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Evaluation of the antioxidant effect of extracted *Juniperus Phoenicia L.* on the neurotoxicity induced by Acetamiprid pesticide exposure in rats

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Abstract

Acetamiprid (ACE), a widely used neonicotinoid pesticide, is effective in pest control but raises health and environmental concerns due to its potential toxicity to non-target organisms like mammals. This study investigated ACE's repercussions on stress parameters in rats and the protective effects of *Juniperus phoenicea L*. (JNP) extract against pesticide-induced neurotoxicity in Albino-Wistar rats.

28 female rats were divided into four groups: control (C), (JNP) extract alone, exposed (ACE), and a combination of (JNP+ACE). The 30-day oral gavage treatment involved 20mg/kg/day of ACE and 200mg/kg/day of JNP decoction. Behavioral assessments (open field, elevated plus maze, forced swim tests) analyzed anxiety, depressive-like behavior, and locomotor activity. Oxidative stress markers glutathione (GSH), glutathione S-transferase (GST), catalase (CAT), and protein levels were measured in brain tissue.

Findings showed ACE exposure reduced body and brain weight, increased anxiety and depressive-like behaviors, and disrupted oxidative balance. This was evidenced by decreased GSH and GST, and elevated CAT activity, indicating oxidative stress and neuronal damage. Crucially, co-administering JNP extract ameliorated these behavioral and physiological impairments.

This study suggests *Juniperus phoenicea* possesses therapeutic potential as a natural agent against pesticide toxicity. It emerges as a promising phytotherapy candidate for enhancing neurological functions and mitigating oxidative stress.

Key words:

Acetamiprid, *Juniperus phoenicea L.*, Oxidative stress, Neurotoxicity, Antioxidants, Biochemical parameters, Stress parameters.

الملخص

يُعد الأسيتاميبريد(ACE) ، وهو مبيد حشري من فئة النيكوتينيات الجديدة، واسع الاستخدام لفعاليته في مكافحة الآفات الزراعية. ومع ذلك، يثير استخدامه مخاوف صحية وبيئية متزايدة بسبب سميته المحتملة على الكائنات غير المستهدفة، بما في ذلك الثدييات. هدفت هذه الدراسة إلى تقييم آثار التعرض للأسيتاميبريد، مع التركيز على مؤشرات التوتر لدى الجرذان، ودراسة التأثيرات الوقائية لمستخلص العرعر الفينيقي (*Juniperus phoenicea L.*) ضد السمية العصبية الناتجة عن المبيد لدى جرذان ويستار البيضاء.

تم تقسيم 28 جرذًا إلى أربع مجموعات: ضابطة، ومجموعة تلقت مستخلص العرعار الفينيقي (JNP) فقط، ومجموعة تعرضت للأسيتاميبريد(ACE) ، ومجموعة تلقت مزيجًا من المستخلص والمبيد .(JNP+ACE) استمر نظام المعالجة لمدة 30 يومًا، حيث تم إعطاء الأسيتاميبريد بتركيز 20 ملغ/كلغ/اليوم ومحلول المستخلص بتركيز 200 ملغ/كلغ/اليوم عن طريق الفم. تم خلال هذه الفترة إجراء اختبارات سلوكية (اختبار الحقل المفتوح، المتاهة المرتفعة ذات الأذرع المتقاطعة، اختبار السباحة القسرية) لتحليل مستويات القلق والسلوك الاكتئابي والنشاط الحركي. بالإضافة إلى ذلك، تم قياس مؤشرات الإجهاد التأكسدي مثل الجلوتاثيون(GSH) ، وإنزيم جلوتاثيون-S-ترانسفيراز(GST) ، وإنزيم الكاتالاز(CAT) ، ومستويات البروتين في نسيج الدماغ.

أظهرت النتائج أن التعرض للأسيتاميبريد أدى إلى انخفاض في وزن الجسم والدماغ، وزيادة في مستويات القلق والسلوكيات الاكتئابية، واضطراب في حالة الأكسدة والاختزال. تجلى ذلك في انخفاض مستويات GSH وGST، وزيادة في نشاط إنزيم CAT، مما يشير إلى حدوث إجهاد تأكسدي وتلف عصبي. في المقابل، أدى الإعطاء المتزامن لمستخلص العرعار الفينيقي مع المبيد إلى تحسن في هذه المعايير السلوكية والفيزيولوجية.

تشير هذه الدراسة إلى أن *Juniperus phoenicea* يمتلك إمكانات واعدة كعامل علاجي طبيعي لمقاومة التأثيرات السامة للمبيدات. ويبرز كخيار محتمل في العلاج بالنباتات لتحسين الوظائف العصبية والحد من الإجهاد التأكسدي.

الكلمات المفتاحية:

أسيتاميبريد، العرعار الفينيقي(*Juniperus phoenicea L.*) ، الإجهاد التأكسدي، السُمية العصبية، مضادات الأكسدة، المؤشرات البيوكيميائية، مؤشرات التوتر.

Résumé

L'acétamipride (ACE), un pesticide néonicotinoïde largement utilisé, est efficace dans la lutte contre les ravageurs agricoles, mais son usage soulève des préoccupations sanitaires et environnementales en raison de sa toxicité potentielle pour les organismes non ciblés, y compris les mammifères. Cette étude a examiné les répercussions de l'exposition à l'ACE sur les paramètres de stress chez le rat, tout en explorant les effets protecteurs de l'extrait de *Juniperus phoenicea L.* (JNP) contre la neurotoxicité induite par le pesticide chez des rats Wistar albinos.

28 rats ont été répartis en quatre groupes : témoin (C), recevant l'extrait de JNP seul, exposé à l'ACE, et une combinaison de JNP+ACE. Le traitement par gavage oral a duré 30 jours, avec 20 mg/kg/jour d'ACE et 200 mg/kg/jour de décoction de JNP. Des évaluations comportementales (test du champ ouvert, labyrinthe en croix surélevé, test de nage forcée) ont été réalisées pour analyser l'anxiété, les comportements dépressifs et l'activité locomotrice. Des marqueurs de stress oxydatif tels que le glutathion (GSH), la glutathion S-transférase (GST), la catalase (CAT) et les niveaux protéiques ont été mesurés dans le tissu cérébral.

Les résultats ont montré que l'exposition à l'ACE entraînait une réduction du poids corporel et cérébral, une augmentation de l'anxiété et des comportements dépressifs, et un déséquilibre de l'état oxydatif. Cela s'est manifesté par une diminution du GSH et de la GST, et une élévation de l'activité de la CAT, indiquant l'induction d'un stress oxydatif et de dommages neuronaux. De manière cruciale, la co-administration de l'extrait de JNP a amélioré ces altérations comportementales et physiologiques.

Cette étude suggère que le *Juniperus phoenicea* possède un potentiel thérapeutique en tant qu'agent naturel contre la toxicité des pesticides. Il apparaît comme un candidat prometteur en phytothérapie pour améliorer les fonctions neurologiques et atténuer le stress oxydatif.

Mots clés :

Acétamipride, *Juniperus phoenicea L.*, Stress oxydatif, Neurotoxicité, Antioxydants, Paramètres biochimiques, Paramètres de stress.

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List of abbreviation

ACE	Acetamiprid.
Ach	Acetylcholine.
ANOVA	analysis of variance.
ATP	adenosine triphosphate.
BBC	Coomassie Brilliant Blue
BSA	bovine serum albumin.
°C	Celsius degree.
C	Control.
CDNB	1-chloro-2,4-dinitrobenzene.
cm	Centimeter.
CNS	central nervous system.
CT	Climbing time.
DDT	Dichlorodiphenyltrichloroethane.
DNA	Deoxyribonucleic Acid.
DO **ΔDO**	Optical density. Variation in optical density.
DTNB	5,5'-dithio-bis-2-nitrobenzoic acid.
ε	Extinction coefficient.
EDTA	ethylenediaminetetraacetic acid.
EPM	Elevated plus maze.
EFSA	European Food Safety Authority.
Fd	Dilution factor.
FST	Forced Swim Test
g	Gramme.
GSH	Reduced glutathione.
GST	Glutathione S-Transferase.
GS-DNB	S-(2,4-dinitrophenyl) glutathione.

HCl	hydrochloric acid.
H_2O_2	hydrogen peroxide.
HVAC	Heating, Ventilation, and Air Conditioning.
I.P. A	Pasteur Institute of Algiers.
I.P.M	Integrated Pest Management.
JNP	*Juniperus Phoenicea.*
kg	Kilogram.
L	Length of the cuvette.
Mg^{2+}-ATPase	Magnesium-dependent Adenosine Triphosphatase.
mg	Milligram.
min	Minute.
ml	Milliliter.
nAChRs	Nicotinic acetylcholine receptors.
N adj	Number of adjustments.
Na^+/K^+-ATPase	Sodium-Potassium Adenosine Triphosphatase.
nm	Nanometer.
NN	Neonicotinoid.
NECA	Number of entries into closed arms.
NEOA	Number of entries into open arms.
OECD TG	Organization for Economic Co-operation and Development.
OFT	Open field test.
pH	potential of hydrogen
ROS	Reactive oxygen species.
SD	standard deviation.
-SH	glutathione's thiol groups.
SSA	sulfosalicylic acid.
ST	Swimming time.
TCP	Time in the center part.

TDT	Total distance traveled.
TI	Time of immobility.
TPP	Time in the peripheral part.
TSC	Time spent in the center.
TSCA	Time spent in the closed arms.
TSCA/D	Time spent in the closed arms: distal part.
TSOA	Time spent in the open arms.
TSOA/D	Time spent in the open arms: distal part.
TSOA/P	Time spent in the open arms: proximity part
U.S. EPA	United States Environmental Protection Agency.
WHO	World Health Organization.
X	Different quantities.

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INTRODUCTION

INTRODUCTION

The growth of the global population has been closely linked to the proportional increase in food production, facilitated in part by the widespread use of pesticides. These substances play a critical role in safeguarding agricultural yields by mitigating losses caused by insects, pests, parasites, and other harmful organisms. However, despite their agricultural benefits, many pesticides are associated with significant environmental and human health risks. They are known or suspected to exhibit toxicity, leading to acute neurological effects, chronic neurodevelopmental disorders, cancer, reproductive impairments, and potential disruptions to the immune and endocrine systems **(Tudi et al., 2021)**.

Neonicotinoids, representing a dominant class of systemic pesticides, constitute approximately 30% of global insecticide sales **(Ihara and Matsuda, 2018)** and are authorized for use in over 120 countries **(Xu et al., 2022)**. Their systemic nature, facilitating plant uptake and prolonged pest control, has contributed to their widespread adoption. However, extensive utilization has precipitated significant concerns regarding their ecological and toxicological implications. Accumulating evidence indicates that neonicotinoids contribute to the decline of pollinator populations and disrupt aquatic ecosystem integrity. Furthermore, investigations suggest potential adverse effects on non-target species, including mammals, through bioaccumulation and the induction of oxidative stress, neurotoxicity, and endocrine disruption **(Ihara & Matsuda, 2018; Xu et al., 2022)**. A central mechanism of pesticide-induced toxicity involves the generation of reactive oxygen species (ROS), leading to oxidative stress and subsequent cellular damage, including lipid peroxidation. Consequently, the identification and characterization of natural compounds with antioxidant properties, capable of attenuating neonicotinoid-mediated toxicity, represents a critical area of research **(Phogat et al., 2022)**.

According to the World Health Organization (WHO), approximately 80% of the African population relies on traditional medicine as their primary healthcare source. The African continent, renowned for its rich biodiversity an estimated 40,000 to 45,000 plant species, of which approximately 5,000 are recognized for their medicinal properties. *Juniperus phoenicea L*, a species within the *Cupressaceae* family, exhibits notable pharmacological properties, including antioxidant, anti-inflammatory, and hepatoprotective activities. The plant's bioactive constituents, such as flavonoids, phenolic acids, and terpenoids, demonstrate significant free radical-scavenging capacity, suggesting a potential role in mitigating oxidative stress induced by xenobiotic agents, including pesticides. Furthermore, the antioxidant and cytoprotective

mechanisms of *Juniperus phoenicea* may attenuate oxidative stress-mediated cellular damage and reduce the neurotoxic effects associated with exposure to acetamiprid, a neonicotinoid insecticide **(Bouyahya et al., 2018; Mechaala et al., 2022)**. These properties highlight its potential therapeutic relevance in counteracting xenobiotic-induced oxidative and neurotoxic insults.

