الجمهورية الجزائرية الديمقراطية الشعبية

PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA

وزارة التعليم العالي والبحث العلمي

MINISTARY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH

جامعة 20 أوت 1955- سكيكدة

UNIVERSITY 20 AOÛT 1955- SKIKDA

Faculty of Sciences

Department of Natural and Life Sciences

A Dissertation Presented to Obtain a Diploma of Master

Branch: Biological Sciences

Option: Applied Biochemistry

Titled

In Vivo Study of the Anti-Inflammatory Effect of Bee Venom and Hesperidin

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University Year 2024/2025

Acknowledgements

In the Name of Allah, the Most Gracious, the Most Merciful

First and foremost, we thank Allah Almighty for granting us the strength, patience, and guidance that enabled us to undertake and successfully complete this work. Without His divine assistance, this journey would not have been possible.

*We would like to express our sincere and heartfelt gratitude to our supervisor, **Dr. Belambri Sahra Amel,** for her continuous support, wise guidance, and unwavering patience throughout this research. Her scientific insight and dedication were instrumental in every stage of this project. We are deeply honored to have worked under her supervision.*

*We extend our special thanks to **Dr. Bouhadouda Nabila**, who honored us by examining this work. Please accept the expression of our sincere appreciation and deepest respect.*

*We are equally thankful to **Dr. Ouamane Souhila** for graciously agreeing to preside over the jury. Her presence and thoughtful evaluation during the defense were greatly appreciated. Please accept the expression of our deepest esteem and warmest thanks.*

*A very special mention goes to **Dr. Aouazal Badis**, whose generous assistance whether through scientific advice, technical help, or providing necessary materials was of immense value throughout the realization of this work. His availability, support, and kindness did not go unnoticed, and we extend to him our heartfelt thanks.*

*Lastly, we would like to acknowledge all **the staff of the Biology Department,** whose direct or indirect contributions, encouragement, and cooperation have supported us during this academic journey.*

To all who have helped us, in ways both great and small, we express our most sincere gratitude.

Dédicace

À moi,
pour cette obsession du détail, cette clarté dans le chaos,
et cette volonté tranquille de faire juste et fort,
là où chaque silence fut promesse de maîtrise.

Papa, Maman, Ines,
Soutiens solides à tous les étages de ce parcours,
par le cœur, par l'attention, par la foi en moi.
Ce mémoire, je le dédie à moi, mais je le partage avec vous.

Sabrine

Dedication

وَمَا تَوْفِيقِي إِلَّا بِاللَّهِ

"My success is only by Allah."
Qur'an, Surah Hud (11:88)

All praise is due to Allah (SWT), the One who never abandoned me.
Through every struggle, doubt, and silent tear, He granted me strength, clarity, and the will to continue.
This journey, with all its challenges and triumphs, is a reflection of His mercy and guidance.

To my beloved parents,
You are my greatest blessing. Your love, sacrifices, and silent prayers have been my shield and my strength.
You stood by me when I stumbled, believed in me when I questioned myself, and carried me with your unwavering support.
This achievement is not mine alone it is yours.
This work is dedicated to you, with all my heartfelt gratitude and deep affection.

To myself,
For enduring the long nights, the silent doubts, and the moments of deep exhaustion.
You chose perseverance when giving up felt easier.
You held on with patience and quiet strength.
And now you've made it.

Hadil

Dedication

الحمد لله رب العالمين، له الشكر أولاً وآخراً، ظاهرًا وباطنًا، على كل نعمة وهداية، وعلى توفيقه وسنده لي في كل خطوة من هذا الطريق.

All praise and thanks be to Allah, Lord of the Worlds for His endless mercy, strength, and presence in my life. Without His guidance and blessings, I would not have reached this stage. Every success I have achieved is by His will, and for that, I am forever grateful.

*I dedicate this work to **myself**, for choosing the path of biological sciences, for holding on through the doubts and challenges, and for continuing to believe in the beauty of science and learning. I am proud of the journey and excited for what lies ahead.*

*To my **dear parents**, thank you for your unconditional love, support, and sacrifices throughout every stage of my education. You are my strength and my peace. I hope this achievement makes you proud.*

*To my **brother and sister**, I wish you a future filled with success and fulfillment. Thank you for being part of this journey.*

*My heartfelt thanks go to **Dr. Sahra Bilambri**, an exceptional mentor and teacher. Your guidance, encouragement, and wisdom especially this past year have shaped not only my academic path but also my personal growth. I will always carry your words with me.*

*To my **cousin Yaknis**, thank you for your help and support with the tools and technical aspects of this work.*

*I also wish to thank **Dr. Bourouis Ahmed** and the entire **Al Amel Laboratory** team for the valuable opportunity and support during my final internship.*

*Special thanks to **Imene**, the biochemist at **EPS Laboratory**, for her generous guidance and willingness to share her experience during my training.*

*Lastly, I thank all the **health professionals** I had the honor of working with your dedication continues to inspire me.*

Aladine

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ABBREVIATIONS LIST

- **AP-1:** Activator Protein 1
- **ATP:** Adenosine Triphosphate
- **BV:** Bee Venom
- **BVT:** Bee Venom Therapy
- **CBP:** CREB-Binding Protein
- **COX:** Cyclooxygenase
- **DCs:** Dendritic Cells
- **DAMPs:** Damage-Associated Molecular Patterns
- **GM-CSF:** Granulocyte-Macrophage Colony-Stimulating Factor
- **GRE:** Glucocorticoid Response Elements
- **HPA:** Hypothalamic-Pituitary-Adrenal Axis
- **HSD:** Hesperidin
- **ICAM-1:** Intercellular Adhesion Molecule 1
- **IFNγ:** Interferon Gamma
- **IL:** Interleukin
- **LB:** B Lymphocyte
- **LT:** T Lymphocyte
- **LTB_4:** Leukotriene B_4
- **LTC_4 / LTD_4:** Leukotriene C_4 / D_4
- **MAPK:** Mitogen-Activated Protein Kinase
- **NaCl:** Sodium Chloride
- **NF-κB:** Nuclear Factor kappa B
- **NK cells:** Natural Killer Cells
- **NO:** Nitric Oxide
- **NSAIDs:** Non-Steroidal Anti-Inflammatory Drugs
- **SAIDs:** Steroidal Anti-Inflammatory Drugs
- **PAMPs:** Pathogen-Associated Molecular Patterns
- **PGE_2:** Prostaglandin E_2
- **PGD_2:** Prostaglandin D_2
- **$PGF_{2}\alpha$:** Prostaglandin F_2 Alpha
- **PGI_2:** Prostacyclin
- **RAST:** RadioAllergoSorbent Test
- **ROS:** Reactive Oxygen Species
- **SEM:** Standard Error of the Mean
- **TLR2 / TLR4:** Toll-Like Receptors 2 and 4
- **TNF-α:** Tumor Necrosis Factor Alpha

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Abstract

This experimental study aimed to evaluate the *in vivo* anti-inflammatory activity of two natural substances: Bee Venom (*Apis mellifera*) and Hesperidin a natural flavonoid. Three acute inflammation models were used in *Wistar* rats: xylene-induced ear edema, (1%)λ-carrageenan-induced paw edema, and (1%)carrageenan-induced acute peritonitis. Bee Venom was administered intraperitoneally twice a week at a dose of (0.8 mg/kg) during one month, while Hesperidin was administered orally at (400 mg/kg), one hour before inflammation induction. Aspirin (400 mg/kg) was used as the reference compound. In the ear edema test, Bee Venom induced a rapid and sustained inhibition, reaching 36% ($p<0.001$) at T2 and remaining around 28% ($p<0.001$) at T3, while Hesperidin showed a progressive effect, peaking at 30% ($p<0.001$) at T3. In the paw edema model, Bee Venom produced moderate inhibition, reaching 28% ($p<0.001$) at T4, while Hesperidin demonstrated activity ranging from 5 to 15%. In the peritonitis model, both Bee Venom and Hesperidin showed strong inhibition of leukocyte recruitment, reaching approximately 85–86%, indicating a marked anti-inflammatory effect. These results suggest that Bee Venom has a significant anti-inflammatory activity at the tested dose, and Hesperidin exerts a moderate anti-inflammatory effect.

Keywords: Bee Vnom, Hesperidin, Acute inflammation, Ear edema, Paw edema, Peritonitis, Carrageenan, Xylene, *Wistar* rats, Anti-inflammatory activity.

Résumé

Ce travail expérimental a été réalisé dans le but d'évaluer l'activité anti-inflammatoire *in vivo* de deux substances naturelles : le venin d'abeille (*Apis mellifera*) et l'hespéridine, un flavonoïde naturel. Trois modèles d'inflammation aiguë ont été utilisés chez le rat *Wistar* : l'œdème de l'oreille induit par le xylène, l'œdème de la patte induit par la λ-carraghénane (1 %), et la péritonite aiguë induite également par la λ-carraghénane (1 %). Le venin d'abeille a été administré par voie intrapéritonéale deux fois par semaine à la dose de (0,8 mg/kg) durant un mois, tandis que l'hespéridine a été administrée par voie orale à (400 mg/kg), une heure avant induction de l'inflammation. L'aspirine (400 mg/kg) a été utilisée comme molécule de référence. Dans le test de l'œdème de l'oreille, le venin d'abeille a induit une inhibition rapide et soutenue, atteignant 36% ($p<0.001$) à T2 et se maintenant autour de 28% à T3, tandis que l'hespéridine a montré une inhibition progressive, culminant à 30 % ($p<0.001$) à T3. Dans le test de l'œdème de la patte, le venin d'abeille a exercé une inhibition de 28% ($p<0.001$) à T4, tandis que l'hespéridine a présenté une activité variant de 5 à 15 %. Dans le modèle de péritonite, le venin d'abeille et la hespéridine ont montré une forte inhibition du recrutement leucocytaire, atteignant environ 85–86 %, indiquant une activité anti-inflammatoire cellulaire marquée. Ces résultats permettent d'attribuer au venin d'abeille un effet anti-inflammatoire considérable à la dose utilisée, et à la hespéridine une activité anti-inflammatoire modérée.

Mots-clés : Venin d'abeille, Hespéridine, Inflammation aiguë, Œdème de l'oreille, Œdème de la patte, Péritonite, Carraghénane, Xylène, Rats *Wistar*, Activité anti-inflammatoire.

ملخص

أُجري هذا العمل التجريبي بهدف تقييم النشاط المضاد للالتهابات داخل الجسم الحي لمادتين طبيعيتين: سم النحل (Apis mellifera) والهسبيريدين، وهو فلافونويد طبيعي. استُخدمت ثلاثة نماذج للالتهاب الحاد في فئران ويستار: وذمة الأذن المُستحثة بالزيلين، وذمة المخلب المُستحثة بـ(1%) λ-carrageenan ، والتهاب الصفاق الحاد المُستحث أيضًا بـ .(1%) λ-carrageenan أُعطي سم النحل داخل الصفاق مرتين أسبوعيًا بجرعة (0.8 ملغ/كغ) لمدة شهر، بينما أُعطي الهسبيريدين فمويًا بجرعة (400 ملغ/كغ) قبل ساعة واحدة من بدء الالتهاب. استُخدم الأسبرين (400 ملغ/كغ) كجزيء مرجعي. في اختبار وذمة الأذن، أحدث سم النحل تثبيطًا سريعًا ومستمرًا، حيث وصل إلى 36% (قيمة الاحتمال <0.001) عند T2 وبقي عند حوالي 28% عندT3 ، بينما أظهر الهسبيريدين تثبيطًا تدريجيًا، وبلغ ذروته عند 30% (قيمة الاحتمال <0.001) عند .T3 في اختبار وذمة المخلب، مارس سم النحل تثبيطًا بنسبة 28% (قيمة الاحتمال <0.001) عندT4 ، بينما أظهر الهسبيريدين نشاطًا يتراوح بين 5 و15%. في نموذج التهاب الصفاق، أظهر كل من سم النحل والهسبيريدين تثبيطًا قويًا لتجنيد كريات الدم البيضاء، حيث وصل إلى حوالي 85-86%، مما يشير إلى نشاط خلوي مضاد للالتهابات ملحوظ. تسمح لنا هذه النتائج بإسناد تأثير مضاد للالتهابات ملحوظ لسم النحل بالجرعة المستخدمة، وللهسبيريدين نشاط مضاد للالتهابات معتدل.

