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M^m^. **CHORFI Khouloud** & M^m^. **CHETTOUH Zoubida**

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Association between exposure to pesticide and disorder on hematological parameters and endocrine function. Possible ameliorative effects of medicinal plants extract

Presenting to the jury:

Pr. **BOUABIDA Hayette**	Lecturer A	Tebessa University	**President**
Dr. **GUEDRI Kamilia**	Lecturer A	Tebessa University	**Examiner**
Dr. **MESSAADIA Amira**	Lecturer B	Tebessa University	**Promoter**

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- **KHOULOUD**

ٱلْحَمْدُ للهِ حُبًّا وشُكْرًا وٱمْتِنَانًا عَلَى ٱلْبِدَايَةِ وَٱلْخِتَامِ

بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيم

{وَآخِرُ دَعْوَاهُمْ أَنِ ٱلْحَمْدُ لِلَّهِ رَبِّ ٱلْعَٰلَمِين}

صَدَقَ ٱللَّهُ ٱلْعَظِيم

Praise be to God, who has brought me to this day where I stand on the threshold of my graduation, reaping the fruits of my labor and raising my hat with pride.
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Abstract

Acetamiprid, a neonicotinoid insecticide, is widely employed in agricultural pest management due to its high efficacy in targeting insect nervous systems. However, both acute and chronic exposure to acetamiprid may exert adverse effects beyond target pests, potentially impairing the immune and endocrine systems in non-target organisms, including mammals.

This study investigates the protective potential of *Juniperus phoenicea* aqueous extract against acetamiprid-induced toxicity. We assessed the impact of acetamiprid exposure on immune function, hormonal balance, and antioxidant defense mechanisms, alongside the ameliorative effects of juniper extract.

28 female rats were randomly divided into four groups (n=7 each) and administered daily oral doses of acetamiprid 20 mg/kg/day, *Juniperus phoenicea* extract 200 mg/kg/day, a combination of both, or vehicle control for 30 days via gavage.

Results demonstrated that acetamiprid exposure induced significant toxicity characterized by hyperglycemia, marked body weight loss, and hematological alterations . Endocrine analysis revealed significant decreases in circulating estrogen and progesterone levels, indicating hormonal disruption. Furthermore, oxidative stress markers in ovarian and adrenal tissues were altered, as evidenced by diminished glutathione S-transferase (GST) activity, reduced reduced glutathione (GSH) levels, and elevated catalase (CAT) enzyme activity, it adversely affects the morphology of the ovaries and the function of the endocrine glands. Co-administration of *Juniperus phoenicea* extract significantly ameliorated these biochemical and physiological disturbances, restoring hematological parameters, hormonal levels, and antioxidant enzyme activities toward normal ranges. The protective effects of juniper are likely mediated by its bioactive phytochemicals exhibiting potent antioxidant and anti-inflammatory properties.

These findings underscore the potential of *Juniperus phoenicea* as a natural therapeutic agent to mitigate pesticide-induced toxicity and oxidative damage.

Keywords: Acetamiprid, *Juniperus phoenicea* , Oxidative stress, Antioxidant defense, Anti-inflammatory , Immune system, Endocrine system .

Résumé

L'acétamipride, un insecticide néonicotinoïde, est largement utilisé dans la lutte agricole contre les ravageurs en raison de son efficacité élevée ciblant le système nerveux des insectes. Cependant, une exposition aiguë ou chronique à l'acétamipride peut entraîner des effets indésirables au-delà des organismes cibles, compromettant potentiellement les systèmes immunitaire et endocrinien chez des organismes non ciblés, y compris les mammifères.

Cette étude examine le potentiel protecteur de l'extrait aqueux de *Juniperus phoenicea* contre la toxicité induite par l'acétamipride. Nous avons évalué l'impact de l'exposition à l'acétamipride sur la fonction immunitaire, l'équilibre hormonal et les mécanismes de défense antioxydants, ainsi que les effets bénéfiques de l'extrait de genévrier.

Vingt-huit rates femelles ont été réparties aléatoirement en quatre groupes (n=7 chacune) et ont reçu quotidiennement par gavage des doses orales d'acétamipride (20 mg/kg/jour), d'extrait de *Juniperus phoenicea* (200 mg/kg/jour), une combinaison des deux, ou un véhicule témoin, pendant 30 jours.

Les résultats ont montré que l'exposition à l'acétamipride a induit une toxicité significative, caractérisée par une hyperglycémie, une perte de poids corporelle marquée et des altérations hématologiques. L'analyse endocrinienne a révélé une diminution significative des concentrations d'œstrogènes et de progestérone circulants, indiquant un dérèglement hormonal. De plus, les marqueurs de stress oxydatif dans les tissus ovariens et surrénaliens ont été altérés, comme en témoignent la diminution de l'activité de la glutathion S-transférase (GST), la réduction des niveaux de glutathion réduit (GSH), et l'augmentation de l'activité de la catalase (CAT). Il affecte négativement la morphologie des ovaires ainsi que la fonction des glandes endocrines.

L'administration concomitante de l'extrait de *Juniperus phoenicea* a significativement atténué ces perturbations biochimiques et physiologiques, rétablissant les paramètres hématologiques, les niveaux hormonaux et les activités des enzymes antioxydantes vers des valeurs normales. Les effets protecteurs du genévrier sont probablement médiés par ses composés phytochimiques bioactifs présentant de puissantes propriétés antioxydantes et anti-inflammatoires.

Ces résultats mettent en évidence le potentiel de *Juniperus phoenicea* en tant qu'agent thérapeutique naturel pour atténuer la toxicité et les dommages oxydatifs induits par les pesticides.

Mots-clés : Acétamipride, Juniperus phoenicea, Stress oxydatif, Défense antioxydante, Anti-inflammatoire, Système immunitaire, Système endocrinien.

ملخص

يُعد الأسيتميبريد، وهو مبيد حشري من فئة النيونيكوتينويدات، من المواد واسعة الاستخدام في مكافحة الآفات الزراعية، وذلك بفضل فعاليته العالية في استهداف الجهاز العصبي للحشرات. غير أن التعرض الحاد والمزمن للأسيتميبريد قد يؤدي إلى آثار سلبية تتجاوز الكائنات المستهدفة، مما قد يُلحق ضررًا بوظائف الجهازين المناعي والهرموني لدى الكائنات غير المستهدفة، بما في ذلك الثدييات.

تهدف هذه الدراسة إلى تقصي الدور الوقائي المحتمل لمستخلص نبات العرعر الفينيقي *(Juniperus phoenicea)* المائي ضد السمية الناتجة عن التعرض للأسيتميبريد. وقد قمنا بتقييم تأثير التعرض للأسيتميبريد على وظائف الجهاز المناعي، والتوازن الهرموني، وآليات الدفاع المضادة للأكسدة، إلى جانب دراسة التأثيرات المخففة لمستخلص العرعر.

تم توزيع 28 أنثى من الجرذان عشوائيًا إلى أربعة مجموعات (n=7) في كل مجموعة(، وتلقّت جرعات فموية يومية عبر التزقيم لمدة 30 يومًا، شملت: أسيتميبريد (20 ملغ/كلغ/يوم)، مستخلص العرعر (200 ملغ/كلغ/يوم)، مزيجًا من المادتين، أو محلولًا شاهدًا (مركبة).

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الكلمات المفتاحية :الأسيتميبريد، العرعر الفينيقي، الإجهاد التأكسدي، الدفاعات المضادة للأكسدة، مضاد للالتهاب، الجهاز المناعي، الجهاز الغدي الصمّ

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Abbreviation list

ROS	Reactive Oxygen Species (implied by oxidative stress)
CNS	Central Nervous System (implied by neuroprotective effects).
nAChRs	Nicotinic Acetylcholine Receptors
J.phoenice	*Juniperus phoenicea*.
DNA	Deoxyribonucleic Acid
OECD	Organisation for Economic Co-operation and Development
EPA	Environmental Protection Agency.
α4β2	Alpha4 Beta2 Nicotinic Acetylcholine Receptor Subtype.
HPGAxis	Hypothalamic-Pituitary-Gonadal Axis.
C	Control Group.
ACE	Acetamiprid-treated Group.
ACE+JNP	Combined Acetamiprid + Juniperus phoenicea Group.
EDTA	Ethylenediaminetetraacetic Acid
CBC	Complete Blood Count
E2	Estrogen
P4	Progesteron
ABEI	Acridinium Ester Brilliant I (conjugate)
ELISA	Enzyme-Linked Immunosorbent Assay
GST	Glutathione S-transferase
CDNB	1-chloro-2,4-dinitrobenzene
GSH	Glutathion.
	Hydrochloric Acid.
	Catalase

HCl

CAT

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Introduction

Introduction

Chemically, insecticides are categorized into several classes, including neonicotinoids (NEOs), carbamates, organochlorines, and pyrethroids, with additional groups such as spinosyns and benzolureas. Among these, neonicotinoids are the most widely utilized, particularly in agricultural and urban settings (Fenibo et al., 2021). However, exposure to NEOs has been associated with a range of adverse health effects, both acute and chronic, including liver diseases, neurological developmental disorders, genotoxic and carcinogenic effects, and reproductive health disruptions (Thompson et al., 2020). Unlike other pesticides, neonicotinoids act rapidly at low doses and are readily absorbed by plants (Kundo t al., 2018). Due to their pervasive environmental presence, they pose significant risks to both human health and ecological balance, negatively impacting ecosystems and disrupting reproductive health, particularly in females (Xu et al., 2021; Liu et al., 2021). Acetamiprid, a newer-generation neonicotinoid, is considered a safer alternative due to its relatively lower toxicity. Despite its intended specificity for nicotinic acetylcholine receptors in insects, the widespread application of acetamiprid has led to harmful effects on non-target organisms, including mammals. At certain doses, neonicotinoids have been linked to oxidative stress in the testes, disruption of spermatogenesis, sperm cell damage, endocrine dysfunction, and impairment of Sertoli and Leydig cell function. In females, acetamiprid induces metabolic alterations in the ovaries and pathomorphological changes in follicles (Lucia et al., 2023).In response to the growing concerns regarding pesticide toxicity, modern medicine has increasingly focused on plant-based solutions, recognizing their potential to mitigate pesticide-induced damage. *Juniperus phoenicea,* commonly known as red juniper, is a prominent plant found in arid and semi-arid regions of the Northern Hemisphere. Native to North Africa, it belongs to the Cupressaceae family and is the second most diverse conifer genus, with over 67 species and 28 variants. Traditionally, the leaves of Juniperus phoenicea have been utilized in decoctions to treat conditions such as diarrhea and rheumatism, underscoring its medicinal significance (Ibrahim Tumen et al., 2012).Scientific studies have demonstrated that Juniperus phoenicea exhibits a wide array of biological activities, including antioxidant properties that help prevent redox damage (Miceli et al., 2020), antimicrobial effects (Tavian et al., 2013), anti-inflammatory actions (Lesjak et al., 2013), anticancer potential (Ben Mrid et al., 2019), diabetes management (Orhan et al., 2012), and neuroprotective benefits (Tavares et al., 2012).

The objective of this study is to assess the impact of the insecticide acetamiprid on the immune and endocrine systems in rats to determine its potential health risks to humans. Additionally, this research aims to explore the potential therapeutic benefits of *Juniperus phoenicea* in mitigating the adverse effects associated with acetamiprid exposure.

1. Neonicotinoids

1.1 Definition and role in agriculture

Neonicotinoids (NEOs) represent a significant class of synthetic insecticides that emerged in the 1990s. They operate by specifically targeting nicotinic acetylcholine receptors, which enables them to induce neurotoxic effects in a distinct manner. These compounds have gradually replaced older insecticides, such as carbamates and organophosphates, due to their superior properties. NEOs are widely utilized because of their broad-spectrum insecticidal activity, low toxicity to mammals, high efficacy, and selective mode of action (Zixiong et al., 2024).

In the agricultural sector, neonicotinoids are preferred over other pesticide classes for several reasons, including:

1. **Versatility in application**: NEOs can be applied through various methods, such as seed treatments, injections, or sprays.
2. **Broad-spectrum insecticidal activity**: They exhibit efficacy against a wide range of insect pests.
3. **Low acute toxicity to non-target aquatic and terrestrial organisms**: NEOs are less toxic to species that are not the intended targets.
4. **High potency in targeting insect pests**: NEOs are highly effective at low doses in controlling insect populations (Thompson et al., 2020).

1.2 Historical usage of Neonicotinoids

Since their introduction in the 1990s, neonicotinoid pesticides have rapidly gained popularity for use in both urban and agricultural environments, not only in the United States but also internationally (Thompson et al., 2020). These insecticides have become the preferred choice due to their high efficacy, selective mode of action, and relatively low toxicity to mammals compared to older pesticide classes. In Algeria, acetamiprid, a prominent neonicotinoid, is extensively used in agriculture to control a wide variety of pests. It is present in nearly 70% of the commercially approved neonicotinoid pesticide formulations within the country, with acetamiprid specifically accounting for 19 of these formulations (Bettiche et al., 2023).

Neonicotinoids can be applied through various methods, including seed treatments, soil drenches, chemigation, tree injections, and foliar applications. These methods involve either aerial or ground spray equipment, offering flexibility in pest control approaches (Anderson et al., 2015).

1.3 Structure of Neonicotinoids

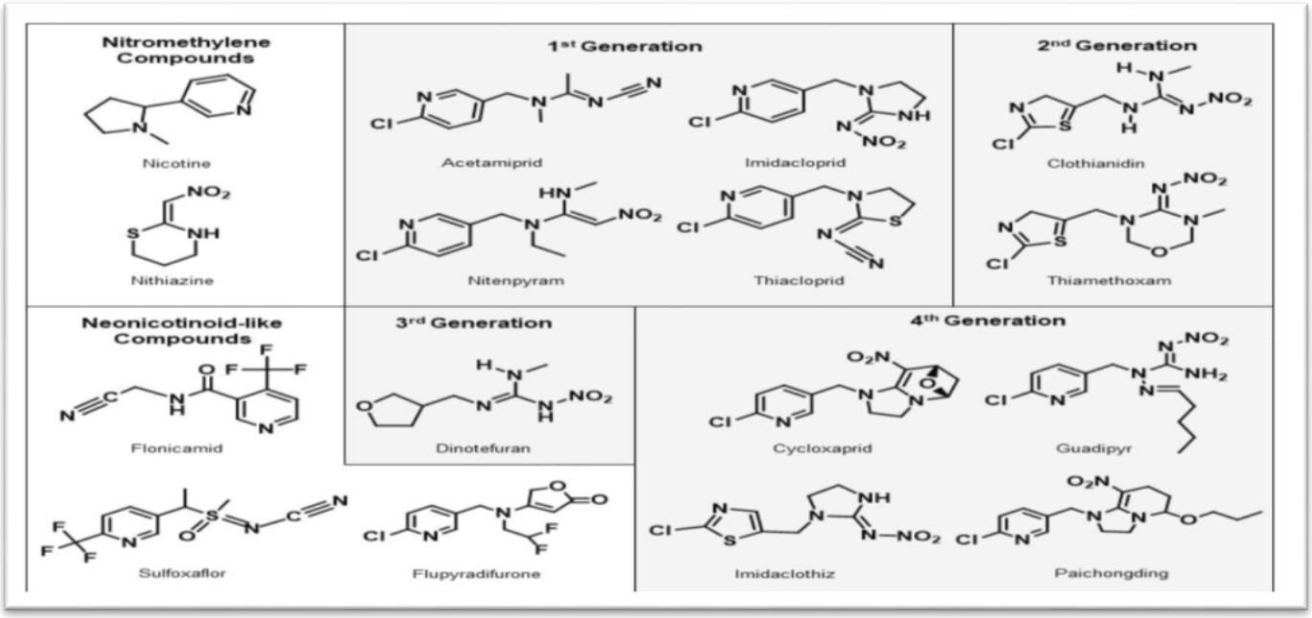


Figure 01: Chemical structures of common neonicotinoids and related compounds (Lucia et al., 2023)

1.4 Environmental exposure to Neonicotinoids water, soil and air

Neonicotinoids (NEOs) were once believed to have a short biological half-life in the environment. However, recent studies have shown that they can persist in the environment for extended periods, up to 19 years (Costas-Ferreira et al., 2021). This prolonged environmental persistence is a concern as only approximately 10% of the applied NEOs actually reach the target organisms. The remaining 90% is released into the environment, where it can accumulate in the soil, evaporate as dust during planting, or migrate into surface and/or groundwater (Thompson et al., 2020). This widespread dispersion poses significant risks to non-target organisms and entire ecosystems (Lin et al., 2020). Neonicotinoids are highly water-soluble, and their presence in surface and groundwater has been documented globally (Thompson et al., 2020). When dissolved in water at pH 7 and 20°C, they can reach concentrations ranging from 184 to 590.0 mg/L. This solubility enables NEOs to be absorbed and distributed throughout the plant system, where they provide insect protection (Ellis et al., 2017).In addition to their mobility in water, tillage and wind contribute to the emission of neonicotinoids as suspended particles in the air, further increasing the potential for environmental contamination (Thompson et al., 2020). These factors highlight the widespread and lasting environmental exposure to neonicotinoids, raising concerns about their impact on ecosystems and non-target species .

