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Evaluation de l'effet anti-inflammatoire de quelques nanoparticules biosynthétisées *-Etude in vivo-*

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Abstract.

While synthetic anti-inflammatory drugs are effective, their side effects necessitate the exploration of safer alternatives, such as bioactive nanoparticles. This study evaluates the in vivo anti-inflammatory potential of zinc oxide nanoparticles (ZnO NPs) biosynthesized from *Myrtus communis L.* leaf aqueous extract, using a carrageenan-induced paw edema model in mice. Groups included untreated control (Inf), standard control (STD: diclofenac 50mg/kg), and both therapeutic (D01 CR: 150 mg/kg; D02 CR: 300 mg/kg) and prophylactic (D01 PR: 150 mg/kg; D02 PR: 300 mg/kg) ZnO NP doses. Edema inhibition was assessed via percentage increase (%AUG) and inhibition (%INH), complemented by FTIR analysis and histological examination of paw tissues. Therapeutic administration revealed a worthy response with D02 CR (300 mg/kg) exhibiting significant edema reduction (%AUG and %INH) comparable to the STD group. Prophylactic treatment showed delayed but notable efficacy at the 5th hour, with the higher dose (D02 PR: 300 mg/kg) also yielding good results. Histological analysis confirmed these findings, demonstrating reduced paw edema and leukocyte infiltration, particularly at the higher therapeutic dose (D02 CR). FTIR analysis confirmed ZnO NP formation and identified characteristic functional groups associated with bioactive phytoconstituents from the extract, likely responsible for the NPs' anti-inflammatory activity.These results highlight the promising anti-inflammatory properties of *Myrtus communis L.*-synthesized ZnO NPs, with therapeutic doses showing efficacy akin to conventional drugs. The prophylactic effect, though slower, suggests potential for preventive applications. Further pharmacological investigations are warranted to elucidate underlying mechanisms.

Key words: Nanoparticles, *Myrtus communis L.*, Zinc Oxide, Biosynthesis, FTIR, Inflammation, In *vivo.*

Résumé

Bien que les médicaments anti-inflammatoires synthétiques soient efficaces, leurs effets secondaires nécessitent l'exploration d'alternatives plus sûres, telles que les nanoparticules bioactives. Cette étude évalue le potentiel anti-inflammatoire in vivo des nanoparticules d'oxyde de zinc (ZnO NPs) biosynthétisées à partir d'extrait aqueux de feuilles de *Myrtus communis L.*, en utilisant un modèle d'œdème de la patte induit par la carraghénine chez la souris. Les groupes comprenaient un contrôle non traité (Inf), un contrôle standard (STD : diclofénac 50 mg/kg), ainsi que des doses thérapeutiques (D01 CR : 150 mg/kg ; D02 CR : 300 mg/kg) et prophylactiques (D01 PR : 150 mg/kg ; D02 PR : 300 mg/kg) de ZnO NP. L'inhibition de l'œdème a été évaluée via l'augmentation en pourcentage (%AUG) et l'inhibition (%INH), complétée par une analyse FTIR et un examen histologique des tissus de la patte. L'administration thérapeutique a révélé une réponse significative, avec D02 CR (300 mg/kg) montrant une réduction significative de l'œdème (%AUG et %INH) comparable au groupe STD. Le traitement prophylactique a montré une efficacité retardée mais notable à la cinquième heure, la dose la plus élevée (D02 PR : 300 mg/kg) donnant également de bons résultats. L'analyse histologique a confirmé ces résultats, démontrant une réduction de l'œdème de la patte et une infiltration de leucocytes, en particulier à la dose thérapeutique plus élevée (D02 CR). L'analyse FTIR a confirmé la formation de ZnO NP et identifié des groupes fonctionnels caractéristiques associés aux phytoconstituants bioactifs de l'extrait, probablement responsables de l'activité anti-inflammatoire des NPs. Ces résultats soulignent les propriétés anti-inflammatoires prometteuses des ZnO NPs synthétisées à partir de *Myrtus communis L.*, les doses thérapeutiques montrant une efficacité comparable à celle des médicaments conventionnels. L'effet prophylactique, bien que plus lent, suggère un potentiel pour des applications préventives. D'autres investigations pharmacologiques sont nécessaires pour élucider les mécanismes sous-jacents

Mots clés : Nanoparticules, *Myrtus communis L.*, Oxyde de Zinc, Biosynthèse, FTIR, Inflammation, In *vivo.*

الملخص

بينما تكون الأدوية المضادة للالتهابات الاصطناعية فعالة، فإن آثارها الجانبية تستدعي استكشاف بدائل أكثر أمانًا، مثل تقيم هذه الدراسة الإمكانات المضادة للالتهابات في الجسم الحي لجسيمات أكسيد الزنك. الجسيمات النانوية النشطة حيويًا المائي، باستخدام نموذج *Myrtus communis L.* التي تم تخليقها حيويًا من مستخلص أوراق (ZnO NPs) النانوية مجموعة تحكم ،(Inf) تضمنت المجموعات مجموعة تحكم غير معالجة. وذمة القدم المحفزة بالكار جينان في الفئران

(كغ/ملغ 300 : D02 CR كغ؛/ملغ 150 : D01 CR) وأيضًا جرعات علاجية ،(كغ/ملغ 50 ديكلوفيناك : STD) قياسية تم تقييم تثبيط الوذمة عبر زيادة النسبة .ZnO NP من) كغ/ملغ 300 : D02 PR كغ؛/ملغ 150 : D01 PR) ووقائية أظهرت الإدارة العلاجية . و ف ح ص نسيجي لأنسجة القدم FTIR مكملةً بتحليل،(INH%) والتثبيط (AUG%) المئوية مقارنة (INH% و AUG%) تقليلًا كبيرًا في الوذمة) كغ/ملغ 300) D02 CR استجابة ذات قيمة، حيث أظهرت أظهر العلاج الوقائي فعالية ملحوظة ولكن متأخرة في الساعة الخامسة، مع تحقيق الجرعة الأعلى .STD بمجموعة أكدت التحليلات النسيجية هذه النتائج، موضحةً تقليل وذمة القدم ووتمثل. ايضًا نتائج جيدة) كغ/ملغ 300 :D02 PR) Zeno NP تشكيل FTIR أكدت تحليلات .(D02 CR) الكريات البيضاء، خاصة عند الجرعة العلاجية الأعلى وحددت مجموعات وظيفية مميزة مرتبطة بالمواد النباتية النشطة حيويًا من المستخلص، والتي من المحتمل أن تكون تسلط هذه النتائج الضوء على الخصائص المضادة للالتهابات. مسؤولة عن النشاط المضاد للالتهابات للجسيمات النانوية حيث تظهر الجرعات العلاجية فعالية ،*Myrtus communis L.* التي تم تخليقها من ZnO NPs الواعدة لجسيمات هناك حاجة إإلى. يشير التأثير الوقائي، على الرغم من كونه أبطأ، إلى إمكانية تطبيقات وقائية. مماثلة للأدوية التقليدية مزيد من التحقيقات الصيدلانية لتوضيح الآليات الأساسية

الكلمات المفتاحية: التهاب، الجسيمات النانوية، أكسيد الزنك، التخليق الحيوي، *Myrtus communis.L* , FTIR,

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Abbreviations List

- **°C** : Degrees Celsius
- **AgNPs** : Silver Nanoparticles
- **AP-1 :** Activator Protein 1
- **AuNPs :** Gold Nanoparticles
- **AUG :** Percentage Increase
- **BCE :** Before Common Era
- **CE :** Common Era
- **cm^{-1} :** Centimeter to the power of negative one
- **C-inf :** Inflammation Control Group
- **COX :** Cyclooxygenase
- **CRD :** Control Group Dose
- **CRP :** C-Reactive Protein
- **DAMPs :** Damage-Associated Molecular Patterns
- **DED :** Dermal Edema
- **EGF :** Epidermal Growth Factor
- **EPI :** Epidermis
- **ESR :** Erythrocyte Sedimentation Rate
- **FGF :** Fibroblast Growth Factor
- **FTIR :** Fourier Transform Infrared
- **g :** Grams
- **GCs :** Glucocorticoids
- **GI :** Gastrointestinal
- **GR :** Glucocorticoid Receptor
- **HF :** Hair Follicle
- **HED :** Hypodermal Edema
- **HPLC :** High-Performance Liquid Chromatography
- **HYPO :** Hypodermis

- **i.g.g :** Intragastric Gavage
- **Ii :** Inflammatory Infiltrate
- **IUPAC** : International Union of Pure and Applied Chemistry
- **LEUK** : Leukocyte
- **LSPR** : Localized Surface Plasmon Resonance
- **mL** : Milliliters
- **MRI :** Magnetic Resonance Imaging
- **NaCl :** Sodium Chloride
- **NCD :** Non-Communicable Diseases
- **NF-kB :** Nuclear Factor kappa-light-chain-enhancer of activated B cells
- **nm :** Nanometer
- **NMs :** Nanomaterials
- **NMRI :** Naval Medical Research Institute
- **NPs** : Nanoparticles
- **OECD** : Organization for Economic Co-operation and Development
- **P** : Probability (statistical significance)
- **PAMPs** : Pathogen-Associated Molecular Patterns
- **PDGF** : Platelet-Derived Growth Factor
- **PET** : Positron Emission Tomography
- **PG** : Prostaglandin
- **PGE2** : Prostaglandin E2
- **RA** : Rheumatoid Arthritis
- **SD** : Standard Deviation
- **SEM** : Standard Error of the Mean
- **SG** : Sebaceous Gland
- **SIRS** : Systemic Inflammatory Response Syndrome
- **SLE :** Systemic Lupus Erythematosus
- **STD** : Standard (treatment)
- **TGF** : Transforming Growth Factor
- **TQ :** Thymoquinone

- **UHC :** Universal Health Coverage
- **UN** : United Nations
- **US :** Ultrasound
- **WHO :** World Health Organization
- **Zn^0 :** Solid Zinc atom
- **Zn^{2+} :** Zinc ion
- **ZnO :** Zinc Oxide
- **λ :** Lambda

Introduction

Introduction

Inflammation is a key defense and healing process in the body, contributing significantly to the recovery process. It can be triggered by a variety of factors, including pathogens, disruption of cells, and exposure to harmful substances. Upon detecting damage or invasive pathogens, the immune system coordinates a complex array of responses. **(Chopra et *al.*, 2024)**

While the inflammatory response is a crucial defense mechanism against pathogens, excessive or prolonged activation can lead to harmful effects. This situation necessitates targeted therapeutic interventions to modulate the inflammatory response. **(Chen et *al.*, 2018)**

Anti-inflammatory therapies currently include steroidal and non-steroidal drugs, specifically synthetic steroidal anti-inflammatory drugs (SAIDs) and non-steroidal anti-inflammatory drugs (NSAIDs). While these treatments are widely used for their effectiveness in managing pain, they can also cause undesirable long-term effects on the physiology of various organs. (**Teroare, 2018)**

In light of these concerns, researchers are increasingly exploring phytochemicals as alternative sources of anti-inflammatory agents. Since antiquity, people have relied on plants as natural remedies for a wide range of illnesses, making herbal medicine one of the most traditional practices recognized across all civilizations. **(Abidullah et *al.*, 2022)**

This growing interest in natural remedies coincides with advancements in nanotechnology, which is emerging as a versatile field of research with numerous applications. Various toxic physicochemical techniques, including gas-phase methods, spray pyrolysis, electrochemical methods, chemical vapor deposition, and laser ablation techniques, have been developed to synthesize nanoparticles, further enhancing the potential for innovative therapeutic solutions. **(Hasan et *al.*, 2021)**

However, these techniques require meticulous process control and involve the use of toxic reagents, expensive instruments, hazardous organic solvents, and non-biodegradable stabilizing agents. As a result, these methods are not only harmful to the environment but also toxic to living organisms. **(Soliman et *al.*, 2023).**

Therefore, Researchers are increasingly favoring the biosynthesis of nanoparticles due to its eco-friendly, cost-effective, and safer nature compared to traditional physical and chemical fabrication methods. **(Rajeswari et *al.*, 2023)**

One of the types of nanoparticle green synthesis is Plant-mediated synthesis which offers a sustainable alternative to conventional techniques, which often rely on toxic chemicals and energy-intensive processes. This approach not only minimizes environmental impact but also enhances the safety profile of nanoparticle production, making it an attractive option for advancing nanotechnology. **(Edo et *al.*, 2025)**

Under this context, our study aimed to evaluate the anti-inflammatory effect of Zinc oxide nanoparticles biosynthesized from *Myrtus communis* leaf extract, using in vivo approach. Before beginning our experimental research, a bibliographical part was developed in three chapters: general information on inflammation, anti-inflammatory treatments (both conventional and alternative), and a final chapter dedicated to nanoparticles and their biomedical applications. The second part of the study focused on experimental work, which included FTIR analysis for characterization, followed by in vivo evaluation using the paw edema measurement protocol to assess anti-inflammatory activity, Statistical analyses and a histological study largely supported the results of this protocol. This second part was reinforced by a discussion and finalized with a general conclusion.

Bibliographic Part

Chapter I:
Inflammation
&
Treatment

1. Overview

Inflammation is a complex and essential defense mechanism for the body, designed to eradicate microbes, pathogens, or any irritants, thus protecting living tissues from infections and injuries **(Eds. of Encyclopaedia Britannica, 2025; Khan, 2024)**. This process plays a crucial role in the immune response, facilitating the repair of damaged tissues and allowing white blood cells to migrate to injured areas **(Barrett et *al.*, 2019; Medzhitov & Janeway, 2000).** When a tissue is injured or infected, a series of biological reactions are triggered, leading to significant changes in local blood flow, including increased vascular permeability, which facilitates the migration of fluids, plasma proteins, and leukocytes from the circulatory system to the site of injury **(Khan, 2024)**.

If the inflammatory response is short-lived, typically lasting a few days, it is referred to as acute inflammation **(Eds. of Encyclopaedia Britannica, 2025).** This form of inflammation is beneficial and represents an immediate reaction to injury or infection. However, when the inflammatory response persists, it becomes chronic inflammation, which can lead to serious health issues such as progressive physiological degradation and organ dysfunction **(Furman et *al.*, 2019; Sherwood & Toliver-Kinsky, 2004).** Chronic inflammation may arise from persistent infections, autoimmune disorders, or prolonged exposure to irritants and has been linked to various diseases, including cardiovascular conditions. **(Garg & Haller, 2018; Friedman, 2019).**

The inflammatory process is characterized by five cardinal signs: (i) rubor (redness), (ii) tumor (swelling), (iii) calor (heat), (iv) dolor (pain), and (v) functio laesa (loss of function) **(Eds. of Encyclopaedia Britannica, 2025).** These signs reflect underlying pathophysiological mechanisms and are crucial for diagnosing inflammatory conditions **(Chandrasoma & Taylor, 1998).** Although inflammation is a necessary protective response, inadequate regulation can lead to tissue damage and chronic diseases.

Understanding the intricacies of inflammation, including the danger model, helps clarify how the body differentiates between harmful and harmless stimuli (**Matzinger, 2002).**

2. The Historical and Scientific Evolution of Inflammation:

Inflammation, derived-from the Latin inflamer, has its roots in antiquity, where

Hippocrates linked imbalances in bodily fluids to this condition. Celsus formalized the four classic signs of inflammation, while Galen advanced the humoral theory, introducing treatments like bloodletting and the concept of "laudable pus." In the 18th century, Albrecht Von Haller challenged this theory by demonstrating that putrid fluids could induce inflammation, an idea further reinforced by François Magendie. The 19th century saw the emergence of Rudolf Virchow's cellular theory, which emphasized cellular changes as the origin of diseases, while Claude Bernard described inflammation as a defense mechanism for maintaining homeostasis. Discoveries in immunology in the 20th century, particularly by Elie Metchnikoff on phagocytosis, revealed inflammation's protective role. Key inflammatory mediators like histamine and prostaglandins were identified, and concepts such as Pathogen-Associated Molecular Patterns (PAMPs) and the Danger Theory deepened our understanding of immune activation and inflammation in various diseases **(Scott et *al*., 2004; Chen et *al*., 2018; Martins et *al*., 2023; Medzhitov, 2010; Gordon, 2016).**

3. Epidemiological Studies of Inflammation:

Chronic inflammation is increasingly recognized as a key driver of major global diseases. Although comprehensive epidemiological studies on mortality and pathologies specifically linked to inflammatory conditions remain limited, recent research continues to emphasize the importance of this area **(WHO, 2021)**. Sustained inflammation contributes to highly prevalent chronic diseases, including cancer through tumor-promoting pathways **(Mantovani et *al*., 2020)**, arthritis via joint tissue destruction, obesity through metabolic dysfunction **(Hotamisligil, 2017)**, and even behavioral disorders via neuroinflammation mechanisms **(Miller & Raison, 2016).** Furthermore, research indicates that anti-inflammatory diets may reduce neuroinflammation, offering a potential nutritional strategy for managing Alzheimer's disease **(Heneka et *al*., 2025; Scheltens et *al*., 2021).**

Beyond the previously mentioned conditions, diabetes, osteoarthritis, and myocardial infarction are also recognized as inflammation-related disorders **(Donath, 2014; Berenbaum, 2013; Libby, 2021).** The World Health Organization **(WHO)** classifies these

diseases as non-communicable diseases (NCDs), which have accounted for a significant proportion of global deaths **(WHO, 2011).** Systemic inflammation is highly prevalent among patients with atherosclerotic cardiovascular disease, particularly those with chronic kidney disease **(Giancarlo Pesce et *al.*, 2025)**, highlighting the need for strategies targeting inflammation reduction for secondary prevention.