الكلمات المفتاحية: سم النحل، هسبيريدين، التهاب حاد، وذمة الأذن، وذمة المخلب، التهاب الصفاق، الكاراجينان، الزيلين، فئران ويستار، النشاط المضاد للالتهابات.

I. Introduction

Introduction

Inflammation is an essential defense mechanism of the immune system, triggered in response to various harmful stimuli such as pathogens, damaged cells, or toxic substances. It plays a crucial role in eliminating the cause of injury, clearing out damaged tissues, and initiating repair processes. This response typically manifests as an acute condition, characterized by rapid onset and short duration, accompanied by classic clinical signs such as redness, heat, swelling, and pain **(Tang *et al.*, 2023).** However, when the inflammatory response is excessive or prolonged, it may contribute to the development of several pathologies such as arthritis, cardiovascular diseases, type 2 diabetes, and certain neurodegenerative disorders **(Hotamisligil, 2011).**

The treatment of inflammatory conditions mainly relies on two classes of drugs: steroidal anti-inflammatory drugs (SAIDs) and non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs are widely used due to their rapid action and accessibility. However, long-term use is associated with significant side effects. Recent studies have shown that chronic NSAID consumption increases the risk of gastrointestinal complications, chronic kidney disease, and cardiovascular toxicity **(Panchal & Sabina, 2023 ; Soliman *et al.*, 2024)**. These therapeutic limitations have prompted growing interest in natural alternatives that offer both efficacy and improved safety profiles.

Phytotherapy, the use of plant-derived substances for medicinal purposes, has emerged as a promising avenue. Plants are rich in bioactive compounds such as flavonoids, polyphenols, and terpenes, many of which have demonstrated anti-inflammatory properties in various experimental models.

Bee Venom (*Apis mellifera*), also known as apitoxin, has been used for therapeutic purposes since ancient times, particularly in Egyptian, Greek, and Chinese medicine. This natural substance is a complex mixture of biologically active peptides (such as melittin and apamin), enzymes (including phospholipase A_2), and amines. In recent years, scientific interest in bee venom has intensified, especially due to its anti-inflammatory potential. Its bioactive components act on key inflammatory pathways, most notably by inhibiting NF-κB signaling **(Son *et al.*, 2018; Stela *et al.*, 2024).**

Similarly, Hesperidin, a flavanone glycoside found abundantly in citrus fruits and first isolated in 1828, has long been used in traditional Mediterranean and Asian medicine. It is now recognized for its antioxidant, vasoprotective, and anti-inflammatory effects. Although many studies have explored the anti-inflammatory effects of Bee Venom and Hesperidin, there is still a need for *in vivo* investigations to confirm their efficacy in living organisms. The aim of our study is to evaluate the potential anti-inflammatory effects of bee venom and hesperidin using experimental models of inflammation in rats. These models include xylene-induced ear edema, carrageenan-induced paw edema, and carrageenan-induced peritonitis. Through these approaches, we aim to better understand the efficacy of these two natural compounds in modulating inflammatory processes.

II. LITERATURE REVIEW

LITTERATURE REVIEW

1. Inflammation

The word inflammation itself comes from the Latin ***inflammare*** (to set on fire). The inflammatory response is an adaptive response generated in response to harmful stimuli such as infection or tissue aggression. It requires a fine regulation, generally beneficial. It leads to the elimination of possible pathogens and the return to homeostasis of the injured tissue. Toxins, pathogens, damaged cells, or radiation can all cause inflammation in the body **(Medzhitov, 2010 ; Rivas, 2010).**

Inflammatory reactions are characterised by four signs: rubor, tumor, calore, and dolore. A few centuries later Galien added a fifth sign «functio laesa» (loss of function). Heat and redness are aused by increased blood flow, whereas swelling is the result of accumulation of fluids. Pain is caused by the release of stimulating chemicals, whereas loss of function is caused by a variety of circumstances **(Zigterman & Dubois, 2022).**

When the body responds well, inflammation stops, but if the stimulation continues, inflammation may continue and even become chronic. Therefore, inflammation is a defense mechanism that is vital to health **(Nathan & Ding, 2010 ; Zhou *et al*., 2016).**

1.1. Factors and causes of inflammation

Inflammatory responses are caused by several factors, including pathogens mentioned in (table 1). These factors determine the cellular and tissue damage that causes inflammation: **(Scott *et al*., 2015)**

Table 1. Causes of Inflammatory Responses (Huang *et al.,* 2024).

Factors	Subtypes / Examples
Infectious agents	• Microorganisms: bacteria, viruses, fungi, parasites • Recognized via PAMPs (Pathogen-Associated Molecular Patterns) • Release of virulence factors
Physical factors	• Mechanical trauma • Heat (burns) • Cold (frostbite) • Radiation (UV, ionizing radiation)
Chemical agents	• Corrosive substances (strong acids, alkalis) • Environmental toxins (e.g., industrial pollutants, pesticides) • Animal toxins (e.g., toxic proteins in snake and scorpion venom)
Foreign materials	• Splinters, sutures, implants • Inhaled particles (asbestos, silica)
Endogenous stimuli	• DAMPs (Damage-Associated Molecular Patterns) • Necrotic cells, ATP, uric acid crystals • ECM fragments, cholesterol crystals

Immune responses	• Autoimmune diseases (e.g., lupus, rheumatoid arthritis) • Allergies (e.g., pollen, food allergens) • Hypersensitivity reactions
Ischemia / Necrosis	• Inflammation secondary to ischemic injury (e.g., myocardial infarction) • Triggered by necrotic tissue and DAMPs

1.2. Types of inflammation

Inflammation can be classified into two types based on the duration and speed of the inflammatory process **(Pahwa *et al*., 2023).**

1.2.1. Acute Inflammation

Acute inflammation is the body's first line of defense. It is part of the innate immune system and develops rapidly, becoming severe within a short period. Symptoms typically last for a few days **(Medzhitov, 2008 ; Pahwa *et al*., 2023).**

Its primary characteristics include the migration of leukocytes into the extravascular compartment and the exudation of fluid and plasma proteins. During inflammatory responses, three primary phases occur (figure 1) increased blood flow to the inflamed area, vasodilation with enhanced vascular permeability leading to plasma leakage from the microcirculation, and the migration of phagocytic leukocytes into surrounding tissues **(Markiewski & Lambris, 2007; Kobayashi *et al*., 2014).**

Acute inflammation is a temporary physiological state, but left unchecked, it can progress to chronic inflammatory disorders **(Zhou *et al*., 2016).**

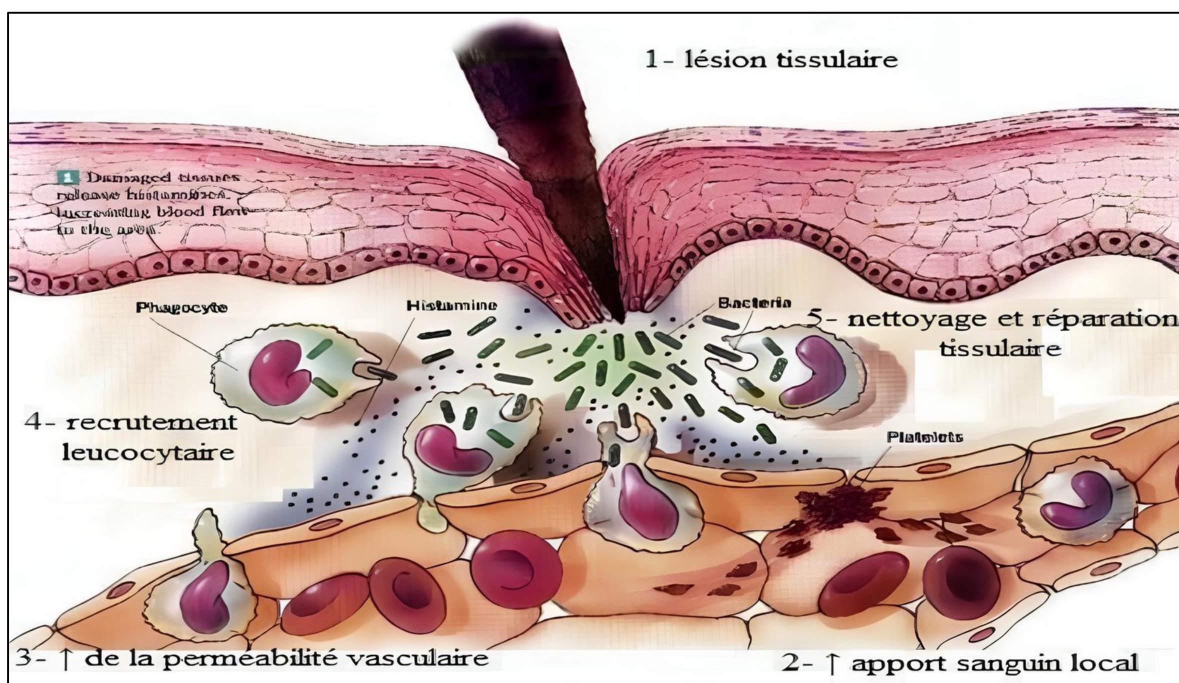


Figure 1. The main steps in the inflammatory reaction (Ghalem, 2014).

a. Vascular phase

Inflammation begins with a brief vasoconstriction, followed by vasodilation involving arterioles and venules. This vasodilation increases capillary blood flow, leading to two of the cardinal signs of inflammation: heat and redness. At the same time, there is an increase in vascular permeability, allowing plasma to leak into the extravascular space as exudate (figure 2). The loss of proteins decreases capillary osmotic pressure and increases interstitial osmotic pressure. In addition, there is an increase in capillary hydrostatic pressure, leading to a marked outflow of fluid and its accumulation in the tissue spaces, resulting in swelling and pain **(Porth, 2011).**

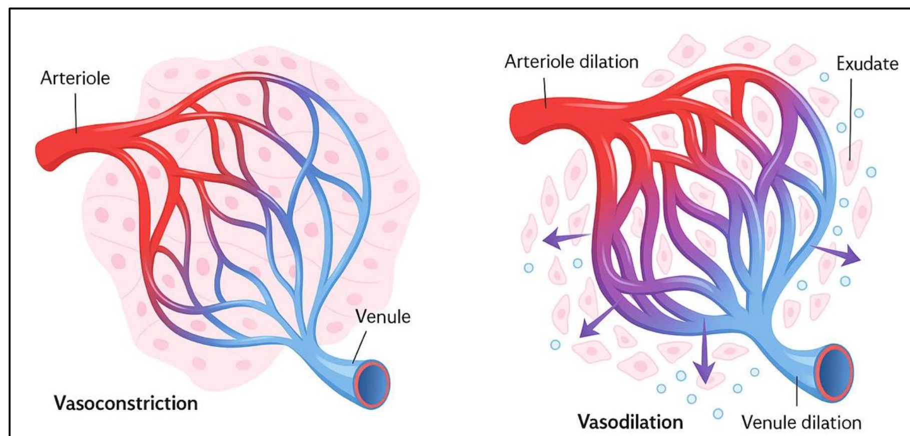


Figure 2. Exudate formation (Porth, 2011).

b. Cell Phase (Amplification)

The cellular response follows the vascular phase. The process is divided into three key stages: the first involves the activation of innate immune cells (neutrophils and monocyte/macrophages), the second involves a non-adaptive response (lymphocytes with antigen receptors), and the third involves the development of an adaptive immune response specific activation of T (LT) and B (LB) lymphocytes) **(Ibsen & Phelan, 2018).**

c. Repair phase (effector)

The resolution phase, known as the repair phase, during which damaged tissue can be repaired. Polynuclear neutrophils and macrophages eradicate aggressors and debris from the inflammatory center, secreting mediators that promote tissue healing. Fibroblast and endothelial cells then create a connective-vascular tissue, resulting in scarring **(Ibsen & Phelan, 2018).**

1.2.2. Chronic Inflammation

Chronic inflammation is also referred to as long-term inflammation and can last anywhere from a few months to several years **(Palavra *et al.*, 2015).**

When the inflammation's resolution phase is poorly managed, acute persistent inflammation turns into a chronic condition. This long-lasting activation may be caused by an inability to remove the inflammatory stimulus, a continuous flow of leucocytes that act as a regulator by producing pro-

inflammatory cytokines and reactive oxygen species (ROS) that continuously damage and modify tissues, or a circumstance that keeps these leucocytes at the site of inflammation **(Roy *et al.*, 2013).**

