصيلة ألبينو ويستر إلى أربع مجموعات، تضم كل مجموعة سبعة فئران. عولجت
ملغ/كغ يوميًا، أو بمستخلص مائي من نبات العرعر الفينيقي بجرعة 200 ملغ/كغ عن الحيوانات إما بالأسيتاميبريد بجرعة طريق الفم لمدة 30 يومًا

أظهرت نتائج التحاليل أن للأسيتاميبريد تأثيرات سلبية واضحة، تمثلت في انخفاض الشهية والقدرة الغذائية، وانخفاض الوزن النسبي للفئران، إلى جانب زيادة الوزن النسبي للكلى. كما سُجلت تغيرات في المؤشرات البيوكيميائية، شملت انخفاض
وارتفاع مستويات حمض اليوريك، اليوريا، والكرياتينين. (eGFR) مستويات الألبومين ومعدل الترشيح الكبيبي التقديري وزيادة (GSH) ومستوى الغلوتاثيون (GST) بالإضافة إلى ذلك، لوحظ انخفاض في نشاط إنزيم غلوتاثيون إس-ترانسفيراز في الكلى (CAT) في نشاط إنزيم الكاتالاز

ومع ذلك، أظهر علاج الفئران بمستخلص العرعر الفينيقي تحسنًا ملحوظًا في هذه المؤشرات، مما يُبرز الخصائص المضادة للأكسدة، والمضادة للالتهابات، والفعالية العلاجية لهذا النبات الطبي في التخفيف من الآثار السمية والتأكسدية التي يُسببها الأسيتاميبريد

الكلمات المفتاحية : الأسيتاميبريد، العرعر الفينيقي(*Juniperus phoenicea*) السمية الكلوية ، المؤشرات البيوكيميائية ، المضادة للأكسدة.

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Abbreviation list

(NNIs)	Neonicotinoid insecticides
(ACE)	Acetamiprid
mNEOs	neonicotinoid metabolites
ACE	Acetamiprid
mNEOs	neonicotinoid metabolites
pNEOs	parent neonicotinoids
nAChR	nicotinic acetylcholine receptors
TCM	traditional Chinese medicine
JNP	*Juniperus phoenicea*
GST	Glutathione S. transferase
GSH	reduced Glutathione level
CAT	Catalase activity
POD	peroxidase
DCBS	dichlorophenol sulfonates
4-AA	4-aminoantipyrine
GlDH	glutamate dehydrogenase
ROS	Reactive oxygen species
H2O2	hydrogen peroxide
CDNB	1-chloro,2.4-dinitrobenzene
DTNB	5,5'-dithio-bis-2-nitrobenzoic acid
EDTA	Ethylene Diamine Tetra Acetic Acid
BSA	bovine serum albumin
NNs	Neonicotinoid
RPM	Revolutions per minute
R	Reagent
CAL	Calibration
BCG	Bromocresol green

SSA	sulfosalicylic acid
eGFR	estimated Glomerular Filtration Rate

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Introduction

Introduction

The use of pesticides in agriculture is crucial for ensuring food security, a topic of enormous worldwide concern, due to the significant increase in food demand brought on by a constantly expanding global population and a decline in the amount of arable land available. In this sense, pesticides are essential for raising agricultural output and enhancing the quality of food **(Lalín-Pousa et al., 2025).**

Neonicotinoid insecticides (NNIs) are the most widely used neuroactive insecticides globally, accounting for nearly 25% of the market share. These chemicals are applied to over 140 varieties of crops in 120 countries. Despite their widespread use, NNIs pose significant risks to non-target organisms, including beneficial insects like bees. Their toxicological effects extend beyond insects, causing hepatic and nephrotoxicity in mammals, oxidative stress in aquatic species, and reproductive toxicity in avian populations. The widespread use of NNIs has raised concerns about their environmental and ecological impacts, disrupting ecosystem processes and services, and potentially threatening human health. Efforts to reassess their environmental safety and explore alternatives are gaining momentum to mitigate these risks **(Zhou et al., 2025).**

A neonicotinoid insecticide, acetamiprid is widely used as a substitute for organophosphates in pest control and is being used more and more frequently to boost crop output. Several foods, water, and soil have been shown to contain traces of ACE and its harmful metabolites **(Messallam et al., 2024).**The insecticide acetamiprid is a chlorinated neonicotinoid. It has been detected in cereal grains, soil, and water sources. Its metabolism has also been shown in human blood, urine, and bodily tissues. Headaches, vertigo, sleep disturbances, vomiting, convulsions, and tachycardia can all result after exposure to ACE. Numerous organisms have been shown to exhibit hematological, neuro, nephro, reproductive, and hepatotoxicity in recent investigations. The primary cause of ACE- induced organ damage is oxidative stress, which modifies cellular biochemistry and impacts enzymes **(Phogat et al., 2023)**.

As a result their many bioactive components, herbal medicinal plants have the potential to revolutionize healthcare by offering natural substitutes for conventional treatments**(Elsherif et al., 2024).**

Phoenicia . *Juniperus*, According to traditional medicine, this plant is used extensively in many nations to cure a variety of conditions, including sunstroke, bronchitis, rheumatism,

eczema, hemorrhoids, dermatitis, diarrhea, bronchitis, acute gonococcal infections, and depurative disinfectants. The berries of *J. Phoenicia* are used as an oral hypoglycemic and to relieve the symptoms of all kinds of coughs, while the leaves are used as a diuretic and to treat bronco- pulmonary illness. Additionally, *J. phoenicia* leaves and berries are combined and used as an oral hypoglycemic medication **(Mansour et al., 2023**). Numerous studies have highlighted that *Juniperus phoenicea L*. contains a wide variety of bioactive compounds, including diterpenoids, flavonoids, lignans, phenylpropanoid glucosides, furanone glucosides, bis-furanone derivatives, norterpenes, and sesquiterpene glucosides **(Elsherif et al., 2024)**. Pharmacological studies have demonstrated that *Juniperus phoenicea* exhibits a wide range of therapeutic properties across its leaves, stem, and root. These include antimicrobial, antioxidant, anti- inflammatory, cytotoxic, hepatoprotective, antimutagenic, antiplasmodial, antiproliferative, antifungal, antiviral, anthelmintic, analgesic, antipyretic, cardioprotective, immunomodulatory, and antiprotozoal activities **(Alagbe., 2022)**.

This study aims to investigate the potential protective effects of a *Juniperus phoenicea* extract against kidney damage caused by acetamprid exposure. By examining biochemical markers and kidney function, the research seeks to determine whether the plant extract can mitigate the adverse effects of the acetamprid. The findings may contribute to developing altemative therapeutic strategies to protect kidney health in cases of toxic exposure.

Bibliographic part

1. Acetamprid

1.1. Definition and historical usage of Acatamprid

Acetamiprid is a well-known neonicotinoid insecticide recognized for its rapid effectiveness in controlling insect populations. However, it poses a risk of residue accumulation in agricultural products, raising concerns about potential impacts on human health **(Ji et al., 2025).**

Nippon Soda Co., Ltd. created the acetamprid in 1995 to combat a variety of agricultural pests **(Tu et al., 2025)**.

The use of neonicotinoid pesticides in Africa has risen significantly due to their effectiveness against invasive crop pests, leading to higher agricultural yields and economic benefits for farmers. However, inadequate data, weak monitoring systems, and insufficient regulations have resulted in widespread challenges, including non-compliance, illegal imports, mislabeled products, excessive application, co-use with other pesticides, and improper usage. In South Africa, over a hundred neonicotinoid-based pesticides, including acetamiprid, have been registered without restrictions. Ghana and Kenya acknowledge the indiscriminate use of these chemicals, while Morocco has banned thiacloprid since 2021. Nigeria is gradually phasing out certain neonicotinoids, including clothianidin, thiacloprid, and thiamethoxam **(Orikpete et al., 2025)**.

1.2. Classification and Usage

Acetamiprid is an odorless neonicotinoid insecticide.With the molecular formula $C_{10}H_{11}ClN_4$ (E-N 1-(6-chloro-3-pyridyl) methyl]-N_2-cyano-N_1-methylacetamidine) **(Jaffar et al., 2025).**

Acetamiprid is a recently developed neonicotinoid insecticide with strong systemic and translaminar activity. It is highly effective against sucking pests like aphids, leafhoppers, thrips, and whiteflies, as well as Homoptera, Thysanoptera, and Lepidoptera, which cause significant crop damage worldwide. Acetamiprid is particularly useful for managing pesticide-resistant pests while maintaining low toxicity to natural predators and mammals, making it a promising option for

integrated pest management with a reduced environmental impact **(Zeeshan et al., 2024).**

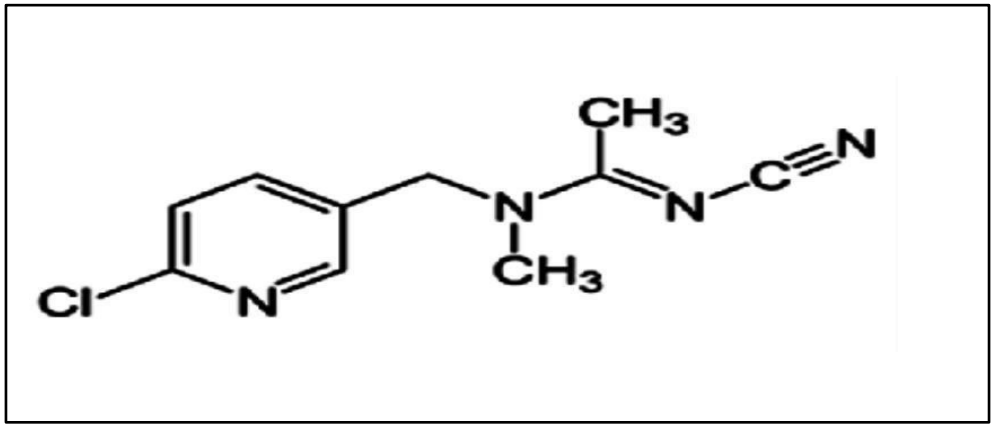


Figure 01. Structure of acetamiprid **(Padmavathi et al., 2020)**

Table 1 . Physical and chemical data of acetamiprid **(web 1).**

Property	**Results**
Physical state and colour	Pure active substance: white powder
Odour	Pure active substance: scentless
Solubility in water including effect of pH	Water solubility at 25 °C: in distilled water: 4.25 × 10° mg/1 pH 5: 3.48 × 10° mg/l pH 7:2.95 x 103 mg/I pH 9:3.96 × 103 mg/l
Solubility in organic solvents	Solubility at 25 °C g/100g Acetone:> 20 Methanol:> 20 Ethanol:> 20 Methylene chloride : > 20 Chloroform :> 20 Acetonitrile :> 20 Tetrahydrofuran : > 20