4. Definition:

Inflammation represents the body's sophisticated defense mechanism against infection and tissue damage, with leukocytes serving as key cellular players in combating pathogens. This biological response is crucial for wound healing and tissue repair. The classic manifestations of inflammation—pain (dolor), heat (calor), redness (rubor), swelling (tumor), and impaired function (functio laesa)—reflect the body's concerted effort to address injury or infection **(Britannica, 2025).**

At the cellular level, neutrophils and macrophages orchestrate the inflammatory response by engulfing and destroying harmful organisms or clearing damaged tissue **(Halpern, 2023).** While inflammation is typically protective, it can become harmful when prolonged or dysregulated. For instance, excessive or persistent inflammation contributes to conditions like atherosclerosis and may progress to chronic inflammation, characterized by granuloma formation, tissue destruction, and eventual organ dysfunction **(Carr, 2023).**

Various inflammatory mediators interact with innate immune pathways to coordinate these responses to harmful stimuli.

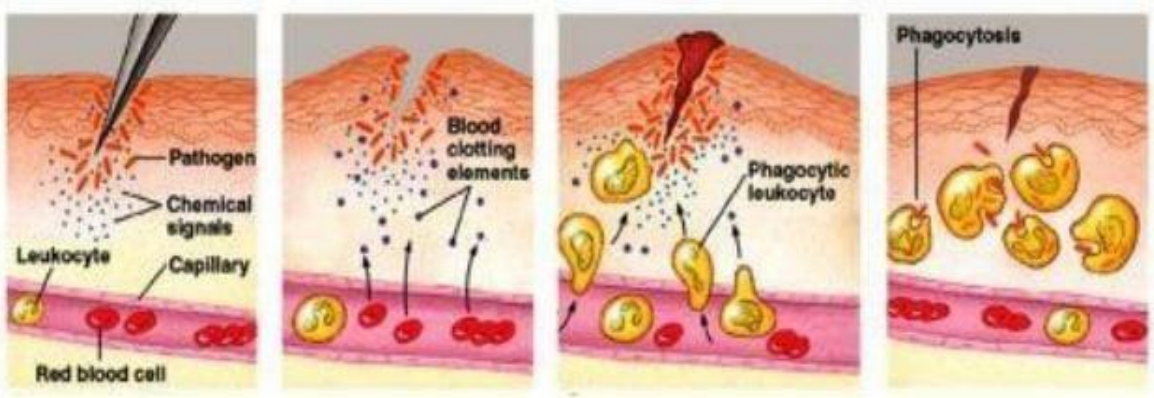

Figure 01: Inflammatory response**. (Karki, 2018)**

5. Inflammatory mechanism:

The inflammatory mechanism manifests locally through several successive stages: the vascular or vascular-exudative phase, the cellular phase, and the repair and healing phase. Concurrently, a systemic reaction occurs. **(Kumar et *al*., 2020; Medzhitov, 2008)**

5.1. Local Level:

The local inflammatory response unfolds through three sequential phases:

5.1.1. Vascular Phase:

This phase involves three phenomena: active congestion, inflammatory edema or exudate, and leukocyte diapedesis. **(Abbas et *al*., 2021; Murphy et *al*., 2022).**

5.1.1.1. Active Congestion in Response to Tissue Injury:

Following tissue injury, active congestion appears very rapidly, corresponding to a change in vascular caliber after a brief vasoconstriction. It consists of arteriolar and then capillary vasodilation in the affected area **(Serhan & Chiang, 2015).** Under the influence of chemical mediators such as histamine and serotonin, as well as neural mechanisms involving vasomotor nerves, there is a local increase in blood supply and a slowing of blood flow. The small vessels become dilated and filled with red blood cells, lined by a swollen endothelium **(Sweeney et *al*., 2022).**

Congestion is triggered by a neural mechanism mediated by vasomotor nerves and the action of chemical mediators. **(Kumar et *al*., 2020; Serhan et *al*., 2010; Medzhitov, 2008)**

5.1.1.2. Inflammatory edema:

Inflammatory edema corresponds to the passage of a fluid called exudate—derived from plasma and composed of water and plasma proteins—into the interstitial connective tissue or serous cavities. Its clinical manifestation is edema, which, by compressing nerve endings, is responsible for pain, also induced by certain chemical mediators such as prostaglandins. Microscopically, the connective tissue appears pale and distended. Inflammatory edema results from increased hydrostatic pressure due to vasodilation and, most importantly, from increased permeability of the walls of small vessels under the

influence of chemical mediators, including histamine. **(Kumar et *al*., 2020; Chen et *al*., 2018; Murphy et *al*., 2022)**

The pathogenesis of inflammatory edema involves two key mechanisms:

1. Elevated hydrostatic pressure resulting from vasodilation
2. Enhanced vascular permeability mediated by chemical factors, particularly histamine **(Kumar et *al*.,2020 ; Chen et al.,2018 ; Murphy et *al*., 2022)**

5.1.1.3. Leukocyte Diapedesis:

Leukocyte diapedesis describes the active migration of white blood cells from blood vessels into injured tissues. This process begins with polymorphonuclear cells (6-24 hours postinjury), followed by monocytes and lymphocytes (24-48 hours). The cells actively cross vascular walls to reach the site of damage **(Abbas et *al*., 2021; Nourshargh and Alon, 2014; Ley et *al*., 2007).**

5.1.2. Cellular Phase:

The cellular phase is characterized by the formation of an inflammatory granuloma, composed of cells derived from both the blood and connective tissue. **(Kumar et *al*., 2020; Robbins & Cotran, 2020).**

5.1.2.1. Cell Migration and Differentiation

The blood-derived cells include polymorphonuclear cells, monocytes, and lymphocytes. Following diapedesis, these cells exit the perivascular area and migrate toward the site of injury through chemotaxis. This movement is directed by chemotactic agents produced by damaged tissues, bacteria, and resident leukocytes in the inflammatory focus. Key chemotactic factors such as leukotriene B4, interleukin-8, and complement component C5a bind to specific membrane receptors on leukocytes, triggering their activation and subsequent mobilization. **(Abbas et *al*., 2021; Nourshargh and Alon, 2014; Ley et *al*., 2007)**

The connective tissue contributes fibroblasts, endothelial cells, mast cells, and resident macrophages. At the local level, these cells undergo proliferation (particularly fibroblasts, lymphocytes, and endothelial cells, with macrophages proliferating to a lesser extent) and various transformations to form the inflammatory granuloma. The following cellular

phenomena are observed .**(Kumar et *al*., 2020; Wynn and Vannella, 2016)**

a. **Neutrophil Accumulation:** Neutrophils, which have a relatively short lifespan of 3-4 days, accumulate at the site and release their enzymatic contents into the inflammatory focus. Their continuous supply during early inflammation is maintained through increased hematopoietic production in the bone marrow **(Nathan, 2006; Kolaczkowska and Kubes, 2013).**

b. **Monocyte Transformation:** Circulating monocytes differentiate into activated macrophages that acquire multiple functional capabilities including phagocytosis, secretion of various inflammatory mediators, and cooperation with lymphocytes for immune response development through antigen presentation. These cells exhibit significantly longer survival compared to polymorphonuclear cells **(Murray & Wynn, 2011; Wynn and Vannella, 2016).**

c. **B Lymphocyte Differentiation:** B lymphocytes undergo transformation into plasma cells, which are specialized for immunoglobulin production and secretion **(Murphy et *al*., 2022; Crotty, 2019).**

d. **T Lymphocyte Activation:** T lymphocytes become activated, gaining the ability to secrete numerous mediators, develop cytotoxic properties, and coordinate with B lymphocytes for immune response regulation **(Abbas et *al*., 2021; Zhu et *al*., 2010).**

e. **Fibroblast Modification:** Resident fibroblasts undergo phenotypic changes into myofibroblasts, acquiring contractile properties and enhancing their capacity for extracellular matrix component synthesis **(Hinz et *al*., 2012; Gabbiani,2003).**

5.1.2.2. Formation of the Inflammatory Granuloma:

The cellular composition of the inflammatory granuloma evolves dynamically over time. During the acute phase of inflammation **(Kumar et *al*., 2020)**, polymorphonuclear cells predominate. However, within days to weeks, the granuloma undergoes a cellular shift, with mononuclear inflammatory cells **(Ramakrishnan et *al*.,2013)**, including macrophages, lymphocytes, and plasma cells **(Eming et *al*., 2014)**. becoming more numerous than polymorphonuclear cells. Progressive changes occur under the influence of growth factors, leading to the incorporation of fibroblasts and endothelial cells that drive

neovascularization, ultimately forming granulation tissue. **(Kumar et *al*., 2020; Ramakrishnan et *al*., 2013).**

The specific cellular makeup of the granulation tissue further varies according to the underlying cause of inflammation, with certain cell types potentially dominating the inflammatory infiltrate depending on the etiology. **(Kumar et *al*., 2020; Eming et *al*., 2014).**

5.1.2.3. Phagocytosis:

The phagocytosis of a microorganism occurs in three stages:

A. Adhesion: This is the contact between the phagocytic cell and the microorganism. It is facilitated by the opsonization of the microorganism, which involves the binding of antibodies to the microorganism. This opsonization subsequently allows the action of complement, leading to better recognition and elimination of the microorganism by the phagocytic cell. **(Flannagan et *al*., 2012; Underhill & Goodridge, 2012)**.

B. Endocytosis: This is the engulfment of the microorganism by the phagocytic cell, using a "zipper" mechanism to form the phagosome **(Masters et *al*., 2013).**

C. Phagosome: This corresponds to the complete engulfment of the microorganism, forming a vacuole. This vacuole fuses with lysosomes in the cytoplasm, which release their lytic enzymes to digest the invading microorganism **(Levin et *al*., 2016).**

The inflammatory reaction is a component of non-specific immunity. It is a response manifested by the four signs of inflammation: redness, heat, pain, and swelling. Heat and redness are due to capillary vasodilation and the slowing of blood circulation. Pain is caused by the compression of nerve fibers by edema, which itself results from plasma exudation due to blood flow congestion. **(Kumar et *al*., 2020; Medzhitov, 2008).**

5.1.3. Repair and Healing:

Tissue repair occurs following complete debridement of damaged tissue and typically results in scar formation. However, certain tissues, such as neurons and myocardial muscle cells, lack regenerative capacity. Similarly, extensive or prolonged tissue destruction (e.g., in severe burns) may also preclude regeneration, leading instead to fibrous scar formation **(Gurtner et *al*., 2008; Eming et *al*., 2014).**

The repair process is orchestrated by numerous growths factors and involves complex interactions between cells and the extracellular matrix to regulate cellular proliferation and biosynthetic activity. Adhesion molecules play a critical role in transmitting activation signals to cells, while specific growth factors can modulate the expression of these adhesion molecules **(Darby & Hewitson, 2016; Hinz, 2016).**

a.Scar Formation:

The scar represents the permanent fibrous remnant of the inflammatory process following the granulation tissue phase. Composed primarily of dense collagenous connective tissue, it replaces areas of irreversible tissue destruction. This scar tissue undergoes progressive structural remodeling over several months as collagen fibers reorganize **(Ogawa, 2019).**

b.Epithelial Regeneration:

Epithelial regeneration occurs alongside connective tissue repair, with intact epithelial cells proliferating and migrating to repopulate damaged areas. The process varies between covering tissues and parenchymal organs **(Blanpain & Fuchs, 2014).**

In covering tissues (e.g., skin, mucous membranes), regeneration progresses from the wound margins toward the center after granulation tissue forms. This may involve metaplasia (e.g., bronchial epithelium becoming squamous) or functional loss (e.g., cilia disappearance) **(Blanpain & Fuchs, 2009).**

In parenchymal tissues (e.g., liver, kidneys), regeneration quality depends on the extent of damage (particularly to the connective framework) and the mitotic capacity of epithelial cells **(Fausto et *al*., 2012).** .

Example of Hepatitis: In common acute viral hepatitis, the supporting connective framework of hepatocytes remains intact, and hepatocyte regeneration from non-necrotic hepatocytes, guided by this connective framework, results in the formation of new, normal hepatocyte cords without scarring. In severe acute viral hepatitis, the initial destruction of hepatocytes and connective tissue is so extensive that hepatocyte regeneration leads to thickened and disorganized hepatic cords, associated with areas of scarring. **(Kumar et *al*., 2020; Fausto et *al*., 2012)**

Table 01: Cells involved in inflammation **(Lakhani et *al.*, 2009).**

Cell category	Cell type	Origin	Percent while cells	Major Function
Circulating Cells Granulocytes (polymorpho nuclear leukocytes)	Neutrophils	Bone marrow	75	Acute inflammatory cell involved in bacterial killing and phagocytosis. Granule contents for increasing vascular permeability, chemotaxis, killing organisms, and digesting extracellular matrix.
	Eosinophils	Bone marrow	1	Acute inflammatory cell particularly common in allergic and parasitic conditions. Granules include major basic protein.
	Basophilis	Bone marrow	<1	Circulating cells that give rise to **mast cells**. Granules include histamine.

Lymphocytes	T cells	Lymphoid organs and thymus	20	Various subtypes involved in antigen recognition and presentation, cell killing, and regulation of immune responses (e.g., helper, suppressor, and natural killer cells).
	B cells	Lymphoid organs and bone marrow	20	On antigen stimulation, proliferate and give rise to specific **plasma cells**, which synthesize specific immunoglobulins.
Macrophage system	Monocytes	Bone marrow	4	Migrate into tissues to become **macrophages** capable of phagocytosis, cytokine production, and antigen processing and presentation.
Non-circulating cells Kupffer cells (liver sinusoids) Macrophages (bone marrow, spleen and lymph nodes				Fixed phagocytic cells lining sinusoids and filtering large molecules/particles from blood or lymph

Megakaryocytes in bone marrow				Produce platelets which contain serotonin, platelet-derived growth factor, etc. Also important in haemostasis
Hepatocytes				Produce proteins important in: ● clotting and fibrinolytic system ● complement system ● kinin system ● acute-phase proteins

6. Types of Inflammation :

Inflammation can divide into two types, as follows:

6.1. Acute Inflammation:

Acute inflammation represents the body's rapid and immediate defensive response to harmful stimuli, typically persisting from several days to a few weeks. This process is marked by sudden onset and prominent vascular changes, progressing through distinct phases from initial vascular alterations to eventual tissue repair. The inflammatory response initiates locally, recruiting immune cells to the site of injury to mediate phagocytosis and pathogen containment, while simultaneously triggering systemic reactions to circulating microbial toxins **(Chernecky and Berger, 2013).**

In optimal conditions, acute inflammation culminates in complete resolution - characterized by total elimination of the inciting agent and restoration of normal tissue architecture. However, persistent infections (e.g., tuberculosis) or localized pathogen survival (e.g., in osteomyelitis or chronic skin infections) may disrupt this resolution, leading to transition into chronic inflammation **(Calhelha et *al*., 2023).**

6.2. Chronic Inflammation:

Chronic inflammation is a prolonged inflammatory response that persists for months to years without resolution. Unlike self-limiting acute inflammation, this persistent state demonstrates no capacity for spontaneous healing and may progressively worsen over time. Two distinct pathogenic pathways underlie chronic inflammation: progression from unresolved acute inflammation or de novo development as a primary chronic process **(Wang et *al*., 2021).**

6.3. Systemic Inflammatory Response Syndrome (SIRS):

SIRS is a generalized inflammatory state triggered by severe insults like trauma, infection, or ischemia. It mobilizes acute-phase reactants to combat threats but can spiral into a harmful cytokine storm, causing multi-organ dysfunction or death due to uncontrolled systemic inflammation (**Chakraborty RK, Burns B., 2023)**

7. The markers of the inflammatory response:

Inflammatory markers are essential blood tests used to detect and monitor inflammation in the body, which can arise from various conditions, including infections and autoimmune diseases. These markers can be either specific including C-reactive protein (CRP), plasma viscosity (PV), and erythrocyte sedimentation rate (ESR) or non-specific blood-based biological markers **(ARC West, 2022).**

7.1. Non-Specific Biological Markers:

The initial diagnostic evaluation involves the identification of non-specific blood-based biological markers, which can indirectly suggest the presence of an inflammatory reaction **(Table02).** These markers are detected through a complete blood count (CBC) and may indicate inflammatory anemia, leukocytosis (elevated white blood cell count), or thrombocytosis (elevated platelet count) **(Crouzilles,Siebert, 2013).**

Table 02: Indirect non-specific inflammatory markers.

Affected Blood Cell	Normal Values	Changes in Inflammation
Red Blood Cells (Erythrocytes)	5 to 6 million/mm^3	Decrease (inflammatory anemia)

White Blood Cells (Leukocytes)	6,000 to 7,000/mm³	Increase (leukocytosis)
Pltelets (Thrombocytes)	150,000 400,000/mm³	Increase (thrombocytosis)

7.2. Specific biological markers:

Specific biological markers, when elevated, indicate the presence of an inflammatory reaction, although they do not specify its origin. These markers include erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and orosomucoid **(Crouzilles,Siebert, 2013).**

7.2.1. Erythrocyte Sedimentation Rate (ESR):

The erythrocyte sedimentation rate (ESR) measures the rate at which red blood cells settle at the bottom of a vertical tube (Westergren tube) when left at rest. It reflects the degree of systemic inflammation, as it varies with blood viscosity, which is partly influenced by antibody levels **(Braun & Anderson, 2023).**

7.2.2. C-Reactive Protein (CRP):

C-reactive protein (CRP) is a protein synthesized by the liver, with a normal blood level of

< 6 mg/L. It is the most reliable marker of inflammation.

- CRP levels rise significantly (10 to 1000 times the normal value) within hours during severe inflammatory processes (e.g., infections, autoimmune diseases, rheumatic inflammation) **(Schuetz et *al*., 2019)**

- Levels decrease rapidly once the inflammatory process resolves.

