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Effect of natural antioxidant extracts on mood, memory and learning in adult stressed mice

Presented by:

- **BENHAMMADA Ikram**
- **SELLAMI Yousra**

Supervised by the jury:

President:	**Mrs. RIHANI Lamia**	**MCA**
Examiner :	**Mr. KEHILI Houssem Eddine**	**MCB**
Supervisor :	**Mr. DERBOUCHE Hilal**	**MAA**

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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

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First of all, we thank Almighty God for granting us health, willpower, patience, and courage to successfully complete this work.

عن النبي صلى الله عليه و سلم : (من لم يشكر الناس لم يشكر الله)

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Dedication

بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

﴿قَدْ جَعَلَهَا رَبِّي حَقًّا﴾

All praise is due to Allah, who has granted us success by His will and grace. To Him belong all merit, perfection, and accomplishment.

I dedicate this success first to myself, To that little girl who never grows up, who still dwells—along with her dreams—deep within each of us...In the hope that we may grow enough to fulfill those dreams.

To the residents of my heart:

*To those who placed their trust in me, to the backbone of our family, my dear father **Azzeddine** ,the one whose strength held us together, who stood tall through every storm, and whose presence made our home feel safe.You have always been by my side, never ceasing to support and encourage me, always striving for my comfort.You are my pillar and my pride.And to the peace of our home, My soul and my love mother **Houria,** who dedicated her life to my success —May Allah bless you both with long life, health, and well-being.*

*" 'To those who loved me unconditionally.To the light that shines through my darkness when the days and life try to extinguishme.To the clouds that shade and nourish me without ever expecting anything in return.To the hands that reach out when I stumble, and push me to carry on despite all that calls me to fall To my beloved sisters and brothers: **Rima, Nabil, Rawia, Rahma, Hala, and DJamil.** My brother's wife I love you, my family'*

" To the one to whom there is no sunset, My refuge and safety "

*To the little jewels of our family**: my princess Raghad**, **My sunshine DJihan**, and the little men of our family: **Raed**, **Charfadine, Abdelraouf**, and **Ishaq**.*

*To my dear cousin, my flower **Yasmin; Aïcha, Aya and mimi l;my precious friends Amina, Roumissa ;**my beautiful worlds, **Telegram and Basty**;childhood friends and classmates —I shall not forget th**e Faditia** family.*

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To all who offered a kind word or a sincere prayer...

This success is yours, from you, and because of you

ikram

Dedication

بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

﴿ وَقُلْ رَبِّ زِدْنِي عِلْمًا ﴾

Praise be to Allah, by whose grace good deeds are completed. Praise be to Allah, who illuminated our path with knowledge and patience, and brought us to this moment of harvest after long nights of struggle and perseverance.

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Thank you for every tearful prayer, every gentle touch, every proud glance.Thank you for being my companions through joy and hardship, the light in my darkest nights.

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And here is the final touch that crowns this dedication with the beauty of self-recognition:

*And finally. **… to myself***

Yousra

Abstracts

This study aims to explore the neuroprotective effects of natural plant extracts rich in antioxidants on behavioral and cognitive functions (mood, learning, memory) in adult mice exposed to chronic stress, used as a model to simulate stress-related disorders in humans.

Three plants known for their physiological and biochemical properties were selected: Aristolochia clematitis, Rosmarinus officinalis L., and Ephedra alata.Various extracts were prepared and administered at different doses, and the mice were subjected to a chronic stress behavioral model. Behavioral and cognitive performance was assessed through five standardized tests: Open Field Test, Elevated Plus Maze, Forced Swim Test, Morris Water Maze, and Novel Object Recognition Test.

Based on the behavioral results obtained in mice , the study revealed a significant variation in the effectiveness of the plant extracts (AC, RO, EA) in alleviating stress-related disorders. *Aristolochia clematitis* extract at 200 mg/kg (AC200) showed overall superiority, recording the highest values in the Open Field Test (56.3 seconds) and the Elevated Plus Maze (44.5%), indicating notable anxiolytic properties. It also showed performance close to the control group in the Forced Swim Test (63.3 seconds) and Morris Water Maze (3 seconds), supporting its potential role as an antidepressant and spatial memory enhancer. These findings make it one of the most promising extracts, warranting further studies to confirm its efficacy and safety.

In contrast, *Ephedra alata* extract at 100 mg/kg (EA100) showed the weakest performance, with increased anxiety indicators (12 seconds in the Open Field Test).Meanwhile, *Rosmarinus officinalis* extract at 200 mg/kg (RO200) demonstrated promising anxiolytic activity (53.3 seconds, 36.6%), positioning it as a potential candidate for supporting psychological and cognitive balance.

Key words : Chronic stress – Antioxidants – Plant extracts – Behavioral and cognitive functions – Behavioral and cognitive tests – Oxidative stress – Neuroprotection – *Aristolochia clematitis L– Rosmarinus officinalis L. – Ephedra alata*.

ملخص

تهدف هذه الدراسة إلى استكشاف التأثيرات العصبية الوقائية لمستخلصات نباتية طبيعية غنية بمضادات الأكسدة على الوظائف السلوكية والمعرفية (المزاج، التعلُّم، الذاكرة) لدى فئران بالغة تعرضت للإجهاد المزمن كنموذج لمحاكاة الاضطرابات المرتبطة بالتوتر عند الإنسان.

تم اختيار ثلاثة نباتات معروفة بخصائصها الفيزيولوجية والبيوكيميائية، وهي: *Aristolochia clematitis L*، *Rosmarinus officinalis L*، و*Ephedra alata*. تم تحضير مستخلصات متنوعة وتجهيزها بجرعات مختلفة، ثم أُخضعت الفئران لنموذج سلوكي للإجهاد المزمن. تمت متابعة الأداء السلوكي والمعرفي عبر خمس اختبارات معيارية: الميدان المفتوح، المتاهة المرتفعة ذات الأذرع، السباحة القسرية، متاهة موريس المائية، واختبار التعرف على الشيء الجديد.

بناءً على نتائج الاختبارات السلوكية المُجراة على القوارض، كشفت الدراسة عن تفاوتٍ ملحوظ في فعالية المستخلصات النباتية (AC ،RO ،EA) في التخفيف من الاضطرابات المرتبطة بالضغط النفسي. تفوّق مستخلص *Aristolochia clematitis* بجرعة 200 مغ/كغ (AC200) بشكل عام، حيث سجّل أعلى القيم في اختبار الحقل المفتوح (56.3 ثانية) والمتاهة المرتفعة (44.5%)، مما يدل على خصائصه المميّزة في تقليل القلق. كما أظهر أداءً قريبًا من المجموعة الضابطة في اختبار السباحة القسري (63.3 ثانية) والمتاهة المائية (3 ثوانٍ)، مما يدعم دوره المحتمل كمضاد للاكتئاب ومحسّن للذاكرة المكانية، مما يجعله من بين أكثر المستخلصات الواعدة ويستدعي إجراء دراسات أعمق لتأكيد فعاليته وسلامته.

في المقابل، أظهر مستخلص *Ephedrla aata* بجرعة 100 مغ/كغ (EA100) أضعف أداء، حيث تفاقمت مؤشرات القلق (12 ثانية) في الحقل المفتوح، أما مستخلص *Rosmarinus officinalis* بجرعة 200 مغ/كغ (RO200)، فقد أظهر فعالية واعدة كمضاد للقلق (53.3 ثانية، 36.6) . ممّا يجعله مرشحًا محتملاً لدعم التوازن النفسي والمعرفي.

الكلمات المفتاحية : الإجهاد المزمن – مضادات الأكسدة – المستخلصات النباتية – الوظائف السلوكية والمعرفية – الاختبارات السلوكية والمعرفية – الإجهاد التأكسدي – الحماية العصبية – *Aristolochia clematitis L*– إكليل الجبل (*Rosmarinus officinalis*) – العلندا (*Ephedra alata*) .

Résumé

Cette étude vise à explorer les effets neuroprotecteurs d'extraits végétaux naturels riches en antioxydants sur les fonctions comportementales et cognitives (humeur, apprentissage, mémoire) chez des souris adultes soumis à un stress chronique, utilisé comme modèle simulant les troubles liés au stress chez l'homme.

Trois plantes connues pour leurs propriétés physiologiques et biochimiques ont été sélectionnées *: Aristolochia clematitis, Rosmarinus officinalis L., et Ephedra alata.*
Des extraits variés ont été préparés et administrés à différentes doses, puis les souris ont été soumis à un protocole de stress chronique. Les performances comportementales et cognitives ont été évaluées à l'aide de cinq tests standards : champ ouvert, labyrinthe en croix surélevé, nage forcée, labyrinthe aquatique de Morris, et test de reconnaissance d'objet nouveau.

Les résultats comportementaux obtenus ont révélé une variation marquée dans l'efficacité des extraits (AC, RO, EA) à atténuer les troubles induits par le stress. L'extrait de *Aristolochia clematitis L* à la dose de 200 mg/kg (AC200) s'est distingué globalement, enregistrant les meilleures performances dans le test du champ ouvert (56,3 secondes) et le labyrinthe surélevé (44,5 %), indiquant un effet anxiolytique notable. Il a également présenté des résultats proches du groupe témoin dans les tests de nage forcée (63,3 secondes) et du labyrinthe aquatique (3 secondes), suggérant un effet potentiel antidépresseur et une amélioration de la mémoire spatiale. Ces résultats en font l'un des extraits les plus prometteurs, nécessitant des recherches plus approfondies pour confirmer son efficacité et sa sécurité.En revanche, l'extrait de *Ephedra alata* à 100 mg/kg (EA100) a montré les résultats les plus faibles, avec une aggravation des indicateurs d'anxiété (12 secondes dans le test du champ ouvert).Quant à l'extrait *de Rosmarinus officinalis L* à 200 mg/kg (RO200), il a montré une efficacité anxiolytique encourageante (53,3 secondes, 36,6 %), ce qui le positionne également comme un candidat intéressant pour le soutien des fonctions psychiques et cognitives.

Mots clés: Stress chronique – Antioxydants – Extraits de plantes – Fonctions comportementales et cognitives – Tests comportementaux et cognitifs – Stress oxydatif – Neuroprotection – Aristolochia clematitis L – Rosmarinus officinalis L. – Ephedra alata .

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List of abbreviations

%: Percentage

°C: Degree Celsius

'02: Singlet oxygen

AC: Aristolochia clematitis L

ACC: Anterior Cingulate Cortex

ACTH: AdrenoCorticoTropic Hormone

AM: Ante Meridiem (before noon)

BALB/c: Laboratory mouse strain used in experiments

BDNF: Brain-Derived Neurotrophic Factor

C_5H_8 Isoprene

CAT : Catalase

Cm : Centimetre

CO_2 Carbon Dioxide

CRH: Corticotropin-Releasing Hormone

Cu^{2+}: Cupric Ion

DI: Discrimination Index

DNA: Deoxyribonucleic Acid

E alata: Ephedra alata subsp.alenda

EPM: Elevated Plus Maze

Fe^{2+}: Ferrous Ion

FST: The Forced Swim Test

g : Gram

GPx: Glutathione Peroxidase

GSH: Glutathione (Reduced)

GSSG: Glutathione Disulfide (Oxidized)

H : Hydrogen

h: hour

H: Hydrogen

H_2O_2: Hydrogen Peroxide

HNO: Nitroxyl

HNO_2: Nitrous acid

HOCI: Hypochlorous acid

Hoc : Hypochlorous acid

HOO: Hydroperoxyl radical

HPA: Axe Hypothalamo-Hypophyso-Surrénalien

L: Ligand (general symbol for an organic ligand)

LDL: Low-Density Lipoprotein

LO: Alkoxyl radical

LOO: Lipid peroxyl

LOOH: Lipid hydroperoxide

LOOO: Likely a Triperoxyl radical (a type of organic peroxide)

Me: Mass of the dry extract (g)

Mg: Milligram

Mg/kg: Milligram per kilogram

Ml: Millilit**re**

Mn: Manganese

Mp: Mass of the plant powder used (g)

MWM: Morris Water Maze

N: Number

N2O2: Dinitrogen dioxide

N_2O_3: Dinitrogen trioxide

NaCl: Sodium chloride

NH_2: Amine Group

NO: Nitric Oxide

NO^-: Nitroxyl anion

NO^+: Nitrosonium ion

NO_2: Nitrogen dioxide

NO_2Cl: Nitryl chloride

NOR: Novel Object Recognition

NOS: Nitric Oxide Synthase

Nrf2: Nuclear factor erythroid 2–related factor 2

O_2: Dioxygen

$O2^-$: Superoxide anion

O3: Ozone/trioxygen

OFT: Open field test

OH: Hydroxyl radical

$ONOO^-$ Peroxynitrite

ONOOH: Peroxynitrous acid

P: Phosphorus

PFC: PreFrontal Cortex

PM: Post Meridiem (after noon)

PNS : Système Nerveux Parasympathique

RNS : Reactive Nitrogen Species

RO: Alkoxyl radicals

RO: Rosmarinus officinalis L

ROO: Alkylperoxyl radical

ROS: Reactive Oxygen Species

RS: Thiyl radical

Se : Selenium

SNS : Système Nerveux Sympathique

SOD: Superoxide Dismutase

v/v : Volume/Volume

Zn : Zinc

Introduction

Introduction

Chronic stress has become one of the most pressing challenges to mental health in modern times, due to the increasing exposure to social and psychological pressures. Stress is defined as a disruption of the body's internal homeostasis caused by exposure to unpleasant or harmful stimuli **(Mohseni-Moghaddam et al., 2022).** Although stress is not considered a disease in itself, its negative effects extend to various bodily systems, including the immune, autonomic, and endocrine systems, as well as behavior **(Mohseni-Moghaddam et al., 2022).**

Numerous studies have shown that chronic stress is a major risk factor in the onset and progression of several mental disorders, such as anxiety and depression, as well as cognitive impairments such as reduced learning and memory capacity **(Mohseni-Moghaddam et al., 2022).** Neurologically, prolonged stress induces neurochemical changes that affect key brain areas, particularly the prefrontal cortex (PFC) and hippocampus, both of which are strongly involved in emotion regulation, learning, and memory formation **(Ashok et al., 2023).**

Evidence shows that chronic stress not only impacts neural structure but also interferes with vital cellular functions, particularly mitochondrial activity. It promotes the overproduction of reactive oxygen species (ROS), which triggers oxidative stress a major contributor to neuronal damage **(Ashok et al., 2023).**

Free radicals are unstable molecules generated during essential biological processes such as metabolism, cellular signaling, and neurotransmitter breakdown. They can also result from external factors such as pollution, smoking, and radiation. When excessively produced, free radicals cause damage to lipids, proteins, and DNA, especially in neurons, which are highly susceptible due to their elevated metabolic rate and limited repair mechanisms **(Ashok et al., 2023).**

To counter these harmful effects, the body relies on its antioxidant defense system molecules that neutralize free radicals and limit cellular damage. However, in chronic stress or neurological conditions, this defense system may become insufficient, necessitating the use of external antioxidants. Studies have shown that natural antioxidants improve bioavailability, enhance neuroprotective efficacy, and help restore oxidative balance **(Ashok et al., 2023).**

In this context, rodents—especially mice—are among the most commonly used models in neuroscience and behavioral research due to their physiological and behavioral responses, which closely mirror those of humans under psychological stress, particularly in conditions such as anxiety, depression, and cognitive dysfunctions **(Murthy & Gould, 2018; Gencturk & Unal,**

2024). These models are developed by exposing mice to various types of acute or chronic stress, such as restraint or unpredictable stress, which activates the HPA axis and increases corticosterone levels, both of which are biomarkers commonly found in psychiatric disorders **(Willner, 2017).**

Behavioral outcomes of such stress can be assessed using standardized and validated tests like the Elevated Plus Maze (EPM), Forced Swim Test (FST), and Tail Suspension Test (TST) to evaluate depressive-like symptoms, in addition to the Morris Water Maze and Novel Object Recognition test to assess learning and memory **(Belzung & Lemoine, 2011).** These behavioral paradigms are widely recognized for their high face and predictive validity **(Belzung & Lemoine, 2011).**

Therefore, using mice in this context represents a powerful tool to evaluate the effectiveness of natural antioxidant compounds in mitigating the behavioral and biological consequences of chronic stress, opening promising perspectives for the development of natural therapies for psychiatric and neurological disorders **(Flandreau et al., 2023)**

Accordingly, this study was carried out on murine models (mice) in order to investigate the significant role and effectiveness of natural antioxidants derived from three medicinal plants—Ephedra alata, Aristolochia clematitis L., and Rosmarinus officinalis—which are traditionally recognized for their high antioxidant content and diverse therapeutic properties.

The main objectives of this study are as follows:

1- Evaluation the effects of natural antioxidants on mood, memory, and cognitive abilities in mice exposed to chronic stress.
2- Analysis of behavioral, cognitive, and learning functions using a set of standardized behavioral tests.
3- Contribution to the development of a natural dietary supplement that enhances cognitive performance and improves mood by reducing the impact of oxidative stress.
4- Proposal for the preparation of a natural calming tea made from a blend of extracts of the three studied plants, as a simple, safe, and effective means to alleviate stress and promote psychological well-being.

Part One. *Literature Review*

Chapter 01:

Understanding Stress: Brain Mechanisms, Cognitive and emotional impact, and Experimental Testing in mice

1. Definition of Stress:

Stress is defined as the nonspecific response of the body to any demand, whether physical, emotional, or environmental. It is a complex physiological and psychological reaction that can be triggered by both internal and external factors. Stress is not limited to emotional tension but includes a wide range of responses involving the nervous, endocrine, and immune systems. Stress can occur even in the absence of a nervous system, as seen in plants and microorganisms, and thus should be viewed as a universal biological response to any kind of challenge or disturbance **(Fink., 2016).**

2. Types of stress:

2.1. Based on the duration of stress:

It can be classified into three main types :

- **Acute Stress:**

Acute stress is a short-term reaction to an immediate perceived threat or challenge, commonly known as the "fight-or-flight" response. It is characterized by an intense but brief activation of the body's stress response systems, particularly the Sympathetic Nervous System (SNS) and the Hypothalamic-Pituitary-Adrenal (HPA) axis. During this response, stress hormones such as adrenaline and cortisol are rapidly released, preparing the body to deal with the situation quickly and effectively. This reaction aligns with the "alarm reaction" stage in the General Adaptation Syndrome **(Selye, 1956)**.

- **Episodic acute stress:**

Episodic acute stress refers to the experience of frequent episodes of acute stress. Individuals with this type of stress often lead chaotic or crisis-prone lifestyles, or they may suffer from persistent worries and negative thinking patterns. As a result, their bodies repeatedly activate the acute stress response, leading to continual surges of stress hormones like adrenaline and cortisol. Over time, this repeated activation can contribute to physical and emotional strain, increasing the risk of stress-related health issues **(Sinha & Kapur, 2021)**.

- **Chronic stress:**

Chronic stress refers to the long-term, persistent activation of the body's stress response due to ongoing or unresolvable demands and situations. Unlike acute stress, which is short-lived, chronic stress keeps the stress response systems particularly the Hypothalamic-Pituitary-Adrenal (HPA) axis and the Sympathetic Nervous System (SNS) continuously active. This leads to a sustained elevation of stress hormones, especially cortisol, which over time can result in dysregulation of these systems. Chronic stress is the most harmful form of stress, as it is strongly associated with serious health issues such as cardiovascular disease, immune suppression, depression, and metabolic syndrome **(McEwen, 1998)**.