The objective of this study is to evaluate the oxidative and neurotoxicity induced by acetamiprid and to determine the potential protective effects of *Juniperus phoenicea L* in mitigating pesticide-induced damage. This will be achieved through the assessment of biomarkers of oxidative stress and biochemical parameters of the nervous system in rats.

Our work is divided into three parts:

-Bibliography research.

-Material and methods.

-Results and discussion.

In the end, we conclude our work with a conclusion.

PART ONE. THEORETICAL SECTION

1. GENERALITIES ON PESTICIDES

1.1. Development of pesticides

Ancient civilizations utilized natural and chemical pest control methods, including fumigation and botanical insecticides like pyrethrum. The late 19th century introduced copper-based fungicides, transforming agriculture. The 1930s and 1940s marked the rise of synthetic pesticides such as DDT, later banned in 1972 due to environmental concerns highlighted by Rachel Carson's Silent Spring (1962), leading to the establishment of the U.S. EPA. Modern agriculture employs Integrated Pest Management (IPM), combining biological, mechanical, and chemical methods to reduce environmental impact. Additionally, genetic engineering has facilitated the development of pest-resistant crops, decreasing reliance on chemical pesticides **(Tudi et al., 2021)**. Despite their known contribution to environmental pollution, including in Algeria, synthetic pesticides remain widely used globally. Algeria's horticultural sector plays a crucial role in the country's agriculture, making it essential to raise awareness and encourage behavioral change among farmers. Improper pesticide use heightens exposure risks for farmers and their families while posing significant threats to human health and the environment **(Soudani et al., 2020)**.

1.2. Definition of pesticides

The word *"pesticide"* is a generic term derived from the Latin words *"caedere"* (to kill) and *"pestis"* (plague) **(Baldi et al., 2013)**. In simpler terms, pesticides are active chemicals molecules used to:

- Protection of plants and their products from harmful organisms.
- Modification of plant life processes by excluding the use of nutrients.
- Preservation of plants by specific regulations.
- Control of undesirable plant growth **(Pretty and Bharucha,2015)**.

1.3. Classification

The table presents the major classification of pesticides in the world based on the target and chemical nature of them.

Table 1: The classification of pesticides on the basis of the target and chemical nature (Boland et al., 2007).

Types of pesticides	Usages
Fungicides **Eg:** Dithiocarbamate, Benzonitrile.	are classified according to their origin and mode of action on preventive fungicides, and curative fungicides. These fungicides employ diverse mechanisms to combat fungal growth, including impeding respiration or cell division, and interfering with the production of essential fungal components like sterols, RNA polymerase.
Herbicides **Eg:** Triazine, Bipyridyls.	constitute the most widely utilized class of pesticides in global agriculture, these chemical agents employ a range of physiological mechanisms to effect weed control. These mechanisms include the disruption of phytohormone regulation, the inhibition of photosynthetic processes, and the interference with critical biosynthetic pathways such as cell division, lipid biosynthesis, cellulose production, and amino acid synthesis.
Insecticides **Eg:** **Neonicotinoids,** Organophosphates.	These agents achieve their efficacy by either directly eliminating insect populations or by disrupting their reproductive cycles. Insecticides exhibit diverse modes of action: Neurotoxins, which target and disrupt the insect nervous system; Respiratory inhibitors, which impede insect respiration; and compounds that interfere with cuticle formation.

Beyond the three major pesticide classes, specialized categories exist to target specific agricultural and environmental pest challenges:

- **Acaricides:** Used to control mites and ticks**.**
- **Nematicides:** Targeting nematodes, a group of parasitic worms.
- **Rodenticides:** Designed to eliminate rodents**.**
- **Moleicides and Molluscicides:** Specifically used against moles, mollusks and slugs.
- **Corvicides and corvifuges:** Used to deter or eliminate crows and other pest birds that damage crops.

1.4. Routes of Pesticide exposure

1.4.1. Effects of pesticides on the environment

In soil: Pesticide application alters soil chemistry and biology, inhibiting essential nutrient production and suppressing microbial activity, which are crucial for plant growth, soil fertility, and ecosystem health. **(Tahar et al., 2017)**.

In water: Pesticides contaminate aquatic environments through drift, runoff, leaching, and direct application, harming aquatic organisms and plants. This contamination also reduces dissolved oxygen levels, disrupting ecosystem balance **(Mahmood et al., 2016)**.

In air: All pesticide spraying methods can be effective but may also cause air pollution and public exposure to residues **(Tudi et al., 2021)**, turning the atmosphere into a reservoir for those compounds that facilitate contamination **(Ouchenane, 2024)**. Pesticide residues can volatilize, travel long distances and react with other pollutants, forming ground-level ozone and smog **(Ludovic et al., 2024)**.Even indoor air currents from HVAC systems can aid in pesticide dispersal **(Kim et al.,2017)**.

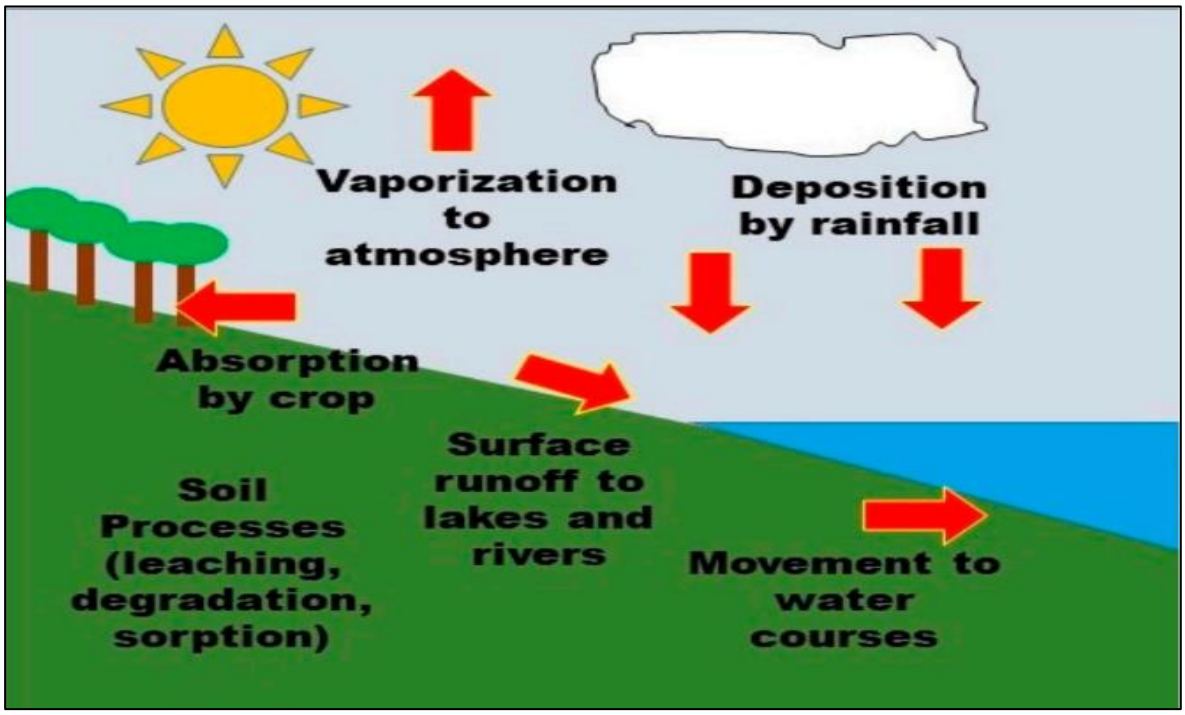


Figure01. Pesticide behavior in the natural environment **(Tudi et al, 2021)**.

1.4.2 The pesticide exposure on humans

Human pesticide exposure, especially among workers, occurs through dermal absorption, ingestion, ocular contact, and inhalation. The toxic effects depend on the exposure route, dose, duration, and chemical toxicity. Pesticide formulations and physicochemical properties also influence absorption and systemic bioavailability **(Kim et al., 2017)**.

1.4.3. The impact of pesticides on human health and oxidative stress

The WHO reports that millions of agricultural workers suffer acute pesticide poisoning annually, while chronic exposure leads to bioaccumulation and health risks. Pesticides induce oxidative stress by disrupting the balance between ROS and antioxidants, causing lipid peroxidation, protein oxidation, DNA damage, and antioxidant depletion. Additionally, some pesticides trigger nitrosative stress, further harming cells **(Sule et al , 2022)**.

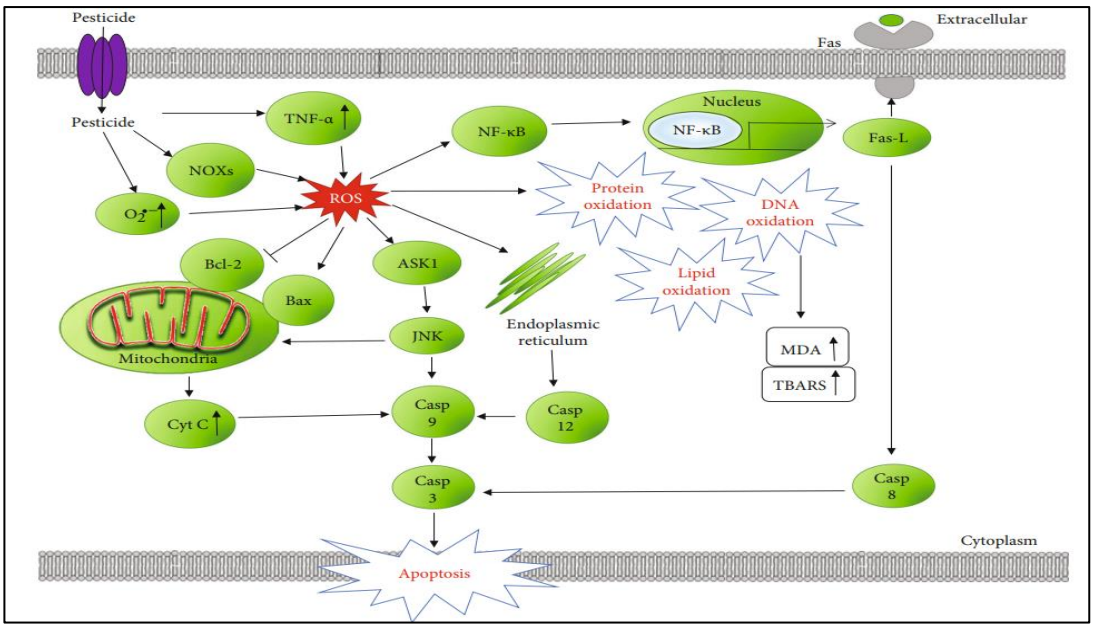


Figure 02. Pesticide-induced apoptosis pathway **(Sule et al., 2022)**.

2.GENERALITIES ON NEONICOTINOIDS

2.1. Definition

Neonicotinoids are a class of insecticides widely used in agriculture and veterinary medicine. This group includes Imidacloprid, **Acetamiprid**, Dinotefuran, Thiamethoxam, and clothianidin. Their chemical structure is designed to selectively interact with insect-specific nicotinic acetylcholine receptors (nAChRs) (**Han et al., 2018).**

2.2. Historical

Neonicotinoid (neonic) insecticides originated from nicotine-based pesticides, with the synthesis of S-nicotine in 1904 enabling structural optimization. The first-generation neonics, including imidacloprid, emerged from these advancements. In 1996, Bayer and Takeda collaborated to develop second-generation neonics, introduced in 2002, offering improved efficacy and safety. That same year, the third-generation neonic, Dinotefuran, was introduced, featuring a furfuryl moiety for greater persistence. To reduce non-target toxicity, newer compounds such as sulfoxaflor, flupyradifurone, and triflumezopyrim were later developed. Despite improvements, concerns about environmental persistence and non-target effects remain **(Zhao et al., 2020)**.

2.3. Mechanisms of Neonicotinoid Toxicity in Mammals

2.3.1. Interaction with Nicotinic Acetylcholine Receptors (nAChRs)

Neonicotinoids act as partial agonists of mammalian nicotinic acetylcholine receptors (nAChRs), leading to prolonged activation, desensitization, and downregulation. This impairs synaptic plasticity and cholinergic transmission, disrupting dopamine release in the prefrontal cortex, glutamate, and serotonin signaling. Consequently, these effects can result in cognitive deficits, mood disturbances, and potential neurodegeneration. Specifically, α2-nAChRs, which are essential for cognitive and emotional regulation, are affected, causing impaired attention, anxiety, and depression-like behaviors, thereby impacting learning and neuroadaptation **(Costas-Ferreira & Faro, 2021)**.

