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Anti-inflammatory and antioxiodant potential of *Artemisia herba-alba*: Application in an innovative topical formulation

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First of all, I dedicate this achievement to myself to the self that endured every pressure and challenge, believed in its dream, and pursued it with endless patience and determination. I proudly say: You did it.

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Dedicatio

"And the end of their prayer will be: Praise be to Allah, Lord of the Worlds."

In the name of Allah, my Creator, the Facilitator of my affairs, and the Protector of my path. To You belongs all praise and gratitude.

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Nihal

Abstract

This study aimed to valorize the medicinal plant *Artemisia herba-alba* through the development of an innovative topical anti-inflammatory formulation combining its aqueous extract and essential oil. and in vivo analyses.

The aqueous extraction yielded 12.04% of dry extract. Phytochemical screening revealed the presence of flavonoids, tannins, alkaloids, sterols, and terpenoids. Quantitative analysis demonstrated a high total phenolic content of 105 ± 0.33 mg GAE/g extract and a flavonoid content of 36.42 ± 1.61 mg QE/g extract.

The antioxidant activity assessed by the DPPH assay showed a strong radical scavenging capacity with a maximum inhibition of 81.42% and an IC_{50} value of 0.018 mg/ml. In vitro anti-inflammatory evaluation based on inhibition of BSA denaturation demonstrated remarkable activity, reaching 101.72 ± 0.01% inhibition at 0.7 mg/ml, with an IC_{50} of 258.91 ± 4.99 µg/ml.

The in vivo study performed on Wistar rats showed that the innovative cream significantly reduced formalin-induced edema, achieving complete inhibition after 48 h and exhibiting efficacy comparable to Diclofenac. Hematological analysis revealed a reduction in inflammatory markers, particularly white blood cells and monocytes, while lipid peroxidation analysis demonstrated a decrease in hepatic MDA levels from 5.13 ± 0.26 µmol/L in the positive control group to 4.00 ±0.14 µmol/L following treatment with the innovative cream.These findings confirm the strong antioxidant and anti-inflammatory potential of *Artemisia herba-alba* and support the development of a promising natural phytopharmaceutical formulationbased on Algerian medicinal flora.

Keywords: *Artemisia herba-alba*, anti-inflammatory activity, antioxidant activity, phenolic compounds, innovative cream, phytopharmaceutical formulation.

Résumé

Cette étude a pour objectif la valorisation de la plante médicinale saharienne *Artemisia herba-alba* à travers le développement d'une formulation topique innovante à activité anti-inflammatoire associant l'extrait aqueux et l'huile essentielle de la plante. et des essais in vivo.

L'extraction aqueuse a permis d'obtenir un rendement de 12,04 % d'extrait sec. Le screening phytochimique a révélé la présence de flavonoïdes, tanins, alcaloïdes, stérols et terpènoïdes.

L'analyse quantitative a montré une teneur élevée en composés phénoliques totaux de 105 ± 0,33mg EAG/g d'extrait ainsi qu'une teneur en flavonoïdes de 36,42 ± 1,61 mg EQ/g d'extrait.

L'activité antioxydante évaluée par le test DPPH a montré une importante capacité de piégeage des radicaux libres avec un pourcentage maximal d'inhibition de 81,42 % et une valeur IC_{50} de 0,018mg/ml. L'évaluation de l'activité anti-inflammatoire in vitro, basée sur l'inhibition de la

dénaturation de l'albumine, a révélé une activité significative atteignant 101,72 ± 0,01 % d'inhibition à la concentration de 0,7 mg/ml, avec une IC_{50} de 258,91 ± 4,99 µg/ml. L'étude in vivo réalisée chez des rats Wistar a montré que la crème innovante formulée à base d'extrait aqueux et d'huile essentielle réduit significativement l'œdème induit par le formol, avec une efficacité comparable à celle du Diclofénac après 48 h de traitement. Les analyses hématologiques ont également montré une diminution des marqueurs inflammatoires, notamment des leucocytes et des monocytes. Par ailleurs, l'analyse de la peroxydation lipidique a révélé une réduction du taux hépatique de MDA de 5,13 ± 0,26 µmol/L chez le groupe témoin positif à 4,00 ±0,14 µmol/L après traitement par la crème innovante.

Ces résultats confirment le fort potentiel antioxydant et anti-inflammatoire d'*Artemisia herba-alba* et soutiennent le développement d'une formulation phytopharmaceutique naturelle prometteuse basée sur les ressources végétales algériennes.

Mots-clés *:* *Artemisia herba-alba*, activité antioxydante, activité anti-inflammatoire, composés phénoliques, formulation topique, phytopharmaceutique.

ملخص

تهدف هذه الدراسة إلى تثمين النبتة الطبية الصحراوية *Artemisia herba-alba* من خلال تطوير تركيبة عالجية موضعية مبتكرة ذات نشاط مضاد للالتهاب تجمع بين المستخلص المائي والزيت العطري للنبات. بالإضافة إلى التجارب الحيوية داخل الجسم الحي.

سمحت عملية االاستخلاص المائي بالحصول على مردود بلغ 12.04٪ من المستخلص الجاف. كما أظهر الفحص الكيميائي النباتي وجود الفلافونويدات، والتانينات، والقلويدات، والستيرويلات، والتربينويدات. وأظهرت التحاليل الكمية محتوى مرتفعا من المركبات الفينولية الكلية قدره 0.33 ± 105 ملغ مكافئ حمض الغاليك/غ من المستخلص، بالاضافة إلى محتوى من الفلافونويدات بلغ 1.61 ± 36.42ملغ مكافئ الكيرسيتين/غ من المستخلص. أظهر تقييم النشاط المضاد الأكسدة باستعمال اختبار DPPH قدرة عالية على تثبيط الجذور الحرة بنسبة قصوى بلغت 81.42٪، مع قيمة IC_{50} تساوي 0.018 ملغ/مل. كما بينت دراسة النشاط المضاد لاللتهاب في المختبر، المعتمدة على تثبيط تمسخ البومين، فعالية معتبرة وصلت إلى 0.01 ± 101.72٪ عند تركيز 0.7 ملغ/مل، مع قيمة IC_{50} بلغت 4.99 ± 258.91ميكروغرام/مل.

وأظهرت الدراسة المنجزة على فئران ويستار أن الكريم المبتكر المحضر من المستخلص المائي والزيت العطري ساهم في التقليل الملحوظ من الوذمة المستحثة بالفورمالين، مع فعالية علاجية مماثلة لدواء الديكلوفيناك بعد 48 ساعة من العلاج. كما كشفت التحاليل الدموية عن انخفاض مؤشرات الالتهاب، خاصة الكريات البيضاء والخاليا الوحيدة. إضافة إلى ذلك، أظهرت تحاليل بيروكسدة الدهون انخفاض مستوى المالون ثنائي الدهيد الكبدي (MDA من 0.26 ± 5.13 ميكرومول/لتر لدى المجموعة الشاهدة الايجابية إلى 0.14 ± 4.00 ميكرومول/لتر بعد العلاج بالكريم المبتكر.

تؤكد هذه النتائج الامكانات العالية المضادة للاكسدة والمضادة للالتهاب لنبات *Artemisia herba-alba*، كما تدعم تطوير تركيبة صيدلانية نباتية طبيعية واعدة قائمة على الموارد النباتية الجزائرية.

الكلمات المفتاحية: *Artemisia herba-alba*، النشاط المضاد للاكسدة، النشاط المضاد للالتهاب، المركبات الفينولية، تركيبة موضعية، مستحضرات صيدلانية نباتية.

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Abbreviation	Full Description
A herba-alba	*Artemisia herba-alba*
Ah	*Artemisia herba-alba*
$AlCl_3$	Aluminum Chloride
BHA	Butylated Hydroxyanisole
BHT	Butylated Hydroxytoluene
BSA	Bovine Serum Albumin
CBC	Complete Blood Count
DPPH	2,2-diphenyl-1-picrylhydrazyl
DPPH•	DPPH Free Radical
EDTA	Ethylenediaminetetraacetic Acid
EXT	Extract
EXT ART	*Artemisia herba-alba* Extract
$FeCl_3$	Iron(III) Chloride
GAE	Gallic Acid Equivalents
GC-MS	Gas Chromatography-Mass Spectrometry
H&E	Hematoxylin and Eosin (Staining)
HCl	Hydrochloric Acid
HRBC	Human Red Blood Cell
IC_{50}	Half Maximal Inhibitory Concentration
LM	Light Microscope
MDA	Malondialdehyde
Na_2CO_3	Sodium Carbonate
Na_2SO_4	Anhydrous Sodium Sulfate
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
NO	Nitric Oxide
NSAIDS	Non-Steroidal Anti-Inflammatory Drugs
PAF	Platelet-Activating Factor
QE / EQUR	Quercetin Equivalents
RBCs	Red Blood Cells
Rpm	Revolutions per minute
SD	Standard Deviation

TBA	**Thiobarbituric Acid**
TCA	**Trichloroacetic Acid**
TNF	**Tumor Necrosis Factor**
TPC	**Total Phenolic Content**
Tris-HCl	**Tris(hydroxymethyl)aminomethane Hydrochloride**
Trolox	**6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid**
UV-Vis	**Ultraviolet-Visible (Spectrophotometer)**
v/v	**Volume per volume**
V_0	**Initial Paw Volume**
Vt	**Paw Volume at time (t)**
WBC	**White Blood Cells**
WHO	**World Health Organization**
w/w	**Weight per weight**
%AUG	**Percentage Increase in Paw Volume**

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Chapter III :Devices And Technikues

Introduction

The evolution of civilizations has always been closely associated with the use of plants and natural medicinal resources, which have played a fundamental role in maintaining human health throughout history **(Dibong et al., 2011)**. Across different civilizations and continents, medicinal plants have occupied a central place in traditional therapeutic practices due to their effectiveness in the treatment of various diseases and their deep integration into cultural heritage and ancestral knowledge systems **(Kidane et al., 2006; Sofowora, 2010).** For centuries, populations have relied on these natural resources not only for primary healthcare but also for the prevention and management of chronic and infectious disorders.

In recent decades, growing interest in alternative medicine and natural therapies has significantly increased the global use of medicinal plants. This renewed attention is mainly driven by the search for safer, more accessible, and less toxic therapeutic alternatives compared to synthetic drugs **(WHO, 2013; Willcox et al., 2012)**. In this context, medicinal plants have become an important field of scientific investigation, particularly due to their richness in biologically active secondary metabolites such as phenolic compounds, flavonoids, alkaloids, and terpenoids, which exhibit diverse pharmacological properties including antioxidant, anti-inflammatory, antimicrobial, and anticancer activities **(Rawani et al., 2011).**

Algeria possesses remarkable plant biodiversity characterized by the spontaneous growth of numerous medicinal and aromatic species adapted to different ecological zones, especially Saharan environments **(Véla & Benhouhou, 2007; Neffati & Sghaier, 2014)**. These plants constitute a valuable natural heritage and an essential component of traditional Algerian medicine transmitted through generations. Moreover, medicinal plants represent a promising source for the discovery of novel bioactive molecules with potential pharmaceutical applications **(Catier & Roux, 2007)**. Several studies have reported the isolation and characterization of active compounds derived from medicinal plants with important therapeutic effects ranging from analgesic and anti-inflammatory activities to anticancer properties **(Da Silva & Meijer, 2012; Saad et al., 2023)**. Furthermore, natural products have attracted considerable scientific attention because they often exhibit lower toxicity and better biological tolerance compared to chemically synthesized drugs **(Djekilamber et al., 2024; Ipona et al., 2023)**. Nevertheless, despite this rich ethnopharmacological heritage, traditional knowledge related to medicinal plants remains insufficiently documented and is progressively threatened by disappearance, emphasizing the urgent need for scientific valorization and preservation.

Introduction

Among the major therapeutic challenges currently facing modern medicine is inflammation, which constitutes a physiological defense mechanism involved in numerous acute and chronic diseases. Although conventional anti-inflammatory drugs are widely used, their prolonged administration is frequently associated with adverse effects and toxic complications, including gastrointestinal, renal, and cardiovascular disorders. Consequently, the search for effective natural anti-inflammatory agents with fewer side effects has become a priority in pharmaceutical and biomedical research.

Within this framework of valorizing Algerian Saharan medicinal plants, the present study focuses on *Artemisia herba-alba*, commonly known as white Artemisia herba-alba, a species traditionally used for its therapeutic virtues. The study investigates both the aqueous extract and the essential oil of the plant through phytochemical and biological approaches. Particular attention was devoted to evaluating their antioxidant and anti-inflammatory activities and to exploiting these bioactive properties in the development of an innovative topical therapeutic formulation.

This dissertation is divided into two complementary sections: a theoretical part and an experimental part. The theoretical section presents a bibliographic synthesis concerning medicinal plants, inflammation and oxidative stress mechanisms, antioxidant and anti-inflammatory compounds, as well as the botanical, phytochemical, and pharmacological characteristics of *Artemisia herba-alba*.

The experimental section describes the plant material and extraction procedures, the phytochemical analyses, the evaluation of antioxidant and anti-inflammatory activities *in vitro* and *in vivo*, the formulation of the innovative topical cream, and finally the interpretation and discussion of the obtained results. This work ultimately aims to contribute to the scientific valorization of Algerian medicinal flora and to explore the therapeutic potential of natural bioactive compounds in the development of innovative phytotherapeutic formulations.

Theoretical Part

Chapter I:
Botanical, Phytochemical and Ethnopharmacological Overview of *Artemisia herba-alba*

Generalities:

A precise understanding of the botanical and environmental characteristics of medicinal plants forms the cornerstone of any research aiming to extract and valorize their bioactive compounds. In this context, this chapter focuses on the study of White *Artemisia herba-alba*, which represents one of the most prominent plant resources in the arid and desert environments of Algeria. Throughout this chapter, we will review the botanical classification of the plant, its geographical distribution, and its morphological adaptations to harsh climatic conditions. This will be followed by an investigation into its traditional ethnopharmacological uses in folk medicine, leading to a deep analysis of its phytochemical composition and secondary metabolite content, which grant the plant its promising therapeutic and biological potentials.

1. Botanical And Environmental Aspects

Complete botanical classification :

1.1 Taxonomy :

Table 1: Taxonomy *Artemisia herba-alba* **(Abou El-Hamd et al., 2010)**

***Artemisia herba-alba* is classified into Kingdom**	**Plantae**
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Asterales
Family	Asteraceae
Subfamily	Asteroideae
Tribe	Anthemideae
Subtribe	Artemisiinae
Genus	Artemisia
Subgenus	Seriphidium
Species	*Artemisia herba- alba Asso*

1.2 Vernacular names :

In Berber and Arabic languages, plant names used in everyday speech do not always provide an exact botanical definition. Therefore, the common names of white *Artemisia herba alba* are presented below. **(Bendas et al., 2022)**

Table 2: Common names of *Artemisia herba-alba* **(Bendas et al., 2022)**

Arabic name	الشيح Chih (Dozy, 1967)
French name	Armoise blanche, Absinthe des steppes (Mahmoudi, p.120), thym des steppes (Trabut, 1935)
English name	*Artemisia herba-alba*
latin name	*Artemisia herba alba*
amazigh name	Izerg, Ifsi ou Zezzaré
In Moroco	Kaisoum

1.3 Distribution :

Artemisia herba-alba exhibits a high degree of adaptation to dry and nutrient-poor soil environments and is found in desert and steppe niches of the Middle East, North Africa, and parts of southwestern Asia. It is native to Morocco, Algeria, Tunisia, Libya, and Egypt, where it constitutes an integral component of the steppe flora. A. herba-alba is abundant in arid habitats throughout Northern Africa, the Arabian Peninsula, and certain regions of southwestern Asia. In the Middle East, it has been reported to form dense and homogeneous shrubland communities in arid areas of Jordan, Palestine, Syria, Iraq, and Saudi Arabia. Additionally, it is found in parts of Pakistan and western Iran, demonstrating its remarkable adaptability to diverse arid environments. **(Aouadhi, 2010)**

To establish a comprehensive ecological and botanical understanding of *Artemisia herba-alba*, it is essential to examine its natural habitats and geographical boundaries. The following **(Figures 01** and **02,03 ,04)** illustrate the global distribution of the species, its specific localization within the Algerian steppes, and its distinctive morphological features adapted to arid environments

Figure 1 : Geographic distribution of *Artemisia herba-alba*

(Salido et al; 2004 in Touane, 2021)

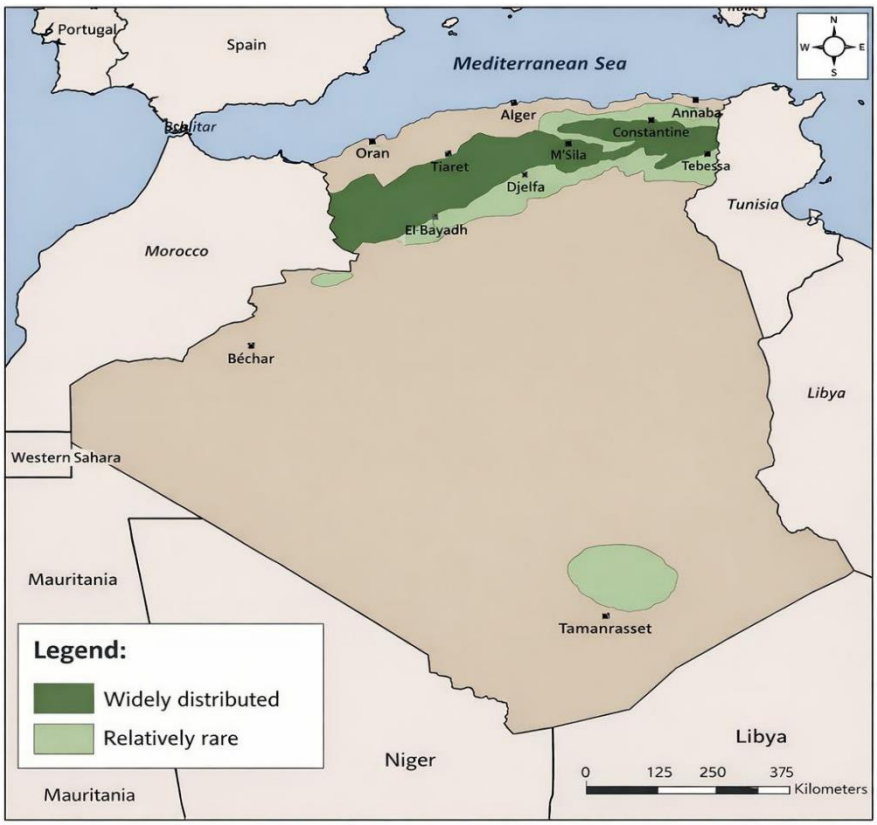

Figure 2: Geographic distribution of *Artemisia herba-alba* in Algeria

(Quezel and Santa, 1963; Aidoud, 1988; Said et al., 2015)

1.4 Environmental conditions :

Three million hectares of the Algerian steppes are covered by white *Artemisia herba-alba*, which has significant potential as a forage resource. *Artemisia herba-alba* thrives in clayey and compact soils with low permeability and is well adapted to desert climates. This species provides a natural defense against erosion and desertification. However, it has been reported that *Artemisia herba-alba* steppes are either replaced by indicator species of vegetation degradation or give way to bare soil, leading to the scarcity or disappearance of this species. **(Abou El-Hamd et al., 2010)**

1.5 Morphological characteristics :

Artemisia herba-alba is a perennial aromatic shrub belonging to the Asteraceae family. The plant generally reaches a height ranging from 20 to 40 cm and is characterized by a highly branched woody stem covered with dense whitish hairs, giving the plant its characteristic silvery appearance. The leaves are small, deeply divided, and covered with fine trichomes that help reduce water loss under arid climatic conditions. The flowers are small, yellowish to whitish, and grouped in compact capitula. The species emits a strong aromatic odor due to its richness in essential oils and volatile compounds. **(Abou El-Hamd et al., 2010)**

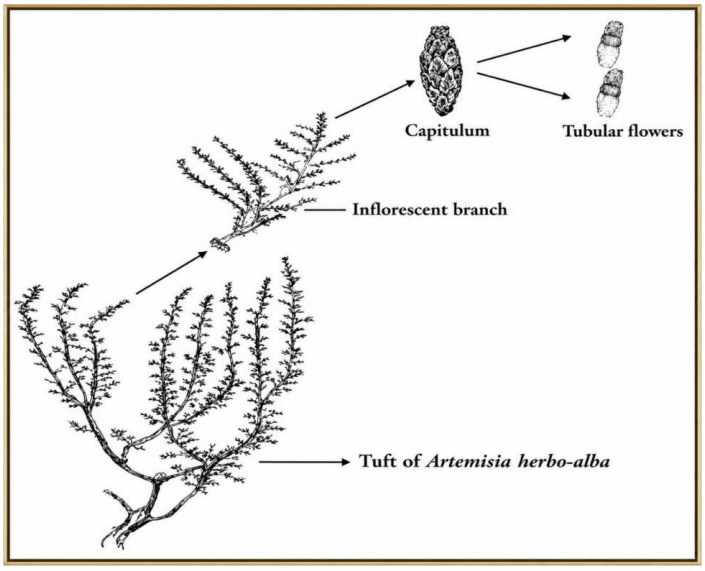

Figure 3 : Morphological description of *Artemisia herba-alba* **(Bougoutaia, 2018)**

1.6 desert adaptations :

Artemisia herba-alba is a species well adapted to arid climates due to its morphological and physiological characteristics.