2.2. Based on the Source of stress :

It can be classified into three main types:

- **Psychological stress:**

Psychological (or psychosocial) stress refers to stress that arises from how an individual perceives and evaluates social or environmental situations as threatening, challenging, or beyond their ability to cope. This type of stress is deeply influenced by cognitive appraisal the mental process of assessing whether a situation is harmful and whether one has the resources to handle it based on the transactional model developed by **Lazarus and Folkman (1984)**. Psychological stress often includes emotional responses such as anxiety, frustration, or helplessness, as the person interprets the situation as overwhelming or uncontrollable.

- **Physical stress:**

Physical stress refers to direct challenges or insults to the body's internal balance (homeostasis), which can arise from various sources. These stressors are typically categorized into three subtypes: physical stressors, such as extreme temperatures, intense physical exertion, injury, pain, noise, and sleep deprivation; chemical stressors, including exposure to toxins, pollutants, drug use or withdrawal, and alcohol abuse; and biological stressors, such as infections, illnesses, inflammation, and parasites. These stressors directly disrupt normal bodily functions **(Chrousos, 2009)**.

- **Oxidative stress:**

Oxidative stress refers to an imbalance between the production of reactive oxygen species (ROS), also known as free radicals, and the body's ability to neutralize them through antioxidants or repair the damage they cause **(Sies, 1991).** While it is not a "source" of stress in the traditional psychological or physiological sense, oxidative stress is a significant biochemical consequence of many types of stressors, both physical and psychological. Chronic psychological stress, in particular, has been shown to elevate oxidative damage by increasing ROS production and weakening the body's antioxidant defenses. This sustained imbalance contributes to cellular aging, inflammation, and the development of various diseases such as cardiovascular disorders, neurodegenerative conditions, and cancer **(Epel et al., 2004)**.

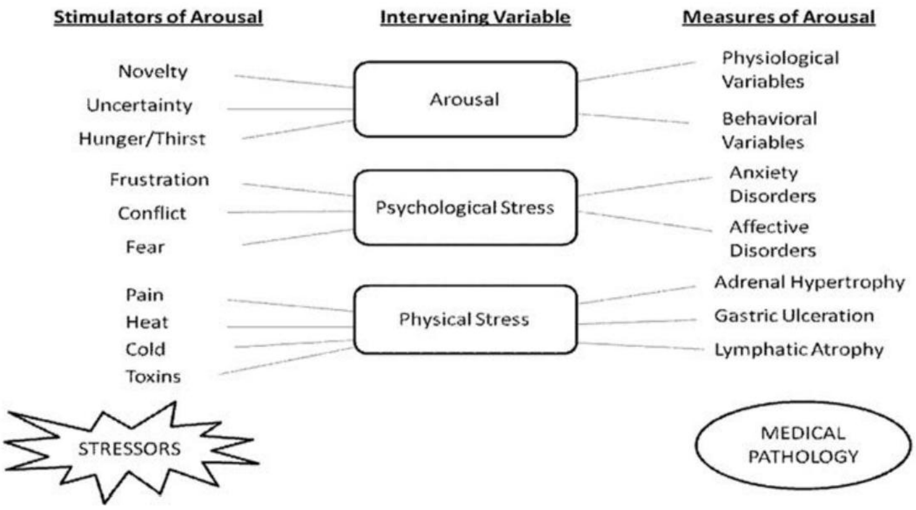

Figure 1: Relationship between arousal, psychological stress, physical stress and pathology **(Kumar et al., 2013).**

3. The dynamics of stress:

The dynamics of stress were defined by Hans Selye within the framework of the General Adaptation Syndrome, which includes three phases:

- **An alarm phase**, during which the body mobilizes all its resources through a trend-type regulation, leading to a continuous increase in the response;
- **A resistance phase**, during which the body adjusts its response to match the actual needs through a constant-type regulation, ensuring the stability of the response;
- **A recovery phase** if the stressor disappears, or an exhaustion phase if the aggression persists and the body is no longer able to maintain the appropriate level of stress. This exhaustion phase may result in the onset of diseases, or even death **(Canini, 2019)**.

4. The physiological pathways of stress:

Although different types of stressors are processed through distinct pathways in the central nervous system, they ultimately lead to a common physiological response involving the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system **(Hardin-Pouzet., 2021)**.

4.1. . The hypothalamic-pituitary-adrenal (HPA) axis :

The hypothalamic-pituitary-adrenal (HPA) axis is a key system involved in the body's stress response. It includes the hypothalamus, pituitary gland, and adrenal glands. When stress occurs, the hypothalamus releases CRH (corticotropin-releasing hormone), which stimulates the pituitary to produce ACTH (adrenocorticotropic hormone). ACTH then travels through the blood to the adrenal glands, triggering the release of cortisol (or corticosterone in rodents) **(Hosseinichimeh et al., 2015)**.

Cortisol helps the body manage stress by directing energy to where it's needed. Once the stress is over, feedback mechanisms reduce the production of CRH and ACTH, allowing the system to return to its normal state **(Hosseinichimeh et al., 2015).**

4.2. Autonomic Nervous System (ANS):

The autonomic nervous system, composed of the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS), is known for its role in maintaining the homeostasis of peripheral organs **(Magnon., 2013)**.

Stress primarily activates the sympathetic nervous system, a branch of the autonomic nervous system responsible for preparing the body to face real or perceived danger. This activation triggers a series of rapid physiological changes known as the "fight or flight" response, such as increased heart rate, elevated blood pressure, enhanced muscle strength, and dilation of the airways and pupils. Meanwhile, functions considered non-essential in emergency situations, like digestion or urination, are temporarily inhibited. On the other hand,

the parasympathetic nervous system, which normally works to restore balance by slowing down bodily functions and promoting recovery, is suppressed. This imbalance between the two systems, when prolonged as in cases of chronic stress can impair the regulation of vital functions, highlighting the direct and lasting impact of stress on the nervous system **(Coon.,2023)**.

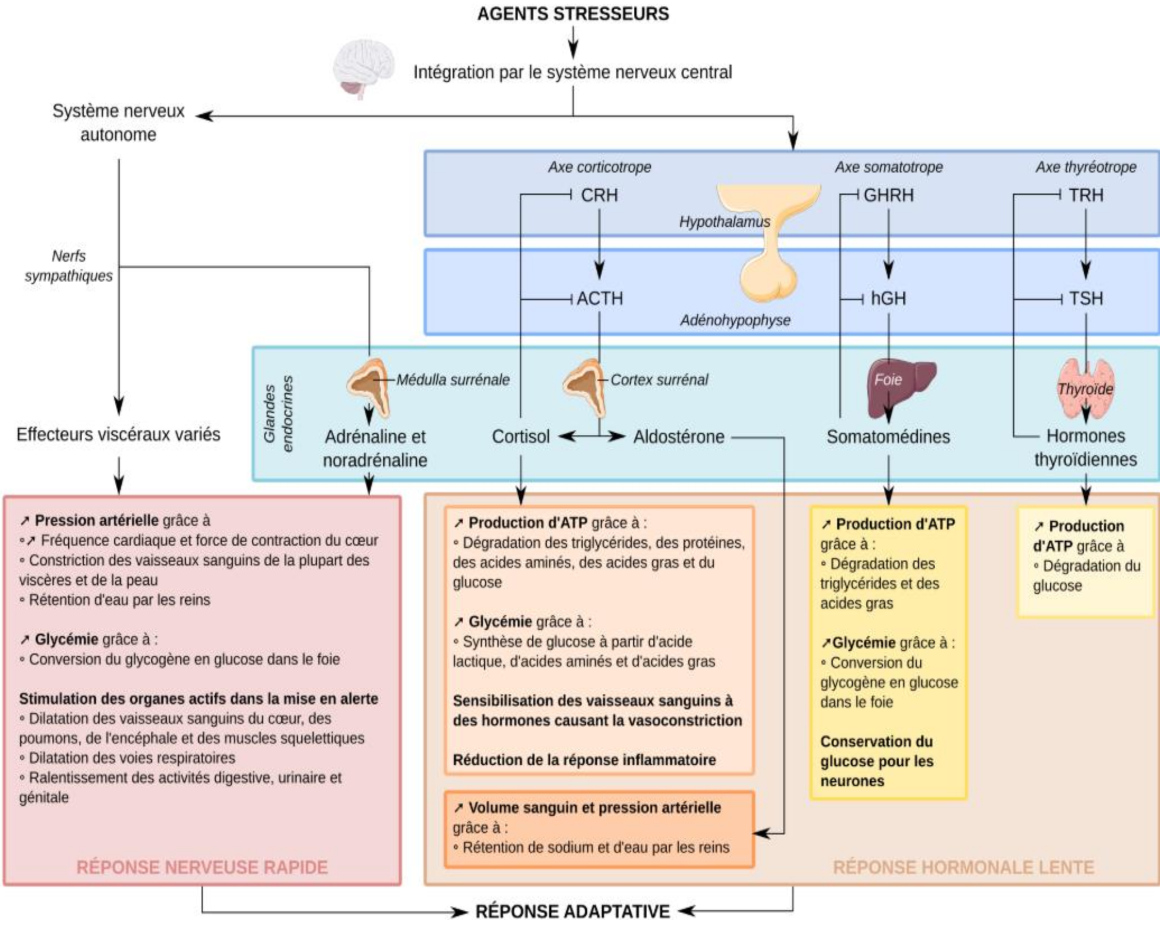

Figure 2: Hormonal and neural responses to stressors enable the organism to adapt **(Hardin-Pouzet., 2021).**

5. Neurological Effects of Stress on Brain Structure and Function:

Prolonged exposure to stress, a growingly common phenomenon, has profound effects on the brain, altering both its structure and function. It causes significant changes in key brain regions involved in emotional regulation, memory, and cognitive functions, such as the prefrontal cortex (PFC), the hippocampus, and the amygdala **(Krupa et al., 2025).** To fully understand these effects, it is essential to consider the course of normal brain development. In early childhood, brain volume increases, followed by a decrease in gray matter and an increase in white matter throughout adolescence. In adulthood, gray matter progressively declines particularly in the frontal and temporal lobes while the ventricles enlarge. The impact of stress varies depending on the stage of life, making the timing of exposure a critical factor in its neurological consequences **(Bremner, 2006)**. Furthermore, research has shown that chronic stress leads to dendritic retraction and decreased spine density, especially in the hippocampus and prefrontal cortex, indicating reduced synaptic connectivity and neural plasticity. These structural changes are associated with impairments in memory, decision-making, and emotional regulation. Stress also disrupts the function of the hypothalamic–pituitary–adrenal (HPA) axis, resulting in hormonal dysregulation and long-term disturbances in the body's stress response systems. Additionally, neurogenesis within the hippocampus a process essential for memory precision and emotional regulation is negatively affected by stress, contributing to fear generalization and increased vulnerability to anxiety disorders **(Krystal and Duman., 2016)**.

These neurobiological changes explain how stress can shift from a temporary state to a chronic risk factor, leading to psychological and neurological disorders such as depression, anxiety disorders, and post-traumatic stress disorder, making its understanding a medic and psychological necessity in today's world **(McEwen, 2007)**.

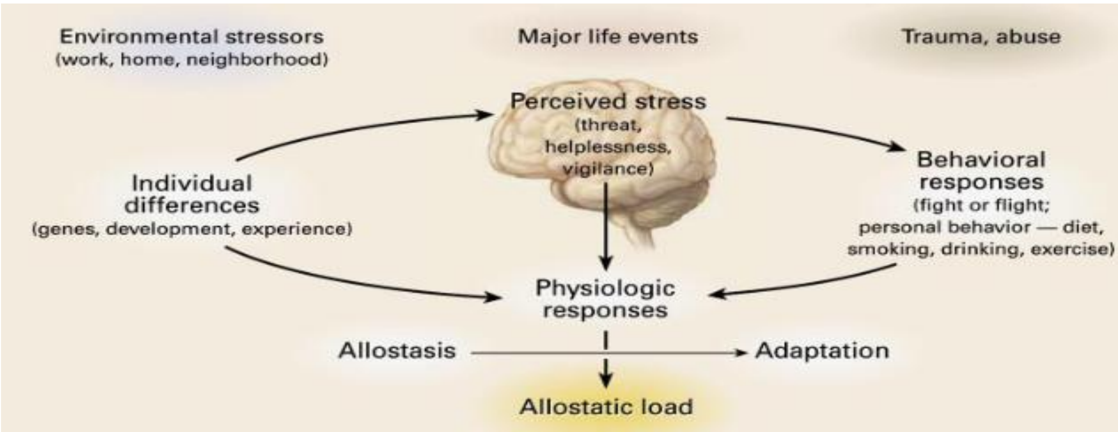

Figure 3: Central role of the brain inallostasis and the behavioral and phys-iological response to stressors **(McEwen, 2007)**.

6. Behavioral and neurobiological consequences of stress on mood , memory and learning :

Chronic stress directly affects the brain, particularly the hippocampus, which is responsible for memory and learning. Prolonged exposure to stress hormones such as cortisol leads to dendritic atrophy, neuronal loss, and an overall reduction in hippocampal volume **(Kumar et al.,2013;Sapolsky,1996)**.

These effects have been observed in patients with depression and post-traumatic stress disorder (PTSD), with a positive correlation between the duration of illness and the degree of hippocampal atrophy.Such neurobiological changes are reflected behaviourally as impairments in declarative memory and spatial learning, along with the emergence of mood disturbances and emotional dysregulation

Therefore, chronic stress not only disrupts cognitive functions but also contributes to emotional dysregulation and the development of mood disorders **(Sapolsky, 1996)**.

6.1. Mood:

6.1.1. Definition:

Mood corresponds to hyperpriors about emotional states that is, long-term expectations about emotional precision. It reflects a prior belief or confidence about how likely and reliable emotional reactions will be, and sets the baseline (or set-point) of neuromodulator systems that control how strongly we respond to prediction errors **(Clark et al., 2021)**.

6.1.2. Brain Regions Underlying Mood Regulation:

Based on **Davidson et al. (2002)** several brain regions are key to mood regulation and are often altered in mood disorders:

- **Amygdala**: Involved in emotions like fear and sadness; its overactivity is linked to depression.
- **Prefrontal Cortex (PFC)**: Some areas show reduced activity or volume in depression, **affecting** emotional regulation.
- **Anterior Cingulate Cortex (ACC)**: Its activity varies with treatment response and is involved in attention and emotional processing.
- **Hippocampus**: Plays a role in memory and emotional context; often reduced in volume in depression due to chronic stress.

6.1.3. Neurobiological Mechanisms:

- **HPA Dysregulation:** Sustained cortisol disrupts glucocorticoid receptor (GR) sensitivity in the PFC/hippocampus, impairing negative feedback **(McEwen, 2007).**
- **Neurotransmitter Imbalance:** Stress reduces prefrontal **serotonin** (5-HT) and **dopamine**, promoting anhedonia and reward dysregulation **(Nestler et al., 2002)**.
- **Inflammation:** Microglial activation and pro-inflammatory cytokines (e.g., IL-6) suppress neurotrophins (e.g., BDNF), exacerbating depressive symptoms **(Miller and Raison., 2016)**.

6.2. Memory:

6.2.1. Definition:

Memory is the brain's ability to record, store, and retrieve information from experiences and various events. Understanding memory, its classification, and the memorization process helps to better understand many memory disorders **(Vuillaume, 2020).**

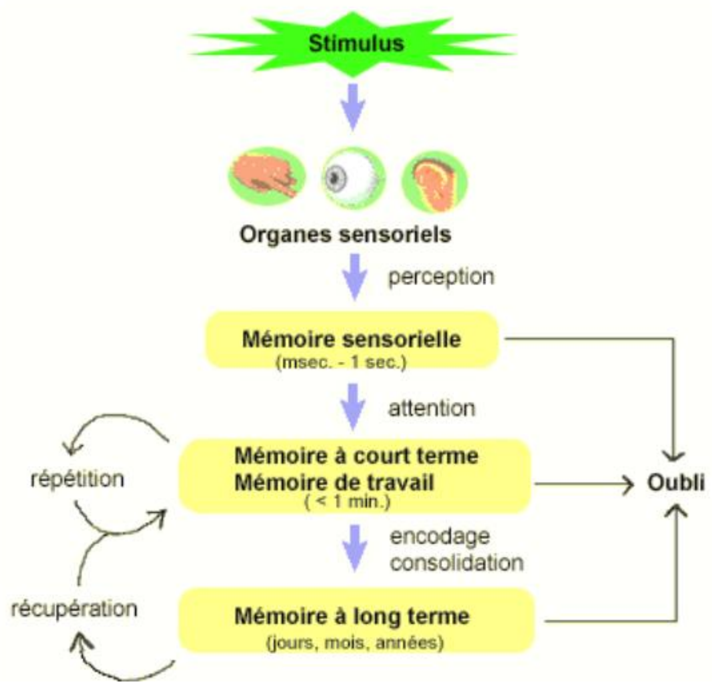

Figure 4: The memorization process **(Vuillaume, 2020).**

6.2.2. Brain Regions Underlying Memory Regulation:

Memory does not rely on a single brain system. In fact, different types of memory involve distinct neural networks distributed across various brain regions.

- **Episodic memory** is governed by the hippocampus and the frontal lobe, particularly the left and right prefrontal cortices, during the encoding and retrieval of memories.
- **Perceptual memory** involves networks located in cortical regions near the sensory areas.
- **Semantic memory** involves the temporal and parietal lobes.
- **Procedural memory** recruits subcortical neural networks and the cerebellum.