The main characteristics of chronic inflammation are : persistence of tissue damage, presence of a chronic inflammatory infiltrate (lymphocytes, plasmocytes, monocytes-macrophages, eosinophilic polynucleate, basophiles, mastocytes) and the existence of fibrosis **(Guedj & Bedossa 2015 ; Ambriz-Pérez *et al.*, 2016).**

1.3. Cells involved in the inflammatory process

The inflammatory response requires the intervention of several cells **(Elaine *et al.*, 2014).**

1.3.1. Tissue resident cells

Tissue-resident immune cells are non-circulating cells that respond rapidly to tissue-specific insults **(Gray & Farber, 2022).**

- **Mast cells**

Mast cells, widely distributed at the host-environment interface, play a crucial role in immunity. Their maturation and function enable them to recognize stimuli and release active mediators, acting as first responders and interacting with other immune cells. Their significance in both innate and adaptive immunity, including immune tolerance, is increasingly recognized **(Elaine *et al.*, 2014).**

- **Macrophages**

Macrophages are key components of the innate immune system, and their activation is crucial for various functions, including immune defense and the inflammatory response **(Sica & Mantovani, 2012).**

In adult mammals, the origin of macrophages are monocytes circulating in the blood, after differentiation they will be present in almost all tissues of the **(Gentek *et al.*, 2014).**

Thus, they perform various regulatory functions in both physiological and pathological processes of the body and also exhibit a phagocytic function **(Cheng *et al.*, 2019).**

- **Dendritic cells**

Dendritic cells (DCs) are recognized as the most powerful professional antigen-presenting cells (APCs), able of triggering adaptive immunity and enhancing the innate immune response **(Lily *et al.*, 2021).**

1.3.2. Cells recruited from the blood

Blood-derived immune cells are recruited to tissues during inflammation, where they differentiate and contribute to pathogen clearance and tissue repair **(Naik & Kaplan, 2022).**

- **Neutrophils**

Neutrophils are cellular components of immune systems, they are typically the first cells to reach sites of inflammation **(Ka *et al.*, 2020 ; Rungelrath *et al.*, 2020).**

They originate from precursors in the bone marrow and enter the bloodstream, where they

circulate, prepared to detect and respond to activating agents. Once detection and activation occur, neutrophils will initiate an immune response against a pathogen or insult, normally using three strategies: phagocytosis, NETosis, and degranulation **(Rosales *et al*., 2020 ; Rungelrath *et al*., 2020).**

Thus, disruptions in neutrophil differentiation or function can impede inflammation resolution and contribute to uncontrolled inflammation **(Lily *et al*., 2021).**

- **Monocytes**

Monocytes possess numerous granules filled primarily with lysosomal enzymes that aid in the breakdown of phagocytosed microorganisms **(Robb *et al*., 2016).**

They originate from hematopoietic stem cells (HSCs) and found in the fetal liver during embryogenesis and in the bone marrow of adults **(Orkin & Zon, 2008 ; Epelman *et al*. 2014).**

Following an injury, monocytes derived from the blood or bone marrow are recruited to the wound site, where they differentiate into mature macrophages or dendritic cells **(Gentek *et al*., 2014).**

- **NK cells**

Natural killer (NK) cells are specialized immune effector cells that play a vital role in activating the immune response against abnormal cells **(KA *et al*., 2020).**

NK cells were identified for their cytolytic activity, enabling them to directly eliminate tumor or virus-infected cells without prior immunization, which inspired their name **(KA *et al*., 2020).**

1.4. Mediators of inflammation

The inflammatory response is actively influenced and modulated by a range of chemical mediators from the circulatory system (table 2), inflammatory cells, and wounded tissue. The chemical mediators that were released consist of vasoactive amines like serotonin and histamine, peptides like bradykinin, and eicosanoids such prostaglandins, leukotrienes, and thromboxanes **(Halliwell & Gutteridge, 2015).**

Table2. Inflammatory mediators (Akdis *et al*., 2016 ; Wautier & Wautier, 2023).

Category	Mediator	Source Cells	Function
Amines	Histamine	Mast cells, basophils	Vasodilation, increased vascular permeability
	Serotonin	Platelets	Vascular changes, modulates pain
Peptides / Kinins	Bradykinin	Plasma-derived (via kallikrein)	Pain, vasodilation, permeability
Cytokines	IL-1, TNF-α	Macrophages, mast cells	Fever, leukocyte recruitment, endothelial activation
	IL-6	Macrophages, T-cells	Promotes acute-phase response
	IFN-γ	T-cells, NK cells	Macrophage activation, chronic inflammation maintenance
	GM-CSF	Macrophages, T-cells	Enhances leukocyte production

Chemokines	IL-8 (CXCL8)	Macrophages	Neutrophil chemotaxis
	CCL2, CCL3 (MIP-1), CCL5 (RANTES)	Macrophages, dendritic cells	Monocyte/T-cell/eosinophil recruitment
Eicosanoids	Prostaglandins (PGE_2, PGD_2, PGI_2, $PGF_{2}\alpha$)	Mast cells, endothelial cells	Pain, fever (PGE_2); bronchoconstriction/allergy (PGD_2)
	Leukotriene B_4; LTC_4, LTD_4	Leukocytes, mast cells	Neutrophil chemotaxis (LTB_4); bronchoconstriction (LTC_4, LTD_4)
Gases	Nitric oxide (NO)	Macrophages, endothelium	Vasodilation, antimicrobial
Enzymes / Proteases	Tryptase	Mast cells	Promotes inflammation, vascular permeability
	Lysosomal enzymes	Granulocytes	Tissue breakdown

1.5. Anti-Inflammatory Agents

An anti-inflammatory is a medication used for the local treatment of inflammation or the general treatment of inflammatory diseases **(Charles & Dinarello, 2010).** They can relieve symptoms without treating the cause of the inflammation. They are recommended when the inflammation becomes bothersome, especially because of the pain it causes **(Wainsten *et al*., 2012).**

Anti-inflammatory drugs are administered orally, injectably or locally. They are divided into two classes: steroidal and nonsteroidal **(Charles & Dinarello, 2010).**

1.5.1. Steroidal Anti-Inflammatory Drugs (SAIDs)

Also known as corticosteroids, these medications (prednisone, prednisolone, and betamethasone) are synthesized from natural corticosteroids, which are hormones produced by the adrenal glands **(Wainsten *et al*., 2012).**

They are highly potent and are used to manage inflammation when it becomes severe or appears without a clear cause, as seen in inflammatory diseases like rheumatoid arthritis and severe allergies **(Charles & Dinarello, 2010).**

a. Mechanism of Action

Glucocorticoids bind to glucocorticoid receptors in the cytoplasm. The formed complex then migrates to the nucleus, where it binds to glucocorticoid response elements (GRE) to activate certain anti-inflammatory genes like IL-10 or lipocortin-1. However, their main anti-inflammatory effect comes from inhibiting pro-inflammatory transcription factors such as NF-κB and AP-1, reducing the expression of cytokines and other inflammatory genes. Glucocorticoids also modify chromatin structure via the CBP (CREB-binding protein) protein, limiting access to DNA and suppressing inflammation **(Oiter & Barnes, 2010).**

b. Adverse Effects

Corticosteroids are effective anti-inflammatory drugs, but they can cause serious side effects.

These include suppression of the hypothalamic-pituitary-adrenal (HPA) axis, weakened immune response, worsened diabetes, high blood pressure, osteoporosis, and growth delay in children. Due to their systemic toxicity, some potent forms are used only topically **(Omar *et al.*, 2008).**

1.5.2. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

Also called NSAIDs (phenyibutazone, Indomethacin, diclofenac, propionic acid derivatives, oxicams), they fall into different categories but all have the ability to inhibit the formation of certain substances, such as prostaglandins, which are chemical mediators essential for the progression of inflammation **(Altman *et al.*, 2015).**

NSAIDs are particularly effective during the acute phases of inflammation and should not be used in combination with each other or with anticoagulants **(Wainsten *et al.*, 2012).**

a. Mechanism of Action

The cyclooxygenase (COX) enzyme is inhibited by NSAIDs, which is their primary mode of action. Arachidonic acid must be converted by cyclooxygenase in order to produce prostacyclins, prostaglandins, and thromboxanes **(Vane, 1971).**

The absence of these eicosanoids is thought to be the cause of NSAIDs' therapeutic effects. In particular, prostaglandins produce vasodilation, raise the hypothalamic temperature set-point, and contribute to anti-nociception, whereas thromboxanes aid in platelet adhesion **(Chaiamnuay *et al.*, 2006).**

COX-1 and COX-2 are two cyclooxygenase enzymes. COX-1 is constantly present and supports kidney function, blood clotting, and protects the stomach lining. COX-2 is produced during inflammation. Most NSAIDs block both enzymes and are nonselective, which can harm the stomach. COX-2 selective NSAIDs, like celecoxib, mainly block COX-2, providing anti-inflammatory effects with less risk to the gastric mucosa **(Chaiamnuay *et al.*, 2006).**

b. Adverse Effects

NSAIDs can cause side effects in the gastrointestinal, kidney, and cardiovascular systems. Still, most patients tolerate short-term, therapeutic use well. However, prolonged use or existing health conditions can increase risks. Therefore, NSAID treatment requires careful consideration of the risk–benefit balance based on the patient's condition **(Harirforoosh *et al.*, 2013)** and there are:

- Gastrointestinal Side Effects
- Serious GI Side Effects
- Renal Side Effects

1.6. Plant-Derived Anti-Inflammatory Agents

Natural or plant-based anti-inflammatory agents are substances found in nature whose chemical structure is not altered during their extraction or preparation processes. Several studies have been conducted on natural substances of plant origin that are rich in bioactive compounds and possess

anti-inflammatory properties (table 3), making them useful in the treatment of certain inflammatory diseases **(Bourkhiss *et al*., 2010).**

Table 3. Anti-inflammatory activity of some medicinal plants (Nunes *et al*., 2020).

Number	Botanical Name	Plant/Family	Parts Used	Constituent Compounds
01	*Acacia catechu*	Mimosaceae	Bark, wood, flowering tops, gum	Tannin, gum, catechuic acid
02	*Azadirachta indica*	Meliaceae	Leaf, root, oil, seed, gum, fruit, flower	Margosine, bitter oil, azadirachtin
03	*Caesalpinia crista*	Caesalpiniaceae	Seeds, root, leaf, root bark	Oleic, linoleic, palmitic, stearic acid, phytosterols
04	*Cassia angustifolia*	Caesalpiniaceae	Pods, dried leaves	Emodin, eathartin, mucilage, senna-picrin, oleanic acid
05	*Coriandrum sativum*	Umbelliferaeapia ceae	Leaf, bark, flower	Tannin, cathartin, malic acid, cathartin, albuminoids
06	*Cuscuta reflexa*	Convolvulaceae	Plant, seed, fruit, stem	Cuscutine, flavonoid, glucoside, bergenin, coumarin
07	*Enicostema littorale*	Gentianaceae	Whole plant	Alkaloids, gentiocrucine

2. Study Extract

This study aims to evaluate the anti-inflammatory effects of two natural extracts: Bee Venom, an apitoxin (a natural biotoxin), and Hesperidin, a natural flavonoid **(Chanchao & Premratanacha, 2014).**

2.1. Bee Venom

Bee venom (BV) (figure 3 B), naturally produced by bees of the *Apis mellifera* species (figure 3 A), has been used for thousands of years in traditional medicine to treat various human ailments **(Goran *et al*., 2024).**

Figure 3. European honey bee *Apis mellifera* (A) and lyophilized bee venom (B) (Goran *et al*., 2024).

As part of apitherapy, ancient civilizations, particularly in China, Egypt, Greece, and Korea, recognized its therapeutic effects in treating pain, inflammation, and immune system imbalances **(Goran *et al*., 2024).**

Bee Venom in an apitoxin (biotoxin) synthesized and secreted by two glands (lubricanting and venomous) present in the abdominal cavity of the bee **(Chanchao & Premratanacha, 2014).**

2.1.1. Species profile of Apis mellifera

The classification is mentioned in the following table (table 4) (**Egum *et al*., 2021**):

Table 4. Species profile of Apis mellifera (Egum *et al*., 2021).