7.2.3. Orosomucoid:

Orosomucoid is a liver-synthesized protein with a normal blood level of 0.5 to 1.5 g/L

(Crouzilles,Siebert, 2013).

Table 03: Specific inflammatory markers.

Specific Inflammatory Proteins	Normal Values	Changes in Inflammation
ESR	**1st hour: < 5 mm** **2nd hour: < 10 mm**	**Increase (2 to 3 times in anemia; up to 120 times in some cancers)**
CRP	**< 6 mg/L**	**Increase (10 to 1000 times)**
Orosomucoid	**0.5 to 1.5 g/L**	**Increase**

Elevated levels indicate an inflammatory stat.

8. Anti-inflammatory treatment:

Anti-inflammatory treatments are designed to reduce inflammation and alleviate associated symptoms. Conventional pharmacological approaches primarily include nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, which work by inhibiting cyclooxygenase enzymes and suppressing immune responses, respectively. However, these treatments are associated with significant adverse effects, particularly with long-term use **(Wongrakpanich et *al*., 2018).**

8. 1. Synthetic Anti-inflammatory Agents:

8.1.1. Non-steroidal Anti-inflammatory Drugs (NSAIDs):

Non–steroidal anti-inflammatory drugs (NSAIDs) is a drug class that gathers drugs that can provide pain-killing/relieving effects (analgesic) and can also reduce fever (antipyretic) and at higher doses, it can present reduce inflammation (anti-inflammatory effects). **(Charles Fokunang, et *al.* 2018)**

NSAIDs reprensets a wide range of drugs with significant structural and functional diversity. Mostly these drugs are weak organic acids that typically feature an acidic moiety

combined with an aromatic functional group. **(S. Bindu, et *al.* 2020)**

The analgesic and anti-inflammatory properties are mainly achieved by the inhibition of two well-known isoenzymes of prostaglandin G/H synthase, commonly referred to as cyclo-oxygenase (COX), more specifically COX 1 and COX 2 **(As shown in fig.02) (Charles Fokunang, et *al.* 2018).**

As for its specific anti-inflammatory activity, it's mainly achieved by the inhibition of the enzyme cyclo-oxygenase (COX) as mentioned previously, these enzymes help in converting a fatty acid known as arachidonic acid into thromboxans, prostaglandins, and prostacyclins, these substances are called eicosanoids, which are in involved in the process of inflammation, Moreover, the therapeutic effects of NSAIDs drugs are attributed to the lack of these eicosanoids.

There are two cyclooxygenase isoenzymes, COX-1 and COX-2. Comparatively, COX-2 is not constitutively expressed in the body; and instead, its inducibly expresses during an inflammatory response, while on the other hand, COX-1 gets constitutively expressed in the body (always active) as it is involved in supporting kidney function, maintaining gastrointestinal mucosa lining and platelet aggregation. Most of the NSAIDs are nonselective and would target both COX-1 and COX-2. In contrast, COX-2 selective NSAIDs such as celecoxib, only target COX-2, hence have a different side effect profile. That's because the enzyme COX-1 is the key mediator for ensuring gastric mucosal integrity while COX-2 is mainly involved in inflammation, therefore COX-2 selective NSAIDs would provide anti-inflammatory relief without causing any damage to the gastric mucosa. (Ida Ghlichloo & Valerie Gerriets , 2019)

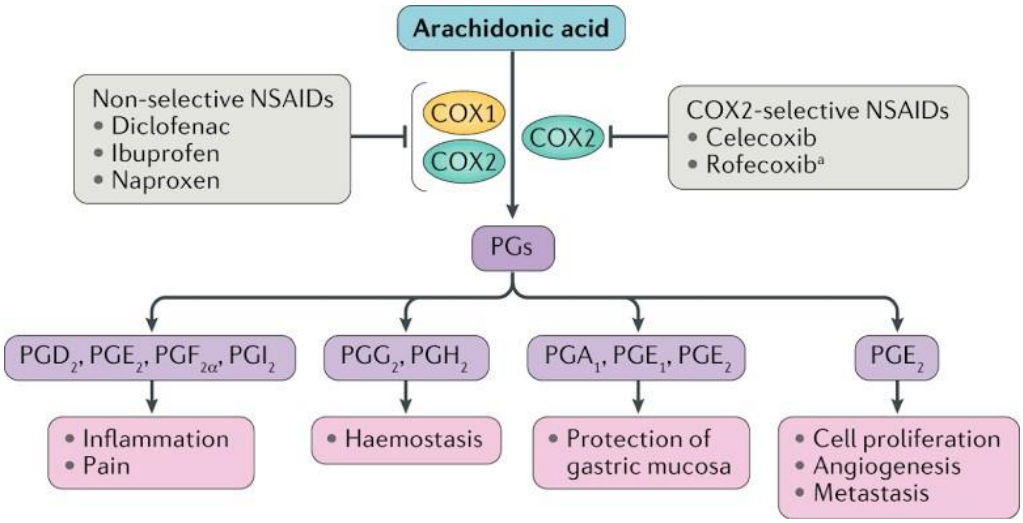

Figure 02: Mechanism of action of NSAIDs. **(Anne-Marie Schjerning et *al.*, 2020)**

8.1.2 Steroidal Anti-inflammatory Drugs (Glucocorticoids):

Glucocorticoids (GCs) are steroid hormones that play a crucial role in the treatment of inflammation, autoimmune diseases, and cancer **(S Timmermans, 2019).** For over 70 years, GCs have been utilized in clinical settings to manage inflammatory diseases and other conditions believed to involve inflammation **(Sybille D Reichardt et *al.*, 2021).** These hormones are fundamental in treating a variety of inflammatory and autoimmune disorders, including bronchial asthma, chronic obstructive pulmonary disease, Crohn's disease, ulcerative colitis, immune-mediated glomerulonephritis, rheumatoid arthritis (RA), systemic lupus erythematosus, and multiple sclerosis **(Simona Ronchetti et *al.*, 2018).**

To achieve their extensive physiological and therapeutic effects, glucocorticoids bind to the glucocorticoid receptor (GR), which is part of the nuclear receptor superfamily of transcription factors **(S Timmermans, 2019).**

By inhibiting transcription factors such as AP-1 and NF-kB, GCs can reduce the expression of cyclooxygenases (COX), particularly COX-2, in inflammatory cells, effectively blocking the synthesis of prostaglandins **(Sébastien Faure, 2009).**

8.1.3. Side effects of synthetic anti-inflammatory drugs:

The toxicity resulting from non-steroidal anti-inflammatory drugs can manifest as: renal damage, hepatotoxicity, and hypertension. **(Ida Ghlichloo & Valerie Gerriets, 2019;**

Rothenberg & JP Holcomb, 2000)

These medications can also result in GI damage that includes gastric ulceration, perforation and their associated complications. It works by inhibiting COX-1 specifically, which causes decreased gastric mucosa production. **(AH Abbas, 2015)**

As of the Nephrotoxicity (renal damage), it occurs with NSAID use because these medications reduce prostaglandin levels, which are crucial for maintaining proper blood flow and function in the kidneys. This reduction can lead to inadequate kidney perfusion and potential damage over time. **(P Ejaz, 2004)**

Lastly, neuro-toxicity can manifest through a range of symptoms, indicating that the drug is affecting the central nervous system and sensory pathways such as drowsiness, confusion, nystagmus, blurred vision, diplopia, headache, and tinnitus. **(Ida Ghlichloo & Valerie Gerriets , 2019; Laura J Hunter et *al.*, 2011).**

As for SAIDs or GCs, Their drawbacks are directly resulted from their biological activities, such as metabolic disorders, Endocrine Disorders, Psychiatric Disorders (Insomnia),Infectious Complications, Digestive Disorders: (Gastroduodenal Ulcer: Risk of perforation of a colonic diverticulum and bleeding, Acute Pancreatitis: Particularly in children) and other (Acute or Chronic Glaucoma, Cataract: Very common; its incidence depends on the dosage administered, Increased Intracranial Pressure, Hypercoagulability: Increased tendency for blood to clot). **(Sébastien Faure, 2009)**

8.2. Natural anti-inflammatory treatment:

Even with the availability of various options of conventional and non-conventional treatment for inflammation, they often present few drawbacks that are related to the safety, effectiveness and the cost.

Herbal remedies are a Natural alternative medicine to synthetic drugs, these Herbal products are typically considered safer for treating various health issues **(Mukta Gupta et *al.*, 2021; Jamshidi-Kia et *al.*, 2018).**

Among these Natural remedies we highlight some plants that are characterized by their anti-inflammatory properties such as **Turmeric (***Curcuma longa L.***), Ginger (***Zingiber officinale Roscoe***),** Rosella (*Hibiscus sabdariffa L.)* **and black seed** *(Nigella sativa L.)*

8.2.1. Turmeric (*Curcuma longa L.*):

Turmeric, also scientifically known as *Curcuma longa L.* is a perennial rhizomatous herbaceous plant, the main part of it is its rhizome which is usually used as a food additive. Scientific research has reported that rhizomes of this plant possess antibacterial, antifungal, anti-inflammatory, antioxidant and antitumor properties. **(Mohammad Basir Khan et *al.*, 2013).**

These therapeutic properties are the result of the presence of secondary metabolites such as curcumin, demethoxycurcumin (DMC), and bisdemethoxy curcumin (BDMC). (**Md Zahorul Islam et *al.*, 2024).**

But the most important secondary metabolite of *C. longa* is curcumin, which is responsible, for plant's the anti-inflammatory properties **(Julie S Jurenka, 2009)**.

More specifically it cures the etiological factors and addresses the pathological changes associated with inflammation.

The anti-inflammatory properties of curcumin were first documented in 1971. **(RC Srimal et *al.*, 1971).**

Fig.03: Curcuma longa plant. **(Ridho& Indriani , 2025)**

Fig.04: Turmeric rhizomes. **(Ridho& Indriani , 2025)**

Fig.05: Powdered Turmeric **(Ridho& Indriani , 2025)**

8.2.2. Ginger (*Zingiber officinale Roscoe*):

Ginger, the rhizome of *Zingiber officinale Roscoe* that belongs the plant family of *Zingiberaceae* originates from the Indo-Malayan region, and in the current time is widely cultivated throughout the tropical areas of Asia, Africa, America, and Australia

(Mirele da Silveira Vasconcelos et *al.*, 2019; Kizhakkayil & Sasikumar, 2011)

Its most commonly used part is the rhizome which is used for numerous therapeutic purposes including anti-inflammatory, antidiabetics, antioxidant, anti-microbial and also curing in vomiting, constipation, indigestion, cold, fever, cough, nausea, respiratory conditions, bronchitis etc. **(D, Mutthuraj et *al.*, 2020)**

Fig. 06 : *Zingiber officinale Roscoe* plant. **(Suliemanet *al.* 2024)**

Fig. 07: Fresh Ginger rhizome. **(Bitari et *al.*, 2023)**

Fig. 08: Dried Ginger rhizome **(Bitari et *al.*, 2023)**

Fig. 09: Powdered Ginger. **(Dharmapala & Amarakoon, 2024)**

There are various (clinical and preclinical) studies which indicate that ginger has a notable efficay in treating inflammation and is comparable to NSAIDs yet it has less side effects especially the drawbacks that are related to the gastrointestinal tract. **(Sonam Shashikala B.V , 2024)**

The mechanism of action of the main secondary metabolites in this plant involves the **Inhibition of Cyclooxygenase and Lipoxygenase Pathways,** Gingerols and shogaols exert their anti-inflammatory action by inhibiting key enzymes such as cyclooxygenase-2 (COX-2) and lipoxygenase (LOX). COX-2 is an enzyme that is crucial in the generating of pro-inflammatory prostaglandins whereas LOX is involved in the production of leukotrienes which is another category of inflammatory mediators enzymes, the synthesis of pro-inflammatory compounds decreases which helps reduce the inflammation. **(Sonam Shashikala B.V, 2024; Bischoff-Kont I & Fürst R., 2021)**

8.2.3. Rosella (*Hibiscus sabdariffa L.*):

Hibiscus sabdariffa L., commonly known as the rosella plant, is a notable herbal plant with significant health benefits. Its petals, leaves, seeds, and stems have all been utilized since ancient times for their medicinal properties. Packed with a variety of beneficial substances, hibiscus is valued in traditional medicine for its positive effects on the human body **(Sri Muliana Putri Bakara et *al*., 2024).**

This plant is recognized for its potential health benefits, attributed to its rich array of bioactive compounds. Notably, it contains flavonoids, phenolic acids, and anthocyanins, all of which are known for their significant therapeutic properties. These compounds contribute to the plant's effectiveness in promoting overall health and wellness, they’re known for their strong anti-inflammatory, antioxidant, and antimicrobial effects. Flavonoids and phenolic acids, in particular, can modulate inflammatory responses by inhibiting key enzymes such as cyclooxygenase (COX), this process leads to a decrease in the production of inflammatory mediators like prostaglandins and leukotrienes, which are

involved in the inflammatory process. By lowering the level of these mediators, this plant has the ability to reduce inflammation and oxidative stress. Moreover, the antioxidant properties of these compounds help protect cells from damage caused by free radicals, further supporting overall health. **(Salsabila Putri Uno et *al.*, 2024)**

Figure 10: Rosella, *Hibiscus sabdariffa L* plant **(Unita & Singarimbun, 2018)** and its flowers.**(Nurnasari & Khuluq, 2018).**

8.2.4. Black seeds (*Nigella sativa L.*):

Black cumin or black seed, is scientifically known as *Nigella sativa L.*, is a native plant in Eastern Europe and Western Asia. Which has naturalized over the decades around North Africa and parts of the Middle East. **(H Abdelhalim & S Arora , 2022 ; Ali, B. H., & Blunden, G. , 2003)**

This medicinal herb is exclusively used in traditional medicine mostly in Western Asia, some scientific researches has proved that it exerts its action by inhibiting both cyclooxygenase (COX) and 5-lipoxygenase pathways of arachidonic acid metabolism. **(Harshal Pise & Sudhir Padwal , 2017)**

It is also rich with Several chemical compounds, with its prime compound as thymoquinone (TQ) /(2-isopropyl-5-methyl-1,4-benzoquinone) , which is known with its Pharmacological properties to reduce asthma symptoms and treat rheumatoid arthritis all by targeting the

inflammatory pathway.

(H Abdelhalim & S Arora , 2022 ; Ahmad Aftab, et *al.*, 2013 ;Amin & Hosseinzadeh, 2016).

Figure 11: *Nigella sativa* flower.

(Verma et *al.*, 2024)

Figure 12: *Nigella sativa* seeds.

(Verma et *al.*, 2024)

Chapter II: Nanoparticles

1. Background:

1.1. Nano etymology:

The prefix nano comes from the Greek word *nanos* which means "a dwarf". At the 14th conference of the International Union of Pure and Applied Chemistry (IUPAC) in 1947, "nano" was officially recognized as a term to describe the one-billionth part ($\mathbf{10^{-9}}$) of a unit. **(Nadeem Joudeh & Dirk Linke, 2022 ; Buzea C et *al*., 2007)**

Nowadays, 'Nano' is a widely recognized term in modern science, with numerous related words appearing in the dictionary, such as nanoscience, nanotechnology, nanostructure, nanoparticle, and nanotube. This concept originated from R. Feynman's speech in December 1959 at the American Physical Society meeting, where he asked: *«What would happen if we could arrange the atoms one by one the way we want them*? » **(Gigault, 2011).**

2. Definitions:

2.1. Nanotechnology:

"technology on the nanoscale" serves as the simplest definition of nanotechnology but this initial, we cannot accurately define nanotechnology without defining what is meant by "nanoscale," that is, a scale covering **1–100 nm. (Bhushan, 2017)**

This branch encompasses the synthesis, engineering, and applications of materials whose size ranges from 1 to 100 nm, which are referred to as nanomaterials. **(Mulvaney P, 2015; Nadeem Joudeh & Dirk Linke, 2022**)

2.2. Nanoparticles:

Nanoparticles are defined as the particulate matter with at least one dimension less than 100 nm. They can be composed of metal, metal oxides, carbon or organic matter. **(Savita Kumari and Leena Sarkar, 2022)**

These particles are viewed as the essential building blocks of nanotechnology. **(P Biswas & CY Wu , 2005)**

They vary in size, shape, and composition, which sets them apart from one another. They exhibit unique physical, chemical, and biological properties in comparison to larger particles. This distinction relies on different factors such as increased chemical reactivity

or stability and a greater surface area relative to volume, all along with greater mechanical strength. Due to these specific properties, nanoparticles have various applications. **(B Yang et *al*., 2021 ; Yousaf Khan et *al*., 2022).**