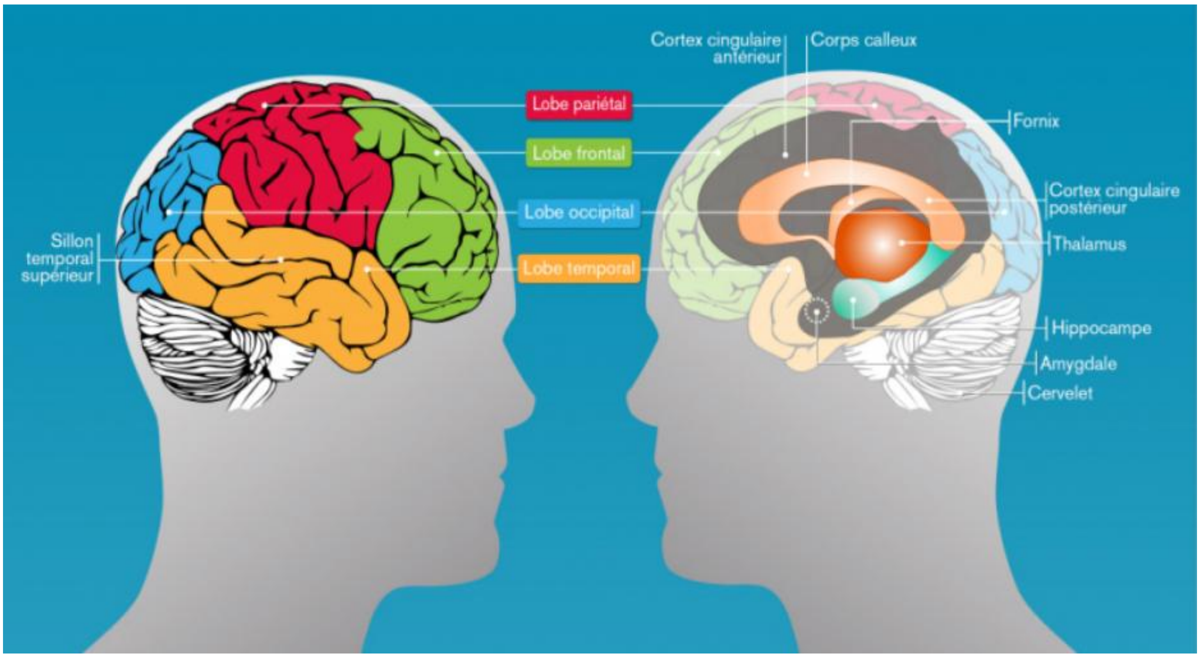

Figure 5: Brain areas involved in memory **(Vuillaume, 2020).**

6.2.3. Neurobiological Substrates:

- **Hippocampal Atrophy:** Chronic glucocorticoid exposure suppresses BDNF, reduces dendritic complexity, and inhibits neurogenesis impaired long-term potentiation (LTP) **(Kim & Diamond., 2002)**.
- **Amygdala-PFC Decoupling:** Stress weakens inhibitory PFC inputs to the amygdala, facilitating **fear** overgeneralization **(Arnsten., 2015)**.
- **Epigenetic Changes:** Histone modifications/DNA methylation in stress-related genes (e.g., *FKBP5*) perpetuate memory dysregulation **(Zannas et West., 2014)**.

6.3. Learning :

6.3.1. Definition:

Learning is the adaptive modification of behavior through experience. Stress shifts cognitive strategies from flexible, hippocampal-dependent learning to rigid, striatum-dependent habit formation **(Schwabe & Wolf, 2013).**

6.3.2. Brain Regions Underlying learning Regulation:

Learning in the brain is regulated through the interaction of three interconnected systems: the metacognitive system, the cognitive control network, and the representation system. In the initial stage of learning, the metacognitive system—particularly the anterior prefrontal cortex (aPFC) is activated to monitor performance and guide attention. After the first few trials, activity in the aPFC decreases, while the cognitive control network becomes more engaged. This network includes the dorsolateral prefrontal cortex (dlPFC), anterior cingulate cortex (aCC), posterior parietal cortex (pPC), and the inferior frontal junction (iFJ), all of which support goal-directed task execution. With continued practice, learning shifts to the representation system, a slower-adapting network that integrates sensory and motor information and stores knowledge through neural associations based on the principle "cells that fire together, wire together." This system includes the hippocampus for fast, declarative learning and the basal ganglia for procedural learning. Over time, it allows for automatic task execution without the need for attentional control, enabling faster responses to familiar stimuli. Brain imaging techniques show how neural activity transitions across these systems during learning, and diffusion imaging reveals internal pathways that coordinate actions within and between them, especially through the episodic memory system **(Chein & Schneider, 2012).**

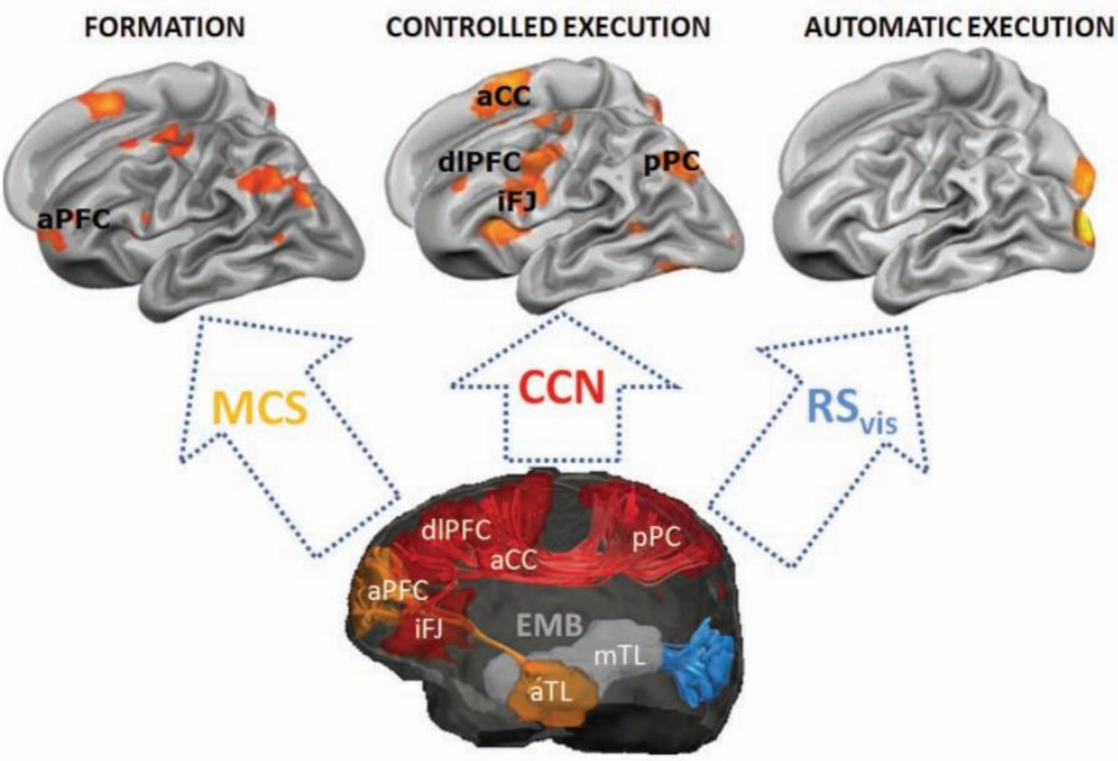

Figure 6: Key brain areas and internal connection pathways of the three learning systems in the brain **(Chein & Schneider, 2012)**

6.3.3. Neurochemical Mechanisms:

- **Glutamate Dysregulation:** Stress increases extrasynaptic glutamate, triggering **excitotoxicity** in the hippocampus **(McEwen** et al**., 2007**).
- **Dopaminergic Shift:** Mesolimbic dopamine surges during stress reinforce amygdala-**striatal** circuits, favoring stimulus-response learning **(Schwabe & Wolf, 2013)**.

- **BDNF Reduction:** Low BDNF in the hippocampus/PFC compromises synaptic plasticity and memory updating **(Duman et al., 2012)**.

7. Behavioral and Cognitive Methods for Assessing Mood, Memory, and Learning :

Behavioral and cognitive tests are an essential part of evaluating animal models in therapeutic research. These tests are employed to accurately analyze behavior across various domains, including motor activity, cognitive and emotional aspects such as learning, memory, mood, depression, anxiety, as well as the assessment of treatment efficacy. The success of these evaluations depends on selecting tests that appropriately align with the stud y's objectives **(Akçay, 2024; AnguBala Ganesh et al., 2023).**

7.1. Test to assess emotional state (mood):

7.1.1. Anxiety-like Behavior :

7.1.1.1. Open Field Test (OFT):

The OFT, originally developed in 1934 as a model for measuring emotional behavior in rodents, is among the widely utilized tools for assessing anxiety-like behavior **(Hall, 1934; Pentkowski et al., 2021).**

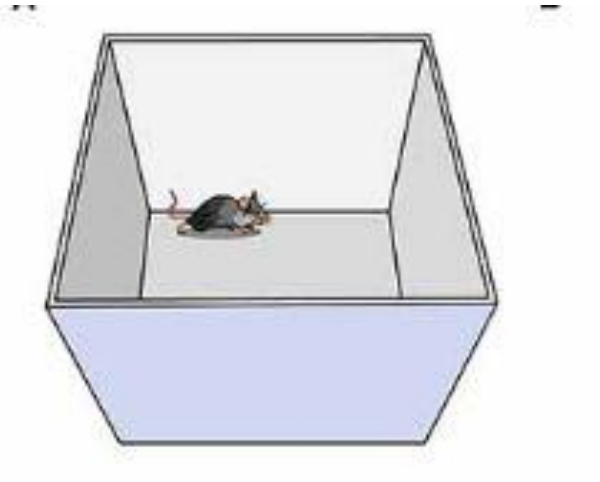

Figure 7: Open Field Test **(Seibenhener & Wooten, 2020).**

7.1.1.2. Elevated Plus Maze Test (EPM):

The EPM test is a popular behavioral test for assessing anxiety-like behavioral changes in rodent models due to its ease, high validity, and cost-effectiveness **(Xu et al., 2025).**

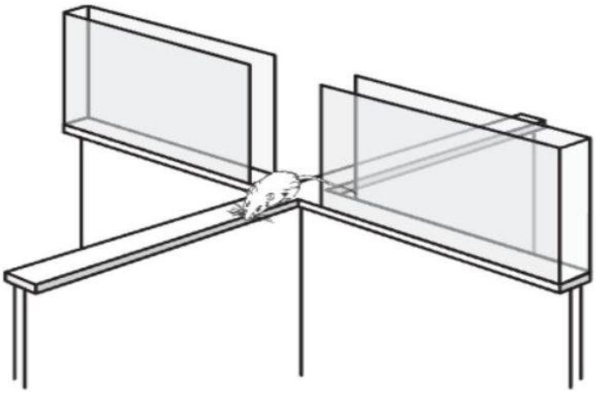

Figure 8: Elevated Plus Maze Test **(Mulder & Pritchett-Corning, 2004)**

7.1.2. Depression-like Behavior

7.1.2.1. Forced Swim Test (FST)

The Forced Swim Test (FST) is one of the most widely used tools for studying depression-like behaviors in rodents. It is considered an essential method in scientific research for evaluating coping strategies under stress due to its simplicity and accuracy of results. The test aims to assess the adaptation strategies of rodents when exposed to a stressful, inescapable environment. It is based on the assumption that the animal, when placed in a water-filled container, initially makes escape attempts but eventually exhibits immobility, which is regarded as an indicator of behavioral despair. Immobility is measured as a marker reflecting this state, representing behavioral aspects linked to stress and depression **(Yankelevitch-Yahav et al., 2015).**

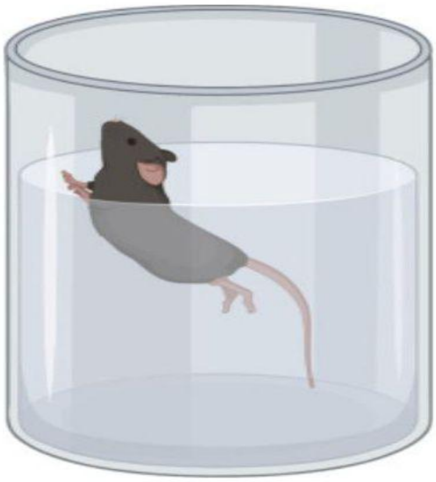

Figure 9: Forced Swim Test **(FST) (Drayson et al., 2023)**

7.2. Learning and Memory Tests:

7.2.1. Morris Water Maze Test (MWM) :

The Morris Maze, created by Richard Morris in 1984, is one of the most commonly used experiments for investigating and measuring spatial learning and memory in laboratory animals (rodents) (**Imran et al., 2021; Morris, 1984).** The test is based on mice's natural desire to avoid water, despite their ability to swim. When exposed to water, they seek a safe haven, which leads them to concentrate on discovering the hidden platform. This behavior is used to evaluate their capacity to learn and remember the location of the platform **(Othman et al., 2022).**

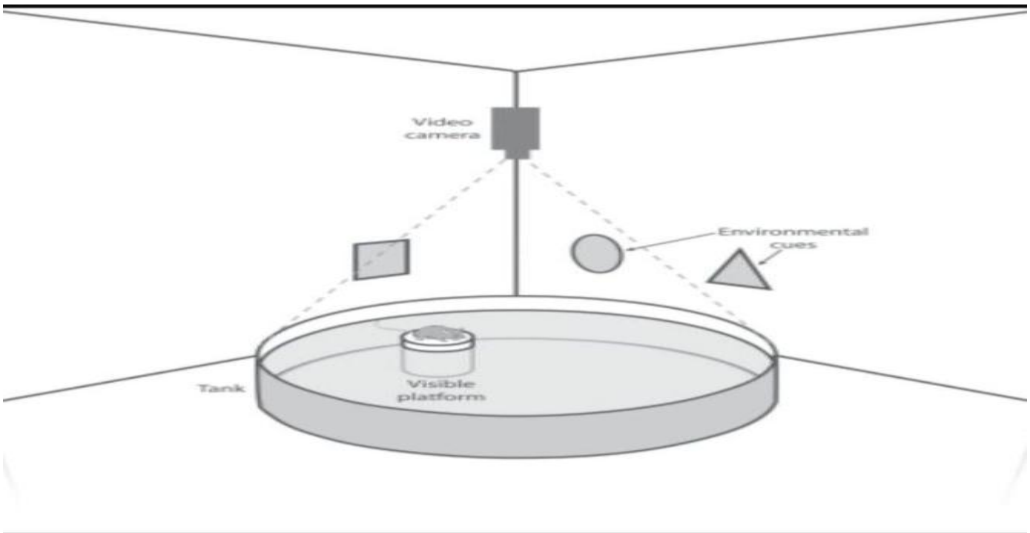

Figure 10: Morris Water Maze Test **(Mulder et al., 2003)**

7.2.2. Novel Object Recognition Test (NOR) :

The Object Recognition Test (ORT) is a widely used behavioral test for studying various aspects of learning and memory in rodents, known for its relative simplicity. This test is conducted over three days, which include: an habituation day, a training day, and a testing day. During the training phase, the rodent is allowed to explore two identical objects within the experimental apparatus, and on the testing day, one of the objects is replaced with a new one **(Lueptow, 2017).**

The design of the Novel Object Recognition (NOR) test is based on assessing short- or long-term memory for object recognition in rodents, relying on their innate preference for novelty. If the rodent recognizes the familiar object, it will spend more time near the novel object **(Bekci et al., 2024).**

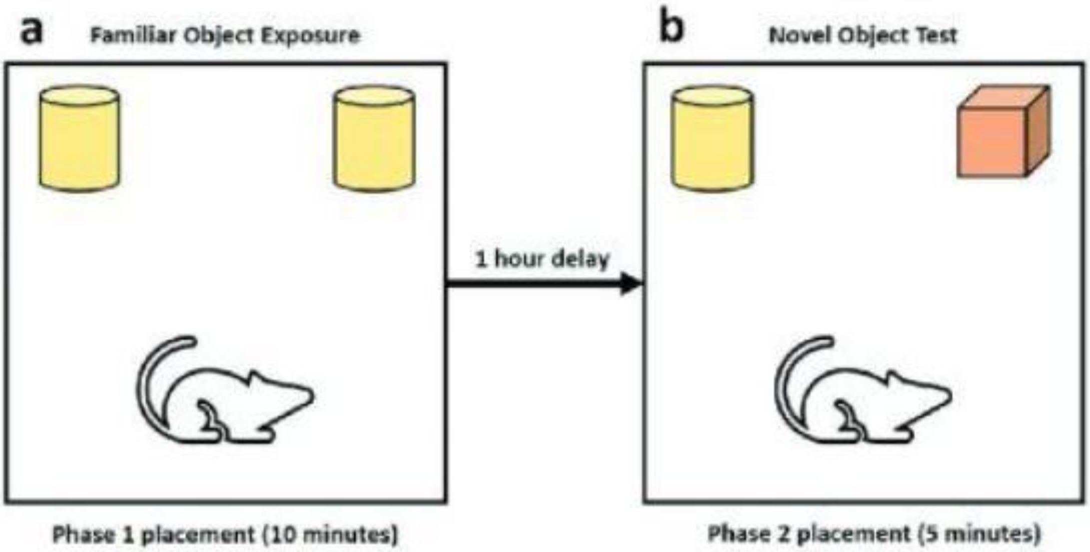

Figure 11: Novel Object Recognition Test **(Barker et al., 2017)**

Chapter 02:

Oxidative Stress and Antioxidant Defense

1. Oxidative stress:

Oxidative stress is defined as a condition arising from an imbalance between reactive oxygen species (ROS) and antioxidant defense mechanisms, favoring the accumulation of ROS and resulting in potential cellular damage. This imbalance, first described by Hsies in 1985, is now recognized as one of the primary contributing factors to the degradation of biomolecules such as proteins, lipids, carbohydrates, and DNA. Furthermore, oxidative stress is acknowledged as a major risk factor in the pathogenesis of numerous chronic diseases, including metabolic disorders, cancer, respiratory diseases, and cardiovascular conditions **(Aydın & Avcı, 2025; Lushchak & Storey, 2021)**.

The severity of this phenomenon is exacerbated by unhealthy lifestyle habits, where factors such as smoking, alcohol consumption, obesity, intense physical exercise, and unbalanced dietary habits contribute to the excessive production of reactive oxygen species. Over time, this may accelerate aging processes and increase the risk of developing serious and chronic illnesses **(Militello et al., 2024).**

2. Free radicals :

Free radicals are chemical substances that can be atoms, ions, or molecules containing one or more unpaired electrons in their valence shell that is, electrons not paired with another of opposite spin placing them in a state of instability. Due to this unique structure, free radicals exhibit high reactivity, seeking to stabilize themselves by extracting electrons from other molecules. As a result, they react non-selectively with surrounding molecules, which can trigger a chain of damaging reactions at the cellular and tissue levels **(Ndior et al., 2022; Wang et al., 2021).**

Despite their potential danger, free radicals play a vital role within the body. When produced in moderate amounts, they contribute to immune defense, cellular signal transmission, and the regulation of various physiological functions. However, excessive production leads to an imbalance in the oxidative state, paving the way for cellular damage and the development of numerous chronic diseases **(Ndior et al., 2022).**

The levels of free radicals in the body are determined by a delicate balance between the rate of their production and the rate at which they are neutralized by antioxidants and protective enzymes. Maintaining this balance is essential to preserve the integrity and proper functioning of cells and tissues **(Valko et al., 2007)**

3. Reactive Oxygen Species:

Reactive Oxygen Species (ROS) are highly reactive derivatives of oxygen, characterized by their strong chemical reactivity. These molecules are primarily generated through natural metabolic processes within cells. In mammalian cells, mitochondria are considered the main source of ROS, contributing to approximately 90% of total intracellular production. Additionally, ROS are also produced in other cellular compartments, including the cytoplasm and subcellular organelles such as peroxisomes and the endo/sarcoplasmic Reticulum **(Kozlov et al., 2024; Tirichen et al., 2021**).