Seasonal variation in its leaves allows the plant to reduce water loss by decreasing the transpiration surface. **(Maghni et al., 2017)**

Figure 4: *Artemisia herba-alba* in nature **(by Pelot 2011)**

2. Ethno-Pharmacological Uses :

Artemisia herba-alba has been widely used in traditional medicine across North African and Middle Eastern countries for the treatment of numerous diseases and health disorders. Traditionally, the plant is employed as an antiseptic, antispasmodic, antidiabetic, anti-inflammatory, antiparasitic, and digestive remedy. Several ethnopharmacological studies have also reported its use in the management of respiratory infections, gastrointestinal disturbances, fever, and skin diseases. These traditional applications are mainly attributed to the richness of the plant in bioactive secondary metabolites, particularly phenolic compounds, flavonoids, and terpenoids, which are known for their important biological activities **(Moufid &Eddouks, 2012; Benamira et al., 2021)**

2.1 Traditional Preparation methods:

Traditional preparation methods for medicinal plants, particularly White *Artemisia herba-alba*, vary according to the targeted therapeutic purpose. According to the field study, the most common preparation methods are:

Infusion and Decoction: These methods are the most widely used for internal applications (oral administration). Preparation involves placing the aerial parts of the plant in hot water or boiling them briefly to extract the active ingredients.

Maceration: This process involves soaking plant parts in water or oil for varying periods to obtain concentrated extracts. It is frequently used for topical massage.

Powder (Poudre): Used after drying the *Artemisia herba-alba* leaves in the shade and crushing them. It is consumed directly, either alone or with the addition of other substances. **(Ould El Hadj et al., 2003)**

Table 3: The medicinal spontaneous species recorded in the region of Ouargla and their therapeutic usages **(Ould El Hadj et al., 2003)**

Scientific Name	*Artemisia herba-alba Asso.*
Therapeutic Indication	Digestive and respiratory disorders, rheumatism, obesity, common cold, aromatic agent.
Part Used	Leaves, stems, and flowers.
Mode of Utilization	Infusion, Maceration, Herbal Tea (Tisane).

2.2 Traditional use limitions and risks:

At high doses, the aroma is abortive, neurotoxic. Thujone is the toxic and bioactive compound in *Artemisia herba-alba*, with α-thujone being the most toxic form. It can cause convulsions **(Aouadhi, 2010)**

3. Phytochemistry And Analysis :

3.1 A general overview of the chemical composition of Artemisia herba-alba:

Chemical composition of the plant: Several secondary metabolites have been isolated and identified from *Artemisia herba alba* Asso, the most significant of which are sesquiterpés lactones, such as eudesmanolides and germacranolides. The flavonoids found in the armoise also show structural diversity, ranging from common flavonoids (flavones glycosides and flavonols) to ethylated flavonoids that are truly indigenous. Flavonoid glycosides include O-glycosides, such as quercitine-3-glucoside, and C-glycosides, which are uncommon in the Artemisia genre and throughout the Astéracée In addition to sesquiterpène lactones and flavonoids,

phytochemical analysis has demonstrated that the essential oils of *Artemisia herba alba* are rich in monoterpenes, triterpenes pentacycliques, santonines, coumarines, and tanins. **(Benamira et al., 2021)**

3.2 Effect of extraction method on components :

The extraction method plays a crucial role in determining the nature and composition of the chemical compounds extracted from *Artemisia herba-alba.*

Phytochemical analyses have demonstrated that the use of different solvents leads to the extraction of diverse classes of secondary metabolites, including phenolic compounds and flavonoids, depending on solvent polarity.

These findings indicate that the polarity £of the solvent significantly influences both the extraction yield and the phytochemical profile of the obtained extracts, highlighting the direct impact of the extraction method on the nature of the extracted compounds. **(Benmeziane et al., 2023)**

3.3 Biological activities of *Artemisia herba-alba*:

- **Antioxidant Activity:**

Medicinal plants represent a major source of natural antioxidants capable of preventing and treating disorders related to oxidativ£e stress. In Algeria,. **(Djeridane et al., 2006)** evaluated the antioxidant capacity of several local medicinal species, including Artemisia herba-alba. The study demonstrated that these plants possess a remarkably high phenolic content and act as potent free radical scavengers, making them highly suitable for medicinal and commercial valorization . **(Djeridane et al., 2006)**

In food preservation, the application of *A. herba-alba* aqueous extract on raw and cooked ground beef patties stored at 4°C for 16 days showed a protective effect against oxidative deterioration, although it was slightly less effective than other herbs like rosemary **(Amr, 1995)** . Similarly, a comparative study of twenty-one Jordanian plants using DPPH and ABTS assays classified *Artemisia herba-alba* as having a moderate antioxidant potential **(Al-Mustafa & Al-Thunibat, 2008).**

Furthermore, the biological benefits of this species were highlighted by Abid et al., who **(Abid et al., 2007)** investigated the long-term effects of an *A. herba-alba* decoction in rats. The

formulation significantly enhanced the total antioxidant status, increased whole blood glutathione peroxidase activity, and successfully mitigated oxidative stress, hyperglycemia, and hyperlipidemia **(Abid et al., 2007)**. However, under specific oxidative challenges, such as the in vitro exposure of erythrocytes to 10 mM H_2O_2 for 60 min, the plant extract did not demonstrate a direct protective effect against lipid peroxidation **(Suboh et al., 2004)**

- **Nematicidal Activity:**

The in vitro nematicidal activity of methanolic leaf extracts (20µg/mL) from twenty Jordanian plant species was evaluated against two species of root-knot nematodes. The extract of A. herba-alba emerged as the most effective, inducing mortality rates of 22%, 51%, and 54% after 24, 48, and 72 hours of exposure, respectively (**Al-Banna et al., 2003) .**

- **Antibacterial Activity:**

The antibacterial potential of *Artemisia herba-alba* has been extensively investigated against various pathogenic strains. Early screening revealed that its essential oil (EO) possesses selective potency against Gram-positive bacteria (Streptococcus hemolyticus and Staphylococcus aureus) and Gram-negative bacteria (Escherichia coli, Shigella sonnei, and Salmonella typhosa). Chromatographic fractionation of the oil identified santolina alcohol as the principal component driving this antimicrobial action **(Yashphe et al., 1979)** .

Comparative studies on different *A. herba-alba* populations demonstrated slight to moderate antibacterial efficacy within a concentration range of 1--2mg/mL against strains including Serratia marcescens and Pseudomonas aeruginosa, with localized chemical variations heavily influencing the baseline activity **(Yashphe et al., 1987) Sherif et al., 1987)**. Additionally, while the EO exhibits a profound inhibitory effect against Staphylococcus species, its activity remains relatively low against enterobacteria **(Charchari et al., 1996)** . Conversely, the aqueous extract of the plant displays weak antibacterial properties and virtually no inhibitory action against the yeast Saccharomyces cerevisiae**(Marrif et al., 1995),** showing non-significant effects when tested directly against Bacillus subtilis and E. coli in crude forms **(Hifnawy et al., 2001).** Significantly, A. herba-alba methanolic extracts demonstrated exceptional in vitro efficacy against 32 clinical isolates of Mycoplasma species atypical bacteria that lack a rigid cell wall and are naturally resistant to beta-lactams. The extract exhibited remarkable Minimum Inhibitory Concentration (MIC) values ranging from 3.125 to 6.25mg/mL, suggesting its potential as a

readily accessible natural alternative to conventional fluoroquinolones, tetracyclines, and macrolides **(Al-Momani et al., 2007).**

- **Antispasmodic Activity :**

The essential oils of various *Artemisia herba-alba* populations have been screened for their smooth muscle relaxant properties using isolated rabbit jejunum models. The experimental findings indicated that the tested oils shared similar relaxation patterns, inducing a 50% relaxation response at low threshold concentrations (approximately 3 $\times 10^{-5}$%), confirming the traditional use of the plant in relieving gastrointestinal spasms **(Yashphe et al., 1987).**

- **Anthelmintic and Antiparasitic Activity:**

The anthelmintic efficacy of powdered *Artemisia herba-alba* shoots was clinically evaluated in Nubian goats experimentally infected with Haemonchus larvae. Treatment with doses of 2, 10, or 30 g successfully suppressed caprine haemonchosis symptoms (such as dullness and soft feces), cleared adult worms from the abomasum, and restored vital serum biomarkers including ammonia, electrolytes, total protein, creatinine, and aspartate aminotransferase (AST/GOT) activities back to physiological baselines **(Idris et al., 1982 , Sherif et al., 1987).** Furthermore, clinical trials evaluating the therapeutic value of *A. herba-alba* aqueous extract in patients suffering from intestinal pinworm infections (Enterobius vermicularis) demonstrated that oral administration successfully eradicated the parasitic infection within 3 days across all treated subjects, underscoring its traditional validation as a potent antiparasitic agent **(Al-Waili, 1988)**

Chapter II:

Biological mechanisms of inflammation, oxidative stress, and toxicological evaluation

Generalities:

Biological This chapter presents the theoretical background related to inflammation, oxidative stress, and toxicological evaluation. It aims to provide the scientific basis necessary for understanding the biological mechanisms involved in inflammatory processes and the methods commonly used to evaluate the efficacy and safety of bioactive compounds. The chapter is organized into three complementary sections addressing inflammation, oxidative stress, and principles of toxicological assessment.

1. Inflammation:

1.1 Definition :

Inflammation is a natural defense mechanism of higher organisms against any external aggression caused by physicochemical factors (irradiation, burns mechanical trauma, etc.), microbial infections (bacterial, viral, or parasitic), or linked to endogenous elements such as compounds resulting from the immune response (immune complexes, cytotoxic antibodies, cytokines, etc.). Its purpose is to eliminate the pathogen and repair tissue damage. **(Yougbaré-Ziébrou et al., 2016 ; Taïba et al., 2017).**

1.2Types of inflammation:

Inflammation is classified into two categories based on the duration and the kinetics of the inflammatory process:

1.2.1 Acute inflammation:

Acute inflammation is the immediate response of the organism to an offending agent Its duration varies from a few days to a few weeks depending on the degree of injury **(Raghavendra et al., 2015).** Its main characteristics are the exudation of fluids and plasma proteins (edema) and the Acute inflammation is a rapid and self-limiting process. Most acute inflammations heal spontaneously or with the aid of pharmacological treatment. However, they may leave permanent sequelae or transition into chronic inflammation if the tissue destruction is significant or if the offending agent persists

- **Vascular phase:**

The vascular phase is immediate and is linked to the emergence of the primary symptoms that lead to congestion and edema **(Sellal, 2009).**

During the vascular phase, a series of reactions occur:

-Platelet activation through the stages of adhesion, aggregation, and degranulation, which allow the release of vasoactive factors such serotonin

- The recruitment and activation of inflammatory cells, such as neutrophils and monocytes, mediated by cytokines and growth factors produced by platelets.
- Activation of endothelial cells and elements of the contact system, along with the release of bradykinin and the initiation of coagulation, leading to the formation of a fibrin clot.
- Activation of fibrinolysis to decompose the fibrin clot and produce plasmin, which activates the complement system and leads to the release of vasoactive chemotactic factors (complement anaphylatoxins C3a, C5a, and C2-kinin).

The latter triggers vasodilation and increased vascular permeability these two reactions are the primary causes of edema formation. **(Ferhat et Mehyach, 2017).**

The biological response to cellular injury involves complex cellular and vascular cascades that restrict tissue damage. The schematic diagrams presented below **(Figure 05,06)** clear up the sequential events of the acute inflammatory initiation phase and outline the pathways of its chronological evolution

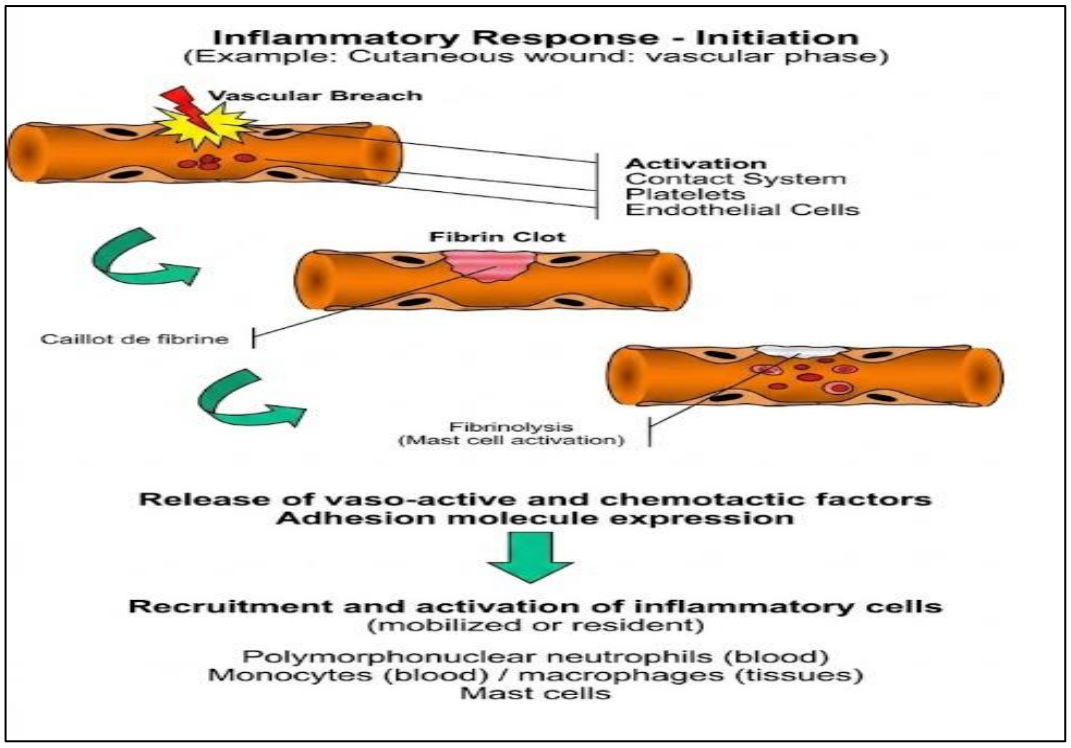

Figure 5: Initiation phase (Ferhatet Mehyach, 2017)

- **The cellular phase:**

The cells found at the inflammatory site originate from two sources:

the blood, such as neutrophils, or the tissue itself, such as phagocytic

cells**. (Duyckaerts et al 2002)**

a) The blood cel:

Chemotaxis is the phenomenon responsible for the migration of polymorphonuclear leukocytes, monocytes, and lymphocytes toward the lesion site. Neutrophils are present from the very first hours of the inflammatory response and are initially the most abundant cell type at the injury site. As the reaction progresses into the subacute and chronic stages, these are replaced by mononuclear cells, and lymphocytic or macrophagic infiltrates are obser.

b) The cells origiating from the tissue :

Histiocytes are macrophages residing within the tissues themselves Kupffer cells in the liver, alveolar macrophages in the lungs, and microglia in the brain). Mast cells, which contain granules rich in histamine and serotonin, also reside within the tissues**. (Duyckaerts et al., 2002)**

- **The resolution and repair phase :**

Once the aggression is controlled, the inflammatory reaction is haltedOnce the aggression is controlled, the inflammatory reaction is halted. Macrophages clean up cellular debris via phagocytosis and secrete growth factors and cytokines that enable tissue repair. This process is mediated by fibroblasts, which synthesize collagen, and endothelial cells which promote neoangiogenesis to restore blood supply. Concurrently anti-inflammatory cytokines, such as IL-10 and TGF-\beta, progressively replace pro-inflammatory mediators and inhibit their secretion and action.

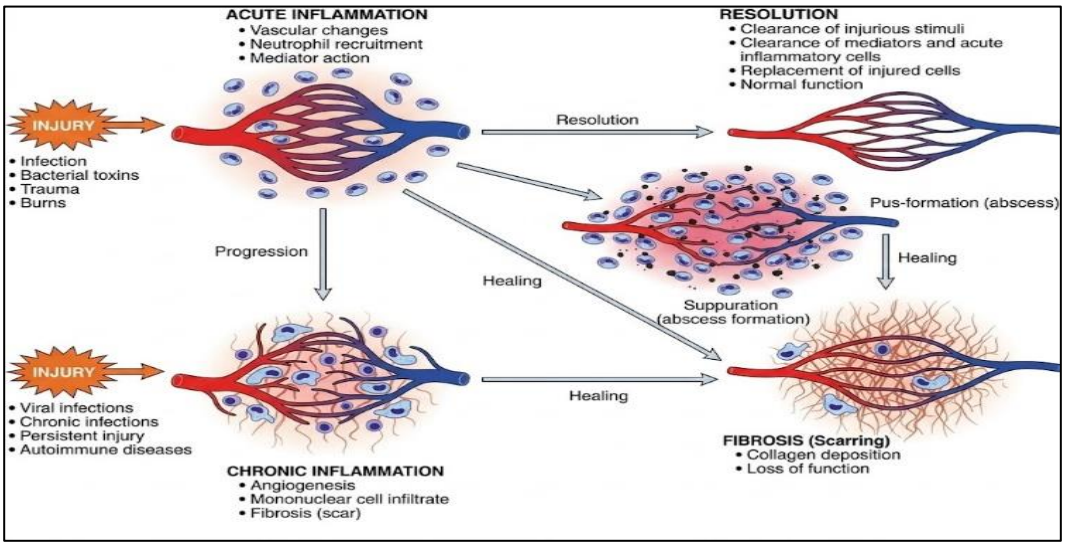

Figure 6 Evolution of acute inflammation (Kada,2018)

inflammatory mediators and inhibit their secretion and action. The inflammation then enters the resolution phase **(Noack and KoloppSarda, 2018).** Once immune cells are no longer required at the inflammatory site, they leave the tissue or die due to the loss of survival signals or through apoptosis. The mechanisms of acute inflammation are identical regardless of the triggering agent**. (Noack and Kolopp-Sarda, 2018).**

1.2.2 Chronic inflammation:

Chronic inflammation represents a failure of the acute inflammatory response and induces numerous pathologies **(Noack andKoloppSarda, 2018).** It is characterized by a prolonged progression that cans pan months or even years; specifically, chronic inflammation is defined by a duration exceeding six weeks **(Heymonet, 2013).**

Causes of Chronic inflammation:

Chronic inflammation may arise as the following:

- Failure to eliminate the agent causing acute inflammation:

This includes infectious organisms that can resist host defenses and persist within the tissue for an extended period **(Pahwa et al., 2020).**

- Exposure to low levels of a specific irritant or foreign material:

Substances that cannot be eliminated through enzymatic degradation or phagocytosis by the body, such as silica dust **(Pahwa et al., 2020).**

- Autoimmune diseases:

Conditions in which the immune system recognizes a normal component of the body as a foreign antigen and attacks healthy tissue, resulting in diseases such as Rheumatoid Arthritis (RA) **(Pahwa et al., 2020).**

- Defects in the cells responsible for mediating inflammation:

Leading to persistent or recurrent inflammation, such as auto-inflammatory disorders **(Pahwa et al., 2020)**

- Pathophysiological Characteristics:

unlike acute inflammation, vascular and cellular phases do not occur sequentially but coexist throughout the progression of the inflammatory process **(Kada, 2018).**

The cellular infiltrate at the inflammatory site persists, thereby contributing to tissue hyperplasia and destruction The microenvironment plays a primordial role in this process. Indeed, the production of cytokines and chemokines promotes the survival and maintenance of cells at the inflammatory site **(Noack & KoloppSarda, 2018)** Common Mediators The mechanisms and mediators involved in the chronic inflammatory process are similar across various chronic inflammatory diseases, such as Rheumatoid Arthritis (RA) ,Psoriasis,Chronic Inflammatory Bowel Diseases (IBD) such as Crohn's Disease (CD) **(Libby, 2007).**

Among these common agents, cytokines represent fundamental factors for the cellular environment, acting both at the local and systemic levels

1.3 Cytokines :

Monokines and lymphokines form a group of proteins that play an essential role in intercellular communication, particularly between the various actors involved in the inflammatory process. They are secreted by lymphocytes, macrophages, fibroblasts, endothelial cells, platelets and other cell types, such as epithelial cells **(Miossec, 2003)**

Table 4: Biological effects of the major inflammatory mediators **(Rinkin, 2004)**.

Mediator Class	Members	Origin	Biological Effect

-Vasoactive (Amines)	-Histamine	-Mast cells -basophils -platelets	-Vasodilation -increase i vascular -permeability -activation
-Lipid mediators (eicosanoids)	-Prostaglandins -Thromboxanes -Leukotrienes	-Arachidoni cacid derivatives	-Vasodilation -increase I vascular permeability -formation of edema-pain, fever -platelet aggregation.
Oxygen and nitrogen Derivatives	Nitric Oxide (NO)-	Macrophages And mast cells	Cytotoxic effect on the aggressor, induction of vasodilation
Cytokines	-lL-1 -lL-6 -TNF-α	-Macrophages, -mast cells and neutrophils	-Endothelial activation, -diapedesis, -recruitment of white blood cells -microbicidal effect to eliminate the pathogenic agent.