	Benzene:2.44 Xylene:4.01 n-Hexane:6.54 ppm Carbon disulfide : 507 ppm

1.3. Routes of Acetamprid Exposure

Common insecticides called neonicotinoids are present in food supplies, soil, and aquatic ecosystems. Through photolysis, hydrolysis, and microbial breakdown, parent neonicotinoids (pNEOs) can yield neonicotinoid metabolites (mNEOs). mNEOs can be generated in agricultural land and are found in food, groundwater, and rivers. According to recent research, the toxicity levels of mNEOs are on par with or higher than those of pNEOs. These levels include negative effects on the human liver and fertility as well as bee mortality. Both human health and ecosystems are at risk from these harmful mNEOs **(Wang et al.,2025).**

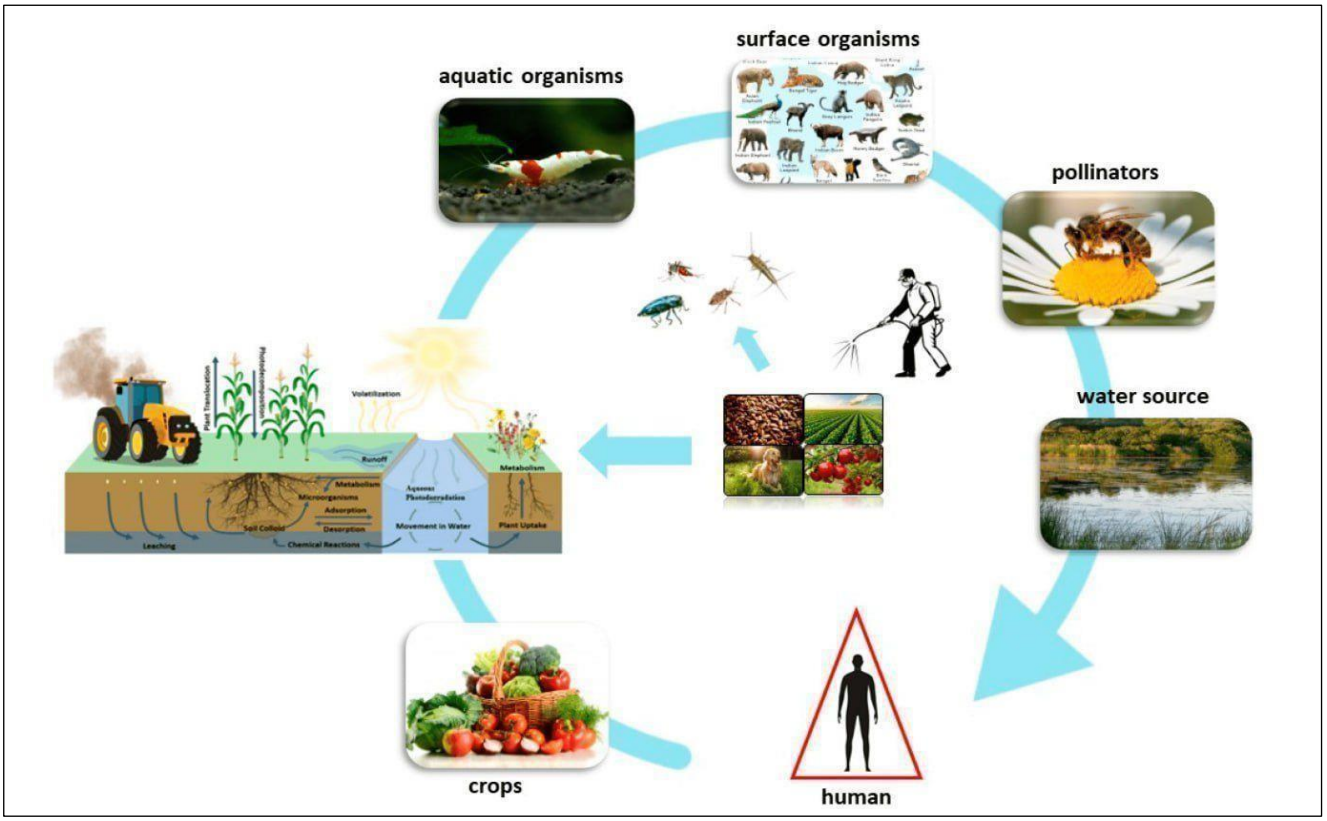


Figure 02. The environmental fate and routes of neonicotinoid pesticide exposure after application **(Zusdiková et al.,2023)**

1.4. Health Impacts of Acetamprid Exposure

Neonicotinoids are common pesticides that target insects that chew and suck. They are utilized in veterinary medications, home gardens, and commercial agriculture. In non-target species, exposure to neonicotinoids can result in immunotoxicity, hepatotoxicity, nephrotoxicity, neurotoxicity, and genotoxicity **(Hou et al., 2025).**

Numerous *in vivo* and *in vitro* studies have indicated that ACE induces an imbalance between the body's antioxidant defense system and oxidants, leading to oxidative stress. This stress not only disrupts cellular functions across various organs and degrades essential cellular macromolecules such as proteins and lipids, but it also reduces antioxidant levels. Consequently, oxidative stress increases lipid peroxidation, DNA damage, and genotoxicity. Research has shown that oxidative stress plays a critical role in liver damage and kidney injury associated with ACE. Additionally, recent findings highlight dysregulation and abnormalities in amino acid and fatty acid metabolism, which alter lipid biochemistry **(Aioub et al., 2024).**

The kidney, an excretory organ, shares the liver's primary function of detoxifying harmful substances. Acetamiprid and other NNs are primarily eliminated in the urine. Although their water solubility and low molecular weights indicate that they flow smoothly through the glomerulus, it is unknown if the renal proximal tubules resorb or excrete them. As a result, the stress of these harmful substances in the body affectes the kidneys and liver. Thus, it becomes critical to monitor the key systems involved in the entire metabolism of acetamiprid and assess its toxicity to these systems **(Toghan et al., 2022).**

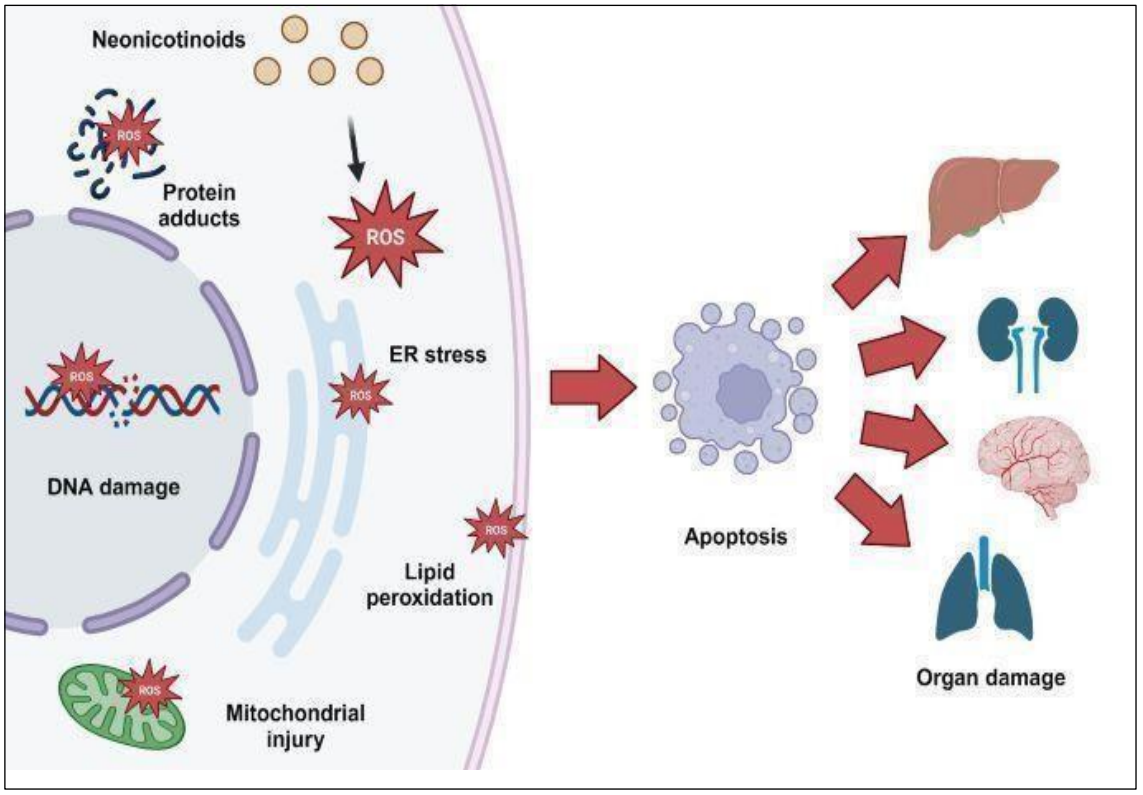


Figure 03. Toxic Effects of Neonicotinoids: Oxidative Stress and Cellular Damage **(Rajak et al., 2024).**

2. Antioxidants and *Juniperus phoenicea*

2.1. Role of antioxidants in counteracting oxidative stress

The human body's physiological homeostasis is essential to maintaining the organs, tissues, and cells' ability to operate. Redox homeostasis is maintained by a system in the body **(Liu et al., 2025)**.

Antioxidants defend against oxidative stress, alter signaling pathways, reduce inflammation, and preserve the intracellular oxidative balance. Even at low dosages, antioxidants efficiently slow down the rate of oxidative damage. Depending on how they work, they can be divided into two groups: primary and secondary. The former is in charge of getting rid of free radicals and stopping the chain reactions that lead to oxidative stress.Hydroperoxides are oxidized and transformed into stable forms by them. Secondary antioxidants decrease singlet oxygen, destroy metal ions, and

promote antioxidant renewal. The body has a sophisticated natural antioxidant system that guards against damage and pro-oxidants. Antioxidant systems are also supported by certain food sources. As a result, there are two types of antioxidant systems: endogenous and exogenous. While endogenous antioxidants are found in the body, exogenous antioxidants are acquired through nutrition (e.g., flavonoids, carotenoids, ascorbic acid, caffeic acid, or lycopene) **(Kamaljeet et al., 2024)**.