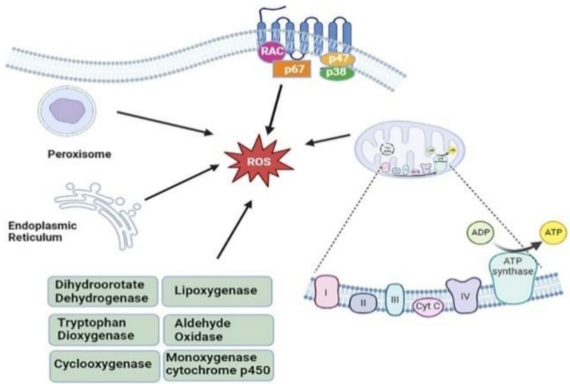

Figure 12: Graphical representation of significant sites for the production of endogenous reactive oxygen species **(Rauf et al., 2024).**

Depending on their nature, ROS play multiple roles in biological systems, contributing to vital processes such as cellular signal transduction and the maintenance of internal homeostasis. However, excessive accumulation of these species can trigger oxidative stress, which is closely linked to the development of various diseases, including neurological disorders. Therefore, precise regulation of ROS levels is essential to preserve cellular integrity and function **(Vitiello et al., 2021).**

Given their dual nature, ROS can be classified into two main categories: radical and non-radical species, as shown in the following table **(Sahoo et al., 2022).**

Table 1: List of radical and non-radical reactive oxygen species **(Sahoo et al., 2022).**

Sl. No.	**Free radicals**	**Non-radicals**
1	Superoxide anion (O2-)	Hydrogen peroxide (H2O2)
2	Hydroxyl radical (OH)	Hypochlorous acid (HOCI)
3	Lipid peroxyl (LOO)	Singlet oxygen ('02)
4	Alkoxyl radicals (RO)	Dinitrogen dioxide (N2O2)
5	Sulfonyl radicals (ROS)	Ozone/trioxygen (O3)

4. Reactive nitrogen species (RNS):

Reactive nitrogen species (RNS) are defined as a group of chemically active molecules that contain nitrogen. They are produced within cells as secondary by-products of various metabolic processes involving nitrogen metabolism, especially through the activity of nitric oxide synthase enzymes (Nitric Oxide Synthases NOS), which catalyze the conversion of the amino acid L-arginine into citrulline in the presence of oxygen, simultaneously producing nitric oxide (NO•), which is considered the most prominent among these types.

RNS include various species such as nitric oxide (NO•) and nitrogen dioxide (NO_2), as well as others that arise either as metabolites of NO degradation or through its reactions with other compounds such as reactive oxygen species (ROS) or carbon dioxide (CO_2). Among these molecules is peroxynitrite ($ONOO^-$), which is formed from the reaction between NO and superoxide anion (O_2^-•), as well as nitroxyl (HNO), which can be generated through the reduction of NO by cellular components or enzymes such as cytochrome C, xanthine oxidase, ubiquinol, hemoglobin, or manganese superoxide dismutase (Mn-SOD) (Salvagno et al., 2024).

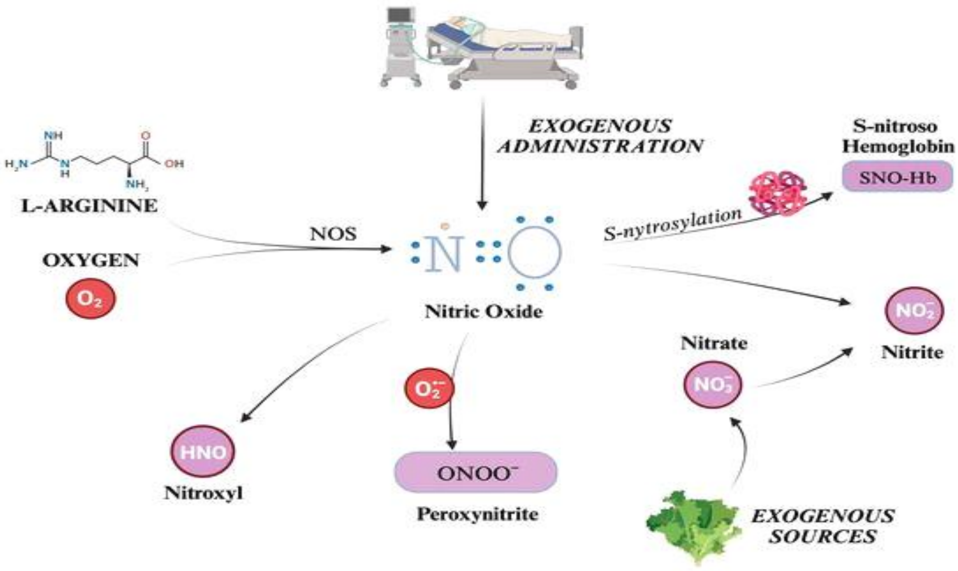

Figure 13: Schematic representation of the main reactive nitrogen species (RNS) and their formation pathways **(Salvagno et al., 2024).**

Table 2: Reactive oxygen species (ROS) and reactive nitrogen species (RNS) **(Gulcin, 2025).**

Reactive oxygen species		Non free-radical species	
• Hydroxyl radical	$HO\cdot$	• Hydrogen peroxide	H_2O_2
• Superoxide radical	$O_2\cdot^-$	• Singlet oxygen	$^1O_2$
• Hydroperoxyl radical	$HOO\cdot$	• Ozone	O_3
• Lipid radical	$L\cdot$	• Lipid hydroperoxide	LOOH
• Lipid peroxyl radical	$LOO\cdot$	• Hypochlorous acid	HOCl
• Peroxyl radical	$ROO\cdot$	• Peroxynitrite	$ONOO^-$
• Lipid alkoxyl radical	$LO\cdot$	• Dinitrogen trioxide	N_2O_3
• Nitrogen dioxide radical	$NO_2\cdot$	• Nitrous acid	HNO_2
• Nitric oxide radical	$NO\cdot$	• Nitryl chloride	NO_2Cl
• Nitrosyl cation	NO^+	• Nitroxyl anion	NO^-
• Thiyl radical	$RS\cdot$	• Peroxynitrous acid	ONOOH
• Protein radical	$P\cdot$	• Nitrous oxide	N_2O

5. Antioxidants:

5.1. Definition of Antioxidants:

Antioxidants are compounds that play a vital role in protecting biomolecules from oxidation caused by free radicals, by delaying or inhibiting oxidative reactions that may damage cells (Halli, 1990).

Antioxidants are widely found in nature, particularly in colorful vegetables, fruits, and medicinal plants, which contain a broad range of active compounds contributing to the maintenance of the crucial redox balance necessary for tissue and cellular functions (Pham-Huy et al., 2008; Sies, 2020).

These compounds protect the body from oxidative damage by neutralizing free radicals, inhibiting chain oxidation reactions, chelating pro-oxidant metals, and activating endogenous defense mechanisms **(Exon Publications, 2025; Gulçin, 2025; Forman et al., 2014).**

5.2. Classification of antioxidants :

5.2.1. Classification of Antioxidants by Origin :

Antioxidants can be classified into two main types based on their origin: those naturally produced by the body and those obtained from dietary or environmental sources. Both types play an integral role in protecting the body from free radicals (Birben et al., 2012; Halliwell & Gutteridge, 2015; Sies, 2020).

5.2.1.1. Endogenous Antioxidants:

Endogenous antioxidants include enzymes and small molecules produced within cells, forming the first line of defense against free radicals.

One.5.2.1.1.1. Antioxidant Enzymes:

- ***Superoxide* Dismutase *(SOD)* :** Converts superoxide radicals ($O_2^{-}\cdot$) into hydrogen peroxide, reducing their toxicity (Valko et al., 2007).
- ***Catalase (CAT)* :** Breaks down hydrogen peroxide into water and oxygen within peroxisomes (Pham-Huy et al., 2008).
- ***Glutathione Peroxidase (GPx)* :** Uses glutathione to reduce hydrogen peroxide, maintaining intracellular redox balance (Birben et al., 2012).

One.5.2.1.1.2. Non-Enzymatic Molecules:

- ***Glutathione (GSH)* :** A sulfur-rich peptide considered one of the most potent cellular antioxidants (Halliwell & Gutteridge, 2015).
- ***Uric Acid* :** A strong antioxidant present in plasma that efficiently scavenges free radicals **(Valko et al., 2007).**
- ***Bilirubin* :** A heme breakdown product exhibiting antioxidant properties at physiological concentrations **(Pham-Huy et al., 2008)**.
- ***Coenzyme Q10 (CoQ10):*** Found in mitochondria, participates in electron transport and acts as an antioxidant (Sies, 2020).

5.2.1.2. Exogenous Antioxidants:

Exogenous antioxidants are compounds acquired from food or supplements, playing a crucial role in enhancing endogenous defenses, especially under oxidative stress conditions (Pham-Huy et al., 2008; Halliwell & Gutteridge, 2015).

- ***Vitamins* :**
- ***Vitamin C* :** Water-soluble antioxidant that donates electrons to neutralize free radicals and regenerates vitamin E **(Valko et al., 2007).**
- ***Vitamin E (α-Tocopherol)* :** Fat-soluble antioxidant that protects membranes from lipid peroxidation **(Sies, 2020).**
- ***Beta-Carotene and Vitamin A* :** Quench singlet oxygen and prevent damage to DNA and proteins **(Pham-Huy et al., 2008).**
- ***Trace Elements* :**
- ***Zinc (Zn)*:** Stabilizes cell membranes and serves as a cofactor for SOD enzyme **(Halliwell & Gutteridge, 2015).**
- ***Selenium (Se)*:** Essential component of the GPx enzyme structure **(Birben et al., 2012).**
- ***Manganese and Copper (Mn, Cu)*:** Involved in regulating antioxidant enzyme activities.
- **Polyphenols :**

Polyphenols are bioactive plant compounds with potent antioxidant properties by scavenging free radicals, reducing inflammation, and protecting biomolecules.

- ***Flavonoids* :** Such as quercetin, catechin, and rutin, inhibit oxidizing enzymes and neutralize free radicals **(Middleton et al., 2000).**
  - ***Phenolic Acids* :** Including ferulic acid, chlorogenic acid, and gallic acid, maintain the integrity of DNA and proteins **(Balasundram et al., 2006).**
  - ***Tannins* :** Plant polymers that bind proteins and metals, preventing LDL oxidation **(Riedl et al., 2001).**
  - ***Lignans* :** Found in flaxseeds, contribute to cellular protection and hormonal regulation **(Thompson et al., 2005).**
- **Other Phytochemicals :**

This group includes non-polyphenolic plant compounds that also contribute to antioxidant defense.

- ***Alkaloids:*** Such as caffeine and theobromine, inhibit reactive oxygen species (El-Shazly & Wink, 2014).
- ***Terpenoids:*** Present in essential oils, stimulate defensive enzymes and protect membranes from damage (Rajeshwar et al., 2005).
- ***Phytosterols:*** Reduce cholesterol absorption and improve redox balance in blood vessels (Plat et al., 2005).

5.2.2. Classification of Antioxidants by Mechanism of Action:

Classifying antioxidants according to their mechanism of action is one of the most important biological classifications, as it highlights how these compounds contribute to combating oxidative stress at the cellular level. These mechanisms are typically grouped into four main categories:

5.2.2.1. Primary Antioxidants (Chain-Breaking) :

These compounds directly neutralize free radicals by donating an electron without becoming harmful themselves, thereby interrupting the oxidative chain reactions—particularly within cell membranes. This category includes Vitamin C, which acts as a water-soluble antioxidant in plasma; Vitamin E, which stabilizes cell membranes by preventing lipid peroxidation; and flavonoids such as quercetin, which contribute to neutralizing reactive oxygen species ***(Sies, 2020; Valko et al., 2007) .***

5.2.2.2. Preventive Antioxidants (Inhibitors of Radical Formation) :

These compounds work by preventing the formation of free radicals in the first place. Notable examples include lycopene, which quenches singlet oxygen; phytic acid, which chelates transition metals like iron and thereby blocks Fenton reactions; and tannins, which stabilize active radicals before they damage cellular components ***(Pham-Huy et al., 2008; Halliwell, 2015.)***

5.2.2.3. Repair Antioxidants :

This group not only helps in prevention but also participates in repairing damage caused by oxidative stress. Key examples include DNA repair enzymes (such as DNA glycosylases), which detect and replace oxidized nucleotides, and the proteasome system, which removes oxidized and damaged proteins from cells ***(Birben et al., 2012).***

5.2.2.4. Catalytic or Regulatory Antioxidants:

Rather than reacting directly with radicals, these compounds activate the genetic expression of endogenous antioxidant enzymes within the cell. Notably, sulforaphane—found in broccoli—activates the Nrf2 transcription factor. Other compounds such as quercetin and organosulfur molecules also promote the expression of enzymes like SOD, CAT, and GPx. ***(Sies & Jones, 2020; Valko et al., 2007).***

5.2.3. Classification of Antioxidants According to Their Chemical Nature :

This classification relates to the molecular structure of antioxidant compounds, which affects their physical and chemical properties such as solubility, efficiency in scavenging free radicals, and their role in cellular protection. This type of classification allows understanding the diversity of mechanisms of action and the effectiveness of these compounds in different biological environments (Halliwell & Gutteridge, 2015; Valko et al., 2007).

Table 3: Classification of Antioxidants Based on Their Chemical Nature .

Chimical class	**Representative Examples**	**References**
Phenolic Compounds	Quercetin, Gallic acid, Tannins	Valko et al., 2007; Middleton et al., 2000
Vitamins	Vitamin C, Vitamin E, Beta-carotene	Sies, 2020; Pham-Huy et al., 2008
Sulfur-containing Compounds	Glutathione, Thiols, Selenium-containing enzymes	Birben et al., 2012; Halliwell & Gutteridge, 2015
Carotenoids	Beta-carotene, Lycopene, Lutein	Sies, 2020
Minerals	Zinc, Selenium, Copper, Manganese	Halliwell & Gutteridge, 2015; Birben et al., 2012
Alkaloids	Caffeine, Theobromine	El-Shazly & Wink, 2014
Terpenoids	Linalool, Carvone, Beta-caryophyllene	Rajeshwar et al., 2005
Plant Sterols	Beta-sitosterol, Campesterol	Plat et al., 2005

5.3. Biological Damages of Oxidative Stress:

Reactive oxygen and nitrogen species (RONS) are molecules naturally produced within cells at tightly regulated levels, where they contribute to the regulation of physiological processes and the maintenance of redox balance. However, any disruption in this equilibrium—whether due to excessive production of these species or a decline in the efficiency of antioxidant defense systems—can lead to oxidative stress. Under such conditions, essential biomolecules including nucleic acids, proteins, lipids, and carbohydrates are subject to structural and functional damage, compromising cellular stability and increasing the likelihood of cellular deterioration (**Aranda-Rivera et al., 2022).**

This harmful effect is considered one of the direct outcomes of free radical activity, which represents a primary factor responsible for cellular damage. These radicals interact directly with sensitive cellular components such as DNA, lipids—particularly through lipid peroxidation—and proteins, resulting in damage at both the cellular and tissue levels. The accumulation of such damage accelerates natural aging processes and increases the risk of developing various chronic diseases, including cardiovascular diseases, cancer, diabetes, and neurodegenerative disorders **(Phaniendra et al., 2015).**

5.4. Impact of Oxidative Stress on the Brain:

The brain consumes approximately 20% of the total oxygen in the body, although it represents only about 2% of the body's weight. This reflects its significant energy demand. This high energy requirement is linked to the complex physiological functions performed by neuronal cells, such as maintaining membrane potential, generating nerve impulses, and releasing and recycling neurotransmitters. All these processes rely primarily on oxidative phosphorylation as the main source of energy, in the absence of sufficient energy reserves. The brain depends almost exclusively on this pathway to meet its energy needs, which increases its reliance on oxygen and makes it one of the organs most susceptible to oxidative stress **(Ebding et al., 2025; Jelinek et al., 2021).**

This high and distinctive metabolic activity of the brain leads to the production of large amounts of reactive oxygen species (ROS), which significantly contribute to oxidative damage in neuronal cells, especially given the relatively weak antioxidant defense mechanisms. Moreover, neuronal membranes contain high levels of polyunsaturated fatty acids (PUFAs), which increases their susceptibility to oxidation **(Ebding et al., 2025; Franzoni et al., 2021).**

Additionally, neuronal cells contain high concentrations of chemically active metals, particularly iron, which plays a crucial role in cellular respiration, neurotransmitter metabolism, and myelin formation. However, free iron can participate in harmful oxidation reactions, such as the Fenton reaction, which leads to the formation of highly toxic hydroxyl radicals.

Since neurons are long-lived and have limited regenerative capacity, oxidative stress-induced damage accumulates gradually over time, which reinforces the brain's long-term vulnerability to this type of damage, especially since dead neurons are difficult to replace **(Ebding et al., 2025).**

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According to **Kim et al. (2024),** oxidative stress contributes to several critical forms of cellular damage in the brain, including:

- **Lipid peroxidation:**

Damages neuronal membranes, reducing their fluidity and function, and disrupts neural signaling.

- **Protein oxidation:**

Leads to structural alterations and accumulation of toxic proteins like beta-amyloid and alpha-synuclein, impairing cellular processes.

- **DNA damage:**

Activates cell death pathways such as apoptosis and necrosis.

- **Blood-brain barrier (BBB) disruption:**

Increases brain permeability to harmful substances, exacerbating neuroinflammation.

- **Increased neuroinflammation:**

Through activation of glial cells and release of inflammatory cytokines, amplifying oxidative damage.

- **Mitochondrial dysfunction:**

Enhances ROS production and weakens cellular energy productionlex physiological functions performed by neuronal cells, such as maintaining membrane potential, generating nerve impulses, and releasing and recycling neurotransmitters. All these processes rely primarily on oxidative phosphorylation as the main source of energy, in the absence of sufficient energy reserves. The brain depends almost exclusively on this pathway to meet its energy needs, which increases its reliance on oxygen and makes it one of the organs most susceptible to oxidative stress **(Ebding et al., 2025; Jelinek et al., 2021).**

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Altogether, these mechanisms highlight oxidative stress not only as a consequence but also as a central driver of neuronal degeneration, contributing to the onset and progression of various neurodegenerative diseases, including Alzheimer’s disease (AD), Parkinson’s disease (PD), and Huntington’s disease (HD). These disorders are characterized by progressive neuronal loss and deterioration of cognitive and motor functions (Kim et al., 2024).

Chapter 03:

Selected Medicinal Plants Used in This Study for Their Therapeutic Potential

1. Ephedra alata:

1.1. Plant Presentation:

The genus *Ephedra*, belonging to the Ephedraceae family, includes around 67 species, mainly found in desert regions of North Africa, Europe, Asia, and America **(Bourgou et *al*., 2020)**.

Among them is *Ephedra alata alenda*, commonly known as *Ephedra alata* or *winged ephedra* in English, and commonly referred to as *Alenda* in Algeria **(Benarba et *al*., 2021) (Mahmoudi et *al*., 2023)**.

1.2. Geographical Distribution:

E. alata is a plant native to temperate regions and subtropical latitudes of:

- **Africa:** Algeria, Egypt, Libya, Morocco, Tunisia, Mauritania, Chad, Mali.
- **Asia:** Saudi Arabia, Iraq, Iran, Palestine, Lebanon, Jordan, Syria, China.
- **Europe:** Spain **(Benarba et *al*., 2021) (Wannes et Tounsi., 2023)**.

1.3. Systematic Classification :

The classification of this species is presented in Table :

Table 4: Taxonomic classification of *Ephedra alata* **(Ozenda, 1991)**.