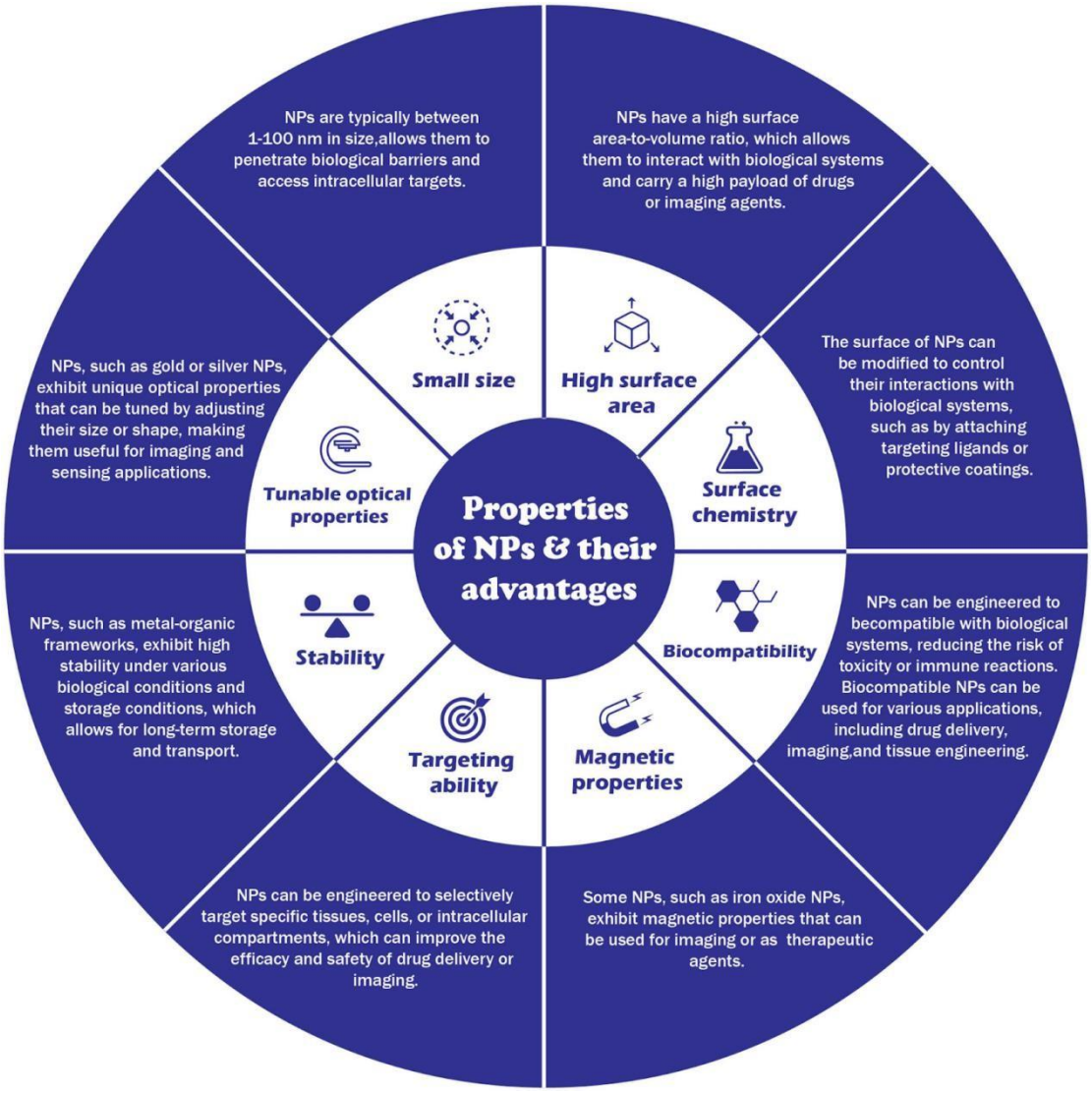

Figure13 : Properties of nanoparticles and their advantages.**(Khadijah A. Altammar, 2023)**

3. Classification of nanoparticles:

The nanoparticles can manifest in different shapes, sizes and structures, they can be spherical, cylindrical, tubular, conical, hollow core, spiral, flat, wire etc. It can also be irregular in shape. They're typically categorized into different classes depending on their morphology, size, physical & chemical properties. Mainly, they're classified into organic, inorganic and carbon-based NPs. **(Savita Kumari & Leena Sarkar , 2022)**

3.1. Organic Nanoparticles:

Organic nanoparticles are solid particles composed of natural or synthetic organic molecules, typically ranging in diameter from 10 to 100 nm, but, However, they can extend up to 1000 nm within this definition. **(Kumar & Lal, 2014)**

Some well-known organic NPs include dendrimers, liposomes, micelles, ferritin etc. These nanoparticles are eco-friendly, biodegradable, non- toxic, cost-effective which makes them more ideal for biomedical applications. **(Savita Kumari & Leena Sarkar , 2022)**

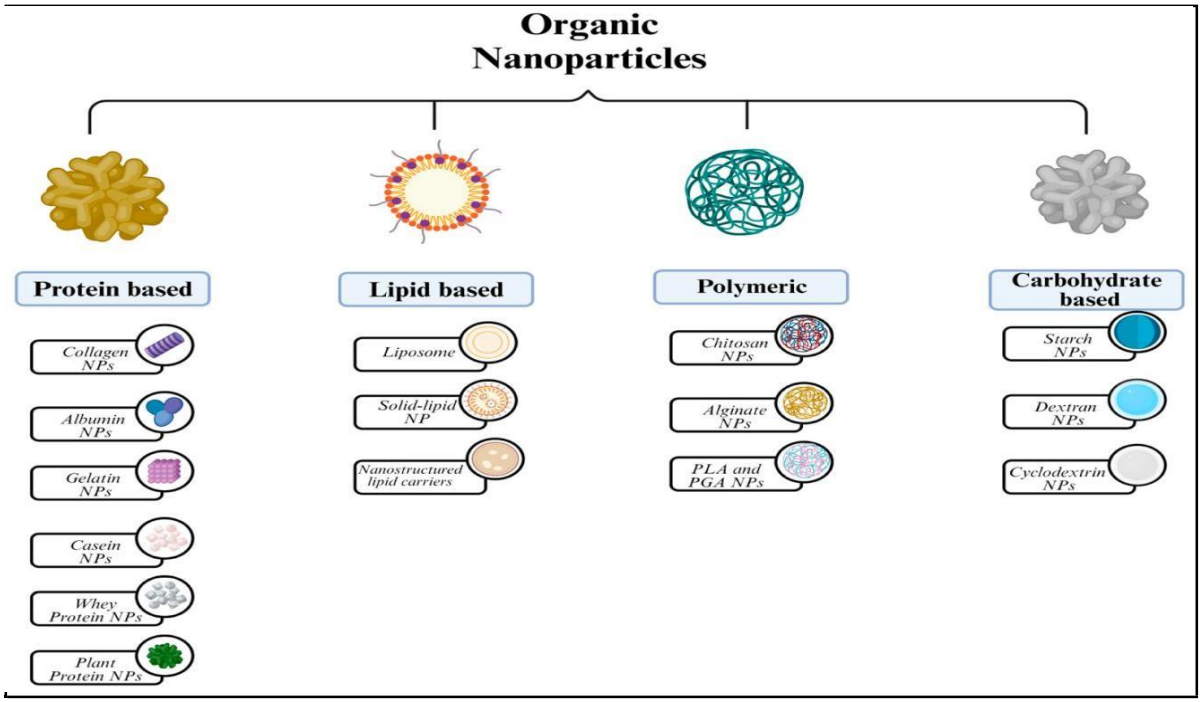

Figure14: Classification and subtypes of Organic NPs. **(Eker et *al.*, 2024)**

3.2. Inorganic Nanoparticles:

Inorganic nanoparticles (iNPs) are the particles that do not contain carbon. Such as metal and metal oxides **(Khalisanni K. et *al.*, 2020)**

Compared to organic nanoparticles, iNP's have received significant research and commercial investment.

They are made up of inorganic atoms bonded by covalent or metallic bonds. They can be synthesized from materials such as semiconductors, ceramics, or magnetic metals **(Savita**

Kumari & Leena Sarkar, 2022)

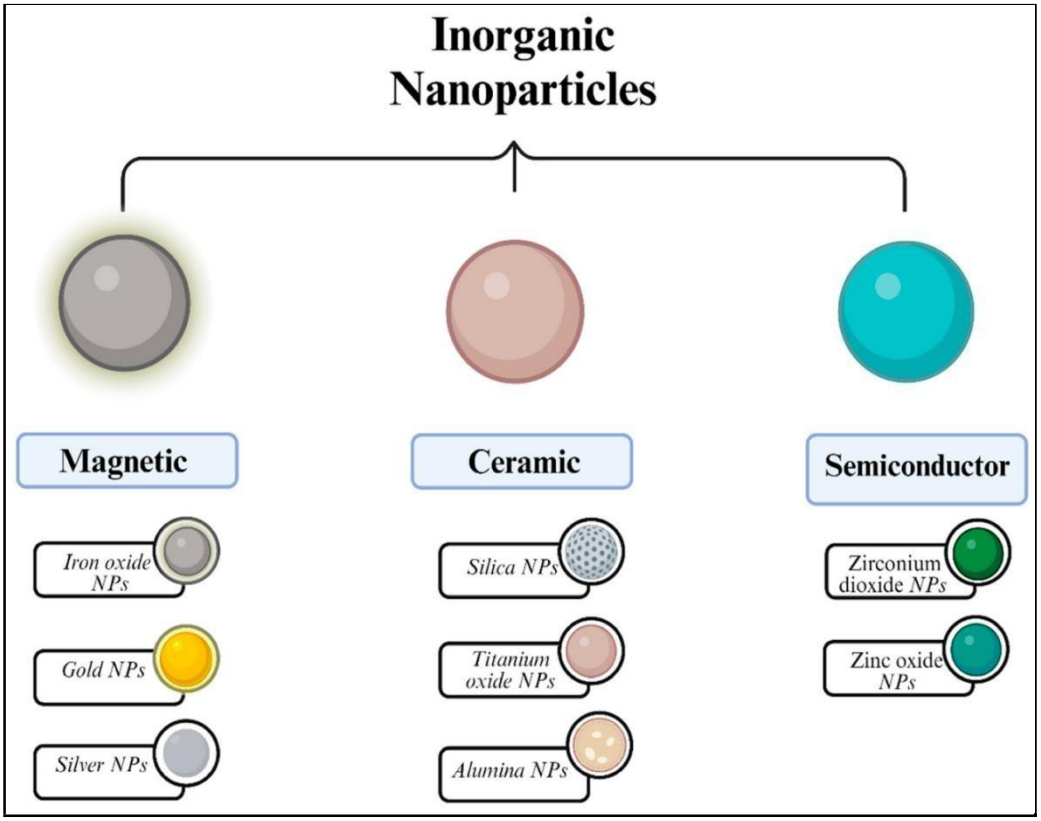

Figure15: Classification and subtypes of iNPs. **(Heuer-Jungemann et *al*., 2019)**

3.2.1. Metal Based Nanoparticles:

Metal-based nanoparticles typically range in size from 10 to 100 nm and can take various shapes, including spherical and cylindrical forms. They exhibit unique properties, such as a high surface area-to-volume ratio, specific pore sizes, surface charge, surface charge density, and can have both crystalline and amorphous structures. Additionally, they display high reactivity and sensitivity to environmental factors like air, moisture, heat, and sunlight. These distinctive properties make them highly valuable in a wide array of research applications. **(Savita Kumari & Leena Sarkar, 2022)**

They can be derived from metals like aluminum (Al), gold (Au), silver (Ag), cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), and zinc (Zn). The most commonly used metals are Ag (silver), Au (gold), Cu (copper), Fe (iron), and Zn (zinc). Transition metals are considered the best candidates for synthesizing **(Elena S. L., et *al*., 2020).**

3.2.2. Metal Oxides Based Nanoparticles:

Metal nanoparticles (NPs) can be oxidized to form metal oxide NPs, which exhibit superior properties compared to their parent metals. Examples include Iron oxide ($Fe_2\ O_3$), Magnetite ($Fe_3\ O_4$), Aluminum oxide ($Al_2\ O_3$), Cerium oxide (CeO_2), Silica (SiO_2), Titanium dioxide (TiO_2), and Zinc oxide (ZnO). **(Savita Kumari & Leena Sarkar, 2022)**

3.3. Carbon Based Nanoparticles:

Nanoparticles made of carbon are referred to as carbon-based NPs. They come in various shapes, such as tube-shaped, horn-shaped, spherical, or ellipsoidal. The main classes of this category include fullerenes and carbon nanotubes (CNTs). Additional classes of carbon-based NPs are graphene, nanofibers, and carbon black.

Due to its ability to form long and resistant chains, Carbon has a crucial place in nanotechnology. This distinctive property of carbon is exploited in the field of NPs, known as carbon-based NPs. They own significant characteristics including high chemical stability, powerful heat and electrical conductivity, high optical absorption, and luminescence **(Kokorina et *al*., 2020)**.

Thus , they are utilized in numerous research fields, including biosensors, drug delivery, cancer, they also play a role in cellular therapy; *in vivo*, *in vitro*, and optical imaging..etc. **(RP Singh & KRB Singh, 2021)**

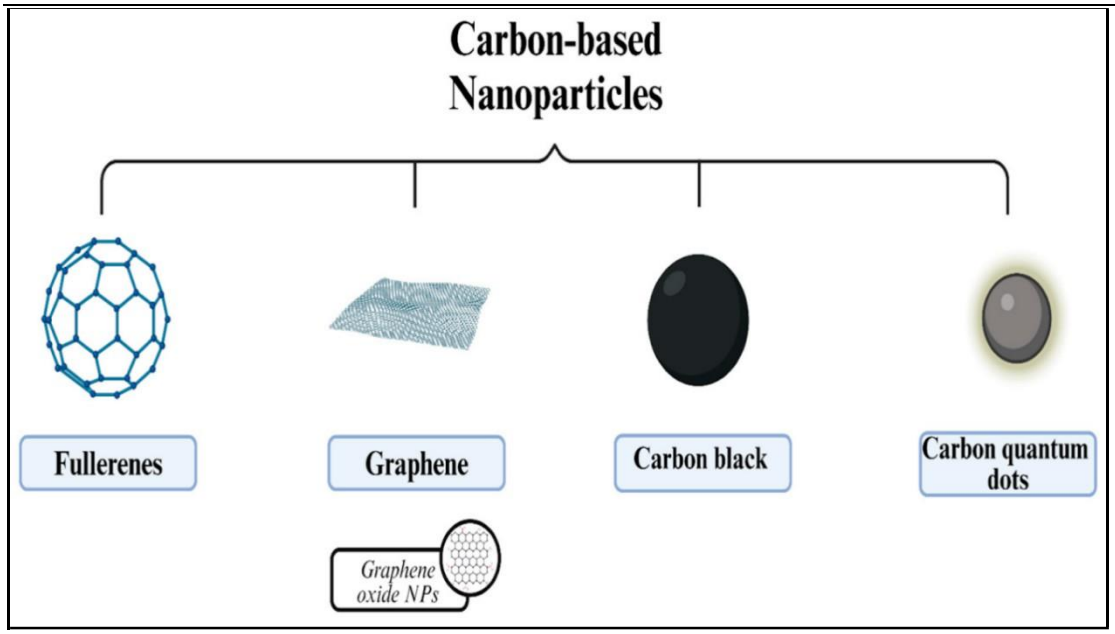

Figure16: Classification and subtypes of carbon-based NPs. **(Eker et *al*., 2024)**

4. Synthesis methods of Nanoparticles:

There are three main approaches for the synthesis of nanoparticles: physical, chemical, and biological. The top-down approach refers to the physical method, in contrast to the bottom-up approach which refers to the chemical and biological approaches collectively. The biological method approach is also named green systems of NPs. Each approach can be further divided into various types based on the employed method**. (Khadijah A. Altammar,2023).**

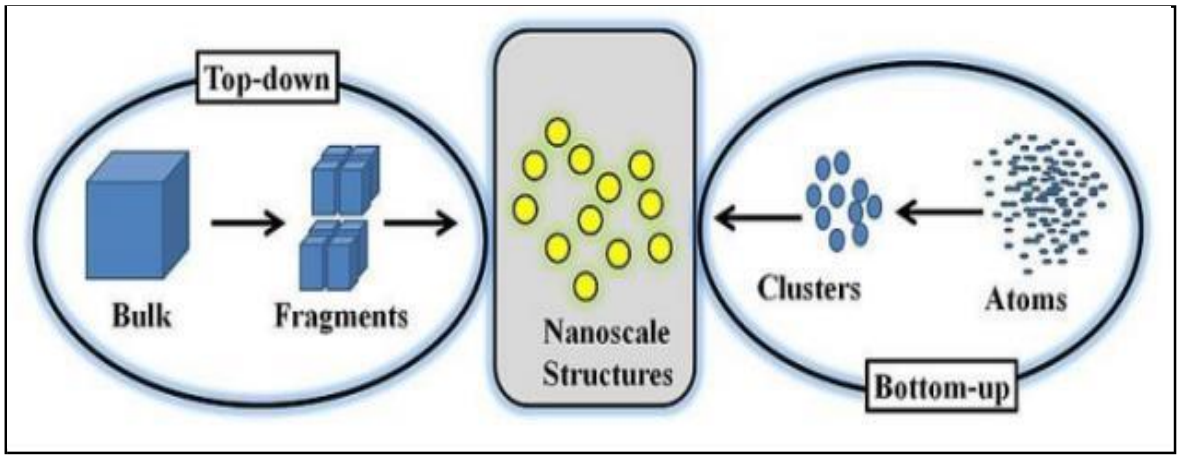

Figure17: Schematic representation of Top- down approach & Bottom-Up approach **(Savita Kumari & Leena Sarkar, 2022)**

4.1. Top-down approach:

In top-down methods Bulk materials are mechanically fragmented to produce nano-structured materials. This approach, also known as the physical method, involves a reduction in size to achieve nanoscale dimensions **(Baig et *al.*, 2021)**. .

The following techniques can achieve a top-down approach.

4.1.1. Mechanical milling:

The mechanical milling process involves the use of balls within containers and can be performed in various types of mills, commonly planetary and shaker mills. This impact process operates with high energy to effectively reduce particle size. **(Gorrasi & Sorrentino, 2015).**

4.1.2. Laser ablation:

Laser beams can fabricate micro-features by vaporizing a single material **(Tran & Wen, 2014).** Similarly, laser ablation synthesis generates nanoparticles through intense laser irradiation that vaporizes the target material. **(Amendola & Meneghetti, 2009)**.