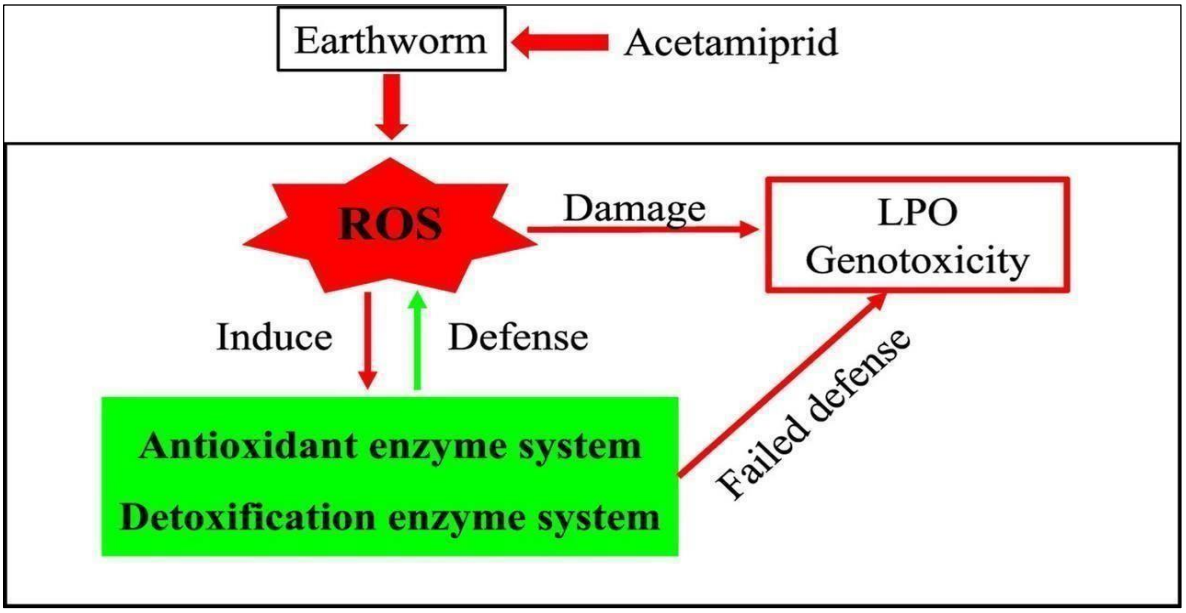


Figure 04.Oxidative Damage and Defense Mechanisms in Earthworms Exposed to Acetamiprid **(Li et al., 2018).**

2.2. Geographic distribution

Juniperus phoenicea L., an evergreen monoecious coniferous tree, is native to the southwestern region. Its distribution spans North Africa, including Morocco, Algeria and Tunisia, extending east to the Arabian coast of the Red Sea and west to the Canary Islands and Madeira. This species thrives in diverse environments, from dunes and hills to arid mountainous regions, with notable populations also present in Spain **(Dakhil et al., 2022 ; Asbabou et al., 2024).**

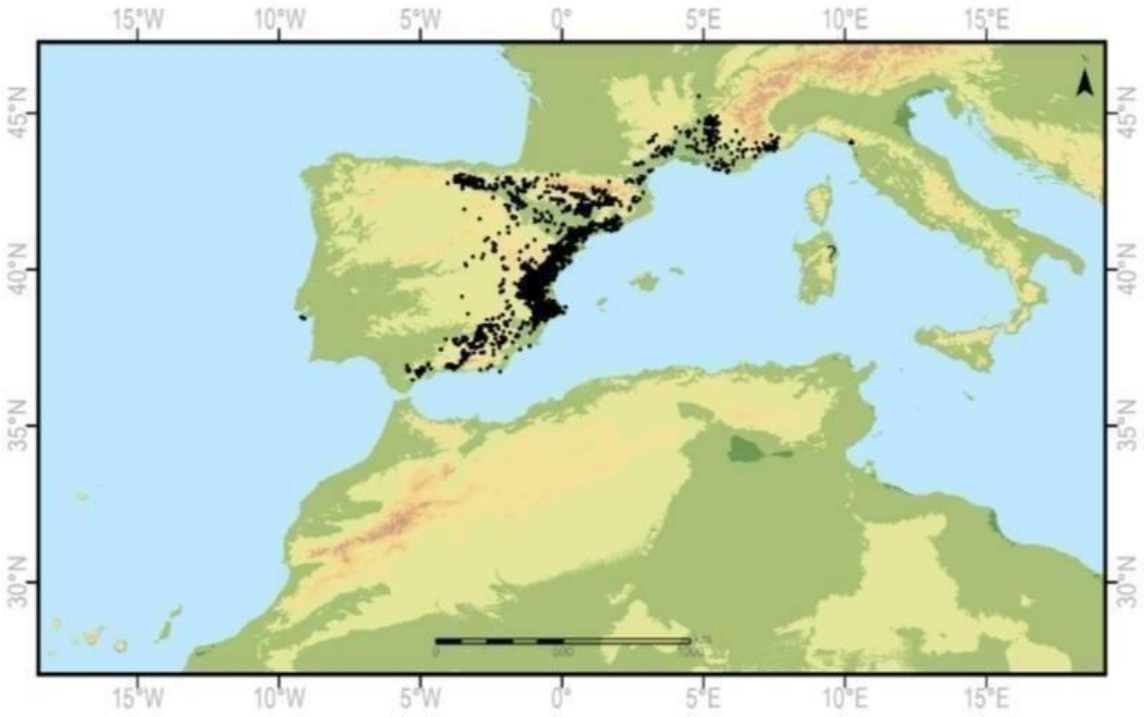


Figure 05. Distribution of *Juniperus phoenicea* **(Boratyński et al., 2024).**

2.3. *Juniperus phoenicea* overview

Juniperus phoenicea is regarded as a significant medicinal plant and is utilized extensively in conventional healthcare. The fruits of *Juniperus phoenicea*, which is indigenous to Egypt's Mediterranean and Sinai regions, have been found in ancient Egyptian tombs, indicating the plant's historical significance. Furthermore, prior studies have demonstrated that *Juniperus phoenicea* possesses strong cytotoxic qualities and can stop the proliferation of several cancer cell types **(Serseg et al., 2024)**.

The genus *Juniperus* is used to prevent or treat several illnesses in traditional Chinese medicine (TCM). The Cupressaceae family includes the plant genus *Juniperus L*, which has 70 species globally and 23 species in China. Numerous species in the genus Juniperus contain a wide range of phytochemicals, including phenolic acids, flavonoids, lignans, diterpenes, and triterpenes. Several *Juniperus* species have been shown to have antibacterial, antioxidant, antitumor, and anticancer properties as a

result of the plants' high phytochemical content, which directly affects their biological activity **(Putera Instal et al., 2024).**

2.3.1 Botanical description:

The *Juniperus phoenicea* is a large shrub that grows to a height of 5 to 8 meters. Its crown is rounded or uneven, and its trunk is 1 to 2 meters in diameter. The bark is dark grayish brown and can be torn into strips. Adult scales, which are 1-2 mm long and green to blue-green, replace the juvenile needle-shaped leaves, which are 5–14 mm long and 1 mm wide, on older plants. On older plants, the leaves are adult scales that are 1-2 mm long and green to blue-green in hue, whereas the leaves on younger plants are 5-14 mm long and 1 mm wide. The leaves are arranged in whorls of three or opposing decussed pairs, Cones diameters of 8–12 mm and 7–11 mm, less than or equal to Typically, a cone holds four to six seeds **(Bouabdallah et al., 2024 ; Boratynski et al.,2024).**



Figure 06. General appearance of one of the *Juniperus phoenicea* **(personal photos).**



Figure 07. Different parts of the *Juniperus phoenicea* **(Boratynski et al.,2024).**

2.3.2.Classification of the J*uniperus phoenicea*

The taxonomy is as follows :

Table 02. Classification of the *Juniperus phoenicea* **(Asbabou et al., 2024)**

Kingdom	Plantae
Class	Equisetopsida
Order	Cupressales
Family	Cupressaceae
Genus	Juniperus
Species	*Juniperus phoenicea L*

2.3.3 chemical compositions of *Juniperus phoenicea*

Several studies on *J. phoenicea's* bioactive ingredients have shown significant differences in the plant's phenolic content, highlighting its wide range of medicinal uses. *J. phoenicea* was found to contain thirteen distinct phenolic compounds: 3-p-Coumaroylquinic acid, Quercetin-O-pentoxide, Myricetin-O-pentoside, Catechin, Myricetin-O-pentoside-O-hexoside, Myricetin-O-dihexoside, Myricetin-O-hexoside, Apigenin-6-C-glucoside, Myricetin-3-O-rhamnoside, Quercetin-3-O-glucoside, Quercetin-O-hexoside, Methyl-biflavone, and Isorhamnetin-O-pentoside **(Serseg et al., 2024).**

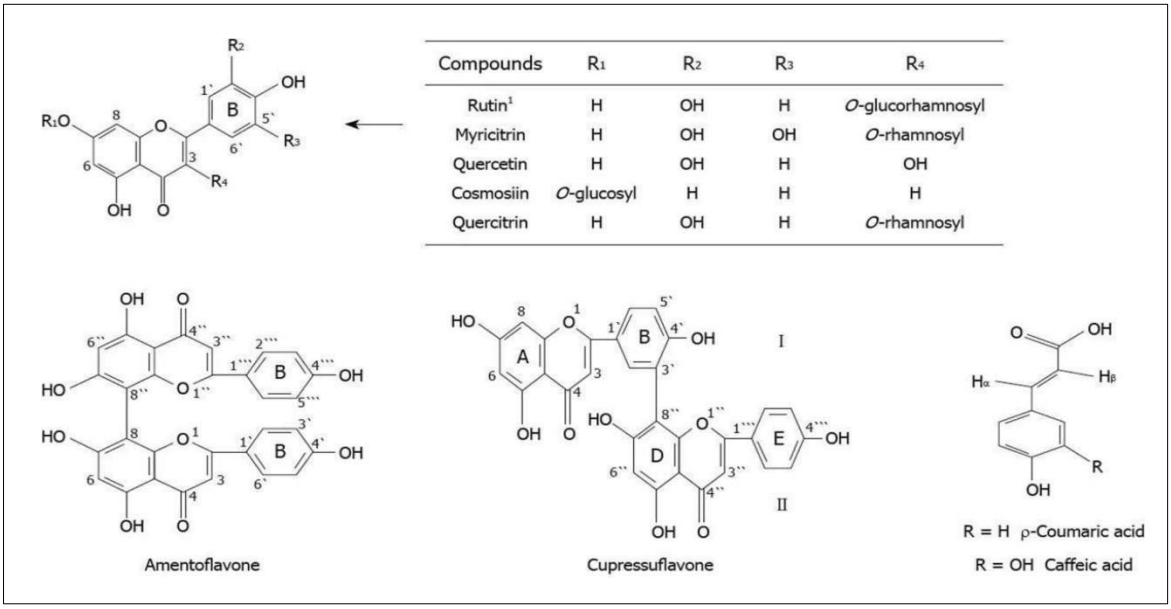


Figure 08. Chemical composition of the methanolic extract of *Juniperus phoenicea* **(Ali et al., 2010).**