Taxonomic Rank	Nomenclature
Kingdom	Plantae
Phylum	Spermaphytes
Division	Gymnospermae
Class	Gnetopsida
Subclass	Ephedroidae
Order	Ephedrales
Family	Ephedraceae
Genus	Ephedra
Species	Ephedra alata
Subspecies	alenda

1.4. Plant Description :

Ephedra alata is one of the rare shrubs found in Saharan regions and is classified as a nanophanerophyte. It is a rigid, perennial, yellow-green shrub, densely branched, measuring 40 to 100 cm in height, and often wider than it is tall **(adjadj et *al*., 2020)**.

Its jointed branches bear reduced, scale-like, opposite leaves at the nodes. The unisexual flowers are grouped in small cones. Male and female flowers usually grow on separate plants, although in some cases both flower types may be present on a single individual **(adjadj et *al*., 2020)**.

Ecologically, *E. alata* is an effective stabilizer of wind-blown sand. However, its charcoal is highly valued by local Saharan populations, which exposes the species to deforestation **(Danciu et al., 2019)**.

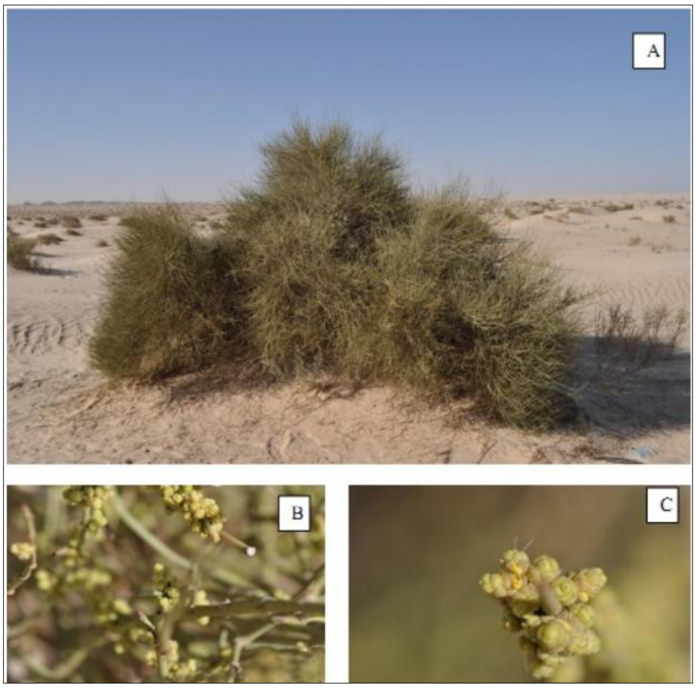


Figure 14: *Ephedra alata* subsp.alenda " A: flowering twig, B: budded twig, and C: flower in full bloom **(adjadj *et al*, 2020)**.

1.5. Therapeutic Uses:

Ephedra alata is a plant commonly used in traditional medicine across various regions of Algeria to treat a wide range of illnesses such as influenza, whooping cough, colds, asthma, and allergies. Additionally, certain parts of the plant are used in the treatment of various types of cancer. Beyond that, it is also employed to address general weakness, circulatory disorders, rheumatism, arthritis, headaches, digestive issues, and skin diseases. These diverse uses highlight the importance of *Ephedra alata* in traditional medicine and its potential for treating numerous medical conditions **(Adjadj et al., 2020)**.

It is typically prepared as an infusion or decoction using the dried aerial parts of the **plant (Hadjadj et al., 2018)**.

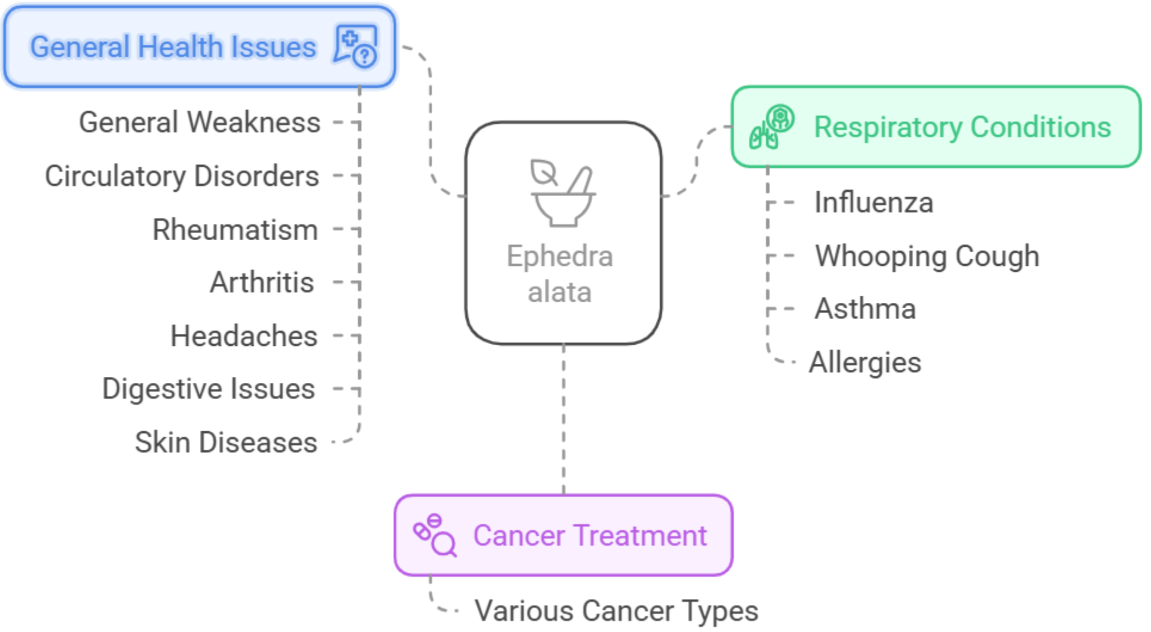


Figure 15: Therapeutic Uses of ephedra alata

1.6. Antioxidant Properties:

Ephedra alata is considered a traditional medicinal plant that is gaining increasing attention due to its biochemical properties, particularly its antioxidant activity. Several studies have shown that methanolic extracts from different parts of this plant such as the fruits, seeds, and pulp contain high levels of phenolic compounds and flavonoids. These compounds are known for their ability to inhibit reactive oxygen species (ROS), which are major contributors to oxidative stress in cells (**Jaradat et *al*., 2021, Mufti et *al*., 2023).**

1.7. Active Compounds and Biological Effects of the Plant:

The preliminary phytochemical analysis of *Ephedra alata alenda* revealed the presence of various bioactive compounds, including:

- **Phenolic compounds :**

- **Phenolic Acids:**

The plant contains several types of phenolic acids, such as trans-cinnamic acid, catechin, syringic acid, epicatechin, symplocoside, kaempferol 3-O-rhamnoside 7-O-glucoside, and isovitexin 2-O-rhamnoside. These compounds contribute significantly to the plant's antioxidant activity **(Amakura et al., 2013).**

- **Flavonoids:**

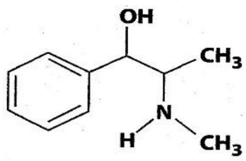
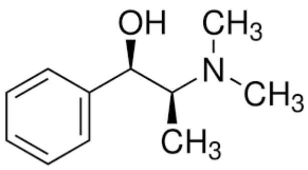
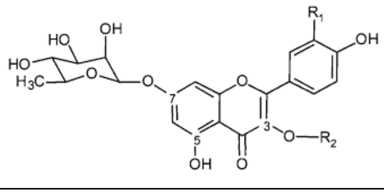
The isolated flavonoids mainly belong to the flavone class, including herbacetin, vicenin II, lucenin, apigenin, kaempferol 3-rhamnoside, quercetin 3-rhamnoside, and herbacetin 7-O-glucoside **(Hegazi et *al* ., 2011)**.

- **Alkaloids:**

Other alkaloids have also been identified, such as pseudoephedrine, norephedrine, and methylephedrine **(Mahmoudi et *al*., 2023)**.

These compounds are known for their antimalarial, analgesic, antispasmodic, bactericidal, and stimulant properties. However, at high concentrations, these molecules may cause acute toxicity **(Jaradat et *al*.,2015).**

Table 5: Chemical structure of some active compounds of this plant

Secondary metabolite	**Structure**	**References**
Ephedrine		**(Limberger et *al* ., 2013).)**
Methylephedrine		**(DONG *et al*., 2015)**
Flavonol-3-O-glycosides		**(Park et *al*., 2019)**

1.8. Ephedra alata effects on Mood, Memory, and Learning:

The study results of **Khattabi et *al* 2023** showed that *Ephedra alata* extract has antioxidant properties that contribute to improving mood, enhancing memory, and boosting cognitive functions. The extract worked by reducing oxidative stress in the brain, which helped improve cognitive and behavioral performance in the treated animals. These effects contributed to a decrease in anxiety and depression markers while enhancing cognitive performance, suggesting that *Ephedra alata* could be used as a natural remedy to support cognitive and mood functions.

2. Rosmarinus officinalis L:

2.1. Plant Presentation:

At present, the demand for *Rosmarinus officinalis* L. (rosemary) is increasing due to its use in traditional medicine, the pharmaceutical and cosmetic industries, agribusiness, and the high quality of its essential oil **(Kadri et *al* ., 2011)**; it is a medicinal and aromatic plant belonging to the Lamiaceae family, well known for its biological activities**(Mersin et Saltan., 2022)**.

2.2. Geographical Distribution:

Rosmarinus officinalis L., a fragrant plant native to the Mediterranean region, belongs to the Lamiaceae family. The province of Murcia in southeastern Spain is recognized as one of the leading centers for the processing and export of rosemary. In both the United States and Europe, rosemary is widely marketed not only as a culinary herb but also as a natural antioxidant, valued for its preservative properties in food products **(Nieto et al ., 2018).**

2.3. Systematic Classification:

The classification of this species is presented in Table

Table 6: Taxonomic classification of *Rosmarinus officinalis* **(Kompelly et *al*.,2019)**.

Taxonomic Rank	Nomenclature
Kingdom	Plantae, herb
Subkingdom	Viridiplantae, green plants
Infra kingdom	Streptophyta
Super division	Embryophyta
Division	Tracheophyta, vascular plants
Sub division	Sprematophytina, seed plants
Class	Magnoliopsida
Subclass	Sympetalae
Order	Lamiales
Family	Lamiaceae
Genus	Rosmarinus
Species	Rosmarinus officinalis

2.4. Plant Description :

Rosemary (*Rosmarinus officinalis* Linnaeus) is a Mediterranean shrub belonging to the Lamiaceae family. It can grow up to 1.5 meters in height and typically thrives in dry, rocky soils. In Corsica, a unique creeping form of rosemary grows flat along the ground and is valued for its ornamental qualities. The plant has persistent, narrow, dark green leaves and blooms twice a year—once in spring and again in autumnproducing flowers that range in color from pale blue to violet. The Corsican and Sardinian rosemary varieties are distinguished from those of Provence, Spain, or Morocco by their smaller, narrower, lighter green leaves and flowers that are closer to mauve in color **(Casanova et Tomi ., 2018)**.

Figure 16: Rosemary (*Rosmarinus officinalis L.)* Plant **(Mersin et Saltan.,** 2022**)**.

2.5. Therapeutic Uses:

Traditionally, *Rosmarinus officinalis* L. has been used for the treatment of various health issues, including renal colic, asthma, spasmogenic disorders, peptic ulcers, inflammatory diseases, hepatotoxicity, atherosclerosis, ischaemic heart disease, cataracts, and poor sperm motility (**Amara et *al*., 2017).**

A population-based study has suggested that regular consumption of herbs, including rosemary, is associated with an overall reduced risk of cancer incidence **(Amara et *al*., 2017).** In particular, the **phenolic constituents** of rosemary have been found to exert protective effects against various types of cancer. However, the precise mechanisms underlying these anticancer effects have not yet been fully elucidated **(Nieto et al., 2018)**.

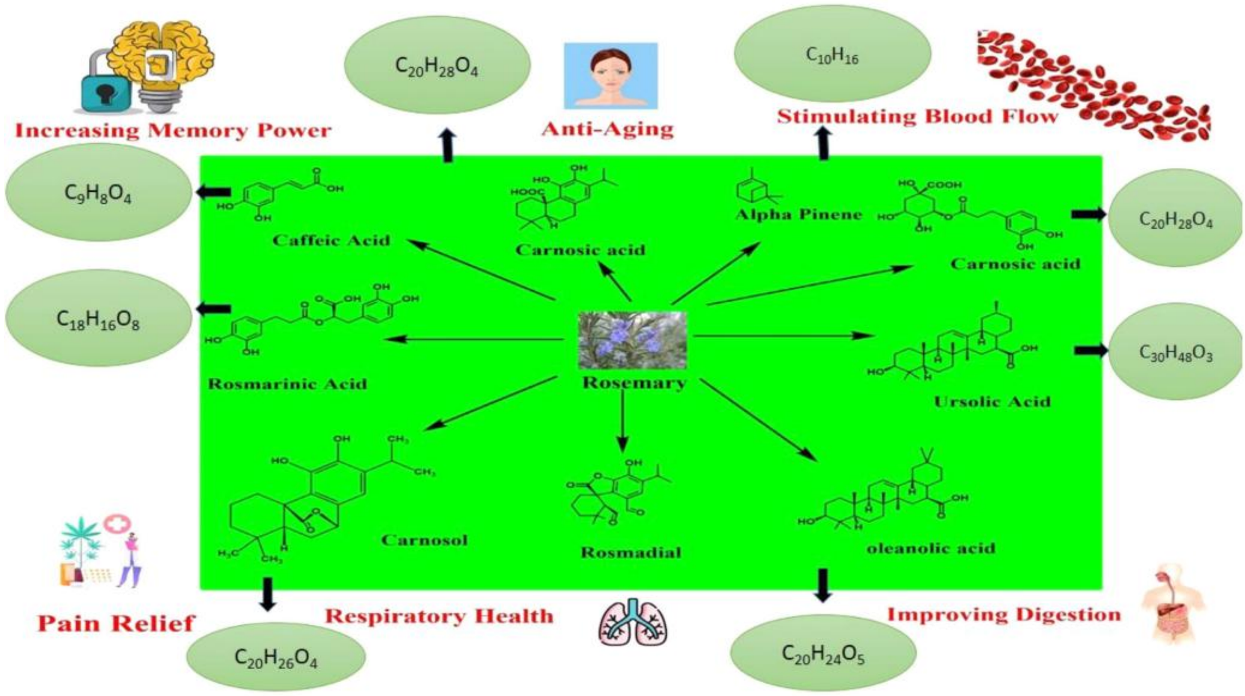


Figure 17: The potential of healthcare impacts of *R. officinalis* on the human body **(Aziz et al., 2023).**

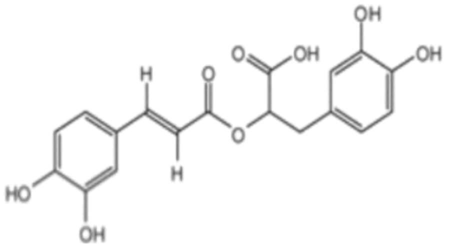
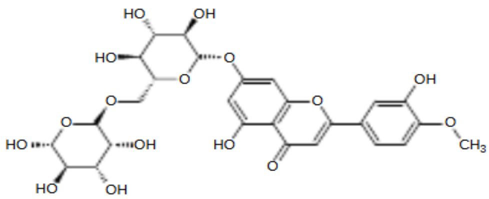
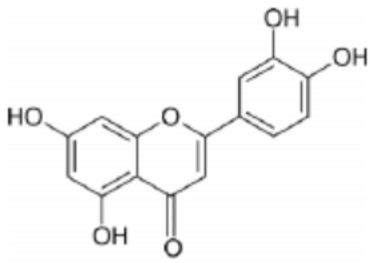
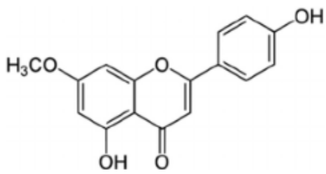
2.6. Antioxidant Properties:

The antioxidant properties of rosemary are largely attributed to its content of isoprenoid quinones, which function as chain terminators for free radicals and chelators of reactive oxygen species. Additionally, the phenolic compounds present in rosemary extracts act as primary antioxidants by reacting with lipid and hydroxyl radicals, converting them into more stable, less reactive molecules. These compounds also have the capacity to bind metal ions, particularly ferrous ions (Fe^{2+}), which helps to reduce the formation of oxygen-derived reactive species. Through these multiple pathways, rosemary contributes effectively to the reduction of oxidative stress in biological systems **(Nieto et al., 2018).**

2.7. Active Compounds of the Plant:

In *Rosmarinus officinalis*, the most common polyphenols are apigenin, diosmin, luteolin, genkwanin, and phenolic acids (>3%), particularly rosmarinic acid, chlorogenic acid, and caffeic acid. Other major compounds found in rosemary are terpenes, which are typically present in essential oils and resins. These terpenes include over 10,000 compounds, categorized into mono, di, tri, and sesquiterpenes, based on the number of carbon atoms and isoprene units (C5H8). In rosemary, terpenes such as epirosmanol, carnosol, carnosic acid, ursolic acid, and oleanolic acid (triterpenes) are found **(Kompelly et *al*., 2019)**.

Table 7: Chemical structure of some active compounds of this plant

Secondary metabolite	**Structure**	**References**
rosmarinic acid		**(Akoury., 2012)**
diosmin		**(Bogucka-Kocka et *al* ., 2013)**
luteolin		**(Cox et *al*.,2003)**
genkwanin		**(El Meníy et al ., 2023)**

2.8. Rosemary Effects on Mood, Memory, and Learning:

Rosemary has shown promising antioxidant effects on mood, memory, and learning. Studies indicate that its active compounds, such as rosmarinic acid and carnosic acid, can help reduce anxiety and depressive symptoms while enhancing memory performance and sleep quality. In particular, rosemary extracts have been associated with reduced stress, improved emotional regulation, and increased cognitive functions in experimental models. These findings suggest that rosemary could serve as a natural alternative to synthetic stimulants often used to boost mental performance, especially among students facing academic stress **(Pawłowska et *al* .,2020)**.

3. Aristolochia clematitis L:

3.1. Presentation of Aristolochia clematitis L:

Aristolochia clematitis L is the most frequent species found in Europe. It is a perennial herbaceous plant that belongs to the family Aristolochiaceae .It is plant distributed throughout Europe, Asia, Minor and Caucasus .It has been used as a medicinal plant since antiquity.

A. clematitisL usually grows in warm, sunlit places , with nutrient-rich soils, in light floodplain forests, on the banks of water-courses, on embankments, wastelands, scrubby slopes, in vineyards and beside road andrailway embankments **(Lerma-Herrera et al., 2022, Brzi´c et al.,2023).**

3.2. Description botanical:

A.clematitis L is a perennial herbaceous plant that can grow up to 1 m .It often propagates vegetatively, through fragile rhizomes. it is indeed a fascinating yet dangerous plant. Its striking appearance with a reedy stem, heart-shaped leaves, and yellow bugle-like flowers can be quite deceptive, given the potent toxins it harbors **(Lerma-Herrera et al.,2022,Katsnelson, 2020).**

The flowers emit from its an unpleasant odor .Its fruits , which is about 5 cm in diameter, is a small pear-shaped capsule. it also possesses underground stolons .Its rhizomes are stimulating and have oily properties.

Figure 18: *Aristolochia clematitis L***(Hranjecet al, 2005).**

3.3. Systematic classification:

According to **Linnaeus, 1753**, the T taxonomic classification of the plant species *Aristolochia clematitis L* is:

Table 8: Chemical structure of some active compounds of this plant.