2.3.2. Neonicotinoid-Induced neurotoxicity

Neonicotinoid (NN) exposure disrupts mitochondrial function by inhibiting electron transport chain activity, reducing ATP production, and increasing reactive oxygen species (ROS), leading to oxidative stress and neuronal damage. Excessive Ca^{2+} influx further triggers mitochondrial permeability transition, cytochrome C release, and apoptosis. Additionally, NN-induced microglial activation and astrocytosis drive neuroinflammation, marked by elevated pro-inflammatory cytokines and nitrosative stress, ultimately exacerbating neuronal degeneration **(Costas-Ferreira and Faro, 2021)**.

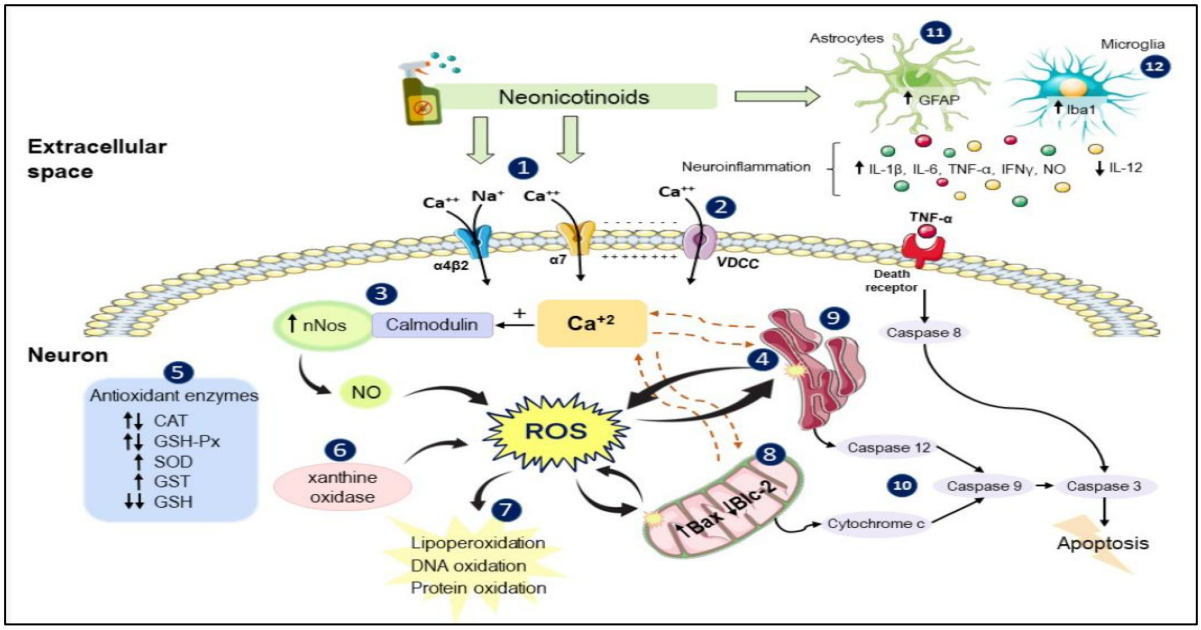


Figure 03. Neonicotinoid-Induced Oxidative Stress and Cellular Damage **(Costas-Ferreira and Faro, 2021).**

3.ANTIOXYDANTS AND PLANT MEDICINAL

3.1. The importance of medicinal plant

Medicinal plants play a crucial role in both traditional and modern healthcare systems, serving as the cornerstone of numerous therapeutic practices around the world. They account for approximately 85% of traditional remedies and are especially significant in indigenous communities, where knowledge of their healing properties has been preserved and passed down through generations. These plants have greatly influenced pharmaceutical development, contributing to the discovery of important drugs. In addition to their therapeutic benefits, medicinal plants are economically valuable. Continued ethnobotanical and phytochemical research is vital for uncovering new bioactive compounds and ensuring the sustainable use and conservation of these natural resources. **(Niazi et al., 2024)**.

3.2. Antioxidants

An antioxidant is a substance that, even at low concentrations, can prevent or delay oxidation by protecting oxidizable substrates from damage **(Brainina et al., 2019)**. Antioxidants are a diverse group of compounds that include enzymatic proteins (such as superoxide dismutase, glutathione peroxidase, and catalase), non-enzymatic proteins with metal-sequestering activity, and small molecules that are either fat-soluble (like vitamin E and beta-carotene) or water-soluble (like vitamin C and uric acid).

3.3. The role of antioxidants

Antioxidants play a crucial role in adapting to oxidative stress, protecting against oxidative damage, and enhancing their effects through synergy. They also contribute to the regulation of apoptosis and cellular signaling, helping in disease prevention by reducing the risk of cardiovascular diseases, cancer, diabetes, and rheumatoid arthritis **(Pincemail et al., 2002).**

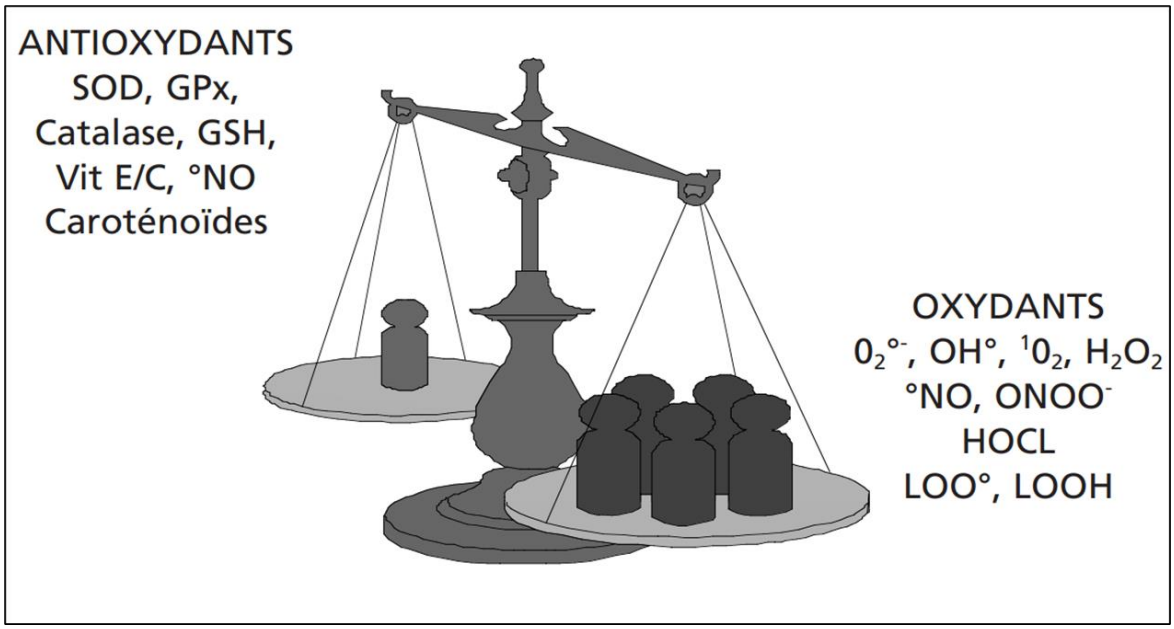


Figure04. The Antioxidant Advantage **(Morena et al., 2002)**.

4. GENERALITIES ON *JUNIPERUS PHOENICEA L.*

Juniperus phoenicea L. is a small Mediterranean tree called red juniper commonly known Arabic names such as **"Arar"**.



Figure 05. The *Juniperus Phoenicia L.* plant **(personal photos)**.

4.1. Taxonomy

Table 02: Taxonomic classification of *Juniperus phoenicea L.* (Haluk and Roussel, 2000)

Scientific Rank	Taxon name
Phylum	*Spermatophytes*
Subphylum	*Gymnosperms*
Class	*Coniferopsida*
Subclass	*Coniferidae*
Order	*Cupressales*
Family	*Cupressaceae*
Subfamily	*Cupressoideae*
Genus	*Juniperus*
Species	*Phoenicea L.*

4.2. Distribution geography

In Algeria, five species of *Juniperus* are documented, among which *Juniperus phoenicea L.* is notably present in semi-arid and arid regions **(Haluk and Roussel, 2000).** It thrives in arid climates and is resistant to harsh environmental conditions such as sea winds and rocky soils and found throughout the Mediterranean Basin, including North Africa, Europe, and parts of the Middle East **(Caudullo and de Rigo, 2016)**.



Figure 06. Geographic range of *Juniperus Phoenicea L.* **(Caudullo et al., 2017).**

4.3. Botanic study

Juniperus phoenicea L. is a shrub or small tree characterized by thick branches with dark green, scale-like leaves arranged in four rows, each measuring approximately 1 mm. The leaves contain a glandular groove on the dorsal side. Its fruit is globular (6–15 mm in diameter), terminal on the branches, and turns red upon maturity **(Telaidji , 2018)**.

4.4. Ethnobotanical Significance and Historical Uses of *Juniperus phoenicea L.*

Juniperus phoenicea L., commonly known as *Phoenician juniper*, holds significant ethnobotanical importance, particularly in North Africa, Southern Europe, and Mediterranean regions. Indigenous populations have historically recognized this plant for its medicinal properties and have widely incorporated it into traditional remedies.

In Algeria, *Juniperus phoenicea L.* has been traditionally used to treat respiratory conditions such as coughs, bronchitis, and asthma. Infusions made from its berries and leaves have been applied to alleviate digestive issues, including stomach cramps and indigestion. Moreover, these parts have been used topically in poultices to promote wound healing and reduce skin inflammation. The plant's antiseptic properties made it a valuable resource for disinfecting wounds and treating skin infections **(Abdelli et al., 2018)**.

Beyond medicinal uses, *Juniperus phoenicea* has cultural significance. In some regions, it has been burned as incense during spiritual rituals due to its aromatic properties. The berries have also been incorporated in traditional cuisine for flavoring purposes, though primarily in limited quantities due to their potent nature **(Innocenti et al., 2007)**.

4.5. Chemical Constituents and therapeutical properties

Juniperus phoenicea L. exhibit a rich chemical profile with various bioactive compounds has garnered scientific interest due to its highest anti-inflammatory activity, notable antioxidant and antimicrobial properties. These bioactivities are primarily attributed to the phytochemical composition which is rich in monoterpenes (α-pinene, limonene, δ-3-carene) **(Abdelli et al., 2018;Elmhdwi et al., 2023)**, fatty acids, minerals, resin, alkaloids, sterols, and carbohydrates ,phenylpropanoid glycosides(**Telaidji,2018)** and flavonoids (cupressoflavone, amentoflavone and hynokiflavone) **(Abdelli et al., 2018)** .The antioxidant potential of *Juniperus Phoenicea* has been linked to its ability to scavenge reactive oxygen species (ROS), thereby protecting biological systems from oxidative stress and associated cellular damage **(Elmhdwi et al., 2023)**.

PART TWO. EXPERIMENTAL SECTION

5. Material and Methods

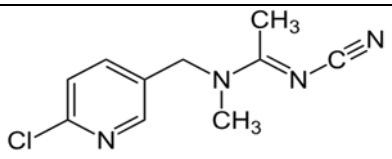
5.1. Chemical and biological material

5.1.1. Definition and use of acetamiprid

Acetamiprid, a first-generation neonicotinoid insecticide, developed by Nippon Soda in the mid-1990s, is designed to offer improved safety and efficacy in controlling sap-sucking insects such as aphids, whiteflies, and leafhoppers. It is particularly effective against pests resistant to organophosphate and other conventional insecticides. While Acetamiprid has not been conclusively classified as genotoxic or carcinogenic, some teratogenic effects observed in animal models highlight the need for further investigation **(Wallace, 2014)**.

5.1.2. Chemical and physical properties

Table 03: Physical and chemical properties of ACE(Wallace,2014).

Properties	Specifications
Chemical Name	(E)-N-[(6-chloro-3-pyridyl) methyl]-N'-cyano-N-methyl acetamidine.
CAS Registry Number	135410-20-7.
Molecular Weight	222.7g.
Synonyms	Assail, Tristar, Mospilan.
Chemical Class	Neonicotinoid (Pyridyl methylamine) Insecticide
Molecular Formula	$C_{10}H_{11}ClN_4$
Structure	

5.1.3. Preparation of Acetamiprid solution

To achieve the intended dose of 20 mg/kg/day of ACE **(Shamsi et al., 2021)**, we prepared the solution by dissolving pesticide powder Rustilan which is a commercial insecticide in Algeria contains 20% ACE as an active compound in distilled water. The solution was administrated with oral gavage.