Table 01: Solubility and half-life of neonicotinoids (Ellis et al., 2017)

Neonicotinoid	Half-Life (DT50) in Soil (Days)	Solubility in Water at 20ºC, pH =7 (mg/L)
Acetamiprid	31-450	2,950
Clothianidin	148 – 6,931	340
Dinotefuran	75-82	39,830
Imidacloprid	100 – 1,250	610
Nitenpyram	8 days	590,000
Thiacloprid	3.4–1,000 days	184
Thiamethoxam	7-335 days	4,100

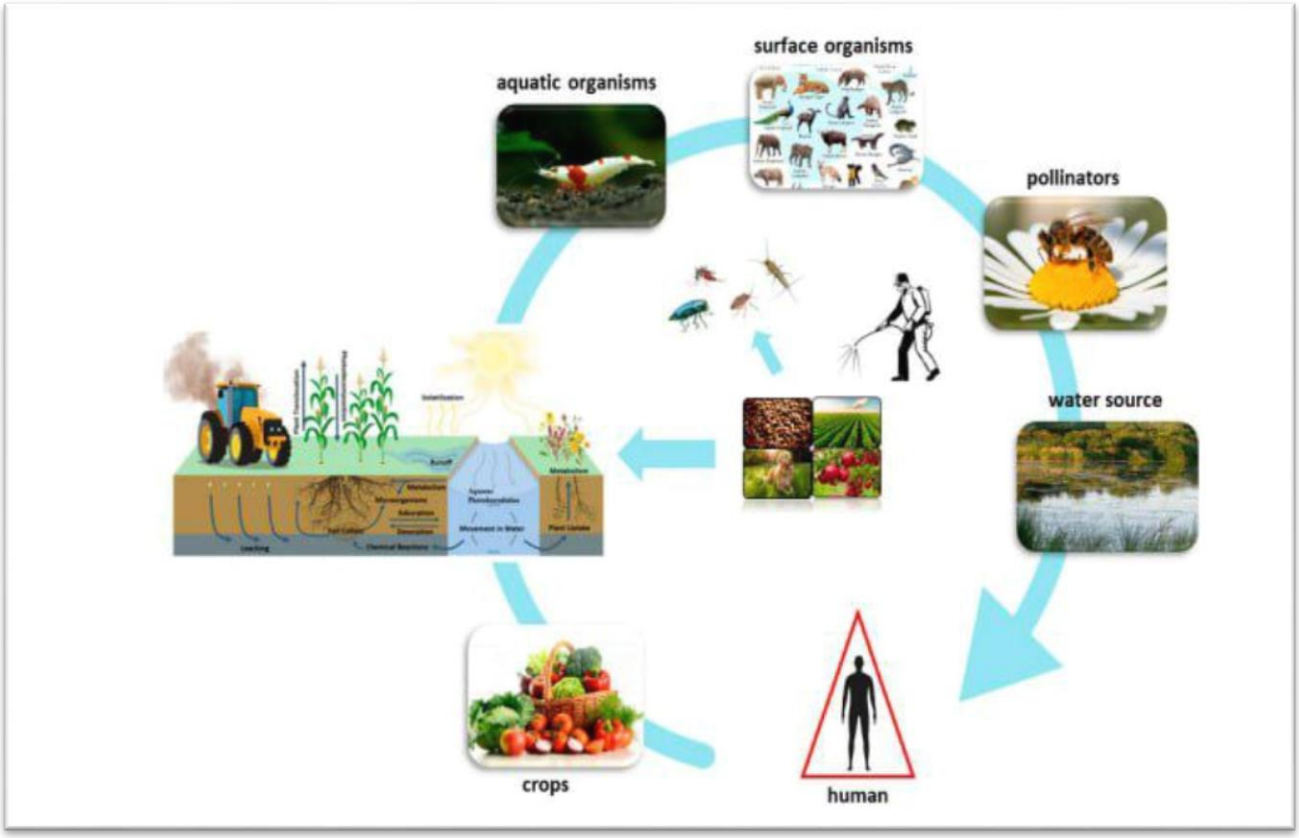


Figure 02: The environmental fate and routes of neonicotinoid pesticide exposure after application (Thompson et al., 2020)

1.5 Classification of Neonicotinoids

Similar to other classes of pesticides, neonicotinoids (NEOs) can be categorized into several chemical subclasses based on their molecular structures. They are primarily classified as hydroheterocyclic guanidines and amidines with functional substituents (Lucia et al., 2023). There are seven

commercially available neonicotinoids: Nitenpyram, Clothianidin, Dinotefuran, Acetamiprid, Thiacloprid, Thiamethoxam, and Imidacloprid. These compounds are grouped into three generations:

- **First-generation (chloropyridinyl) chemicals**: This group includes Thiacloprid, Imidacloprid, Nitenpyram, and Acetamiprid.
- **Second-generation (chlorothiazolyl) chemicals**: This category includes Thiamethoxam and Clothianidin.
- **Third-generation (tetrahydrofuryl) compounds**: An example of this group is Dinotefuran (Wang et al., 2004).

1.6 Occupational and dietary exposure pathways of Neonicotinoid

Agricultural workers, particularly those handling and applying neonicotinoid-treated seeds, are at risk of occupational exposure through dermal contact and inhalation (LaDuca et al., 2018). The U.S. Environmental Protection Agency (EPA) classifies neonicotinoids as having low acute toxicity when applied topically or inhaled (Toxicity Category III) and moderate toxicity when ingested orally (Toxicity Category II). They do not cause skin or eye irritation or dermal sensitization (Christina et al., 2017). Dietary exposure can occur through the consumption of processed foods or contaminated drinking water, posing additional risks to human health (Christina et al., 2017).

1.7 Effects of Neonicotinoid pesticide exposure on human health

Neonicotinoids have been shown to affect mammalian nicotinic acetylcholine receptors (nAChRs) in a manner similar to nicotine (Kimura et al., 2012). These pesticides have a high affinity for the α4β2 nAChR subtype, which is densely concentrated in the thalamus (Chen et al., 2014; Li et al., 2011). Alterations in the density of this neuroreceptor subtype have been associated with various central nervous system disorders, including Alzheimer's disease, Parkinson's disease, schizophrenia, and depression. In the developing brain, α4β2 nAChRs are involved in crucial processes such as neural proliferation, apoptosis, migration, differentiation, synapse formation, and neural circuit development (Chen et al., 2014).Research has also revealed adverse effects on mammalian development and reproduction, including reduced sperm function and production, lower pregnancy rates, increased embryo mortality, stillbirths, premature births, and decreased offspring weight (Abou-Donia et al., 2008; Gibbons et al., 2015; Gu et al., 2013).Acetamiprid, a neonicotinoid, is a potent agonist of the nicotinic acetylcholine receptor, leading to neuromuscular paralysis and potentially fatal outcomes. Ingestion of larger amounts can result in severe symptoms such as nausea, vomiting, respiratory failure, tachycardia, hypotension, and dry mouth. Acute poisoning with acetamiprid may also lead to muscle weakness, hypothermia, convulsions, and significant changes in the electrocardiogram (Selladurai et al., 2021).Chronic exposure to acetamiprid has been linked to

disruptions in hormone production and gene expression within the hypothalamic-pituitary-gonadal axis, potentially leading to reproductive failure. These findings highlight the potential endocrine-disrupting effects of acetamiprid (Rodriguez-Paar et Jocelyn 2022).

1.8 Systems affected by Neonicotinoid and their impact on oxidative stress

Neonicotinoids have the potential to induce oxidative stress, leading to the production of reactive oxygen species (ROS) and subsequent harmful effects. Exposure to neonicotinoids can trigger oxidative stress, which may enhance antioxidant defenses while simultaneously damaging essential macromolecules within cells, including proteins, lipids, and DNA. This oxidative damage can result in both necrotic and apoptotic cell death. During this process, protein degradation, increased lipid peroxidation, and DNA damage are potential outcomes (Xu Wang et al., 2017).

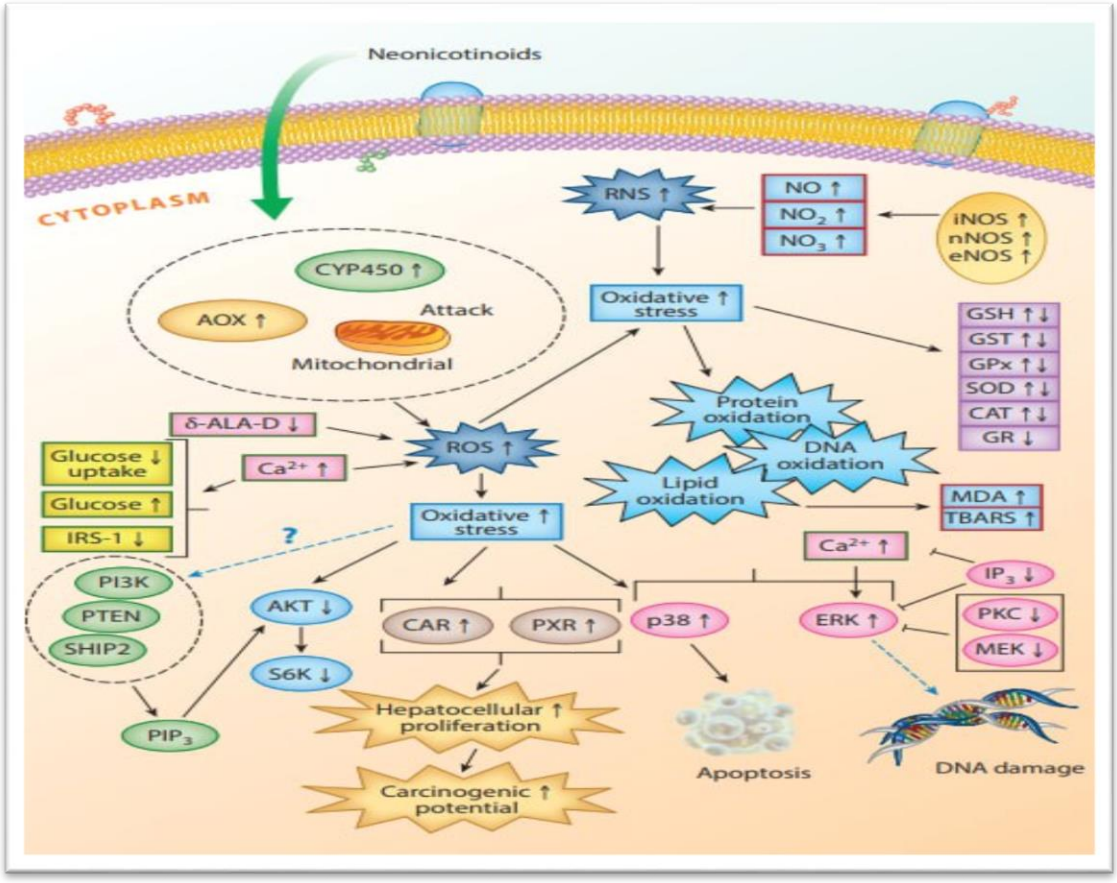


Figure 03: Oxidative stress–mediated mode of action for neonicotinoids (Xu Wang et al., 2017)

2. *Juniperus phoenicea*

2.1 Role of antioxidants in counteracting oxidative stress

Antioxidants play a crucial role in mitigating oxidative stress by neutralizing free radicals and preventing their harmful effects and excessive accumulation. These compounds are essential in protecting against diseases associated with oxidative damage. Numerous studies have highlighted the protective value of plant-derived antioxidants in preventing degenerative diseases, such as cancer, Parkinson's disease, Alzheimer's disease, and atherosclerosis, which are primarily driven by oxidative stress (Imge et al., 2020).

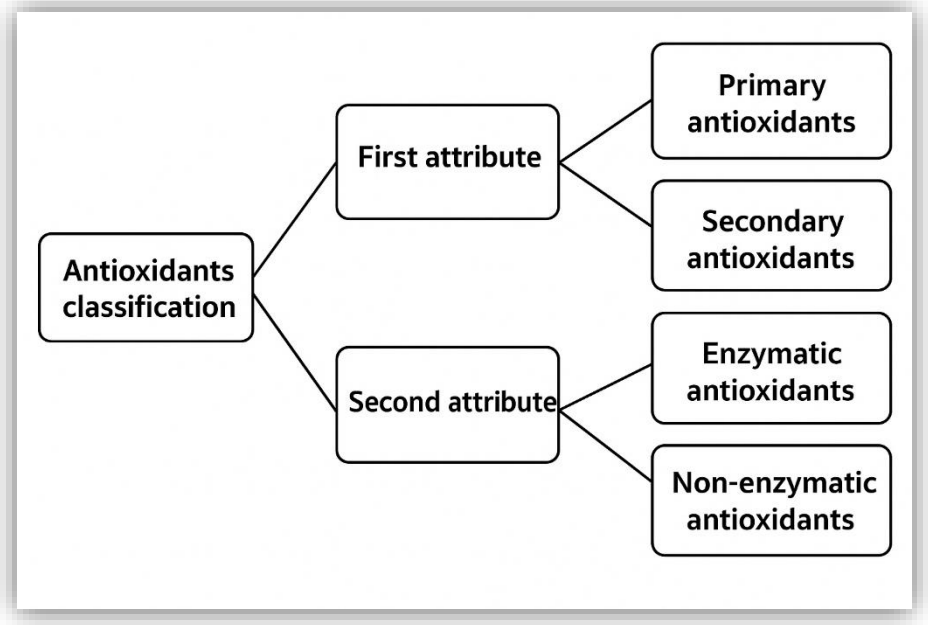


Figure 04: Antioxidant classification based on two (Imge et al., 2020)

2.2 Definition, origin and historical uses of *Juniperus phoenicea*

Juniperus phoenicea, commonly known as red juniper or locally as "Araar," is a shrub belonging to the Cupressaceae family. It thrives on arid hills and slopes throughout the Mediterranean region, including northern Algeria, where it is more commonly found in forests (Terfaya et al., 2021). This plant has significant medicinal value in traditional medicine, where its leaves are commonly used in decoctions to treat conditions such as diabetes, rheumatism, and diarrhea. Additionally, the leaves of Juniperus phoenicea are employed as a diuretic and to address broncopulmonary diseases. A

combination of its berries and leaves is also utilized in the preparation of oral hypoglycemic remedies (Terfaya et al., 2021).



Figure 05: *Juniperus phoenicea* (Terfaya et al., 2021)

2.3 Chemical constituents and bioactive compounds of *Juniperus phoenicea*

Juniperus phoenicea contains a diverse array of chemical constituents, as evidenced by numerous studies. These include diterpenoids, flavonoids, norterpenes, furanone glucosides, furanone derivatives, phenylpropanoid glucosides, lignans, and sesquiterpene glucosides. The plant's bioactive compounds exhibit significant antioxidant properties, which enable it to mitigate or prevent the oxidation of other molecules by free radicals. Through the reduction of oxidative stress, these compounds contribute to the prevention and treatment of various diseases, such as cancer, diabetes, hypertension, and Alzheimer's disease (Amalich et al., 2016).