Taxonomic Rank	Nomenclature
Kingdom	Plantae
Phylum	Tracheophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Magnoliidae
Order	Piperales
Family	Aristolochiaceae
Genus	Aristolochia L
Espèce	Aristolochia clematitis

3.4. Therapeutic uses:

Aristolochia clematitis L is used as stomachic, an astringent in dentistry, used against gastric disorders, headache, snake bite poisoning, toothache and fever. In addition, the decoctate of the plant is used for the treatment of the ulcer. Furthermore, Theophraste (372-286 before J.-C.) reported that *A. clematitis L* is popularly used for treating uterus dysfunction, snake bite, stomach complaints and the wound. Moreover, this plant is used for a long time as disinfectant and helps in childbirth. In South Algeria, and in addition to the medicinal uses of *A. clematitis* described above, the population uses this plant as antitumor and anticancer **(Iserin et al., 2001, Benmehdi, 2017).**

3.5. Antioxidant Properties of Aristolochia clematitis :

Aristolochia clematitis L is a medicinal plant traditionally used in folk remedies, and recent studies have confirmed its significant antioxidant properties. Methanolic and ethanolic extracts from the roots and leaves have shown high levels of phenolic and flavonoid compounds. Various in vitro assays such as DPPH, TBARS, and β-carotene bleaching have demonstrated the plant's ability to neutralize free radicals and reduce oxidative stress, highlighting its potential in preventing oxidative damage and related disorders **(Ćujić et al., 2023; Bibi et al., 2023).**

Advanced analytical techniques like HPTLC-DPPH bioautography have further identified bioactive constituents such as tannins and alkaloids that contribute to the plant's antioxidant capacity **(Tariq et al., 2023).**

Despite these beneficial effects, caution is advised when using *A. clematitis* extracts, as the plant contains aristolochic acids, which have been associated with nephrotoxicity and DNA mutations in long-term exposure. Therefore, isolating beneficial antioxidant compounds from potentially toxic components is recommended to ensure safe application **(Jelaković et al., 2012).**

3.6. Active Compounds and Biological Effects of Aristolochia clematitis :

The phytochemical analysis of *Aristolochia clematitis L* reveals a rich diversity of biologically active compounds, including alkaloids, flavonoids, phenolic acids, and terpenoids, which contribute to the plant's antimicrobial, antioxidant, and anti-inflammatory properties **Pricop et al., 2024).**

- **Alkaloids:**

The plant contains aristolochic acids I and II (AA-I, AA-II), which are nitrophenanthrene derivatives, as well as related compounds such as aristolactam N-β-D-glucoside, magnoflorine, and aristolone. While these compounds exhibit strong antimicrobial and cytotoxic effects, aristolochic acids are associated with nephrotoxicity and genotoxicity, raising concerns regarding their safety **(Rastrelli et al., 2019; Pohodina et al., 2021).**

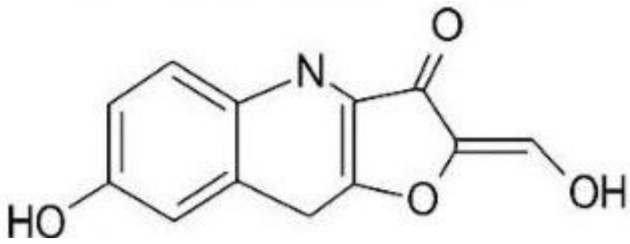
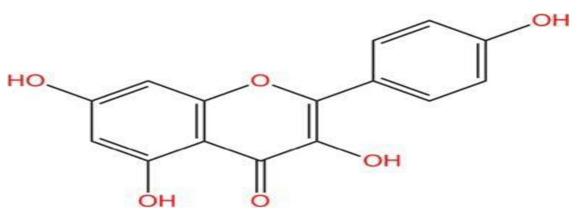
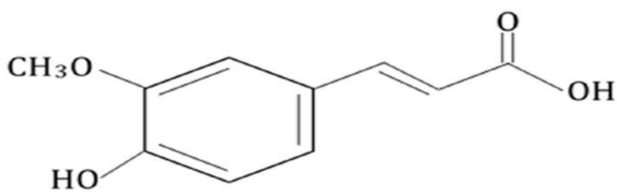
- **Phenolic Compounds and Flavonoids:**

Several phenolic acids, including ferulic acid, syringic acid, and 4-coumaric acid, have been identified in A. clematitis, alongside flavonoids such as kaempferol and rutin. These compounds play a significant role in the plant's antioxidant capacity and protection against oxidative stress **(Burda et al., 2021; Pricop et al., 2024).**

- **Terpenoids:**

Among the terpenoid compounds isolated from A. clematitis is aristolone, known for its anti-inflammatory properties and in vitro cytotoxic activity, supporting its pharmacological potential **(Pohodina et al., 2021).**

Table 9: Chemical structure of some active compounds of this plant .

Secondary metabolite	**Structure**	**References**
Aristolochic acid I (Alkalold)		**Rastrelli et al., 2019**
Aristolactam N-β-D-glucoside)		**Pohodina et al., 2021**
Kaempferol		**(Hussain et al., 2024)**
Ferulic acid		**(Sgarbossa et al., 2015)**

Part Two. The practical part

Chapter 01: Material and method

1. Objectives

The objective of this study is to evaluate the antioxidant effect of natural extract from specific medicinal plants on mood, memory, and learning in adult BALB/c mice subjected to chronic psychological stress. These concentrated, antioxidant-rich plant extracts have been utilized in earlier research and will be used in this study as an alternate treatment for behavioral and cognitive impairments brought on by stress. The extract (treatment) will be administrated by intraperitonial injection into mice and monitoring their behavioral reactions through a battery of behavioral and neurological tests that have been widely used; the results will be recorded and discussed.

2. Vegetal material :

The study focused on three natural plants that were meticulously selected based on their biological properties and the high concentrations of antioxidants in their extracts. These plants are:

- ***Rosmarinus officinalis*** (rosemary) .
- ***Aristolochia clematitis L .***
- ***Ephedra alata*** (alanda).

Figure 19: The medicinal plants used

A: *Rosmarinus officinalis L* **B:** *Aristolochia clematitis L* **C:** *Ephedra alata*

2.1. Harvesting :

The active parts of each plant species were carefully selected, taking into account the optimal harvest time to ensure the preservation of their chemical properties.

For *Ephedra alata*, the stems were collected during spring (March 2025) from Ain Amenas, located in the Tamanrasset region.

For *Aristolochia clematitis* L., the roots and leaves were harvested after the end of the floweringperiod (February 2025) from a region in Mila province.

For *Rosmarinus officinalis*, the leaves were collected during the flowering period in late spring from the mountainous areas of the Mila governorate during the 2025 season.

2.2. Preparation of plants:

- **Washing:** It is imperative that all plant parts (roots, leaves, or stems) be thoroughly washed with water to ensure the removal of any impurities.

- **Drying and Grinding:** Plant parts are dried in a shaded, well-ventilated area; the drying time ranges from 7 to 15 days depending on the type of plant and drying conditions.

Subsequently, the appropriate parts of the plant should be cut, and then the plant parts should be ground using an electric grinder to obtain a fine and homogeneous powder.

Figure 20: The powder of the plants used

A: *Rosmarinus officinalis L* **B:** *Aristolochia clematitis L* **C:** *Ephedra alata*

- **Storage:** The powder should be stored in airtight glass containers in a dark place away from moisture.

2.3. Extraction by maceration:

2.3.1. The principle:

The process of maceration entails submerging the ground plant material in an airtight container that is filled with an appropriate solvent; it is often a hydro alcoholic solution, such as 80% ethanol. This solvent is highly effective in extracting antioxidant compounds. The samples are kept at room temperature for no less than **72** hours, with regular stirring to ensure the solvent's ability to extract the bioactive compounds present in the plant material**. (Chongo, 2025; Gonfa et al., 2020; Ni'maturrohmi et al., 2025).**

After the maceration period, the resulting mixture is filtered to separate the liquid extract from solid residues and remove any impurities. Then, the filtrate is evaporated using a rotary evaporator at 50 °C **(Bensam et al., 2023).**

2.3.2. Method:

80 g of *Rosmarinus officinalis*, 80 g of *Ephedra alata*, and 80 g of *Aristolochia clematitis* L., each previously dried, ground, and accurately weighed, were placed separately into individual Erlenmeyer flasks.

A hydroalcoholic mixture (ethanol/water at a ratio of 80/20 v/v) was then added to each flask.

The flasks were covered with aluminum foil to protect the contents from light exposure and preserve light-sensitive compounds.

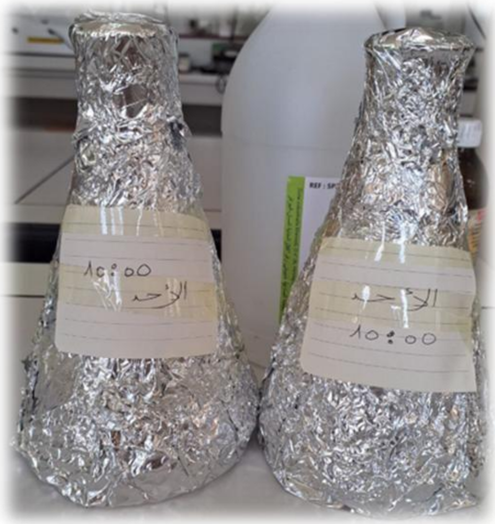


Figure 21: Maceration

The mixtures were left at room temperature for 72 hours under continuous stirring using a stirrer, in order to enhance the extraction of biologically active compounds.

After the maceration period, each mixture was filtered using filter paper, and the resulting filtrates were collected for subsequent use.

Figure 22: Filtration assembly

2.4. Evaporation:

2.4.1. Principle:

The principle of a rotary evaporator is based on vacuum (partial) distillation. The solution is rotated to increase the surface area for evaporation, and the pressure is reduced, typically using a water pump. The combination of rotational speed and reduced pressure allows evaporation to occur at temperatures lower than the normal boiling points of the solutions being evaporated **(Nyarko, Larbie, Anning, & Baidoo, 2019)** .

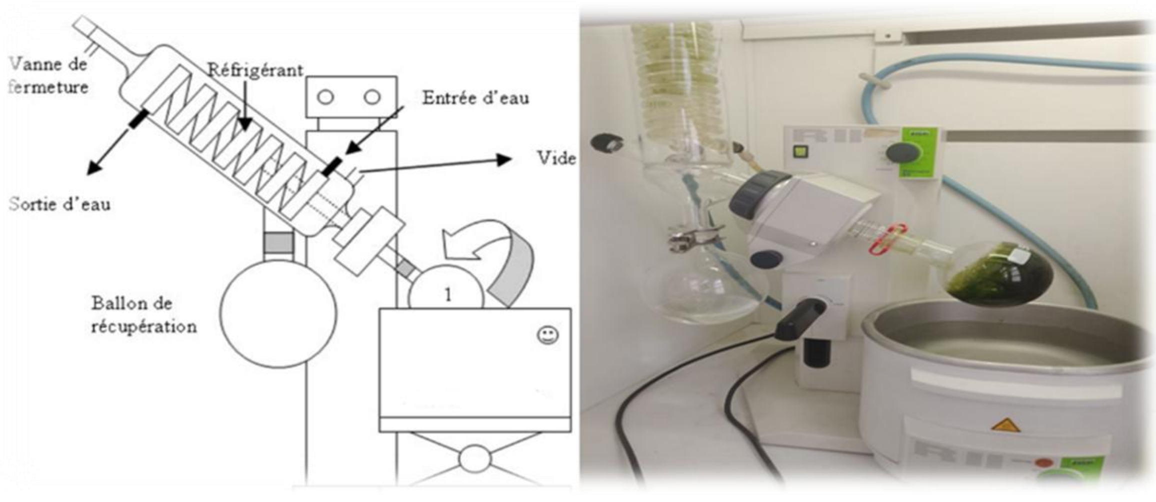


Figure 23: The assembly of a rotary evaporator

2.4.2. Method:

The extract was placed in the rotary evaporator flask. The temperature of the device was set to 65 °C. The operation was carried out continuously until all the ethanol had evaporated. The dry extract remained adhered to the walls of the flask and was then recovered into a glass Petri dish using dichloromethane (a solvent that easily evaporates in open air). After complete drying of the extract, the extraction yield was determined.

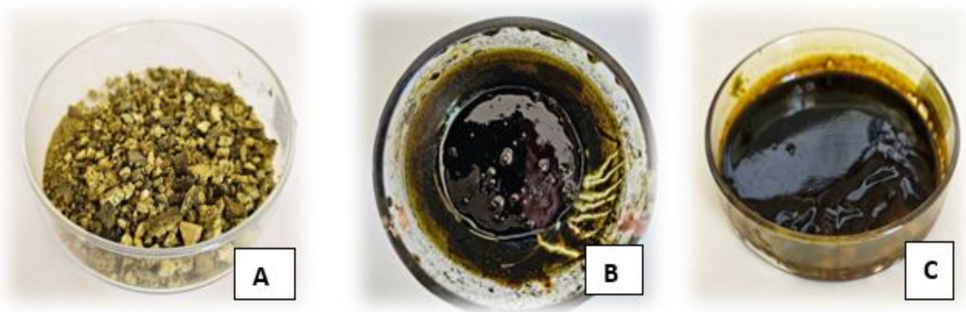


Figure 24: The dry extract obtained for each plant

A: *Rosmarinus officinalis L* **B**: *Aristolochia clematitis L* **C**: *Ephedra alata*

2.5. Determination of Extraction Yield of Natural Antioxidant Extracts:

The extraction yield was calculated to determine the amount of dry matter extracted from each plant, expressed as a percentage of the initial mass of the plant powder used **(Tourabi et al., 2025).**

The following formula was used to calculate the yield:

$$\boxed{\textit{Yield}\ (\%) = (Me\ /\ Mp) \times 100}$$

Where:

Me: Mass of the dry extract (g).

Mp: Mass of the plant powder used (g).

3. Animal material:

For this research work, *in vivo* experiments were conducted on 28 adult male and female *Mus musculus* BALB/c mice, aged 7 to 8 weeks and weighing between 18 and 25 grams. This strain was selected because it is commonly used in behavioral studies related to stress and cognitive function **(Garcia & Esquivel, 2018)**.

Thirty mice were purchased from the Pasteur Institute ; Before the start of the experiment, the animals were housed in clean plastic cages in a temperature and humidity controlled environment (approximately 22 ± 3 °C, 40–60% humidity), under a natural photoperiod (12/12 h light/dark cycle). During the 7-day adaptation period, they were provided with an adequate amount of standard food pellets and water ad libitum.

Figure 25: *Mus musculus* BALB/C mouse (personal photo, 2025).

3.1. Distribution of experimental groups:

To satisfy the requirements of the study, 30 mice female and male BALBc were utilized for all experimental procedures. These animals were randomly assigned to three distinct lots, with each lot comprising six mice (n=6).

Each group is submitted to a specific life conditions and behavioral tests outlined below:

- **group 1 : Control Group**

This group had six adult mice in good health who had not received any treatments (stress and antioxidant extracts therapy), allowing them to remain in their typical physiological and behavioral states. The subjects had unlimited access to food and water and were kept in their natural habitat. This group functioned as the study's control group and was used to compare the other groups. To evaluate behavior and memory in typical circumstances, they were subjected to the same behavioral tasks.

- **Group 2 : Stress-exposed and antioxidant-treated group**

This group consisted of 18 adult BALB/c mice, which were divided into three subgroups (a, b), with each subgroup containing 6 mice. All animals were subjected to psychological stress induced by the sound of a predator (dog or cat) for 12 consecutive days, with the aim of inducing a state with the intention of creating a chronic stress state that would lead to behavioral abnormalities. Immediately after each daily stress session, a distinct plant extract was administered to each subgroup:

- **Subgroup A:** Rosemary extract.

Three mice were administered a dose of 100 mg/kg, and three others received a dose of 200 mg/kg of rosemary extract. These doses were selected based on a previous study by **Noori Ahmad Abadi et al. (2016) and Rasoolijazi et al. (2015).**

- **Subgroup B*:*** *Ephedra alata* extract.

Three mice were administered a dose of 100 mg/kg, and three others received a dose of 200 mg/kg of *Ephedra alata* extract, that is, the permissible dose reported in previous studies by **Bensam et al. (2023).**

- **Subgroup C:**

Aristolochia clematitisL extrait Three mice were administered a dose of 100 mg/kg, and three others received a dose of 200 mg/kg of *Aristolochia clematitisL* extract.

- **Group 3: Stress-exposed but not treated by antioxidant group (injected by physiological water)**

This group consisted of 5 adult BALB/c mice that were exposed to psychological stress but did not receive any antioxidant treatment.

This group served as a negative control to assess the behavioral and cognitive effects of stress alone, without any antioxidant intervention. It allowed comparison with both the control group (no stress, no treatment) and the antioxidant-treated groups. Behavioral tests were conducted to evaluate changes in mood, anxiety, depression, and memory.

3.2. Stress protocol:

A psychological stress protocol was applied to adult BALB/c mice to induce behavioral and cognitive changes resembling anxiety, allowing for the evaluation of the effectiveness of plant-based antioxidants in alleviating these symptoms.

The protocol involved exposing the mice to acoustic stimuli that simulated predator sounds, such as cat meowing, which were obtained from a YouTube video and modified to fit the experimental conditions for 15 minutes daily; the control group was not exposed to these stress-inducing sounds and was kept in comfortable housing conditions in a separate location. The exposure sessions continued for 12 consecutive days **(Balatskyi et al., 2025)**.

Figure 26: Stress method.

3.3. Treatment:

Two doses were prepared from each plant extract; a dose with a concentration of 100 mg/kg and another with 200 mg/kg.

Given that the average weight of the mice is approximately 26 g and that the injected volume per mouse is 0.2 mL and administered intraperitoneally to all animals daily, immediately after each stress exposure session **(Noori Ahmad Abadi et al., 2016)**, the doses were prepared as follows:

- **100 mg/kg dose:**

An amount of 2.6 mg of the extract was dissolved in 200 mL of physiological saline solution (0.9% NaCl), and the solution was thoroughly mixed to ensure homogeneity.

- **200 mg/kg dose:**

Twice the amount of extract used in the previous dose was dissolved in the same volume of physiological saline, followed by thorough mixing to obtain a homogeneous us solution.

The mice were then subjected to a series of behavioral tests to assess changes in mood, learning, and memory. This treatment aims to evaluate the potential effectiveness of antioxidant-rich plant extracts in mitigating the behavioral and cognitive effects of chronic stress.

Figure 27: Treatment bottle.