4.1.3. Electron explosion:

This technique requires the use of a thin metal wire that is subjected to a high current pulse that will result in an explosion, evaporation, and ionization. The metal vaporizes and ionizes, then expands and cools as it reacts with the surrounding gas or liquid medium. This condensed vapor ultimately forms nanoparticles**. (Joh et *al.*, 2013)**

4.2. Bottom-up approach:

In the bottom-up method small atoms and molecules are assembled to form nano-structured particles **(Baig et *al*., 2021).** These include chemical and biological approaches as follows;

4.2.1. Chemical synthesis:

Numerous Chemical synthesis methods of NPs has been used over the years, we cite mainly:

4.2.1.1. Sol-gel process:

The sol-gel method is a widely used wet-chemical approach for the synthesis of nanomaterials **(Das and Srivasatava, 2016; Baig et *al.*, 2021).**

This technique is used for synthesizing nanostructures (particularly metal oxide nanoparticles). It involves dissolving a molecular precursor (e.g., metal alkoxide) in water/alcohol, converting it to gel via hydrolysis/alcoholysis with heat and stirring. The resulting wet gel is dried (e.g., through solvent combustion for alcoholic solutions), powdered, and calcined as clarified in (**Figure 18)** This low-temperature, cost-effective process enables precise control over product composition. **(Bokov, 2021)**

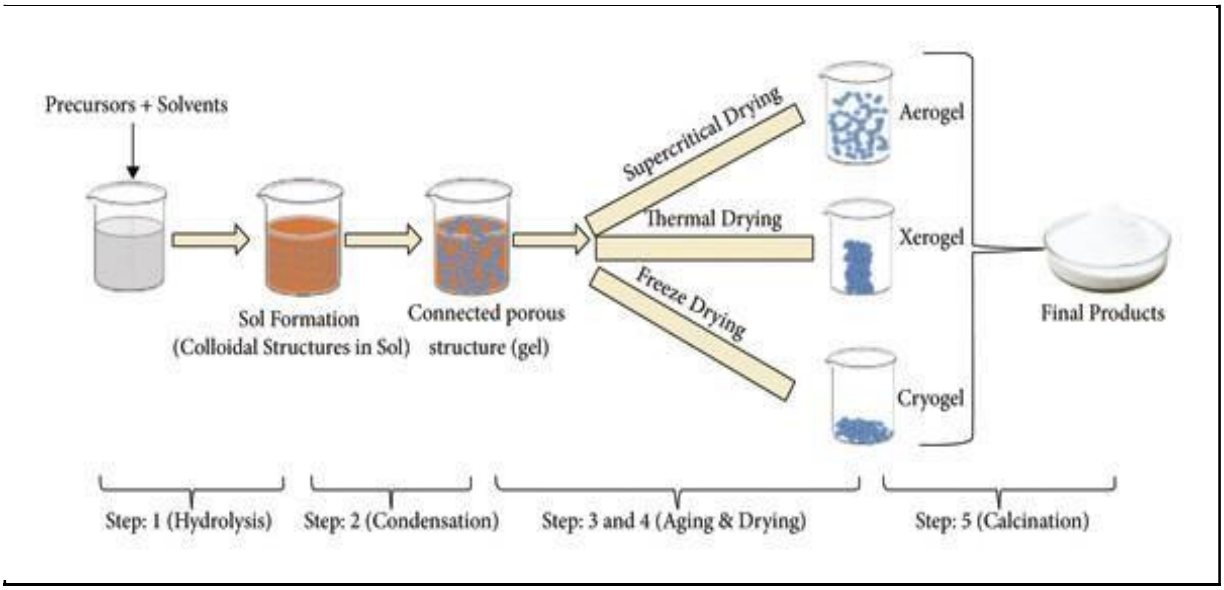

Figure18: Schematic of different stages of sol-gel process **(Bokov, 2021)**

4.2.1.2. Co-precipitation:

This method involves a solvent displacement technique as part of a wet chemical process. Examples of solvents used include ethanol, acetone, hexane, and non-solvent polymers. The polymer phases can be synthetic or natural. When the polymer solution is mixed, the polymer-solvent diffuses quickly into the non-solvent phase, creating interfacial stress between the two phases, which ultimately results in the formation of nanoparticles. **(Das & Srivasatava, 2016).**

4.2.2. Green/biological synthesis:

The synthesis of various metal nanoparticles using bioactive agents, including plant materials, microbes, and various biowastes like vegetable scraps, fruit peels, eggshells, and

agricultural residues, is known as "green" or "biological" nanoparticle synthesis and it goes as shows in the following scheme (**Figure 19**.) **(Kumari et *al.*, 2021).**

Developing reliable and sustainable green synthesis technologies is essential to avoid the creation of undesirable or hazardous byproducts. The green synthesis of nanoparticles offers numerous advantages, such as simplicity, cost-effectiveness, the production of highly stable nanoparticles, minimal time requirements, the generation of non-toxic byproducts, and the ease of scaling up for large-scale production. **(Malhotra & Alghuthaymi, 2022).**

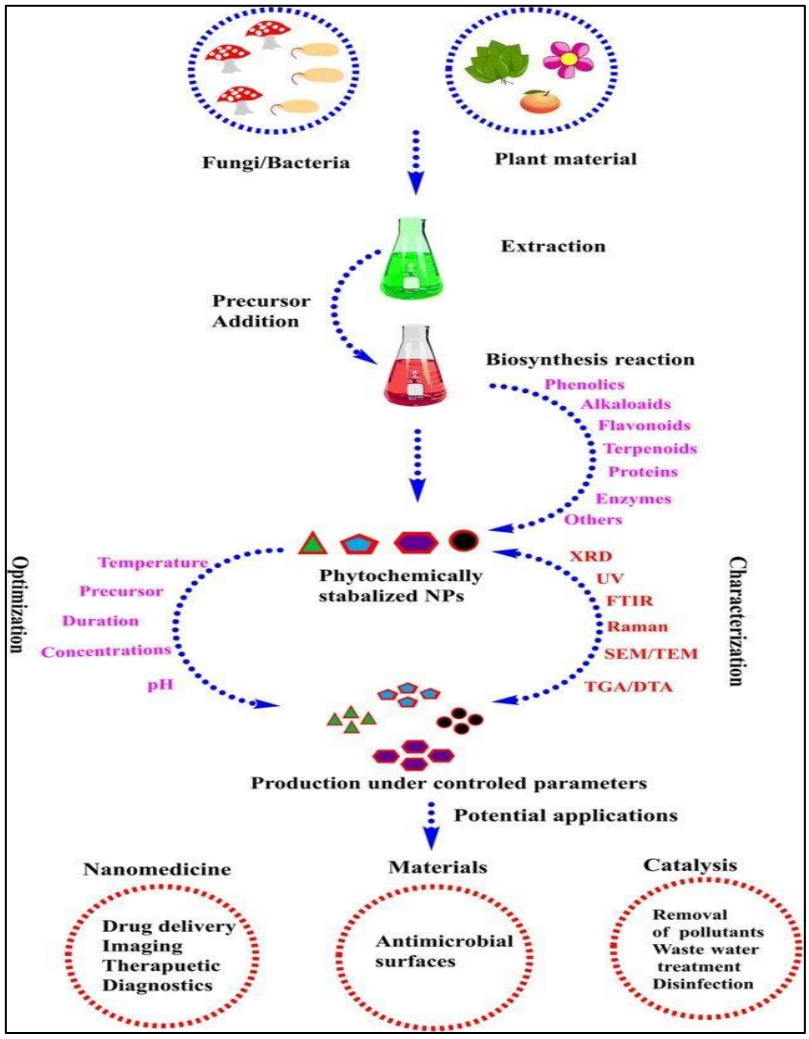

Figure19: Biosynthesis, characterization, and application of metal nanoparticles.(**Ovais et *al.*, 2018)**

4.2.2.1. Biological synthesis using microorganisms:

Microbes generate nanoparticles through metal capture, enzymatic reduction, and capping processes. Metal ions are first trapped on the surface or inside the microbial cells before enzymes convert them into nanoparticles **(Ghosh et *al.*, 2021)**.

Some microorganisms, particularly marine microbes, are used for the synthesis of metallic nanoparticles is environmentally friendly, rapid, and economical. **(Patil & Kim, 2018).**

4.2.2.2. Biological synthesis using plant extracts:

Plant extract is the substance or active ingredient of a desired quality obtained from plant tissue through specific treatment for a particular purpose. **(Jadoun et *al.*, 2021)**.

Plant extracts are mixed with a metal salt solution at room temperature for the sole purpose of creating nanoparticles. The reaction is achieved within minutes. nanoparticles of silver, gold, and many other metals has been created by using this technique. **(Li X. et *al.*, 2011).**

5. Applications of Nanoparticles:

Being the fundamental component of nanotechnology, nanoparticles have a wide spectrum of applications such as:

5.1. Environmental applications:

NPs appeal to various environmental applications such as:

5.1.1. Bioremediation:

Nanoparticles can eliminate environmental pollutants like heavy metals from water and organic substances from soil **(Zhuang & Gentry, 2011)**. As an example, silver nanoparticles (AgNPs) are effective in breaking down certain pollutants, such as organic dyes and other compounds present in wastewater. In addition to AgNPs. **(Khadijah A. Altammar, 2023)**

5.1.2. Sensors in environment:

Nanoparticles have been and still are currently used to enhance water quality and support in environmental clean-up activities **(Pradeep, 2009).**

They can be engineered to bind selectively to specific types of pollutants, enabling their detection even at low concentrations. For example, gold nanoparticles (AuNPs) have been used to detect mercury in water **(Theron et *al.*, 2010; Khadijah A. Altammar, 2023)**

5.2. Applications in medicine:

Nanomaterials (NMs) are crucial in modern medicine, with applications ranging from enhancing contrast in imaging to serving as carriers for targeted drug and gene delivery into tumors. **(Joseph et *al.*, 2023)** as we note below:

5.2.1. Cancer diagnosis and therapy:

Metallic and semiconductor NPs have great potential in cancer diagnosis and therapy, thanks to their enhanced light scattering and absorption properties from the LSPR effect. For instance, AuNPs can efficiently absorb light and convert it into localized heat, making them suitable for selective photothermal therapy that induces cancer cell death through the heat produced in tumor tissues. **(Huang et *al.*, 2007)**

5.2.2. Drug delivery:

Targeted drug delivery is also an important potential application of NPs. ZnO and Fe3O4 NPs were efficiently used for targeted drug delivery and selective destruction of tumor cells **(Rasmussen et *al*., 2010).**

5.2.3. Imaging:

Nanoparticles have been successfully used in different medical applications such as cellular imaging **(Hutter & Maysinger, 2011)**

Nanotechnology holds great promise for rapid, high-resolution imaging of tissue microstructures and accurate lesion characterization. This can be achieved through the development of non-toxic contrast agents with extended circulation times such as Nanoparticle-based contrast agents which are useful across a range of widely used biomedical imaging techniques, including fluorescence imaging, Magnetic Resonance Imaging (MRI), Computed Tomography (CT), Ultrasound (US), Positron Emission Tomography (PET), and Single Photon Emission Computed Tomography (SPECT), each with unique structural features, benefits, and limitations. **(Han et *al.*, 2019)**

Experimental Part

Materials
&
Methods

I. Plant Material:

Myrtus communis L. also commonly known as Myrtle was obtained from the region of Souk-ahras, North east Algeria. **(Fig 20&21)**

Myrtle leaves were collected. The fresh leaves were first washed several times with distilled water to remove impurities and dust. The obtained leaves were then dried in the shade at ambient temperature for 5 to 7 days.

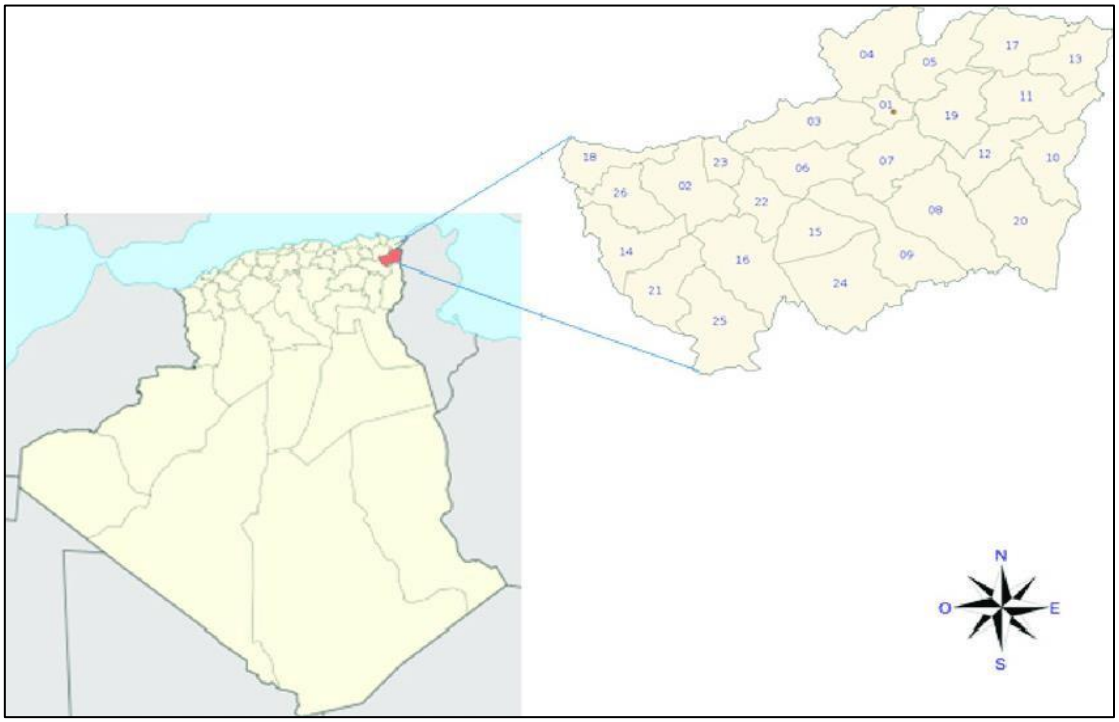

Figure 20: Geographic localization of Souk Ahras.

Figure 21: *Myrtus communis L.* plant.

I.1. Preparation of the leaf extract:

Myrtle leaves were collected. The fresh leaves were first washed several times with distilled water to remove impurities and dust. The obtained leaves were dried in the shade at ambient temperature for 5 to 7 days, then ground to a fine powder, which was stored in a container away from air.

To prepare the extract, we mixed 20 g of powder with 200 ml of distilled water in a flask then stirred the mixture for 24 hours at room temperature. The extract was then filtered by vacuum filtration using Buchner funnel and stored in an airtight bottle at 6 °C for immediate use.

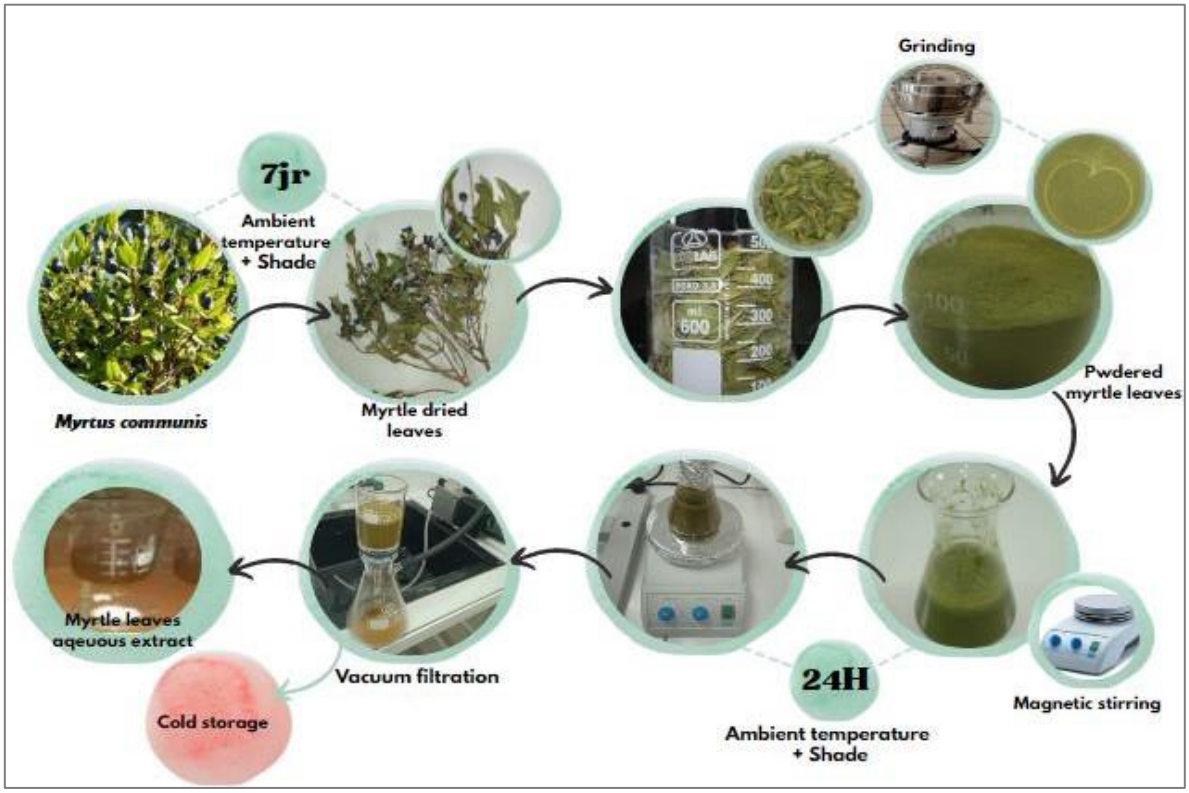

Figure22: Preparation of *Myrtus communis L.* aqueous leaf extract.