Level	**Classification**
Kingdom	Animalia
Phylum	Arthropodes (Arthropoda)
Class	Insecta
Order	Hyménoptères (Hymenoptera)
Family	Apidés (Apidae)
Genus	*"Apis"*
Species	*"Apis mellifera"* (Abeille domestique)

2.1.2. Anatomy of the venomous apparatus in the honeybee (*Apis mellifera*)

In the honeybee (*Apis mellifera*), the venom apparatus is located in the posterior part of the abdomen. It consists mainly of two components (figure 4): the venom glands and the storage reservoir. The venom glands are elongated, thin, and distally bifurcated structures that open into an oval reservoir **(Bridges *et al*., 1984).**

Each gland is composed of secretory units including secretory cells, ductal cells, ducts, and terminal apparatuses. These units are responsible for producing venom components. The proximal region of the glands, close to the reservoir, contains secretory units with a funnel-like structure between the duct and the terminal apparatus, probably to protect the secretory cells from the cytolytic effects of the venom **(Roat *et al.,* 2006).**

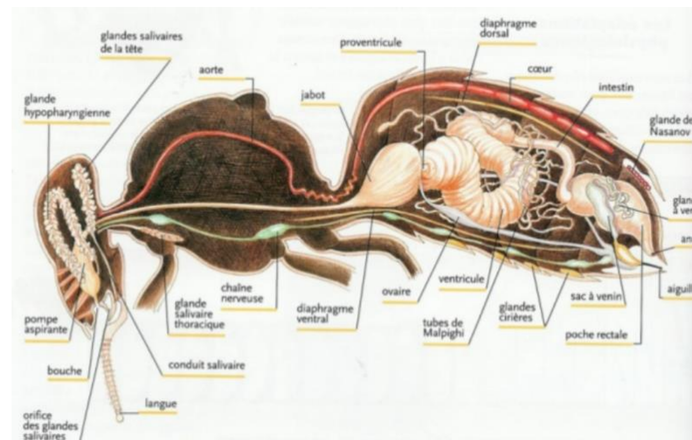


Figure 4. The glandular system of the bee (Chapman, 1998).

2.1.3. Venom Production and Secretion Mechanism

Bee venom glands belong to the type 3 exocrine glands, composed of two cell types: a secretory cell and a microduct-forming cell. Unlike hypopharyngeal glands, which produce secretions on demand, venom glands produce and store venom in a reservoir. This difference is reflected in the cytoskeletal structure of the secretory cells: venom glands have an actin-rich brush border surrounding a short and narrow terminal apparatus, facilitating continuous secretion into the reservoir **(Kheyri *et al*., 2012).**

Venom secretion is an active process, involving the synthesis of various peptides and enzymes. Secretory cells have abundant rough endoplasmic reticulum, vacuoles, and granules, indicating intense secretory activity. In virgin queens, these cells are particularly active, while in fertilized queens, the glands show degeneration, reflecting reduced venom utilization **(Arruda *et al.,* 2007).**

2.1.4. Introduction to Bee Venom Therapy (BVT)

Bee venom therapy (BVT) is a form of complementary and alternative medicine. It relies on the use of live bee stings, applied directly to the patient's skin, to benefit from the biological effects of bee venom **(Khalil *et al*., 2021).**

The active compounds in bee venom give it anti-inflammatory, analgesic, and immunomodulatory properties. These properties have led to the use of BVT to relieve pain and reduce inflammation in chronic and often painful conditions **(Goran *et al*., 2024).**

Traditionally, BVT aims to restore normal body functions by stimulating certain beneficial physiological responses, among the pathologies it treats: rheumatoid arthritis, tendonitis, burns and

infected wounds **(Khalil *et al.*, 2021).**

In recent years, researchers have begun to explore the anticancer potential of bee venom, particularly through the ability of melittin to target certain tumor cells **(Goran *et al.*, 2024).**

2.1.5. Physicochemical property

Bee venom is a colorless, bitter-tasting liquid, composed of approximately 88% water. The remaining 12% (dry matter) contains a variety of peptides, enzymes, biogenic amines, lipids, sugars, amino acids, and minerals, giving the venom its many biological and pharmacological properties **(Kadry *et al.*, 2024).**

The composition of bee venom can vary depending on several factors **(El Mehdi *et al.*, 2021):**

- Bee species and subspecies: For example, *Apis mellifera* intermissa exhibits specific variations.
- Season and geography: Environmental conditions influence venom production and composition.
- Bee age: Older bees generally produce more concentrated venom.

2.1.6. Major constituents of bee venom

Bee venom contains biologically active compounds with unique properties, which are grouped in the following table (table 5) **(Chen *et al.*, 2010):**

Table 5. Composition of dry bee venom (BV) expressed as type of molecule, components, and weight percentages (Chen *et al.*, 2010).

Component	Approximate Proportion	Nature / Type	Properties / Role
Melittin	50–60%	Main peptide	Cytolytic, causes pain, promotes histamine release, induces inflammation.
Phospholipase A_2	10–12%	Enzyme	Destroys cell membranes, acts synergistically with melittin to amplify inflammatory effects.
Apamin	2–3%	Small neurotoxic peptide	Blocks certain potassium channels in the central nervous system.
Mast Cell Degranulating Peptide (MCD)	2–3%	Peptide	Induces histamine release from mast cells.
Hyaluronidase	1–2%	Enzyme	Facilitates the diffusion of other components through tissues.
Histamine, dopamine, norepinephrine	Trace / small quantities	Biogenic amines	Involved in pain, inflammation, and allergic responses.

Other enzymes and peptides	Not specified	Various (amino acids, enzymes..)	

This complex cocktail gives bee venom its powerful pharmacological effects, both in local reactions (pain, redness) and systemic reactions (allergic reactions, potential therapeutics) **(Chen *et al.*, 2010).**

2.1.7. Analgesic, anti-nociceptive and anti-inflammatory effects

The analgesic efficacy of bee venom relies on complex biological mechanisms that involve the central nervous system, the peripheral nervous system, and several molecular pathways. This section explores how bee venom works to reduce pain at different levels of the body **(Sung *et al.*, 2021).**

a. Molecular mechanisms of analgesia

Bee venom contains several bioactive compounds that: **(Sung *et al.*, 2021)**

- Regulates the cytokine and inflammatory mediator production.
- Modulates the activity of ion channels involved in pain transmission.
- Inhibits certain pro-inflammatory enzymes (e.g., COX-2).
- Stimulates of the release of natural analgesic substances (e.g., endogenous opioids).

b. Bee venom and the central nervous system

BV influences the nerve centers involved in pain processing and perception by: **(Sung *et al.,* 2021).**

- Activating certain brain regions associated with pain control.
- Modulating the release of neurotransmitters (serotonin, dopamine, GABA, etc.).
- Stimulating descending inhibitory pathways that reduce the transmission of pain signals to the brain.

c. Bee venom and the peripheral nervous system

Near the injection site:

Bee venom temporarily blocks the sensory nerve fibers responsible for pain, reduces local inflammation, and inhibits the sensitization of peripheral receptors, thereby reducing pain perception **(Sung *et al.*, 2021).**

d. Anti-nociceptive (pain-relieving) effects

Experimental studies have shown that bee venom, when injected in small doses into specific areas of the body, can reduce the perception of pain. This phenomenon, called antinociceptive effect, means that BVT can reduce the nervous system's sensitivity to pain **(Chen *et al.*, 2010).**

By acting on the nerve circuits responsible for transmitting pain signals, bee venom may modulate or block certain pain mechanisms, thus reducing the intensity perceived by the body **(Wehbe *et al.*, 2019).**

e. Anti-inflammatory effects

Active compounds in the venom, such as melittin and peptides, appear to inhibit the production of inflammatory cytokines, modulate nervous and immune pathways involved in chronic pain, and stimulate natural pain regulation mechanisms **(Chen *et al*., 2010).**

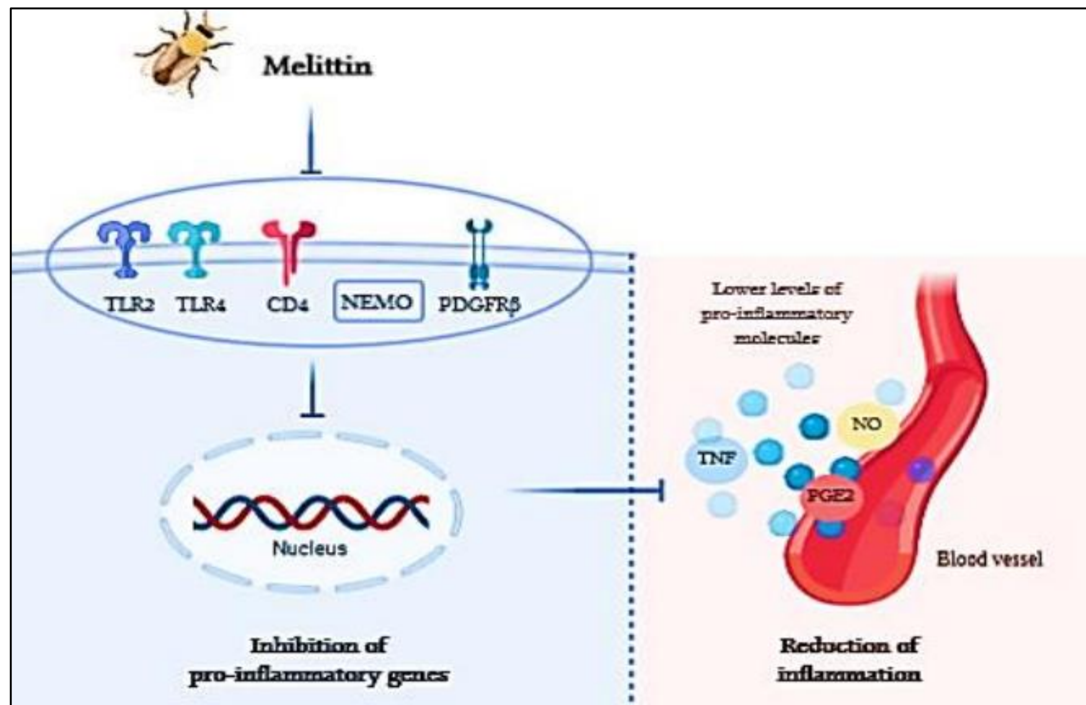


Figure 5. Action mechanism of anti-inflammatory effects of melittin (Carpena *et al*., 2020).

Melittin inhibits the routes of TLR2, TLR4, CD4, NEMO and PDGFRβ and therefore, inhibits the action of pro-inflammatory genes (figure 7) **(Carpena *et al*., 2020).**

This process results in lower levels of pro-inflammatory molecules and the reduction of inflammation **(Carpena *et al*., 2020).**

2.1.8. Allergic risks linked to bee venom

One of the main obstacles to integrating bee venom therapy (BVT) into conventional medicine is the risk of allergic reactions, sometimes very serious **(Haddad *et al*., 2023).**

In some sensitized individuals, stings can trigger severe allergic reactions, including:

- systemic reactions (generalized urticaria, edema, hypotension).
- life-threatening anaphylactic reactions (respiratory distress, anaphylactic shock).

According to epidemiological data:

- Approximately 3% of adults in developed countries have experienced a systemic reaction after a sting.
- Up to 25% may have a latent allergic risk, detected by skin tests or specific antibody assays (RAST tests).

The problem is that even people with no apparent signs of allergy can react violently to a new bite (antibody levels measured by RAST do not always predict a reaction) **(Rosiek-Biegus *et al*.,**

2022).

2.1.9. Preservation of Honeybee Venom (*Apis mellifera*)

Honeybee venom is a fragile bioactive compound that requires specific conditions to preserve its integrity, pharmacological activity, and stability during storage. It can be stored under the following conditions **(Kim, 2021):**

- Lyophilized venom (freeze-drying) is stable for several years at room temperature if stored under appropriate conditions (moisture-free, in airtight containers). This form facilitates transport and use in laboratories or clinics **(El Mehdi *et al*., 2021).**
- Storage in solution: The venom can be dissolved in buffered solutions (e.g., phosphate saline) to prolong stability, but this form is less stable than freeze-drying.

Shelf life:

- When freeze-dried, the venom retains its biological properties for several years (up to 5 years or more), provided it is properly stored.
- In frozen solution (-20°C or -80°C), stability is generally 6 months to 2 years.
- In solution at room temperature, degradation is rapid, within a few days to weeks.