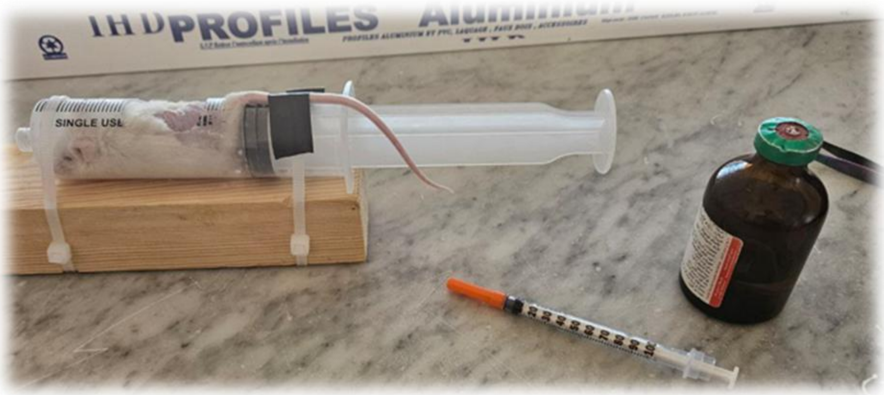


Figure 28: Injecting mice with the treatment.

3.4. Behavioral and Cognitive Tests in Stressed mice:

In this study, we selected the following appropriate tests:

- Open field test .
- Elevated plus maze test .
- Forced swim test.
- Morris water maze test.
- Novel object recognition—NOR .

Behavioral experiments were conducted on all mouse groups after 14 days, following the completion of the treatment program using plant extracts in the two designated groups. The tests were performed during the morning hours, between 8:30 AM and 12:00 PM, in a quiet room with constant white-light conditions to eliminate any unwanted effects caused by noise or inappropriate lighting.

These tests were designed to evaluate the antioxidant effects of the plant extracts on mood, memory, and learning, in order to assess their potential to improve cognitive and behavioral performance in stress-exposed rodents.

3.4.1. Test to assess emotional state (mood):

3.4.1.1. Anxiety-like behavior:

- **open field test:**

The protocol:

The experimental protocol was to place each mouse individually into a corner of a square box with dimensions of 30 cm (length) × 30 cm (width) × 40 cm (height), which is divided into two zones: a central zone (21 × 21 cm) and a peripheral zone. Test sessions typically last 5 minutes and are conducted under a bright white light positioned directly above the box. After each experiment, the floor and inner walls of the box were cleaned with a cotton swab soaked in 70% ethanol to minimize odor stimulation and ensure the same testing conditions for each animal. It is expected that animals will show a tendency to avoid the exposed central area and stay in the peripheral area, which can be used as an indicator of anxious behavior. All behaviors of the mice were recorded by a camera. **(Asgharzadeh et al., 2025; Figueiredo Cerqueira et al., 2023).**

Evaluation Criteria:

Among the parameters used to assess anxiety-like behavior, one key metric is the duration (in seconds) that mice spend in the central and peripheral zones **(Asgharzadeh et al., 2025; Figueiredo Cerqueira et al., 2023).**

The experimental setup used in this study is shown in **Figure 29.**

Figure 29: Open field test (personal photo, 2025).

- **Elevated plus maze test:**

Protocol:

The protocol involves placing the animal on a device made of acrylic glass (Plexiglas), shaped like a plus sign (+) and raised 40 cm from the ground. The maze consists of four arms, two open arms (30 x 5x 0.5 cm) and two closed arms (30×5 x 15 cm) that start from a central point (5 x 5 cm). Each mouse is placed in the center of the maze with its face towards one of the closed arms. The total time each mouse spends in both the open and closed arms is then recorded over a period of 5 to 7 minutes. Entry into the arm is counted when all four legs of the animal are fully inside the arm. After each trial, the apparatus is thoroughly cleaned with a cloth dampened with 10% ethanol **(Adnan et al., 2020; Agrawal et al., 2024).**

Evaluation criteria:

In the EPM test, the total time spent in the open arm and total times spent in the closed arm were recorded. The percent of time spent on the open arms was then determined as follows:

% = 100x Number of seconds spentonopenarms /300 total seconds (5 min observation time) (Agrawal et al., 2024) .

The experimental setup used in this study is shown **in Figure 30.**

Figure 30: Elevated plus maze test (personal photo, 2025).

3.4.1.2. Depression like behavior:

- **Forced swim test:**

Protocol :

The protocol involves filling a container with tap water at room temperature (25°C). The container should have a height of at least 50 cm and a diameter of 20 cm for mice, with the water level set at 10 cm. This depth prevents the mice from touching the bottom with their hind limbs or tail and from attempting to escape. The test session lasts for 5 minutes, during which behaviors are manually recorded, including the time spent swimming, struggling, and immobile floating. At the end of the session, the animals are dried with a towel and then placed in a container with rice hulls to prevent hypothermia. Animals exhibit immobility by floating without resistance, with minimal movements to keep their heads above the water **(Yankelevitch-Yahav et al., 2015; Nguyen et al., 2025)**

Evaluation Criteria:

Immobility time (in seconds) is measured during the last four minutes of the session (from minute two to the end of minute five).

The experimental setup used in this study is shown in **Figure 31.**

Figure 31: Forced swim test (personal photo, 2025).

3.4.2. Tests learning and memory:

- **Morris water maze test:**

Protocol:

The test protocol involved a circular pool (diameter ranging from 150 to 210 cm, height 50 cm) with a smooth inner surface, filled with water at a temperature of 20±1°C to a depth of 25–32 cm. An opaque substance such as milk or a non-toxic dye was added to the water to hide the platform from the rats' sight, ensuring that they relied on spatial cues rather than direct vision.

The pool was divided into four quadrants marked with visible proximal cues (, ◦, —, N) and distal cues placed around the pool to provide consistent spatial references. The escape platform (dimensions: 10×10 cm) was placed 1–2 cm below the water surface, usually in the southwest (SW) quadrant **(He et al., 2024; Imran et al., 2021)**.

Figure 32: Morris water maze test (personal photo, 2025).

On the first day of the experiment, an acclimatization phase was conducted, during which each rat was placed in the pool for 60 seconds without the platform to allow adaptation to the water environment. This was followed by the acquisition phase, which lasted four days (from day 2 to day 6), during which three training trials were conducted daily for each rat. Each trial lasted a maximum of 150 seconds, and animals were given 120 seconds to find the hidden platform. If successful, the animal remained on the platform for 10 seconds to reinforce learning;

if not, it was gently guided to the platform and kept there for the same duration. A 5-minute inter-trial interval was maintained.

On day 6, the probe test (retention test) was conducted by removing the platform and recording the time the rats spent in the target quadrant and the number of times they crossed the former platform location over a 60–120 second period. The protocol included video monitoring of the animals’ behavior using a camera placed above the pool to record performance during the various phases the of test **(Kandeda et al., 2025).**

Evaluation Criteria:

The time spent by the animal in the target quadrant was recorded, as well as the time it took to locate the platform's position **(Kandeda et al., 2025).**

The experimental setup used in this study is shown in **Figure 32.**

- **Novel object recognition NOR:**

Protocol:

The protocol involves the use of square testing boxes with dimensions of 40 cm × 40 cm × 40 cm, where the NOR test was conducted.

On the first and second days (training phase), two identical objects measuring 5 cm × 5 cm × 5 cm were placed inside the experimental apparatus. After 24 hours of training, one of the objects was replaced with a new object to assess the rodents' preference between the new and the familiar objects. The objects used were similar in size and shade, with slight differences in shape to ensure that the rodents could distinguish between them.

Figure 33: the Novel Object Recognition test (personal photo, 2025).

Each mouse was placed at one side of the arena with its back facing the objects, then allowed to freely explore for two minutes. To ensure a clean experimental environment free from unwanted sensory influences, the arena and objects were cleaned with a 70% ethanol solution after each trial. All sessions were recorded using a mobile phone.

During the analysis, the total time spent by the rodents exploring the two objects (new and familiar) was recorded for each animal. The animal was considered to be exploring an object when it engaged in direct sniffing, head or whisker rubbing, biting, or licking. Other behaviors such as looking without contact, sitting on the object, standing on it, or sniffing the air nearby were not counted as exploration **(Jin et al., 2022).**

Evaluation criteria:

The Discrimination Index (DI) was measured to assess the ability of animals to discriminate between novel and familiar objects.

DI = (Time spent exploring the novel object – Time spent exploring the familiar object) / Total exploration time **(Bekci et al., 2024).**

The experimental setup used in this study is shown in **Figure 33.**

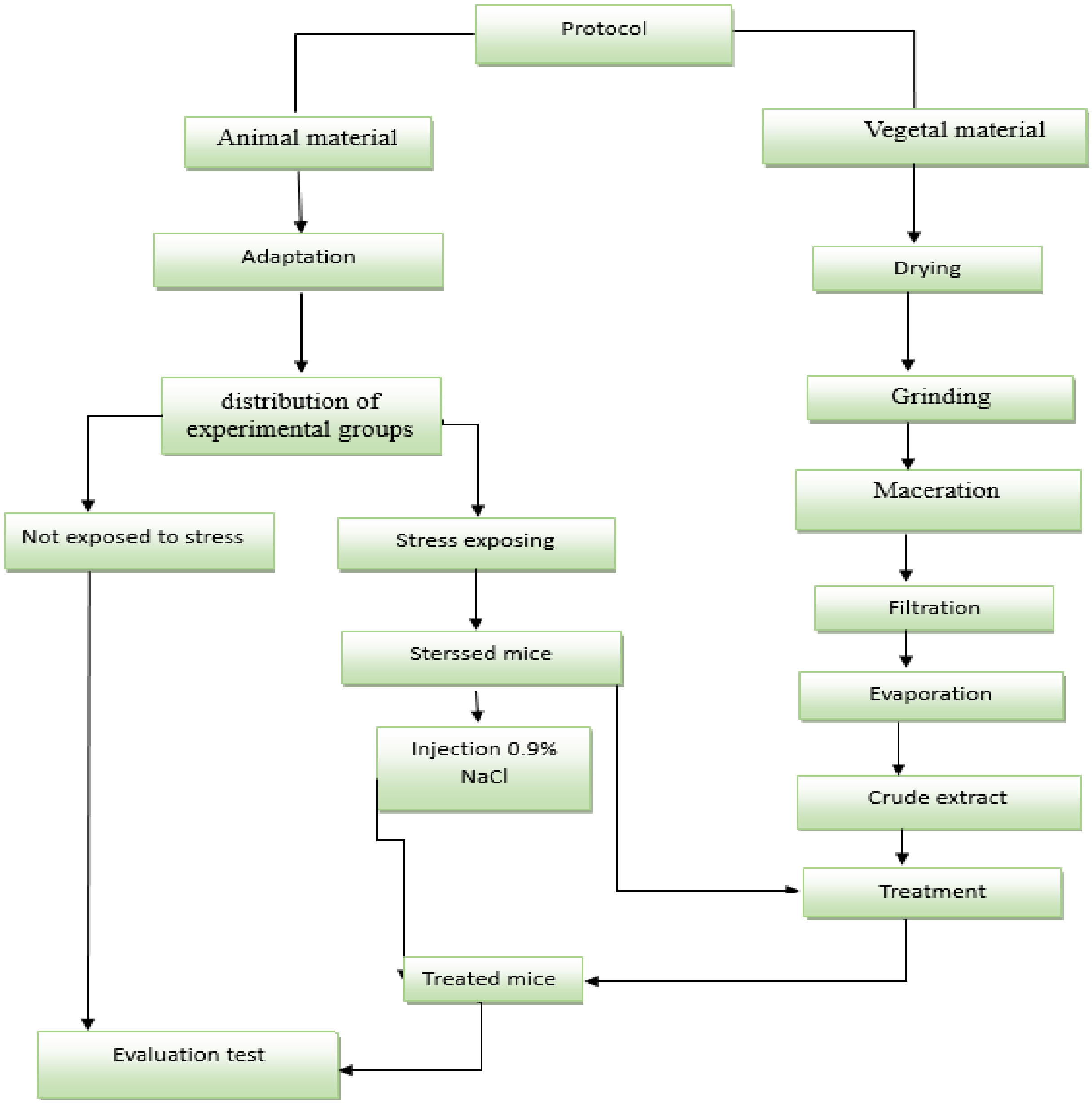


Figure 34: Summary Flowchart of the Experimental Procedures

Chapter 02: Results and Discussion

1. Results:

1.1. Open Field Test results :

The results presented below represent data from the Open Field Test, a behavioral test used to evaluate anxiety-related indicators in laboratory animals. The time spent by the mice in the central zone was measured as an indicator of anxiety levels. The results showed variations among the studied groups, which can be attributed to differences in the type of plant extract used and the applied dosage.

Table 10: Average Time Spent in the Central Zone (in seconds) During the Open Field Test for Different Experimental Groups

Groups	Control	Sterssed	AC100	AC200	RO100	RO200	EA100
Average	39,33±5,6	23,333±7,02	34±3,6	56,333±1,5	35,66±15,50	53,3±5,5	12±20,78

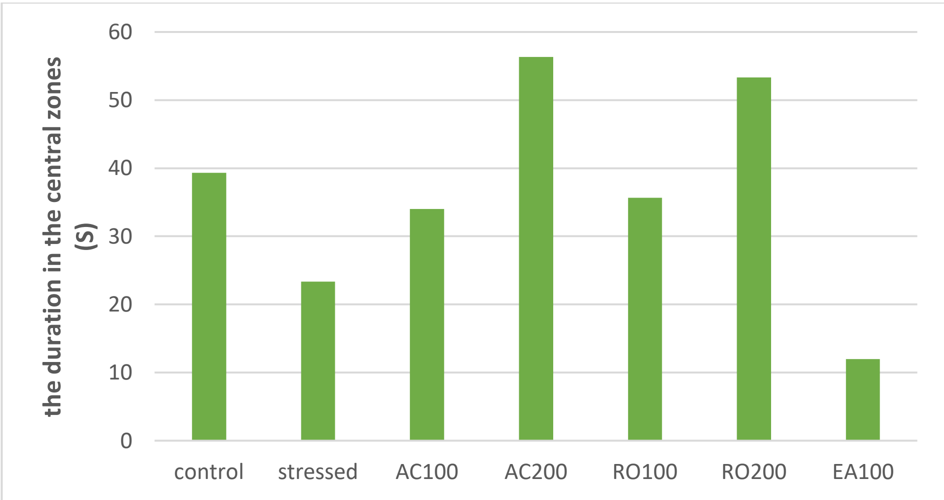


Figure 35: Graphical Representation of the Average Duration Spent in the Central Zone (s) in the Open Field Test.

1.2. Elevated plus maze test:

This section presents the results of the Elevated Plus Maze Test, which is used to assess anxiety levels in laboratory animals by measuring the time spent in the open arms. The results revealed behavioral differences among the studied groups, indicating that the type of plant extract and the applied dosage had varying effects on anxiety-related behavior. This reflects the differences in the anxiolytic or stimulant efficacy of each treatment.

Table 11: The Average percent of time spent on the open arms for different experimental group.

Groups	control	stressed	AC100	AC200	RO100	RO200	EA100
Average	42,6±2	21±6,9	44,5±2,12	44,5±30,4	32±8,71	36,6±0,57	7,3±12,7

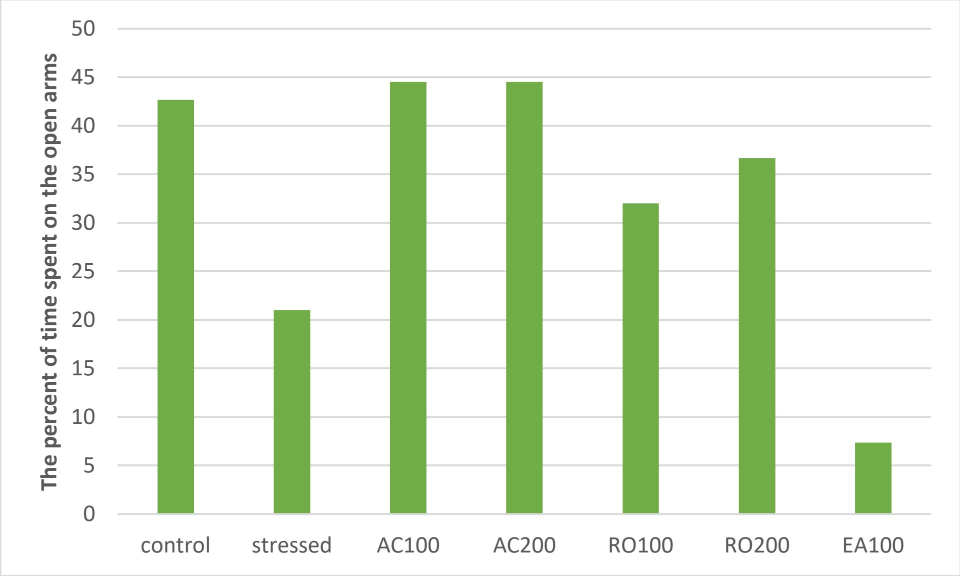


Figure 36: Graphical Representation of the percent of time spent on the open arms

1.3. Forced swim test results :

The following results represent data from the Forced Swim Test, which is used to evaluate depressive-like behavior by measuring the duration of immobility. The stressed group showed a noticeable increase in immobility time compared to the other groups, reflecting a depressive-like behavioral state. In contrast, the treated groups recorded results similar to the control group, which may suggest a potential role of the plant extracts in reducing stress-induced depressive behavior.

Table 12: Average Time Spent on Immobility time (in seconds) during the Forced swim test for Different Experimental Groups.

Groups	Control	Sterssed	AC100	AC200	RO100	RO200	EA100
Average	67±21,3	110±18,02	59,6±5,5	63,3±9,2	62,3±27,3	73,6±10,5	23,3±40,4

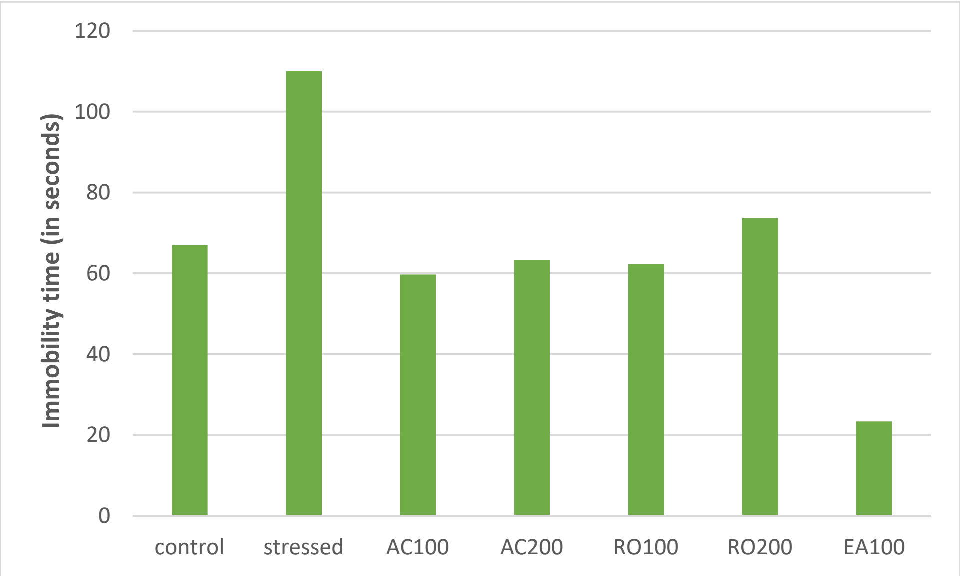


Figure 37: Graphical Representation of Average Time Spent on Immobility time (in seconds) during the Forced swim test for Different Experimental Groups.