2.4. Therapeutic Properties of the *Juniperus phoenicea*

Numerous pharmacological effects, including as diuretic, anti-inflammatory, anti-fungal, hepatoprotective, antidiabetic, and anti-hyperlipidemic qualities, have been found for several species in this genus. Furthermore, in individuals with Parkinson's disease, several species have been demonstrated to possess neuroprotective, antibacterial, and antioxidant qualities. The phytochemical components present in these species are primarily responsible for these activities **(Abdul Majid et al., 2024).**

Hepatocellular carcinoma (HCC) ranks as the sixth most prevalent cancer worldwide, posing a significant global health challenge. The juniper plant has demonstrated potent liver-protective and anti-hyperlipidemic properties **(Saleh et al., 2025).**

Experimental part

Material and methods

3 Materials and methods

3.3. Chemical materiel

3.3.1 Definition and usage

Acetamiprid is a commonly utilized insecticide, recognized for its systemic properties, selectivity, and efficacy. However, it is essential to apply it correctly and take necessary precautions to reduce potential environmental hazards. Its effectiveness extends to a broad range of pests.

3.1.2 Physiochemical properties

- Chemical Formula: $C_{10}H_{11}ClN_4$

- Molecular Weight: 222.68 g/mol

- Appearance: White crystalline solid

- Melting Point: 98-101°C

- Solubility: - Water: 4.2 g/L at 25°C - Organic solvents: Soluble in acetone, ethanol, and methanol

- Stability: - Remains stable under standard conditions - Decomposes in the presence of strong acids or bases, or with extended exposure to UV light

- Partition Coefficient (Log P): Approximately 0.8

- Vapor Pressure: 1.5×10^{-6} Pa at 25°C

Mechanism of Action

Functions as an agonist at nicotinic acetylcholine receptors (nAChR) within the insect nervous system, leading to excessive stimulation, paralysis, and ultimately death.

3.1.4. Prepartion of the acetamprid solution

We used acetamiprid 20%, dissolving 20 mg/kg **(Shamsi et al., 2021)** in distilled water and administering it to the rats via oral gavage.



Figure 09. Prepartion of the *acetamprid* solution **(personal photos).**

3.2. Biological materials

3.2.1 Plant materials

Our study focuses on the Phoenician juniper *(Juniperus phoenicea),* a species of juniper belonging to the Cupressaceae family. This plant is found in arid and semi-arid regions, particularly in the Mediterranean Basin and North Africa. It holds ecological and medicinal significance, playing a role in environmental balance and containing compounds with various therapeutic applications. Its morphological and physiological characteristics are studied, along with its applications in environmental and pharmaceutical fields.

3.2.1.1. Harvesting and identification

In February 2025, The aerial part of the *Juniperus phoenicea (Phoenician juniper*) plant was collected from the region of the Sefsaf El Ousra Southeast of Tebessa.



Figure 10. The upper part ofthe *Juniperus phoenicea* plant and its specific location on Google Maps **(personal photos).**

3.2.1.2 Preparation of decoction

We added a quantity of *Juniperus phoenicea* plants to boiling distilled water, covered it, and left it for 30 minutes. The mixture was then stored at 4°C in a light-blocking container. 200 mg/kg **(Soltani et al., 2018).**



Figure 11. Preparation of decoctions **(personal photos)**.

3.2.2.Animal care and experimental design

This study was conducted on 28 female Wistar rats, all healthy and six weeks old, obtained from the Pasteur Institute in Algiers. The animals were housed in the animal breeding facility of the Faculty of Exact Sciences and Natural and Life Sciences at the University of Tebessa, in accordance with approved environmental standards.

The rats underwent an adaptation period of month before the experiment began to acclimate to the laboratory environment. During this period, they were provided with a balanced diet (croquettes) and clean drinking water without restriction. The rats were housed in cages filled with wood shavings, which were replaced daily to maintain a high level of cleanliness and ensure the animals' well-being.Environmental conditions were carefully regulated, maintaining a constant temperature of (22 ± 2°C) along with a regular light cycle of 12 hours of light followed by 12 hours of darkness, ensuring optimal living conditions,And the average weight of the rats before the experiment was about: 240g +/- 10g.

Table 03. composition of 1 kg of food.

	Quanntity in g/kg of food	%
Protein	140-180	14-18
Grains	500-600	50-60
Fats	40-80	4-8
Fiber	80-120	8-12
Minerals &Vitamins	10-30	1-3
Veggies &Herbs	50-100	5-10
Extras	5-10	0.5-1

After an acclimatization period, the rats were divided into four groups (three treatment groups and one control group), with each group consisting of seven rats (Table 04). All treatments lasted for 30 consecutive days. During the experiment, the body weight of both treated and untreated rats was measured and recorded twice a week. Additionally, daily weight measurements were conducted to ensure accurate dose adjustment for each rat based on its individual weight.

Table 04.The Experimental design of the study.

Experimental Groups	**Treatment**
Control	Received distilled water by oral gavage.
JNP	Received *Juniperus phoenicea* decoction (200 mg/kg bodyweight) by oral gavage.
ACE	Received acetamiprid (20 mg/Kg body weight) by oral gavage.
ACE+JNP	Received acetamiprid (20 mg/kg body weight), and after 4 hours Received *Juniperus phoenicea* decoction (200 mg/kg body weight) by oral gavage.



Figure 12. Animal care **(personal photos)**.

3.2.2 Blood sampling

Following a 30-day treatment period, the rats Decapitation was executed with a sharp blade, and arterial-venous blood was collected in heparinized tubes, which were clearly labeled for group identification. The blood samples were then transported to the laboratory in temperature-controlled coolers to maintain their integrity. Afterward, the samples underwent centrifugation at 3,000 RPM for 20 minutes to separate the plasma, which was subsequently analyzed for levels of creatinine, urea, uric acid, and albumin.



Figure 13. Blood sampling **(Personal photos)**.

3.2.3 Kidneys sampling

After sacrificing the rats, the kidneys were carefully extracted using specialized equipment. Subsequently, all kidneys from each rat in the group were accurately weighed. After weighing, they were stored in a refrigerator at -20°C to preserve their biological properties and will be used to determine biomarkers of oxidative stress.

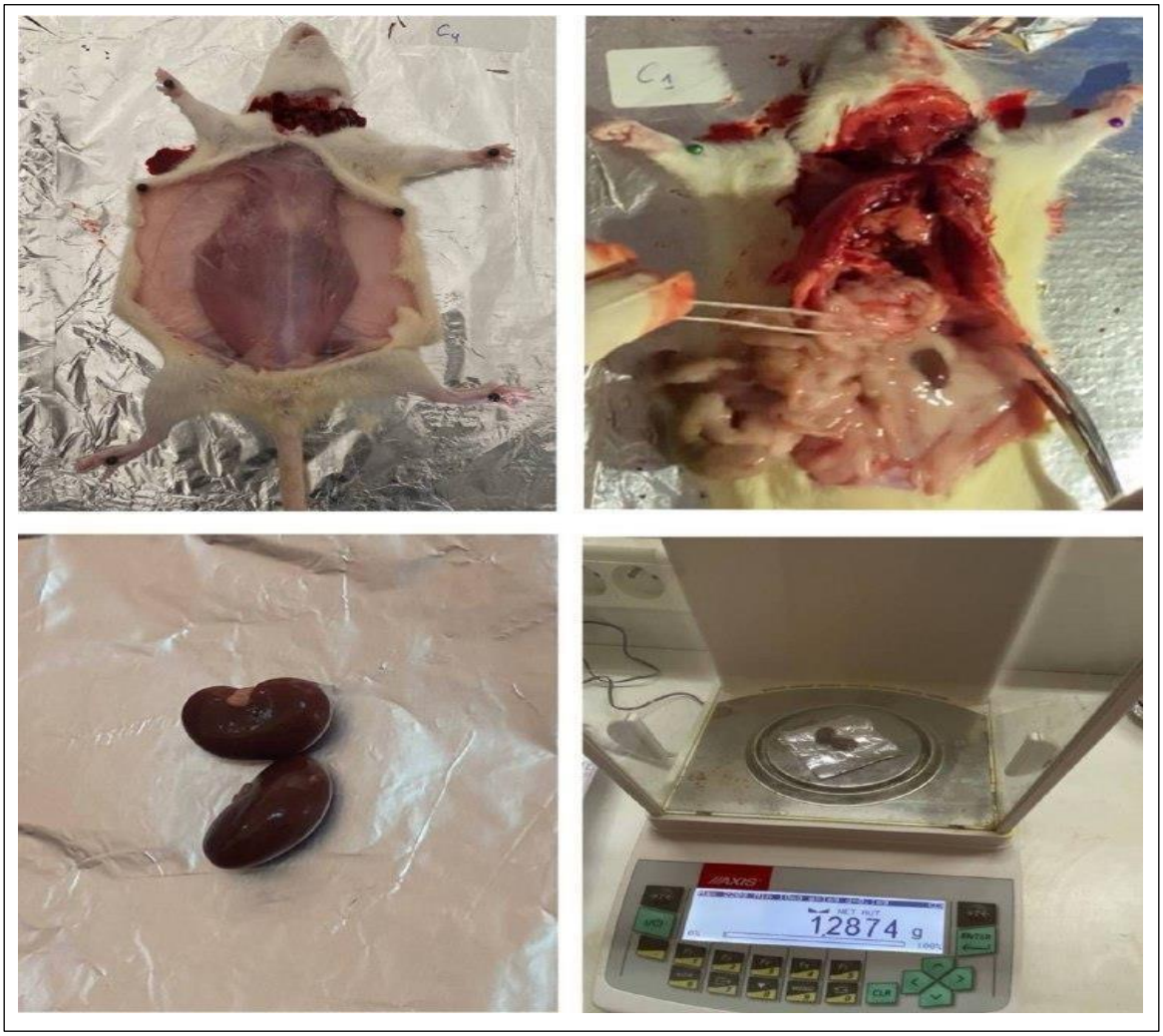


Figure 14. Organs sampling **(personal photos)**.