2 Anti-Inflammatories:

2.1. Steroidal Anti-inflammatory Drugs (SAIDs):

Steroidal anti-inflammatory drugs (SAIDs) constitute a large family of drugs derived from cortisol, the main adrenal glucocorticoid. Glucocorticoids are cholesterol-derived substances whose production is stimulated by ACTH, released according to a circadian rhythm by the anterior lobe of the pituitary gland**. (Muster, 2005)**. SAIDs act according to the following mechanism **(Figure 7**):

- Glucocorticoids (GCs) inhibit the synthesis of numerous pro-inflammatory proteins (cytokines, chemokines, adhesion molecules, etc.); they bind to their receptors (GCRs) and form a GC-GCR complex; this complex blocks the transcription of genes encoding pro-inflammatory proteins by inhibiting the binding of transcription factors (NF-κB, AP-1). They also inhibit the activity of histone acetylation enzymes (HAT) and activate histone deacetylation enzymes (HDAC) **(Devillier, 2004)**

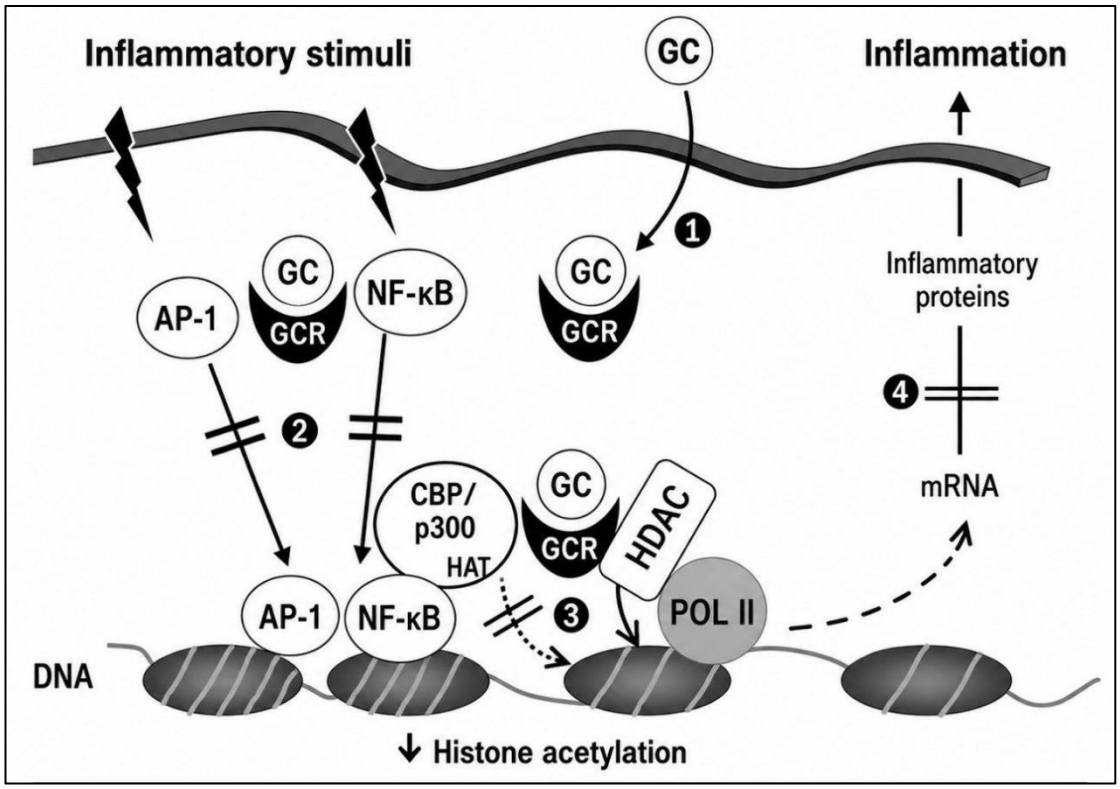

Figure 7 : Mechanism of action of SAIDs**(Devillier, 2004)**

2.2. Non-steroidal anti-inflammatory drugs (NSAIDs):

have been used successfully for the relief of pain, fever, and inflammation for over 3,000 years and are still used daily by millions of patients worldwide. They are drugs with anti-inflammatory, antipyretic, and analgesic properties. They exhibit significant chemical heterogeneity **(Table)**, but share the common characteristic of non-selective inhibition of the cyclooxygenase enzyme. However, their long-term therapeutic use is often associated with adverse effects such as gastrointestinal ulcers and renal failure **(Mansour, 2015)**

Table 5 : Examples of non-steroidal anti-inflammatory drugs (Mansour, 2015).

Structural Class	Scientific Name	Trade Name
Salicylates	Acetyl salicylic acid Diflusinal	Aspirin® Dolobid®
Propenic acid derivatives	Ibuprofen Fenoprofen calcium Flurbiprofen Ketoprofen	Ibuprofen® Nalfon® Ansaid®
Acetic acid derivatives	Diclofenac	Voltarene®
Indoles	Indoles Indomethacin Tolmetin Sulindac	Indocine® Tolectine® Clinoril®

2.3. Plant-based anti-inflammatories:

Natural or plant-based anti-inflammatories are those found in nature that do not undergo any alteration of their chemical structure after the processes involved in their extraction. Several studies have been conducted on natural plant-based substances with anti-inflammatory properties, thanks to the presence of bioactive natural molecules that allow the treatment of certain inflammatory diseases **(Bourkhiss et al., 2010).**

3 Oxidative Stress and its Relationship To Inflammatory:

1. Oxidative stress:

This is an imbalance between reactive oxygen species and antioxidants. This imbalance can be caused by several factors, including:

Excessive production of reactive oxygen species Dietary deficiency of antioxidants External factors (smoking, alcohol abuse, obesity, etc.).

Increased oxidative stress can lead to an increased risk of age-related diseases, heart disease, cancer, and diabetes. **(Preiser, 2012)**

2. Free radicals:

Atoms in molecules are held together by strong bonds held by opposing helical electrons. These electrons carry enough energy to break these bonds, resulting in the formation of chemical units with unbonded electrons in their outer shell. These chemical units are called free radicals. **(BOURAS ET HOUCHI, 2013)**

A free radical is any independent molecule that contains one or more unbonded electrons derived from oxygen (Ros) or nitrogen (Nos)**. (Berger, 2006).**Their primary source of production is the mitochondria.

They damage cells, tissues, and organs. They are involved in many acute and chronic diseases such as cancer, heart disease, and arthritis. **(Preiser, 2012)**

3.2.1 Types of free radicals:

- **Reactive Oxygen Species (ROS):**

Reactive oxygen species are the most common type of free radicals produced within cells. They mainly originate from the partial reduction of molecular oxygen during metabolic reactions. The principal ROS include **superoxide anion $O_2^{\bullet -}$**, **hydroxyl radical •OH**, and hydrogen **peroxide H_2O_2**. When present in excessive amounts, these species can induce oxidative damage to lipids, proteins, and nucleic acids, leading to cellular dysfunction**.(HAMIDI, 2013)**

- **Reactive Nitrogen Species (RNS) :**

Reactive nitrogen species consist of molecules derived from nitrogen-related reactions within the cell. The most important of these is **nitric oxide •NO**, which is enzymatically produced from the amino acid arginine in the presence of oxygen. This group also includes other compounds resulting from the reactions of nitric oxide with oxygen or superoxide radicals, such as nitrous

oxide and **Peroxynitrite $ONOO^-$**, which are characterized by their high reactivity with cellular components. **(BOULDJADJ, 2009)**

- **Reactive Sulfur Species (RSS):**

Reactive sulfur species arise from redox reactions involving sulfur-containing compounds, particularly thiol groups present in proteins and small peptides. These species are involved in the regulation of protein structure and function; however, their accumulation may disrupt cellular homeostasis and contribute to oxidative stress. **.(MOHAMMEDI, 2006)**

3.3 Functional Link Between Oxidation and Inflammation:

3.3.1 Role of Free Radicals in Activating Inflammatory Pathways (NF-κB):

Reactive oxygen species (ROS) act as secondary signaling molecules capable of activating major inflammatory pathways within the cell. The most well-studied mechanism is the ability of ROS to activate the NF-κB pathway. Under normal conditions, NF-κB is bound to an inhibitory protein (IκB) in the cytoplasm. Oxidative stress triggers a series of reactions leading to the degradation of this inhibitory protein, thereby freeing NF-κB to translocate to the nucleus. There, NF-κB acts as a transcription factor that stimulates the gene expression of a wide range of pro-inflammatory cytokines (such as TNF-α, IL-1β, and IL-6), thereby igniting a potent inflammatory response **(Morgan & Liu, 2011).**

3.3.2 The Vicious Cycle: The Reciprocal Relationship Between Stress and Inflammation:

A complex, reciprocal relationship exists between oxidative stress and inflammation, best described as a vicious cycle. On one hand, as mentioned above, oxidative stress activates inflammatory pathways (via NF-κB). On the other hand, inflamed cells, particularly macrophages, produce large quantities of ROS as a defense mechanism against pathogens. This increased production of free radicals, in turn, deepens oxidative stress, leading to further inflammatory activation and tissue damage. Thus, oxidative stress and inflammation feed into each other, forming a positive feedback loop that exacerbates chronic pathological conditions and drives their progression **(Biswas, 2016).**

4 Toxicity:

4.1 Definition of toxicity :

Toxicity is defined as the degree to which a substance (chemical physical, or biological) can cause harm to aliving organism. It is a fundamental concept in toxicology, describing the

relationship between the dose of a substance and the resulting adverse effects.(Atsdr agency for toxic substances and disease registry)

4.2 Determination of the lethal dose LD_{50}:

In its simplest form, the LD_{50} is the dose of a compound that causes 50% mortality in a test animal population. This involves administering a single dose of the product under strictly defined experimental conditions The evaluation is based on the 'all-or-nothing' response: the death or survival of the animalThe experimental protocol consists of conducting trials on 5 to 6 groups, each containing 10 to 20 animals, to which increasing doses of the test substance are administered so that the mortality percentage ranges between 0% and 100%. This is because it is impossible to achieve exactly 50% mortality instantaneously within a single group Constructing a curve that plots the mortality percentage against the logarithm of the dose leads to the determination of the LD_{50} **(Wallace Hayes, 2008)**. This dose is frequently used as a starting point for toxicity studies as it provides fundamental baseline knowledge

4.2.1 Differentes methods for determining LD_{50} :

LD_{50} can be determined using two calculation methods: the Dragstedt and Lang method (1957) and the Karber and Behrens method (1935). Additionally, it can be determined through two graphical methods: the Miller and Tainter method (1944) and the Litchfield and Wilcoxon method (1949).

- **Manifestation of toxicity :**

The effect of a toxicant on an organism depends essentially on the quantity of the toxicant or the reactive substances it produces (reactive metabolites, free radicals) that bind to the site of action (enzymes, cytoplasmic receptors, DNA). This effect depends not only on the amount of active toxicity reaching the site of action but also on its toxicity reaching the site of action but also on its affinity for that site **(Law Willis, 2003)**. The four main biological factors influencing the concentration of the active toxicant at the receptor level are.

a) Absorption (Entry):

The process by which a product enters the body is called absorption. This is a critical stage because a product does not cause systemic toxic effects until it has entered the bloodstream meaning it has moved away from the initial point of exposure Several factors affect the absorption process: the nature of the product, its solubility, the permeability of the biological tissue at the point of contact, and the duration and frequency of contact **(Lapointe et al., 2005)**.

While dermal and pulmonary routes exist, molecules absorbed via the digestive tract generally must pass through the liver before being diluted into systemic circulation. Consequently, it is not surprising that the liver is often a target organ for ingested toxinsparticularly in cases of acute poisoning, as it is situated directly on the necessary transport pathway. The degree of absorption of a foreign substance by the gastrointestinal tract is itself subject to various influences **(Diamond et al., 1970).**

b) Distribution (Partitioning):

Once it reaches the bloodstream, the product can be transported throughout the entire body; this phenomenon is known as distribution. During the period between absorption and excretion, the substance may distribute into different body tissues and undergo metabolic transformations. Certain tissues may preferentially store specific substances, even if those tissues are not necessarily the primary site of toxic action **(Lauwerys, 2007)**

In addition to transporting oxygen, essential nutrients, and waste, the blood can also carry toxic substances. These toxins may come into contact with cells and bind to specific tissues. For example, organochlorine pesticides like DDT concentrate in adipose (fat) tissue, where they can remain stored for extended periods without causing toxic effects. However, they may induce toxic effects in other tissues or organs where they are present in smaller quantities **(Lapointe et al., 2005)**. The nature, intensity, and location of these internal disturbancesFollowing transport in the blood, the toxin may come into contact with various cells in the body that possess the enzymatic capacity to transform its chemical structure. This metabolictransformation process is called biotransformation, and the resulting chemical products are known as metabolite.

c) Biotransformation (Metabolism):

Following transport in the blood, the toxin may interact with various cells in the body that have the capacity to transform it. This transformation process is called biotransformation, and the resulting .products are called metabolites Biotransformation can lead to the formation of less toxic products (detoxification) or more toxic products (activation/toxification), as well as the accumulation or elimination of the toxin and its metabolites **(Lapointe et al., 2005).** The liver is the primary organ responsible for the biotransformation of toxins. It acts as the body's chemical laboratory and contains numerous enzymes, which are specialized proteins that catalyze chemical reactions. The liver enriches the blood with nutrients andpurifies it by concentrating and eliminating many substances. Other organs, such as the lungs and kidneys, can also participate in the biotransformation of toxins **(Lapointe et al., 2005)**

d) Excretion:

Excretion is the process by which the toxin or its metabolites are eliminated from the body. It can occur through various routes, including renal (urine), gastrointestinal (feces), pulmonary (expired air), cutaneous (sweat), or mammary (milk). However, urinary andbiliary tracts are the primary pathways for the excretion of foreign substances The relative proportion of these two elimination routes depends on the metabolic transformations the toxin undergoes within the body. Various endogenous factors can modify excretion rates and, consequently, the concentration of the toxin at its site of action (Reidenberg, 1974). For instance, the kidneys play an essential role in eliminating metabolic products. They filter the blood, helping to maintain the balance of blood components, and ensurethe elimination of numerous toxic products **(Lapointe et al., 2005).**

Finally, because the therapeutic value of any natural extract or chemical compound remains incomplete without determining its biological safety margin, the third axis is dedicated to toxicological evaluation and biosafety criteria. This section discusses the principles of toxicology and dose-response relationships, with a specific focus on the toxicological profiling of *Artemisia herba-alba.* It details standard international protocols used to evaluate acute toxicity and determine the lethal dose (LD_{50}), alongside in vitro cell viability assays, the selectivity index, and criteria for local and dermal safety.Ultimately, through these three axes, this chapter aims to establish a solid scientific foundation that bridges pathological mechanisms (inflammation and oxidation) with therapeutic and toxicological evaluation tools, thereby providing a logical framework for interpreting the subsequent experimental results

Part

Experimental Study

Chapter III :
Devices And Technikues

Materials And Methods :

This work was completed entirely at the level of Laboratories of the Faculty of Natural and Life Sciences (University of Echahid Hamma Lakhdar - El Oued) during the two periods (15/02/2026)to(12/03/2026) and (12/04/2026) to (07/05/2026) .

.1. Materials :

1.1. Plant materials :

The aerial parts of the white plant *Artemisia herba-alba* Asso were collected. The collected plant material was cleaned of impurities and dried in the shade at room temperature until a constant weight was reached. The dried parts were then ground into a fine powder using an electric grinder and stored in airtight, dark containers at room temperature until later use in extraction.

The experimental procedures began with the collection and preparation of the plant material, followed by the extraction of its active volatile components. Figure [07] displays the authentic sample of the collected *Artemisia herba-alba*, while Figure [09] shows the operational hydro-distillation setup using a Clevenger-type apparatus

Figure 8: plante *Artemisia herba-alba* (personal photo, 2026)

1.2. Animal Materials:

Male Wistar albino rats, weighing between 180 and 250 g at the beginning of the experiment, were obtained from Inspector Houssam and used in this study. The rats were housed in plastic cages with stainless steel covers, with six rats per cage. They had free access to water and a standard diet throughout the study.

Before the experiment, the rats underwent a two-week acclimatization period in the animal facility. Environmental conditions were controlled to maintain room temperature (20–24°C) and a 12-hour light/dark cycle to align with their natural circadian rhythms.

Wood shavings were used as bedding material and were replaced three times weekly to maintain a clean and hygienic environment for the animals.

1.3. Chemicals and Reagents :

All chemicals, solvents, and reagents used in this experimental study were of analytical grade. These materials were supplied by the Toxicology Laboratory (Laboratory 09) of the Faculty of Natural and Life Sciences, University of Echahid Hamma Lakhdar - El Oued. Distilled water was used in all extraction and dilution procedures.

The following table summarizes the main chemicals and reagents used in this study:

Table 6: List of Chemicals and Reagents.

Chemical Reagents & Materials	Category
Distilled Water, Methanol, Ethanol, Chloroform, Hexane, Glacial Acetic Acid.	Solvents & Extraction
Wagner Reagent, Sodium Hydroxide (NaOH), Sulfuric Acid (H2SO4), Ferric Chloride (FeCl3), Fehling Reagent.	Screening Phytochemical
Folin-Ciocalteu, Sodium Carbonate (Na2CO3), Aluminum Chloride (AICI3), Gallic Acid, Quercetin.	Quantitative Analysis
DPPH, Ascorbic Acid.	Antioxidant Activity
Trichloroacetic Acid (TCA), Thiobarbituric Acid (TBA), Hydrochloric	MDA Assay

Acid (HCI).	
Bovine Serum Albumin (BSA), Tris-HCI Buffer, Diclofenac Sodium, Diclofenac Gel, Formalin, Salicylic Acid.	Anti-inflammatory
Anhydrous Sodium Sulfate (Na2SO4), Potassium Hydroxide (KOH), Phenolphthalein.	Buffers & Salts

1.4. Apparatus and Equipment :

The following analytical and laboratory instruments were used in this study:

- UV-Visible Spectrophotometer .
- Rotary Evaporator (Rotavapor).
- GC-MS System (Gas Chromatography-Mass Spectrometry).
- Refractometer.
- Analytical Precision Balance.
- Magnetic Stirrer.
- Vortex Mixer.
- Clevenger typ apparatus.
- Centrifuge.
- Water Bath (Bain-marie)
- Electric Hand Blender.
- pH-meter.
- Laboratory Glassware: (Burettes, Reflux condensers, Volumetric flasks, Pipettes).
- Pycnometers.
- Electric Grinder.
- Drying oven.
- Micropipettes .
- Syringes.
- Digital caliper.

2. Methods :

I. PART A

1. Aqueous Extraction of *Artemisia herba-alba*:

This step was conducted at the Laboratories of the Faculty of Natural and Life Sciences of the Faculty of Natural and Life Sciences, University of Echahid Hamma Lakhdar - El Oued.

- **objective**

was to extract the maximum amount of bioactive secondary metabolites from the aerial parts of the plant.

- **The solid-liquid extraction was performed as follows:**

Maceration: A mass of 50 g of the plant powder was macerated in 500 ml of distilled water. The process was carried out at room temperature with continuous mechanical stirring for 24 hours to ensure optimal diffusion of the chemical compounds.

Filtration: After the maceration period, the mixture was filtered using Whatman filter paper. The resulting filtrate (liquid) was collected, while the plant residues (marc) remained on the filter.

Evaporation: The obtained filtrate was then subjected to evaporation using a Rotary Evaporator (Rotavapor) at a controlled temperature of 45°C. This process was continued until a concentrated crude extract was obtained.

Storage: The final crude extract was stored in a hermetically sealed, dark glass container and protected from light at the refrigerator until further use in phytochemical and biological assays.

1.1 Extraction Yield :

The extraction yield represents the percentage of secondary metabolites obtained using an aqueous or organic solvent during the extraction process **(Abe et al., 2010).** It is defined as the ratio between the mass of the dry extract obtained after solvent evaporation and the initial mass of the plant powder used. The yield is calculated using the following equation:

$$\text{Yield}(\%) = \frac{M_{ext}}{M_{dry}} \times 100$$

Where :

M_{ext}: The mass of the final dry extract (obtained after evaporation or drying process), measured in grams (g).

M_{dry}: The initial mass of the dried plant material (powder of *Artemisia herba-alba*) used for extraction, measured in grams (g)

1.2 Phytochemical Screening of *Artemisia herba-alba:*

The phytochemical screening of the aqueous extract *of Artemisia herba-alba* .The following tests were conducted:

Test for Alkaloids (Wagner's Test):

In a test tube, 1 ml of the aqueous extract was mixed with 5 drops of Wagner's reagent.

The formation of a brown precipitate indicates the presence of alkaloids. (**Kanoun, 2011)**

Test for Flavonoids:

A volume of 2 ml of the aqueous extract was mixed with 1 ml of Sodium hydroxide (NaOH, 0.5 M).

The appearance of a yellow color confirms the presence of flavonoids in the plant sample. **(Kanoun, 2011)**

Test for Sterols and Terpenoids (Liebermann-Burchard Test):

A volume of 10 ml of the extract was placed in a beaker and allowed to evaporate completely. The residue was then dissolved in 5 ml of Glacial acetic acid and 5 ml of Chloroform. Using a pipette, 1 ml of concentrated Sulfuric acid (H_2SO_4) was carefully added along the side of the tube. After 30 minutes. the appearance of a reddish-brown ring at the junction of the two layers indicates the presence of sterols and terpenoids. **(Zegheb, 2013)**

Test for Tannins:

To 2 ml of the extract in a test tube, 0.4 ml of a 1% Ferric chloride ($FeCl_3$) solution was added.

The development of a blue-black color indicates the presence of gallic tannins **. (Kanoun, 2011)**

Test for Saponins (Foam Test):

In a test tube, 2 ml of the extract was diluted with a small amount of distilled water. The tube was shaken vigorously for 15 seconds.

The formation of a persistent foam lasting for 20 minutes indicates the presence of saponins. **(Kanoun, 2011)**

Test for Reducing Compounds (Fehling's Test):

A volume of 2 ml of the extract was mixed with 1 ml of Fehling's reagent. The mixture was heated in a water bath.

The absence of a brick-red precipitate indicates that reducing compounds are not present in the plant sample. (**Kanoun, 2011)**

1.3 Quantitative Determination of Polyphenols and Flavonoids:

The quantitative determination of the bioactive compounds in the aqueous extract of *Artemisia herba-alba* was carried out as follows:

1.3.1 Determination of Total Phenolic Content (TPC):

The total phenolic content was determined spectrophotometrically using the Folin-Ciocalteu colorimetric reagent, following the protocol described by **(Wong et al., 2006).**

Principle:

The total phenolic content is generally determined by a colorimetric method using a UV-Visible spectrophotometer, employing either the Folin-Denis assay or, more commonly, the Folin-Ciocalteu assay. This method is based on the reduction of the Folin reagent a mixture of phosphotungstic (WO_4^{-2}) and phosphomolybdic (MoO_4^{-2}) acids by the oxidizable groups of phenolic compounds in an alkaline medium. This reaction results in the formation of reduced blue-colored complexes, which exhibit maximum light absorbance at a wavelength of 765 nm. Theintensity of this absorbance is directly proportional to the total concentration of polyphenols present in the analyzed sample **(Georgé et al., 2005).**

Procedure:

A volume of 0.2 ml of the aqueous extract (at various concentrations) was mixed with 1 ml of the Folin-Ciocalteu reagent (diluted 1:10). After shaking, 0.8 ml of Sodium Carbonate (Na_2CO_3, 7.5%) was added to the mixture to create the necessary alkaline environment for the reaction. The tubes were then incubated in the dark at room temperature for 30 minutes to allow for full color development (the shift from yellow to blue). The absorbance was measured at 765 nm

using a UV-Visible Spectrophotometer. Quantification: Gallic acid was used as the standard for the calibration curve. The total phenolic content was expressed as milligrams of Gallic Acid Equivalents per gram of dry extract (mg GAE/g extract).