1.4. Morris water maze test:

The results presented below represent data from the Morris Water Maze Test, which is used to assess spatial memory and learning in laboratory animals. The time taken to reach the hidden platform was measured as an indicator of cognitive performance. The stressed group showed an increase in search time, indicating a decline in cognitive abilities. In contrast, most of the treated groups demonstrated performance similar to the control group, suggesting a potential role of the plant extracts in mitigating the effects of stress on memory and learning.

Table 13: Average Time to access to the Platform on the fifth day of learning

Groups	control	stressed	AC100	AC200	RO100	RO200	EA100
Average	3±1	5±1	3,6±0,5	3±1	2,3±0,5	3±1	1±1,7

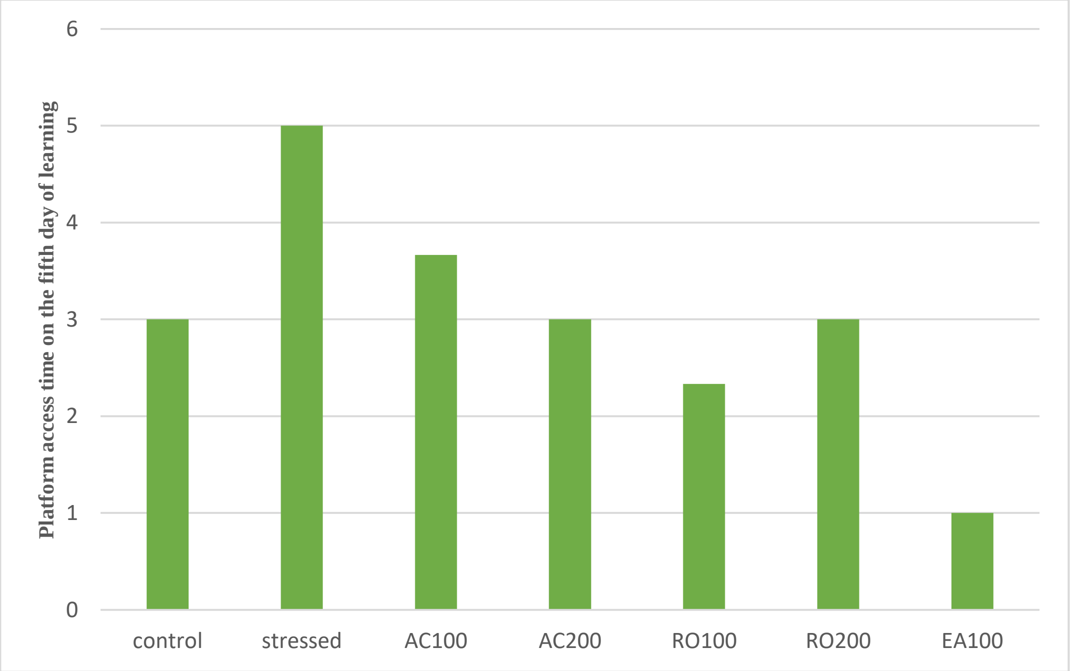


Figure 38: Graphical Representation of average Time to access to the Platform on the fifth day of learning

1.5. Novel object recognition NOR:

These results represent data from the Novel Object Recognition Test, which is used to assess recognition memory in mice. The Discrimination Index was measured across the different experimental groups, including the control group, the stressed group, and the groups treated with plant extracts.

Table 14: Discrimination Index (DI) Results

Groups	control	stressed	AC100	AC200	RO100	RO200	EA100
Average	0,267±0,153	0,35±0,308	0,297±0,333	0,173±0,077	0,257±0,132	0,383±0,232	0,133±0,231

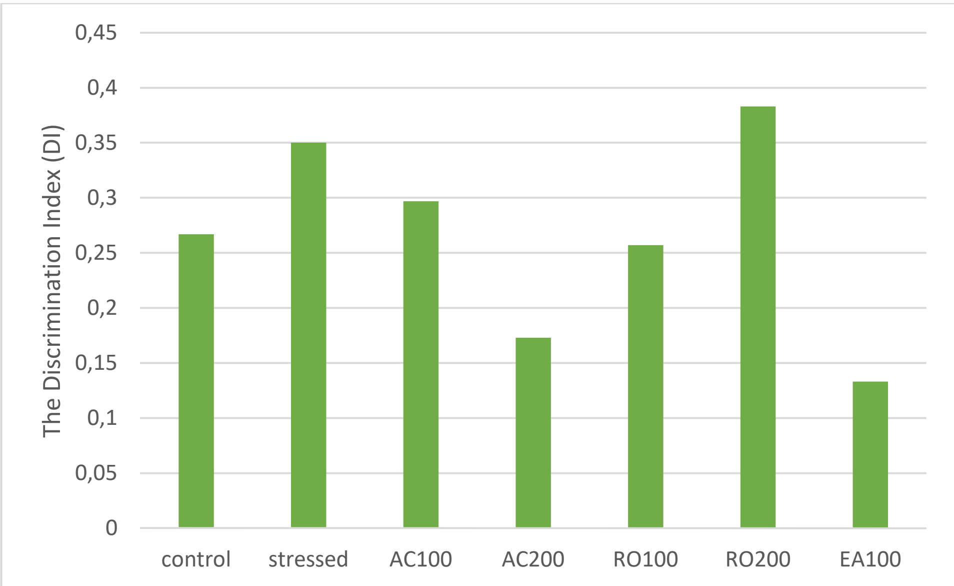


Figure 39: Graphical Representation of the Discrimination Index (DI)

2. Discussion

2.1. Open Field Test Discussion :

The results of the open field test in this study provide strong evidence of the varying efficacy of plant extracts in modulating anxiety-related behavior in stressed mice. The stressed group showed a significant reduction in the time spent in the central **zone (23.33 ± 7.02 seconds)** compared to the control group **(39.33 ± 5.6 seconds),** reflecting heightened anxiety-like behavior due to chronic stress exposure **(Omam et al., 2022).**

At a dose of **200 mg/kg**, the *Rosmarinus officinalis L* extract **(RO200)** yielded a time of **53.3 ± 5.5 seconds**. This is in line with the findings of **Meftah et al. (2023),** who reported that rats treated with rosemary extract **at 200 mg/kg** spent **51.4 ± 3.1** seconds in the central area compared to **29.7 ± 4.6** seconds in the stressed group. This supports the anxiolytic properties of rosemary, attributed to compounds such as rosmarinic acid and essential oils that modulate oxidative stress and neuroendocrine responses.

Similarly, the *Aristolochia clematitis L* extract **(AC200)** recorded **56.33 ± 1.5 seconds**, which is comparable to the findings of **Saffidine et al. (2023)**, where rats treated with a **300 mg/kg** ethanolic extract spent **58.1 ± 3.7** seconds in the central zone versus **26.9 ± 5.1 seconds** in the stressed group. This indicates strong agreement despite a slight dose variation.

In contrast, the *Ephedra alata* extract **(EA100)** produced unexpectedly low results at **12 ± 20.78 seconds**, diverging notably from **El Hachimi et al. (2018)**, who reported that mice treated with **100 mg/kg** methanolic extract stayed in the center for **36.5 ± 9.2 seconds** compared to **22.3 ± 5.8 seconds** in the stressed group. This substantial discrepancy suggests a need to re-evaluate extraction methods and experimental parameters.

Furthermore, findings from **Nguyen et al. (2019)** reinforce the dose-response relationship of plant-based anxiolytics, as they observed an increase from **33.2 ± 4.3 seconds (100 mg/kg)** to **50.7 ± 5.9 seconds (200 mg/kg),** which mirrors our observations of improved responses at the higher dose.

The observed differences, especially concerning *Ephedra alata*, can be attributed to variations in preparation methods and experimental conditions compared to existing literature. In our study, the ethanolic extract was prepared via maceration for **72 hours** at room temperature, administered at **100 mg/kg** for a short duration prior to behavioral testing. In contrast, **El Hachimi et al. (2018)** utilized a Soxhlet-extracted methanolic preparation, administered daily for 15 days, allowing bioactive compounds to accumulate and exert a measurable effect.

Additionally, our study used **BALB/c mice**, a strain known for heightened sensitivity to stress and anxiety, whereas comparison studies often employed the less reactive **Wistar strain**. This could partly explain the pronounced reduction in central zone activity in our **EA100** group.

As for *Rosmarinus officinalis L* and *Aristolochia clematitis L*, despite comparable results, previous studies often employed higher doses **(e.g., 300 mg/kg** for AC in **Saffidine et al., 2023)** or more concentrated extraction techniques such as methanolic or distilled methods. The similarity of outcomes despite these differences strengthens the credibility of our extracts and suggests that active compounds may be effective under milder experimental conditions. This supports the potential application of our protocol as a cost-effective and accessible anxiolytic intervention.

2.2. Elevated Plus Maze (EPM) Test Discussion :

The results of the Elevated Plus Maze test provide valuable insights into the behavioral effects of the studied plant extracts on BALB/c mice exposed to chronic stress. As established in the literature, anxious animals tend to avoid the open arms of the maze due to their elevated and exposed nature, making this a reliable indicator of anxiety-like behavior **(Lister, 1987; Pellow et al., 1985)**. In our study, stressed mice (negative control group) exhibited a significant reduction in open-arm exploration, spending only **9.6% (± 2.33%)** of their time in these areas—indicative of a heightened anxiety state. In contrast, the non-stressed and untreated control group spent **13.66% (± 1.53%)** of the time in the open arms, reflecting baseline exploratory behavior.

The extract of *Rosmarinus officinalis L* (rosemary) exhibited a clear dose-dependent anxiolytic effect. Mice treated with **a 200 mg/kg** dose **(RO200)** spent **24% (± 2.3%)** of the time in the open arms, compared to **15.33% (± 3.05%)** in the **100 mg/kg** group **(RO100).** These results are in line with those reported by **Machado et al. (2013),** who demonstrated that rosmarinic acid, a major component of rosemary, improves anxiety-related behavior through inhibition of monoamine oxidase A and modulation of neurotrophic factors. **Similarly, Khalil et al. (2020)** reported significant behavioral improvements at a **300 mg/kg** dose, reinforcing the anxiolytic potential of this extract.

The extract of *Aristolochia clematitisL* at **200 mg/kg (AC200)** also demonstrated anxiolytic-like effects, as shown by the increased time spent in the open arms **(25.3% ± 2.6%). Although Saffidine et al. (2023)** did not assess anxiety behavior using the EPM, their phytochemical analysis confirmed the presence of flavonoids and polyphenols with known antioxidant and neuroprotective properties. These bioactive compounds may contribute to reduced anxiety through modulation of oxidative stress and inflammation, suggesting potential anxiolytic effects that warrant further investigation in behavioral models.

In contrast, the *Ephedra alata* extract at **100 mg/kg (EA100)** produced an anxiogenic effect, with mice spending only **7.6% (± 0.57%)** of their time in the open arms—less than even the stressed control group. These findings differ from those reported **by El Hachimi et al. (2018),** who observed a moderate anxiolytic effect with a methanolic Soxhlet extract. Several factors may explain this discrepancy:

- Extraction method: Our ethanol-based maceration may have favored the extraction of stimulating alkaloids such as ephedrine, while the Soxhlet method could yield more anxiolytic compounds.
- Treatment duration: A 14-day post-stress treatment was used in our study, versus a 15-day preventive protocol in the reference study.
- Animal model: BALB/c mice, known for their high sensitivity to stress, were used in our experiment, whereas Wistar rats, commonly less stress-reactive, were used in the reference.

These variables highlight the need for standardized protocols when comparing plant-based interventions. Despite its antioxidant content, the limited anxiolytic effect of the EA extract indicates that further optimization of dosage and extraction methods may be required to enhance its therapeutic efficacy.

2.3. Forced Swim Test (FST) Discussion

The Forced Swim Test (FST) is a widely used behavioral model to assess antidepressant-like activity in rodents, where increased immobility time is considered an indicator of behavioral despair. In the current study, the stressed untreated group exhibited a significantly elevated immobility time **(110 ± 18.02 s)** compared to the non-stressed control group **(67 ± 21.3 s),** reflecting the impact of chronic stress on inducing depressive-like behaviors. This result is consistent with the classical model described by **Porsolt et al. (1977),** which correlates increased immobility time with depressive states in animals.

Rosmarinus officinalis extract demonstrated a moderate antidepressant-like effect, with a more pronounced response at the lower dose. The group treated with **100 mg/kg (RO100)** recorded an immobility time of **(62.3 ± 27.3 s),** while the **200 mg/kg** dose **(RO200)** showed a time of **(73.6 ± 10.5 s)**. These results align with findings by **Ghareib et al. (2022),** who reported that Origanum majorana extract significantly reduced immobility time in the FST through its antioxidant and neuromodulatory properties, primarily attributed to its rich content in rosmarinic acid and polyphenolic compounds.

Similarly, *Aristolochia clematitis L* extract exhibited behavioral improvement. The **100 mg/kg** dose (**AC100)** resulted in an immobility time of **(59.6 ± 5.5 s),** while **the 200 mg/kg** dose **(AC200)** recorded **(63.3 ± 9.2 s).** These results suggest a mild antidepressant-like effect, which is consistent with findings by **Alamri et al. (2021),** who reported that Pimpinella anisum extract produced a dose-dependent reduction in immobility in mice, likely due to its flavonoid and phenolic constituents.

In contrast, the *Ephedra alata*-treated group **(EA100)** displayed a surprisingly low immobility time **(23.3 ± 40.4 s)**, accompanied by high variability. Although this may initially suggest an antidepressant effect, the inconsistency could be attributed to the central nervous system stimulants such as ephedrine found in the plant. Previous studies **(Bent et al., 2003; Gurley et al., 2000)** have warned that excessive stimulation by ephedrine may lead to anxiety, hyperactivity, and behavioral instability, which could distort performance in behavioral tests.

Overall, both *Rosmarinus officinalis L* and *Aristolochia clematitis L* demonstrated promising mild-to-moderate antidepressant-like effects. However, further investigation is required for *Ephedra alata* to better determine its behavioral profile, effective dosage, and optimal extraction method.

2.4. Morris water maze test:

The Morris Water Maze **(MWM)** test is one of the most widely used paradigms to evaluate spatial learning and long-term memory in rodents. In the present study, the non-stressed control group exhibited an average target quadrant retention time of **(3 ± 1)** seconds, reflecting normal spatial memory performance. Interestingly, the stressed (untreated) group spent more time in the target quadrant **(5 ± 1)** seconds, which may be explained by increased arousal or anxiety induced by acute stress. This phenomenon has been documented in previous studies reporting that short-term stress can temporarily enhance performance in spatial tasks through glucocorticoid-mediated mechanisms **(Sandi & Pinelo-Nava, 2007).**

Regarding the treated groups, *Rosmarinus officinalis* (rosemary) extract showed promising efficacy, particularly at the **100 mg/kg** dose, which demonstrated improved performance **(2.3 ± 0.5)** seconds. The **200 mg/kg** dose **(RO200)** yielded values comparable to the baseline **(3 ± 1)** seconds. These findings are consistent with the study by **Rasoolijazi et al. (2015),** which showed that administration of rosemary extract to aged rats significantly improved performance in the MWM test. The treated animals spent more time in the target quadrant compared to the control group, suggesting antioxidant effects on the hippocampus.

In contrast, *Ephedra alata* extract showed the poorest performance **(1 ± 1.7)** seconds, reinforcing the hypothesis that excessive stimulation from ephedrine compounds may disrupt neural and behavioral balance.

As for *Aristolochia clamatitis*, the **AC100** group showed a relatively improved retention time **(3.6 ± 0.5 seconds),** while the **AC200** group did not differ much from the baseline. These results are comparable to findings reported by **Hosseinzadeh & Nassiri-Asl (2013**), where high doses of certain plant extracts were associated with sedative effects or neurotoxicity, potentially impairing cognitive performance due to suppressed behavioral activity.

2.5. Novel object recognition (NOR) :

The Novel Object Reco gnition (NOR) test evaluates recognition memory in rodents by measuring their innate preference for novel objects over familiar ones. Results showed that the non-stressed control group recorded a discrimination index of (**0.267 ± 0.153),** reflecting normal cognitive performance. In contrast, the stressed control group (untreated) exhibited a relatively higher value **(0.35 ± 0.308)**, which was unexpected. This result may be attributed to high variability in behavioral responses or individual differences in stress sensitivity. Some studies suggest that acute stress may occasionally cause a temporary enhancement of certain cognitive functions, which could explain such unexpected findings in recognition tasks **(Sandi & Pinelo-Nava, 2007).**

Effects of Rosemary Extract (*Rosmarinus officinalis* – RO):

- The **100 mg/kg** dose showed a slight improvement **(0.257 ± 0.132)** compared to the control group.
- The **200 mg/kg** dose recorded the highest discrimination index **(0.383 ± 0.232),** even surpassing the control groups, indicating strong efficacy in memory enhancement.

Effects of *Ephedra alata* Extract (EA):

- The **100 mg/kg** dose of *Ephedra alata* showed the lowest discrimination index **(0.133 ± 0.231)** among all groups. Although *Ephedra* is known to contain stimulant compounds such as ephedrine, these stimulants may cause overstimulation of the central nervous system when the dosage is not carefully controlled, leading to adverse behavioral effects such as anxiety, irritability, and behavioral confusion **(Bent et al., 2003; Gurley et al., 2000)**.
- Furthermore, some studies have reported that high or chronic use of ephedrine may lead to mood disturbances, including anxiety, irritability, and even psychotic-like symptoms in rare cases, highlighting the need for precise dosage control **(Cohen & Ernst, 2010)**.

Effects of *Aristhilosia climatitis* Extract :

- **AC100: (0.297 ± 0.333),** demonstrated a performance close to that of the non-stressed control group.
- **AC200: (0.173 ± 0.077),** showed a reduction in the discrimination index, which could suggest that the higher dose was ineffective or had a sedative effect that suppressed exploratory behavior. Some studies have indicated that high doses of certain stimulants may

paradoxically inhibit behavioral activity rather than enhance it, especially in tasks related to exploratory cognition **(Hosseinzadeh & Nassiri-Asl, 2013).**

Conclusion

Conclusion

This study demonstrated the potential neuroprotective and psychotropic effects of three natural plant extracts—*Rosmarinus officinalis L, Aristolochia clematitis L, and Ephedra alata*—in a murine model of chronic stress. Through a series of behavioral tests (Open Field, Elevated Plus Maze, Novel Object Recognition, and Forced Swim Test), we observed significant behavioral changes that reflect the differential effects of each extract on anxiety, cognition, and depressive-like symptoms.

Rosmarinus officinalis L exhibited moderate anxiolytic and antidepressant effects, particularly at lower doses, which may be attributed to its high content of rosmarinic acid and antioxidant compounds. *Aristolochia clematitis L* also showed favorable results in modulating mood and behavior, especially in the NOR and FST, supporting its potential as a natural neuroprotective agent. On the other hand, *Ephedra alata* presented inconsistent effects, with possible overstimulation likely linked to its alkaloid content, warranting further pharmacological investigation.

Overall, the findings highlight the importance of dose selection, extraction methods, and stress model conditions when evaluating phytochemical interventions. These plant-based treatments show promise as complementary strategies for managing stress-induced behavioral disorders. However, more in-depth studies including molecular analyses, histological validation, and safety profiling are necessary before translating these results into clinical or therapeutic applications.

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