28 female albino wistar rats

30 days adaptation

30 days Treatment

Lot T

Lot JNP

Lot ACE

Lot ACE+JNP

Blood harvesting

Sacrifice and beheading

Organ harvesting: kidneys

Biochemical parameters assays :**Urea,Creatinine, Uric acid, Albumin**.

Assay of oxidative stress biomarkers : **CAT , GSH ,GST.**

Figure 15. Diagram summarizing the experimental protocol

3.3 Methods

3.3.1 Biochemical analyses

Following blood collection and its placement in heparinized tubes, the samples were promptly transported to the laboratory for centrifugation to obtain plasma. The extracted plasma was subsequently utilized for the analysis of the following biochemical parameters: creatinine, uric acid, bilirubin, and albumin.

.



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

1. **Creatinine**

Principle

This method is based on a modification of the original reaction (Jaffe) to picrate. Creatinine in an alkaline medium reacts with picrate ions to form a reddish complex. The rate of complex formation, measured by the increase in absorbance over a predefined time interval, is proportional to the concentration of creatinine in the sample

REAGENT COMPOSITION

R1 **Picric acid:** Picric acid 25 mmol/L

R2 **Alkaline buffer:** Phosphate buffer 300 mmol/L pH 12.7, SDS 2.0 g/L (w/v). X_i R: 36/37/38.

CAL **Standard creatinine:** Creatinine 2 mg/dL (177 µmol/L). Primary organic matrix standard: Concentration value traceable to Standard Reference Material 909b.

SAMPLES

Heparinized serum or plasma. Creatinine in serum or plasma is stable for up to 24 hours at 2-8°C. Freeze for long-term storage.

2. Uric acid

Principle

Uric acid is oxidized by uricase to allantoin, with the formation of hydrogen peroxide. In the presence of peroxidase (POD), a mixture of dichlorophenol sulfonates (DCBS) and 4-aminoantipyrine (4-AA) is oxidized by hydrogen peroxide to form a quinoneimine dye that is proportional to the concentration of uric acid in the sample.

REAGENT COMPOSITION

R1 **Monoreactive.** Phosphate buffer 100 mmol/L pH 7.8, uricase > 0.5 KU/L, peroxidase > 0.5 KU/L, ascorbate oxidase > 1 KU/L, 4-aminoantipyrine 0.5 mmol/L, DCBS 2 mmol/L, non-ionic surfactants 2 g/L (w/v). Biocides.

CAL **Standard uric acid**. Uric acid 6 mg/dL (357 µmol/L). Organic matter based on primary standard. Concentration value traceable to Standard Reference Material 909b.

SAMPLES

However, possible medication should be suspended 12 hours before sample collection. Non-hemolytic serum, plasma under EDTA or heparin, and urine (see Notes).Uric acid in serum or plasma is stable for up to 5 days at 2-8°C and for 6 months at -20°C.

3. Urea

Principle

Urea is hydrolyzed by urease into ammonia and carbon dioxide. The ammonia thus produced is converted into glutamate by glutamate dehydrogenase (GlDH) in the presence of NADH and oxoglutarate.The decrease in absorbance of the reaction measured at 340 nm, resulting from the oxidation of NADH to NAD+, is proportional to the concentration of urea present in the sample.

Reagent composition

R1 **Urease/GlDH buffer:** TRIS buffer 125 mmol/L pH 7.4, 2-oxoglutarate 10 mmol/L, urease > 140 U/mL, glutamate dehydrogenase > 120 U/mL, biocides.

R2 Coenzyme: NADH 1.50 mmol/L.

CAL **Standard Urea:** Urea 50 mg/dL (8.3 mmol/L). Organic matter based on primarystandard. Concentration value traceable to Standard Reference Material 909b.

Samples

Non-hemolytic heparinized serum or plasma and urine (see Notes). Other anticoagulants (ammonium heparinate or double potassium ammonium oxalate) must not be used.

Urea in serum, plasma, or urine is stable for 7 days at 2-8 °C. Freeze for long-term storage.

4 Albumin

Principle

The method1 is based on the specific binding of bromocresol green (BCG), an anionic dye, and the protein at acid pH with the resulting shift in the absorption wavelength of the complex. The intensity of the color formed is proportional to the concentration of albumin in the sample.

Reagent composition

R1 **Bromocresol reagent**. Succinate buffer 75 mmol/L pH 4.2,

BCG 0.12 mmol/L, tensioactive 2 g/L (w/v).

CAL **Albumin standard**. Bovine serum albumin 5 g/dL (50 g/L) Concentration value is traceable to Standard Reference Material 927c.

Samples

Serum, EDTA plasma unhemolyzed. Albumin in serum and plasma is stable for 2 weeks at 2-8°C, and For up to 4 months at –20°C.

5. estimated glomerular filtration rate (eGFR)

Plasma creatinine ≥ 52 umol/L: eGFR = 5862 ×W0.695 x $C^{-1.150}$ x $U^{-0.391}$ **(Basseling et al., 2021).**



Figure 17. Mindray BS-240: Automated Biochemistry Analyzer **(personal photos).**

3.3.2. Oxidative stress analyses

3.3.2.1. Assay of glutathione S-transferase GST enzymatic activity

Principle

The measurement of GST (Glutathione S-Transferase) activity involves providing the enzyme with a substrate—generally 1-chloro-2,4-dinitrobenzene (CDNB)—which readily reacts with various forms of GST and glutathione. The conjugation reaction between these two compounds leads to the formation of a new molecule, GS-DNB, along with HCl.

The method used in this study to assay GSTs is that of Habig et al. (1974). It consists of allowing the GSTs contained in the homogenate to act on a mixture of GSH (glutathione) and CDNB at a temperature of 25°C and a pH of 7. The change in optical density, resulting from the formation of the GSH-CDNB complex, is measured every minute over a period of 5 minutes at a wavelength of 340 nm.(**Habig et al.,1974**)

Preparation of the homogenate

100 mg tissue + 1 ml Phosphate buffer → → → → cold grinding → → → → homogenate. **Note:** keep the homogenates cold until the end of homogenization.

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

Operating mode

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

The reagents used	**Blanc (µl)**	**Sample (µl)**
Phosphate buffer (0.1 M ,PH 7)	850	830
CDNB (0.02 M)	50	50
GSH (0.1 M)	100	100
Supernatant		20

Calcula: the GST concentration is obtained bythe following formula.

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

OD Sample / min : Optical densityof sample per minute.

OD blank / min : Optical densityof blank per minute.

9.6 : Extinction coefficient ofGSH-CDNB expressed in mm–1.cm–1.



Figure 18. GST assay**(personal photos)**.

3.3.2.2. Assay of Catalase enzymatic activity

Principle

The method used is that of Aebi (1984). Catalase activity was measured at 240 nm using a spectrophotometer by monitoring the change in optical density due to the decrease in hydrogen peroxide (H_2O_2) at 25°C in the presence of an enzymatic source from the cytosolic fraction. The absorbance reading is taken after a 15-second delay and measured over 60 seconds.

Catalase activity decreases rapidly, so it is important to maintain the same timing between pipetting and placing the cuvette in the spectrophotometer.(**Aebi.,1984**)

Operating mode

Table 06. The dosage of the Catalase enzymatic activity.

The reagents used	Blanc (µl)	Sample (µl)
Phosphate buffer (0.1 M ,PH 7)	800	780
H2O2 (0.5 M)	200	200
Supernatant		20

Calcula: the catalase activity is calculated according to the following equation.

$$\text{CAT Activity(mmol H2O2 / min/mg protein)} = \frac{\Delta \text{ DO} \times 10}{\varepsilon \times L \times X \times Fd}$$

ΔDO : Variation in optical density per minute.

ε : Extinction coefficient of H202 = 0.04 Mml. Cm1

L : Tank length= 1 Cm

X : Protein quantity in mg/ml

Fd : H202 dilution factor in buffer (0.02)



Figure 19. Assay of Catalase enzymatic activity **(personal photos)**.

3.3.2.3. Assay of glutathione reduces GSH

Principle

The quantification of glutathione was carried out according to the method of Wekbeker and Cory (1988). This assay is based on the measurement of the optical absorbance of 2-nitro-5-thiobenzoic acid, which results from the reduction of 5,5'-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent, DTNB) by the sulfhydryl (-SH) groups of glutathione. For this purpose, deproteinization of the homogenate is essential to preserve only the thiol groups specifically associated with glutathione. (**Weckbercker et cory.,1988**)

Preparation of the Homogenate

In a tube (No. 1), place: 100 mg of tissue + 4 mL of Ethylenediaminetetraacetic Acid (EDTA) → cold grinding → homogenate. No centrifugation required.

Operating mode

In tube (No. 2), add:

- Take 0.8 mL of the homogenate.
- Deproteinize byadding 0.2 mL of 0.25% sulfosalicylic acid (SSA) solution.
- Mix the solution and leave it in an ice bath for 15 minutes.
- Centrifuge at 1000 rpm for 5 minutes. (This is the step where centrifugation is required).

In tube (No. 3), add:

- Take 0.5 mL of the supernatant.
- Add 1 mL of Tris-EDTA buffer (0.02 M EDTA), pH 9.6.
- Mix and add 0.025 mL of 0.01 M 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB).
- Let the reaction sit for 5 minutes at roomtemperature to stabilize the instantly developed color.Measure the optical density at 412 nm against the blank.

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

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

OD : Optical density at 412 nm.

1 : Total volume of solutions used in deprotonization.

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

13100 : Absorption coeficient du groupement.

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

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



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

3.3.2.4. Protein assay

Principle

This method uses Coomassie Blue as a reagent, which reacts under acidic conditions with arginine and, to a lesser extent, with histidine, lysine, and aromatic amino acids. Coomassie Blue binds to proteins through non-covalent interactions (hydrogen bonds, hydrophobic interactions, and ionic interactions). Its maximum absorption wavelength shifts from 465 nm (red) to 595 nm (blue). (**Bradford.,1976**)

Operating mode

- Take 0.1 ml (100 µl) of the sample (supernatant).
- Add 4 ml of Bradford reagent.
- Mix and let it stand for 5 minutes to allow color stabilization.
- Measure the optical density at 595 nm, using a blank (4 ml of Bradford reagent + 0.1 ml of distilled water) as reference.
- The obtained optical density is then compared to a previously prepared calibration curve.
- The protein concentration is determined by comparison with a standard range of bovine serumalbumin (BSA) (1 mg/ml), prepared under the same conditions.