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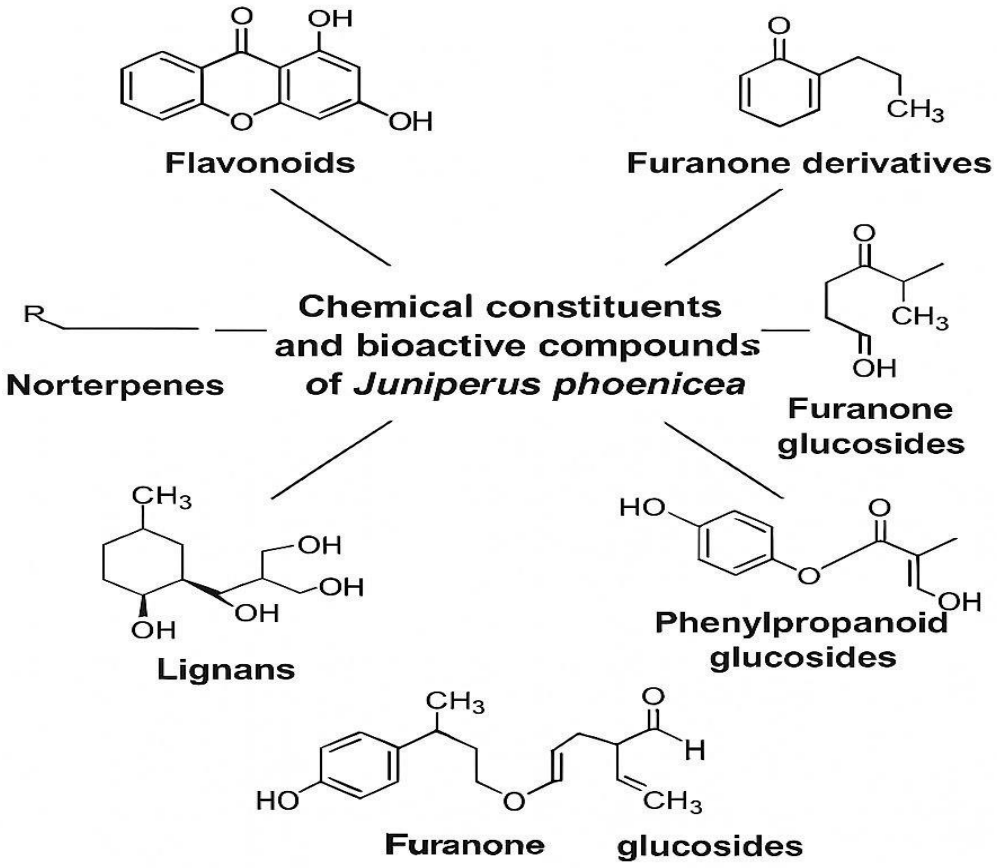


Figure 06: Chemical constituents and bioactive compounds of *Juniperus phoenicea* (Amalich et al., 2016)

Materials and methods

3. Materials

3.1 Chemical materials

- **Insecticide**

Acetamiprid, a neonicotinoid insecticide commercially marketed under the trade name *RUSTILAN*, is supplied in aluminum sachets containing 100 grams of powder with a purity of 20%. In our study, it was prepared as an aqueous solution by dissolving the appropriate quantity of Acetamiprid in distilled water. The solution was administered orally to rats at a dose of 20mg/kg /day.



Figure 07: Acetamiprid



Figure 08: Preparation of an Acetamiprid solution

3 .2 Biological material

- **Plant :**

Juniperus phoenicea was selected due to its well-established antioxidant properties. The plant material was harvested from the Safsaf El Ouesra mountain region in Tebessa in February 2025. The aerial parts of the plant were utilized to prepare an infusion with distilled water. This prepared solution was administered orally to the rats, following accurate weighing, at a daily dose of 200 mg/kg/day**.**



Figure09: *Juniperus phoenicea* plant of the Safsaf El Ouesra Tebessa



Figure10: The plant decoction process

- **Rat treatment :**

In our study, we used 30 female Wistar rats, obtained from the Pasteur Institute in Algiers, with a weight ranging between 180 and 225 grams. These rodents belong to the mammalian, and they are widely used in scientific research.The rats underwent an adaptation period of approximately 30 days under controlled environmental conditions, maintaining a suitable temperature and implementing a lighting system following a 12-hour light/12-hour dark cycle.They were housed in cages lined with wood shavings as bedding, ensuring regular cleaning and bedding replacement every two days until the end of the experiment. Additionally, they were provided with a balanced rodent-specific diet along with water supplied in bottles.

In the context of our study, the rats were randomly assigned to 4 groups, with each group comprising 7 animals. The designated dose of the solution was administered to each group via oral gavage, following accurate measurement of the body weight of each rat :

- **Group (C)**: Control rats were administered a daily dose of distilled water for a period of 30 days.

- **Group (ACE)**: Rats in this group were treated daily with acetamiprid at a dose of 20mg/kg/day for 31 days.

- **Group (JNP)**: Rats in this group were given daily doses of *Juniperus phoenicea* at 200 mg/kg/day for 30 days.

- **Group (ACE + JNP)**: This group was treated with a combination of acetamiprid and *Juniperus phoenicea*. The rats received a dose of acetamiprid in the early morning, followed by a dose of the plant extract after four hours, for a total duration of 31 days.



Figure11: Oral gavage technique for dose administration in rats

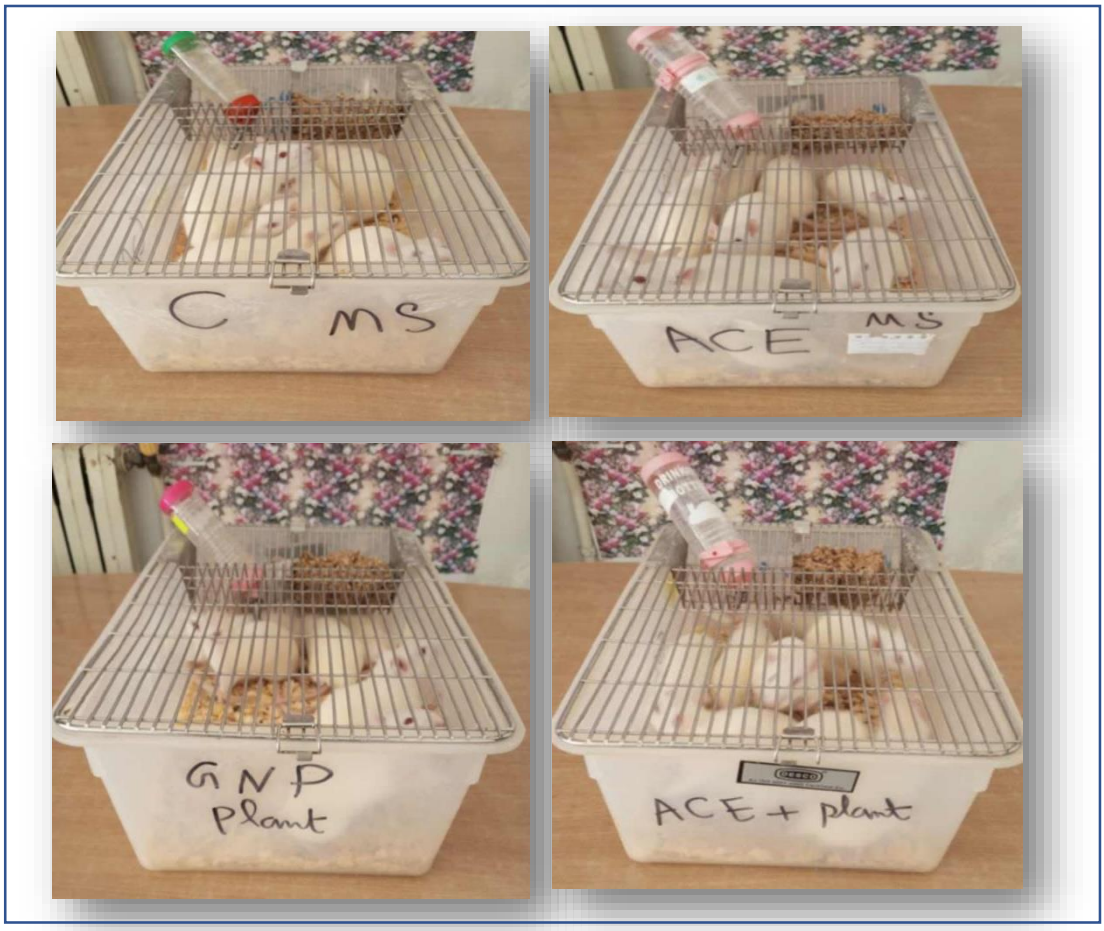


Figure 12: 4 groups of rats

3.3 Sacrifice and dissection

The sacrifice was performed by quick decapitation using a surgical scalpel, without anesthesia, between 9 and 11 a.m to avoid hormonal fluctuations throughout the day.

For Progesterone (P4) and Estrogen measurement, blood was collected in heparinized tubes, and EDTA tubes were also used for comprehensive blood analysis CBC. After the sacrifice, the rats were dissected to obtain the necessary organs for our study, which included the ovaries and adrenal glands. Some of these organs were placed in a formalin aqueous solution to preserve them and prevent degradation until histological sectioning. The remaining organs were weighed and stored in a refrigerator for future use in oxidative stress marker measurements.



Figure 13: Sacrifice and dissection

3.4 Assessment of Hormonal Parameters

We performed tests for the biochemical parameters of the blood collected on the sacrifice day. The parameters involved include complete blood count (CBC) , Progesterone (P4) and Estrogen and blood glucose levels, using the specific devices for each analysis.

3.4.1 Measurement of Progesterone (P4) and Estrogen

- **Principle of the methods**

The estrogen assay is based on the principle of a competitive chemiluminescent immunoassay. In this method, the test sample is incubated with a monoclonal anti-E2 antibody conjugated to acridinium ester (ABEI) and magnetic particles coated with purified E2 antigen. During incubation, antigen–antibody complexes form as the estrogen in the sample competes with the immobilized antigen for binding to the labeled antibody. The amount of chemiluminescence generated is inversely proportional to the concentration of estrogen (E2) in the sample.Following incubation, the mixture is subjected to a magnetic field to precipitate the complexes, and the unbound substances are removed through a decantation and wash step. Subsequently, chemiluminescent substrates (Starters 1 and 2) are introduced to trigger a light-emitting reaction. The emitted light is quantified by a photomultiplier and expressed in relative light units (RLU). The intensity of the signal is inversely proportional to the estradiol concentration in the sample or control, meaning higher estradiol levels result in lower RLU values (MAGLUMI Estradiol et al.,2020).

The Progesterone ELISA kit operates on the principle of a competitive enzyme-linked immunosorbent assay (ELISA). Microtiter plate wells are pre-coated with a polyclonal antibody specific to the Progesterone molecule. During the assay, endogenous Progesterone present in the patient's sample competes with a horseradish peroxidase (HRP)-conjugated Progesterone analog for a limited number of antibody binding sites.Following an incubation period that allows for competition and binding, unbound components are removed through a series of washing steps. The amount of HRP-conjugated Progesterone bound to the antibody is inversely proportional to the concentration of Progesterone in the sample.Subsequent addition of a chromogenic substrate results in a colorimetric reaction catalyzed by HRP. The intensity of the color developed is measured spectrophotometrically and is inversely proportional to the concentration of Progesterone in the tested sample(IBL International GmbH et al.,2020).

- The analysis was conducted using the Cobas® e 411 medical analyzer.



Figure 14: Measurement of P4 and Estrogen

3.4.2 Measurement of complete blood count

- **Principle of the method**

The blood cell counter automates the analysis of blood components (red blood cells, white blood cells, platelets) by aspirating and diluting a sample. It measures hematocrit, hemoglobin concentration, and establishes the leukocyte formula. Using advanced technologies, the device provides accurate and reproducible results, offering time-saving and reducing human errors compared to manual methods. The results are presented numerically and can be used for medical diagnosis (Biologie Sans Frontières et al.,2012).

- The analysis was conducted using the mindray BC-30s .



Figure 15: Measurement of CBC

3.4.3 Measurement of Glycemia

- **Principle of the Test :**

The glucose in the blood sample reacts with the glucose oxidase enzyme present in the test strip, producing a weak electric current. The intensity of this current varies depending on the amount of glucose in the sample. The Vital Check MM1200 device measures the current intensity, calculates the blood glucose level, displays the result, and stores it in memory (Vital Check et al.,2020).

The analysis was conducted using the Vital Check® MM1200



Figure 16 : Measurement of Glycemia

3.5 Quantitative analysis of oxidative stress biomarkers

3.5.1 Quantitative analysis of Glutathione S-transferase (GST) enzymatic activity

- **Principle**

Glutathione S-transferase (GST) activity is commonly assessed using the substrate 1-chloro-2,4-dinitrobenzene (CDNB), which readily undergoes conjugation with reduced glutathione (GSH) in the presence of various GST isoforms. This enzymatic reaction results in the formation of a conjugated product, S-(2,4-dinitrophenyl)glutathione (GS-DNB), accompanied by the release of hydrochloric acid (HCl).In the present study, GST activity was quantified following the method described by Habig

et al. (1974). Briefly, the assay involves incubating the enzyme source (tissue homogenate) with a reaction mixture containing CDNB and GSH, under controlled conditions (25°C, pH 7.0). The progress of the reaction is monitored spectrophotometrically by measuring the increase in absorbance at 340 nm, corresponding to the formation of the GS-DNB conjugate. Absorbance readings are taken at 1-minute intervals over a 5-minute period to determine the enzymatic activity (Habig WH et al., 1988).

○ Experimental procedure

The required reagents for this study, including phosphate buffer and reduced glutathione (GSH), were prepared in advance and stored at 4°C to preserve their stability and reactivity. The 1-chloro-2,4-dinitrobenzene (CDNB) reagent was freshly prepared on the day of the Glutathione S-transferase (GST) activity assay to ensure the reliability and accuracy of the enzymatic quantification.

A tissue sample weighing 100 mg was excised from the **ovaries and adrenal glands**, then transferred into test tubes. A phosphate buffer solution was added, and the samples were subsequently homogenized to obtain a uniform suspension. The homogenates were subjected to cold centrifugation using a refrigerated centrifuge, leading to the separation of the mixture into two distinct phases: a pellet and a supernatant. The supernatant, containing the soluble protein fraction, was carefully collected and reserved for the quantitative determination of Glutathione S-transferase (GST) activity.

Upon completion of reagent preparation, defined volumes were accurately dispensed into test tubes according to the protocol detailed in the accompanying table. Subsequently, absorbance measurements were performed using a UV-Vis spectrophotometer to facilitate the quantitative assessment of enzymatic activity.

Table 02: Experimental procedure of GST

Les réactifs utilisés	**Blanc (µl)**	**Echantillon (µl)**
Tampon phosphate (0.1M, pH 7)	850	830
CDNB (0.0 2M)	50	50
GSH (0.1M)	100	100
Surnageant	-	20

Calcula: GST activity is calculated using the following equation:

GST activity (nmol/min/mg protein) = (OD_sample/min – OD_blank/min) / (9.6 × mg of protein)

- **OD_sample/min:** Optical density of the sample per minute.
- **OD_blank/min:** Optical density of the blank per minute.
- **9.6:** Molar extinction coefficient of the GSH-CDNB conjugate, expressed in $mM^{-1} \cdot cm^{-1}$.

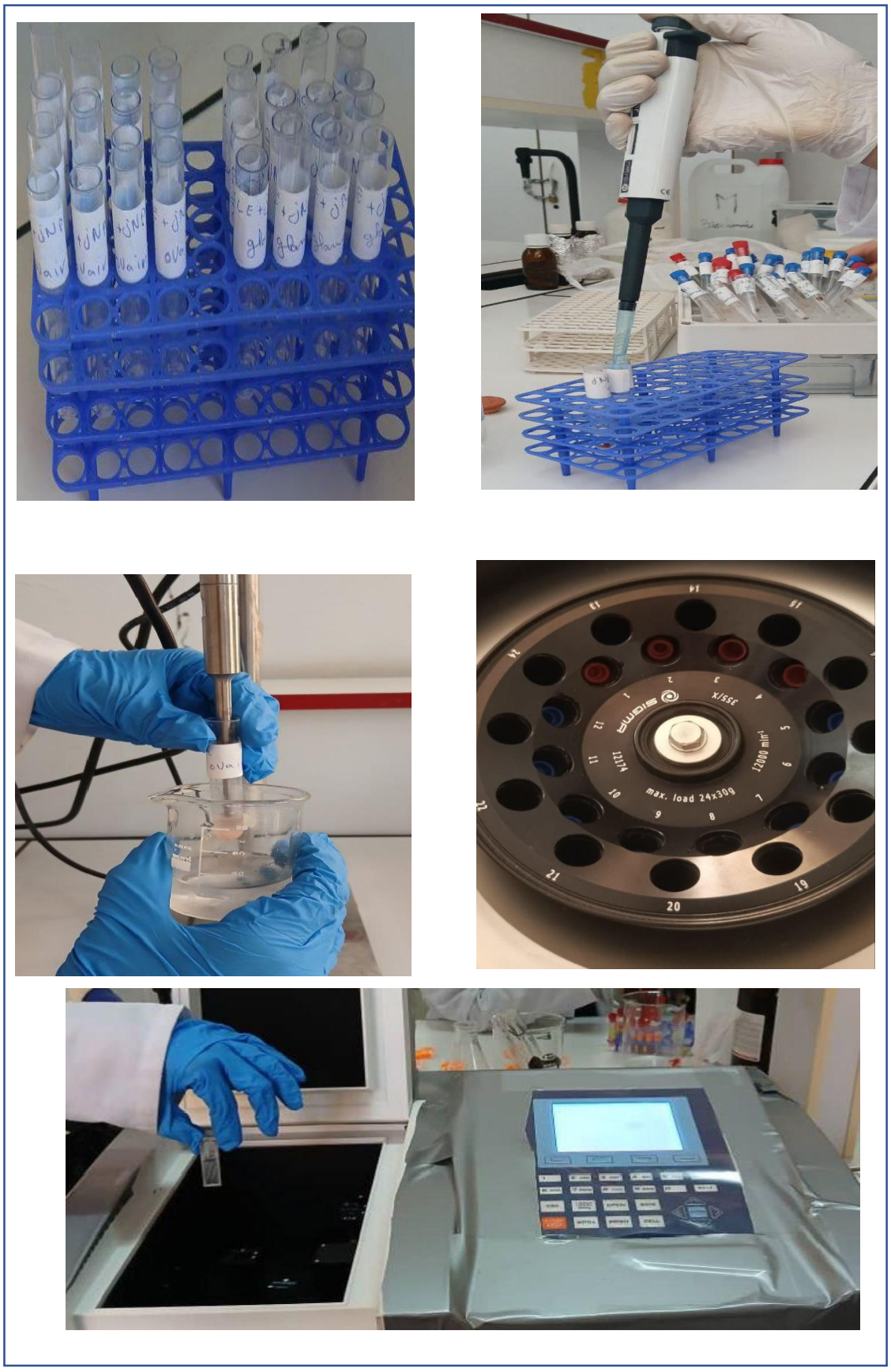


Figure 17: Quantitative analysis of GST

3.5.2 Quantitative analysis of Catalase enzymatic activity

○ Principle

Catalase activity was determined following the method described by Aebi (1984), which involves monitoring the decomposition of hydrogen peroxide (H_2O_2) spectrophotometrically at 240 nm. The assay was performed at 25°C using the cytosolic fraction as the enzymatic source. The decrease in absorbance, corresponding to the reduction of H_2O_2 concentration, was recorded after an initial 15-second lag phase and continued for a 60-second measurement period. Given the rapid decline in catalase activity, strict timing was maintained between the addition of reagents and the placement of the cuvette in the spectrophotometer to ensure reproducibility and accuracy of the results (Aebi H ., 1984).