2.2. Hesperidin

Hesperidin (3,5,7-trihydroxyflavanone 7-rhamnoglucoside), also known as hesperetin-7-O-rutinoside) (figure 6, table 6) is a flavanone molecule and a subclass of flavonoids. It has been widely researched for its health-promoting and pharmacological properties **(Khan & Zill-E-Huma 2014).**

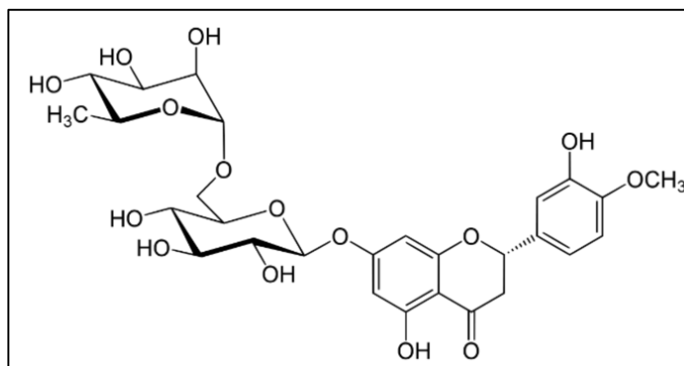


Figure 6. The chemical structure of Hesperidin (Khan & Zill-E-Huma 2014).

Hesperidin is transformed to glucuronides in the large intestine after being hydrolysed by gut bacteria rhamnosidases in the small intestinal and colon. Hesperetin is present in plasma as glucaronides (87%) and sulphaglucooronides (13%), peaking between 5 and 7 hours post-ingestion **(Pla-Pagá *et al*. 2019).**

2.2.1. Sources of Hesperidin

Hesperidin and its derivatives are chemicals found in citrus fruits (Rutaceae family), including orange (Citrus sinensis), grapefruit (Citrus paradisi), tangerine (Citrus reticulata), lime (Citrus aurantifolia), and lemon (Citrus limon). Their concentration in citrus fruits varies according to the fruit variety (table 6), portion of the fruit, climate, and degree of maturity **(Gattuso *et al*. 2007).**

Furthermore, it has been suggested that the hesperidin concentration of green fruits varies as they grow. Hesperidin levels correlate with seed germination, indicating that light exposure promotes its synthesis **(Rouseff *et al*. 1987).**

Table 6. Natural sources and quantity of Hesperidin (Gattuso *et al*. 2007).

Citrus Fruit Juices	**Quantity**
Grapefruit concentrate juice	1.55 mg/100 mL
Pure grapefruit juice	0.65 mg/100 mL
Juice from the concentrate of lemon	24.99 mg/100 mL
Lemon juice, pure	17.81 mg/100 mL
Pure juice, lime	13.41 mg/100 mL
Orange [Blond], concentrate juice	52.68 mg/100 mL
Pure orange [Blond] juice	25.85 mg/100 mL
Orange [Blond], concentrate juice	51.30 mg/100 mL
Orange [Blond] juice, undiluted	43.61 mg/100 mL
Tangerine, concentrate juice	36.11 mg/100 mL
Herbs	**Quantity**
Dried, peppermint	480.65 mg/100 g FW
Fresh welsh onion	0.02 g/100 g FW

2.2.2. Toxicity of Hesperidin

Hesperidin is generally considered safe, with few known adverse effects, even during pregnancy: **(Garg & Garg, 2001)**

- Dietary doses range between 0.3% and 5% without toxic effects.
- Phosphorylated hesperidin at high doses (100–125 mg/kg/day) shows antifertility effects.
- About 10% of patients taking Daflon (hesperidin + diosmin) reported mild to moderate side effects.
- Hesperidin can interact with drugs like daunorubicin and vincristine; caution is advised with such medications.
- Animal studies show phosphorylated hesperidin is non-toxic, with no effects on weight, behavior, or mortality.
- Methylhesperidin showed no carcinogenic or mutagenic effects in rats, even after long-term exposure.

2.2.3. Mode of Action of Hesperidin as an Anti-inflammatory Agent

Inhibition of Pro-inflammatory Mediators such :

a. Hesperidin reduces the production of pro-inflammatory cytokines

- TNF-α (Tumor Necrosis Factor-alpha).
- IL-1β (Interleukin 1 beta).
- IL-6 (Interleukin 6).

These cytokines are key molecules in triggering and propagating inflammation **(Jagetia, 2022).**

b. Blockade of the NF-κB Pathway

The NF-κB (Nuclear Factor kappa B) signaling pathway plays a central role in regulating pro-inflammatory gene expression. Hesperidin inhibits the activation of NF-κB, thereby preventing the expression of genes encoding cytokines, inflammatory enzymes (such as COX-2), and cell adhesion proteins **(Jagetia, 2022).**

c. Reduction of COX-2 Expression and Prostaglandin Synthesis

COX-2 is an enzyme that promotes the synthesis of pro-inflammatory prostaglandins. Hesperidin decreases COX-2 expression, thus reducing the production of prostaglandins such as PGE2, a powerful mediator of inflammation and pain **(Jagetia, 2022).**

d. Antioxidant Effect

Inflammation is often worsened by oxidative stress (accumulation of free radicals). Hesperidin neutralizes free radicals and increases the activity of antioxidant enzymes (SOD, catalase, glutathione peroxidase), thereby reducing oxidative damage related to inflammation **(Jagetia, 2022).**

e. Stabilization of Cell Membranes

Hesperidin helps protect the membranes of immune cells (such as macrophages) against lysis or overactivation **(Jagetia, 2022).**

f. Additional biological functions

Beyond its well-documented antioxidant and anti-inflammatory properties, hesperidin exhibits several additional biological functions that contribute to its therapeutic potential (table 7) (**Aalikhani *et al.*, 2021**), including:

Table 7. Biologicl activities of Heperidin (Aalikhani *et al.*, 2021).

Biological Activities	Effect
Anticancer	Modulation of antioxidative enzymes, induced apoptosis, suppression of cancer cell proliferation and invasiveness..
Prevention of cardiovascular diseases	Analysis of biochemical, histopathological, ultrastructural and immunohistochemical studies of rat heart after isoproterenol induced cardiac hypertrophy. Evaluation of the effect of orange peel extract on streptozotocin-induced diabetic nephropathy.
Neurodegener-ative properties	Prevention of the cognitive deficits, biochemical anomalies and apoptosis associated with neuro-degenerative diseases, including Alzheimer's disease, induced by AlCl3 treatment. Preventing memory impairment in the Morris water maze test and depressive-like behavior in the tail suspension test. Hesperidin attenuates the induced reduction in glutathione peroxidase, catalase activity and total reactive antioxidant potential.

2.2.4. Pharmacokinetics

- Absorption: Hesperidin has limited oral bioavailability regardless of whether it is consumed in water, orange juice, or grapefruit juice **(Ji *et al.*, 2024).**

- Metabolism: It is metabolized into hesperetin by intestinal bacterial enzymes such as β-glucosidase **(Kim *et al*., 1999).**
- Intestinal transport: Pure hesperidin shows poor absorption through intestinal cells (Caco-2 model), whereas its glycosides are absorbed more effectively **(Ameer *et al*., 1996).**
- Excretion: There is considerable inter-individual variability; metabolites, including glucuronides, are excreted in urine **(Erlund *et al*., 2001).**
- Biological activity: Metabolites exhibit stronger antiplatelet and cytotoxic effects than hesperidin itself **(Srinivasan, 2001).**

2.2.5. Therapeutic uses of hesperidin

Hesperidin, a flavonoid predominantly found in citrus fruits, has been studied for its potential therapeutic applications in various diseases **(Tran, 2024).**

- **Blood Pressure Regulation**: A clinical trial involving 159 participants with prehypertension or stage 1 hypertension found that consuming orange juice rich in hesperidin for 12 weeks significantly reduced systolic blood pressure and pulse pressure **(Tran, 2024).**
- **Alzheimer's Disease**: Preclinical studies suggest hesperidin inhibits β-amyloid aggregation, reduces neuroinflammation, and enhances antioxidant defenses, potentially offering neuroprotection in Alzheimer's disease **(Han *et al*., 2023).**

Wound Healing in Diabetic Ulcers: Studies on diabetic rats indicate that hesperidin supplementation improves wound healing by enhancing angiogenesis, reducing inflammation, and lowering blood glucose levels **(Mirzaei *et al*., 2023).**

III. MATERIAL and METHODS

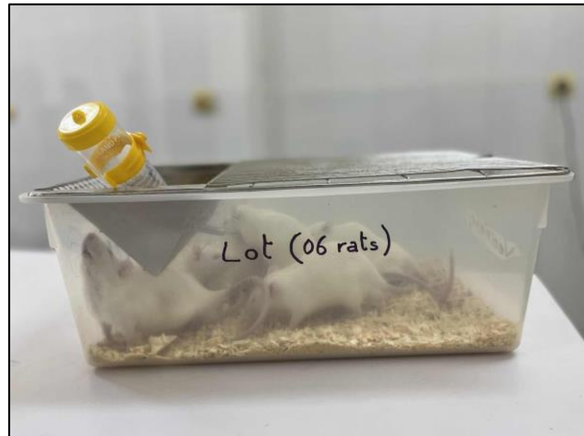
MATERIAL & Methods

1. Material

1.1. Study animals

This research was conducted on male *Wistar albino* rats weighing between 120 and 220 g (figure 7 A) These animals were purchased from the Pasteur Institute in Algiers, Algeria. All animals were kept to acclimate under laboratory conditions for two weeks and received a standard rodent diet and water ad libitum. The animals were selected based on their weight for different experimental groups (6 rats/group) (figure 7 B). All procedures were carried out in accordance with the European Union Guidelines for Animal Experimentation.

(A)



(B)

Figure 7. Albino mice in the pet store (original photos, 2025).

1.2. Working solution

- Hesperidin was dissolved in 0.9% NaCl solution.
- λ-Carrageenan (1%): dissolved in sterile 0.9% NaCl.
- Aspirin was dissolved in distilled water.
- Sodium chloride (NaCl 0.9%, sterile).
- Türk's solution prepared by mixing 1 ml of gentian violet with 1 ml of acetic acid, and the volume is made up to 100 ml with distilled water.
- Bee venom purchased from a production company in Béjaia: dissolved in sterile 0.9% NaCl.

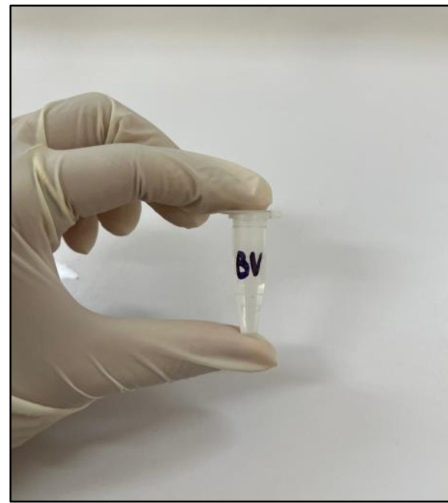
2. Methods

In this study, we evaluated the anti-inflammatory propeties of Bee Venom and Hesperidin *in vivo*, by using several models of experimental inflammation.

- **Preparation of bee venom:** The bee venom used was diluted to a concentration of (**0.8 mg/kg)** in sterile (0.9% NaCl). The diluted bee venom dosage was selected based on previous laboratory reports **Choi *et al*. (2018)**, which showed that a dose of (0.8 mg/kg) of bee venom produced the maximum anti-inflammatory effect without any significant side effects, such as allergic reactions or nociceptive behaviors.

A group of 6 rats received a cure of (**0.8 mg/kg)** of Bee Venom administered by intraperitonial injection of 100uL twice a week during 30 days (figure 8 A and B). After the 30 days of treatment, we applied the different tests mentioned in the following protocol.

(A)

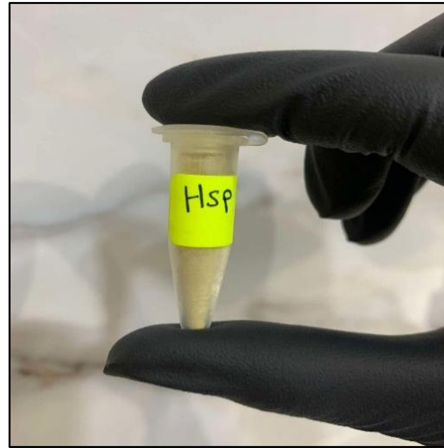


(B)

Figure 8. Bee Venom cure injection (original photos, 2025).

- **Preparation of Hesperidin:** it was diluted to a concentration of **(400 mg/kg)** in sterile (0.9% NaCl). The Hesperidin dosage was selected based on previous laboratory reports **Bai *et al*. (2017)**, who showed that a dose of **(400 mg/kg)** of bee venom produced the maximum anti-inflammatory effect without any significant side effects.