I.2. Green synthesis of Zinc oxide nanoparticles:

The aqueous extract of myrtle leaves (100mL) was reacted with 0.6g of zinc acetate in a water bath system with continuous stirring at 85 °C for 1 hour. The formation of zinc oxide nanoparticles was indicated by a color change of the mixture solution from green to dark brown. The obtained products were collected and dried at 100 °C before being

calcined at 200 °C for 2 hours.

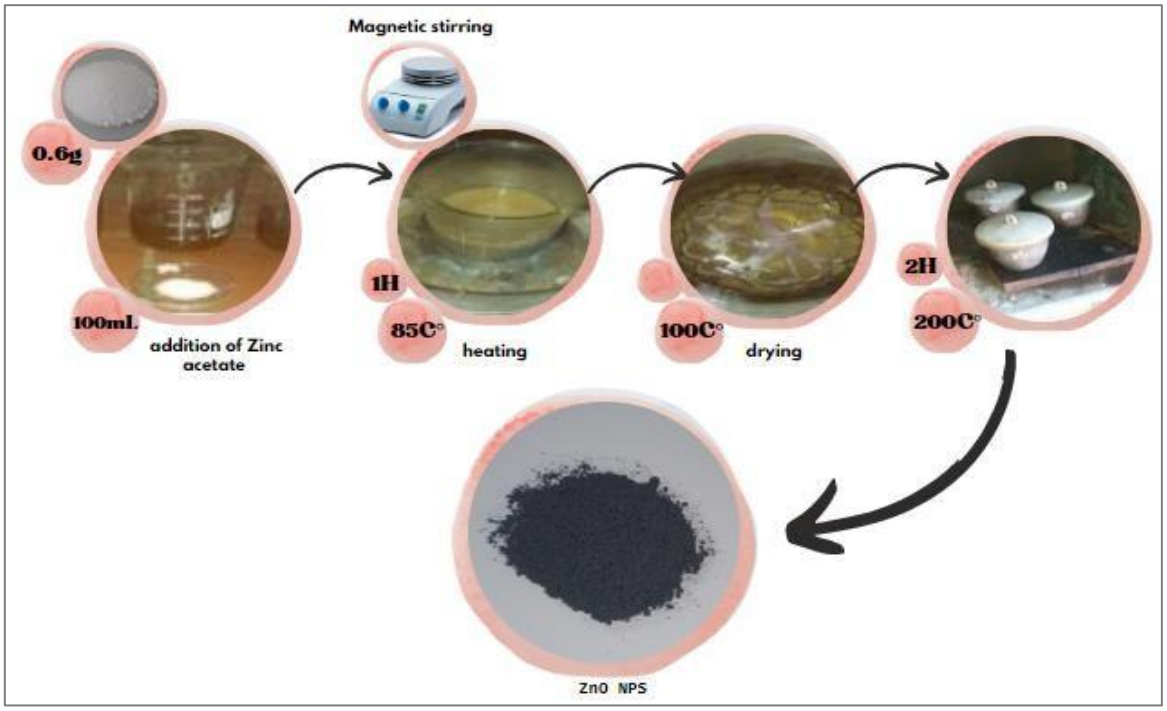

Figure23: Green synthesis of ZnO NPs from *Myrtus communis L.* leaf extract.

I.3. Structural Properties Characterization by Fourier Transform Infrared (FTIR) Spectroscopy of Green Synthesized Nanoparticles via *Myrtus communis L.*:

I.3.1. Principle:

Fourier-transform infrared (FTIR) spectroscopy is based on the principle that molecules absorb infrared light at specific frequencies corresponding to the vibrational modes of their chemical bonds. The resulting absorption spectrum acts as a molecular fingerprint, allowing for the identification and characterization of various organic and inorganic substances. Functional groups, which determine the main chemical properties of compounds, absorb IR radiation at similar wavenumbers, even across different compounds. This establishes a clear relationship between the wavenumber of IR absorption and the material's structure, enabling identification through the recognition of absorption bands that correspond to the functional groups present. Consequently, infrared spectroscopy is a valuable tool in chemical analysis, particularly in the mid-infrared (mid-IR) region, which ranges from 4000 to 400 cm^{-1}. **(Smith, 2011; Pasieczna-Patkowska et *al*., 2025).**

Figure24: Fourier Transform Infrared Spectroscopy.

I.3.2. General Procedure for Spectroscopy (FTIR):

Sample Preparation:

Representative samples are taken in a clean, dry container. Ensuring that the sample is at room temperature to avoid any condensation. A thin layer of the sample is spread on an appropriate surface (usually a salt crystal window or Br) for measurement. **(El-Mansy & El-Shafey, 2022).**

Calibration of the Spectrometer:

Data Acquisition: Place the prepared sample in the FTIR spectrometer and ensure it is correctly positioned. Select the appropriate acquisition parameters, such as spectral resolution, wavelength range, number of scans, etc. Then, initiate the measurement by recording the infrared absorption spectra of the sample.

Data Analysis: Once the data is acquired, perform a Fourier transform to convert the raw data into an FTIR spectrum. Analyze the FTIR spectrum by identifying characteristic absorption bands corresponding to the molecular vibrations present in the sample. Compare the obtained spectra with spectral libraries or known references to identify the chemical components present in the tested sample.

Interpretation of Results: Interpret the FTIR data based on known information about the chemical composition of the extract/sample. Finally, identify the characteristic functional groups such as O-H, C-H bonds, sugars, amino acids, etc. **(Science Info, 2023)**

II. Evaluation of In *Vivo* Anti-Inflammatory Activity:

II.1. Animal Material :

Fifty female NMRI mice (Mus musculus; mean body weight 25 ± 5 g) were procured from the Pasteur Institute (Algiers, Algeria) and maintained under standardized laboratory conditions at the **Pharmacognosy and Api-Phytotherapy Research Laboratory** (University of Mostaganem).

Following arrival, animals underwent a 10-day acclimatization period in polypropylene cages with environmental parameters strictly maintained Standard housing conditions (12:12 light: dark light cycle system)

Throughout the study, mice received ad libitum access to drinking water and standard rodent chow (15 ± 5 g/mouse/day), with cage changes performed twice weekly.

II.2. Acute toxicity Test:

II.2.1. The Pprinciple:

The toxicological evaluation of the extracts was conducted in accordance with Test No. 425 of the guidelines established by the Organization for Economic Co-operation and Development **(OECD, 2008).**

A comprehensive clinical monitoring protocol was implemented, evaluating toxicological manifestations across all major physiological systems at predetermined intervals (1, 24, and 48 hours) post-administration, with subsequent daily observations maintained throughout the 14-day experimental period.

2.2.2. The Protocol:

For the nanoparticle extract evaluation, a dose-range finding study was designed utilizing four distinct concentration levels (150, 300, 1000, and 2000 mg/kg body weight) The doses were administered by intragastric gavage with a unique dose, each administered to separate experimental cohorts to ensure independent assessment of dose-response relationships.

II.2.3.Distribution of mice:

The experimental groups, designated as :

***Groupe01(n=3)** was administered a unique dose of ZnO NPs extract at a concentration of 150 mg/kg via intragastric gavage.

***Groupe02(n=3)** was administered a unique dose of ZnO NPs extract at a concentration of 300 mg/kg via intragastric gavage.

***Groupe03(n=3)** was administered a unique dose of ZnO NPs extract at a concentration of 1000 mg/kg via intragastric gavage.

***Groupe04(n=3)**was administered a unique dose of ZnO NPs extract at a concentration of 2000 mg/kg via intragastric gavage.

II.3. In *vivo* anti-inflammatory activity:

To evaluate the in vivo anti-inflammatory activity of nanoparticle suspensions, the paw edema test induced by carrageenan was selected, following the method described by **Winter et *al*.** (1962). The injection of carrageenan under the plantar aponeurosis of the mice triggers an inflammatory response that can be alleviated by an anti-inflammatory agent. Diclofenac was selected as the reference drug due to its well-established pharmacological profile. Prior to the experiment, the mice were food-deprived for 16 hours. Two protocols, curative and preventive, were implemented to measure the activity as follows:

II.3.1. Curative Testing (Therapeutic Treatment):

Sixty minutes after inducing edema with carrageenan, the mice received nanoparticle suspensions via intragastric gavage, as detailed below:

- **Group C (Control Group)**: Represents the control group.
- **Group Inf (Inflammatory Group)**: Includes mice with inflammation that received distilled water by i.g.g.
- **Group STD**: Corresponds to the STANDARD group, which received 50 mg/kg of Diclofenac by i.g.g.
- **Group D01 Cr (Curative Group Dose 01)**: Includes mice that received 150 mg/kg of *Myrtus communis L.* extract containing ZnO nanoparticles by i.g.g.

- **Group D02 Cr (Curative Group Dose 02)**: The mice received 300 mg/kg of *Myrtus communis L*. extract with ZnO nanoparticles by i.g.g.

II.3.2. Preventive Testing (Prophylactic Treatment):

For the preventive protocol, 25 mice were divided into five groups and received the nanoparticle suspensions by intragastric gavage (i.g.g) using a sterile feeding tube, as detailed below:

- **Group C (Control Group)**: This group received distilled water via intragastric gavage.
- **Group Inf (Inflammatory Group)**: This group consisted of mice with inflammation that also received distilled water via gastric gavage.
- **Group STD**: This group represents the STANDARD, with an administration of 50 mg/kg of Diclofenac by i.g.g.
- **Group D01 Pr (Preventive Group Dose 01)**: This group received 150 mg/kg of *Myrtus communis L.* extract containing ZnO nanoparticles by i.g.g.
- **Group D02 Pr (Preventive Group Dose 02)**: The mice received 300 mg/kg of *Myrtus communis L.* extract with ZnO nanoparticles by i.g.g.

One hour after intragastric gavage i.g.g, edema was induced by intraplantar injection of 0.1 ml of a 1% λ-carrageenan solution (freshly prepared in a 0.9% NaCl solution) into the right hind paw. The paw volumes were measured using a caliper one hour prior to λ-carrageenan injection and hourly for six hours.

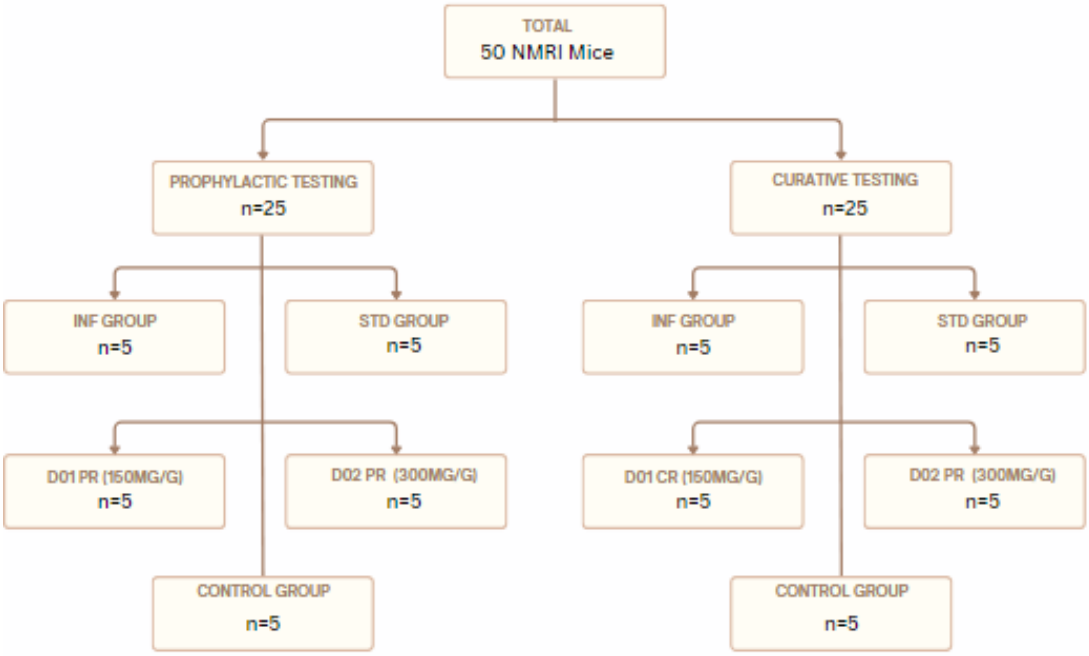

Figure25: Distribution of experimental groups.

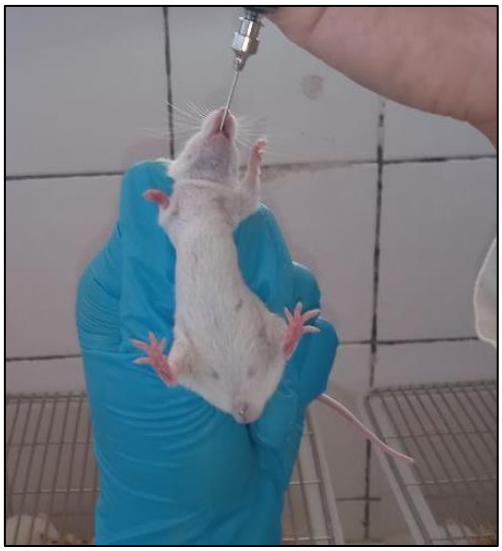

Figure26: Intragastric gavage administration.

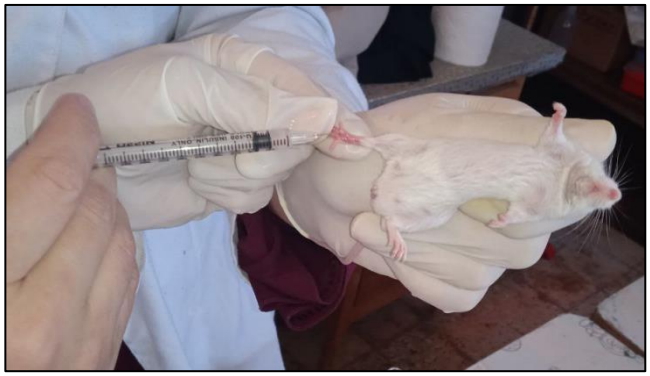

Figure27: Inflammation induction with carrageenan injection.

II.4. Studied Parameters:

II.4.1. Paw Edema Measurement:

The diameter of the mice's paw volume was measured using a digital caliper before and after inflammation induction by carrageenan at one-hour intervals for six successive hours.

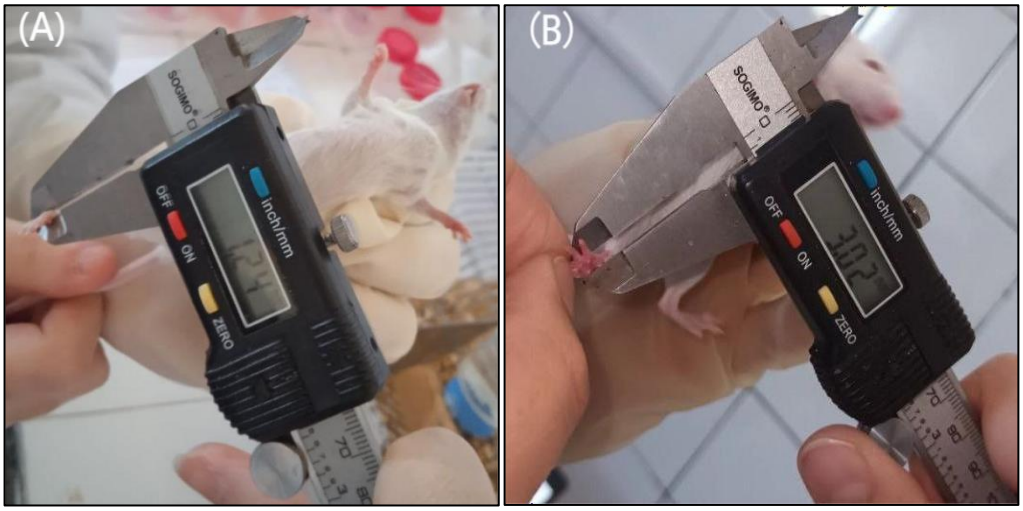

Figure28: Measurement of the paw volume before(A) the inducing inflammation and after(B).

II.4.1.1. Percentage Increase in Paw volume (%AUG):

The Percentage increase (%AUG) in the edema was calculated for each mouse, which is determined by the following formula **(Ndiaye et al, 2006)**

$$\%AUG = (Dx-Do) \times 100/Do$$

DX: Diameter of the paw at x hour after the injection of carrageenan.

Do: Diameter of the paw before the injection of carrageenan .

II.4.1.2. Percentage Inhibition of Paw volume (%INH):

The percentage inhibition (%INH) of paw edema was calculated for each mouse that is treated compared with inflammatory mice (Inf), which is determined by the following formula **(Ndiaye et al, 2006).**

$$\%INH = (\%AUG\ Inf - \%AUG\ treated) \times 100/\%AUG\ Inf.$$

Inf: Inflamed mice group.