○ Experimental procedure

All reagents necessary for the enzymatic assay, including the phosphate buffer, were prepared in advance and stored under refrigerated conditions to maintain their physicochemical stability and enzymatic compatibility. To ensure maximal reactivity and analytical precision, the hydrogen peroxide (H_2O_2) solution was freshly prepared on the day of catalase activity quantification.

The subsequent steps of the quantitative analysis were performed in accordance with the protocol established for the GST enzyme assay, with the sole modification being the use of a different type of spectrophotometer.

Table 03: **Experimental procedure of catalase**

Les réactifs utilisés	**Blanc (µl)**	**Echantillon (µl)**
Tampon phosphate (0.1M, pH 7)	800	780
H2O2 (0.5 M)	200	200
Surnageant	-	20

The activity of catalase was determined according to the following equation:

$$\text{CAT activity (µmol } H_2O_2\text{/min/mg protein)} = (\Delta OD * 10) / (\varepsilon * L * X * Fd)$$

- **Δ OD**: Change in optical density per minute.
- **ε:** molar extinction coefficient of H_2O_2 = 0.04 $mM^{-1} \cdot cm^{-1}$.

- **L:** Longueur de la cuve = 1 cm.
- **X :** Protein concentration in mg/ml.
- **Fd :** Dilution factor of H_2O_2 in the buffer (0.02).



Figure 18: spectrophotometer for quantitative analysis of Catalase

3.5.3 Quantitative Determination of Glutathione (GSH)

○ Principle

Glutathione quantification was conducted following the protocol established by Wekbeker and Cory (1988). This assay is based on the spectrophotometric detection of 2-nitro-5-thiobenzoic acid, a yellow-colored compound formed upon the reduction of 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB, also known as Ellman's reagent) by the free thiol (-SH) groups of glutathione. To ensure assay specificity and eliminate potential interference from protein-bound thiol groups, a preliminary deproteinization step of the homogenate was performed, allowing selective measurement of glutathione-derived thiols (Weckbercker G et Cory JG ., 1988).

○ Experimental procedure

The analytical procedure for glutathione quantification begins with sample preparation, wherein 0.8 mL of tissue homogenate is combined with 0.2 mL of 0.25% sulfosalicylic acid (SSA) to induce

protein precipitation. The mixture is vigorously vortexed and incubated on ice for 15 minutes to ensure effective protein denaturation. Subsequently, the sample is subjected to centrifugation at 1,000 rpm for 5 minutes to isolate the supernatant, which contains the low molecular weight thiol compounds, including reduced glutathione (GSH).

For the colorimetric determination, 0.5 mL of the clarified supernatant is transferred into a clean test tube. To this, 1.0 mL of Tris-EDTA buffer (pH 9.6, 0.02 M EDTA) is added to maintain the optimal pH and chelate divalent metal ions that could interfere with the assay. Following homogenization, 0.025 mL of 0.01 M 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), commonly referred to as Ellman's reagent, is introduced to initiate the chromogenic reaction with thiol groups. The reaction mixture is incubated at room temperature for 5 minutes to allow complete color development. The absorbance is then recorded at 412 nm using a UV-Vis spectrophotometer against a reagent blank, facilitating the quantitative assessment of glutathione content.

Calcula: The determination of glutathione concentration is performed according to the following formula:

$$[GSH]\ (nM\ GSH/mg\ total\ protein) = (OD \times 1 \times 1.525) / (13{,}100 \times 0.8 \times 0.5 \times mg\ total\ protein)$$

- **DO:** Absorbance measured at a wavelength of 412 nm.
- **1:** Total volume of solutions used during deproteinization (0.8 mL of homogenate + 0.2 mL of SSA).
- **1.525:** The total volume of solutions used in the GSH assay consisted of 0.5 mL of supernatant, 1 mL of Tris-EDTA buffer, and 0.025 mL of DTNB.
- **13100:** The absorbance coefficient of the thiol group (-SH) at 412 nm.
- **0.5:** Volume of the supernatant present in 1.525 ml.
- **0.8:** Volume of the homogenate present in 1 ml.



Figure 19: Quantitative Determination of (GSH)

3.5.4 Quantitative analysis of proteins

- **Principle**

This method employs Coomassie Brilliant Blue as a dye reagent, which, under acidic conditions, exhibits strong affinity for arginine residues and, to a lesser extent, for histidine, lysine, and aromatic amino acids. The dye binds to proteins through non-covalent interactions, including hydrogen bonding, hydrophobic forces, and ionic interactions. Upon binding, the dye undergoes a spectral shift in its maximum absorbance from 465 nm (red form) to 595 nm (blue form), facilitating quantitative protein determination (Bradford ., 1976).

- **Experimental procedure**

- An aliquot of 0.1 ml (100 µl) of the supernatant sample was transferred into a clean test tube.
- Subsequently, 4 ml of Bradford reagent was added to the sample.
- The mixture was gently agitated and allowed to stand for 5 minutes at room temperature to ensure complete color development.
- The absorbance was then measured at 595 nm using a spectrophotometer, with a reagent blank (comprising 4 ml of Bradford reagent and 0.1 ml of distilled water) serving as the reference.

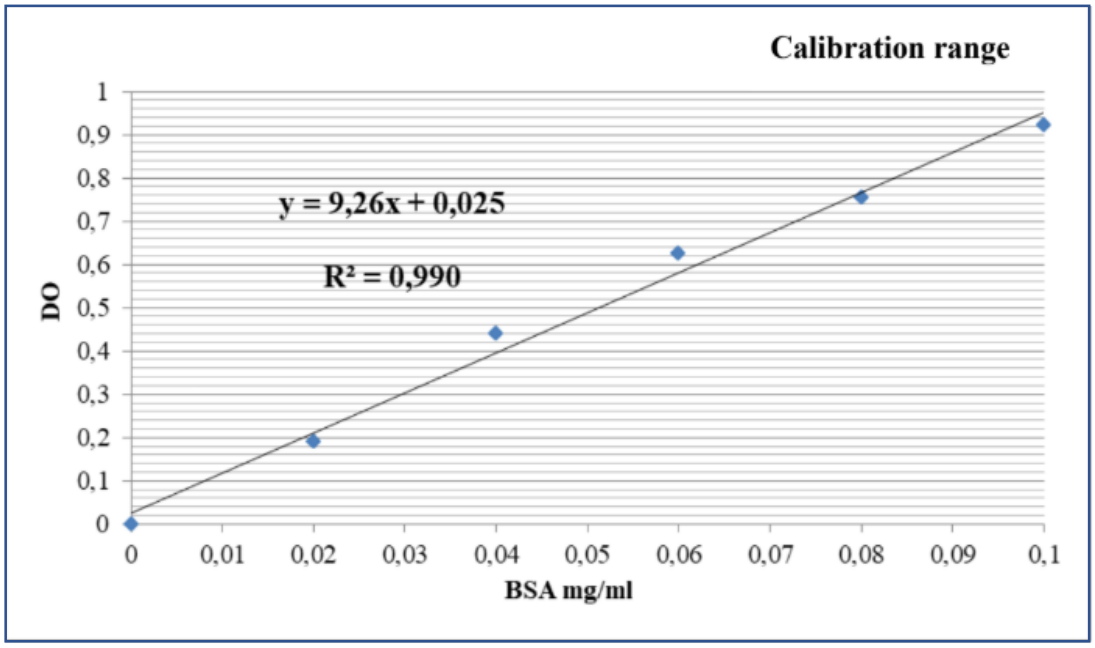
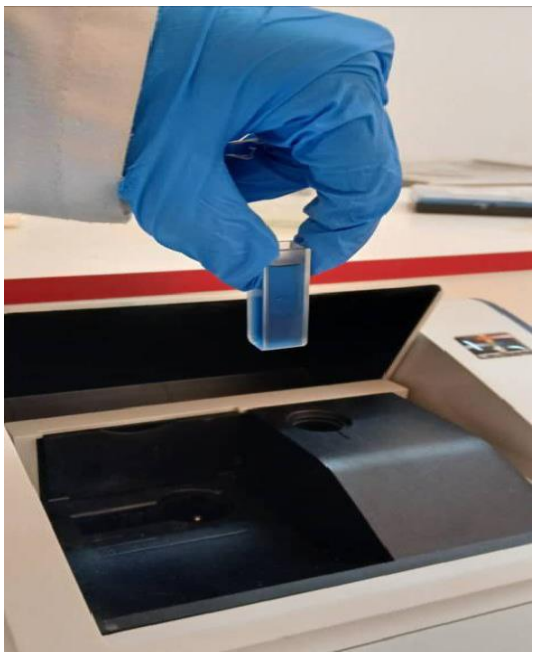


Figure 20: Bovine Serum Albumin (BSA) Calibration Curve







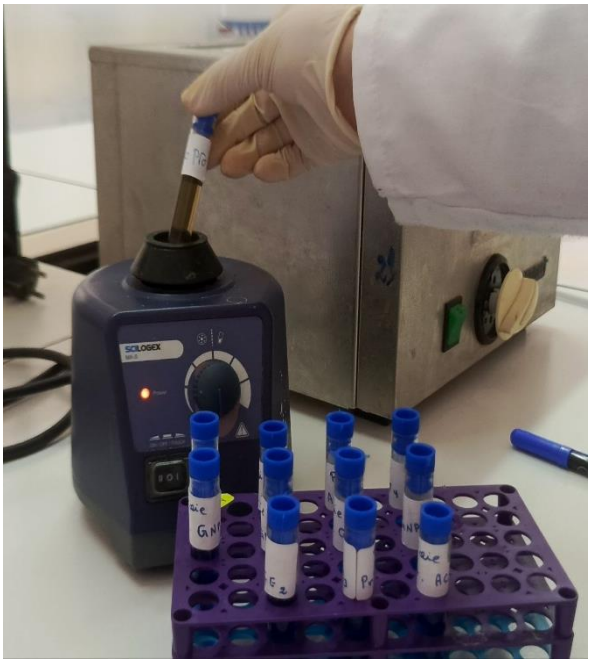


Figure 21: Quantitative analysis of proteins

3.6Histological study

Histological sections were processed at the Department of Anatomical Pathology, Ibn Rochd Hospital,Annaba. The methodology employed was based on the protocol established by Houlot (1984), comprising the following sequential steps:

3.6.1 The fixation

This process aimed to preserve cellular architecture while maintaining tissue morphology. Timely fixation is a critical determinant of histological technique success and was therefore performed immediately following the decapitation of the rats and excision of the target organs. Tissue fragments from the ovaries and endocrine glands, measuring approximately 1–2 cm^2 in surface area and 2 mm in thickness, were immersed in alcoholic Bouin's fixative. This solution facilitates optimal tissue hardening without compromising structural integrity. After 48 hours of fixation, the samples were removed from the fixative and thoroughly rinsed with distilled water. Transverse sections were subsequently prepared by a pathologist, and the specimens were placed in perforated histological cassettes to ensure efficient fluid exchange during the following processing stages.

3.6.2 Dehydration

As paraffin is immiscible with water, the samples must undergo complete dehydration prior to embedding in paraffin. Furthermore, paraffin is insoluble in the alcohol used during the dehydration process, thus necessitating a substitution with xylene. Dehydration is achieved through an automated system, which progressively immerses the samples in ethanol solutions of increasing concentrations (70%, 95%, and 100%). This is followed by immersion in xylene baths, which serve as a clearing agent, providing the tissue with enhanced transparency. Subsequently, the xylene evaporates from the tissue specimens in an oven. This step is facilitated by an apparatus known as the circulator.

3.6.3 Embedding and Block Preparation

Successful embedding is contingent upon the complete dehydration of the sample and the absence of any residual intermediate solvents (e.g., alcohol). The specimens are immersed in molten paraffin baths at 60°C for a period of two hours to ensure thorough impregnation. Once fully saturated with paraffin, the samples are placed in molds, specifically Leuckart bars, and filled with additional paraffin. This process is carried out using specialized embedding apparatus, which consists of a paraffin reservoir maintained in a liquid state by a heating system, a small valve, and a refrigerated metal plate, facilitating the rapid solidification (within 10 to 15 minutes) of the paraffin block containing the tissue.

3.6.4 Histological Sectioning

Paraffin-embedded tissue blocks were precisely trimmed and sectioned using a rotary microtome to produce thin sections of 4–5 µm thickness. The resulting paraffin ribbons were carefully transferred onto pre-cleaned glass slides. To facilitate optimal tissue adhesion and smooth unfolding, the slides were placed on a gelatin-coated water bath maintained at 40 °C. Each slide was then accurately labeled with the corresponding experimental group using a silver marking pencil and subsequently dried in a thermostatically controlled oven at 100 °C for one hour to ensure proper fixation and adhesion of tissue sections.

3.6.5 Staining and Mounting

Several staining techniques are available depending on the type of tissue analyzed. The Hematoxylin and Eosin (H&E) method is the most widely used in histological analysis. The staining procedure follows these steps:

- **Deparaffinization:** Tissue sections are immersed in a xylene bath for 10 minutes to remove paraffin and allow the penetration of dyes.
- **Rehydration:** The slides are passed through ethanol for 10 minutes, followed by hydration under running tap water.
- **Hematoxylin Staining:** Sections are immersed in hematoxylin for 10 minutes, which stains basophilic structures (such as cell nuclei) a purplish-blue color. The slides are then rinsed under running water.
- **Eosin Staining:** The second dye, eosin, is applied to stain acidophilic structures (such as cytoplasm) pink, followed by rinsing in running water.
- **Mounting:** A glass coverslip is placed over the stained tissue sections to ensure:
  - Mechanical protection of the sections.
  - Chemical preservation of the stains.



Figure 22: Histological evaluation (Grx100, H&E coloration)

3.7Comparative Statistical Analysis of Experimental Groups

To assess the differential effects of the various treatments, a statistical comparison was conducted among the experimental groups: control (C), juniper extract-treated (JNP), acetamiprid-exposed (ACE), and the co-treated group (ACE+JNP). All quantitative data were presented as mean ± standard deviation (SD). Statistical significance was determined using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison post hoc test. A p-value of less than 0.05 was considered indicative of statistical significance. The results demonstrated significant intergroup differences, highlighting the cytotoxic impact of acetamiprid and suggesting a potential ameliorative effect of juniper extract in mitigating acetamiprid-induced toxicity.






7 Control rats

7 rats ACE

7 rats ACE+JNP

7 rats JNP

Adaptation 30 days

Treatment for one month



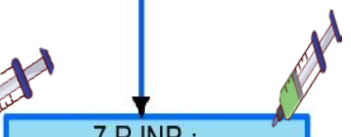
7 CR : distilled water



7 RACE : Force-feeding by pesticide



7 RACE+JNP: Force-feeding by pesticide + plant



7 RJNP : Force-feeding by plant

Sacrifice and dissection

Sang



EDTA : CBC



Heparin : P4+E2

Glycemia



Organs

80% for dosage of OSP

GST activity

CAT activity

Glutathione (GSH)

20% in formalin

Histological study

Results and discussion

4.1 Physiological parameters

4.1.1 Effect of treatments on body weight

Quantitative analysis revealed statistically significant differences in body weight progression among the experimental groups. The control group (C) exhibited a mean body weight increase of +4.40%. Rats administered *Juniperus phoenicea* extract (JNP) showed an increased body weight gain of +6.02%. In contrast, exposure to acetamiprid (ACE) resulted in a significant decrease in body weight (−4.57%). The group receiving the combined treatment of acetamiprid and *Juniperus phoenicea* (ACE+JNP) displayed a relatively stable body weight profile, with a minimal reduction of −0.53%.