Figure07. Preparation of ACE solution and oral gavage **(personal photos)**.

5.1.4 Plant materials and selection of medicinal plants

In an effort to better valorize the plant biodiversity of Eastern Algeria our study focused on *Juniperus Phoenicea L.* The choice of these species was informed by a literature review and a simple survey conducted among the local population familiar with traditional medicine.

The selection criteria for this plant are as follows:

- wide distribution across various region of Algeria.
- The presence of bioactive compounds reported in previous phytochemical studies, which suggests potential therapeutic effects and justifies further investigation.
- Traditional use in treating a variety of illnesses.
- Non-toxicity of the plant ensuring its safety and suitability for use in herbal tea preparation.

We took the aerial part *of Juniperus phoenicea L. (*stem, leaves, fruits) in February from Safsaf El Ouesra, Tebessa, Algeria.



Figure08. The place of taking the aerial part of *Juniperus Phoenicia L.* **(personal photos)**.

5.1.5. Preparation of the plant decoction

To achieve a dose of 200 mg/kg/day **(Soltani et al., 2019)**, we weighed *Juniperus phoenicea L.* plant material then we dissolved it in boiling distilled water. After cooling, we filtered the solution in sterile bottle. Finally, we stored the prepared infusion at 4°C in a light-protected container.



Figure 09. The preparation of the decoction **(personal photos)**.

5.2. Method

5.2.1. Animals care and treatment

For this study, we obtained 28 female Albino Wistar rats from the Pasteur Institute of Algiers (I.P.A.). The rats were housed in the animal facility of Larbi Tébessi University - Tébessa. They were kept in polyethylene cages labeled with information such as the group name, treatment received, and experimental dates. The cages were lined with wood shavings, which were cleaned 4 times in the week.

The rats underwent an adaptation period of approximately one month to acclimate to the animal facility conditions, maintained at a temperature of 22 ± 2°C with a 12/12-hour natural photoperiod. They were fed a nutritionally balanced diet consisting of kibble, water, and bread. The average body weight of the rats at the start of the experiment was approximately 200g.

Table 04 diet: Composition of 1 kg of feed.

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

After acclimatization period, the animals were randomly assigned to four groups, each consisting of seven rats. Body weight monitoring was conducted to ensure accurate dosage administration.

Batch C: control group, the seven rats received drinking water and a standard diet.

Batch JNP: The rats received drinking water and 200 mg/kg/day prepared from *Juniperus Phoenicia* via oral gavage

Batch ACE: The rats received by oral gavage 20 mg/kg/day of Acetamiprid via oral gavage.

Batch ACE+JNP: The rats undergo oral gavage with a dose of Acetamiprid at 20 mg/kg/day followed by the administration of 200 mg/kg/day of *Juniperus Phoenicia* decoction 3 hours later.

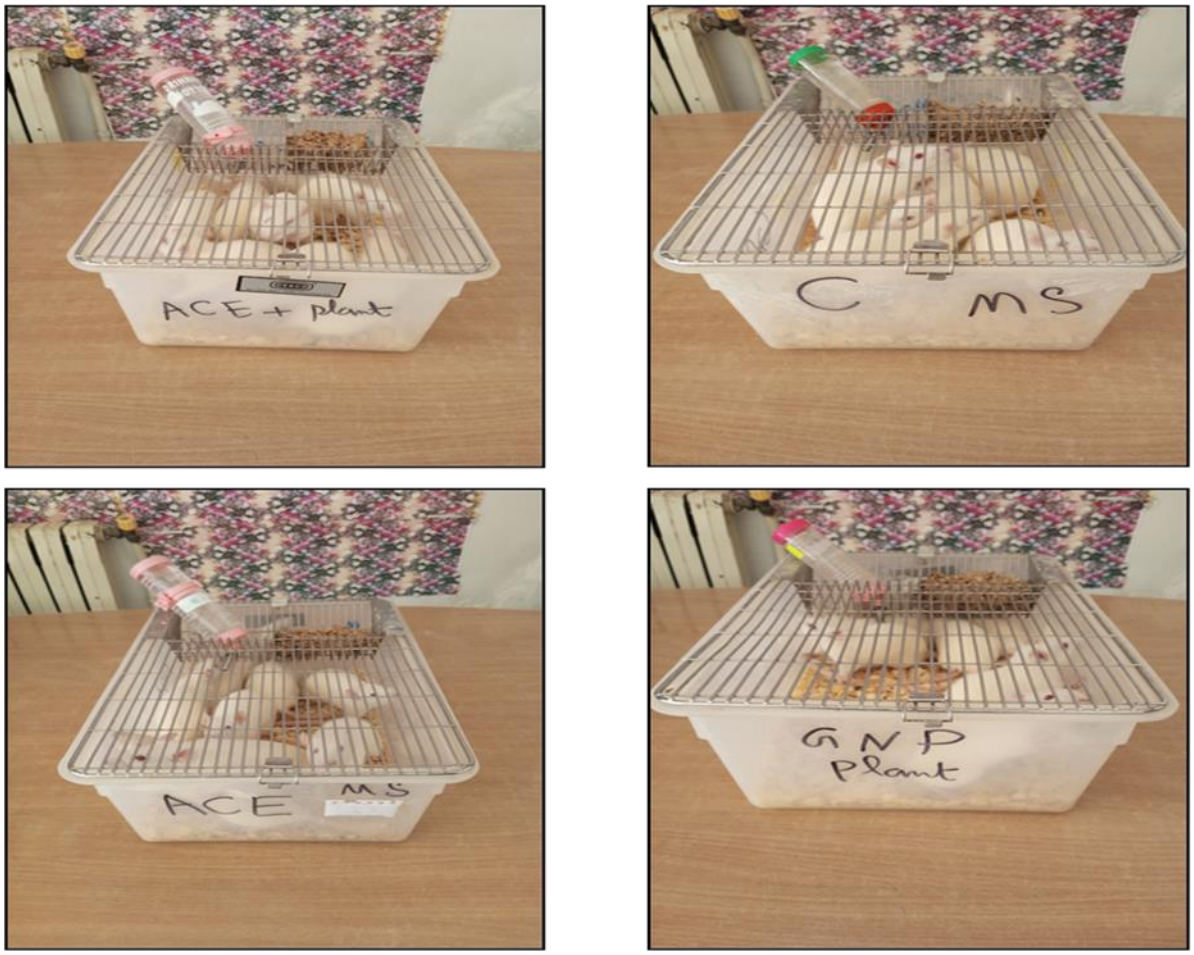


Figure 10. Photo gallery Showing the different groups of the experimental rats.

5.2.2. Behavioral study

Behavioral tests were conducted in the animal facility at Larbi Tébessa University – Tébessa to assess the deleterious effect of Acetamiprid and the protective effect of Juniperus phoenicea on locomotor activity, anxiety levels and depressive behavior. To achieve these objectives, the following tests were performed: the Open Field test (OFT), the Elevated Plus Maze test (EPM) and the Forced Swim Test (FST) with 15min of adaptation phase and 5 min recording session.

5.2.2.1. Elevated Plus Maze (EPM) Test

The Elevated Plus Maze (EPM) is a widely used test to evaluate anxiety-related behavior in rodents described by **Pellow et al in 1985**. The apparatus consists of two open arms and two closed arms, elevated 50 cm above the ground. During the 5-minute test, the rat is placed facing an open arm and allowed to explore. Rats naturally prefer enclosed spaces due to their fear of open areas. Therefore:

- Spending more time in the open arms indicates lower anxiety.
- Staying mostly in the closed arms reflects higher anxiety.

Key observations include the number of entries and time spent in each type of arm.



Figure 11. Photo gallery Showing the anxiety study.

5.2.2.2. Open Field (OF) Test

The Open Field (OF) test is a common method in neuroscience for assessing emotional reactivity in rodents and evaluating the potential anxiolytic effects of a substance described by **Hall in 1934**. The apparatus consists of a 70 cm x 70 cm plexiglass square arena with 40 cm-high sidewalls. The floor is divided into a peripheral zone and a central zone, each subdivided into 10 cm^2 squares to facilitate movement tracking.

In general:

- Anxious rats tend to avoid the central area, preferring to stay in the peripheral zone.
- Less anxious rats are more likely to explore the central area.

At the start of the test, the rat is placed in the central zone and allowed to explore freely for 5 minutes. During this time, key parameters such as the number of entries into the central area, the time spent immobile and the total distance traveled are recorded.

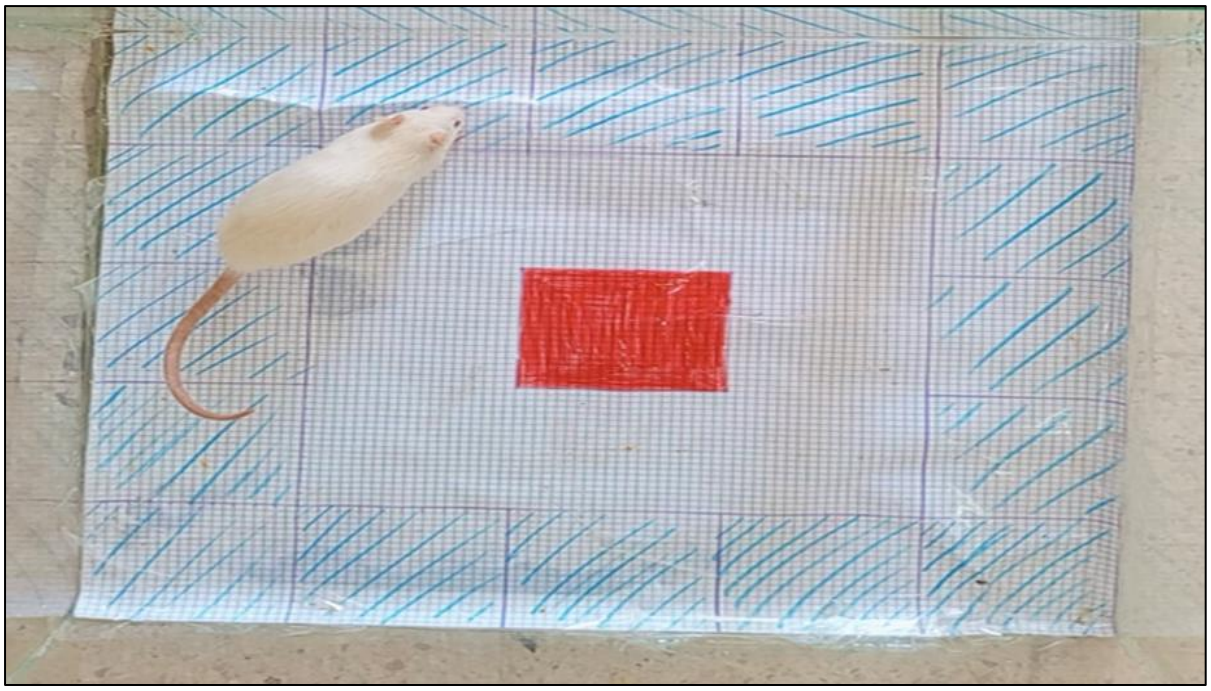


Figure 12. Photo gallery depicting the locomotor activity assessment.

5.2.2.3. Forced Swimming Test (FST)

The Forced Swimming Test (FST), originally described by **Porsolt in 1977**, is widely used in pharmacology to evaluate the antidepressant properties of new molecules. It induces a depressive-like state. The rat is placed in a 35 cm wide and 50 cm deep plexiglass tank filled with 26°C water up to a height of 40 cm. These conditions force the rat to swim without touching the bottom to stay afloat. After a few minutes of active swimming, the rat typically becomes immobile, performing only minimal movements to keep its head above water. This test lasts 5 minutes, during which three key parameters are recorded using a video camera:

- Immobility time, which reflects the animal's resigned behavior.
- Climbing time, considered an active behavior.
- Swimming time, also regarded as an active response.

In this test, increased immobility is linked to depressive-like behavior, while enhanced swimming or climbing activity suggests a reduced depressive state, often influenced by antidepressant treatments.



Figure 13. Photo gallery depicting the depressive behavior assessment.

5.2.3. Brain Sampling

following 30 days of treatment, the brain was immediately collected, rinsed with physiological saline, and stored in a freezer at -20°C for the assessment of oxidative stress parameters (GST, GSH, and catalase). The photos below are summering the sacrifice and the brains sampling procedure:



Figure 14. Personal photos summarizing the steps of the sacrifice and collect of the different organs.