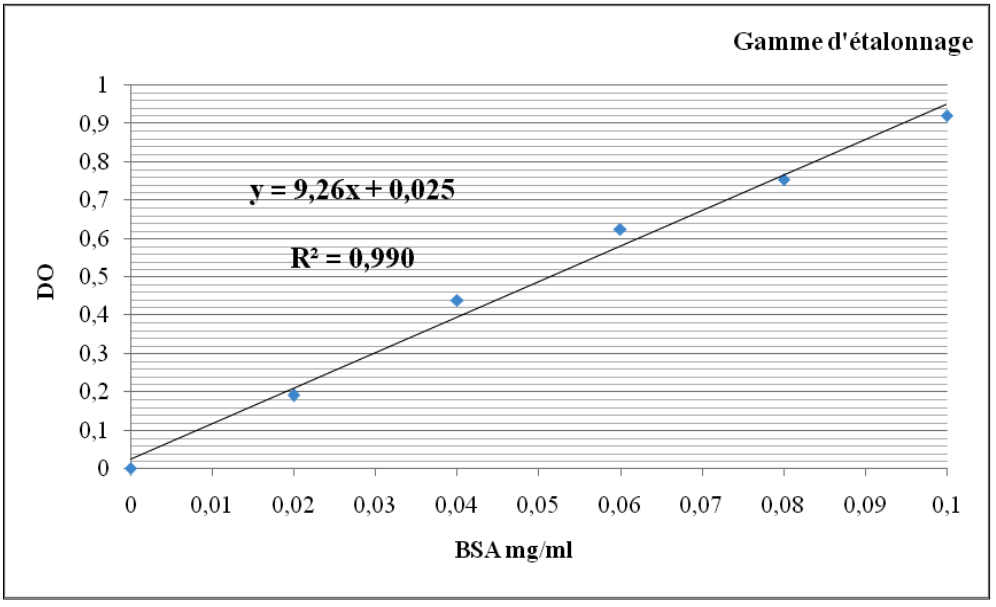


Figure 21. Calibration curve of bovine serum albumin



Figure 22. Preparation of Bradford reagent **(personal photos)**.



Figure 23. Protein assay **(personal photos).**

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 (JNV), acetamiprid-exposed group (ACE), and the co-treated group (ACE+JNV). 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 results of weight changes show clear differences between the experimental groups depending on the type of treatment. The witness group (C) recorded a weight gain of (+4.40%). The JNP group showed an increase in weight (+6.02%). In contrast, the ACE group experienced a decrease in weight (-4.57%), and the group that received the combined treatment (ACE + JNP) showed a slight weight reduction (-0.53%).

The dietary consumption of the animals remained consistent during the treatment phase in both the control group and the group administered with *Juniperus phoenicea*. In contrast, the groups subjected solely to acetamiprid or in conjunction with the aqueous extract of the plant exhibited a decline in both food intake and appetite compared to the control group.

Table 7. Effect of aqueous extracts of *juniperus phoenicea* leaves and/or Acetameprid treatment on body weight gain after 30 days of experience **(values represent the mean±SEM of seven rats).**

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**

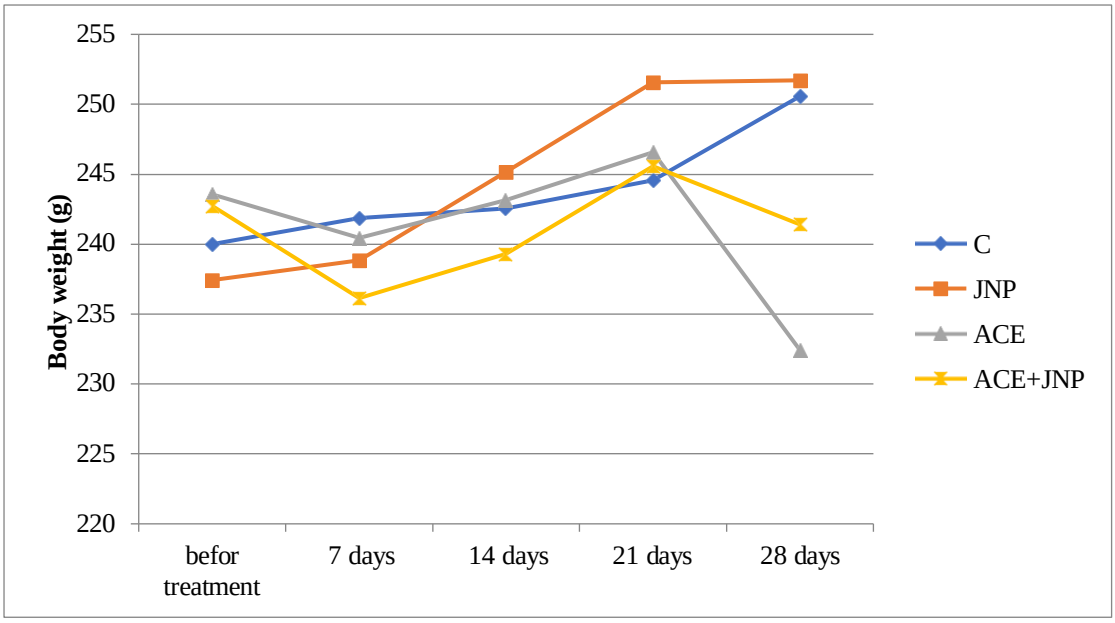


Figure 24. Effect of aqueous extracts of *juniperus phoenicea* leaves and/or Acetameprid treatment on body weight gain after 30 days of experience.

Acetamiprid is the second most prevalent neonicotinoid, after imidacloprid. This pesticide exhibits unique physiological and toxicological characteristics and has been documented to induce toxicity in mammals. Recent research has highlighted the detrimental effects of acetamiprid on multiple organ systems**(Phogat et al., 2024).**

Current research suggests that subchronic exposure to acetamiprid for 30 days resulted in a decrease in body weight gain compared to a control group of mice. This decrease is likely due to the toxic effect of acetamiprid on physiological functions, which may include disruption of normal metabolic processes or organ function, leading to anorexia and a general deterioration in the animals' health, growth rate, and food consumption. Our results support the findings of **Aioub et al. (2024)**, An investigation was conducted on the toxicity of acetamiprid at a concentration of 30 mg/kg over 35 days. A subsequent investigation by **Phogat et al. (2024)** revealed that changes in body weight metrics suggested potential organ toxicity and damage.

The findings indicated that the average body weights of rats treated with acetamprid were considerably lower compared to those in the control group. Control and JNP groups had similar weight gains, considerably higher than the ACE group. Nevertheless, the addition of an aqueous extract from *Juniperus phoenicea* leaves to the acetamprid-treated rats effectively brought their body weights back to levels close to normal, suggesting some mitigation by JNP of ACE adverse effects on weight. Our results support the findings of **Alzergany et al.(2017)** The results indicate that extract from Phoenician juniper could mitigate the adverse impacts of toxins on body weight, owing to its active compounds that are recognized for their antioxidant characteristics, particularly flavonoids and phenolics.

4.1.2. Effect of treatment on kidney weight

Table 08 results show a statistically significant increase ($p < 0.01$) in kidney weight in the Acetamiprid-treated group compared to control groups. The groups treated with acetamiprid and *Juniperus phoenicea* showed a slight improvement in weight, although the weight remained significantly higher than the control group.

Table 8. Effect of aqueous extracts of *Juniperus phoenicea* leaves and/or Acetameprid treatment on kidney weights after 30 days of experience.

Experimental groups				
Groups	**C**	**JNP**	**ACE**	**ACE+JNP**
Absolute weight (g)	1.332±0.04	1.348±0.10	1.487±0.06**	1.367±0.08
Relative weight (%)	0.513±0.03	0.537±0.06	0.692±0.07**	0.587±0.05**

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

Our results show a significant increase in absolute kidney weight in rats treated with acetamiprid. These changes might be due to oxidative damage to the lipids and proteins, as well as structural changes. According to studies by **Nur et al. (2022)**, the application of acetamiprid leads to an increase in enzymatic activity, resulting from the kidneys being overworked, which ultimately causes an increase in both the weight and volume of the kidneys.

Our observations indicated that there was no alteration in kidney weight within the combined acetamiprid and JNP group when compared to the acetamiprid-only groups, contrary with the results reported by **Lafraxo et al. (2024)**,The findings of this research indicate that high doses of juniper leaf extract could lead to elevated weights of the liver and kidneys, along with adverse impacts on kidney function. Consequently, it is recommended to exercise caution when utilizing juniper extracts.

The results indicate that exposure to acetamiprid caused clear disturbances in certain biochemical markers of kidney function. A significant decrease in albumin levels was observed in the acetamiprid group ($p < 0.01$), while the combined treatment with

4.1.2.1.1. Biochemical profile*:*

Juniperus phoenicea showed partial improvement ($p < 0.05$). However, levels did not return to normal. Creatinine levels were significantly elevated in both the acetamiprid and combined groups compared to the control group ($p < 0.05$), indicating impaired kidney function. Uric acid levels were similar among the four groups, with only a slight increase in the acetamiprid and combined groups. The acetamiprid group also recorded the highest urea levels ($p < 0.001$), while the combined group showed moderately elevated levels, still above normal. A significant increase in estimated glomerular filtration rate (eGFR) was also observed in the acetamiprid group ($p < 0.001$), possibly reflecting renal hyperfiltration. The presence of *Juniperus phoenicea* reduced this increase, though not to baseline levels. Overall, the findings suggest that acetamiprid induces adverse effects on liver and kidney functions, while *Juniperus phoenicea* provides partial protective effects against this toxicity.

Table 9. Effect of aqueous extracts of *juniperus phoenicea* leaves and/or Acetameprid treatment on several biochemical parameters after 30 days of experience.

Parametrs	C	JNP	ACE	ACE+JNP
Albumin (g/L)	43.33±1.53	41.00±1.00	33.00±1.00**	35.67±1.52*
Creatinin (mg/dl)	5.183±0.28	5.097±0.24	6.573±0.07*	6.597±0.5*
Uric acid (mg/dl)	25.34±2.43	25.64±3.73	29.33±0.58	28.00±1.00
Urea (g/L)	0.250±0.01	0.260±0.02	0.453±0.03***	0.436±0.01***
eGFR (mL/min)	1708.3±80.7	1800.3±87.8	946.3±49.8**	1075±53.0*

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

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

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

Our study showed that acetamiprid reduced albumin levels significantly, which aligns with the results observed by **Khovarnagh et al. (2021)**, The observed decline may be linked to the harmful impact of reactive oxygen species (ROS) produced by ACE within the metabolic pathway, which adversely affects cellular structure and function. Conversely, this phenomenon occurs as ACE leads to a reduction in protein metabolism and the impairment of the protein synthesis machinery.