Throughout the experimental period, no statistically significant alterations in food intake were observed in the control group. In contrast, the group treated with *Juniperus phoenicea* extract exhibited a statistically significant increase in food consumption. Conversely, animals exposed to acetamiprid, either alone or in combination with *Juniperus phoenicea*, showed a marked reduction in food intake and appetite compared to controls. Importantly, all animals survived the entirety of the study duration, and no observable clinical signs of toxicity were recorded during the monitoring phase.

Table 04: Effect of aqueous extracts of *Juniperus phoenicea* aerial parts and/or Acetameprid treatment on body weight gain after 30 days of experience (values represent the mean±SEM of seven rats)

	Experimental group			
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**

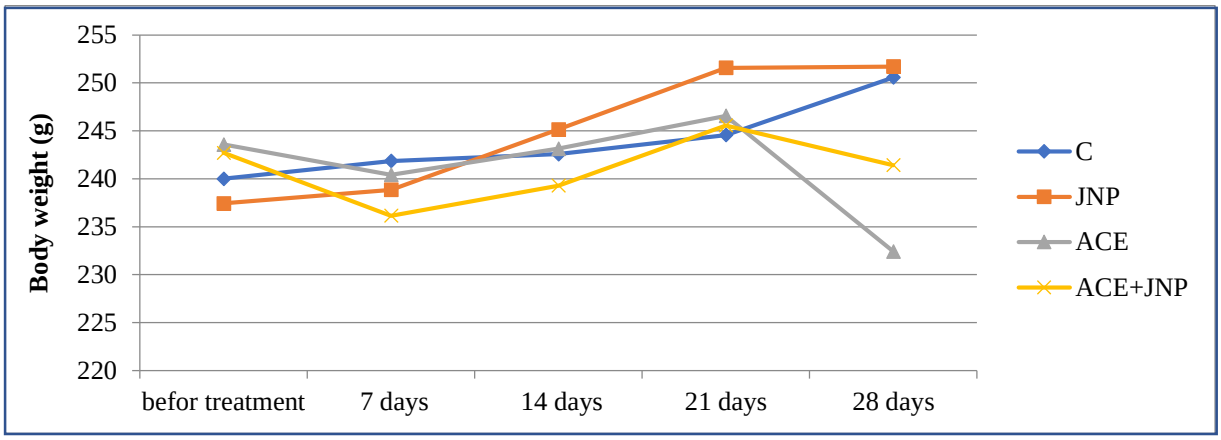


Figure 23: Effect of *Juniperus* leaf extract and/or Acetamiprid on body weight Over 30 days

In the present study, we observed that chronic exposure to acetamiprid for a duration of 30 days resulted in a significant reduction in body weight gain compared to control rats. This decrease in weight gain may be indicative of direct toxicity or stress-induced effects on the gastrointestinal system, leading to a reduction in both appetite and nutrient absorption. Additionally, the observed weight loss could be attributed to the appetite-suppressing properties inherent to acetamiprid (Samiran et al., 2015) .

The findings of the present study are consistent with those reported by (Imen et al., 2024), who investigated the subacute toxicity of acetamiprid at doses of 21.7 and 43.4 mg/kg over a 30-day period, and observed a significant reduction in body weight. Similarly , Annu (2024) demonstrated that acetamiprid exposure leads to notable body weight loss in rats. These results are further supported by the study of (Sana et al., 2016), which also reported comparable outcomes. Moreover, Shipra (2010) reported that oral administration of imidacloprid, a neonicotinoid insecticide, at a dose of 20 mg/kg/day in female rats resulted in a significant decrease in body weight, reinforcing the current findings regarding the weight-reducing effects of neonicotinoid compounds.

Preparations derived from *Juniperus* species may be classified as traditional herbal medicinal products, supported by both direct and indirect evidence of their medicinal use spanning at least three decades. Among their most commonly documented traditional applications is the management of dyspeptic disorders (Abdel-Salam et al., 2006). Furthermore, the pseudo-fruits of *Juniperus* are traditionally employed as appetite stimulants, potentially enhancing food consumption and thereby contributing to weight gain (EMA 2012) .

In our study, we observed a significant increase in body weight in rats treated with *Juniperus phoenicea*, which aligns with the results reported by Al-Ahdab (2017). Their research demonstrated that oral administration of *Juniperus phoenicea* leaf extract at doses of 200 and 400 mg/kg body

weight led to significant increases in both body weight gain (BWG) compared to the control group. However, other studies, such as that by Lui (2023), have reported a reduction in body weight following treatment with *Juniperus* extract, indicating variability in the effects.

4.1.2 Effect of treatment on adrenal gland and ovary weights

- With respect to the ovaries, treatment with ACE led to a highly significant reduction in absolute weight ($P < 0.01$). A non-significant decrease in ovarian weight was also observed in the group receiving both ACE and JNP, whereas the JNP group alone did not exhibit any notable changes. Similarly, a significant reduction in relative ovary weight was detected in the ACE group ($P < 0.05$), while no statistically significant differences were found in the JNP or ACE+JNP groups compared to the control.
- As indicated by the data presented in the table, administration of ACE alone resulted in a statistically significant increase in the absolute weight of the adrenal glands ($P < 0.05$) when compared to the control group. In contrast, no significant alterations were observed in the groups receiving JNP alone or the combination of ACE and JNP. Although the relative adrenal gland weight showed a modest elevation in the ACE-treated group, this difference did not reach statistical significance.

Table 05: Effect of aqueous extracts of *Juniperus phoenicea* aerial parts and/or Acetameprid treatment on adrenal gland and ovary weights after 30 days of experience (values represent the mean±SEM of seven rats)

Groups	**C**	**JNP**	**ACE**	**ACE+JNP**
Adrenal gland				
Absolute weight (g)	0.054±0.011	0.059±0.010	0.070±0.005*	0.058±0.008
Relative weight (%)	0.021±0.005	0.024±0.011	0.030±0.008	0.025±0.006
Ovary				
Absolute weight (g)	0.204±0.06	0.172±0.08	0.122±0.05**	0.132±0.05
Relative weight (%)	0.079±0.03	0.068±0.04	0.053±0.02*	0.056±0.02
*P< 0,05 **P<0,01 relative to the control group (C)				

Exposure to acetamiprid has been documented to cause significant pathomorphological alterations in ovarian follicles, accompanied by metabolic dysfunction within ovarian tissue. These adverse effects are largely attributed to oxidative stress and disruptions in endocrine regulation. Notably, acetamiprid-induced follicular deformities have been associated with a decline in embryo quality, underscoring its potential to impair female reproductive health (Zuscikova et al., 2023). Consistent with these observations, a reduction in ovarian weight in ACE-treated animals suggests the occurrence of follicular atresia and inhibited development of ovarian structures, likely mediated by elevated oxidative stress and suppression of critical reproductive hormones, including estrogen and progesterone (Sulaiman et al., 2014; Kapoor et al., 2011). Collectively, these findings highlight the deleterious impact of acetamiprid on ovarian physiology and reproductive competence.

In our study, a decrease in ovarian weight was observed in mice treated with acetamiprid, which aligns with the findings of Kapoor (2011), who examined the effects of imidacloprid, a neonicotinoid, on ovarian weight in female rats. Their results demonstrated a significant reduction in the relative ovarian weight at a dose of 20 mg/kg/day, indicating a potential reproductive toxicity of this compound.

In the ACE+JNP treatment group, a partial amelioration in ovarian weight was noted relative to the group exposed solely to acetamiprid; however, the ovarian weight remained below the established physiological baseline. This limited recovery is plausibly attributable to the antioxidant and anti-inflammatory properties of *Juniperus phoenicea*, which may attenuate, but not fully counteract, the oxidative and endocrine disruptions induced by acetamiprid exposure. Several studies have documented the presence of bioactive constituents in *Juniperus* species namely flavonoids and terpenoids that exhibit potent free radical scavenging capacity and the ability to modulate pro-inflammatory signaling cascades (Ertas et al., 2015; El-Sawi et al., 2010). Such phytochemical-mediated effects may contribute to the preservation of ovarian follicular architecture under oxidative stress conditions. Nonetheless, the persistent downregulation of pivotal reproductive hormones by acetamiprid continues to impair the endocrine regulation essential for normal ovarian function (Kapoor et al., 2011; Sulaiman et al., 2014). Therefore, despite the cytoprotective potential of *J. phoenicea*, its efficacy appears insufficient to fully restore ovarian morphological and functional homeostasis following toxicant insult.

Although direct investigations into adrenal gland weight in female subjects exposed to acetamiprid remain sparse, existing literature suggests that acetamiprid may exert neuroendocrine effects, including modulation of nicotinic acetylcholine receptors and alterations in catecholamine profiles. These changes may potentially lead to secondary structural or weight alterations in the adrenal glands

(Matsuda et al., 2021). The significant increase observed in the relative weight of the adrenal glands in female rats exposed to acetamiprid may be attributed to the chronic activation of the hypothalamic-pituitary-adrenal (HPA) axis, or to hypertrophic changes in adrenal cortical cells, potentially mediated by oxidative stress and endocrine-disrupting mechanisms (Kapoor et al., 2011). Furthermore, the observed increase in adrenal gland weight may also be the result of adrenal cortical hypertrophy, a response to stress, which is characterized by an enhanced production of glucocorticoids such as cortisol (Zhang et al., 2011; Martyniuk & Feswick, 2018).

In our study, we observed a significant increase in the relative weight of the adrenal glands in female rats treated with acetamiprid. This finding aligns with the results of Kapoor (2011), which reported a notable increase in the relative adrenal gland weight in female rats exposed to subchronic doses of imidacloprid, a neonicotinoid, over a period of 90 days.

To date, the available scientific literature does not provide direct evidence of increased adrenal gland weight in rats treated with *Juniperus* extracts. Nevertheless, certain studies suggest that *Juniperus phoenicea* extracts may exert modulatory effects on the hypothalamic-pituitary-adrenal (HPA) axis, which could indirectly influence both the functional activity and morphological characteristics of the adrenal glands (Ghaleb et al., 2023).These observations are in line with our experimental findings, which demonstrated a slight yet measurable increase in adrenal gland weight in rats administered *Juniperus phoenicea* extract.

4.2 Effect of treatment on blood glucose levels assessment

As presented in Table 06, no significant difference in blood glucose levels was observed between the group treated with *Juniperus phoenicea* alone and the control group. However, exposure to acetamiprid alone resulted in a significant increase in glycemia (1.46 ± 0.08 g/L, $p< 0.05$). Notably, the group receiving a combination of acetamiprid and *Juniperus phoenicea* demonstrated a reduction in blood glucose levels (1.223 ± 0.12 g/L), which was lower than that observed in the control group.

Table 06: Effect of aqueous extracts of *Juniperus phoenicea* aerial parts and/or Acetameprid treatment on blood glucose levels after 30 days of experience (values represent the mean±SEM of seven rats)

Parametrs	**C**	**JNP**	**ACE**	**ACE+JNP**
Glycemia (g/L)	1.280±0.09	1.274±0.21	1.46±0.08*	1.223±0.12
***(P< 0.05):** Significant difference compared to control.				

Acetamiprid exerts multifaceted toxicological effects that contribute to significant disturbances in glucose metabolic homeostasis. Through the induction of oxidative stress, acetamiprid may enhance hepatic gluconeogenesis, thereby promoting elevated blood glucose levels (El-Sayed et al., 2024).Chronic exposure to this neonicotinoid compound has been shown to impair pancreatic endocrine function, specifically by reducing the insulin responsiveness of β-cells and lowering circulating insulin concentrations. Such dysfunctions disrupt the physiological regulation of glycemia and may predispose exposed organisms to glucose intolerance.Furthermore, acetamiprid has been characterized as a potential endocrine-disrupting agent, capable of interfering with hormonal pathways involved in glucose regulation, including insulin and glucagon signaling. This hormonal dysregulation further compromises metabolic stability (El-Sayed et al., 2024). In addition, acetamiprid-induced hepatotoxicity has been documented, with evidence pointing to hepatic

structural and functional impairments. These hepatic alterations may negatively affect glucose metabolism, thereby exacerbating the risk of hyperglycemia (ElMazoudy et al., 2020).

In the context of our study, a significant elevation in blood glucose levels was observed in mice treated with acetamiprid. This finding aligns with previous research that has documented the toxic effects of acetamiprid on glucose homeostasis.

Evidence from various studies indicates that, *Juniperus phoenicea* may exert hypoglycemic effects through the stimulation of insulin secretion in diabetic rats, thereby facilitating improved glycemic control (Abdel-Rahim et al., 2011). *Juniperus phoenicea* has been shown to contain high levels of phenolic and flavonoid compounds, which contribute to its potent antioxidant properties. According to Ben Ali (2015), the administration of *J. phoenicea* berry extract conferred significant hepatoprotective effects against carbon tetrachloride (CCl_4)-induced oxidative damage in rats, suggesting a potential cytoprotective effect on pancreatic β-cells through the attenuation of oxidative stress. Additionally, findings by Yousef (2017) reported that *J. phoenicea* extract exerts a notable inhibitory effect on α-amylase activity an enzyme involved in carbohydrate hydrolysis thereby potentially delaying glucose absorption in the intestine and contributing to the attenuation of postprandial hyperglycemia.

Our findings demonstrated a significant reduction in blood glucose levels in rats co-treated with *Juniperus phoenicea* extract and acetamiprid, consistent with prior evidence supporting the hypoglycemic activity of *J. phoenicea*. This observation reinforces the potential of *J. phoenicea* as an adjuvant therapeutic agent for modulating glycemic homeostasis under conditions of chemically induced metabolic disruption.

4.3 Effect of treatment on blood parameters levels assessment

As shown in Table (07), no statistically significant differences in hematological parameters were observed between the group treated with *Juniperus phoenicea* (JNP) alone and the control group. Conversely, administration of acetamiprid (ACE) alone resulted in a significant elevation in both white blood cell (WBC) count (17.95±1.17 ×10³/µl; $p<0.05$) and lymphocyte count (12.35±2.16 ×10³/µl; $p<0.05$) compared to the control. Furthermore, hemoglobin concentration (15.15±0.30g/dl;$p<0.05$) and hematocrit level (36.26 ± 0.72%; $p<0.01$) were significantly reduced in the ACE-treated group relative to the control. A significant increase in mean corpuscular volume (MCV) (60.05 ± 2.48 fl; $p<0.05$) was also recorded in the ACE group. Notably, coadministration of acetamiprid and *Juniperus phoenicea* resulted in hematological values WBC and lymphocyte counts, hemoglobin concentration, hematocrit, and MCV that, although significantly different from the control group, were less pronounced than those observed in the group treated with acetamiprid alone.