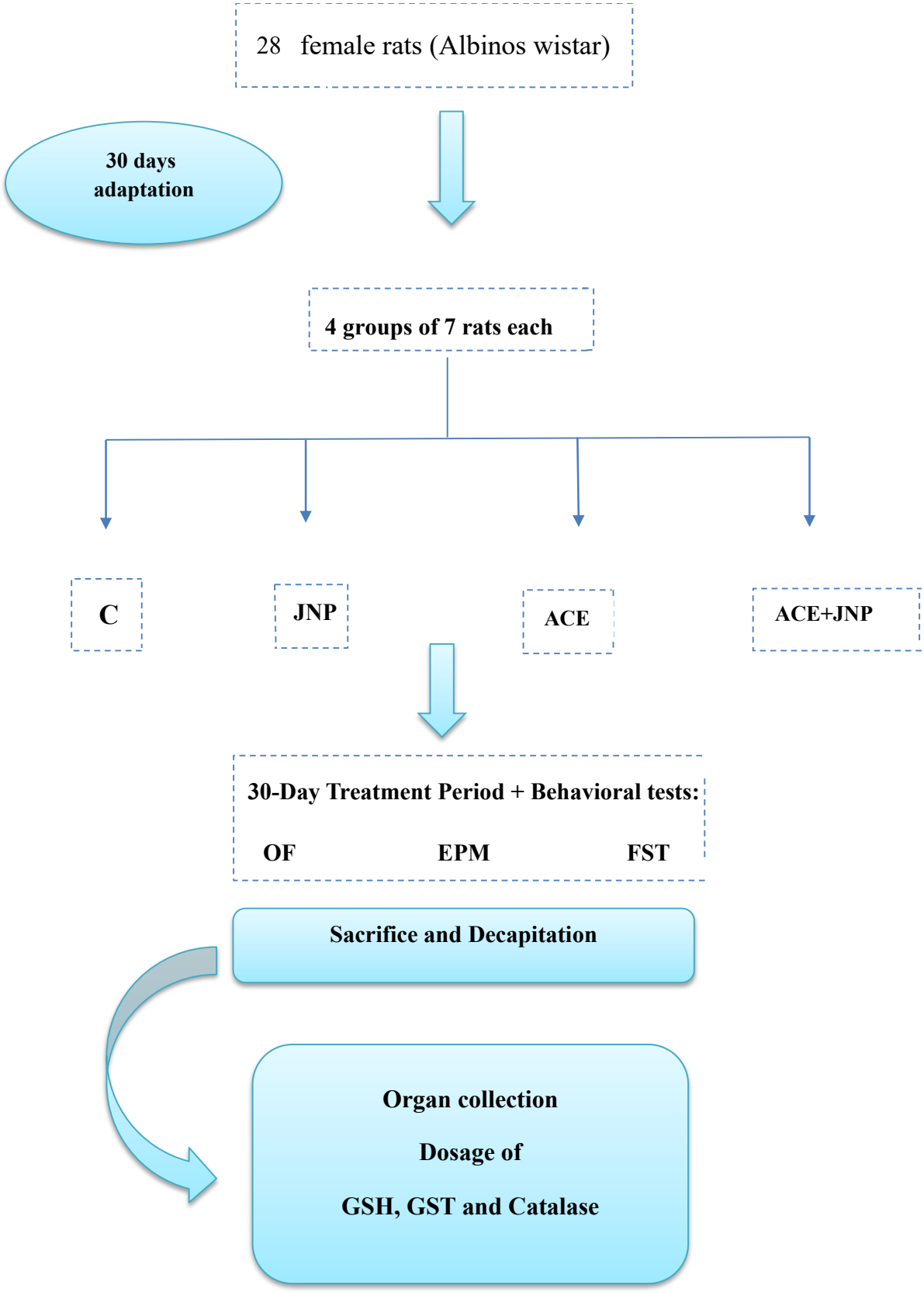


Figure 13: Diagram summarizing the experimental protocol.

5.2.4. Parameters of GSH, GST, Catalase activity and Proteins essay

5.2.4.1. GSH reduced glutathione assay

Principe

The quantification of glutathione (GSH) was perfmed following the spectrophotometric method described by **Wekbeker and Cory in 1988**. The fundamental principle of this assay lies in the measurement of the optical absorbance of 2-nitro-5-mercapturic acid. This chromophore is generated through the reduction of 5,5'-dithio-bis-2-nitrobenzoic acid (Ellman's reagent, DTNB) by the thiol (-SH) groups present in glutathione. To ensure specific measurement of glutathione's thiol groups, a deproteinization step of the tissue homogenate is crucial.

Homogenate Preparation

100 mg of tissue was combined with 4 mL of ethylenediaminetetraacetic acid (EDTA) solution in a designated tube1. This mixture was then subjected to cryogenic homogenization to yield the tissue homogenate. Subsequent centrifugation is required to remove proteinaceous components and obtain a protein-free supernatant containing glutathione for analysis.

Operating Mode

-In tube 2 pipette 0.8 ml of the homogenate.

-Deproteinize by adding 0.2 ml of 0.25% sulfosalicylic acid (SSA) solution.

-Mix the solution and place it in an ice bath for 15 minutes.

-Centrifuge at 1,000 rpm for 5 minutes.

-In tube 3 transfer 0.5 ml of the supernatant.

-Add 1 ml of Tris + EDTA buffer (containing 0.02 M EDTA), pH 9.6.

-Mix and add 0.025 ml of 0.01 M DTNB (5,5'-dithiobis-(2-nitrobenzoic acid)).

-Allow the reaction to proceed for 5 min at room temperature to stabilize the color, which develops instantly.

-Measure the optical densities at 412 nm against a blank.

For the calculation of GSH, we used the equation bellow:

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

DO : Optical density 412 nm.

1 : Total volume of solutions used in deproteinization.

1.525 : Total volume of solutions used in the determination of GSH

13100 : is the molar absorption coefficient of the group (-SH) at 412 nm.

0.5 : Volume of supernatant in 1.525 ml.

0.8 : Volume of supernatant in 1 ml.



Figure 15. Personal photos presenting GSH preparation.

5.2.4.2. Determination of Glutathione S-Transferase (GST) Activity

Principe

The determination of Glutathione S-transferase (GST) activity is based on the enzyme's ability to catalyze the conjugation of reduced glutathione (GSH) with a substrate commonly 1-chloro-2,4-dinitrobenzene (CDNB). This reaction results in the formation of a conjugated product, S-(2,4-dinitrophenyl) glutathione (GS-DNB), along with the release of hydrochloric acid (HCl).

In this study, the method described by **Habig et al. (1974)** is employed. The assay involves incubating the GSTs present in the homogenate with a mixture of GSH and CDNB at 25°C and pH 7. The enzymatic activity is monitored by measuring the increase in absorbance at 340 nm, which corresponds to the formation of the GSH-CDNB complex. Absorbance is recorded every minute over a 5-minute period.

Homogenate preparation

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

Note: keep homogenates cold until homogenization is complete.

Centrifugation of the homogenate at 9000 rpm for 15min →→→→→ recovery of the

supernatant for GST and mg protein determination.

Operating mode

Table 05: The table presents the operating mode of Glutathione S-transferase activity.

Reagents Used	Blank (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

For the calculation of GST, we used the equation bellow:

$$\textbf{GST (n mol GST / min / mg protein)} = \frac{(DO\ echantillon/min - DO\ blanc/\text{min})}{9.6 * mg\ proteine}$$

DO Cantillon / min: Optical density of the sample per minute.

DO blank / min: Optical Density of blank per minute.

9.6: Extinction coefficient of GSH-CDNB expressed in m.M^{-1}.Cm^{-1}.

5.2.7.3 Catalase (CAT) Assay

Principe

The method employed is that of Aebi (1984). Catalase activity was measured at 240 nm using a spectrophotometer by monitoring the change in optical density resulting from the decomposition of hydrogen peroxide (H_2O_2) at 25°C in the presence of an enzymatic source within the cytosolic fraction. Absorbance readings were taken after a 15-second delay, with measurements recorded over a 60-second period.

Due to the rapid decline in catalase activity, it is crucial to maintain consistent timing between pipetting and placing the cuvette in the spectrophotometer.

Mode operation

Table 06: The table presents the operatory mode of Catalase activity.

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

To calculate the catalase activity, we used the equation bellow:

$$\textbf{The catalase Activity CAT (µ mol } H_2O_2\textbf{/ min/ mg protein)} = \frac{\Delta \textbf{DO}*10}{\varepsilon*\textbf{L}*\textbf{X}*\textbf{Fd}}$$

ΔDO: Variation in optical density per minute.

ε: Extinction coefficient of the H_2O_2 = 0.04 M$m^{-1}.Cm^{-1}$.

L: Length of the cuvette 1cm.

X: Quantity of protein in mg/ml.

Fd: Dilution factor in H_2O_2 in the buffer (0.02).

5.2.4.3. Protein Assay in a Sample

Principe

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

Operating mode

- Pipette 0.1 ml (100 µl) of the sample (supernatant).
- Add 4 ml of Bradford reagent.
- Mix and allow to stand for 5 minutes for color stabilization.
- Read the optical density at 595 nm against a blank (4 ml of BBC + 0.1 ml distilled water).
- The obtained optical density is plotted against a previously prepared calibration curve. The protein concentration is determined by comparison to a bovine serum albumin (BSA) standard curve (1 mg/ml) performed under the same conditions.

5.2.4.3.1. The calibration curve of proteins

To construct the calibration curve, a stock solution of bovine serum albumin (BSA) was prepared by dissolving 1 mg of BSA in 1 ml of distilled water with agitation. Six tubes were then prepared using 4 ml of Bradford reagent (BBC) and varying quantities of BSA and distilled water to create different protein concentrations. After mixing, the optical density of each tube was measured at 595 nm. These readings were used to plot the calibration curve, allowing the determination of protein concentration in unknown samples based on the linear equation:

$$y = ax + b.$$

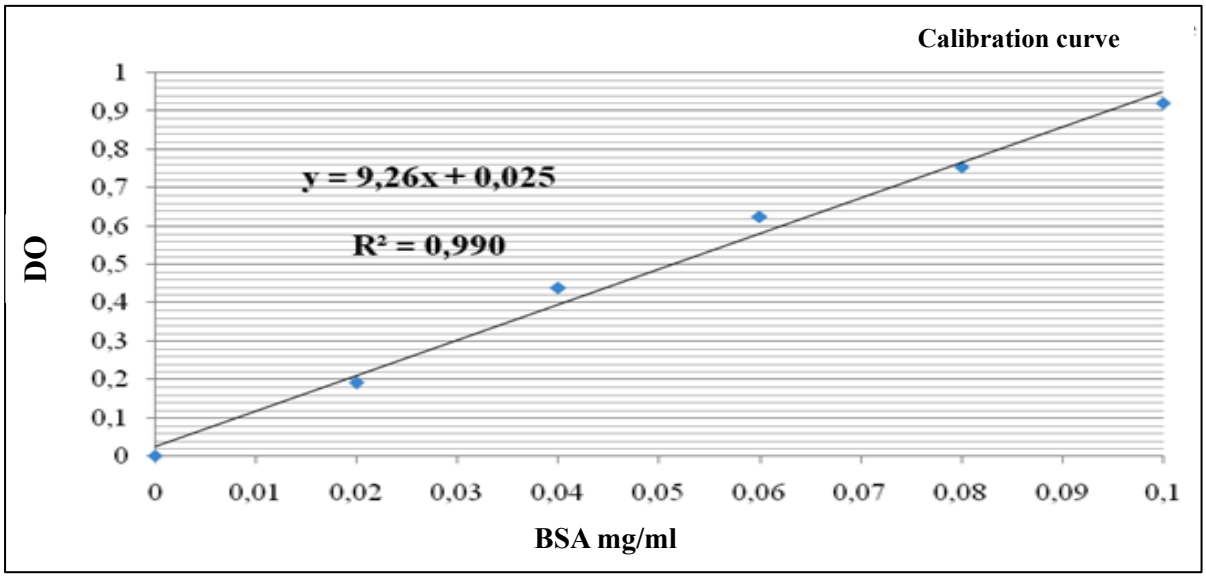


Figure 16. Calibration curve of bovine serum albumin.