Elevated levels of blood albumin were observed in the groups administered acetamiprid and *Juniperus phoenicea* (JNP) leaves. These leaves are recognized for their significant presence of various classes of secondary metabolites, including flavonoids, phenolic compounds, and terpenoids. These metabolites exhibit a range of biological activities, including antibacterial, anti-inflammatory, and antiviral properties **(Jemai Ben Ali et al., 2015).**These findings are similar to those of **Laouar et al. (2017)** who demonstrated that JNP has elevated the concentrations of plasma total protein and albumin, suggesting its hepatoprotective properties. The enhancement of protein synthesis has been proposed as a key mechanism contributing to hepatoprotection, facilitating the regeneration process and the production of hepatic cells.

Creatinine, a metabolic byproduct of creatine produced and stored in the liver, is formed from the conversion of creatine in skeletal muscle. Reduced levels of creatinine in the bloodstream can indicate liver failure and a decrease in skeletal muscle mass. Conversely, elevated blood creatinine levels may be linked to renal pathologies **(Karaca et al., 2019).**

Our study showed that acetamiprid elevated blood creatinine levels significantly, which aligns with the results observed by **Noaishi et al. (2016)**. Significant renal impairment will result in elevated creatinine concentrations. According to **Ibrahim et al. (2024)**, The increased level of creatinine suggests a deterioration in kidney function as a result of exposure to acetamiprid, thereby supporting the hypothesis that this pesticide could exert harmful effects on renal health.

The results of the groups treated with the combination of acetamiprid and *Juniperus phoenicea* showed a Very slight asignificant increase in serum creatinine levels. Contrary to our results **Zeima et al. (2023)**, Following the administration of *J.Phoenicea* leaf extract, a notable reduction was observed compared to group C, likely

attributable to its antioxidant properties. The antioxidant and anti-diabetic effects of *J. Phoenicea* leaf extract mitigate

Uric acid is a byproduct generated from the breakdown of purines. In a healthy individual, the kidneys filter uric acid from the bloodstream and eliminate it through urine. Given that various kidney disorders can influence uric acid concentrations, measuring uric acid levels is essential for the diagnosis and assessment of kidney diseases **(Noaishi et al., 2016).**

Our research showed a non-significant increase in serum uric acid levels as a result of acetamiprid exposure.When the body breaks down substances known as purines, a chemical called uric acid is produced. Under normal conditions, the kidneys filter this acid from the blood and eliminate it through urine. However, if the kidneys are not functioning properly, excess amounts of uric acid can accumulate in the body, which may indicate a health problem. For this reason, measuring uric acid levels is considered an important test that helps detect and accurately diagnose kidney issues **(Hosseini et al., 2019).**

We did not observe any change in uric acid levels in the combined group compared to the other groups, reflecting the results obtained by **Gdoura et al. (2013)**, found a statistically significant increase when adding *Juniperus phoenicea.*

Elevated blood urea levels were observed in rats administered acetamiprid when compared to the control group, suggesting a possible nephrotoxic impact of the pesticide on renal function. This implies that acetamiprid may hinder glomerular filtration in the affected subjects **(Mondal et al., 2015)**.

Our observations indicated that there was no improvement in urea levels within the combined acetamiprid and JNP group, And they remained high very significant compared to the control group, contrary with the results reported by **Ahmed Ali et al. (2010)**, who noted a statistically significant reduction upon the addition of *Juniperus phoenicea.*

The results obtained from the group exposed to acetamiprid only showed a reduction significantly in eGFR levels in the kidney. Our findings are consistent with those of **Kabata et al. (2016).** Statistical analyses revealed significant correlations between pesticide exposure and a reduction in estimated glomerular filtration rate

(eGFR), as well as an elevated risk of chronic kidney disease (CKD). Furthermore, it was noted that prolonged exposure to pesticides throughout the applicator's lifetime could be linked to a decline in renal function and filtration efficiency **(Shearer et al., 2022)**. according to **Ma et al. (2025)**, A reduced estimated glomerular filtration rate suggests that the pesticide induces oxidative stress through the generation of reactive oxygen species (ROS), including free radicals. This process results in the impairment of proteins, DNA, and lipids within renal cells. Additionally, it contributes to inflammatory and immune system disruptions, ultimately resulting in the decline of kidney function via pathways such as mitochondrial dysfunction and renal fibrosis. These processes culminate in damage to tubular cells and a failure in filtration.

Additionally, in rats treated with a combination of acetamiprid and *Juniperus phoenicea*, we observed a significant increase in eGFR levels. The findings suggest that *Juniperus phoenicea* . serves as a valuable source of bioactive compounds, and its aqueous extract can facilitate the synthesis of nanoparticles with potential applications in antioxidant, anti-inflammatory, and antibacterial treatments **(Zemmouli et al., 2024)**.

4.3. Effect of a treatment on oxidative stress parameters

4.3.1. Glutathione S. transferase (GST)

According to the results obtained, we observe a significant decrease ($P< 0.05$) in the level of GST in the kidney of the rats receiving lacetamprid compared to the control rats. In the presence of JNP the GST increases considerably to reach values close to those of the control rats.

4.3.2. Reduced Glutathione level (GSH)

We observed a significant idecrease in GSH levels in acetamiprid-treated group compared to the control group. However, treatment with *Juniperus phoenicea* extract restored GSH levels to almost normal, with no significant difference compared to the control group.

4.3.3. Catalase activity (CAT)

According to the results obtained, we observe a very highly significant increase ($P < 0.001$) in the level of CAT in the kidneys of the rats receiving acetamiprid compared to the control rats. In the group combination of acetamiprid and JNP, a improvement in activity was observed, but it was still higher than the activity recorded in the control (C) groups, with a significant difference ($p < 0.01$). The JNP group showed almost similar activity to the control group, with no significant differences.

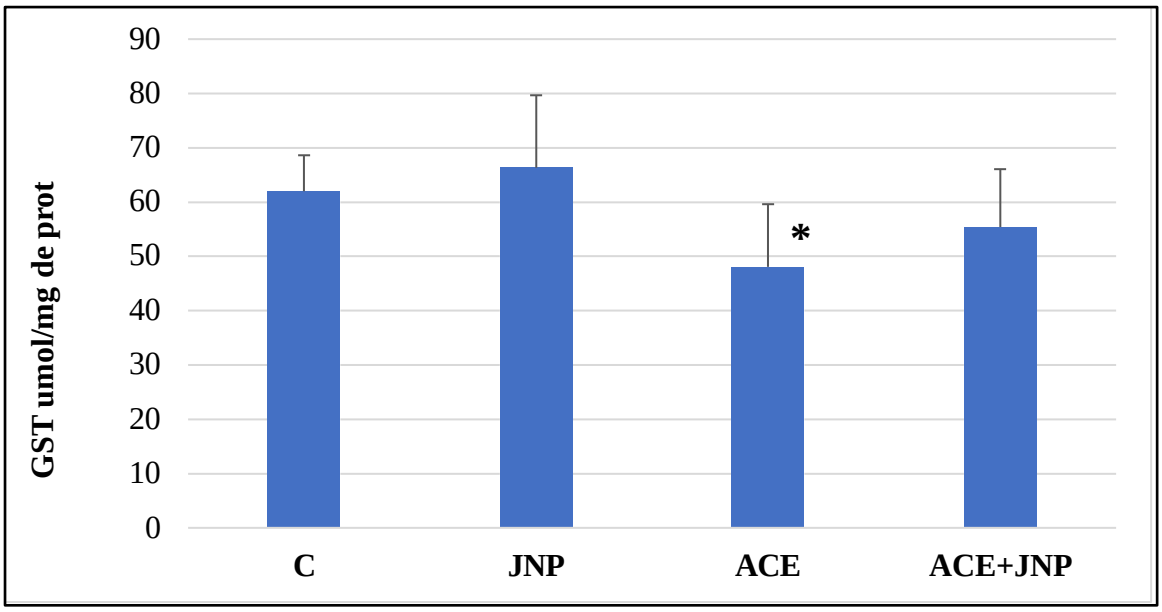


Figure 25. Effect of aqueous extracts of *Juniperus phoenicea* leaves and/or Acetameprid treatment on Glutathione S. transferase (GST) activity in the kidney after 30 days of exposure.

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

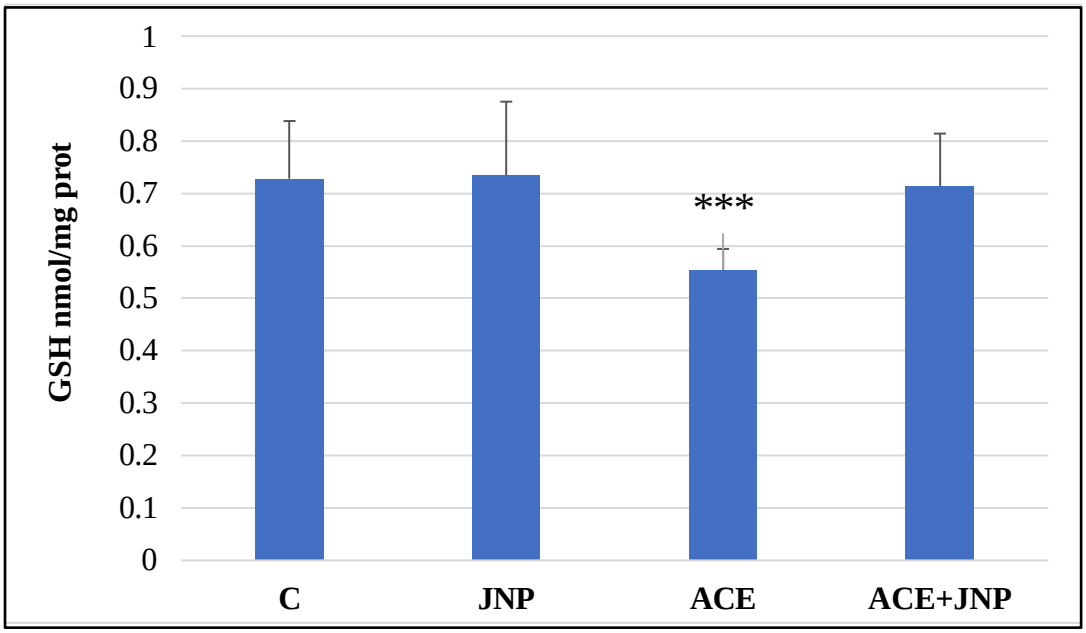


Figure 26. Effect of aqueous extracts of *Juniperus phoenicea* leaves and/or Acetameprid treatment on reduced Glutathione level (GSH) in the kidney after 30 days of exposure.