Hesperidin at a concentration of **(400 mg/kg)** was administered orally to the groups one hour before the following tests (figure 9 A and B).



(A) **(B)**

Figure 9. Hesperidin administrated orally (original photos, 2025).

2.1.Xylene-Induced Ear Edema in Rats

To evaluate the anti-inflammatory activity of Bee Venom and Hesperidin, the xylene-induced ear edema model was used, following the protocol described by Guardia *et al*. (2003) with slight modifications. This model is a standard method for assessing acute topical inflammation.

An application of 60 µL of xylene to both the inner and outer surfaces of the left ear of each rat (figure 10 A). Ear thickness (left and right) was measured using a digital caliper before xylene application (t_0) and then at 1-hour intervals for 4 hours (t_1, t_2, t_3, t_4) to monitor the inflammatory response (figure 10 B).

Rats were randomly assigned into :

- **Positive control group** (+) **:** six rats received **60 µL** of xylene without any anti-inflammatory treatment.
- **Test group :**
  - After the cure, six rats received only xylene application.
  - Six rats received hesperidin **(400 mg/Kg)** orally, one hour before xylene application.
- **Reference group:** six rats received aspirin **(400 mg/kg)** orally, one hour prior to xylene application.

(A) **(B)**

Figure 10. Ear Edema Test (original photos, 2025).

2.2. λ-Carrageenan-Induced Paw Edema in Rats

To further assess the anti-inflammatory effect of Bee Venom and Hesperidin, we conducted a paw edema model in rats induced by (**1**%) λ –carrageenan (figure 11 A), following the method described by **Srebro *et al*. (2023),** with slight modifications.

Rats were divided into four groups **:**

- **Positive control group** (+) **:** six rats received only the injection of **100uL** of (**1%**) λ-carrageenan.
- **Negative control group (-) :** six rats received only a sterile injection of **100uL** of (**0.9%** NaCl) physiological saline.
- **Test group :**
  - After the course, six rats received directly the carrageenan injection.
  - Six rats were given **1 ml** of Hesperidin solution **(400 mg/kg)** orally one hour before the carrageenan injection.
- **Reference group :** six rats were given **1 ml** of aspirin solution **(400 mg/kg)** orally one hour prior to the induction of paw edema.

Paw thickness or volume was measured at regular intervals to assess inflammation (figure 11 B).

The anti-inflammatory effect was determined by comparing the degree of swelling between groups (figure 11 C and D).

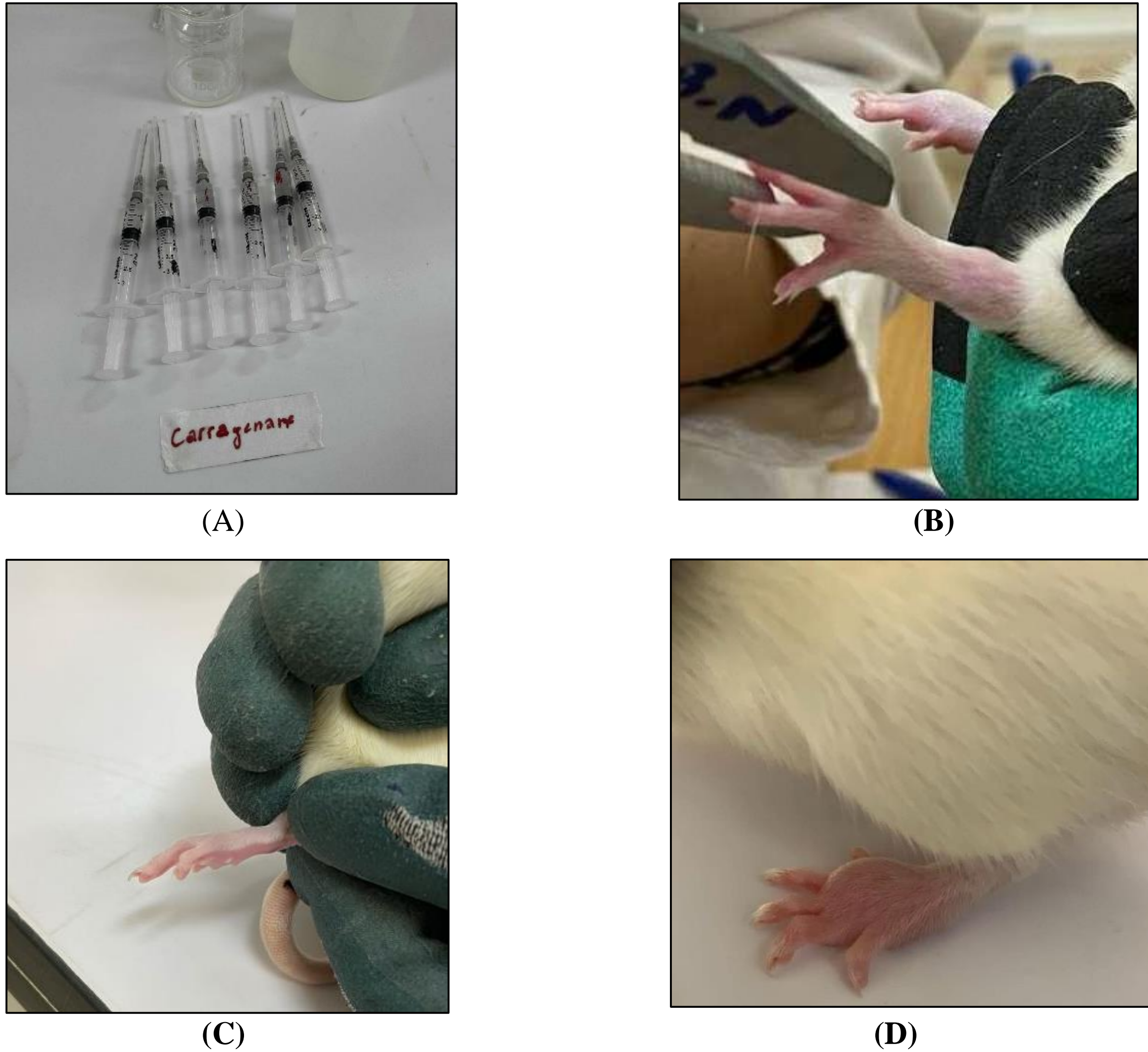


Figure 11. Paw Edema Test (original photos, 2025).

2.3. λ-Carrageenan-Induced Peritonitis in Rats

To investigate the systemic anti-inflammatory effect of Bee Venom and Hesperidin, we used a (**1%**) λ-carrageenan-induced peritonitis model adapted from **Barth *et al*. (2016)** with modification. Peritonitis was induced by intraperitoneal injection of 200 uL of a (**1%**) λ-carrageenan solution.

Rats were divided into four groups:

- **Positive control group** (+) **:** six rats received an injection of **200uL** of (**1%**) λ-carrageenan solution without any treatment.
- **Negative control group (−) :** six rats received a sterile injection of **200uL** physiological saline.
- **Test group :**
  - After the cure, six rats received directly the carrageenan injection.
  - Six rats received **1 mL** of hesperidin solution **(400 mg/mL),** orally one hour before carrageenan injection.

- **Reference group:** six rats received **1 mL** of aspirin solution **(400 mg/kg**) orally, one hour prior to peritonitis induction.

Four hours after the injection, rats were sacrificed by chloroform asphyxiation.

The peritoneal cavity was washed with 3 mL of sterile (0.9% physiological saline) (figure 13 A and B). The peritoneal lavage fluid was collected using a micropipette and stained with Türk's solution (figure 12).

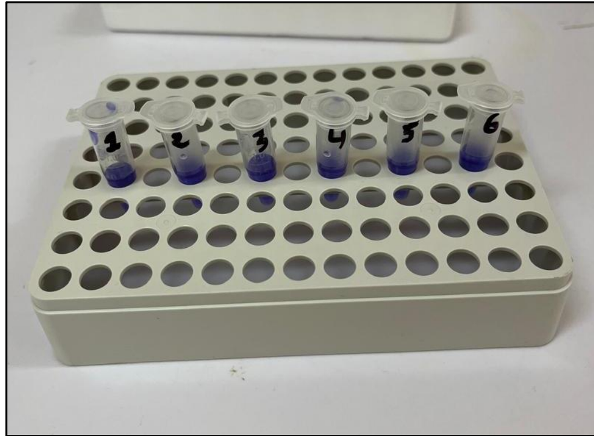


Figure 12. Türk's solution (original photos, 2025).

Leukocytes were then counted using a Malassez counting chamber to determine the number of neutrophils present (figure 13 C).

The total leukocyte count was calculated using the following formula:

$$\mathbf{Nb = N \times F \times 1000 \times V}$$

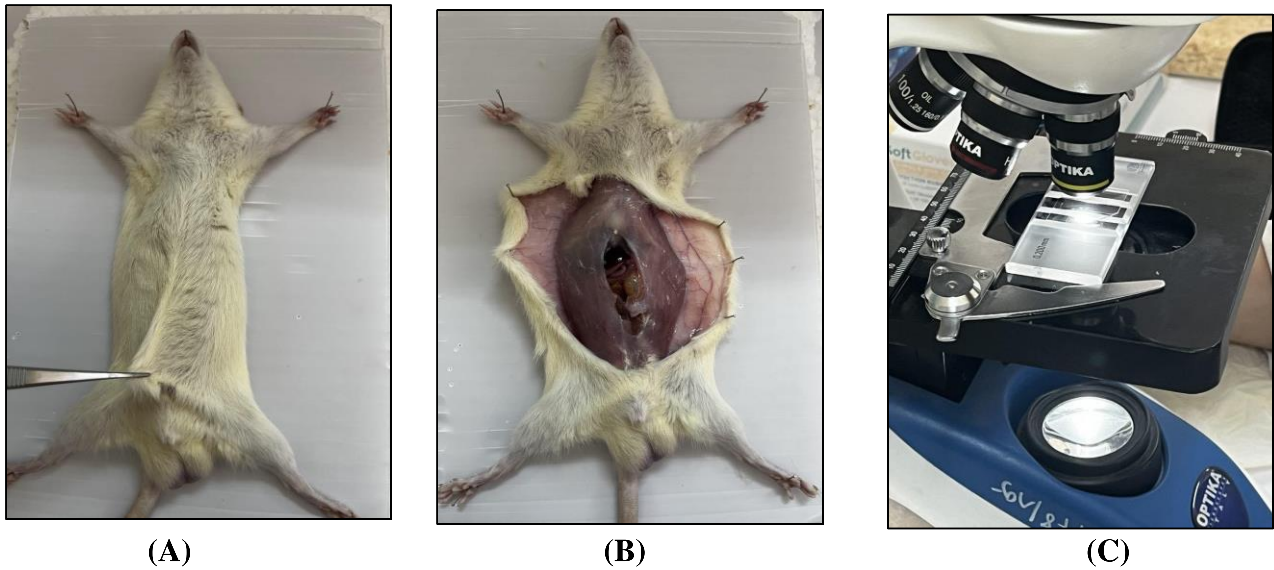
Where:

• Nb: Total number of leukocytes

• N: Number of leukocytes per field of view

• V: Volume of fluid aspirated from the peritoneal cavity (in mL)

• F: Dilution factor



(A) **(B)** **(C)**

Figure 13. Peritonitis Test (original photos, 2025).

3. Statistical Analysis

The *in vivo* results are presented as the arithmetic mean (M) of the n obtained values ± the standard error of the mean (SEM) [M ± SEM], with n = 6. Student's t-test was used to evaluate the significance of the effects of the different substances tested in vivo. Differences were considered statistically significant at $p < 0.05$ (**), $p < 0.01$ (*) and $p < 0.001$ (***).

IV. RESULTS & DISCUSSION

RESULTS & Discussion

1. Results

This research aimed to evaluate the anti-inflammatory properties of Bee Venom and Hesperidin. To this end, we tested the activity of Bee Venom (0.8 mg/kg) and Hesperidin (400 mg/kg) both dissolved in 0.9% NaCl solution, using three models of acute inflammation in rats: carrageenan-induced paw edema, xylene-induced ear edema, and carrageenan-induced peritonitis.

1.1. Results of bee venom

Bee venom is a natural secretion from honeybees known for its anti-inflammatory and immunomodulatory effects, are presented and interpreted to assess its biological impact and therapeutic potential.