1.3.2 Determination of Total Flavonoids Content (TFC)

The determination of flavonoids in the extracts was carried out according to the method described by **(Bahorun et al.,1996).**

Principle:

This method is based on the formation of yellowish complexes between flavonoids and metals (such as Fe^{2+} and Al^{3+}). The mechanism involves the loss of two electrons by the metal, which then binds to two oxygen atoms of the phenolic molecule acting as an electron donor. The resulting yellow coloration exhibits a maximum absorbance at 430 nm. The intensity of this coloration is directly proportional to the concentration of flavonoids present in the extract **(Ribéreau-Gayon, 1968).**

Procedure:

A volume of 0.5 ml of each aqueous extract was mixed with 0.5 ml of a 2% $AlCl_3$ solution (prepared in ethanol). The reaction tubes were shaken thoroughly and then incubated at room temperature for one hour to allow the formation of the yellow flavonoid-aluminum complex. The absorbance of the resulting mixture was measured at a wavelength of 420 nm using a UV-Visible Spectrophotometer. Quercetin (dissolved in distilled water/ethanol) was used as the standard to establish the calibration curve. Expression of Results: The results were expressed as milligrams of Quercetin Equivalents per gram of dry extract weight (mg QE/g extract).

4.1 Biological Activities :

4.1.1 Evaluation of Antioxidant Activity :

- **DPPH Free Radical Scavenging Assay:**

Principle:

The DPPH (2,2-diphenyl-1-picrylhydrazyl) method is the most widely used technique for analyzing antioxidant activity **(Ouafa Medjoujda, 2012).**

The DPPH free radical possesses an unpaired electron on one of its nitrogen atoms. The reduction of this radical by an antioxidant can be monitored by UV-Visible spectrometry, by measuring the decrease in absorbance at 517 nm induced by the presence of radical scavengers **(Ouafa Medjoujda, 2012**). This process leads to the formation of the non-radical form (2,2-diphenyl-1-picrylhydrazine), resulting in a color change from violet to yellow. The intensity of this decolorization reflects the antioxidant's ability to trap free radicals by donating a hydrogen atom. Standard antioxidants such as α-tocopherol, BHT, or BHA are commonly used as references in this assay **(Blois, 1958).**

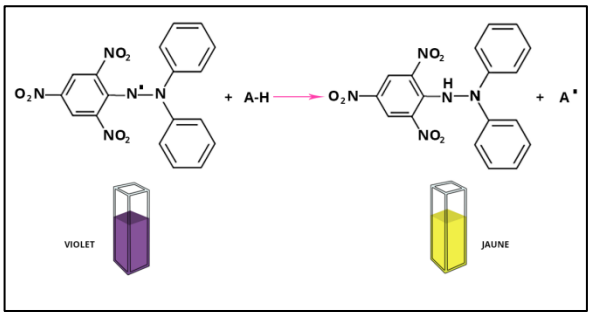

Figure 9 : Mécanisme de réduc tion du radical libre DPPH **(BENYOUCEF, 2020)**

Procedure:

The antioxidant activity was determined using the DPPH scavenging assay according to the following steps:

- A methanolic solution of DPPH (0.1 mM) was prepared by dissolving 4 mg of DPPH powder in 100 ml of methanol. The solution was stirred thoroughly before use to ensure complete dissolution.
- A series of concentrations of the aqueous extracts were prepared.
- A volume of 200 µl of each extract concentration was mixed with 800 µl of the prepared DPPH solution.
- The reaction mixtures were incubated in the dark at room temperature for 30 minutes.
- The absorbance was measured at 517 nm using a UV-Visible spectrophotometer. Ascorbic acid was used as a positive reference standard.

Expression of Results (IC_{50}) :

The antioxidant capacity was expressed as the inhibition percentage (I%) of DPPH radicals, calculated using the following formula:

$$I\% = \left(\frac{A0 - A1}{A0}\right) \times 100$$

Where:

A_0**:** Is the absorbance of the control (DPPH solution without extract).

A_1**:** Is the absorbance of the sample (DPPH solution with the extract).

The IC_{50} value (Half Maximal Inhibitory Concentration) was determined. It is defined as the concentration of the extract required to inhibit 50% of the DPPH radicals. This value was calculated graphically from the curve representing the inhibition percentage (I%) as a function of the extract concentrations.

4.1.2 Evaluation of in vitro the Anti-inflammatory Activity

- **Inhibition of Protein Denaturation Assay (BSA):**

Principle:

The anti-inflammatory potential of the plant extracts is evaluated in vitro using the protein denaturation inhibition method. This assay is based on the ability of the extracts to prevent the thermal denaturation of Bovine Serum Albumin (BSA).

Protein denaturation is a well-documented cause of inflammation. When proteins are exposed to heat (72°C in this protocol), they lose their tertiary and secondary structures. In biological systems, such denaturation leads to the release of auto-antigens, which triggers inflammatory responses. Therefore, the ability of a plant extract to protect the protein structure against heat-induced damage serves as a reliable indicator of its anti-inflammatory efficacy **(Kandikattu et al., 2013).**

Procedure:

The anti-inflammatory activity was evaluated based on the inhibition of protein denaturation using the following procedure:

- Preparation of Samples A range of concentrations for each plant extract was prepared, ranging from 0 to 10 mg/ml.
- For each concentration, 1 ml of the extract was mixed with 1 ml of 0.2% BSA (Bovine Serum Albumin) solution prepared in Tris-HCl buffer (0.05 M, pH 6.6).

- The mixtures were first incubated at 37°C for 15 minutes, followed by heating at 72°C for 5 minutes to induce protein denaturation.
- After incubation, the samples were vortexed and cooled rapidly. The turbidity (absorbance) was then measured at 660 nm using a UV-Visible spectrophotometer.
- To account for the natural color of the extracts, a specific blank was prepared for each concentration by mixing 1 ml of the extract with 1 ml of Tris-HCl buffer. The absorbance of this blank was subtracted from the sample results.
- Diclofenac was used as a standard anti-inflammatory reference drug and was tested under the same experimental conditions.

II. PART B :

1. Extraction of Essential Oil from *Artemisia herba-alba*

Extraction of essential oil clavenger apparatus :

Principle:

The extraction of the essential oil from *Artemisia herba-alba* was carried out by hydro-distillation using a Clevenger-type apparatus, following the official method described in the European Pharmacopoeia (2.8.12). The principle of this method relies on the distillation of the plant material with water. The heat generated during boiling causes the steam to penetrate the plant tissues, leading to the rupture of the volatile oil-containing structures. The mixture of water vapor and essential oil is then cooled in a condenser, leading to its liquefaction. The Clevenger apparatus allows for the continuous separation of the oil from the aqueous phase based on the difference in their densities, where the oil is collected in a graduated tube while the water is automatically returned to the distillation flask **(European Pharmacopoeia, 2010).**

Procedure:

The extraction was performed using the apparatus and method standardized by the European Pharmacopoeia (2.8.12). A quantity of 100 g of powder of *Artemisia herba-alba* was placed in a 1000 ml round-bottom flask. Distilled water was added as the distillation liquid. A few fragments of porous porcelain (boiling chips) were added to ensure even boiling. The flask was connected to the Clevenger-type apparatus. The graduated tube was filled with water through the filling orifice until it began to overflow into the flask. The mixture was heated to boiling, and the distillation was maintained at a rate of 2 ml/min for a duration of 4 hours. After the distillation was complete and the apparatus had cooled for 30 minutes, the volume of the essential oil

collected in the graduated tube was read. The essential oil was then separated, dried over anhydrous sodium sulfate (Na_2SO_4), and stored in an amber glass vial at 4°C until analysis.

Calculation of Essential Oil Yield :

The essential oil yield was calculated as a percentage relative to the dry weight of the plant material, using the following formula:

$$\text{Yield}(\%) = \frac{m_{EO}}{m_{dry}} \times 100$$

Where:

m_{EO}: Mass of the extracted essential oil measured (g).

m_{dry}: Mass of the dry aerial parts of the plant used for extraction, measured in grams (g). **(AFNOR, 1982)**

Figure 10 :Essential oil extraction of *Artemisia herba-alba* using a Clevenger-type apparatus. (personal photo, 2026)

1.1. Physico-chemical Characterization of Essential Oils :

Each essential oil is characterized by various physical properties that allow for its identification and the verification of its geographical origin, as well as its authenticity and purity. The parameters determined according to the European Pharmacopoeia, ISO standards, and AFNOR standards include density, solubility in alcohol, refractive index, acid value, and ester value **(Faucon, 2012; Franchomme et al., 2007).**

1.1.1 Physico Analysis:

- **Organoleptic Assessment:**

According to the European Pharmacopoeia (Ph. Eur.), evaluating the organoleptic properties of an essential oil (EO) is a fundamental part of the preliminary identification process. Although these qualitative tests are non-normative and insufficient for full identification on their own, they provide essential diagnostic indicators **European Directorate for the Quality of Medicines & HealthCare [EDQM], 2023** . This assessment includes:

Visual Appearance: A clear, limpid liquid, free from sediment or turbidity.

Color: Varies depending on the botanical species.

Odor: Characteristic and typical of the source plant.

Taste: Less common for EOs, except in oral aromatherapy, and therefore rarely tested.

- **Relative Density (d_{20}):**

The determination of relative density can be considered a purity criterion for EOs . **(Kaloustian & Hadji-Minaglou, 2012)** Relative density was determined using a 5 mL pycnometer at 25 °C, with weighing performed using an OHAUS ADVENTURER precision balance. We weighed the empty pycnometer, the pycnometer filled with water, and the pycnometer filled with EO, in accordance with the Ph. Eur. **. (Council of Europe, 2019)**

Density, expressed without units, corresponds to:

$$\text{Density} = \frac{m_2 - m_0}{m_1 - m_0}$$

with:

m_0: mass of the empty pycnometer (g)

m_1: mass of the pycnometer filled with water (g)

m_2: mass of the pycnometer filled with EO (g).

Procedure :

Preparation: The pycnometer is thoroughly cleaned, rinsed with ethanol, and dried to ensure no residue remains.

Weighing the empty pycnometer: The empty, dry pycnometer with its stopper is weighed using the analytical balance to the nearest 0.1 mg (m_0).

Weighing with Water (Calibration): The pycnometer is filled with distilled water at 20°C (or 25°C). The stopper is inserted, excess water is wiped off, and it is weighed (m1).

Weighing with Essential Oil: The pycnometer is emptied, dried again, and then filled with the essential oil of *Artemisia herba-alba*. Care is taken to avoid air bubbles. After inserting the stopper and cleaning the outside, it is weighed (m_2).

Temperature Recording: The temperature of the laboratory (T_{exp}) is recorded during the measurement to ensure the results are adjusted to the standard reference temperature (usually 20°C).

- **Calculation:**

The relative density (d_{20}^{20}) is calculated using the following formula:

$$\mathbf{d} = \frac{m_2 - m_0}{m_1 - m_0}$$

- **Refractive Index (Norme NF T 75 – 112, 2000) :**

Principle:

In toxicology, the refractive index (ŋD_{20}) serves as a primary purity indicator for Artemisia herba-alba essential oil. It is based on the Snell-Descartes Law, which describes the relationship between the angles of incidence and refraction:

$$ŋ = \frac{\sin i}{\sin r}$$

Where:

I :is the angle of incidence

r : is the angle of refraction.

The refractive index of an essential oil is the ratio between the sine of the angle of incidence and the sine of the angle of refraction of a light beam of a specific wavelength passing from the air into the essential oil maintained at a constant temperature. The wavelength used is the sodium D-line (589.3 nm). The correction to 20°C is then performed using the following formula:

$$ŋD_{20} = ŋD_T + 0.00045(T - 20)$$

Where:

ŋD_{20}: Refractive index at 20°C.

ŋD_T: Refractive index at ambient or measurement temperature.

T: Ambient temperature.

Procedeur :

Before each measurement, the prisms are cleaned with ethanol to avoid cross-contamination between samples. A volume of 1-2 drops of the essential oil is applied to the stationary prism using a Pasteur pipette to avoid any physical contact with the glass surface. The prisms are closed, and the system is allowed to stabilize at 20°C (using the integrated thermostatic circulation) to ensure results consistent with **AFNOR (NF T 75-112)** and European Pharmacopoeia standards. The border line between the dark and light fields is adjusted to the crosshairs, and the refractive index value is recorded directly from the digital or optical scale.

1.1.2 Chemical Analysis:

- **Determination of Acid Value (I_A) :**

Principle :

The acid value (I_A) is defined as the number of milligrams of potassium hydroxide (KOH) required to neutralize the free fatty acids present in one gram of essential oil. According to the AFNOR standard (NF T 75 – 103, 2000), this parameter serves as a primary indicator of the oil's quality, reflecting the extent of hydrolysis and oxidation of its constituents. **(Chaabane et al., 2001)**

Procedure:

Weigh approximately 0.2 g (test portion, PE) of the essential oil into a 250 ml Erlenmeyer flask. Add 5 to 10 ml of the solvent (ethanol) to dissolve the oil sample completely. Add 2 to 3 drops of phenolphthalein indicator. Titrate the mixture with the KOH solution (0.01 N) from the burette, shaking constantly until a faint pink color persists for at least 15 seconds. Record the volume (V) of the KOH solution used for the titration.

Calculation

The acid value (I_A) is calculated using the following formula:

$$I_A = \frac{V \times N \times 56.1}{PE}$$

Where:

V: Volume of KOH solution used (ml).

N: Normality of the KOH solution (0.01 N).

56.1: Molar mass of potassium hydroxide (KOH) in g/mol.

PE: Mass of the test portion of essential oil (g).

- **Ester Value (I_E):**

Principle:

According to the Ph. Eur., the ester value I_E is the number that expresses, in milligrams, the quantity of potassium hydroxide required to saponify the esters present in 1 g of substance **(EDQM, 2013)** . It is calculated from the acid value I_A and the saponification value I_S:

$$I_E = I_S - I_A$$

Procedure:

To the neutralized oil (after the acid value test), 25 ml of ethanolic KOH (0.5 N) are added. The mixture is heated under reflux in a water bath for 60 minutes to ensure complete hydrolysis of esters. After cooling, the excess KOH is titrated with HCl (0.5 N) using phenolphthalein as a color indicator. A blank test is conducted under identical conditions without the essential oil.

- **Saponification Value I_S:**

According to the Ph. Eur., the saponification value I_S is the number that expresses, in milligrams, the quantity of potassium hydroxide required to neutralize the free acids and saponify the esters present in 1 g of the substance **(EDQM, 2013).**

The determination method consists of dissolving 1.014 g of EO (≈ 1 g) in 25 mL of potassium hydroxide (KOH 0.5 M) in a 100 mL flat-bottomed borosilicate glass flask equipped with a reflux condenser. Then, the condenser is started, and the flask is heated to reflux using a hot plate under magnetic stirring at 90 °C until boiling. After boiling, the mixture is cooled for 45 mins. Subsequently, 20 mL of distilled water and 3 drops of phenolphthalein solution are added (the solution turns pink), followed by immediate titration with a hydrochloric acid solution (HCl 0.5 M) using a graduated burette and a magnetic stirrer until the pink color disappears. A blank test was also carried out under the same conditions (HCl 0.5 M).

The saponification value I_S, expressed without units, corresponds to:

$$I_E = \frac{28.05 \times (V_0 - V_1)}{m}$$

Where:

V_0**:** Volume of the blank test (HCl 0.5 M)

V_1**:** Volume of the titrant solution (HCl 0.5 M)

m: Mass of the substance to be examined (in g)

C: Concentration of the titrant solution (HCl 0.5 M)

56.1: Molar mass of potassium hydroxide (KOH) in g/mol

3. **PART C:**

1. **Evaluation Method of In Vivo Anti-inflammatory Activity:**

- Induction of Acute Inflammatory Edema in the Mouse Paw by Formalin:

Principle:

The anti-inflammatory activity (the curative effect of the innovative *Artemisia herba-alba* cream) was evaluated using the Formalin-induced mouse paw edema method, according to the procedure described by **(Rahmani et al.,2016).**

Inflammation is induced by injecting 1% formalin into the plantar arch of the right hind paw, causing swelling in the plantar fascia. The swelling resulting from this phlogistic agent is translated into measurable volume, allowing for the monitoring of the inflammatory process **(Rahmani et al., 2016)**. The control group is injected with the same concentration.

Procedure:

The rats were divided into 5 groups. Groups 1 and 2 contained 4 mice each, while the remaining groups contained 6 rats (total: 4 normal rats and 22 formalin-treated rats total: 26 rats). The animals were fasted for 4 hours before the experiment.

Initial Volume Measurement (V_0): The volume of the right hind paw was measured for each mouse before any intervention.

Induction of Inflammation: All rats (except the healthy control) were injected with 0.1 ml of 1% formalin solution into the plantar fascia of the right paw arch.

Induction Period: The rats were left for 30 minutes to ensure the stabilization of the first phase of inflammation and the formation of edema.

Treatment (After 30 Minutes):

Group 01 (Healthy Control): Not injected with formalin and received no treatment.

Group 02 (Positive Control): Injected with 1% formalin and received a placebo treatment of physiological saline via intraperitoneal injection.

Group 03 (Extract): Injected with formalin and then received the aqueous extract (100 mg/kg) via intraperitoneal injection.

Group 04 (Innovative Cream): Injected with formalin and then the cream was applied topically to the paw arch (at a rate of 100 mg/kg).

Group 05 (Diclofenac): Injected with formalin and then 1% Diclofenac gel was applied topically to the paw arch (at a rate of 100 mg/kg).

Measurements:

The edema volume was measured at the following time intervals: 30 minutes,and 1,2,3 hours as well as at 24 hours, 48 hours after the formalin injection.

The severity of the edema was assessed by determining the average percentage increase (%AUG) in the volume of the mouse according to the following formula:

$$\%AUG = \frac{(V_t - V_0)}{V_0} \times 100$$

Where:

V_t: Volume of the paw at any given time t.

V_0: Initial volume of the paw (T_0: before formalin injection)

The anti-inflammatory activity was evaluated by calculating the percentage of inhibition (%INH) of the edema according to the formula:

$$\%INH = \frac{(\%AUG_{Control} - \%AUG_{Treated})}{\%AUG_{Control}} \times 100$$

The data were analyzed using to compare the treated groups with the negative control group.

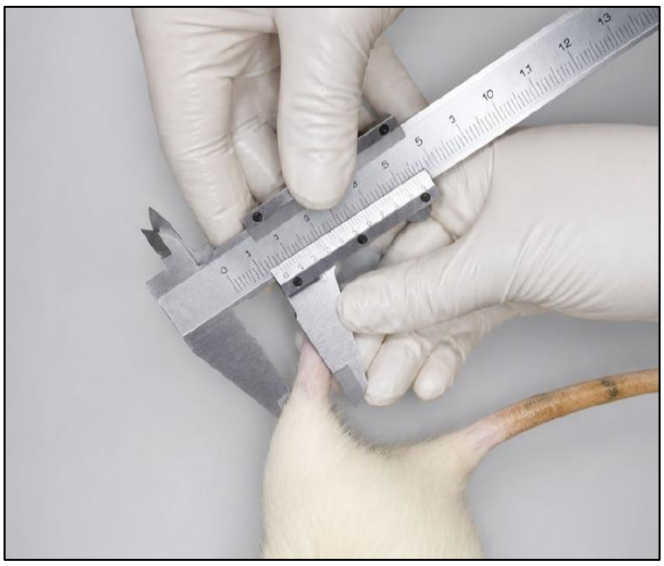

Figure 11 : Measuring the edema thickness in the rat's right paw. (personal photo, 2026)

2. Measurement of Hematological Parameters :

The hematological parameters (hemoglobin, red blood cells, granulocytes, etc.) are determined using the Coulter principle. This is performed via a Medonic automated hematology analyzer, specifically designed for Full Blood Count (FBC/CBC) analysis.

3. Method for Determining Oxidative Stress Parameters :

3.1 Preparation of Organ Homogenates :

To assess oxidative stress biomarkers, liver and skin tissue samples were processed as follows:

1. Tissue Preparation and Homogenization:

- Approximately 0.5 g of each tissue (liver and skin) was accurately weighed and rinsed with cold Phosphate Buffered Saline (PBS) to remove excess blood.
- The tissues were then homogenized in 5 ml of cold Phosphate Buffer (50 mM, pH 7.4) uniform suspension was obtained for each sample.

2. Centrifugation and Storage:

- The resulting homogenates were centrifuged at 4000 rpm for 10 minutes at 4°C to separate cellular debris.
- The supernatant (the clear liquid) was carefully collected and stored at 4°C for the subsequent biochemical assays.

Preparation of Buffers and Solutions :

1. Phosphate Buffered Saline (PBS) :

The PBS was prepared by dissolving the following salts in distilled water:

- 360 mg of Na_2HPO_4 (Disodium phosphate).
- 120 mg of KH_2PO_4 (Monopotassium phosphate).

The salts were dissolved in approximately 400 ml of distilled water. The volume was then adjusted to 500 ml. The final pH was adjusted to the physiological range (7.2–7.4) using NaOH or HCl (0.1 N).