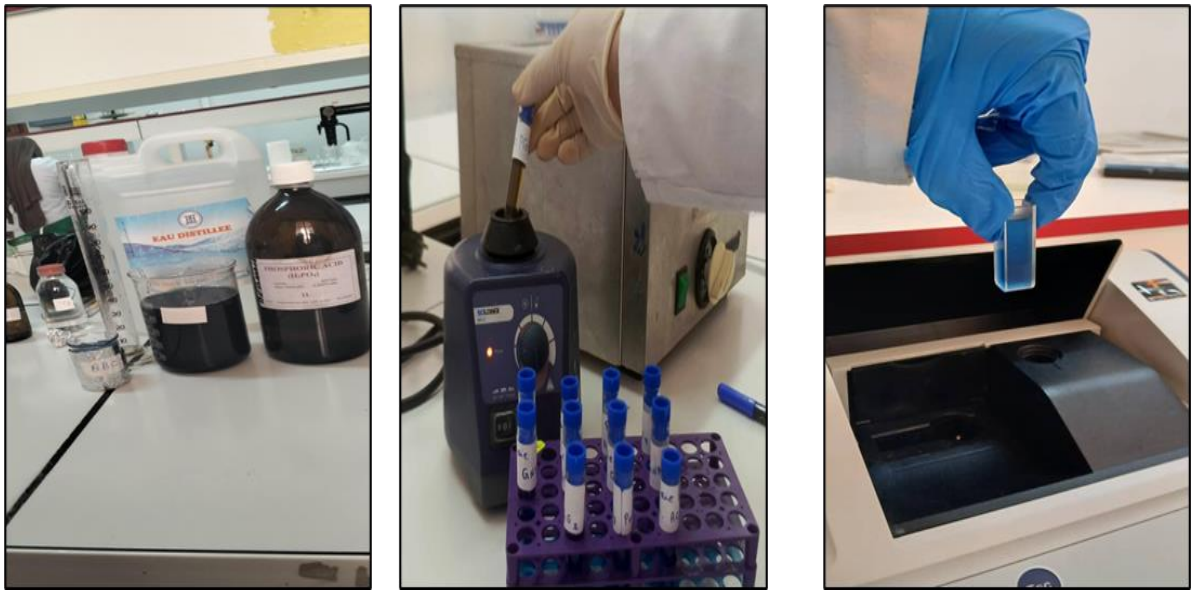


Figure17. Personal photos present preparation of proteins.

5.2.5 Statistical analysis

A comparative statistical analysis was conducted to evaluate the differential effects of various treatments on experimental groups. The study comprised a control group (C), a group treated with *Juniper* extract (JNP), a group exposed to acetamiprid (ACE), and a group subjected to co-treatment with Acetamiprid and *Juniper* extract (ACE+JNP).

Quantitative data are presented as the mean ± standard deviation (SD). To ascertain statistical significance among the groups, a one-way analysis of variance (ANOVA) was employed, followed by Tukey's post hoc test for pairwise comparisons. The threshold for statistical significance was set at $p<0.05$. The results of this analysis demonstrated statistically significant variations across the experimental groups, providing evidence for the toxicological impact of Acetamiprid and suggesting a potential protective effect associated with the application of *Juniper* decoction.

PART THREE.RESULTS AND DISCUSSION

6. Results and discussion

6.1. Physiological parameters

6.1.1. Effect of treatments on body weight

In our findings the monitoring of body weight variation in animals during the treatment period revealed a significant decrease in the weight of the groups exposed to ACE (-4.57) and the combination of ACE+JNP (-0.53) compared to the control group. However, a considerable increase in weight gain (6.02) was observed in the JNP group.

Table 07: The table presents the effect of aqueous extracts of *Juniperus* aerial parts and/or Acetamiprid treatment on body weight gain after 30 days of experience.

Experimental groups				
Parameters	**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**

The diagram represents more details of the development of weights for the four groups through the days. Compared to C group, which showed a steady increase in body weight over the period, the JNP group followed a similar growth pattern, indicating no adverse effects from the extract. In contrast, the ACE group exhibited a decline in body weight after day 21, highlighting its potential toxic impact. Notably, rats co-treated ACE+JNP experienced a less pronounced weight loss than the ACE group alone, suggesting that *J. phoenicea* may partially mitigate Acetamiprid-induced toxicity, though not to the level observed in the control group.

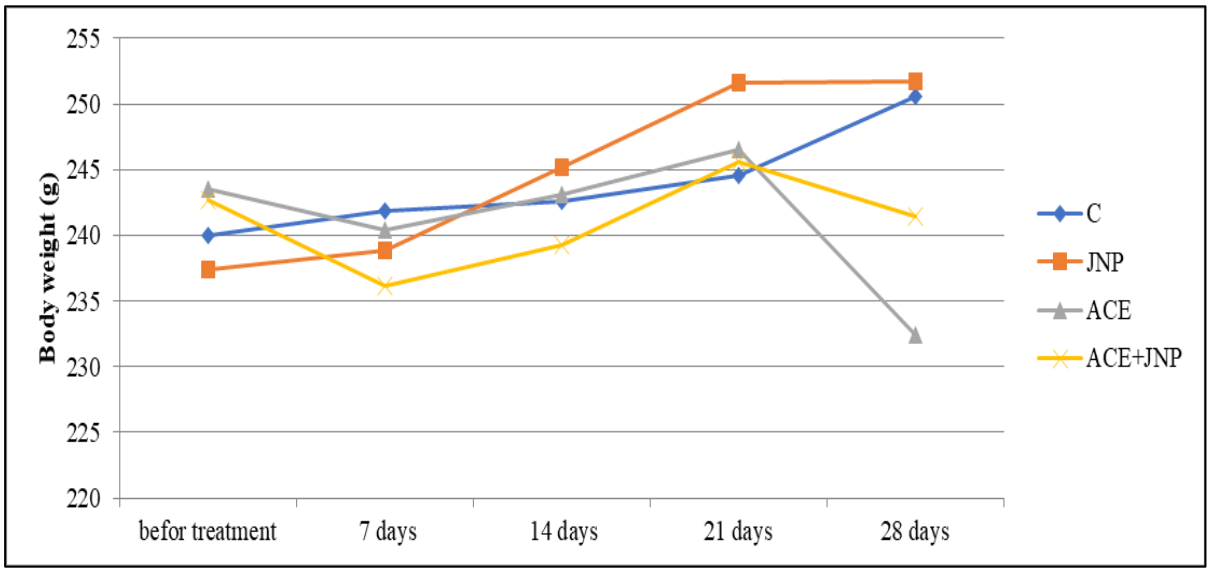


Figure 19. Body weight development (g) in control and treated rats after 30 days of treatment.

The −4.57% reduction in body weight observed in the ACE group indicates systemic toxicity and metabolic dysregulation resulting from Acetamiprid exposure. This is consistent with established literature demonstrating that various pesticides can induce adverse health outcomes and metabolic disturbances. Specifically, pesticide exposure has been shown to disrupt bile acid metabolism and trigger systemic inflammation. Furthermore, evidence suggests that exposure to pesticide residues can significantly alter gut microbiota composition, leading to host metabolic changes and contributing to the pathogenesis of various diseases. This observation aligns with findings by **Xu et al. (2022)**, who reported that neonicotinoids exert broad toxic effects on non-target organisms, including mammals. Consequently, the observed body weight reduction likely reflects compromised physiological functions and decreased metabolic efficiency. These findings support the conclusion that acetamiprid exposure negatively impacts overall health and growth, with weight loss serving

as a quantifiable indicator of its systemic toxicity. Moreover, the European Food Safety Authority **(EFSA, 2024)** report underscores the significance of body weight changes as potential indicators of developmental toxicity.

Conversely, the group administered with Juniperus phoenicea (JNP) alone exhibited a notable increase in body weight (+6.02%). This finding suggests the absence of toxic effects and points towards a potential health-promoting or protective role for JNP. The observed body weight preservation in the treated cohorts, alongside the mitigation of gastric lesions, further supports its potential as a gastroprotective agent. These results substantiate the traditional use of *J. phoenicea* in gastrointestinal therapeutic contexts, specifically highlighting its efficacy in ameliorating gastric damage, as reported by **Hennia et al. (2024)**.

Interestingly, the ACE+JNP co-treated group exhibited only a slight decrease in body weight (-0.53%) compared to the ACE group, suggesting that *J. phoenicea* may mitigate the adverse effects of Acetamiprid. This protective effect is likely due to the antioxidant properties of *J. phoenicea*, which have been documented in various studies highlighting its ability to scavenge free radicals and improve redox status **(Tavares et al., 2012; Bouassida et al., 2018; Chelouati et al., 2023; Asbabou et al., 2024)**. Therefore, the near-normal weight profile in the co-treated group reinforces the potential of *Juniperus Phoenicia* to counteract Acetamiprid-induced systemic toxicity.

6.1.2. Effect of treatment on brain weight

The results indicated that Acetamiprid exposure significantly reduced absolute brain weight ($P < 0.01$) compared to the control group. In contrast, treatment with the decoction of *Juniperus* alone did not induce significant changes in brain weight. The absence of a marked difference in relative brain weight (%) across groups implies that the observed reduction in absolute brain mass was not merely due to overall body weight variations.

Table 08: The table presents the effect of decoction of *Juniperus* and/or Acetamiprid treatment on brains weights after 30 days of experience.

Weights	Control	JNP	ACE	ACE+JNP
Absolute weight (g)	1.829±0.06	1.763±0.05	1.518±0.07**	1.616±0.05**
Relative weight (%)	0.710±0.06	0.703±0.05	0.672±0.05	0.687±0.05
****P<0.01 relative to the control group (C).**				

The significant reduction in absolute brain weight observed in Acetamiprid-treated rats in this study aligns with the **OECD Test Guideline 424** (Neurotoxicity Study in Rodents). This guideline recognizes both absolute and relative brain weight measurements as critical endpoints in the assessment of chemical-induced neurotoxicity. According to **OECD TG 424**, a significant reduction in brain weight, even without overt behavioral changes, is indicative of potential neurotoxic effects when supported by pathological or mechanistic evidence.

Given that Neonicotinoids are known to accumulate in brain tissue and induce oxidative stress, neuronal degeneration, and apoptosis **(Costas-Ferreira & Faro, 2021)**, the observed reduction in brain mass likely reflects direct structural damage to central nervous system tissue. Therefore, our findings are consistent with internationally accepted toxicological standards for neurotoxicity assessment and support the interpretation of Acetamiprid as a neurotoxic compound in mammals.

6.2. Effects of treatment on oxidative stress parameters

6.2.1. The results of GST activity following ACE and JNP Treatment

The data reveal that C group exhibited the highest GST activity, indicative of basal GST levels in the absence of Acetamiprid exposure. ACE group resulted in a significant reduction in GST activity ($p < 0.01$), suggesting a compromise in the brain's detoxification capacity. Notably, co-administration of ACE+JNP group partially attenuated the Acetamiprid-induced reduction in GST activity; GST activity in the ACE+JNP group was significantly higher than that observed in the ACE group ($p < 0.01$). JNP did not differ significantly from the control group.

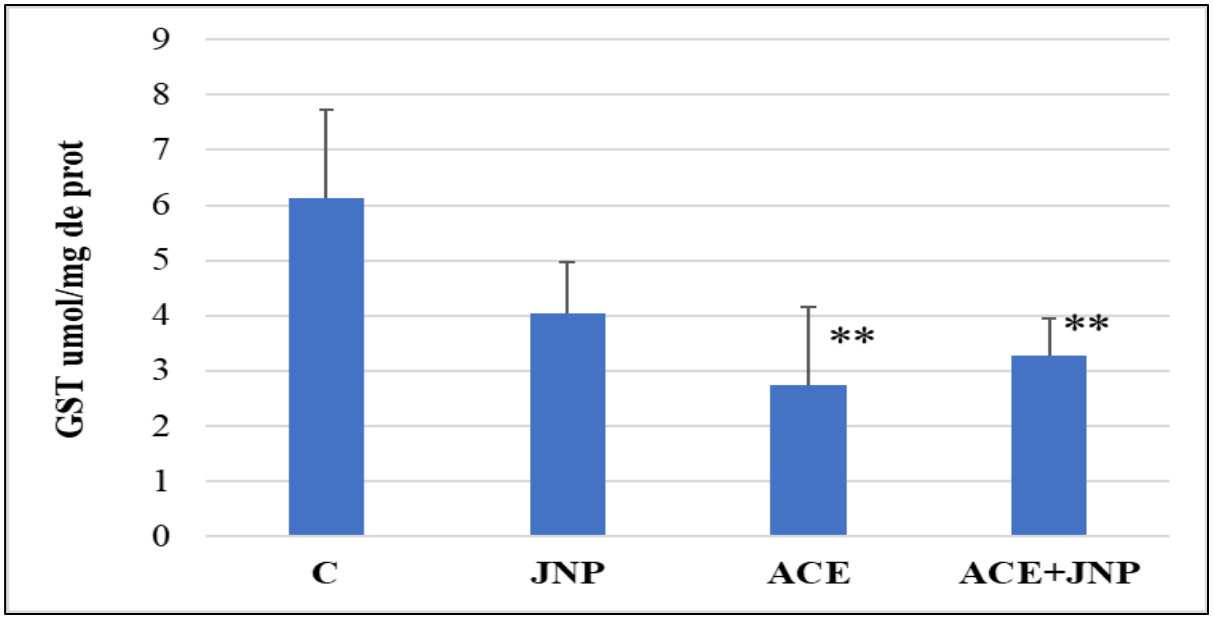


Figure 20. GST activity on various treatments.