AUG: treated

II.4.2. Histological Analysis:

The histological study was carried out according to the Anatomical and Cytopathology Techniques Manual by **Marck, (2010).**

It was performed at the Pharmacognosy & Api-Phytotherapy Research Laboratory at the University of Mostaganem. At the end of the experiment, the mice were anesthetized by inhalation with Chloroform. Their paws were then removed and immediately placed in 10% formaldehyde. **(Annexe)**

Following several steps in the right order **(Tab.04)** Pictures shown in **Annexe**

Table 04: Histological study steps

Paws	
Post-fixation	The collected paws are drained in a solution of 10% formalin.
Decalcification	Due to the hardness of bone tissue, chemical decalcification is required to remove calcium and soften the tissue. This process facilitates the preparation of thin histological sections suitable for microscopic examination. During decalcification, the paws are immersed in a decalcifying solution, which is 10% hydrochloric acid.
Macroscopic	A detailed macroscopic examination is an essential part of the study of an operative piece, so our pieces are examined, measured, weighed, palpated and then dissected. Preparation of the fragments, and then we introduce these fragments into inclusion cassettes. The cassettes were

Paws		
	marked on their edge.	
Circulation	**Dehydration**	*1 Glass staining dish of 96% ethanol for 1 hour *1 Glass staining dish of 96% ethanol for 1 hour *1 Glass staining dish of acetone for 2 hours
	Substitution	*1 Glass staining dish of toluene / xylene for 2 hours
	Impregnation	*1 Glass staining dish of paraffin at 70°C for 1 hour.
Inclusion and coating	Placing the sampled part in a steel mould and coating it with liquid paraffin. Once the block is prepared, it is stored in a freezer (-20°C).	
Microtomy	This step allows the cuts to be made on the block using a microtome. All the slices obtained from a ribbon of very thin quality (2 to 4 µm). The sections are then spread on microscope slides using a hot plate to avoid the formation of wrinkles and streaks.	
Coloration	Before the dewaxing step, the slides must be dried to facilitate the adhesion of the sections to the microscope slide. This firing is carried out in a laboratory oven at 58°C for 1 hour.	
	Dewaxing	The first step in any staining of a histological section is to remove the paraffin from the tissue so that the dyes can penetrate it. *1 Glass staining dish of toluene / xylene for 10 min.

Paws		
	Rehydration	Consists of gradually replacing the solvent of the tissue with ethanol baths to bring it to the water. *1 Glass staining dish of 70% ethanol for 5 minutes *1 Glass staining dish of 80% ethanol for 5 minutes *1 Glass staining dish of 96% ethanol for 5 minutes Rinse with water for 10 minutes
	Coloring; Staining with Hematoxylin/Eosin	*1 Glass staining dish of Harris hematoxylin for 5-10 minutes *1 Glass staining dish of acidified water *1 Glass staining dish water tank * 1 Glass staining dish of 96% ethanol * 1 Glass staining dish of eosin for 5 minutes * 2 Glass staining dish of acetone, 5 min each * 1 Glass staining dish of toluene or xylene until assembly.
Montage	This operation consists of using a synthetic resin (EUKITT solution) to fix a coverslip on the section (the slide) in order to protect it from the chemical degradation of the dyes which oxidize in the air and mechanical breakage.	
Micro-scopic observation	The microscopic examination was performed using a photonic microscope, and each section was photographed.	

II.5. Statistical Analysis:

The results obtained from mice paw Edema measurements (%AUG and %INH) were presented as mean (an average value) ± SD. Then they have been analyzed using Student's t-test with XLstat.

The values of $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ has been considered as :

-Significant (*), Very significant (**) and highly significant (***) compared to the inflammatory group (Inf) respectively.

-Significant (#), Very significant (##) and highly significant (###) compared to STD Respectively as well.

Results
&
Discussion

II. Evaluation of *in vivo* anti-Inflammatory activity:

II.1. Acute Toxicity Test:

No signs of toxicity (behavioral, neurological, respiratory abnormalities...) were observed in mice during the 14-day observation period following intra gastric gavage administration of ZnO nanoparticles at doses of (150, 300,1000 and 2000mg/kg). **(Tab07)**

Table 07: The results of toxicity signs observed during the 14 days after the administration of ZnO nanoparticles.

Doses	General Physical Signs (Macroscopic Observation) : • Hunched posture • Pilocrection (fur standing up) • Hypothermia	Gastrointestinal & Excretory Signs : • Diarrhea • Abdominal distension • Bloody stools	Skin & Fur : • Alopecia (hair loss) • Swelling • Skin ulcers	Severe Toxicity : • Death
NP Extract : Lot 01 " 150mg "	-	-	-	-
NP Extract : Lot 02 " 300mg "	-	-	-	-
NP Extract : Lot 03 " 1000mg "	-	-	-	-
NP Extract : Lot 04 " 2000mg "	-	-	-	-

(-): No signs

Researchers are increasingly interested in phytochemicals as natural anti-inflammatory agents, in connection with advancements in nanotechnology **(Abidullah et *al*., 2022).** Traditional methods of nanoparticle synthesis are often toxic and pose environmental risks, making biosynthesis, which is more eco-friendly and safer, increasingly favored **(Hasan et *al*., 2021).** Plant-mediated synthesis is considered a sustainable approach **(Edo et *al*., 2025).** In this context, a study was conducted to evaluate the anti-inflammatory efficacy of biosynthesized zinc oxide nanoparticles (ZnO NPs) in vivo.

Before conducting in vivo experiments, it is crucial to assess the safety profile of zinc oxide nanoparticles (ZnO NPs) due to their potential applications in medicine and industry. Toxicity testing is an essential first step in identifying any adverse effects that may result from the administration of these nanoparticles **(Oberdörster et *al*., 2005).**

In our study, we performed an acute toxicity test by administering varying doses of *Myrtus communis L*. biosynthesized ZnO NPs via oral gavage. The results showed that no behavioral changes or mortality were observed in mice, even at high doses up to 2000 mg/kg body weight over a 14-day observation period. These observations suggest that ZnO NPs, at the tested concentrations, do not have immediate toxic effects, which is promising for their future use **(Zhang et *al*., 2020).**

The absence of acute toxicity is consistent with other studies that have also reported low toxicity of ZnO NPs at similar doses **(Rajeswari et *al*., 2023; Soliman et *al*., 2023).** For instance, **Ahamed et *al*. (2010)** demonstrated that zinc oxide nanoparticles do not induce DNA damage in human cells at specific concentrations.

It is important to note that the safety of nanoparticles can vary depending on their size, shape, and synthesis method, highlighting the importance of rigorous evaluation for each type of nanoparticle. These results pave the way for further studies on the anti-inflammatory efficacy of ZnO NPs and reinforce their potential as safe therapeutic agents in medical applications **(Gopinath et *al*., 2014; Hussain et *al*., 2005).**

II. Evaluation of *in-vivo* Anti-Inflammatory Activity:

Evaluation of anti-inflammatory activity in vivo by Paw edema measurement:

The carrageenan-induced mouse paw inflammation model was used to evaluate both the preventive (pre-treatment) and curative (post-treatment) anti-inflammatory effects of zinc oxide nanoparticles. The quantification of paw edema through caliper or plethysmometry measurement provides a reliable and reproducible method to assess subcutaneous inflammation in the well-established carrageenan-induced mouse model. This standardized experimental approach remains one of the most practical and widely adopted systems for screening the anti-inflammatory potential of pharmacological compounds, as demonstrated in numerous studies **(Cuzzocrea et *al.*, 1998; Gilroy et *al.*, 1999; Chauhan et *al.*, 2018)**

II.1. Curative assessment:

II.1.1. Percentage Increase in Paw Edema in the curative group treated with Zinc oxide nanoparticles extract (%AUG):

The histogram illustrates the anti-inflammatory effects of intraplantar carrageenan across four groups: C-INF (inflammation control), STD (diclofenac 50 mg/kg), NP's CRD1 (ZnO NPs 150 mg/kg), and NP's CRD2 (ZnO NPs 300 mg/kg). The C-INF group showed a significant increase in %AUG from the first hour, peaking before a slight decline at 6 hours. In contrast, both ZnO NP groups (CRD1 and CRD2) demonstrated statistically significant-inflammatory effects versus control ($p<0.001$) starting at the first hour. The higher dose (CRD2) exhibited rapid onset, achieving maximal significance ($p<0.001$) by the third hour, with progressively enhanced efficacy through later timepoints. The STD group displayed extremely significant effects from hour 2 ($p<0.001$), sustaining this through hours 5–6. Comparative analysis revealed CRD2 matched diclofenac's anti-inflammatory efficacy in the late phase (both $p<0.001$), with ZnO NPs showing marginally earlier numerical improvement at intermediate stages ($p<0.05$ vs. STD from hour 4 onward). Annotations highlight highly significant reductions in %AUG for both ZnO NPs versus control ($p<0.001$). These results underscore ZnO nanoparticles particularly CR D02 as a promising therapeutic alternative, offering comparable efficacy to standard diclofenac with potential

early-phase advantages in mitigating inflammatory responses. **Fig (31)**

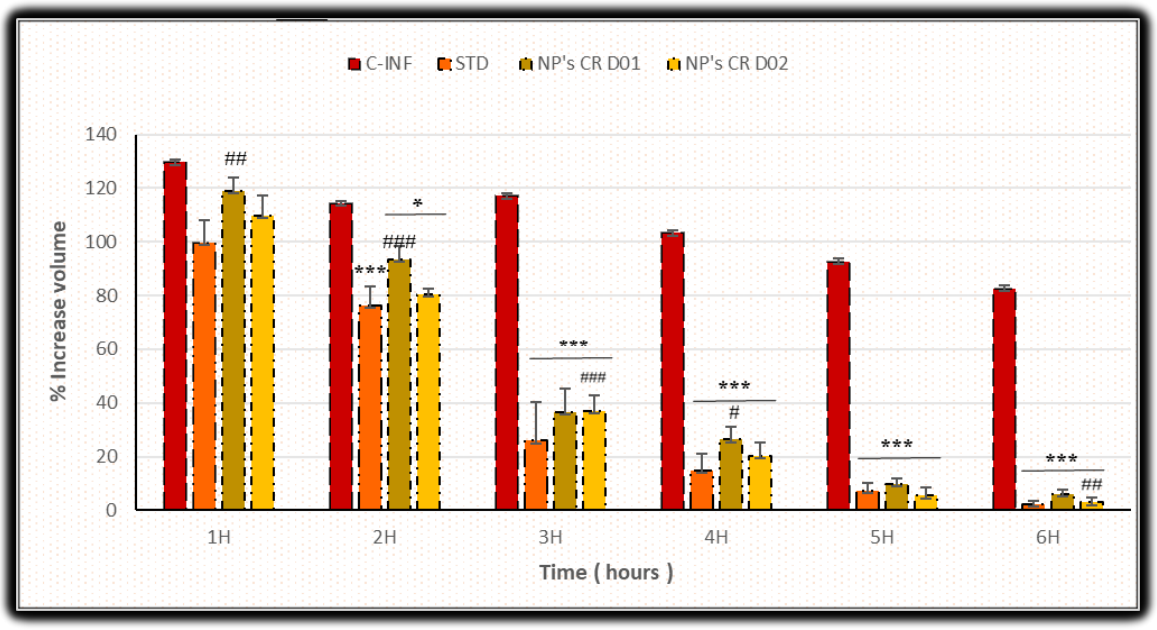

Figure 31: Percentage increase in paw edema (%AUG) over the six-hour period following inflammation induction. C-INF: Inflammation control group; groups treated with Zinc oxide nanoparticles (ZnO NPs): ZnO NPs CRD1 (150 mg/kg), CRD2 (300 mg/kg); Standard group treated with Diclofenac at 50 mg/kg (STD). Values are expressed as means ± SEM in each group (n=5). *P < 0.05 (significant), **P < 0.01 (highly significant), ***P < 0.001 (extremely significant) compared to the C-inf group. #P < 0.05 (significant), ##P < 0.01 (highly significant), ###P ≤ 0.001 (extremely significant) compared to the standard (STD) group.

II.1.2. Percentage Inhibition of Inflammatory Edema in the curative group treated with zinc oxide nanoparticles extract (%INH):

The anti-inflammatory efficacy of zinc oxide nanoparticles (ZnO NPs) was evaluated in a carrageenan-induced murine paw edema model. Treatment with 150 mg/kg ZnO NPs (D01 CR) demonstrated highly significant inhibition (P < 0.01) at multiple timepoints compared to the standard group, though with intermittent periods of non-significant activity. The higher-dose ZnO NP group (D02 CR, 300 mg/kg) showed more consistent but nonsignificant effects throughout the observation period when compared to STD. The reference standard diclofenac group (STD, 50 mg/kg) maintained extremely significant

anti-inflammatory effects ($P \leq 0.001$) from hour 2 through hour 6. Comparative analysis revealed that while CR 01 achieved statistically comparable effects to STD at selective timepoints, from the 4th hour up till the 6th , neither ZnO NP's formulation matched the sustained, extremely significant suppression demonstrated by the standard treatment throughout the entire observation period. **Fig (32)**

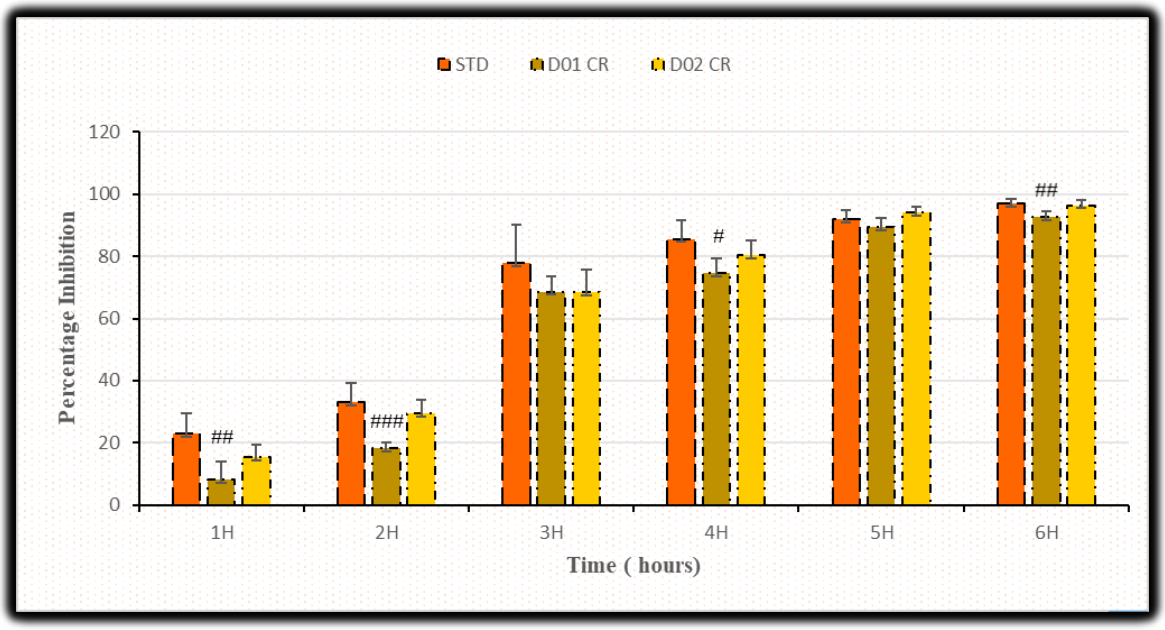

Figure 32: Percentage inhibition of paw edema (%INH) during the six-hour period following inflammation induction. Groups treated with zinc oxide nanoparticles (ZnO NPs): CRD1 (150 mg/kg), CRD2 (300 mg/kg), and the standard group treated with diclofenac (STD) at 50 mg/kg. Values are expressed as means ± SEM for each group (n=5). #P<0.05 (significant), ##P<0.01 (highly significant), ###P<0.001 (extremely significant) compared to the standard group (STD).

II.2. Prophylactic assessment:

II.2.1. Percentage Increase in Paw Edema in the preventive (prophylactic) group treated with zinc oxide nanoparticles extract (%AUG):

The intraplantar administration of carrageenan induced significant edema in the control group (INF). In contrast, zinc oxide nanoparticles (ZnO NPs) at doses of 150 mg/kg (D01 Pr) and 300 mg/kg (D02 Pr) demonstrated modest but statistically significant anti-inflammatory effects, with maximum efficacy observed between hours 5 and 6. The D02 Pr group showed a significant reduction ($P<0.05$) in %AUG from the 2nd hour till the third, while D01 Pr exhibited a highly significant reduction ($P<0.01$). Both groups achieved extremely significant reductions in %AUG ($P<0.001$) between hours 5 and 6. Comparatively, the standard diclofenac treatment (STD) displayed extremely significant anti-inflammatory effects ($P<0.001$) from hours 2 to 6, establishing a robust benchmark. The histogram illustrates these results, showing significant reductions in the ZnO NP groups compared to the control group and confirming the effectiveness of the standard treatment. **Fig (38)**

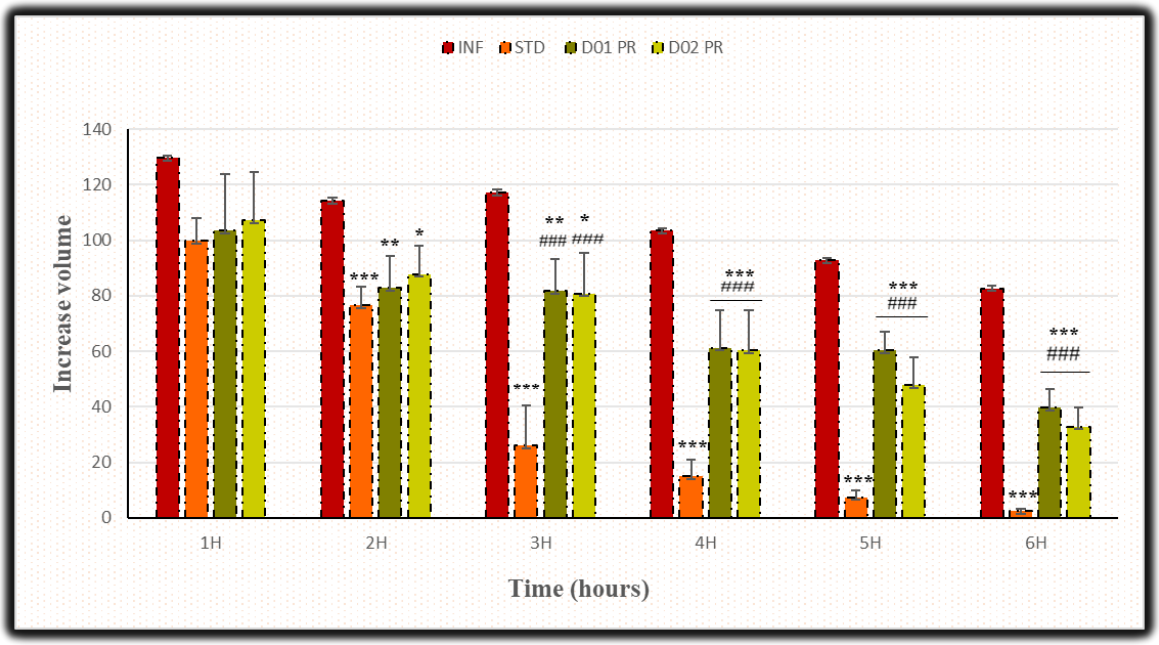

Figure 38: Percentage increase in paw edema (%AUG) over the six-hour period following inflammation induction. C-INF: Inflammation control group; groups pre-treated with Zinc oxide nanoparticles (ZnO NPs): ZnO NPs PRD1 (150 mg/kg), PRD2 (300 mg/kg); Standard group treated with Diclofenac at 50 mg/kg (STD). Values are expressed as means

± SEM in each group (n=5). *P < 0.05 (significant), **P < 0.01 (highly significant), ***P < 0.001 (extremely significant) compared to the C-inf group. #P < 0.05 (significant), ##P < 0.01 (highly significant), ###P ≤ 0.001 (extremely significant) compared to the standard (STD) group.