Table 07: Effect of aqueous extracts of *Juniperus phoenicea* aerial parts and/or Acetameprid treatment on blood parameters after 30 days of experience (values represent the mean±SEM of seven rats)

Parameters	C	JNP	ACE	ACE+JNP
WBC 10^3/µl	11.39±2.27	10.13±0.32	17.95±1.17**	15.45±2.97
Lym 10^3/µl	08.17±2,23	06.30±0.98	12.35±2.16*	10.78±1.89
RBC 10^6/µl	07.63±1.69	06.83±0.22	06.47±0.96	06.51±0.13
Hb g/dl	16.65±0.72	16.98±0.49	15.15±0.30*	15.25±0.62*
HTC %	41.62±1.80	42.44±1.23	36.26±0.72**	38.12±1.56*
MCV fl	55.42±1.93	55.03±1.47	60.05±2.48*	59.22±1.83*
MCHC g/dl	379.25±15.81	397.50±14.20	426.00±17.76	419.25±11.90
PLT 10^6/µl	877.00±87.13	784.00±28.96	987.25±23.54*	956.25±42.91
*P< 0,05 **P<0,01 relative to the control group (C)				

Acetamiprid, a neonicotinoid insecticide extensively applied in agriculture, has been consistently implicated in the induction of oxidative stress through the generation of reactive oxygen species

(ROS) within biological systems. This mechanism was substantiated by Kim et al (2024), who reported that excessive ROS levels result in significant cellular injury, subsequently activating the immune response. One of the key immunological consequences observed is an elevated production of leukocytes, particularly lymphocytes, as an adaptive response to oxidative damage. Chronic exposure to acetamiprid has been associated with histopathological alterations in several organs, most notably in hematopoietic tissues. Phogat (2023) documented that such exposure leads to persistent inflammatory states, which in turn stimulate the bone marrow to enhance leukopoiesis, often resulting in lymphocytosis. These findings suggest a dual impact involving both oxidative stress and immune activation.In addition to these indirect effects, acetamiprid has been shown to interact directly with nicotinic acetylcholine receptors (nAChRs) expressed on immune cells, potentially disrupting immune homeostasis. Kim (2024) emphasized that this receptor-level interference may contribute to dysregulated lymphocyte proliferation, thereby posing a risk for immune system imbalance and related pathologies.From a hematological perspective, oxidative stress induced by acetamiprid may also affect red blood cell (RBC) morphology and integrity. Brugrana (2003) demonstrated that such stress could lead to an increase in the mean corpuscular hemoglobin concentration (MCHC), likely due to a decrease in RBC volume not proportionally matched by hemoglobin loss, or due to membrane damage-induced cellular dehydration.Additionally, increased platelet counts (PLT) have frequently been reported following acetamiprid exposure. This hematological shift is attributed to an inflammatory cascade involving interleukin-6 (IL-6), a pro-inflammatory cytokine that stimulates megakaryopoiesis, as indicated by (Ishibashi et al., 2002). Moreover, elevated platelet production may also represent a compensatory mechanism to counteract endothelial injury linked to the vasculotoxic effects of the compound.Changes in mean corpuscular volume (MCV) have likewise been observed, potentially reflecting disrupted erythropoiesis within the bone marrow. Kaferle and Strzoda (2009) noted that elevated MCV values could be due to the premature release of macrocytic erythrocytes, and may also be associated with hepatic dysfunction or deficiencies in vitamin B12 and folic acid, both of which are commonly perturbed in cases of chronic toxic exposure.Furthermore, acetamiprid exhibits myelotoxic properties, as shown by El-Shenawy (2022), who reported that it suppresses the proliferation of erythroid progenitor cells, ultimately leading to anemia, marked by reductions in RBC count and hemoglobin levels (Hb).Compounding this effect, oxidative stress can accelerate erythrocyte destruction (hemolysis) by damaging the cellular membrane, a process confirmed by Badr (2024). This hemolytic activity further exacerbates anemia by reducing both RBC concentration and total hemoglobin content.

The results of the present study are in concordance with previously published findings, thereby substantiating the validity of our observations and reinforcing the scientific basis of the conclusions derived.

Juniperus species are known to contain a variety of bioactive compounds, particularly monoterpenes, such as α-pinene, and flavonoids. These compounds have been extensively studied for their pharmacological activities, including anti-inflammatory, antioxidant, and immunomodulatory effects. However, certain bioactive molecules within *Juniperus* have also been implicated in potential adverse effects, particularly when administered at high doses or over prolonged periods. Notably, such effects may involve the inhibition of hematopoiesis, which consequently affects the production of blood cells, including white blood cells (WBC), red blood cells (RBC), and platelets, within the bone marrow (Amrani et al., 2024; Al-Rehaily et al., 2001). The bone marrow serves as the primary site for the production of blood cells, including leukocytes, lymphocytes, erythrocytes, and platelets. Bioactive compounds capable of impairing bone marrow function can lead to a suppression of blood cell production through mechanisms of direct cytotoxicity on hematopoietic stem cells or by disrupting the microenvironment required for their differentiation (Tiwari & Chauhan, 2020). Hematopoietic suppression may result from interference with the proliferation and maturation of hematopoietic stem cells, leading to a reduction in the levels of blood cells in the peripheral circulation (Al-Snafi, 2015).

The findings of the present study are in concordance with those reported in previous research, thereby supporting the reliability and validity of the observed outcomes.

4.4 Effect of treatment on hormonal parameters levels assessmen

Following a 30-day treatment period, a significant reduction in serum concentrations of estrogen (E2) and progesterone was recorded in the group exposed to Acetamiprid (ACE), with values of 23.67 ± 8.08 pg/mL ($p<0.01$) and 12.33 ± 2.52 ng/mL ($p<0.05$), respectively, compared to the control group (40.66 ± 5.13 pg/mL for E2 and 27.00 ± 5.13 ng/mL for progesterone). Administration of *Juniperus phoenicea* (JNP) extract alone did not produce statistically significant alterations in hormonal levels relative to the control. In contrast, combined treatment with ACE and JNP resulted in a partial recovery of hormone concentrations, as evidenced by increased levels of estrogen (30.00 ± 3.00 pg/mL; $p<0.05$) and progesterone (19.33 ± 2.52 ng/mL), compared to the ACE-treated group.

Table 08: Effect of aqueous extracts of *Juniperus phoenicea* aerial parts and/or Acetameprid treatment on hormonal parameters after 30 days of experience (values represent the mean±SEM of seven rats).

Parametrs	**C**	**JNP**	**ACE**	**ACE+JNP**
Estrogen (E2) (pg/mL)	40.66±5.13	34.70±5.64	23.67±8.08**	30.00±3.00*
Progesterone (ng/mL)	27.00±5.13	29.00±6.24	12.33±2.52*	19.33±2.52
*P< 0,05 **P<0,01 relative to the control group (C)				

Acetamiprid, a systemic neonicotinoid insecticide, has been implicated in the disruption of the hypothalamo-pituitary-gonadal (HPG) axis, thereby exerting deleterious effects on female reproductive endocrinology. Evidence indicates that acetamiprid exposure leads to a significant downregulation of gonadotropin secretion namely luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary. This suppression compromises the physiological stimulation of ovarian follicles, resulting in attenuated biosynthesis of key steroid hormones such as estrogen and progesterone (Chandra et al., 2017; Ochoa-Acuña et al., 2018; Liao et al., 2016). Mechanistically, acetamiprid acts as an agonist of neuronal nicotinic acetylcholine receptors (nAChRs), thereby interfering with normal neurotransmission within the central nervous system. This neural dysregulation can extend to neuroendocrine pathways, impairing the hypothalamic and pituitary regulation of gonadotropin release, and thus indirectly diminishing ovarian steroidogenic capacity (Matsuda et al., 2021; Kapoor et al., 2011). Furthermore, acetamiprid exposure has been shown to induce a state of oxidative stress, marked by an overproduction of reactive oxygen species

(ROS) and a concomitant reduction in antioxidant defense mechanisms. This oxidative imbalance contributes to structural and functional damage within ovarian tissue, particularly in granulosa and theca cells, ultimately impairing steroidogenesis and leading to decreased serum concentrations of estrogen and progesterone (Kapoor et al., 2011; Zhang et al., 2011). Collectively, these findings underscore the multifaceted mechanisms by which acetamiprid may compromise female reproductive health through endocrine disruption and oxidative tissue injury.

The findings of our study are consistent with those of prior research, which demonstrate that acetaminophen induces a reduction in the levels of both progesterone and estrogen.

Several bioactive compounds present in *Juniperus* have been identified to exhibit moderate phytoestrogenic activity, which may contribute to the stabilization of estrogen levels during periods of hormonal decline. A study by Hussein and Elsayed (2015) demonstrated that extracts of *Juniperus* possess such properties, influencing the hormonal equilibrium, particularly with respect to estrogen. Additionally, Murray (1995) highlights in his comprehensive work that *Juniperus* is among the plant species capable of modulating estrogenic activity, suggesting that its bioactive constituents may interact with estrogen receptors to help maintain optimal hormonal balance. This hypothesis is further supported by Sadeghi and Torkzadeh (2019), whose review indicates that *Juniperus* extracts positively affect estrogen levels in laboratory animals, thereby reinforcing the plant's potential role in endocrine regulation.Beyond its estrogenic effects, recent evidence suggests that *Juniperus* extracts may also modulate progesterone levels through indirect endocrine mechanisms. Hussein and Elsayed (2015) reported that, in addition to their phytoestrogenic activity, the bioactive components of *Juniperus* species may influence the hypothalamic-pituitary-gonadal (HPG) axis, a central regulatory system governing the secretion of reproductive hormones, including progesterone. This modulation is hypothesized to be mediated by the presence of flavonoids and terpenoids—bioactive compounds known for their regulatory effects on glandular functions. Supporting this, a systematic review by Sadeghi and Torkzadeh (2019) demonstrated that *Juniperus* extracts enhanced the functionality of the corpus luteum in animal models, thereby promoting endogenous progesterone synthesis. These findings collectively suggest that *Juniperus* may support hormonal homeostasis, particularly under conditions of physiological stress or following exposure to xenobiotic agents.Furthermore, the therapeutic potential of *Juniperus* is further strengthened by its potent antioxidant and anti-inflammatory properties. According to Sadeghi and Torkzadeh (2019), the phytochemical profile of *Juniperus* includes radical-scavenging compounds that mitigate oxidative stress and reduce cellular damage. These antioxidative and anti-inflammatory mechanisms likely contribute synergistically to the regulation of hormonal balance by preserving the structural and functional integrity of endocrine

tissues. As such, *Juniperus* emerges as a promising multi-target botanical agent with potential applications in supporting reproductive health and maintaining endocrine balance.

The findings of our study align with previous research, which demonstrates that Juniperus exerts a moderate influence on the modulation of estrogenic and progesterone activity. This may play a role in restoring hormonal balance in conditions of pesticide exposure.

4.5Effect of treatment on oxidative stress parameters

4.5.1 Glutathione S-Transferase (GST)

- **In the ovary and adrenal gland**

The enzymatic activity of glutathione S-transferase (GST) was significantly decreased in both ovarian and adrenal tissues following exposure to Acetamiprid (ACE), compared to the control group ($p < 0.05$). Co-administration of ACE with the aqueous extract of *Juniperus phoenicea* (ACE+JNP) resulted in a significant restoration of GST activity in the ovary relative to the ACE-treated group; however, enzyme levels did not fully return to those observed in the control. In contrast, GST activity in the adrenal gland did not exhibit statistically significant differences among the ACE+JNP and JNP groups when compared to controls, although a slight, non-significant reduction was noted in both cases. Similarly, administration of *J. phoenicea* extract alone induced a marginal, non-significant decrease in GST activity in ovarian tissue relative to control values.

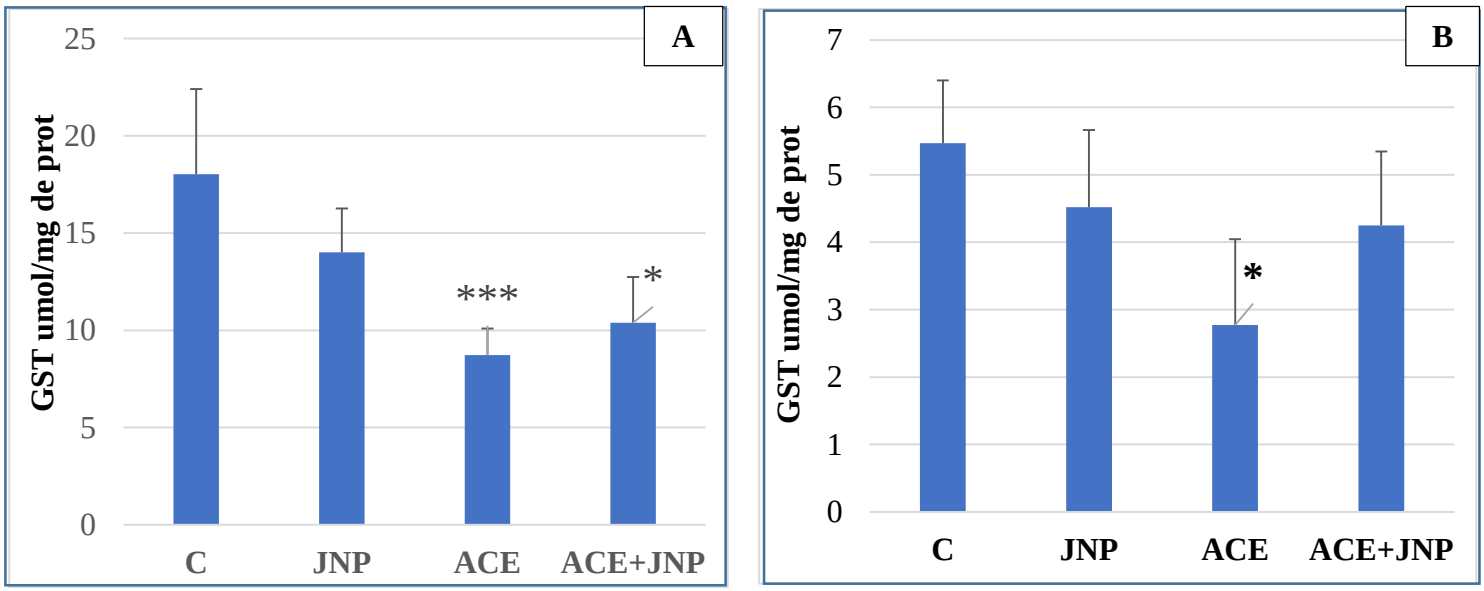


Figure 24: Effect of aqueous extracts of *Juniperus phoenicea* aerial parts and/or Acetameprid treatment on Glutathione S-Transferase (GST) activity in the ovary (A) and the adrenal gland (B) after 30 days of exposure (values represent the mean±SEM of seven rats).

Acetamiprid (ACE), a widely applied neonicotinoid insecticide, has been extensively reported to induce oxidative stress in various biological systems, thereby disrupting the activity of key antioxidant defense mechanisms, including glutathione S-transferase (GST) (Ibrahim et al., 2020). While specific data on the direct impact of acetamiprid on GST activity within ovarian tissue of female rats remain scarce, prior investigations have demonstrated significant suppression of GST activity in other organs, such as the testes of male rats, following exposure to this compound. These observations suggest a broader potential for acetamiprid to impair GST-mediated detoxification processes across reproductive tissues, regardless of sex.The current study corroborates this hypothesis, as a marked decrease in GST activity was detected in the ovarian tissue of rats exposed to acetamiprid, indicating a deleterious effect on the enzymatic detoxification pathway. Moreover, existing literature highlights that acetamiprid exposure may downregulate the expression of genes encoding GST in adrenal cortical cells, thereby diminishing the synthesis of this vital detoxifying enzyme. As a member of the neonicotinoid class, acetamiprid is also increasingly recognized for its endocrine-disrupting potential, which could interfere with hormonal regulatory networks. Such interference may further dysregulate the transcriptional control of genes responsible for antioxidant defense. These mechanisms are supported by multiple experimental studies in rodent models, which have documented significant impairments in antioxidant enzyme activities and modulation of oxidative stress-related gene expression following neonicotinoid exposure (Singh et al., 2018; Ibrahim et al., 2020; Warner et al., 2019; Zhang et al., 2020).

The findings of this study demonstrate that acetamiprid induces a significant decrease in glutathione S-transferase (GST) activity, thereby supporting the hypothesis that this neonicotinoid insecticide contributes to oxidative stress. The observed enzymatic inhibition is likely linked to the downregulation of gene expression involved in detoxification pathways, providing further insight into the compound’s biochemical mechanisms of toxicity.

Currently, there is no direct evidence in the literature demonstrating that *Juniperus phoenicea* specifically decreases glutathione S-transferase (GST) activity within ovarian tissue. Nonetheless, *J. phoenicea* is acknowledged for its antioxidant and antifertility properties, which may indirectly influence the activity of detoxification enzymes such as GST (Benariba et al., 2018). In the present study, a reduction in ovarian GST activity following administration of *J. phoenicea* extract was observed, thereby supporting the proposed hypothesis regarding its modulatory effect on detoxification enzymes. Although investigations focusing on the impact of *Juniperus* extracts on GST gene expression in the adrenal gland are limited, analogous research on phytoestrogens such as genistein and daidzein compounds with structural and functional similarity to bioactive constituents of *Juniperus* has revealed significant modulation of adrenal steroidogenesis. These phytoestrogens

have been shown to inhibit 21-hydroxylase (P450c21), a pivotal enzyme in glucocorticoid biosynthesis, resulting in decreased cortisol production and enhanced androgen synthesis (Cao et al., 2017). Such alterations in steroid hormone profiles may affect the expression and functionality of antioxidant enzymes, including GST (Cui et al., 2020).Furthermore, while *Juniperus* extracts exhibit well-established antioxidant properties, emerging evidence indicates that prolonged or high-dose exposure may paradoxically induce oxidative stress. This phenomenon is attributed to the overaccumulation of bioactive compounds, which promote excessive reactive oxygen species (ROS) generation, thereby overwhelming cellular antioxidant defenses in tissues such as the adrenal gland. The resultant oxidative imbalance may deplete intracellular glutathione (GSH) reserves, consequently impairing GST enzymatic activity. Recent experimental studies have substantiated that both acute and chronic oxidative stress markedly diminish the activity of critical antioxidant enzymes, including GST, particularly within the adrenal cortex (Fenton Navarro et al., 2024).