6.2.2. The results of GSH following ACE and JNP treatment

The data reveal that while the C group exhibited a baseline GSH level, Acetamiprid treatment ACE group significantly reduced GSH levels ($p < 0.001$), indicative of oxidative stress induction. JNP group did not significantly alter GSH levels compared to the control. However, co-administration of ACE+JNP group resulted in a less pronounced reduction in GSH levels than Acetamiprid alone, suggesting a potential mitigating effect of *Juniperus* extract on Acetamiprid-induced GSH depletion.

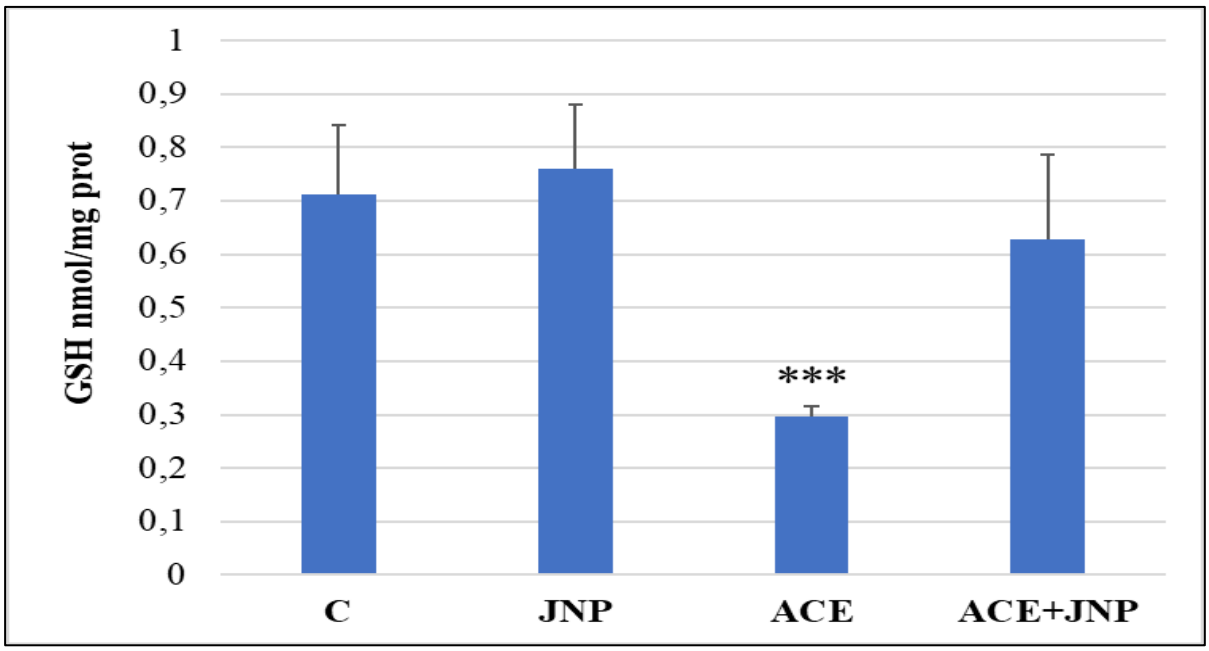


Figure 21. GSH levels on various treatments.

6.2.3. The results of Catalase following ACE and JNP treatment

The data reveal that ACE group significantly increased Catalase activity ($p < 0.001$) compared to the control group C, indicative of a compensatory response to Acetamiprid-induced oxidative stress. In contrast, JNP group did not significantly alter Catalase activity. The co-administration of ACE+JNP group showed a trend towards normalization of Catalase activity, although the activity level did not differ significantly from the control group.

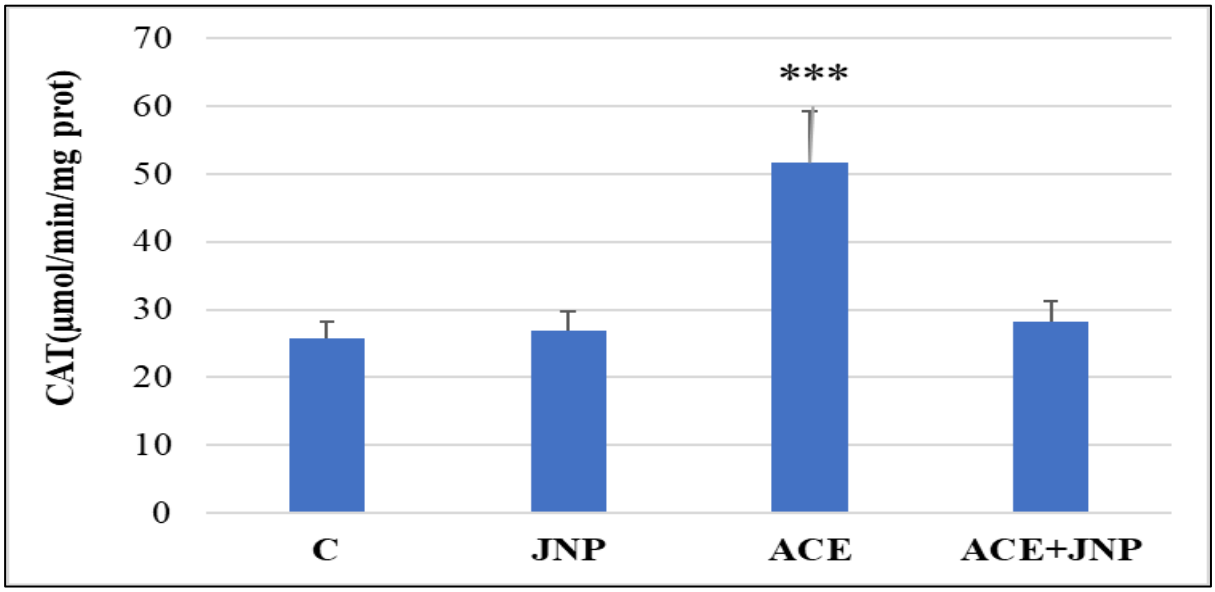


Figure 22. CAT levels on various treatments.

Our study provides strong evidence that exposure to acetamiprid induces oxidative stress in the rat brain, a key mechanism in neonicotinoid neurotoxicity and neuronal apoptosis. We observed significant alterations in the brain's antioxidant defense system, making it more vulnerable to damage.

Specifically, we found a decrease in glutathione S-transferase (GST) activity and glutathione (GSH) levels. This reduction in GST and GSH is direct evidence of oxidative stress, as GST is crucial for detoxifying foreign compounds by conjugating them with GSH, and GSH itself is a primary antioxidant that directly neutralizes free radicals. This compromised antioxidant defense can lead to the activation of apoptosis in brain cells, aligning with findings by **Gasmi et al. (2017) and Pal et al. (2023)**. Conversely, we observed a compensatory increase in

catalase activity, consistent with **Gasmi et al. (2017)**. This upregulation of catalase, an enzyme that breaks down hydrogen peroxide (H2O2) into water and oxygen, likely represents the brain's attempt to counteract the increased H2O2 production caused by acetamiprid-induced oxidative stress. Further mechanistic studies by **Gasmi (2018)** delineate the intricate pathways through which chronic acetamiprid exposure culminates in neurotoxicity. A pivotal event involves the inhibition of acetylcholinesterase (AChE), leading to an accumulation of acetylcholine (ACh) within the synaptic cleft. This sustained elevation of ACh results in cholinergic receptor hyperstimulation. Subsequent cellular events include the desensitization and functional blockade of Na+/K+ -ATPase channels, critical for maintaining neuronal membrane potential, and the inhibition of Mg2+ -ATPase, an enzyme indispensable for oxidative phosphorylation and intracellular calcium homeostasis. These disruptions collectively impair neuronal membrane potential, impede nerve impulse transmission, and ultimately compromise synaptic communication, thereby contributing to the observed neurotoxic effects. In developmental contexts, Acetamiprid exposure reduces neuronal cell number, alters gene transcription related to the cell cycle, and promotes neuronal apoptosis **(Gasmi, 2018; Jeschke et al., 2011; Ramirez-Candoa lenin J, 2023)**. Collectively, these mechanisms suggest that Acetamiprid contributes to neural oxidative damage not only through mitochondrial pathways but also via direct interference with neurotransmission and neurodevelopment, coupled with decreased antioxidant and enzyme activities.

In contrast, *Juniperus phoenicea*, a plant species rich in various antioxidant compounds, has demonstrated potential neuroprotective effects. The compounds present in Juniperus phoenicea, as documented by **Bouassida et al. (2018) and Chelouati et al. (2023)**, may act to counteract the acetamiprid-induced oxidative stress and the associated disruptions in GST, GSH, and catalase levels so the phytochemicals present in *Juniperus*, including phenolic compounds, exhibit strong antioxidant properties. Studies have shown that extracts from *Juniperus* can restore antioxidant enzyme activities and mitigate oxidative damage in neural tissues due to its variety of essential trace elements, including zinc, selenium, magnesium, and iron **(Nedjimi et al., 2015)**. These minerals may contribute synergistically to the plant’s antioxidant and neuroprotective effects, acting as cofactors in enzymatic defenses against oxidative stress.

Furthermore, given the findings of **Xu et al. (2022)** on mitochondrial damage, it is plausible that *Juniperus phoenicea* may also exert protective effects at the mitochondrial level, potentially preserving mitochondrial function and neuronal survival.

6.3. Treatment effect on rat behavior

6.3.1. The results of elevated plus maze (EPM) test

In the Elevated Plus Maze (EPM) test, the control group exhibited typical exploratory behavior, while JNP treatment alone produced no significant changes. ACE treatment significantly increased anxiety-like behavior, as indicated by reduced time in the center and open arms and increased time in the closed arms. Co-administration of JNP with ACE partially attenuated these effects, with most behavioral parameters returning to control levels, except for the time spent in the distal open arms, which remained significantly decreased. These findings suggest that JNP may exert a protective effect against ACE-induced anxiety-like behavior.

Table 09: Variation in parameters linked to the Elevated Plus Maze test in control rats and those subjected to different treatments for 30 days.

Settings	C	JNP	ACE	ACE+JNV
TSC (s)	41.00±4.47	33.86±8.19	27.71±5.19**	38.14±9.96
TSCA (s)	196.86±7.99	186.43±6.87	243.71±5.50***	192.57±9.69
TSCA/P (s)	108.71±5.31	98.57±6.71	126.71±6.37***	105.86±11.78
TSCA/D (s)	87.43±5.32	86.14±2.63	116.71±4.42***	89.86±5.51
TSOA (s)	66.71±13.02	89.43±9.47	30.86±8.28***	60.29±12.37*
TSOA/P (s)	36.14±4.29	46.86±5.98	25.14±3.34***	39.29±4.68
TSOA/D (s)	30.57±6.76	42.29±4.82	5.71±2.29***	21.00±2.71***
N adj	13.86±2.94	14.14±2.27	8.14±2.61**	11.43±1.40*
NEOA	5.71±2.62	6.14±3.08	2.57±1.27**	4.57±2.37
NECA	9.57±2.44	8.43±3.09	13.43±2.64*	9.29±2.69
***(P< 0.05) **(P< 0.001) ***(P< 0.001): significant difference compared to control (C).**				

Our results of the Elevated Plus Maze (EPM) test demonstrate that Acetamiprid exposure induces significant anxiety-like behavior in rats, as evidenced by a substantial decrease in the time spent in the open arms and center, and a marked increase in time spent in the closed arms. This behavioral shift is a classic indicator of anxiety **(Carobrez & Bertoglio, 2005).**

Mechanistically, the anxiogenic effect of ACE appears to be mediated through several neurotoxic pathways. While its primary mechanism of action involves targeting insect nicotinic acetylcholine receptors (nAChRs), acetamiprid also interacts with mammalian nAChRs, and despite exhibiting lower affinity, the diversity and widespread distribution of these subtypes suggest a potential contribution to neurological and behavioral disturbances. However, the neurotoxic effects of neonicotinoids extend beyond nAChR activation, encompassing more complex neurophysiological impacts **(Xu et al., 2022)**. This aligns with findings by **Arafa et al. (2024)**, who observed that ACE induces oxidative stress and inflammation in human epithelial cells, processes known to affect central nervous system (CNS) function when extrapolated in vivo.