2. Phosphate Buffer :

The buffer was prepared by dissolving:

- 4.05 g of Na_2HPO_4 (Disodium phosphate).
- The required amount of NaH_2PO_4 (Monosodium phosphate) to achieve the target concentration.

The salts were dissolved in distilled water, and the final volume was adjusted to 500 ml. The pH was verified to ensure stability for enzymatic activity.

3.2 Determination of Tissue Malondialdehyde (MDA) Levels :

Principle :

The quantification of lipid peroxidation is based on the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA). MDA is a secondary end-product generated from the oxidative degradation of polyunsaturated fatty acids (PUFAs) containing three or more double bonds.

Under acidic conditions and high temperature, one molecule of MDA reacts with two molecules of TBA to form a stable, pink-colored chromophore (TBA-MDA complex). This complex exhibits maximum light absorption at a wavelength of 532 nm, providing a highly sensitive and established method for assessing lipid peroxidation *in vitro*. The assay was conducted according to the colorimetric methods described by (**Yagi ,1976) and Esterbauer et al., (1992).**

Procedure:

Preparation of Reagents :

Trichloroacetic Acid (TCA) 20%: This reagent is used for protein precipitation to obtain a clear supernatant for analysis. It was prepared by dissolving 10 g of TCA in 40 ml of distilled water, then adjusting the final volume to 50 ml. The solution was stored at 4°C.

Thiobarbituric Acid (TBA) 0.375%: This reagent reacts with MDA to produce the characteristic pink color. It was prepared by dissolving 0.1875 g (or your measured 0.335 g for a different concentration/volume) of TBA in 50 ml of distilled water using a magnetic stirrer with heating. Once cooled, the flask was wrapped in aluminum foil (to protect it from light) and stored at 4°C.

MDA Assay Procedure :

The MDA levels were determined according to the following steps:

Reaction Mixture: In each test tube, 0.5 ml of the tissue homogenate was mixed with 0.5 ml of TCA (20%) and 0.5 ml of TBA (0.375%).

Homogenization: The tubes were vortexed or shaken well to ensure a homogeneous mixture.

Incubation: The mixture was heated in a water bath at 95°C for 30 minutes.

Cooling: After heating, the tubes were immediately placed in an ice bath for 5 minutes to stop the reaction.

Centrifugation: The mixture was centrifuged at 3,000 rpm for 10 minutes to precipitate the proteins and debris.

Measurement: The absorbance of the resulting clear supernatant was measured at 532 nm using a UV-Visible spectrophotometer.

4. Study of Histological Sections :

Preparation of Histological Sections of the Liver :

For anatomo-histopathological examination, the livers fixed in 10% formalin were placed in plastic cassettes. These histological sections were prepared in Laboratory 6, Faculty of Natural and Life Sciences, University of Echahid Hamma Lakhdar – El Oued. The technique used is that described by **Houlot (1984).** The preparation was examined under a light microscope at X400 magnification.

Steps of the Histological Technique :

1) **Sampling :**

- It should be performed as soon as possible after death, sometimes on a living subject (biopsy).
- Avoid crushing or kneading the organ and detach it with a very sharp instrument.
- Cut thin slices taking into account the general orientation of the organ.

2) **Fixation**

The sample is fixed in order to preserve the tissues and slow down tissue degradation. Tissue fixation is a critical step that preserves tissue and cellular components and maintains their natural structure.

During fixation, formaldehyde binds to primary amines, such as those found on the side chains of the amino acids lysine and glutamine, to form a stable cross-link called a methylene bridge. This process is inherently slow and can take up to 1 to 2 days.

Essential for preserving biological structures:

- Should be performed as soon as possible after excision.
- Small organ blocks are immersed in a large volume of fixative liquid.

3) **Embedding :**

Tissue water is replaced by paraffin. However, these media are not miscible with each other. Therefore, this is done step by step by replacing water with alcohol (70°, 95°, 100°), then alcohol with an organic solvent (toluene), then toluene with paraffin. The sample is impregnated with molten paraffin to harden it so that it can be cut afterwards.

4) **Sectioning :**

Performed with a microtome to obtain section slices (histological sections) 2 to 5 µm thick. The sections are collected on glass slides. An ultra-microtome is a very high-precision device that allows sections of 60 to 100 nm to be used for electron microscopy.

5) **Staining :**

In order to distinguish between different tissues, different stains can be used:

- Acidic stains, Basic stains.
- Acidic substances in the cell are stained with a basic stain, and basic substances in the cell are stained with an acidic stain.
- Routine staining uses one stain (hematein) or two different stains: Hematoxylin-Eosin (H&E), where hematoxylin stains nuclei purple, and eosin stains cytoplasm pink.

6) **Mounting and Observation :**

The stained sections are mounted between a slide and a coverslip using a synthetic resin whose refractive index is close to that of glass. This yields a "microscopic preparation" (called a "slide") ready to be observed under a light microscope (LM).

Chapter IV :

Results and discussion

1. Extraction Yield :

The yields and physical characteristics of the aqueous extract of the studied plant are presented in Table 5. According to the obtained results, the extraction yield reached 12.04%, with the extract appearing as a (mention color, brown) powder.

Table 7 :Yield and physical characteristics of the aqueous extract

Yield (%)	Weight of dry extract (g)	Color	Aspect	Extract	Weight of plant material (g)
12.04%	6.02	Brown	Powder	Aqueous Extract	50

These results do not agree with those recorded by **Cherif and Louzini (2016)** and **Beddazekri and Ghemam (2017**), who recorded higher yields for their studied plants. However, our results for the aqueous extract (12.04%) are relatively consistent with those published by **Habera and Laoudi (2019)**, who recorded a yield of 15.68 \pm 0.40\%.

Extraction yields depend on several factors, namely the method and the experimental conditions under which the extraction was carried out (maceration time, temperature, and choice of solvent) (**Telli et al., 2010; Dent et al., 2013).** The chemical compound content, geographical origin (climate and soil), harvesting season, storage conditions, and the specific plant parts used in the extraction also significantly influence the final extraction yield **(Fellah et al., 2006)**

2. Phytochemical screening:

Phytochemical screening of the leaves and fruits of *Artemisia herba-alba* revealed the presence of specific chemical groups, identified using specialized reagents that produce distinct color changes. The preliminary assessment indicated that the composition of *Artemisia herba-alba* is limited to the chemical constituents listed in the table below. The presence of flavonoids is confirmed by a light yellow color; these compounds are often recognized for their anti-inflammatory, anti-allergic, hepatoprotective, and antispasmodic properties. They also act as cholesterol-lowering agents, diuretics, and exhibit antibacterial and antiviral activities in vitro. **(Bruneton, 2009).** The appearance of a blackish-blue color confirms the presence of gallic tannins, which exert antiseptic, antibacterial, and antifungal effects. These molecules are typically utilized in treating conditions such as infectious diarrhea and dermatitis.

The formation of a brown precipitate confirms the presence of alkaloids in the *Artemisia herba-alba* extract. However, this test is strictly qualitative and does not allow for the determination of the actual quantity of these compounds, as the color intensity or precipitate density does not accurately reflect the concentration of alkaloids. This result suggests that alkaloids are present in trace to moderate amounts.

We also observed the presence of steroids and terpenes, confirmed by the appearance of a reddish-brown ring. Additionally, the emergence of a brick-red color confirms the presence of reducing compounds, albeit in small quantities. Finally, the tests conducted for saponosides yielded negative results.

Table 8: Results of Phytochemical Screening of *Artemisia herba-alba* Extract

Test performed	Results
Flavonoid	+++
Saponoside	-
Sterol and triterpene	+
Tannins	++
Alkaloid	+

Strongly positive reaction: + + + **Positive reaction:** +

Moderately positive reaction: + + **Negative:** -

The phytochemical study of *Artemisia herba-alba* revealed the presence of flavonoids, alkaloids, tannins, sterols, and triterpenes, which confirms the internal work of (**Fadili et al., 2017).** A previous study conducted by **(Medour et al., 2011)** demonstrated the presence of flavonoids, alkaloids, and tannins, which is comparable to our results. (**Chamam et al., 2014)** also showed the presence of flavonoids.

3. Quantitative Characterization of Plant Extracts:

3.1 Polyphenol Content in the Extracts:

The total polyphenol content was determined using the spectrophotometric method with the Folin-Ciocalteu reagent. The concentration of phenolic compounds in the aqueous extract of *Artemisia herba-alba* was calculated from a gallic acid calibration curve, expressed in micrograms per milliliter µg/mL. Absorbance was measured at a wavelength of 765 nm .

In order to quantify the bioactive secondary metabolites present in the aqueous extract, standard calibration curves were constructed using reference standards. The linear regression equations and the quantitative distribution of total phenolics and flavonoids are graphically demonstrated below

gallic acid: y = 0.0339x - 0.0655 with a correlation coefficient R2= 0.9938 As shown below.

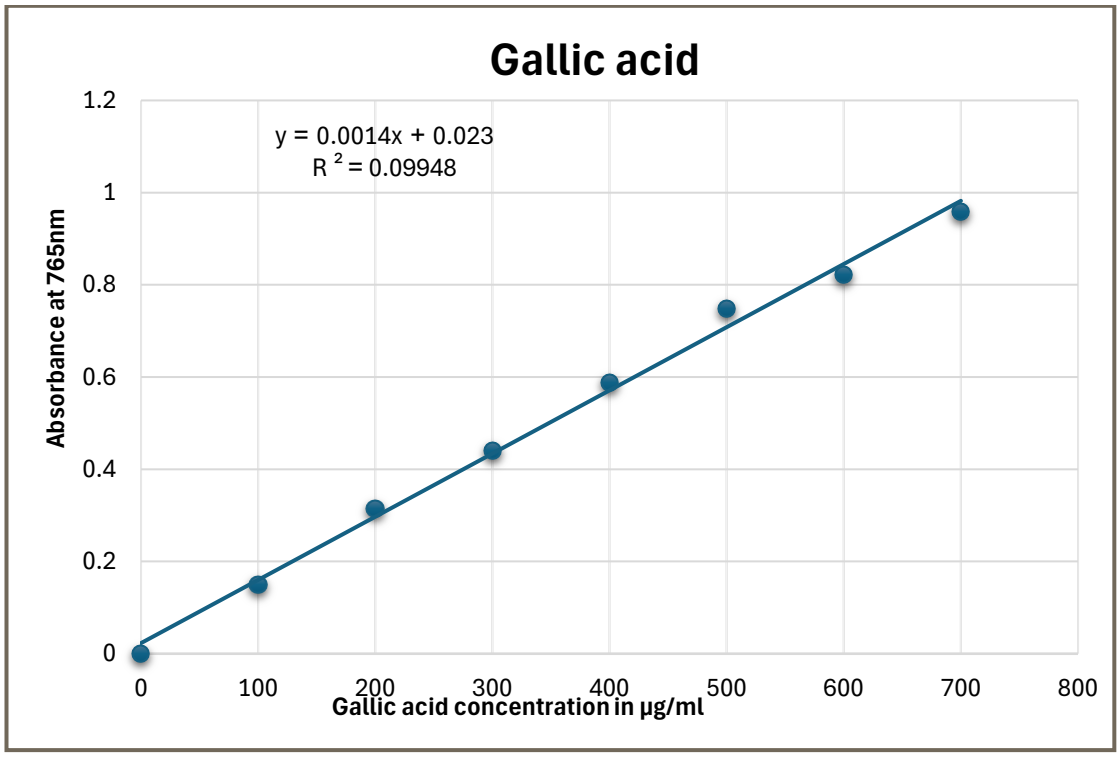

Figure 12 : Gallic Acid Calibration Line (Mean ± SD of three trials)

Polyphenol Content:

The figure 07 The results show the polyphenol content in the *Artemisia herba-alba* extract, where we obtained a value of 105±0.33 mg GAE /g EXT. Based on the recorded values, we observed that it is rich in polyphenols.

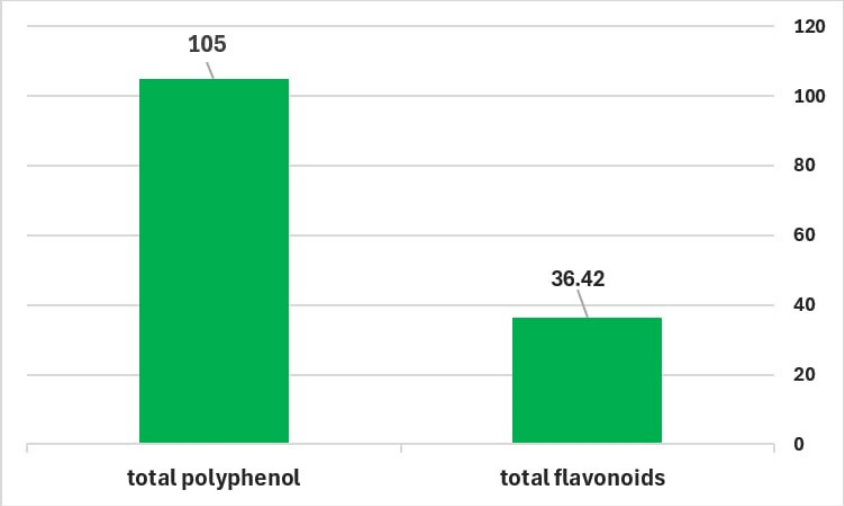

Figure 13 :Total Phenolic Content (TPC) of *Artemisia herba-alba* extract expressed as Gallic Acid Equivalent (GAE) and Total flavonoid content in aqueous extracts expressed as quercetin equivalents

The level of phenolic compounds we recorded for the extract is in agreement with **(Cherif & Louzini ,2016)** (0,821±0,061 mg GAE/g EXT) and **(Habera & Laoudi , 2019)** (97,17 mg GAE/g EXT).

The determined total polyphenol contents are not absolute measurements of the quantities of phenols in the raw material; rather, they are based on the reducing capacity relative to gallic acid equivalents (GAE). The values obtained by the colorimetric method provide direct information regarding the quantity of antioxidant phenolic groups in the extract, which primarily depends on the number of hydroxyl groups present (**Balasunderam et al., 2006**).

The polyphenolic profile of plant extracts can vary under the influence of various factors, including:

Plant species Environmental and climatic conditions Storage conditions Harvest period Maturity stage, etc actors Influencing Polyphenolic Profiles **(Falleh et al., 2008).**

3.2 Flavonoid Content in the Extracts:

The flavonoid content in the aqueous extract of Artemisia herba-alba was calculated using a quercetin calibration curve, expressed in micrograms per milliliter (µg /mL). Absorbance was measured at a wavelength of 415nm, and the results are presented in the calibration curve Querectine: y = 0.0339x - 0.0655 with a correlation coefficient R^2= 0.9938 As shown below.

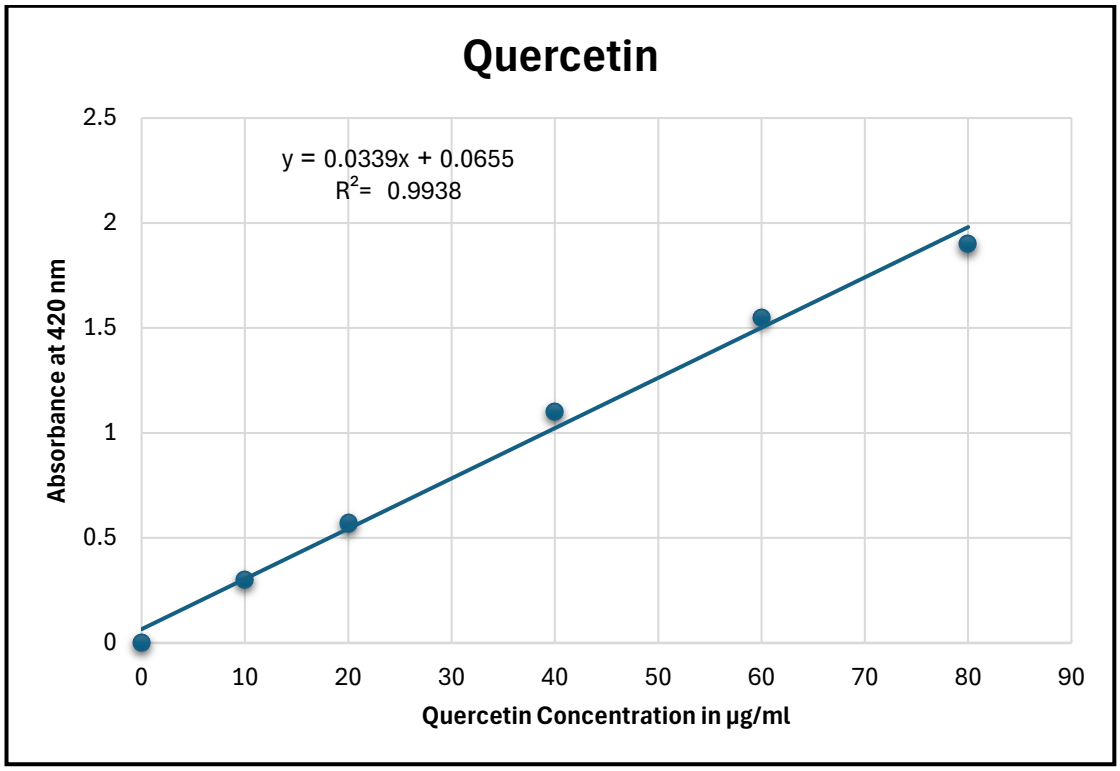

Figure 14 : Querectine Calibration Line (Mean ± SD of three trials)

Results and Analysis Flavonoid Content:

The figure 09 illustrates that the aqueous extract of *Artemisia herba-alba* contains (36.42±1.61 mg EQUR/g EXT), and these results are consistent with the polyphenol findings.

The results of the *Artemisia herba-alba* extract align with those published by (**Habera & loudie 2019**), where they recorded a level of(35.61 mg EQUE/g EXT). This confirms the plant's richness in flavonoids; however, these results remain significantly lower than the findings of (**Saoudi et al., 2010)** (131,89mg EQUE/1g EXT).

Factors Influencing Flavonoid Quantification:

According to (**Lapronik et al.,2005)** , the determination of flavonoid quantity depends on the solubility of flavonoid compounds in solvents. This solubility is contingent upon the number, type, and position of carbohydrate bonds within the flavonoids.

Numerous studies have demonstrated that external factors such as geographical and climatic conditions, genetic factors, plant maturity stage, and storage duration can strongly influence the flavonoid content in extracts .(**Aganga & Mosase, 2001).**

Furthermore, the concentration of flavonoids in the extracts depends on the type of standard used (Quercetin), which can also alter the results **(Ghedadba, 2015).**

4. **Evaluation of Antioxidant Activity:**

4.1 DPPH Free Radical Scavenging Assay:

The antioxidant activity of the extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical assay. This method is based on the reduction of the DPPH radical, which results in a color change from purple to yellow, measured via spectrophotometry at 517 nm. The results are expressed in terms of the half-maximal inhibitory concentration (IC_{50}), calculated from the linear regression curve of the Antioxidant Activity Evaluation (DPPH Assay)

The antioxidant potential of the aqueous extract was evaluated based on its ability to scavenge DPPH free radicals. The results, obtained by measuring absorbance at 517 nm, demonstrated a clear dose-dependent inhibitory effect.

Inhibition Percentage (I%): The scavenging activity increased proportionally with the concentration of the extract. The maximum inhibition reached 81.42% at a concentration of 0.04 mg/ml. In comparison, the positive standard (Ascorbic acid) exhibited a higher inhibition of 98.48% at the same concentration.

IC50 Determination: Based on the linear regression analysis, the IC50 value for the plant extract was determined to be approximately 0.018mg/ml. This value represents the concentration required to neutralize 50% of the DPPH radicals.

The in vitro biological potential of the plant extract was evaluated through antioxidant radical scavenging and anti-inflammatory protein stabilization assays. The charts below depict the dose-dependent percentage of inhibition obtained for the DPPH and BSA denaturation assays

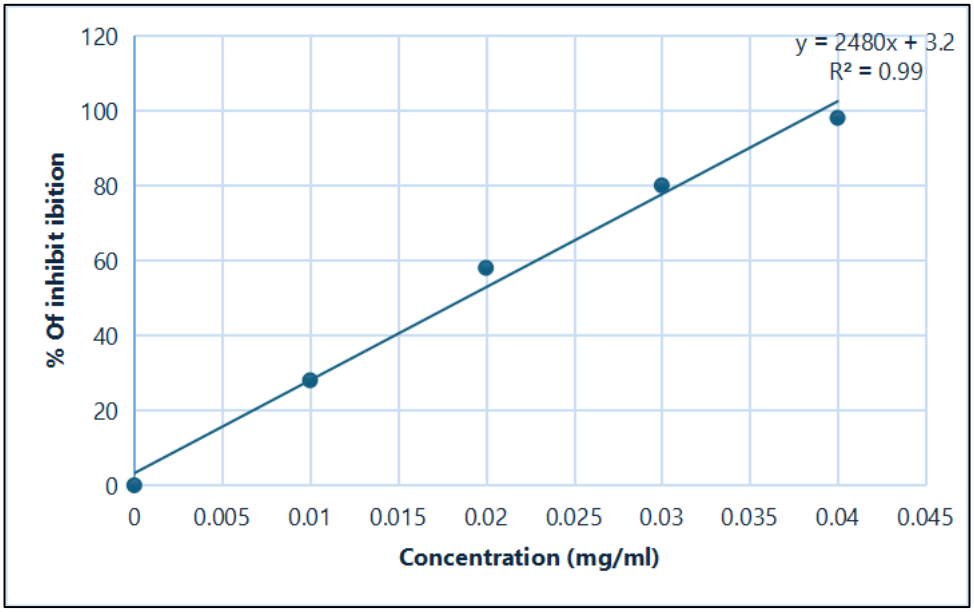

Figure 15 :Percentages Evaluation of Antioxidant Activity

DPPH

Table 9 : IC_{50} values of DPPH radical scavenging activity of *Artemisia herba-alba* aqueous extract.