Consistent with these findings, the present study demonstrates that *Juniperus* extracts contribute to a significant reduction in GST activity in the adrenal glands and the ovary , thereby enhancing our understanding of the complex biochemical effects exerted by this plant.

4.5.2 Reduced glutathione (GSH)

- **In the ovary**

The quantification of reduced glutathione (GSH) levels in ovarian and adrenal tissues demonstrated a statistically significant decline in the Acetamiprid-treated group (ACE) relative to the control group ($p < 0.01$), indicating that Acetamiprid induces oxidative stress associated with depletion of this critical intracellular antioxidant. In contrast, treatment with *Juniperus phoenicea* aqueous extract alone (JNP) resulted in a modest, non-significant elevation of GSH concentrations in both tissues, suggesting a limited intrinsic antioxidant capacity. Notably, co-administration of Acetamiprid and *Juniperus phoenicea* extract (ACE+JNP) led to a partial restoration of GSH levels compared to the ACE group. However, these levels remained significantly lower than those observed in the untreated controls. Collectively, these results imply that *Juniperus phoenicea* may confer a degree of protective efficacy against Acetamiprid-induced oxidative damage, although its modulatory impact on intracellular GSH homeostasis appears to be incomplete.

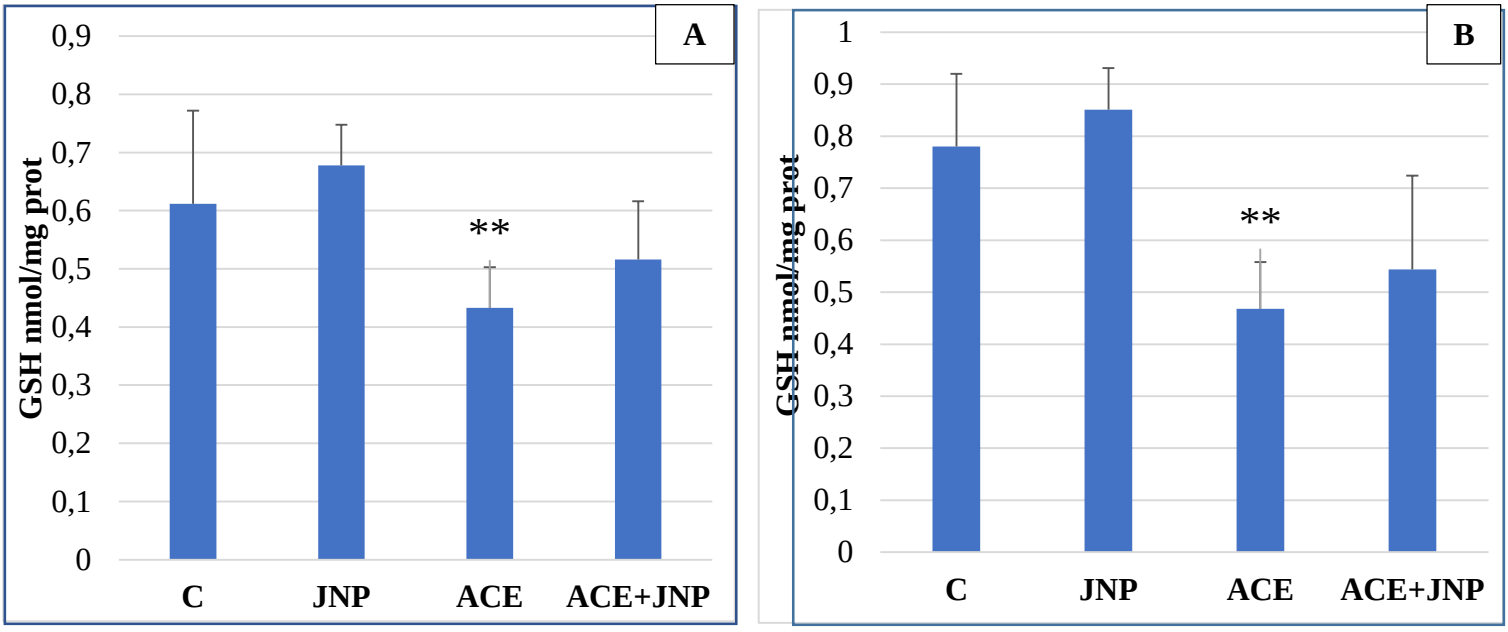


Figure 25: Effect of aqueous extracts of *Juniperus phoenicea* aerial parts and/or Acetameprid treatment on reduced Glutathione level (GSH) in the ovary (A) and the adrenal gland (B)after 30 days of exposure (values represent the mean±SEM of seven rats).

Acetamiprid, a widely utilized neonicotinoid insecticide in agricultural practices, has been increasingly recognized for its role in eliciting oxidative stress and perturbing redox homeostasis within mammalian biological systems. Its toxicodynamic properties are primarily attributed to the overproduction of reactive oxygen species (ROS), which surpass the buffering capacity of endogenous antioxidant mechanisms. A principal indicator of oxidative insult is the marked reduction in intracellular levels of reduced glutathione (GSH), a pivotal non-enzymatic antioxidant essential for preserving cellular redox equilibrium and safeguarding tissues from ROS-mediated cytotoxicity (Costa et al., 2008). Substantial evidence has demonstrated that Acetamiprid exposure significantly diminishes GSH concentrations in various organs, notably the ovaries and adrenal glands, implicating oxidative mechanisms in both its cytotoxic and endocrine-disruptive activities (Abd El-Ghany & Ismail, 2014; Kapoor et al., 2011; Marzban et al., 2021; Eltoweissy et al., 2022).

In ovarian tissues, depletion of GSH compromises the antioxidant defense network, potentially undermining reproductive competence and follicular integrity. Parallel effects have been documented in the adrenal cortex, where Acetamiprid-induced GSH depletion is concomitant with increased lipid peroxidation, denoting a pronounced oxidative challenge (Marzban et al., 2021). Furthermore, the observed oxidative stress is often accompanied by an upregulation of key enzymatic antioxidants, including catalase (CAT) and superoxide dismutase (SOD), which likely reflect an intrinsic compensatory mechanism aimed at mitigating redox disequilibrium (Eltoweissy et al., 2022). These outcomes are consistent with the toxicological profiles of other neonicotinoid compounds such as clothianidin, which similarly disrupt antioxidant systems and impair cellular homeostasis (Bal et al., 2012). Collectively, these findings reinforce the assertion that Acetamiprid poses a significant risk to reproductive and endocrine health via oxidative stress-mediated pathways.

The findings of the present study corroborate existing literature, revealing a marked decrease in reduced glutathione (GSH) levels in both ovarian and adrenal tissues following a 30-day exposure to Acetamiprid. This supports the classification of Acetamiprid as a pro-oxidant compound capable of inducing significant oxidative stress, thereby implicating it in both reproductive and endocrine toxicity.

Juniperus phoenicea has been increasingly recognized for its significant antioxidant properties, particularly in its ability to preserve or enhance intracellular levels of reduced glutathione (GSH), a fundamental component of the cellular redox defense system. Evidence from multiple studies indicates that both the aqueous extract and essential oil of *J. phoenicea* contribute to the mitigation of oxidative stress by stimulating antioxidant enzymatic activity such as glutathione peroxidase and by reducing lipid peroxidation products (Khalil et al., 2017; Zou et al., 2018; El-Massry et al., 2019).

Moreover, recent investigations have demonstrated that administration of *J. phoenicea* extract leads to a marked elevation in GSH concentrations within adrenal tissues, suggesting a protective mechanism that reinforces the endogenous antioxidant defense network (Ganguly et al., 2020). This effect is likely attributed to the plant's phytochemical constituents, including flavonoids and terpenes, which are known for their capacity to neutralize reactive oxygen species and stabilize cellular membranes (Khan et al., 2021). Collectively, these findings support the potential therapeutic application of *J. phoenicea* in the prevention of oxidative damage, particularly in organs vulnerable to redox imbalance such as the ovaries and adrenal glands.

The present study's results are consistent with previous research demonstrating the potent antioxidant properties of *Juniperus phoenicea*, evidenced by a significant increase in glutathione (GSH) levels in ovarian tissues following treatment. This enhancement of intracellular GSH highlights the plant's capacity to modulate oxidative stress and provide cellular protection against oxidative damage. Correspondingly, in adrenal tissues, *J. phoenicea* extract effectively mitigates lipid peroxidation and facilitates the re-establishment of redox equilibrium, thereby counteracting pesticide-induced oxidative injury. These protective effects are attributed to the bioactive compounds present in *Juniperus phoenicea*, which contribute to maintaining cellular integrity and functionality. Overall, these findings underscore the potential therapeutic application of *Juniperus phoenicea* as a natural antioxidant agent for safeguarding reproductive and endocrine tissues from oxidative stress-related damage.

4.5.3 Catalase activity (CAT)

- **In the ovary**

The results demonstrated a significant elevation in catalase (CAT) activity in both the ovaries and adrenal glands of the Acetamiprid-treated group (ACE) compared to the control group (C), indicating an oxidative stress-induced upregulation of antioxidant defense mechanisms. In contrast, the groups treated solely with the aqueous extract of *Juniperus phoenicea* exhibited CAT activity levels comparable to or slightly lower than those of the control group, without reaching statistical significance. Notably, in the co-treated groups (ACE+JNP), CAT activity remained elevated relative to the control group but was significantly reduced compared to the ACE group, suggesting a partial normalization of oxidative status due to the modulatory effect of *Juniperus phoenicea* extract.

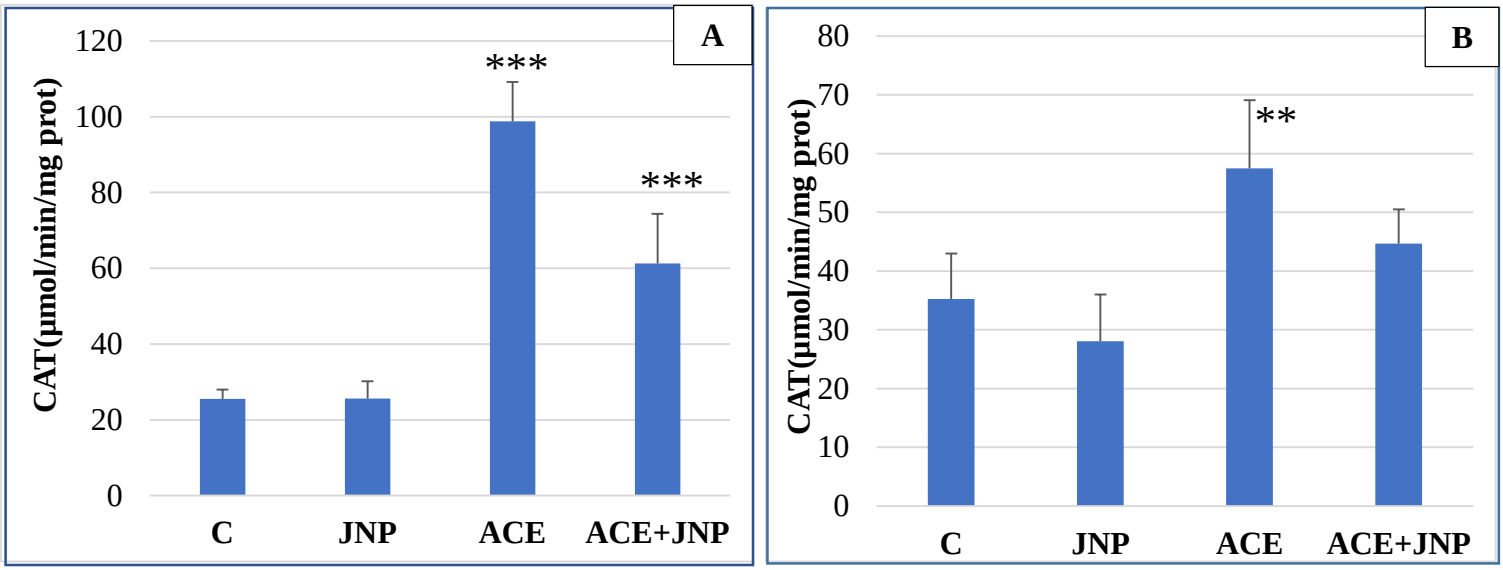


Figure 26: Effect of aqueous extracts of *Juniperus phoenicea* aerial parts and/or Acetameprid treatment on Catalase activity (CAT) in in the ovary (A) and the adrenal gland (B) after 30 days of exposure (values represent the mean±SEM of seven rats).

Acetamiprid, a commonly utilized neonicotinoid insecticide, has been extensively documented to elicit oxidative stress in various mammalian tissues, including the female reproductive system and the adrenal glands. Its mechanism of toxicity involves the disruption of mitochondrial function, resulting in excessive generation of reactive oxygen species (ROS). This redox imbalance subsequently activates cellular antioxidant defense mechanisms. Among these, catalase (CAT) is a key enzymatic antioxidant that catalyzes the decomposition of hydrogen peroxide (H_2O_2) into water and molecular oxygen, thereby mitigating oxidative injury.Several studies have reported significant upregulation of CAT activity in response to Acetamiprid exposure, reflecting a compensatory response to oxidative insult. In ovarian tissues, increased CAT activity has been interpreted as a physiological attempt to counteract oxidative stress and maintain redox equilibrium (Eltoweissy et al., 2022; Gasmi et al., 2019). Similarly, in the adrenal glands, elevated CAT activity is associated with Acetamiprid-induced oxidative burden and may serve to protect adrenal cells from ROS-mediated cytotoxicity and apoptosis (Kapoor et al., 2020; Ibrahim et al., 2022; Karmakar et al., 2009). These findings highlight catalase as a critical biomarker of oxidative perturbation in both reproductive and endocrine tissues following exposure to Acetamiprid.

The present study demonstrates tissue-specific alterations in catalase (CAT) activity following exposure to acetamiprid, consistent with previous findings. In ovarian tissue, a significant reduction in CAT activity was observed, indicative of impaired redox homeostasis and a compromised antioxidant defense system. In contrast, a marked elevation in CAT activity was detected in endocrine glands, particularly the adrenal glands, suggesting an adaptive enzymatic response to acetamiprid-induced oxidative stress. This upregulation likely reflects a compensatory mechanism aimed at counteracting the increased generation of reactive oxygen species (ROS) and mitigating cellular oxidative damage. Collectively, these results highlight the differential modulation of antioxidant defenses by acetamiprid across distinct tissue types.

The combined administration of *Juniperus phoenicea* extract with acetamiprid resulted in a notable attenuation of catalase (CAT) activity in both ovarian and adrenal tissues when compared to the acetamiprid-only group. This reduction suggests a partial restoration of oxidative balance and highlights the protective role of *J. phoenicea* against acetamiprid-induced oxidative stress in endocrine organs. The extract's high content of polyphenolic and flavonoid compounds, recognized for their potent antioxidant and free radical scavenging activities, likely underpins this protective effect. Although direct studies on ovarian and adrenal tissues remain limited, previous research has demonstrated the systemic antioxidant potential of *J. phoenicea*. For example, Ben Hsouna et al. (2017) reported a reduction in hepatic oxidative damage, while Elshazly et al. (2020) documented normalization of antioxidant enzyme activities across multiple organs following oxidative stress

induction. Despite these promising effects, the persistent elevation of CAT activity in the co-treated group relative to controls indicates that the ameliorative capacity of *J. phoenicea* is partial, possibly reflecting the severity of oxidative insults induced by acetamiprid exposure (Bouaziz et al., 2020; Hmidani et al., 2019; Ibrahim et al., 2022; Kapoor et al., 2020).

The present data corroborate the findings of our study, demonstrating that co-administration of *Juniperus phoenicea* extract significantly mitigates the deleterious oxidative stress induced by acetamiprid exposure. This suggests a partial protective effect of the extract, highlighting its potential efficacy in attenuating oxidative imbalance and associated cellular damage.

4.6Histological Evaluation

4.6.1 Histological evaluation of the Ovary

Histopathological analysis of ovarian sections revealed significant structural alterations in rats exposed to acetamiprid, in comparison with both the control and the protective treatment groups.

In the control group (C) and the group receiving the aqueous extract of *Juniperus phoenicea* (JNP) (Figures 27(A–B)), the ovarian architecture was well maintained. Multiple follicles at various developmental stages (primordial, primary, secondary, and antral) were observed, exhibiting a normal organization of granulosa cells, an intact zona pellucida, and well-preserved ovocytes. The ovarian stroma appeared homogeneous and moderately vascularized, with no evidence of inflammatory cell infiltration or vascular congestion.