II.2.2. Percentage Inhibition in Paw Edema in the preventive (prophylactic) group treated with zinc oxide nanoparticles extract (%INH):

The percentage inhibition (%INH) of paw edema showed distinct patterns among treatment groups. The standard diclofenac (STD) group exhibited significant efficacy, with extremely significant inhibition at 2 hours (##P < 0.01) till 6 hours (###P < 0.001), but only marginal effects at other times. In contrast, zinc oxide nanoparticles (ZnO NPs) PRD1 (150 mg/kg) showed weaker inhibition without statistical significance, while PRD2 (300 mg/kg) achieved significant inhibition by 5hr (#P < 0.05), though still less effective than STD (###P < 0.001). The STD group had a rapid onset of action, whereas both ZnO NP groups required prolonged exposure for detectable effects, with PRD2 showing delayed but dose-responsive inhibition.

The histogram highlighted these differences, illustrating the quick response of Diclofenac compared to the gradual increase in inhibition with ZnO NPs. **Fig (34)**

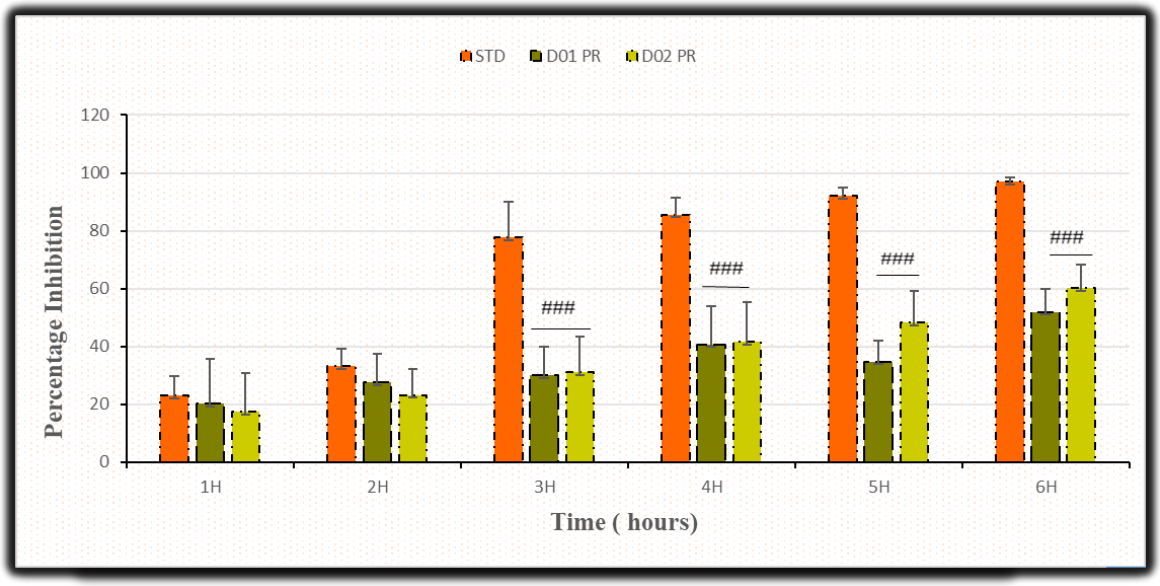

Figure 39: Percentage inhibition of paw edema (%INH) during the six-hour period following inflammation induction. Groups pre-treated with zinc oxide nanoparticles (ZnO NPs): PRD1 (150 mg/kg), PRD2 (300 mg/kg), and the standard group treated

with diclofenac (STD, 50 mg/kg). Values are expressed as means ± SEM for each group (n=5). #P < 0.05 (significant), ##P < 0.01 (highly significant), ###P < 0.001 (extremely significant) compared to the standard group (STD).

Conclusion

Conclusion

Green synthesis, especially plant-mediated methods, offers a sustainable and safe approach to nanoparticle production, reducing environmental impact and costs while advancing nanotechnology.

Our experiment was primarily based on the *in vivo* evaluation of the anti-inflammatory activity of the aqueous extract ZnO NPs biosynthesized from *Myrtus communis L.* leaf extract.

The biosynthesized ZnO NPs exhibited no acute toxicity at doses up to 2000 mg/kg, indicating a favorable safety profile for in vivo applications.

The carrageenan-induced paw edema model confirmed that ZnO NPs, particularly at both doses 300 mg/kg D02 Cr for the Curative assassement and and 300mg/kg D02 Pr for the prophylactic assassement, exerting an anti-edematous activity, comparable to the reference drug diclofenac (50 mg/kg). This effect was statistically significant. D02 Pr showed a delayed effect at the late hours of the experiment starting from the 5th hour.

Histopathological analyses substantiated these findings, revealing reduced edema, minimal leukocyte infiltration, and restored tissue architecture in treated groups, especially with the D02 Cr (300 mg/kg) dose, as for D02 Pr (300mg/kg) showed a reduced but yet persistent edema inflammatory infiltration. The presence of flavonoids, alkaloids, tannins, and terpenoids (confirmed through FTIR spectroscopy) suggests a synergistic mechanism between the ZnO core and bioactive plant compounds, enhancing anti-inflammatory efficacy.

These results propose that ZnO NPs synthesized from *Myrtus communis L.* not only offer a biocompatible and eco-friendly alternative to conventional anti-inflammatory drugs but also benefit from the intrinsic therapeutic properties of plant-derived compounds. The observed pharmacological activity is likely attributed to both the metallic core and the phytochemical surface coating, reinforcing the concept of synergy in green nanomedicine.

In conclusion, the biosynthesized ZnO NPs represent a promising and multifunctional nanomaterial for anti-inflammatory therapy, warranting continued research and potential

development into safe, plant-based nano pharmaceuticals.

Further investigations are recommended to elucidate the precise molecular mechanisms underlying this anti-inflammatory activity and to isolate, quantify, and characterize the bioactive phytoconstituents responsible for nanoparticle formation and stabilization. Advanced analytical techniques such as X-Ray Diffraction (XRD), Transmission Electron Microscopy and Scanning Electron Microscopy (TEM/SEM) image analysis techniques should be employed to complement FTIR data and deepen our understanding of the structure-activity relationships.

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Annexe

Annexe 01: Percentage Increase in Paw Edema in the curative and prophylactic group treated & pre-treated with Zinc oxide nanoparticles extract (%AUG)

Mean				
%AUG	C-INF	STD	NP's CR D01	NP's CR D02
1H	129,5827368	99,67620765	103,5449988	107,1725188
2H	114,2721663	76,44226075	82,89973792	87,80600496
3H	117,0872969	26,00478162	81,82362295	80,84261356
4H	103,3888154	14,90012166	61,28948733	60,44289949
5H	92,66377911	7,441221488	60,46060378	47,8190644
6H	82,59512673	2,409117834	39,70153678	32,91569331

Standard deviation				
%AUG	INF	STD	NP's CR D01	NP's CR D02
1H	28,62021177	8,36398076	5,12421407	7,63233892
2H	16,18478765	6,87906521	4,88264551	2,28917588
3H	20,71680155	14,3821706	8,57433997	5,71715606
4H	5,032474386	6,18947241	4,95507785	4,85557369
5H	11,28430816	2,62833817	1,79474209	2,91025151
6H	11,77630176	0,99835649	1,36997036	1,67779143

Mean				
%AUG	C-INF	STD	NP's PR D01	NP's PR D02
1H	129,5827368	99,67620765	118,9521041	109,6747472
2H	114,2721663	76,44226075	93,40875695	80,45152387
3H	117,0872969	26,00478162	36,69122042	36,95818626
4H	103,3888154	14,90012166	26,33351195	20,29659313
5H	92,66377911	7,441221488	9,936710368	5,533735307
6H	82,59512673	2,409117834	6,149081913	2,929278468
Standard deviation				
%AUG	INF	STD	NP's PR D01	NP's PR D02
1H	28,62021177	8,363980764	20,31838156	17,22542373
2H	16,18478765	6,879065212	11,39751927	10,35978571
3H	20,71680155	14,38217064	11,27160106	14,69566208
4H	5,032474386	6,189472414	13,46598114	14,39511891
5H	11,28430816	2,628338167	6,570197046	9,812487653
6H	11,77630176	0,99835649	6,621972772	6,69211719

Annexe 02: Percentage Inhibition of Inflammatory Edema in the curative and prophylactic group treated & pre-treated with Zinc oxide nanoparticles extract (%INH)

Mean			
%INH	**STD**	**NP's D01 CR**	**NP's D02 CR**
1H	**23,0790998**	**8,203741452**	**15,36314953**
2H	**33,10509178**	**18,25764751**	**29,59657064**
3H	**77,79026222**	**68,66336366**	**68,43535785**
4H	**85,58826543**	**74,52963182**	**80,3686762**
5H	**91,96965464**	**89,27659711**	**94,02815711**
6H	**97,08322037**	**92,55515167**	**96,45344879**
Standard deviation			
%INH	**STD**	**NP's D01 CR**	**NP's D02 CR**
1H	**6,45454863**	**5,88993496**	**3,95439562**
2H	**6,01989569**	**2,00326638**	**4,27282134**
3H	**12,2832886**	**4,88281497**	**7,32303179**
4H	**5,98659767**	**4,69642066**	**4,79266334**
5H	**2,83642454**	**3,14065705**	**1,93683239**
6H	**1,20873534**	**2,03134434**	**1,65865762**

Mean			
%INH	**STD**	**NP's D01 PR**	**NP's D02 PR**
1H	**23,0790998**	**20,09352377**	**17,29413853**
2H	**33,10509178**	**27,45412936**	**23,16063673**
3H	**77,79026222**	**30,11742083**	**30,95526526**
4H	**85,58826543**	**40,71942204**	**41,53826095**
5H	**91,96965464**	**34,75271098**	**48,39508505**
6H	**97,08322037**	**51,93234958**	**60,14814116**

Standard deviation			
%INH	**STD**	**NP's D01 PR**	**NP's D02 PR**
1H	**6,454548632**	**15,67985217**	**13,29299269**
2H	**6,01989569**	**9,974011727**	**9,065887214**
3H	**12,28328864**	**9,626664342**	**12,55103027**
4H	**5,986597668**	**13,02460144**	**13,9232845**
5H	**2,836424536**	**7,090361638**	**10,58934542**
6H	**1,208735344**	**8,017389202**	**8,102314816**

Annexe 03 : Animal Material, Female NMRI mice.

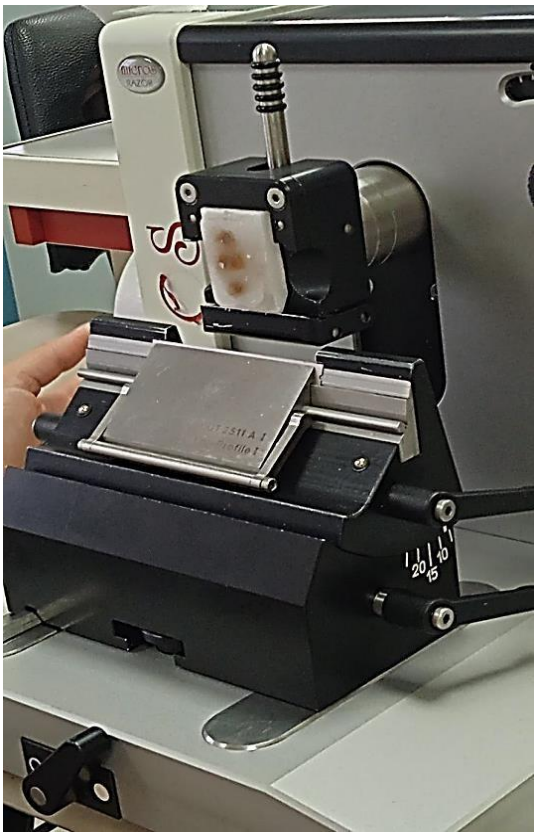
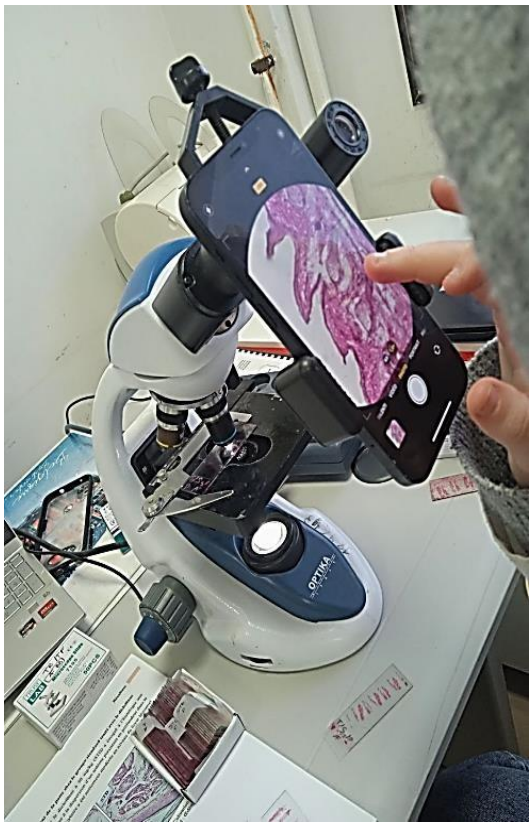
Annexe 04 : Caliper.

Annexe 05 : Histology steps.

Post-fixation

Dehydration

Inclusion

Microtomy	Microscpic analysis
	

الجمهورية الجزائرية الديمقراطية الشعبية

وزارة التعليم العالي والبحث العلمي

جامعة عبد الحميد بن باديس-مستغانم-

كلية علوم الطبيعة و الحياة

تصريح شرفي خاص بالالتزام بقواعد النزاهة العلمية لإنجاز البحث

أنا الممضي أدناه،

الطالب(ة): بلحول فراح .... رقم التسجيل الجامعي: 202037031311

الحامل لبطاقة التعريف الوطنية رقم: 11002111002419000 والصادرة بتاريخ: 2025/06/19

عن: بلدية عين تادلس - مستغانم

المسجل ب كلية علوم الطبيعة و الحياة/ قسم البيولوجيا

شعبة علوم بيولوجية./ التخصص علم الصيدلة و السموم

والمكلف بإنجاز مذكرة ماستر بعنوان:

Evaluation de l'effet anti-inflammatoire de quelque nanoparticules biosynthétisés : étude in vivo

أصرح بشرفي أني ألتزم بمراعاة المعايير العلمية والمنهجية ومعايير الأخلاقيات العلمية والنزاهة الأكاديمية المطلوبة في إنجاز البحث ، وأتحمل المسؤولية الشخصية عن كل المحتوى المتضمن في البحث المذكور أعلاه .

التاريخ: 10/07/2025

إمضاء المعني

* ملحق القرار الوزاري رقم 933 المؤرخ في 28 جويلية 2016 الذي يحدد القواعد المتعلقة بالوقاية من السرقة العلمية ومكافحتها.

الجمهورية الجزائرية الديمقراطية الشعبية

وزارة التعليم العالي والبحث العلمي

جامعة عبد الحميد بن باديس-مستغانم-

كلية علوم الطبيعة و الحياة

تصريح شرفي خاص بالالتزام بقواعد النزاهة العلمية
لإنجاز البحث

أنا الممضي أدناه،

الطالب(ة): بن عودة أسماء رقم التسجيل الجامعي: 202037034538

الحامل لبطاقة التعريف الوطنية رقم 110020064034 والصادرة بتاريخ: 2025/07/07

عن بلد بئر ماسرى

المسجل ب كلية علوم الطبيعة و الحياة/ قسم البيولوجيا

شعبة علوم بيولوجية./ التخصص علم الصيدلة و السموم

والمكلف بإنجاز مذكرة ماستر بعنوان:

Evaluation de l'effet anti-inflammatoire de quelques nanoparticules biosynthétisées - étude in vivo -

أصرح بشرفي أني ألتزم بمراعاة المعايير العلمية والمنهجية ومعايير الأخلاقيات العلمية والنزاهة الأكاديمية المطلوبة في إنجاز البحث ، وأتحمل المسؤولية الشخصية عن كل المحتوى المتضمن في البحث المذكور أعلاه .

التاريخ: 2025/07/10

إمضاء المعني

* ملحق القرار الوزاري رقم 933 المؤرخ في 28 جويلية 2016 الذي يحدد القواعد المتعلقة بالوقاية من السرقة العلمية ومكافحتها.