IC50± Standard Deviation (in mg/ml)	
AQ EXT *Artimisia herba alba*	0.0018±1.2

The findings indicate that the aqueous extract possesses significant reductive capacity. Although the IC_{50} of the extract is higher than that of Ascorbic acid (indicating that the pure standard is more potent), the result is considered highly effective for a crude plant extract .

This robust antioxidant activity is primarily attributed to the presence of phytochemicals, such as phenols and flavonoids, which act as hydrogen donors to stabilize free radicals. These results strongly support the efficacy of the innovative formulation being developed. The extract serves as a protective agent against oxidative stress, thereby enhancing the overall anti-inflammatory properties of the final pharmaceutical product.

5. **Inhibition of Thermal Protein Denaturation:**

Protein denaturation is considered among the causes of inflammation, and the production of autoantigens in inflammatory diseases may be attributed to in vivo protein denaturation. The potential mechanism of this denaturation includes the alteration of electrostatic, hydrogen, hydrophobic, and disulfide bonds that maintain the three-dimensional structure of proteins.

Non-steroidal anti-inflammatory drugs (NSAIDs), such as phenylbutazone and indomethacin, have been proven not only to inhibit the synthesis of pro-inflammatory prostaglandins but also to inhibit protein denaturation **(Sangeetha et al., 2011).** They prevent the denaturation of heat-treated albumin at physiological pH.

Figure 13 illustrates the change in the percentage of protection against thermal damage to Bovine Serum Albumin (BSA) according to different extract concentrations. These results are compared with those recorded for Diclofenac, which was considered the reference molecule in this experiment.

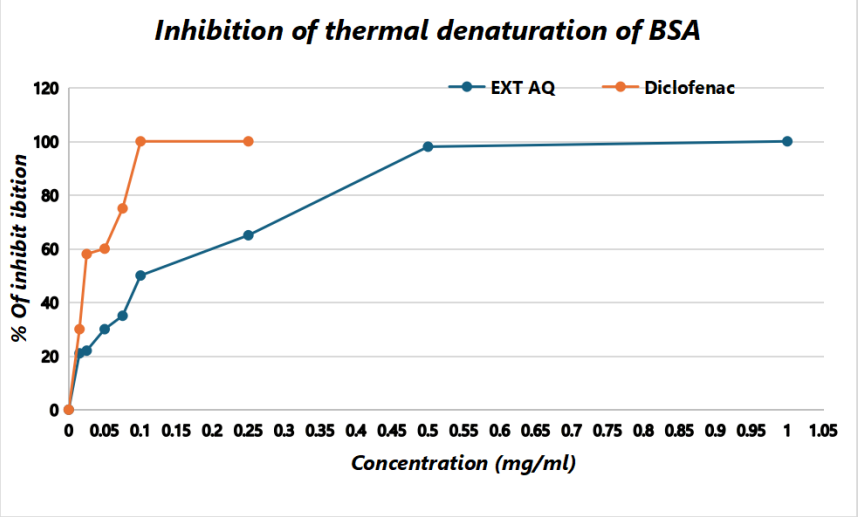

Figure 16 : Percentages of Inhibition of BSA Denaturation

According to the results, there appears to be a proportional relationship between the increase in concentration and the percentage inhibition of Bovine Serum Albumin (BSA) denaturation by both Diclofenac and the aqueous plant extract under study.

Our results showed that the percentage inhibition of BSA denaturation for the extract was lower than that of Diclofenac at all concentrations used. At a concentration of 0.1 mg/mL, Diclofenac exhibited the highest inhibition percentage of BSA denaturation, reaching 99.37 ± 0.30%. As for

the extract, at a concentration of 0.7 mg/mL, the inhibition percentage of BSA denaturation reached 101,72 ± 0,01% .

The anti-inflammatory capacity of the extract was determined using the IC_{50} value, which is the concentration required to reduce the level of BSA denaturation by 50%. We determined the concentration required for a 50% reduction, or the IC_{50} value, using linear regression equations. The values are presented in the following table:

Table 10 : In vitro Anti-inflammatory Potency (Expressed as IC_{50} in µg/ml) of a Reference Anti-inflammatory Drug and the Tested Extract

IC50± Standard Deviation (in µg/ml)	
Diclofenac	42.0±13.29
Artimisia herba alba	258.91±4.99

6. **essential oil Extraction Yield :**

The essential oil of *Artemisia herba-alba* was extracted using laboratory-scale steam distillation. A mass of 100 g of dried plant material underwent the extraction process, yielding 2 mL of essential oil.

$$\text{Yield}(\%) = \frac{2ml}{100g} \times 100 = 2\%$$

This result indicates an essential oil yield of 2%, a value considered high compared to the data reported in the literature, which typically ranges between 0.5% and 1.5%. This elevated yield can be attributed to several factors, including:

Quality of the plant material: (growth stage, collection and drying conditions).

Optimal distillation conditions: (duration, temperature, and water-to-plant ratio).

Richness of the sample: potential high concentration of volatile compounds.

Consequently, this result reflects both the superior quality of the plant sample and the efficiency of the extraction process employed.

7. **Experimental Result The Physical and Chemical Indices of *Artemisia herba-alba* Essential Oil**

- **Organoleptic Assessment:**

The extracted essential oil obtained is a clear, pale yellow, mobile liquid with a distinctive aromatic odor reminiscent of camphor. These organoleptic properties are consistent with the standard sensory profiles established for the *Artemisia herba-alba* species.

Table 11 : The Physical and Chemical Indices of *Artemisia herba-alba* Essential Oil

Test Applied	Experimental Result
Relative Density	0.935
Refractive Index	1.470
Acid Value (I_a)	1.52 mg Koh/g
Ester Value (I_e)	95.32 mg Koh/g
Saponification Value(I_s)	96.84 mg Koh/g

- **Relative Density:**

This result indicates that the EO is less dense than water (water density of 1.000 at 25 °C) and will therefore float on the surface when poured into an aqueous medium, reflecting the predominantly organic, hydrophobic, and volatile nature of the EO constituents, such as monoterpenes and sesquiterpenes. According to the standard (NF T 75-006) **(AFNOR, 2000)**. the relative density of white Artemisia herba-alba EOs must be between 0.910 and 0.940. The value obtained in this study remains compliant with this reference range, which bears witness to the quality of the extracted EO. testifying to the quality and purity of the extracted EO. Such compliance is essential to ensure the efficacy of our innovative formulation (anti-inflammatory cream).

- **Refractive Index:**

The corrected refractive index measured at 20 °C for *Artemisia herba-alba* essential oil is 1.470. This value is in full agreement with the data reported in the literature, where the refractive index for this species typically ranges between 1.465 and 1.485, according to AFNOR standards **(AFNOR, 2000).** This index reflects the purity and chemical composition of the essential oil,

particularly its high content of oxygenated compounds such as monoterpenes and sesquiterpenes, which directly influence its optical properties.

- **Determination of Acid Value (I_A) :**

The measured acid value of the essential oil of *Artemisia herba-alba* is 1.52 mg KOH/g, which is very close to the data mentioned in the scientific literature, where the acid value for essential oils of this species is generally less than 2 mg KOH/g **(AFNOR, 2000).** This value indicates that the distillation process using the Clevenger apparatus was performed correctly and did not lead to the degradation of the components. It also signifies that the oil was not exposed to oxidation or light in a way that would lead to the decomposition of esters into free acids.

- **Ester Value (I_E):**

The measured ester value for the white *Artemisia herba-alba* essential oil reached 96.32 mg KOH/g, which is a good value compared to the average mentioned in the references, ranging between 80 and 110 mg KOH/g **(AFNOR, 2000)**. This value indicates that the essential oil is rich in compounds with specific therapeutic potential, such as aromatic esters or keto-esters.

- **Saponification Value(I_S):**

This value of 96.84 mg KOH/g is consistent with the average mentioned in the literature, which ranges between 90 and 120 mg KOH/g **(AFNOR, 2000).** This value indicates the presence of a significant amount of esters, which enhances its therapeutic value.

8. Anti-edematous Effect (In vivo anti-inflammatory activity):

Acute inflammation is characterized by classic local symptoms, such as heat, redness, pain, and edema or swelling. Indeed, the various pro-inflammatory mediators secreted at the inflammatory site, notably prostaglandins, exert their action locally by increasing capillary permeability, thereby causing the infiltration of an exudate **(Okombe and Nzuzima, 2019).** Consequently, the formed edema compresses nerve endings and acts on the hypothalamic center, which often results in a sensation of pain **(Etameloe et al., 2018).**

Induced paw edema constitutes a reproducible experimental model and provides good predictive value for the screening of anti-inflammatory agents **(Lu et al., 2006; Cabrini et al., 2011; Okombe & Nzuzima, 2019).**

In this study, the induction of paw edema in mice using formalin allowed us to evaluate the anti-edematous effect of the white *Artemisia herba-alba* extract and the formulated cream by monitoring the rate of increase in paw volume over time. The change in edema was measured at time intervals of 30, 60, 120, 180 minutes, as well as 24, 48 hours following the intraperitoneal and intraplantar injections of the different products. The results obtained were compared with those of Diclofenac, a non-steroidal anti-inflammatory drug (NSAID), and with physiological control groups.

Table 12 : Effect of the different extracts and Diclofenac on formalin-induced edema

		Right paw volume (Mean ± SD) ml					
Treatment	**Dose**	**0h**	**1h**	**2h**	**3h**	**24h**	**48h**
Healthy Control	**0**	**0.931± 0.081**	**1.097± 0.081**	**1.236± 0.062**	**1.312± 0.114**	**1.087± 0.100**	**0.936± 0.055**
Positive Control	**1%**	**0.672 ± 0.008^{NS}**	**0.937 ± 0.203***	**0.957 ± 0.200****	**1.102 ± 0.149*****	**1.130 ± 0.121*****	**1.222 ± 0.086*****
Diclofenac	**100 mg/ml**	**0.925± 0.277$^{NS\ NS}$**	**1.008± 0.245^{**A}**	**0.990± 0.248^{*B}**	**0.970± 0.254^{NSC}**	**0.960 ± 0.2505^{NSC}**	**0.956± 0.254^{NSC}**
AQ Ext of Artemisia Hb	**100 mg/ml**	**0.670± 0.070$^{NS\ NS}$**	**1.070± 0.110^{**A}**	**0.940± 0.060^{*B}**	**0.800± 0.100^{*B}**	**0.785± 0.085$^{NS\ C}$**	**0.660± 0.600$^{NS\ C}$**
innovative cream	**100 mg/ml**	**0.745± 0.183^{NSNS}**	**0.846± 0.195^{**A}**	**0.826± 0.186^{*B}**	**0.766± 0.179$^{NS\ C}$**	**0.748 ± 0.182$^{NS\ C}$**	**0.745± 0.183^{NSC}**

Values represent means ± SD.

ns: non-significant.

p < 0.05 * significant.

p < 0.01 ** very significant.

p < 0.001 *** highly significant.

Comparison with the Healthy Control

Ap < 0.05 * significant.

Bp < 0.01 ** very significant.

Cp < 0.001 *** highly significant.

Comparison with the Positive Control

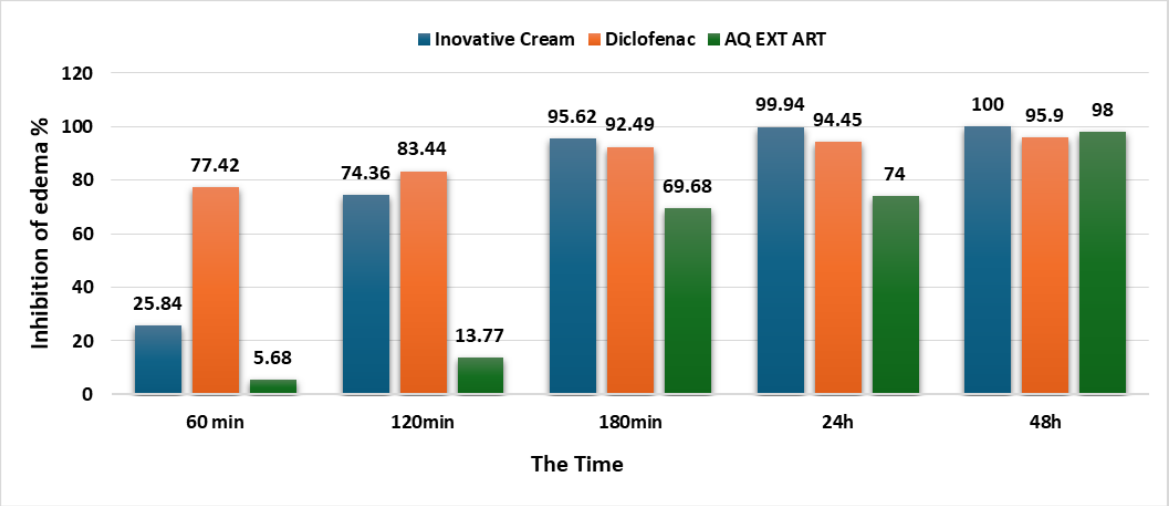

Figure 17 : Effect of the aqueous extract, the innovative cream, and Diclofenac on formalin-induced edema

Percentage of paw edema inhibition (%INH)

AQ Ext of *Artemisia herba-alba*

For the extract-treated group, a statistically significant inhibition of rat paw edema was observed during the first hour of the experiment with inhibition percentages of 5%. A statistically significant effect ($p<0.05$) was also observed during the second hour with inhibition percentages of 13%, followed by a higher statistically significant inhibition ($p<0.01$) at the third hour with inhibition percentages of 69%. The effect remained highly statistically significant ($p<0.001$) at 24 and 48 hours, with inhibition percentages of,74%, and 100%, respectively (Figure 18). Compared with the positive control group, the extract showed a reduction in rat pawedema throughout the experimental period.

Diclofenac

For the group treated with Diclofenac, a statistically significant inhibition ($p<0.05$) of rat paw edema was observed during the first hour of the experiment, with an inhibition percentage of 77.42%. A higher statistically significant effect ($p<0.01$) was observed during the second hour, with an inhibition percentage of 83.44%. This effect remained highly statistically significant ($p<0.001$) at 3, 24, and 48 hours, with inhibition percentages of 92.49%, 94.95%, and 95.90%, respectively (Figure 18). Compared with the positive control group, diclofenac showed a marked reduction in rat paw edema throughout the experimental period.

Innovative cream

For the group treated with the formulated cream, a statistically significant inhibition ($p<0.05$) of rat paw edema was observed during the first hour of the experiment, with an inhibition percentage of 25%. A higher statistically significant effect ($p<0.01$) was observed during the second hour, with an inhibition percentage of 74%. This effect remained highly statistically significant ($p<0.001$) at 3, 24, and 48 hours, with inhibition percentages of 95%, 99%, and 100%, respectively (Figure 18). Compared with the positive control group, the formulated cream showed a marked reduction in rat paw edema throughout the experimental period

In the positive Control group, the injection of 1% formalin into the right hind paw of the mice induced an edema with a volume increasing from 0.672 ± 0.008 ml to 0.937 ± 0.203 ml in the first hour, reaching 0.957 ± 0.200 ml in the second hour, and peaking at 1.102 ± 0.149 ml by the third hour (Table 8). These values correspond to respective edema volume increase percentages of 39.43%, 42.41%, and 63.98%. These results indicate that the maximum edema was reached at the 3rd hour.

Formalin induces local inflammation when injected into the plantar aponeurosis of the paw **(Singh et al., 1997; Suzuki et al., 1996)**, similar to carrageenan **(Bhatt et al., 1977; Ossipov et al., 1995)**. This inflammatory reaction is primarily caused by tissue injury, which triggers the synthesis of various mediators such as histamine, prostaglandins, leukotrienes **(Ammon et al., 1993),** platelet-activating factor (PAF), cytokines, nitric oxide (NO), and tumor necrosis factor (TNF) **(Clarke et al., 1996).**

In the presence of Diclofenac, we recorded a significantly lower increase in paw volume, with a maximum increase of only 4.86% at the third hour. Diclofenac effectively prevented edema

starting from the first hour with an inhibition percentage of 77.42%, reaching a maximum inhibition of 92.49% three hours after the 1% formalin injection.

Regarding the effect of the aqueous extract of *Artemisia herba-alba* and the innovative cream, the results demonstrated significant inhibition of inflammation. For the cream, the edema inhibition percentage progressed from 25.84% in the first hour to 95.62% in the third hour, proving the efficacy of the pharmaceutical formulation in the sustained release of active compounds.

Furthermore, the long-term evaluation at 24 and 48 hours post-injection revealed a sustained anti-inflammatory effect. While the edema in the positive Control group continued to rise, reaching an increase of 81.84% at 48 hours, the innovative cream demonstrated a superior recovery, achieving an inhibition rate of 100%, thereby outperforming the reference drug Diclofenac, which maintained an inhibition rate of 95.9%. This remarkable performance confirms the stability of the pharmaceutical formulation and its ability to provide a more prolonged and effective release of the bioactive compounds from *Artemisia herba-alba* compared to both the aqueous extract and the standard treatment.

The administration of the innovative cream formulated from *Artemisia herba-alba* extract induced a progressive and remarkable inhibition of paw edema, evolving from 25.84% in the first hour to reach 95.62% by the third hour, and peaking at 100% at the 48th hour. This result demonstrates a superior efficacy compared to the reference drug, Diclofenac, which maintained an inhibition rate of 95.9% at the end of the experiment.

This anti-inflammatory potential can be attributed to the presence of bioactive compounds within the Artemisia genus, such as phenolic compounds, flavonoids, and saponins (**Santengelo, 2007).** Numerous in vivo studies have shown that flavonoids, such as quercetin and rutin, possess potent anti-inflammatory activity in both acute and chronic inflammation models by inhibiting lipid peroxidation, platelet aggregation, and capillary permeability **(Falleh et al., 2008; Hussain et al., 2016; Li et al., 2016).**

Indeed, formalin induces neurogenic inflammation when applied locally, triggering the release of pro-inflammatory substances. The superiority of our innovative formulation (cream) compared to the aqueous extract alone could be explained by enhanced skin penetration and the controlled release of active principles. This allows for a more effective blockade of the biosynthesis of vasoactive mediators via the inhibition of phospholipase A2 (PLA2) and the enzymes

cyclooxygenase (COX) and lipoxygenase (LOX) **(Selloum et al., 2003; Rotelli et al., 2003; Ghedira, 2005).**

The results obtained in this test confirm that the anti-inflammatory activity of the cream is linked to its ability to reduce the infiltration of inflammatory cells as well as the production of key cytokines such as TNF-α and IL-1 **(Li et al., 2016).**

9. Study of Hematological Parameters in Rats :

Effect of the Cream and Aqueous Extract of *Artemisia herba-alba* on Immune Cells :

Table 13 : Hemoglobin levels, red blood cell count, lymphocytes, monocytes, and platelets in the control and treated groups

	means ± SD				
FNS	**healthy control**	**positive control**	**AQ EXT**	**Inovative Cream**	**Diclofenac**
White Blood Cells ($\times 10^3/\mu L$)	**7.355±0.31**	**13.175±0.47*** **	**2.77±1.9 *C**	**4.425±0.49NS C**	**3.275±2.5NS C**
Monocytes ($\times 10^3/\mu L$)	**0.85±0.35**	**1.575±0.71****	**0.80±0.31*C**	**0.83±0.38NS C**	**0.88 ±0.48 NS C**
Lymphocytes ($\times 10^3/\mu L$)	**4.62±1.28**	**7.55 ±1.47****	**6.32±1.75*A**	**5.43±0.48NS B**	**6.13±1.32NS A**
Granulocytes ($\times 10^3/\mu L$)	**1.775±0.82**	**4.05±2.97****	**1.55±0.15* C**	**2.3±0.20NS C**	**2.68±1.28NS NS**
Red Blood Cells (RBCs) ($\times 10^3/\mu L$)	**7.65±0.31**	**6.85±0.79 NS**	**6.57±0.87NS NS**	**7.52±0.55 NS NS**	**7.73±0.40NS NS**
Platelets ($\times 10^3/\mu L$)	**974±107**	**917±165 ***	**695±301*A**	**927±198 NS NS**	**929±97 NS NS**

Hemoglobin g/dL	**15.125±0.44**	**13±1.23** NS NS	**14.58±0.99**NS NS	**14.63±0.61**NS NS	**13.6 ±1.9** NS NS

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Values represent means ± SD.

ns: non-significant.

p < 0.05 * significant.

p < 0.01 ** very significant.

p < 0.001 *** highly significant

Ap < 0.05 * significant difference Comparison with the Positive Control

Bp < 0.01 ** very significant difference Comparison with the Positive Control

Cp < 0.001 *** highly significant difference Comparison with the Positive Control

- **WhiteBloodCells (WBC) :**

The complete blood count (CBC) results revealed a highly significant increase in white blood cell count (P < 0.001) in the positive control group injected with formalin, reaching 13.175±0.47×10^3/µL, compared with the healthy control group, which recorded 7.355±0.31×10^3/µL. This elevation may be attributed to the severe inflammatory response induced by formalin administration. On the other hand, the group treated with the aqueous extract of *Artemisia herba-alba* showed a significant decrease (P < 0.05) in white blood cell count, with a reduction of approximately 2.77±1.9×10^3/µL compared with the positive control group. In addition, the groups treated with the Inovative Cream and Diclofenac also exhibited a noticeable decrease in WBC count, estimated at 4.425±0.49×10^3/µL and 3.275±2.5×10^3/µL respectively; however, these reductions were not statistically significant.