Conversely, the acetamiprid-exposed group (ACE) (Figure 27(C)) demonstrated pronounced disruption of ovarian tissue architecture. Severe follicular atresia (represented by the plus symbol in the figure), marked vascular congestion, and extensive degeneration of follicular cells (indicated by yellow arrows) were evident. These degenerative changes were characterized by cytoplasmic vacuolization, nuclear pyknosis, and focal hemorrhagic areas (denoted by yellow stars). The stromal tissue was densely infiltrated and showed considerable structural disorganization.

Notably, in the group treated with the aqueous extract of *Juniperus phoenicea* following acetamiprid exposure (Figure 27(D)), substantial restoration of ovarian architecture was observed. Despite the persistence of mild stromal atrophy and moderate follicular atresia, the majority of ovarian follicles displayed near-normal morphology. The stroma remained moderately vascularized, with minimal signs of inflammatory infiltration, indicating a partial protective effect of juniper extract against acetamiprid-induced ovarian toxicity.

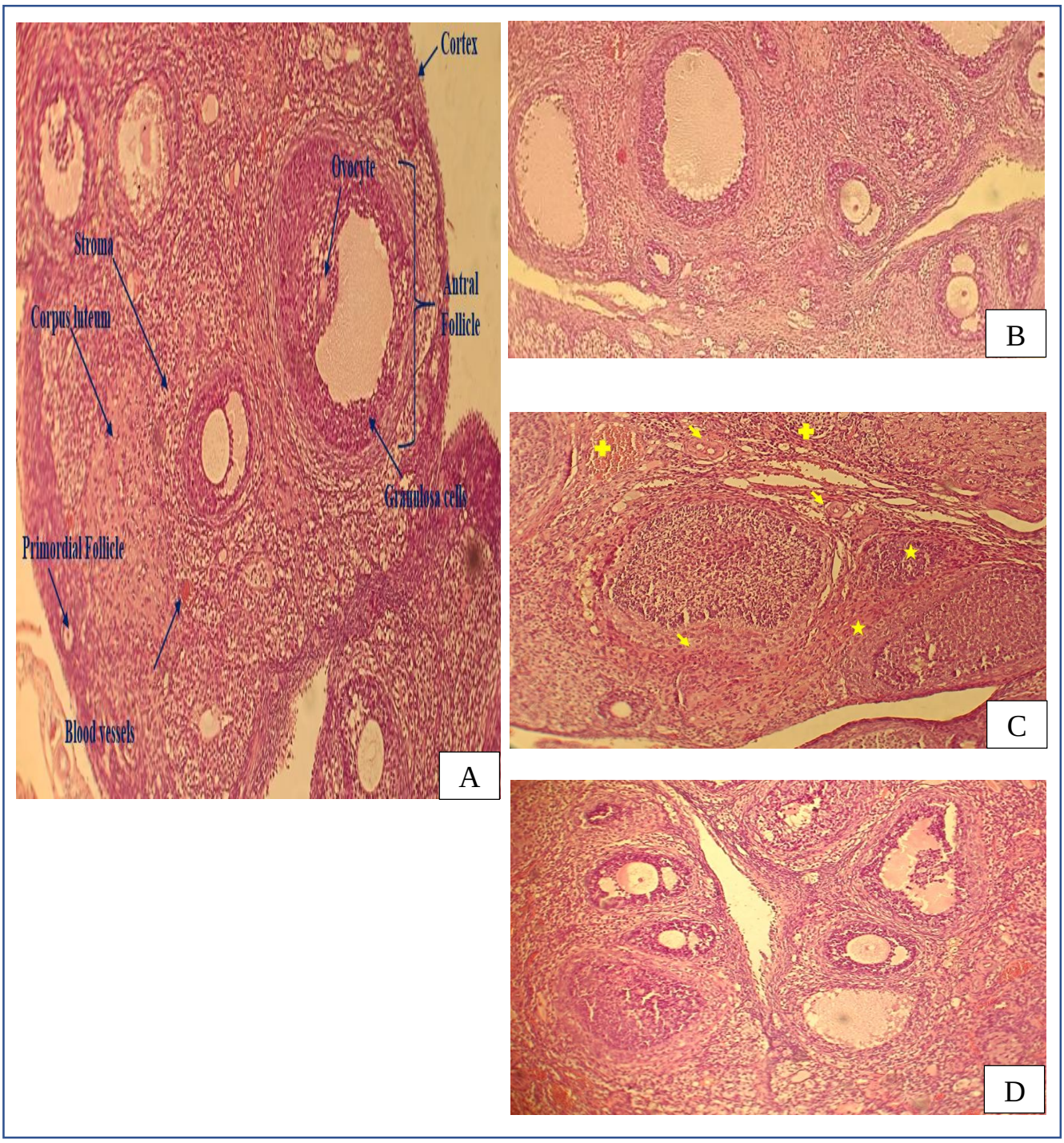


Figure 27: Histological Evaluation of the Ovary

The present study demonstrates that chronic exposure to acetamiprid exerts significant toxic effects on ovarian tissue through a multifaceted disruption of endocrine function, and histological integrity. Biochemical assays revealed a marked depletion of glutathione (GSH) levels accompanied by a compensatory increase in catalase (CAT) activity, indicative of an adaptive antioxidative response to elevated reactive oxygen species (ROS) accumulation, consistent with findings by (Ghazouani et al., 2023) This redox imbalance precipitates damage to essential cellular macromolecules, including proteins, lipids, and nucleic acids, thereby impairing ovarian cellular function. Concurrent endocrine assessments revealed a significant reduction in circulating progesterone and estrogen concentrations, reflecting disruption of the hypothalamic-pituitary-ovarian axis and resulting in impaired folliculogenesis characterized by follicular atresia or early arrest of follicular development (Kumar et al., 2011). Histopathological analysis corroborated these biochemical and hormonal alterations, revealing hallmark features of cellular toxicity such as nuclear pyknosis, karyorrhexis, focal hemorrhages, and cytoplasmic vacuolization, indicative of activated apoptotic and necrotic pathways (Veterinary World, 2014). Additionally, pronounced vascular congestion and infiltration of inflammatory cells within ovarian stroma suggest a secondary inflammatory response, supporting the premise that oxidative stress acts as a principal mediator of chronic inflammation in affected tissues (Sakr et al., 2014). Collectively, these findings substantiate that acetamiprid exposure leads to profound structural and functional compromise of ovarian tissue, posing a significant risk to female reproductive health.

Histopathological examination of ovarian tissue demonstrated that administration of *Juniperus phoenicea* extract following acetamiprid exposure significantly ameliorated ovarian structural integrity compared to the group exposed solely to acetamiprid. Histological sections (Figure D) revealed follicles at various developmental stages with near-normal morphology, accompanied by a marked reduction in follicular atresia and an absence of pronounced inflammatory cell infiltration or hemorrhagic lesions, indicating the extract's protective efficacy. This histological improvement is likely mediated by the potent antioxidant properties of flavonoids and terpenoids present in *Juniperus phoenicea*, which mitigate reactive oxygen species (ROS)-induced oxidative stress and consequent cellular damage (Boudjelal et al., 2015; Bakour et al., 2020). Furthermore, the extract's anti-inflammatory effects contribute to restoring tissue homeostasis and promoting repair mechanisms, as evidenced by the enhanced organization of granulosa cells and preservation of vascular structures in treated specimens (El-Bahr et al., 2021). Collectively, these findings underscore the therapeutic potential of *Juniperus phoenicea* extract as a protective agent against pesticide-induced ovarian toxicity and emphasize the relevance of medicinal plants as complementary strategies in mitigating environmental toxicants.

4.6.2 Histological evaluation of the Adrenal Gland

The histopathological analysis of adrenal gland sections demonstrated distinct morphological differences across the four experimental groups.

In the control group (C) and the group treated with the aqueous extract of *Juniperus phoenicea* (JNP), the adrenal glands retained normal histological architecture. The cortical regions including the zona glomerulosa, zona fasciculata, and zona reticularis were well delineated and structurally organized, while the medulla remained intact. Cortical cells appeared orderly and exhibited no signs of cytoplasmic vacuolization or cellular abnormalities (Figures 28(A–B)).

Conversely, adrenal tissues from rats exposed solely to acetamiprid (ACE) showed severe structural disruption. Histological alterations included extensive cytoplasmic vacuolization, pronounced disorganization of cortical zonation (indicated by yellow stars), vascular congestion or focal hemorrhages, and evidence of cellular degeneration or necrosis (yellow arrows), indicating significant adrenocortical toxicity (Figure 28(C)).

In the group co-administered acetamiprid and the *Juniperus phoenicea* aqueous extract (ACE+JNP), partial histological recovery of the adrenal cortex was observed. Although mild vacuolization persisted, the cortical zones appeared relatively restored with improved tissue organization. These findings suggest a moderate protective effect of the juniper extract against acetamiprid-induced adrenal toxicity (Figure 28(D)).

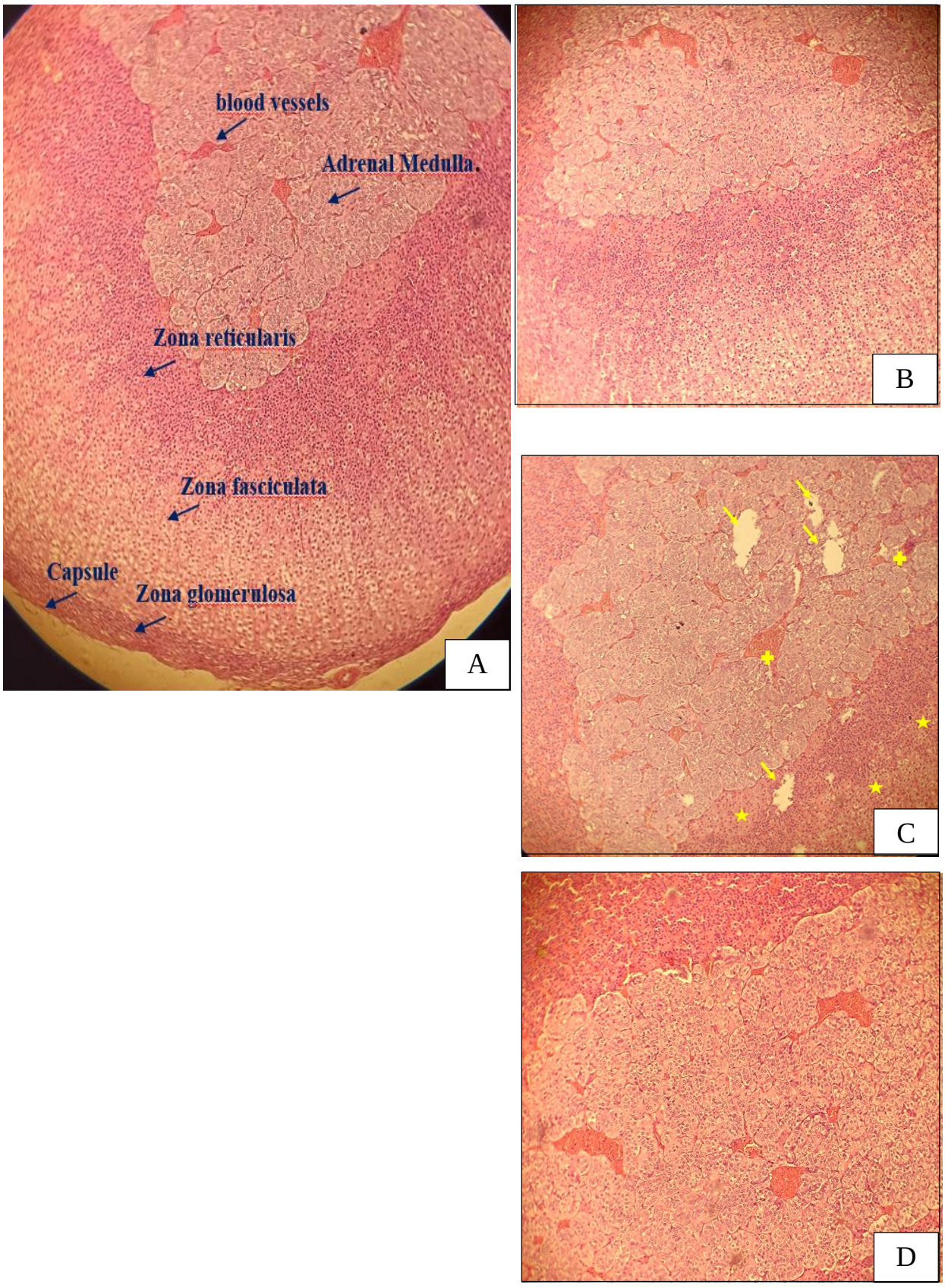


Figure 28: Histological Evaluation of the Adrenal Gland

The findings of our study corroborate previous reports demonstrating that chronic exposure to acetamiprid elicits pronounced structural alterations in adrenal gland tissue through multiple interconnected pathogenic mechanisms, chiefly oxidative stress, mitochondrial dysfunction, and disruption of neuroendocrine homeostasis. Acetamiprid, a neonicotinoid insecticide, functions as a potent agonist of nicotinic acetylcholine receptors (nAChRs), resulting in their sustained hyperactivation and elevated intracellular calcium influx. This dysregulation precipitates excessive generation of reactive oxygen species (ROS) and consequent mitochondrial impairment (Tomizawa & Casida, 2005). The ensuing oxidative damage targets lipids, proteins, and nucleic acids, manifesting histopathologically as cytoplasmic vacuolation, disorganization of the adrenal cortical layers, vascular congestion, and prominent necrotic lesions (Bal et al., 2017; El-Murr et al., 2020). Moreover, persistent oxidative insult compromises the activity of steroidogenic enzymes within the adrenal cortex zones (glomerulosa, fasciculata, and reticularis), thereby disrupting steroid hormone biosynthesis. The observed vascular congestion and hemorrhagic events are plausibly linked to increased vascular permeability and endothelial injury secondary to inflammation induced by oxidative stress (Zhang et al., 2021; Sakr et al., 2014). Collectively, these mechanisms elucidate the acute adrenal toxicity of acetamiprid, as evidenced by the severe and multifaceted histopathological alterations documented in our experimental observations.

In the cohort of rats receiving combined treatment with acetamiprid and aqueous extract of *Juniperus phoenicea*, histopathological examination demonstrated a notable amelioration in the structural integrity of the adrenal cortex. Specifically, a partial reconstitution of the cortical zones was observed despite the persistence of mild cytoplasmic vacuolization (Eidi et al., 2006). The observed protective effects of *Juniperus phoenicea* are primarily attributed to its documented antioxidant, anti-inflammatory, and membrane-stabilizing properties, as corroborated by recent phytotherapeutic research (El Kharrassi et al., 2011; Zhang et al., 2021). The bioactive constituents, including flavonoids, terpenoids, and phenolic compounds, play a critical role in attenuating oxidative stress through the neutralization of reactive oxygen species (ROS), thereby preserving cellular homeostasis and function (Ahmad et al., 2019; Kumar et al., 2022).

Moreover, these phytochemicals contribute to the stabilization of cellular membranes and mitochondrial protection, thereby mitigating the cytotoxic effects induced by acetamiprid exposure (Bal et al., 2017; Singh et al., 2023). Collectively, these findings substantiate the hypothesis that *Juniperus phoenicea* extract confers partial histoprotective effects against acetamiprid-induced adrenal toxicity, underscoring the therapeutic potential of medicinal plant extracts as adjunctive interventions to counteract pesticide-induced oxidative damage (Tomizawa & Casida, 2005; Li et al., 2020).

Conclusion and perspective

This study provides compelling evidence of the deleterious effects of the neonicotinoid insecticide acetamiprid on the hematological and endocrine systems in a rat model. Subchronic exposure to acetamiprid induced significant physiological alterations, including reduced body weight, statistically significant changes in the masses of reproductive organs and adrenal glands, hyperglycemia, and perturbations in the serum levels of estrogen and progesterone. Furthermore, oxidative stress assessments and histopathological examinations revealed a decline in antioxidant systems alongside marked morphological alterations in ovarian and adrenal tissues.

Therapeutically, the aqueous extracts of *Juniperus phoenicea* exhibited a marked efficacy in ameliorating these biochemical and physiological parameters, highlighting its antioxidant and anti-inflammatory properties and its potential role in mitigating the adverse effects induced by acetamiprid toxicity.

In light of these results, *Juniperus phoenicea* extract emerges as a promising natural candidate for protecting immune and endocrine functions against neonicotinoid-induced toxic insults. Nevertheless, further investigations are essential to elucidate the precise molecular mechanisms involved, validate its efficacy in clinical settings, and extend biochemical and histological analyses to fully characterize its protective effects and safety profile.

However, further comprehensive studies are necessary to:

- The advancement and refinement of extraction methodologies to improve the efficiency and specificity of isolating active acetamiprid constituents from environmental and agricultural matrices.
- The implementation of detailed analytical investigations utilizing state-of-the-art techniques for accurate qualitative and quantitative characterization of these bioactive compounds.

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