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

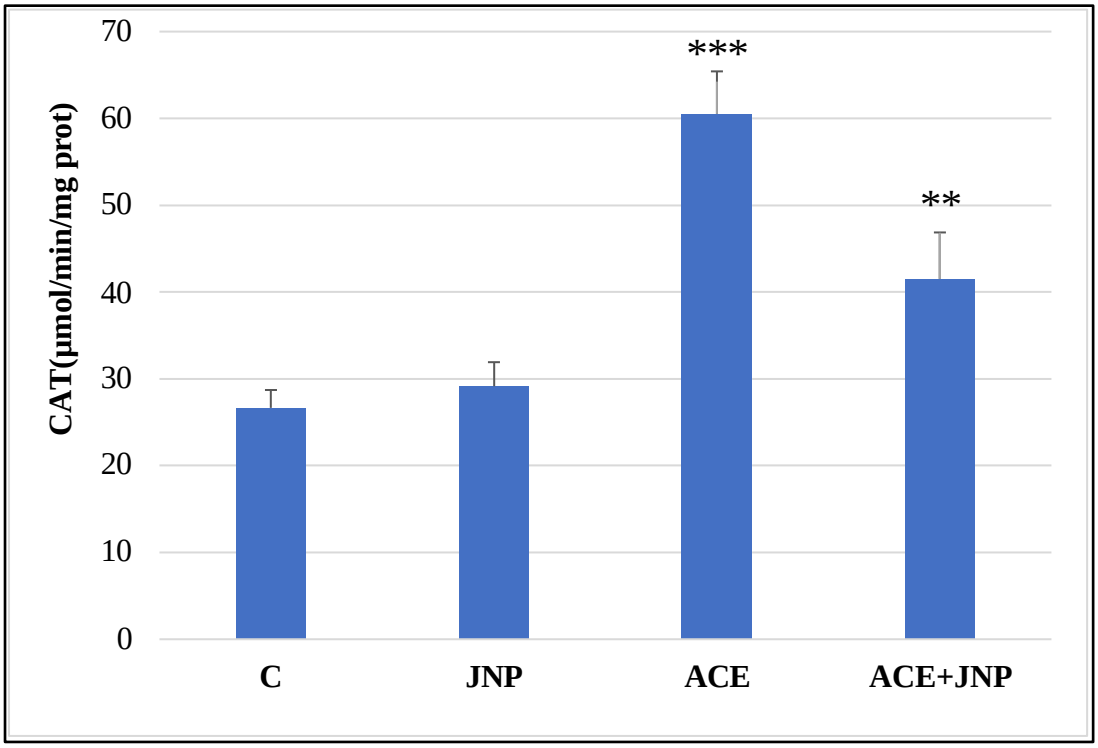


Figure 27. Effect of aqueous extracts of *Juniperus phoenicea* leaves and/or Acetameprid treatment on Catalase activity (CAT) in the kidney after 30 days of exposure.

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

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

Oxidative stress is a detrimental state resulting from an overabundance of reactive oxygen species (ROS) and/or a reduction in antioxidant levels, which can inflict tissue damage through physical and/or chemical mechanisms, ultimately contributing to various diseases. This process is a primary mechanism through which numerous pesticides exert their effects. One significant consequence of oxidative stress induced by pesticides is the damage to membrane lipids **(Devan et al., 2015 ; Noaishi et al., 2016).**

Glutathione S-transferase (GST) is an enzyme that facilitates the elimination of foreign and toxic compounds from the bloodstream via covalent or non-covalent interactions. Furthermore, it plays a crucial role in phase II detoxification processes of xenobiotics and demonstrates antioxidant properties by mitigating stress induced by organic hydroperoxides through its peroxidase-like function **(Balci et al., 2019).**

The results obtained from the group exposed to acetamiprid only showed a clear reduction in GST levels in the kidney. Our findings are consistent with those of **Abdelrahman et al. (2024)** Diminished GST activity suggests a compromised capacity of cells to detoxify, thereby exacerbating oxidative damage.According to **Ahmad et al. (2018)**, The reduction in GST activity observed in our study may significantly contribute to a decline in glutathione synthesis within tissues due to the compromised regulation of redox homeostasis in response to an oxidative environment.

Consumption of *Juniperus phoenicea* increases the level of GST in rats' kidneys, making it similar to the control group. This is in line with the results of **Laouar et al. (2017)**. The administration of *J. Phoenicea* to experimental rats successfully corrects this imbalance and reinstates the activity of antioxidant enzymes. The influence of J. Phoenicea on GST activity may be attributed to an upregulation of the gene responsible for GST, as it has been documented that antioxidant compounds significantly enhance the expression of genes coding for phase II enzymes.

Glutathione (GSH), a tripeptide made up of γ-glutamic acid, cysteine, and glycine, is the predominant small-molecule thiol found within cells, serving essential functions in numerous cellular activities. As the principal antioxidant within cells, GSH is vital for sustaining redox balance, shielding cells against oxidative damage, and engaging in detoxification mechanisms **(Lin et al., 2024).**

The data from the cohort subjected solely to acetamiprid indicated a significant decrease in glutathione (GSH) concentrations within the renal tissue. Our results align with those of **Devan et al. (2015)**. Glutathione (GSH) functions as a substrate within the glutathione peroxidase and reductase system, utilizing NADPH as a reducing agent to influence lipid peroxidation. Changes in the levels of reduced glutathione during oxidative stress may arise from alterations in GSH synthesis and/or depletion. Furthermore, the decrease in GSH levels may be attributed to its role in the scavenging of hydrogen peroxide (H2O2). Similar results were also found by **Ahmad et al.(2018)**, who showed reducing GSH activity levels in animals exposed to pesticides signifies a disruption in their antioxidant defense mechanisms.

Juniperus phoenicea. is abundant in a variety of compounds, such as diterpenoids, flavonoids, lignans, phenylpropanoid glucosides, furanone glucosides, bis-furanone derivatives, norterpenes, and sesquiterpene glucosides, which exhibit a broad spectrum of pharmacological effects, including antioxidant, antiviral, anticancer, antibacterial, antifungal, and antiparasitic activities. Its antioxidant properties, specifically its ability to inhibit or reduce the oxidation of other molecules by free radicals, confer significant therapeutic advantages **(Elsherif et al., 2024)**. According to **Zouari Bouassida et al., (2018)** *J. phoenicea* may enhance the activity of glutathione (GSH) by strengthening a cellular antioxidant defense mechanism. This suggests that the extract of *J. phoenicea* has a protective role by promoting the expression and function of antioxidant enzymes during the inflammatory response.

Catalase (CAT) is an essential enzyme that significantly contributes to cellular defense systems by reducing oxidative stress induced by reactive oxygen species (ROS). It demonstrates one of the highest catalytic efficiencies of all enzymes, with a single catalase molecule capable of transforming millions of H2O2 molecules into water and oxygen each second **(Anwar et al., 2024).**

Our study results showed that the treatment of rats with the pesticide acetamiprid led to a significant increase in catalase (CAT) enzyme activity. This is consistent with the findings of the study by **Khovarnagh et al. (2021)**. The elevation of catalase activity is likely attributable to the antioxidant defense mechanisms that counteract prooxidants. Specifically, the antioxidant defense system mitigates the rise of H2O2 and the resultant tissue damage induced by ACE through the upregulation of catalase.

Moreover, short-term exposure to ACE can generate reactive oxygen species (ROS), prompting the organism's defense system to counteract the toxic effects by enhancing catalase activity. Many studies, including the research of **Eltoweissy et al. (2022)**, The elevated CAT ratio may indicate a greater availability of H2O2, which can generate the highly reactive hydroxyl radical through the Fenton reaction.

Consumption of *Juniperus phoenicea* leaf extract reduced the level of CAT in the kidneys of rats, which indicates its antioxidant activity. This action may result from heightened expression and/or activity of antioxidant enzymes or the neutralization of circulating free radicals and reactive oxygen species **(Gdoura et al., 2013)**.

Conclusion and perspective

Acetamiprid is an insecticide from the neonicotinoid group, used to control crop pests. Despite its effectiveness, studies have shown that it may have potential toxicity to the kidneys, which may cause kidney damage, leading to cell injury and impaired kidney function with repeated exposure, due to its toxic effects and oxidative stress.

In this study, our objective was to assess the nephrotoxicity of acetamiprid in rat models and to illustrate its health implications. We concentrated on investigating the therapeutic potential of *Juniperus phoenicea*, an aromatic and medicinal plant known for its abundance of secondary metabolites that possess therapeutic and pharmacological benefits.

To complete our research, we measured body weight, biochemical parameters, and markers of oxidative stress in control rats and rats treated with acetamiprid (20mg/kg/day) and the plant *Juniperus phoenicea* for 30 days.

Our results showed the effect of acetamiprid in:

- Relative weight loss in mice;
- Increased relative weight of the kidneys;
- Relative change in the biochemical parameter (decreased in albumin,eGFR and increased in uric acid, urea, and creatinine);
- Changes in markers of oxidative stress.

Our results showed significant changes in antioxidant levels in the kidneys where we observed decreased Glutathione S-transferase (GST), reduced Glutathione level (GSH), and increased Catalase activity (CAT).

Nevertheless, the incorporation of *Juniperus phoenicea* (200 mg/kg) enhanced the observed parameters. This enhancement illustrates the antioxidant, anti-inflammatory, and therapeutic properties of these medicinal plants in countering the disruptive and oxidative impacts of acetamiprid.

Recommendations for further research:

- It is recommended to conduct future in-depth studies aimed at evaluating the toxic effects of acetamiprid on various organs, taking into account the change in the doses administered, in order to understand the relationship between dose and toxic response more accurately.
- It is also proposed to focus on determining the biological characteristics of the plant used and identifying the active ingredient responsible for reducing the toxic effects of this pesticide and other similar pesticides, to find its mechanism of action and its potential role in prevention or treatment.
- Accurate histologic examinations of kidney tissue are necessary to determine the extent of cellular damage caused by acetamiprid exposure. This includes the use of histological sectioning techniques and special stains to assess structural and functional changes in renal cells, which will help elucidate the microscopic effects of acetamiprid at the cellular level.

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Webographic

Web 1

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