- **Monocytes :**

According to the (**Figure19**), the positive control group showed a highly significant increase (P<0.01) in monocyte count, reaching 1.575±0.71×10^3/µL compared with the healthy control group, which recorded 0.85±0.35×10^3/µL. This increase may be attributed to the inflammatory response induced by formalin injection. Moreover, the group treated with the aqueous extract of *Artemisia herba-alba* showed a

statistically significant decrease ($P < 0.05$) in monocyte count, with a value of $0.80\pm0.31\times10^3/\mu L$ compared with the healthy controlgroup. In contrast, the group treated with the Inovative Cream exhibited a non-significant decrease, with a value of $0.83\pm0.38\times10^3/\mu L$, whereas the group treated with Diclofenac showed a slight non-significant increase, reaching $0.88 \pm0.48\times10^3/\mu L$. compared with the healthy control group .

- **Lymphocytes :**

Regarding lymphocytes, the positive control group showed a highly significant increase ($P<0.01$), with a count of $7.55\pm1.47\times10^3/\mu L$ compared with the healthy control group, which recorded $4.62\pm1.28\times10^3/\mu L$.A statistically significant increase ($P < 0.05$) was also observed in the group treated with the aqueous extract of *Artemisia herba-alba*, where the lymphocyte count reached$6.32\pm1.75\times10^3/\mu L$.

In addition, the groups treated with the Inovative Cream and Diclofenac showed non-significant increases, with values of $5.43\pm0.48\times10^3/\mu L$ and $6.13\pm1.32\times10^3/\mu L$ respectively.

- **Granulocytes :**

The results showed a significant increase ($P < 0.01$) in granulocyte count in the positive control group, reaching $4.05\pm2.97\times10^3/\mu L$ compared with the healthy control group, which recorded $1.775\pm0.82\times10^3/\mu L$. This indicates a clear inflammatory response induced by formalin injection. In contrast, the group treated with the aqueous extract of *Artemisia herba-alba* showed a statistically significant decrea ($P < 0.05$) compared with the healthy control group, with a value of $1.55\pm0.15\times10^3/\mu L$, However, the groups treated with Inovative Cream and Diclofenac showed non-significant increases, with values of $2.3\pm0.20\times10^3/\mu L$ and $2.68\pm1.28\times10^3/\mu L$ respectively, compared with the healthy control group.

- **RedBloodCells(RBCs) :**

According to the graphical analysis, the positive control group showed a non-significant decrease in red blood cell count, reaching $6.85\pm0.79\times10^3/\mu L$ compared with the healthy control group, which recorded $7.65\pm0.31\times10^3/\mu L$. This suggests that formalin-induced inflammation had a limited effect on erythrocyte levels.

Similarly, the groups treated with the aqueous extract of *Artemisia herba-alba* and Inovative Cream showed non-significant decreases in RBC counts, with values of $6.57\pm0.87\times10^3/\mu L$ and

7.52±0.55×10^3/µL respectively, compared with the healthy control group . In contrast, the group treated with Diclofenac showed a non-significant increase in red blood cell count, reaching 7.73±0.40×10^3/µL compared with the healthy control group.

- **Platelets :**

The results showed a statistically significant decrease ($P < 0.05$) in platelet count in the positive control group, reaching 917±165×10^3/µL compared with the healthy control group, which recorded 974±107×10^3/µL. This indicates a slight effect of formalin-induced inflammation on platelet levels. Similarly, the group treated with the aqueous extract of *Artemisia herba-alba* showed a significant decrease ($P < 0.05$), with platelet values around 695±301×10^3/µL, suggesting a noticeable effect of the extract on this hematologicalparameter. In contrast, the groups treated with Inovative Cream and Diclofenac showed non-significant decreases, with values of 927±198×10^3/µL and 929±97×10^3/µL respectively, compared with the healthy control group.

- **Hemoglobin :**

The results showed a non-significant decrease in hemoglobin levels in the positive control group, reaching 13±1.23g/dL compared with the healthy control group, which recorded 15.125±0.44g/dL. This suggests a slight effect of formalin-induced inflammation on this parameter. Similarly, the groups treated with Inovative Cream, Diclofenac, and the aqueous extract of *Artemisia herba-alba* showed non-significant decreases in hemoglobin levels, with mean values of 14.58±0.99g/dL, 14.63±0.61g/dL, and 13.6 ±1.9g/dL respectively, compared with the healthy control group.

The obtained results demonstrated that formalin injection induced a marked inflammatory response characterized by a significant increase in total white blood cells (WBCs), lymphocytes, monocytes, and granulocytes compared to the healthy control group. This finding reflects activation of the immune system due to the irritant effect of formalin. The formalin test is widely recognized for its ability to stimulate inflammatory responses through the release of inflammatory mediators and pro-inflammatory cytokines, leading to recruitment and activation of immune cells at the site of injury **(Tjolsen and et al.,, 1992).**

The observed elevation in white blood cells also indicates activation of the innate immune response, as these cells play an essential role in defense mechanisms through the production of reactive oxygen species and inflammatory cytokines such as TNF-α and IL-6, which are closely associated with oxidative stress during inflammation **(Bellantiand et al., 2025).**

In contrast, the group treated with *Artemisia herba-alba* cream showed a relative reduction in inflammatory markers and values closer to the normal group compared with the positive control group, suggesting an anti-inflammatory activity of the cream. This effect may be attributed to the presence of several bioactive compounds in *Artemisia herba-alba,* including flavonoids, phenolic compounds, and terpenoids, which are known for their anti-inflammatory and antioxidant properties **(Hoang, N et al., 2012).**

Flavonoids are among the most important plant-derived compounds involved in the inhibition of inflammatory pathways and oxidative stress. They contribute to reducing free radical formation and suppressing inflammatory signaling pathways within cells, which may explain the decrease in immune inflammatory markers observed in the treated groups **(Bellantiand et al., 2025).**

The therapeutic efficacy of the cream may also be explained by the presence of the essential oil of *Artemisia herba-alba*, which is rich in volatile bioactive compounds such as monoterpenes and sesquiterpenes known for their anti-inflammatory and antioxidant activities. Previous studies demonstrated that essential oils are capable of inhibiting inflammatory mediators and reducing oxidative damage associated with inflammation **(Long, Zand et al., 2008).**

Furthermore, the lipophilic nature of the essential oil enhances skin penetration and improves the permeability of active compounds into the inflammatory site, thereby increasing the effectiveness of the cream compared to the crude aqueous extract. Incorporation of the oil into a cream base may also improve the bioavailability and chemical stability of active constituents while ensuring gradual release at the site of application **(Kathuria, H et al.,2025)** Although the aqueous extract exhibited anti-inflammatory activity, its effectiveness appeared lower than that of the cream, possibly due to reduced absorption or limited availability of certain active compounds at the site of inflammation.

On the other hand, Diclofenac showed an anti-inflammatory effect relatively close to that of the cream. This effect is mainly attributed to its well-known mechanism involving inhibition of cyclooxygenase (COX) enzymes and reduction of prostaglandin synthesis responsible for pain

and inflammation **(Bang, U,2024).** The similarity between the effects of the cream and diclofenac suggests that *Artemisia herba-alba* cream may represent a promising natural anti-inflammatory formulation.

Regarding red blood cells (RBCs) and hemoglobin levels, no significant changes were observed, indicating that the formalin-induced inflammation remained localized and did not significantly affect erythropoiesis or general hematological balance. Similarly, platelet stability suggests the absence of obvious toxic effects of the tested compounds on coagulation mechanisms and blood homeostasis **(Kumar et al., 2018).**

The hematological analysis results showed that formalin injection induced a clear inflammatory response characterized by a significant increase in white blood cells (WBCs), lymphocytes, monocytes, and granulocytes compared to the healthy control group. This reflects the activation of the immune system as a consequence of the inflammatory effect of formalin. This experimental model is known for its ability to stimulate the release of inflammatory mediators and pro-inflammatory cytokines, leading to the recruitment of immune cells to the site of injury **(Tjolsen et al., 1992)**. The observed elevation in immune cell counts indicates a strong activation of the innate immune response, as white blood cells play an essential role in inflammation defense through the production of reactive oxygen species and inflammatory cytokines such as TNF-α and IL-6, which are closely associated with oxidative stress and inflammatory progression (**Lavrovsky, 2000**). In contrast, the treated groups showed a reduction in inflammatory markers compared to the formalin-injected group, indicating the anti-inflammatory potential of the treatments. This reduction was more pronounced in the group treated with the white Inovative Cream, whose values were closer to those of the healthy control group compared to the aqueous extract-treated group. These findings suggest a better efficacy of the cream in restoring immune balance and reducing inflammation severity. Moreover, the diclofenac-treated group exhibited results comparable to those of the cream-treated group, as it also reduced inflammatory parameters relative to the formalin group, reflecting its well-known anti-inflammatory activity.

The effectiveness of the white Inovative Cream may be attributed to its richness in biologically active compounds such as flavonoids, phenolic compounds, and terpenes, which are recognized for their antioxidant and anti-inflammatory properties. Previous studies have demonstrated that these compounds can inhibit inflammatory signaling pathways and decrease the production of

cytokines and inflammatory mediators, in addition to their ability to neutralize free radicals associated with oxidative stress **(Rahman & MacNee, 1998; Barnes et al., 2005)**.

The anti-inflammatory activity of the cream may also be related to the presence of the essential oil of *Artemisia herba-alba*, which is rich in volatile bioactive compounds such as monoterpenes and sesquiterpenes known for their anti-inflammatory and antioxidant effects **(Abad et al., 2012)**. Furthermore, the lipophilic nature of the essential oil may enhance skin penetration and improve the permeability of active compounds to the inflamed area, thereby increasing the therapeutic effectiveness of the cream compared to the crude aqueous extract **(Bakkali et al., 2008).**The relatively superior efficacy of the cream compared to the aqueous extract may also be explained by the pharmaceutical formulation itself, since the cream base enhances the bioavailability of active compounds and allows their gradual and sustained release at the skin level, thereby improving topical therapeutic activity **(Barry, 2001).** Although the aqueous extract exhibited anti-inflammatory activity, its effectiveness appeared lower than that of the cream, which may be related to limited absorption or reduced bioavailability of some active constituents at the inflammation site. On the other hand, the anti-inflammatory effect of Diclofenac is mainly attributed to its ability to inhibit cyclooxygenase (COX) enzymes and reduce prostaglandin synthesis, which explains the similarity observed between diclofenac and the white Inovative Cream **(Vane & Botting, 1998)**. Regarding red blood cells (RBCs) and hemoglobin levels, no significant variations were observed among the different groups, suggesting that the formalin-induced inflammation remained localized and did not markedly affect erythropoiesis or general hematological balance. Likewise, the stability of platelet counts indicates the absence of obvious toxic effects of the tested treatments on coagulation functions and blood homeostasis**(Kumar et al., 2018).** Overall, the hematological findings demonstrate that the white Artemisia herba-alba cream possesses a more effective anti-inflammatory activity than the aqueous extract, with therapeutic effects relatively comparable to the reference drug Diclofenac, supporting its potential development as a promising natural topical antiinflammatory formulation.

10. Evaluation of Tissue Oxidative Stress Markers :

10.1 Study of Lipid Peroxidation Malondialdehyde (MDA) Assay :

The results of the lipid peroxidation assay in liver tissue are summarized in Table 13.

Table 14 : Tissue Malondialdehyde (MDA) Concentrations in Control and Treated Groups.

		MEAN ±SD				
Parameters		**healthy control**	**Positive Control**	**Diclofenac**	**Innovative Cream**	**AQ EXT**
MDA (µmol/L)	**Liver**	**3.9±0.25**	**5.13±0.26*** **	**4.33±0.25* B**	**4.00±0.14 NS C**	**4.89±0.09** A**

- Values are expressed as Mean ± SD (n = 4 to 6).
- p < 0.001***: Significant difference compared to the Healthy Control group.
- p < 0.01**: Highly significant difference compared to the Healthy Control group.
- p < 0.05*: Very highly significant difference compared to the Healthy Control group.
- NS: Non-significant difference compared to the Healthy Control group.
- Ap < 0.05 * significant difference Comparison with the Positive Control
- Bp < 0.01 ** very significant difference Comparison with the Positive Control
- Cp < 0.001 *** highly significant difference Comparison with the Positive Control

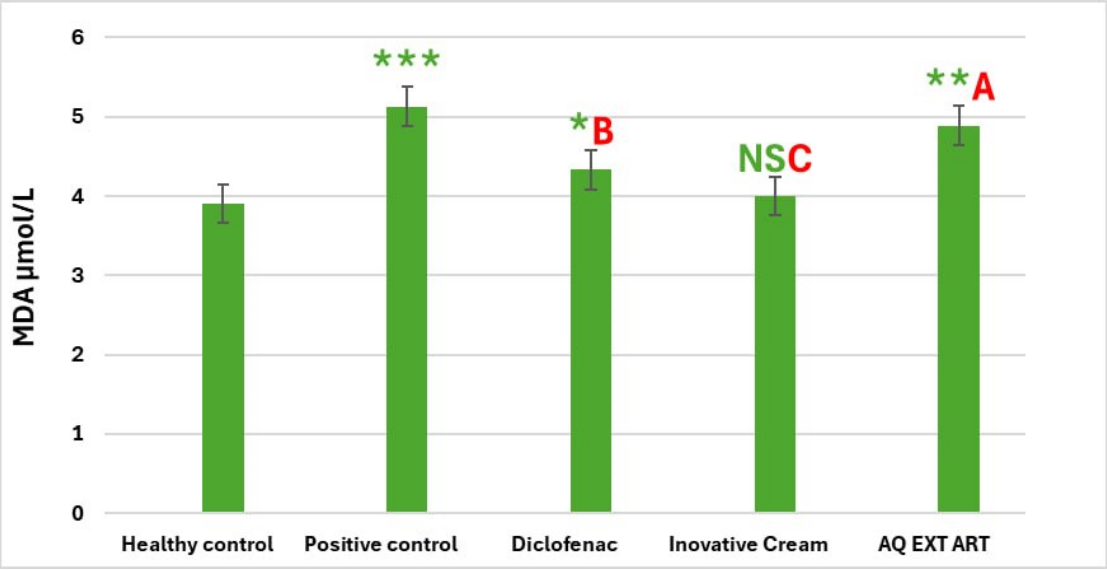

Figure 18 :Tissue concentrations of Malondialdehyde (MDA) in the control group and treated groups.

- Values are expressed as Mean ± SD (n = 4 to 6).
- $p < 0.001$***: Significant difference compared to the Healthy Control group.
- $p < 0.01$**: Highly significant difference compared to the Healthy Control group.
- $p < 0.05$*: Very highly significant difference compared to the Healthy Control group.
- NS: Non-significant difference compared to the Healthy Control group.
- Ap < 0.05 * significant difference Comparison with the Positive Control
- Bp < 0.01 ** very significant difference Comparison with the Positive Control
- Cp < 0.001 *** highly significant difference Comparison with the Positive Control

The results show a highly significant increase in MDA concentrations in the liver of the negative control group (Formalin) compared to the healthy control group. In contrast, the results obtained after treatment with the Innovative Cream show a highly significant decrease in MDA concentrations compared to the negative control group, reaching levels with no significant difference (NS) relative to the healthy control. On the other hand, the Diclofenac $p < 0.05$ for the Aqueous Extract $p < 0.01$ groups showed a reduction in MDA levels, but they maintained a significant difference compared to the healthy control .

Oxidative stress results from an imbalance between the production of reactive oxygen species (ROS) and their elimination by antioxidant defenses, contributing to the initiation and progression of several diseases. In fact, the inflammatory agent formalin is associated with the production of free radicals. The reaction of these free radicals with membrane lipids leads to the formation of degradation products, including malondialdehyde (MDA) **(Singh et al., 2010).**

In the present study, we recorded an increase in hepatic MDA levels in the negative control group compared to the healthy control group after exposure to formalin. MDA has been widely used for many years as a biomarker of lipid peroxidation. It is one of the most common indicators for determining oxidative stress in clinical situations, due to its high reactivity and toxicity **(Ayala et al., 2014).**

Our results show a very highly significant decrease in MDA levels in the groups treated with the Innovative Cream and Diclofenac compared to the positive Control group. These treatments proved to be cytoprotective compounds possessing anti-lipid peroxidation potential, acting as a shield against the deleterious effects of free radicals. Indeed, previous studies have suggested that flavonoid-rich extracts, such as those from *Artemisia herba-alba*, interfere with lipid peroxidation processes and protect cell membrane integrity **(Silvia, 2008; Ayala et al., 2014**).

This ability to enhance antioxidant defenses confirms the high potential of our formulation in protecting hepatic tissues.

The ability of the Innovative Cream to restore MDA levels to normal, similar to the healthy control group, indicates a complete normalization of oxidative stress markers. This potent antioxidant effect can be attributed to the bioactive compounds present in the extract of *Artemisia herba-alba*, such as flavonoids and phenolic acids, which are known for their ability to scavenge free radicals and inhibit lipid peroxidation. These results prove that the Innovative Cream formulation effectively protects hepatic tissues from oxidative damage induced by inflammatory agents.

This laboratory-proven efficacy makes this Innovative Cream a promising product in the pharmaceutical market for treating inflammation and its associated side effects.

- **Histopathological Analyses :**

To confirm the biological safety margin and examine the localized anti-edematous efficacy of the formulated innovative cream, histopathological analyses were executed. The microscopic cross-sections of the liver and skin tissues across the different experimental rat groups are detailed in the figures 19 to 28 below

11.1 Histological Analysis of the Liver :

a) Healthy Control Group :

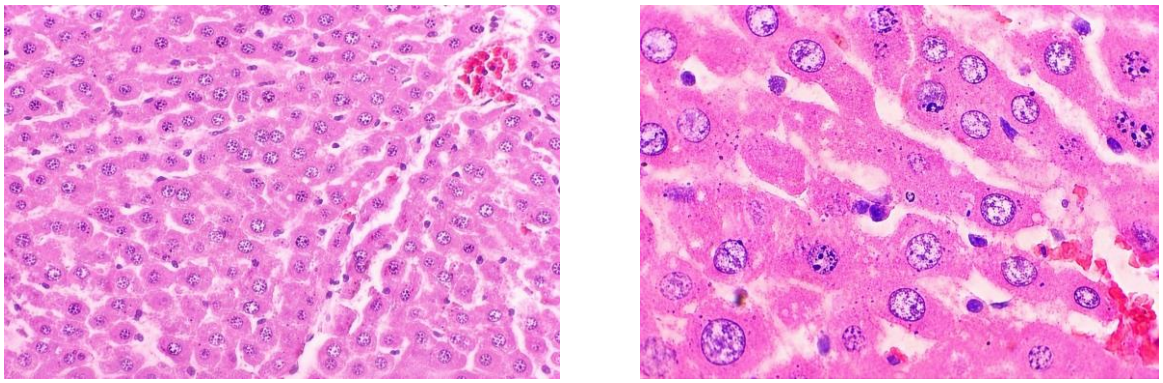

Figure 19 : Liver of the healthyControl Group(Staining: 5 µm section of Hematoxylin-Eosin, ×40 & ×100)

Microscopic examination of the liver tissue section from the normal control group shows a normal lobular central vein and cords of mono-and binucleated hepatocytes exhibiting a regular radial arrangement, separated by normal, non-dilated hepatic sinusoids .

b) Positive Control :

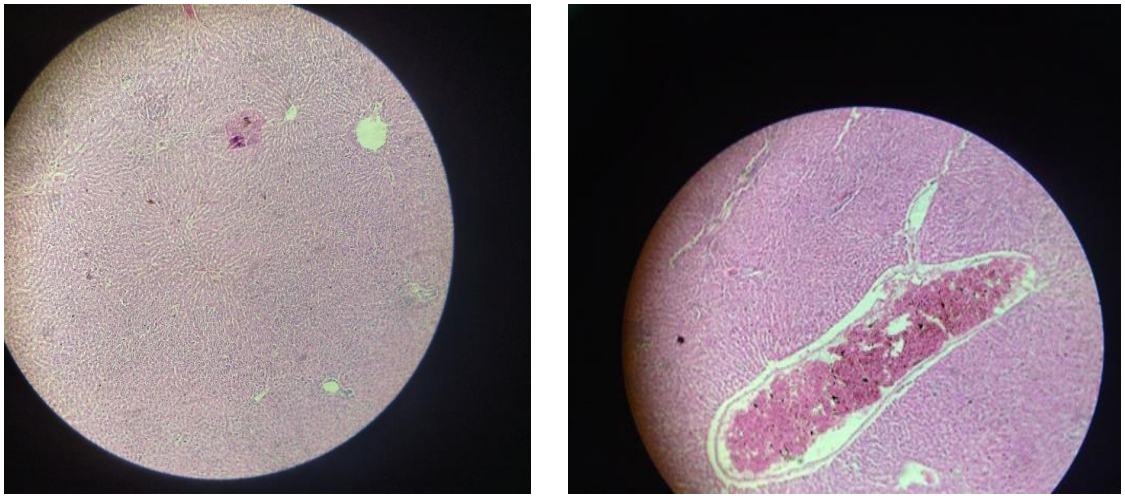

Figure 20 : Liver of the positive controlGroup(Staining: 5 µm section of Hematoxylin-Eosin, ×40 & ×100)

Liver sections in the formalin-induced group showed acute systemic toxicity, characterized by severe, massive vascular congestion. Large blood vessels appeared significantly dilated and engorged with densely packed red blood cells, causing mechanical compression on the surrounding hepatocytes and loss of the typical radial organization .

c) Aqueous Extract Group :

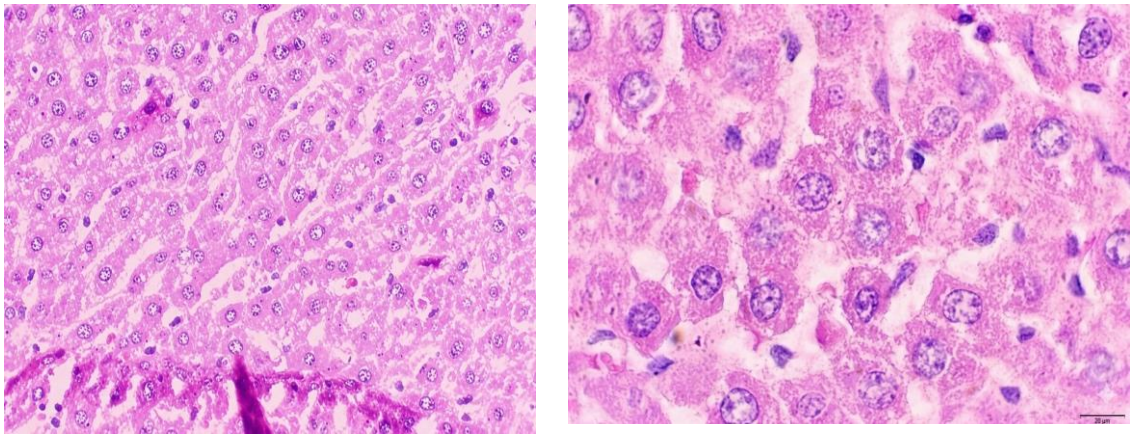

Figure 21 : Liver of the *Artemisia herba-alba*Aqueous Extract Group(Staining: 5 µm section of Hematoxylin-Eosin, ×40 & ×100)

Examination reveals satisfactory cellular protection; hepatocytes maintain their structural integrity with healthy nuclei and defined cellular boundaries, limiting severe cellular degeneration, despite a slight granular appearance in the cytoplasm and localized sinusoidal constriction .

d) The Innovative Cream Group :

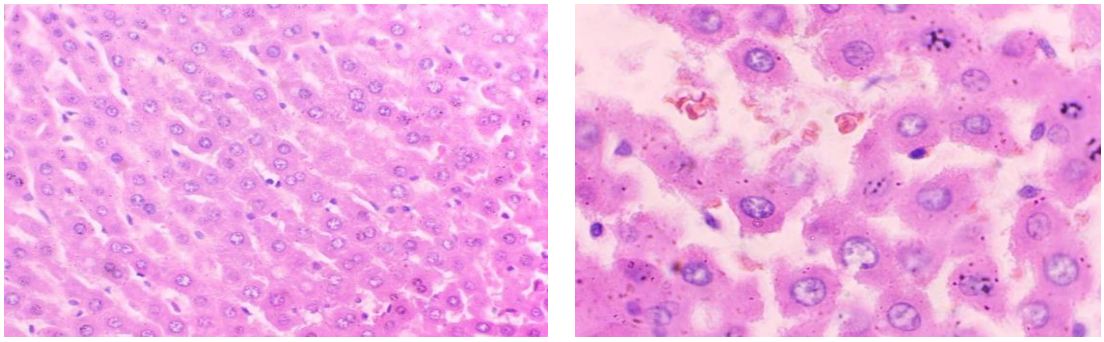

Figure 22 : Liver of the Innovative Cream Group(Staining: 5 µm section of Hematoxylin-Eosin, ×40 & ×100)

The liver sections demonstrate a remarkable and complete restoration of the normal lobular architecture.