1.1.1. Effect of bee venom on xylene-induced ear edema

Acute inflammation caused by xylene is manifested by typical symptoms such as redness, warmth, swelling, and pain. Thus, measuring edema is an effective method to quantify xylene-induced skin inflammation. The ear thickness of each rat was determined using a digital caliper. Measuring this edema is therefore an excellent way to quantify skin inflammation. This method is widely used to study the inflammatory process.

Results from the positive group showed (figure 14) that the ear thickness of the rats, measured before xylene application (time t_0), was approximately 0.43 mm. Following application of 60 µL of xylene, localized edema developed, reaching a peak of inflammation at the 2nd hour (t_2), with a mean ear thickness reaching 0.59 ± 0.03 mm. The thickness then decreased slightly, reaching 0.50 ± 0.03 mm at the 3rd hour (t_3), and 0.46 ± 0.02 mm at the 4th hour (t_4), indicating a gradual but partial resolution of the edema.

In rats treated with bee venom (0.8 mg/kg), the thickness of the ear reached (figure 14) its peak at t1 (1 h), the measurement was 0.37 ± 0.01 mm with a highly significant difference ($p < 0.001$). After one hour of that, precisely at t3 (3 h), a marked reduction was noted with a value of 0.36 ± 0.009 mm, highly significant ($p < 0.001$), a trend that continued at t4 (4 h) with 0.35 ± 0.02 mm ($p < 0.01$). These results suggest a strong and statistically robust temporal dynamic. This corresponds to a maximum inhibition of edema of around 40% at the 1st hour (Figure 15).

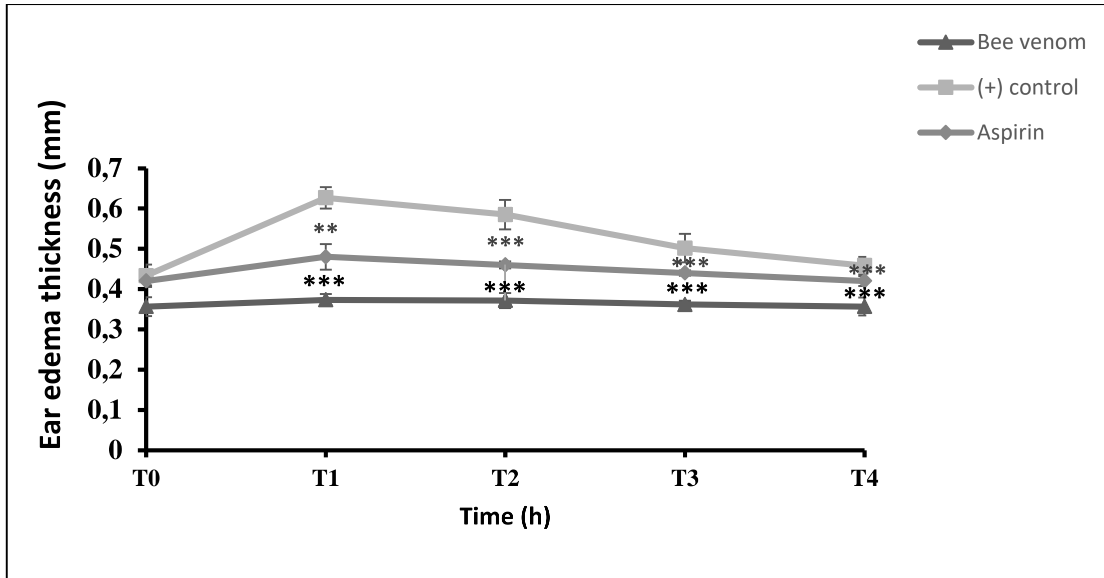


Figure 14. Evolution of ear thickness following the appearance of xylene-induced edema after the use of bee venom as a function of time, the results are presented as mean ±SEM for n=6; * if p<0.001, ** if p<0.01 and *if p<0.05 compared to the control + (student test).**

Comparing to the inhibition of the reference (aspirin) (400 mg/kg) which has reached its maximum at T1 with a percentage of 23%, the effect of bee venom is more powerful, which showed highly significant and consistent inhibition of inflammation.

These results suggest highly significant topical anti-inflammatory activity of bee venom, possibly related to the modulation of inflammatory mediators in the skin.

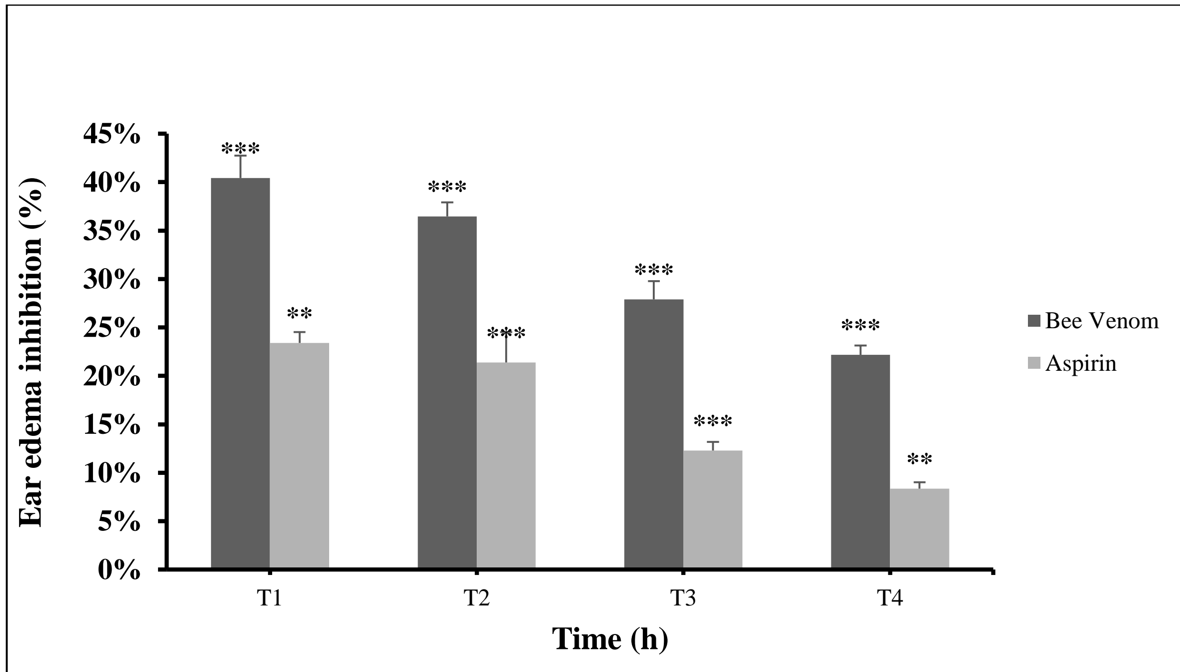


Figure 15. Inhibition percentage of Bee Venom compared to Aspirin in ears edema test, the results are presented as mean ±SEM for n=6; * if p<0.001, ** if p<0.01 and *if p<0.05 compared to the control + (student test).**

Comparing to the inhibition of the reference (aspirin) (400 mg/kg) which has reached its maximum at T1 with a percentage of 23%, the effect of bee venom is more powerful, which showed highly significant and consistent inhibition of inflammation.

These results suggest highly significant topical anti-inflammatory activity of bee venom, possibly related to the modulation of inflammatory mediators in the skin.

1.1.2. Effect of bee venom on λ-carrageenan-induced paw edema

Injection of 100 μL of 1% λ-carrageenan into the paws of rats in the positive group resulted in a progressive increase in plantar thickness, indicating acute inflammatory edema:

A clear increase in thickness was observed (figure 16) after injection, reflecting the onset of edema. At t_0 (before injection), the baseline value was 2.50 mm. At t_1 (1 h), the thickness increased to 5.56 ± 0.69 mm, then increased significantly at t_2 (2 h) to reach 6.20 ± 0.55 mm, then increased at t_3 (3 h) to 6.68 ± 0.49mm. The maximum was observed at t_4 (4 hours) with value of 6.84 ± 0.57 mm, no decrease is observed.

In rats treated with bee venom, the increase in the thickness of the leg was successive (figure 16). From t_1 (1 hour), it was observed at (3.92 ± 0.65 mm; $p < 0.05$), which increased at t_2 (2 hours) with a value of 4.53 ± 0.04 mm ($p < 0.001$). This trend was confirmed at t_3 (3 hours) with 5.22 ± 0.12 mm ($p < 0.01$). At t_4 (4 hours), the thickness of the paw has highly significantly decreased and reached 4.91 ± 0.39 ($p < 0.001$), reflecting a sustained and highly significant treatment effect.

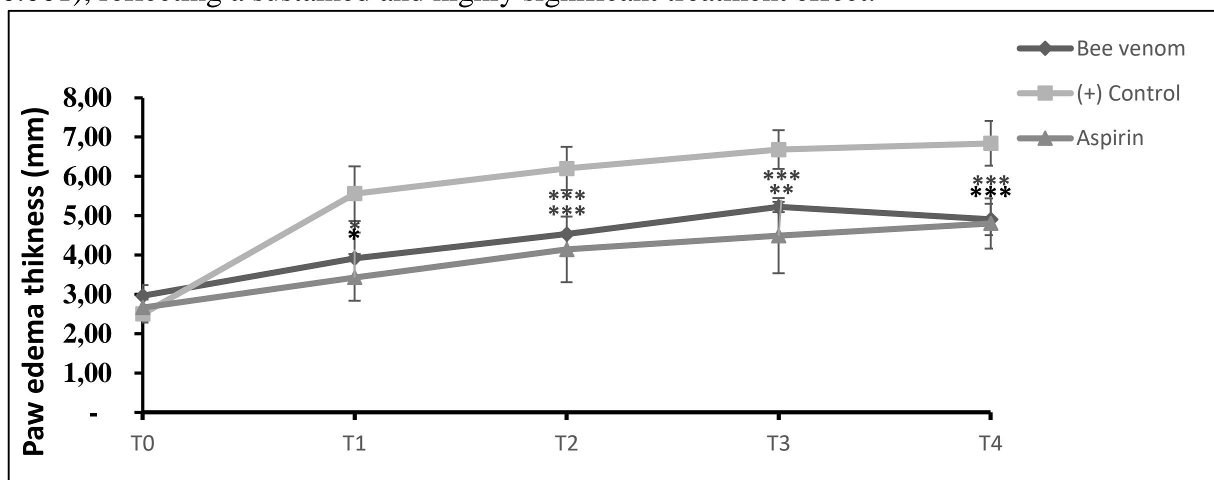


Figure 16. Evolution of Paw thickness following the onset of carrageenan-induced edema after the use of bee venom as a function of time, the results are presented as mean ±SEM for n=6; ***if p<0.001, ** if p<0.01 and *if p<0.05 compared to the control + (student test).

The percentage of edema inhibition by bee venom reached 28% at the 4th hour, which is very close to the effect obtained with aspirin 30%, considered the reference treatment. These data (Figure 17) confirm the systemic efficacy of the venom in this model of acute inflammation.

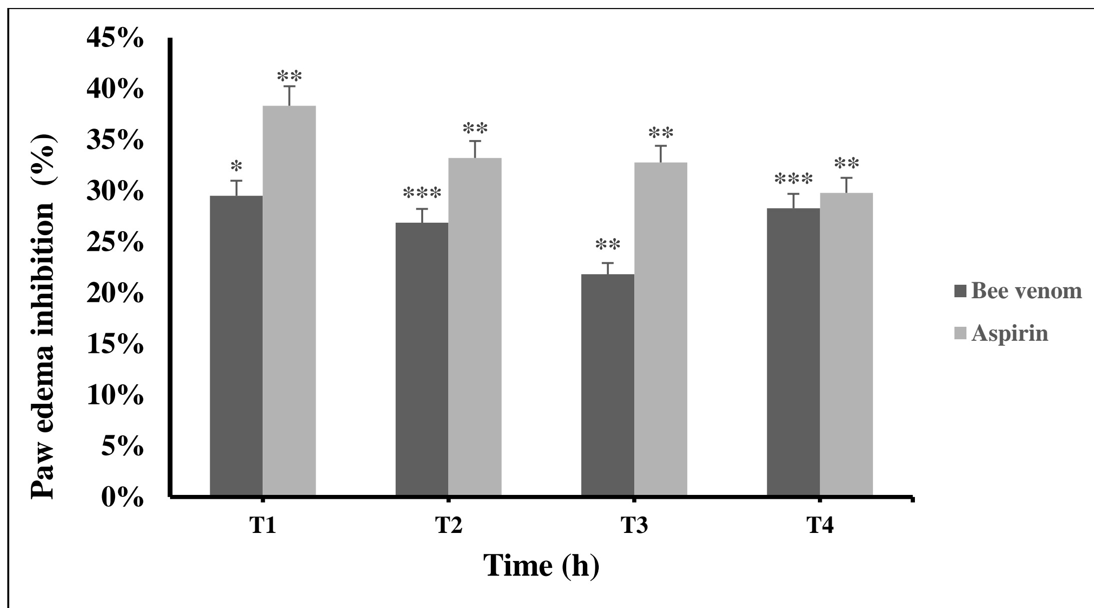


Figure 17. Inhibition percentage of Bee Venom compared to aspirin in paw edema test, the results are presented as mean ±SEM for n=6; * if p<0.001, ** if p<0.01 and *if p<0.05 compared to the control + (student test).**

1.1.3. Effect of bee venom on λ-carrageenan-induced peritonitis

Intraperitoneal injection of 200 µL of 1% λ-carrageenan in the positive control group resulted in an acute systemic inflammatory reaction characterized by massive accumulation of leukocytes in the peritoneal cavity. The mean number of leukocytes counted using a Malassez cell in rats in the positive control group was approximately $333 \pm 63 \times 10^6$ cells/mL (figure 18).