Moreover, **Gasmi et al. (2017)** provided direct evidence that chronic low-dose ACE exposure compromises brain mitochondrial integrity and respiratory function. In neurons, such mitochondrial dysfunction can disrupt energy metabolism, interfere with neurotransmitter synthesis, and increase oxidative stress factors closely linked to anxiety-like behaviors. These mitochondrial alterations align with the behavioral changes observed in our study.

Complementing these findings, a recent study by **Hirai et al. (2024)** demonstrated that ACE exposure in mice elevated hippocampal serotonin levels and increased activity in the raphe nuclei, further implicating dysregulated serotonergic signaling in ACE-induced anxiety.

Interestingly, co-administration of JNP decoction with ACE appeared to partially mitigate these effects. Although the time spent in open arms remained significantly lower than in controls, other EPM parameters were not significantly different, suggesting a potential anxiolytic or neuroprotective action of JNP. This effect may be attributed to the antioxidant and anti-inflammatory properties of bioactive compounds in *Juniperus phoenicea* linked to its rich content of bioactive phytochemicals, such as flavonoids and terpenoids, which possess strong antioxidant and anti-inflammatory activities **(Zubi et al., 2025)**.These compounds likely contribute to the attenuation of oxidative stress and neuronal damage induced by acetamiprid, thus helping to restore more normal exploratory behavior in the EPM test which can counteract oxidative stress and support neurotransmitter balance.

6.3.2. The results of open field test

The ACE group exhibited significant alterations across all measured behavioral parameters in comparison to the C group, indicative of anxiety-like and hypoactive behavior, as evidenced by a significant decrease in total distance traveled (TDT, $p < 0.05$), demonstrating reduced locomotor activity; a significant elevation in time spent immobile (TI, $p < 0.001$), reflecting increased freezing behavior; a significant increase in time spent in the peripheral part (TPP, $p < 0.001$), suggestive of thigmotaxis; a sharp decrease in time spent in the central part (TCP, $p < 0.001$), indicating diminished exploratory behavior and heightened anxiety and a significant reduction in the number of adjustments (Nbr Adj, $p < 0.001$), suggestive of decreased reactivity or adaptability. In contrast, JNP group did not differ significantly from the control group in the majority of measures, displaying a slight enhancement in locomotion (higher TDT) and a reduction in immobility (lower TI), potentially indicative of mild stimulant or anxiolytic effects; finally, the ACE+JNP co-treatment group demonstrated a clear attenuation of the effects induced by ACE treatment, evidenced by improved TDT, TCP, and Nbr Adj values, a marked reducer in TI than the ACE group alone, and a trend towards normalization, with values approximating those of the control group for most parameters, although not fully restored.

Table 10: Variation in parameters linked to the Open Field test in control rats and those subjected to different treatments for 30 days.

Settings	C	JNP	ACE	ACE+JNV
TDT	1154.29±119.54	1221.43±90.63	1000.00±138.44*	1040.00±139.70
TI	92.43±10.87	54.57±12.97***	198.57±33.25***	87.14±14.61
TPP	269.43±14.73	274.86±9.44	322.86±4.57***	277.14±10.30
TCP	24.86±3.63	20.86±7.07	8.57±3.82***	20.00±4.43
Nbr Adj	26.71±6.34	20.43±5.29	13.14±3.43***	15.29±3.64***
***(P< 0.05) ***(P< 0.001): significant difference compared to control (C).**				

The Open Field Test (OFT) was employed to assess locomotor activity and anxiety-like behavior in rats following exposure to Acetamiprid ACE, *Juniperus phoenicea* JNP, and their combination. The OFT is widely recognized for its utility in measuring exploratory behavior and anxiety in rodents, with a preference for the periphery (thigmotaxis) being indicative of heightened anxiety **(Walsh & Cummins, 1976; Prut & Belzung, 2003)**.

Rats treated with ACE exhibited significant alterations in open field behavior compared to controls, characterized by reduced time spent in the center, lower numbers of entries into the center zone, and decreased overall locomotor activity. These findings suggest increased anxiety-like behavior and possible psychomotor retardation. The anxiety-inducing effect of ACE aligns with prior studies reporting that neonicotinoid exposure can disturb neurotransmitter balance, particularly impacting the glutamatergic systems **(Kimura-Kuroda et al., 2012; Shamsi et al., 2021)**. The reduction in exploratory behavior may be linked to oxidative stress and neuroinflammation induced by ACE, as shown in studies involving mitochondrial dysfunction and hippocampal damage **(Gasmi et al., 2017; Shamsi et al., 2018).**

Interestingly, co-administration of JNP with ACE appeared to partially reverse these behavioral changes. Rats in the ACE+JNP group showed improved exploratory activity and slightly increased center time compared to the ACE only group, though not fully restored to control levels. This ameliorative effect is potentially due to the antioxidant and neuroprotective properties of JNP, as demonstrated in studies highlighting the plant's rich content of phenolic compounds and essential oils with free radical-scavenging abilities **(Chelouati et al., 2023; Zubi et al., 2025)**. These bioactive compounds may help counteract ACE-induced oxidative damage and inflammation in the brain and as demonstrated by **Lafraxo et al. (2024)**, *J. phoenicea* hydroethanolic extracts significantly inhibited inflammation in vivo. This effect is likely mediated by active constituents such as gallic acid, ferulic acid, and linalool, which are known to reduce pro-inflammatory cytokine production and modulate immune cell activity.

In addition to its antioxidant and neuroprotective properties, *Juniperus phoenicea* has been reported to possess hypoglycemic activity, which may contribute to improved energy availability and neural function. This could partially explain the reduction in immobility time observed in the OFT, as adequate glucose metabolism is essential for sustaining motor behavior and adaptive responses **(Sánchez de Medina et al., 1993).**

ACE exposure induced anxiety-like and hypoactive behaviors in the OFT, likely through oxidative stress, neurotransmitter disruption, and mitochondrial impairment. JNP showed promise in attenuating these effects, possibly due to its antioxidant properties. Further research is warranted to clarify the molecular mechanisms underlying these interactions and to explore the therapeutic potential of JNP in pesticide-induced neurotoxicity.

6.3.3. The results of forced swimming test

The ACE group exhibited a significant increase in immobility time (IT; 70.57 ± 10.44 sec, $P < 0.05$) and a significant decrease in swimming time (ST; 188.14 ± 18.27 sec, $P < 0.001$) compared to the C group (C; IT: 57.00 ± 6.61 sec; ST: 203.86 ± 12.40 sec). These findings are indicative of increased despair-like behavior and reduced active coping strategies in response to acetamiprid exposure. In contrast, the JNP group, while showing no significant difference in IT or ST from the control, displayed a significant increase in climbing time (CT; 66.00 ± 7.21 sec, $P < 0.001$) compared to the control (CT: 39.14 ± 8.95 sec), potentially suggesting a mild anxiolytic or stimulant-like effect of *Juniperus* alone. Importantly, co-administration of ACE+JNP group largely attenuated the Acetamiprid-induced behavioral alterations; immobility time (52.00 ± 8.20 sec) and swimming time (203.29 ± 6.43 sec) in this group were comparable to control levels, indicating a protective effect of *Juniperus* better than acetamiprid-induced behavioral despair. Additionally, climbing time in the ACE+JNP group (49.86 ± 4.07 sec) showed a significant increasing than the ACE group ($P < 0.05$), further supporting the anxiolytic-like effects of *Juniperus*.

Table 11: The table presents the variation in parameters linked to the Forced Swimming Test in control rats and those subjected to different treatments for 30 days.

Settings	C	JNP	ACE	ACE+JNP
Immobility time	57.00±6.61	49.57±11.12	70.57±10.44*	52.00±8.20
Swimming time	203.86±12.40	193.00±14.32	188.14±18.27**	203.29±6.43
Climbing time	39.14±8.95	66.00±7.21**	37.43±4.84	49.86±4.07*
***(P< 0.05) **(P< 0.001) ***(P< 0.001): significant difference compared to control (C).**				

The Forced Swimming Test (FST) is a widely used behavioral paradigm for assessing depressive-like states in rodents, based on the animal's response to an inescapable stressor **(Porsolt et al., 1977)**. In this test, increased immobility is interpreted as a sign of behavioral despair, while increased swimming or climbing indicates active coping or antidepressant-like responses. In our study, rats exposed to Acetamiprid exhibited a marked increase in immobility time, alongside reduced swimming and climbing behavior, indicating a shift toward behavioral despair and a depressive-like phenotype.

The reduced exploratory activity and increased immobility observed in FST may be attributed to the disruption of cholinergic signaling by Acetamiprid. According to **Gasmi (2018)**, chronic exposure to acetamiprid leads to excessive accumulation of acetylcholine and subsequent desensitization of cholinergic receptors, as well as inhibition of Na^+/K^+-ATPase. These changes likely impair neuronal excitability and motor coordination, contributing to the observed hypoactivity.

In addition to oxidative damage, Acetamiprid-induced neuroinflammation may contribute to the depressive-like behaviors observed in the FST. Oxidative stress in neuronal tissue is known to activate microglia and astrocytes, triggering the release of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 according to **Costas-Ferreira and Faro (2021)**, This inflammatory response is primarily mediated through activation of the NF-κB signaling pathway and contributes to neuronal dysfunction and cell death. Inflammation also disrupts synaptic plasticity and affects neurotransmitter balance, further exacerbating behavioral abnormalities such as depressive-like symptoms. These cytokines can impair neurogenesis, synaptic plasticity, and neurotransmitter signaling particularly in the hippocampus and prefrontal cortex regions critically involved in mood regulation and behavioral adaptation **(Gasmi et al., 2018; Costas-Ferreira & Faro, 2021).**

Notably, co-treatment with *Juniperus phoenicea* decoction significantly attenuated these behavioral effects, suggesting a neuroprotective role potentially mediated by anti-inflammatory action. The plant's essential oils and phenolic compounds have been shown to inhibit inflammatory cytokine production, suppress NF-κB signaling, and modulate glial activity **(Chelouati et al., 2023; Zubi et al., 2025)**. These effects may help preserve synaptic integrity and neurochemical balance, contributing to the reduced immobility and improved behavioral outcomes observed in the ACE+JNP group.

CONCLUSION AND PERSPECTIVES

CONCLUTION AND PERSPECTIVES

In conclusion, our findings of this research underscore the significant impact of Acetamiprid on the physiological, biochemical, and behavioral status of the Wistar rats. Exposure to Acetamiprid led to a marked decrease in body and brain weights, accompanied by disruptions in the brain's antioxidant defense system specifically reflected by decreased levels of glutathione (GSH) and glutathione S-transferase (GST). These biochemical imbalances are strong indicators of oxidative stress, which likely plays a central role in triggering the observed behavioral disturbances. Indeed, the oxidative and systemic stress induced by Acetamiprid appears to be directly linked to the emergence of anxiety- and depressive-like behaviors, as demonstrated by altered performance in the Elevated Plus Maze (EPM), Open Field Test (OFT), and Forced Swimming Test (FST). Such behavioral changes reflect neurochemical imbalances and neuronal dysfunction likely stemming from acetamiprid's neurotoxic effects, particularly its impact on the cholinergic system, glutamate, and serotonin systems.

Conversely, the co-administration of *Juniperus phoenicea L.* extract exhibited a protective effect. The extract partially restored antioxidant balance, mitigating oxidative stress and leading to noticeable improvements in behavioral responses across all tests. These findings support our hypothesis that *Juniperus phoenicea L.* possesses potent antioxidant and neuroprotective properties, likely due to its rich content of bioactive phytochemicals.

Based on these results, we propose several future research directions by conduct a detailed phytochemical analysis of *Juniperus phoenicea L.* using Gas Chromatography-Mass Spectrometry (GC-MS) to identify and quantify its active constituents; perform histological examinations of brain tissues to better understand cellular and structural damage caused by acetamiprid and the restorative effects of *Juniperus phoenicea L.* treatment; undertake long-term exposure studies to evaluate the chronic toxicity of acetamiprid and determine whether compensatory mechanisms arise over time; promote eco-friendly pest management solutions by developing phytosanitary products based on essential oils and plant extracts as safer, sustainable alternatives to synthetic pesticides.

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