Hepatocytic cords regain their regular radial arrangement around the central vein, and sinusoids remain clear, functional, and uncompressed, with healthy hepatocytes and consistent nuclei .

e) **The Diclofenac Group :**

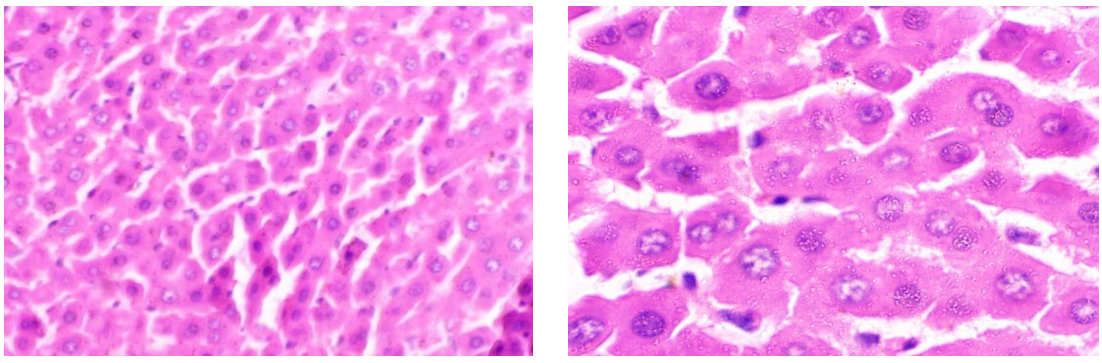

Figure 23 : Liver of the Diclofenac Group(Staining: 5 µm section of Hematoxylin-Eosin, ×40 & ×100)

The hepatic tissue showed good overall protection and a generally well-preserved lobular architecture.

Hepatocytes appeared healthy with distinct nuclei, indicating the success of Diclofenac in preventing severe damage, although slight sinusoidal constriction remained visible in localized areas .

11.2 Histological Analysis of the Skin :

a) Helthycontrol Group :

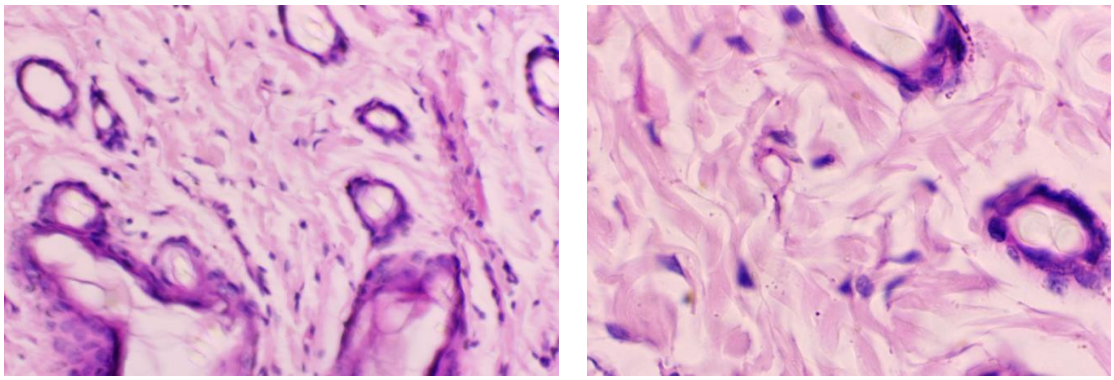

Figure 24 : Skin of the helthyControl Group(Staining: 5 µm section of Hematoxylin-Eosin, ×40 & ×100)

Microscopic examination of the dermis shows a healthy, normal architecture. Collagen fiber bundles appear dense, wavy, and regularly organized, with a distinct dermo-epidermal junction and absence of any edema or inflammatory infiltration .

b) Positive Control :

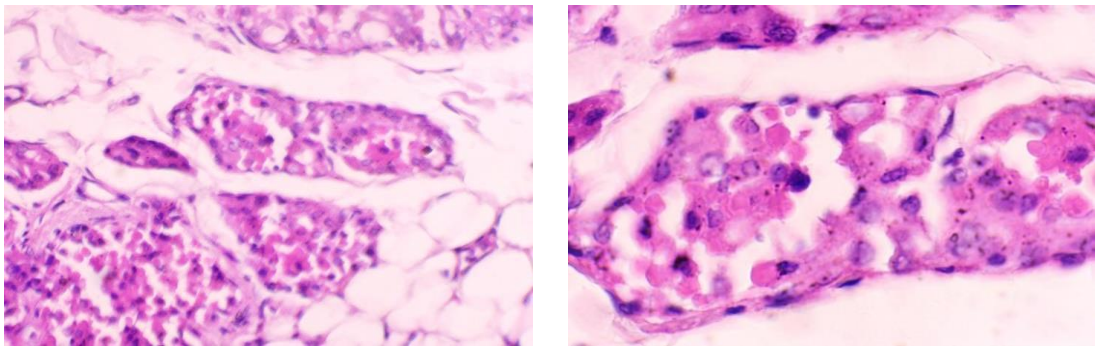

Figure 25 : Skin of the positive control(Staining: 5 µm section of Hematoxylin-Eosin, ×40 & ×100)

Local formalin injection resulted in significant histopathological changes in the dermal tissue. Microscopic observation revealed severe, persistent acute edema, characterized by extensive

separation and disorganization of the collagen fiber bundles, leaving wide empty spaces and causing significant structural breakdown .

c) Aqueous Extract Group :

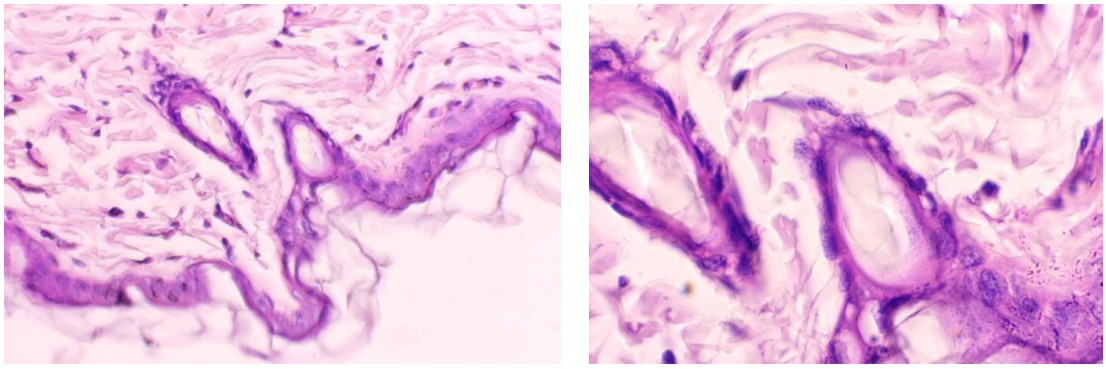

Figure 26 :Skin of the *Artemisia herba-alba*Aqueous Extract Group(Staining: 5 µm section of Hematoxylin-Eosin, ×40 & ×100)

The skin tissue shows partial recovery with a clear dermo-epidermal junction and reduced inflammation. In the dermis, collagen fibers are partially reorganized and begin to show their normal texture, with only a small number of scattered inflammatory cellsand significantly less residual edema compared to the untreated positive group .

d) The Innovative Cream Group :

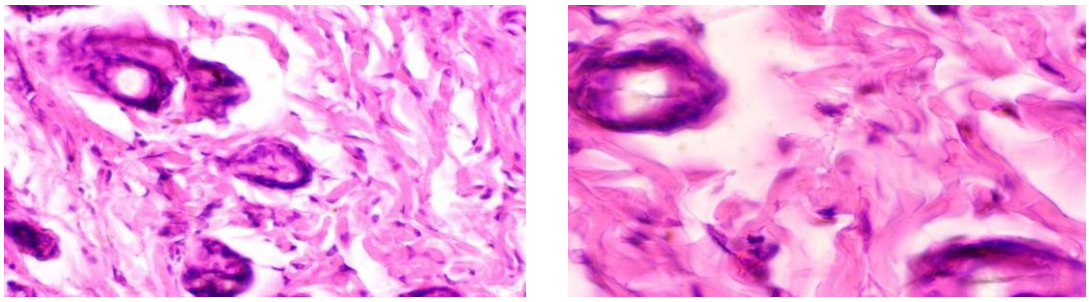

Figure 27 : Skin of the Innovative Cream Group(Staining: 5 µm section of Hematoxylin-Eosin, ×40 & ×100)

Microscopic examination of the dermis reveals excellent protection. Collagen fiber bundles show extensive remodeling and perfect organization, appearing dense, structured, and free of edema. Associated skin structures, such as hair follicles and glands, are well-preserved, accompanied by the total inhibition of acute inflammatory infiltration .

e) The Diclofenac Group :

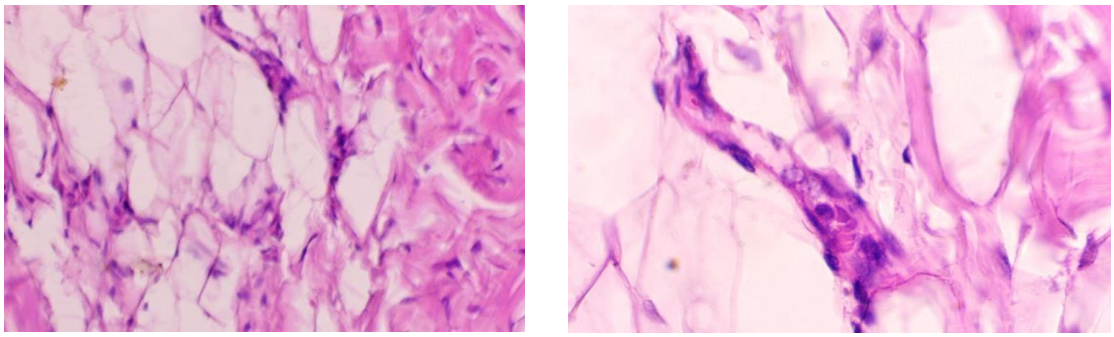

Figure 28 : skin of the Diclofenac Group(Staining: 5 µm section of Hematoxylin-Eosin, ×40 & ×100)

the dermal tissue shows a clear reduction in the acute inflammatory response. Collagen fibers are generally reorganized but still show some localized separation due to a persistent residual edema, accompanied by a scattered, mild to moderate number of inflammatory cells .

- **The Liver :**

Although formalin administration was strictly localized to the right hind paw, its systemic absorption through the bloodstream inevitably triggers secondary visceral metabolic stress and systemic toxicity, particularly in the liver—the primary organ responsible for xenobiotic detoxification **(Fiore et al., 2011).** Microscopic findings from the positive control group (Figure 22) exposed a major systemic vascular failure, manifested as intense, massive vascular congestionengorged with densely packed erythrocytes. This severe congestion confirms the occurrence of acute systemic oxidative stress and endothelial damage caused by circulating formaldehyde metabolites **(Kumari et al., 2022).**The treated groups exhibited varying degrees of hepatocyte preservation. The Diclofenac group (Figure 25) successfully maintained nuclear integrity but showed a slight compaction of the cellular cords and sinusoidal narrowing.This is a well-known side effect reflecting the additional metabolic workload and mild hepatotoxic stress that non-steroidal anti-inflammatory drugs (NSAIDs) frequently impose on the hepatic parenchyma **(Borges et al., 2015).** On the other hand, evaluating the aqueous extract of *Artemisia herba-alba* (Figure23) demonstrated satisfactory cellular protection, validating the traditional hepatoprotective reputation of this medicinal plant. The natural antioxidants and polyphenols present in the Shiah extract act as effective free-radical scavengers, thereby stabilizing the hepatocyte plasma membranes against systemic chemical insults **(Bougandoura & Marouf, 2013).**In a highly synergetic manner, the innovative creamformulation (Figure 24) achieved a complete restoration of the normal hepatic lobular architecture. The hepatocytic cords

fully regained their typical radial arrangement around the central vein, and the sinusoids remained perfectly open, clear, and uncompressed. This outstanding systemic result proves that the therapeutic action of the developed innovative cream extends far beyond a simple topical effect on the paw. By drastically reducing circulating inflammatory cytokines and maintaining a balanced systemic oxidative status, the formulation offers profound organoprotective properties **(Zaid et al., 2024).**

- **The skin :**

The sub-plantar injection offormalinin to the rat right hind paw induces a classic biphasic local inflammatory response, widely documented as a standard experimental model for evaluating potential anti-inflammatory agents **(Tjolsen et al., 1992).**

Histopathological analysis of the untreated positive control group (Figure 28) revealed severe acute dermal edema, characterized by a pronounced separation, fragmentation, and marked disorganization of the collagen fiber bundles, accompanied by an infiltration of inflammatory cells around skin appendages**.** These structural disruptions result directly from the massive release of local pro-inflammatory mediators (such as histamine, serotonin, and prostaglandins) triggered by the acute chemical irritation of formalin, which significantly increases microvascular permeability **(Damien et al., 2018).**Treatment with the reference drug, Diclofenac(Figure 30), led to a noticeable reduction in inflammatory cell infiltration. However, a localized residual edemapersisted within the dermal layers of the right paw, which can be explained by its targeted, single-pathway chemical mechanism of action focusing primarily on cyclooxygenase (COX) inhibition **(Cryer & Feldman, 1998)**. Conversely, topically applying the aqueous extract of *Artemisia herba-alba* (Figure 28) promoted a gradual structural reorganization of the dermal collagen matrix. This natural anti-inflammatory potential is closely linked to the rich phytochemical profile of the "Shiah" plant, which contains bioactive polyphenols, thuyones, and flavonoids (such as seltamin and hispidulin) known to suppress inflammatory signaling pathways and attenuate local oxidative stress **(Khaled et al., 2021)**.The most exceptional histopathological recovery was recorded in the group treated with the innovative cream formulation (Figure 29). Dermal sections of the right hind paw demonstrated optimal extracellular matrix remodeling, denseand compactcollagenbundles, andthecomplete clearanceoflocalized edema.This evident structural superiority of the cream over the raw aqueous extract strongly indicates that an optimized galenic vehicle (cream) markedly enhances the

transdermal penetration and bioavailability of the active principles of *Artemisia herba-alba* through the thickened stratum corneum of the rat paw, thereby maximizing its synergistic tissue-regenerating and anti-inflammatory efficacy **(Amorim et al., 2020).**

These histopathological observations strongly validate the biochemical and hematological findings of this study. The severe dermal edema in the right hind paw and the massive hepatic vascular congestion in the positivecontrol group directly correlatewiththeelevated Malondialdehyde(MDA)levels (indicating intense lipid peroxidation) and the alterations found in blood analysis(reflecting acute inflammatory responses).Conversely, the optimal tissue repair and reduction of edema achieved by the innovative creamgroup perfectly mirror the successful reduction of MDA levels and the normalization of hematological parameters, confirming the superior therapeutic efficacy of the developed formulation.

Preparation Procedure of the Topical Emulsion (HerbaPharm)

The innovative topical formulation was developed as an Oil-in-Water (O/W) emulsion incorporating the bioactive fractions of *Artemisia herba-alba*. To ensure intellectual property protection for the potential startup project, the formulation is described qualitatively through its components and preparation technique.

Aqueous Phase: Consists of purified water, a standard humectant (Glycerin), and the dry Artemisia herba-alba aqueous extract.

Oily Phase: Composed of a blend of cosmetic-grade emollient vegetable oils, fatty alcohol co-emulsifiers, and stabilizing surfactants.

Active & Volatile Phase: Consists of *Artemisia herba-alba* essential oil and Tocopherol (Vitamin E) as a natural antioxidant.

Method of Preparation:

Both phases were processed using the hot emulsification technique. The oily phase components and the aqueous phase components were heated separately on a water bath until complete melting and homogeneity were achieved. The aqueous phase was then added progressively to the oily phase under continuous mechanical homogenization using a laboratory stirrer.

To preserve the thermolabile volatile compounds of the Artemisia herba-alba essential oil, it was strictly incorporated along with Vitamin E during the cooling phase at a lowered temperature.

Stirring was maintained until the cream reached room temperature and developed a smooth, stable, and homogeneous texture. The final pH was adjusted to match skin physiology.

General Conclusion

Today, phytotherapy represents a true heritage for humanity in the field of public health, given its therapeutic efficacy and growing importance in both traditional and modern medicine. Our work aimed to evaluate the anti-inflammatory activity, both in vitro and in vivo, of the aqueous extract of *Artemisia herba-alba*.

In the first part of our study, the quantitative evaluation of total phenolic compounds using the Folin-Ciocalteu method revealed a significant percentage of polyphenols. Furthermore, the flavonoid content was estimated using the aluminum chloride method, where the results showed that *Artemisia herba-alba* is rich in flavonoids, which are compounds well-known for their antioxidant and anti-inflammatory properties.

In the second part of this work, the in vitro evaluation of anti-inflammatory activity, conducted through the protein denaturation inhibition assay, demonstrated that the aqueous extract of *Artemisia herba-alba* possesses potent anti-inflammatory efficacy.

We also extracted the plant's essential oil using steam distillation, followed by physical and chemical analysis. The results indicated that this medicinal plant, widely distributed in Algeria, is rich in oxygenated terpenoid compounds. The biological efficacy of *Artemisia herba-alba* essential oil is attributed to its active compounds with anti-inflammatory, antioxidant, and antimicrobial properties.

The most remarkable outcome of this research is the successful development of an innovative anti-inflammatory cream enriched with *Artemisia herba-alba* extract. This formulation demonstrated an extraordinary inhibitory effect, reaching 100% inhibition of edema in Wistar rats after 48 hours, thereby outperforming or matching conventional treatments. This highlights the potential of our product as a potent, bio-based alternative in the pharmaceutical industry, marking a significant step toward the valorization of Algerian medicinal flora in modern therapy.

On the other hand, formalin injection induced noticeable changes in certain hematological parameters in rats, while treatment with the cream formulation contributed to restoring many of these parameters to near-normal levels, indicating the preparation's therapeutic

effect. Furthermore, the results showed that the formulation helped reduce oxidative stress caused by formalin exposure by neutralizing free radicals and minimizing oxidative damage.

L'analyse histopathologique a fourni une confirmation microscopique explicite de ces résultats ; l'examen du tissu cutané de la voûte plantaire a démontré la haute capacité de la crème à réduire considérablement l'infiltration des cellules inflammatoires et à diminuer l'œdème du tissu dermique, tout en préservant totalement l'intégrité et la structure des couches épidermique et dermique. De plus, l'examen histologique du tissu hépatique a confirmé la biosécurité totale de la formulation topique, les hépatocytes ayant conservé leur architecture normale et les sinusoïdes hépatiques sans aucun signe de toxicité ou d'infiltration inflammatoire résultant du traitement

Finally, future studies and additional biological evaluations remain essential to confirm the efficacy and safety of this formulation, with the ultimate goal of developing a natural phytopharmaceutical drug for the treatment of joint pain and inflammation, in alignment with the requirements of both traditional and modern medicine.

Reference

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