In rats treated with bee venom (0.8 mg/kg, administered intraperitoneally twice a week for 30 days), a significant reduction in leukocyte recruitment was observed (figure 18). The mean leukocyte count in this group was measured at $49.81 \pm 9.58 \times 10^6$ cells/mL ($p < 0.001$, highly significant). This corresponds to an inhibition of leukocyte infiltration of approximately 85%, (figure 24). This decrease suggests a marked attenuation of systemic inflammation, likely via modulation of inflammatory signaling pathways and inhibition of cell migration.

In comparison, aspirin (400 mg/kg), used as a reference substance, a significant reduction in leukocyte recruitment was observed (figure 18). The mean leukocyte count in this group was measured at $45.5 \pm 9.16 \times 10^6$ cells/mL showed a similar effect, with a nearly 86% reduction in leukocyte count, reflecting its well-established efficacy as a nonsteroidal anti-inflammatory drug.

Bee venom therefore demonstrated significant systemic anti-inflammatory activity, almost similar to that of aspirin, and sufficiently pronounced to justify its potential therapeutic interest in acute inflammation.

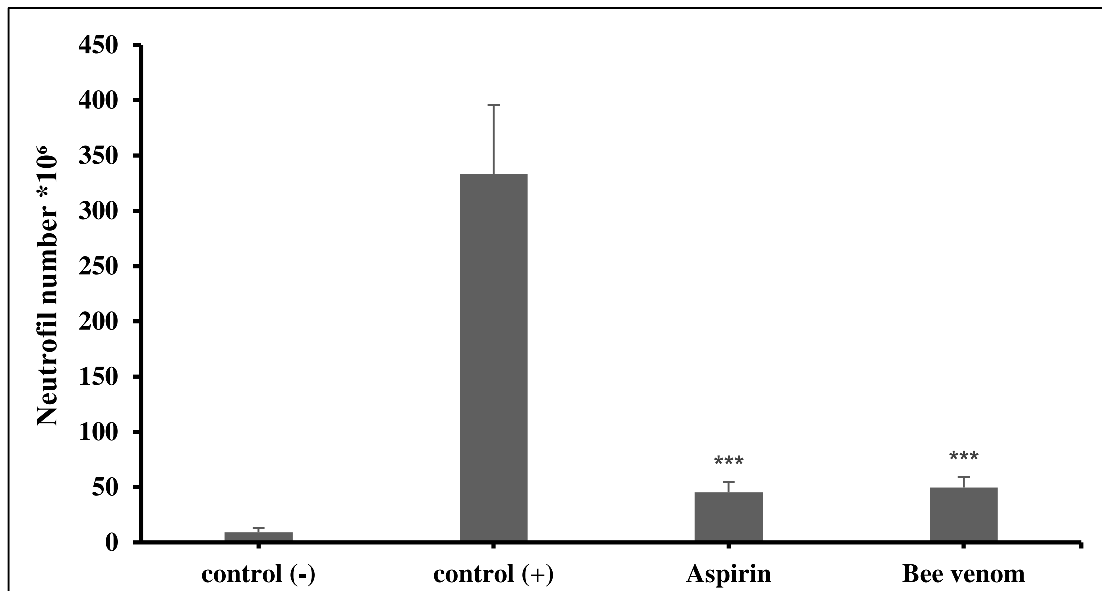


Figure 18. Effect of bee venom on the number of leukocytes recruited to the peritoneal cavity after intraperitoneal injection of 0.2 ml of 1% λ-carrageenan. Both histograms represent the mean (n=6) ±SEM; ***if p<0.001, ** if p<0.01 and *if p<0.05 compared to the control + (student test).

1.2. Results of hesperidin

Hesperidin is a citrus flavonoid with well-documented antioxidant and anti-inflammatory properties, are presented and interpreted to assess its biological impact and therapeutic potential.

1.2.1. Effect of Hesperidin on xylene-induced ear edema

The xylene-induced ear edema model was used to evaluate the topical anti-inflammatory activity of Hesperidin in rats. Xylene application to the inner and outer surfaces of the left ear induces rapid inflammation characterized by ear swelling.

Ear thickness was measured at five time points (T0 to T4) using a digital caliper. In the positive control group, ear thickness increased from 0.43±0.03 mm at T0 to a peak of 0.58±0.02 mm at T2, then gradually decreased to 0.49±0.02 mm at T4 (Figure 21), confirming an acute inflammatory reaction to xylene. The aspirin results marked 0.42±0.01 mm at T0 to 0.46±0.008 mm at T2, and a decrease to 0.42±0.01 mm at T4(Figure 21), reflecting a strong inhibition of inflammation. The Hesperidin-treated group also exhibited reduced ear edema, with measurements rising from 0.26±0.1 mm at T0 to 0.48±0.04 mm at T2, then decreasing to 0.29±0.02 mm at T4 (Figure 21).

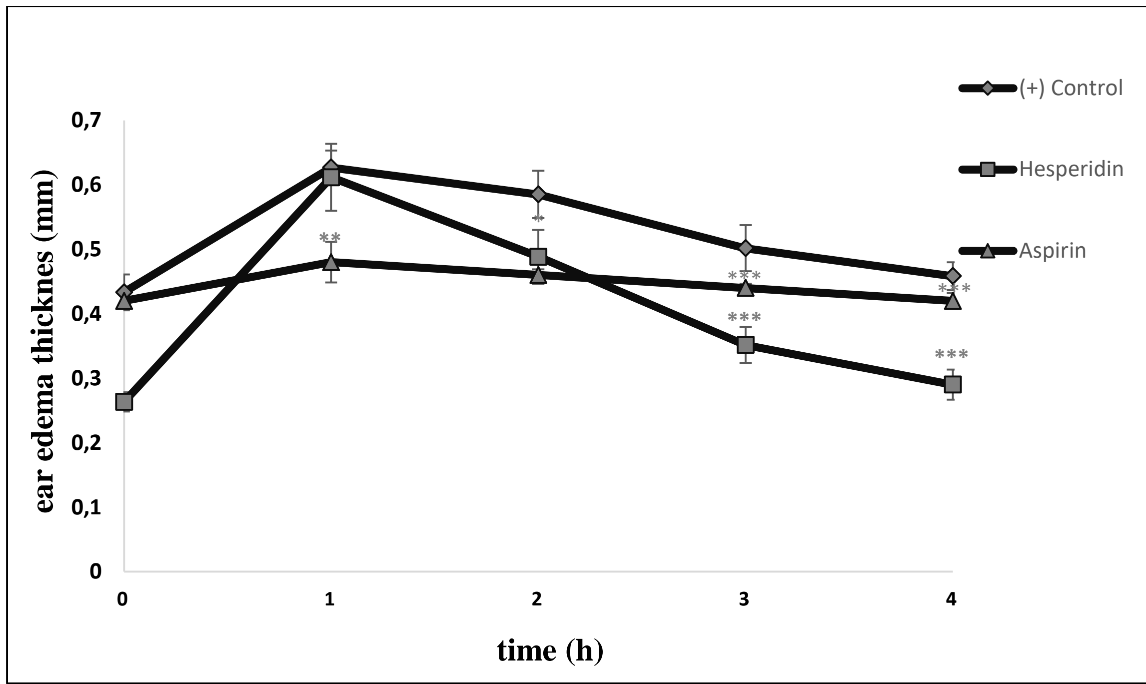


Figure 19. Evolution of ear thickness following edema induced by xylene after administration of Hesperidin and Aspirin over time. Results are presented as mean ± SEM for n = 6; ** if p < 0.01; * if p < 0.05; * if p< 0.001 compared to the positive control group (Student's t-test).**

Calculated inhibition percentages for the Hesperidin group were 2% at T1,17% at T2**,** 30% **(**p < 0.01**)** at T3, and 32% (p< 0.001**)** at T4. In comparison, Aspirin produced 23% inhibition at T1**,** peaked at 21% at T2 (Figure 22), and declined to 8% at T4. Notably, the anti-inflammatory effect of Hesperidin surpassed that of Aspirin at later time points (T3 and T4) (Figure 22) , suggesting a more prolonged action.

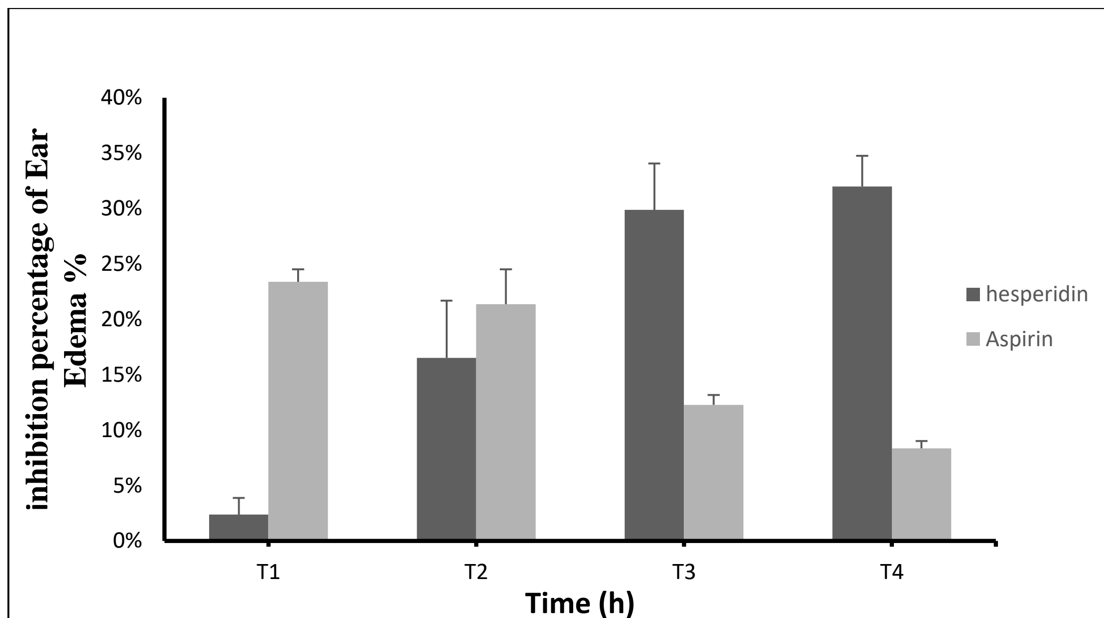


Figure 20. Inhibition percentage of ear edema induced by xylene following administration of Hesperidin and Aspirin. Results are presented as mean ± SEM for n = 6; ** if p < 0.01; * if p < 0.05; * if p < 0.001 compared to the positive control group (Student's t-test). test**

1.2.2. Effect of Hesperidin on λ-carrageenan-induced paw edema

Paw thickness was measured at five time points: T0 (baseline), and then hourly up to T4. The positive control group exhibited a clear increase in paw edema, starting from 2.50 ±0.21 mm at T0 and reaching 6.84 ± 0.57mm at T4 (Figue 19), confirming a strong inflammatory response. In the Aspirin-treated group the paw thickness increased more moderately, from 2.67 ±0.21 mm at T0 to 4.80 ± 0.63 mm at T4 (Figue 19) , corresponding to inhibition rates of 38% ($p< 0.001$) at T1, 33% at T2 and T3, and 30% ($p< 0.001$) at T4 (Figure 20).

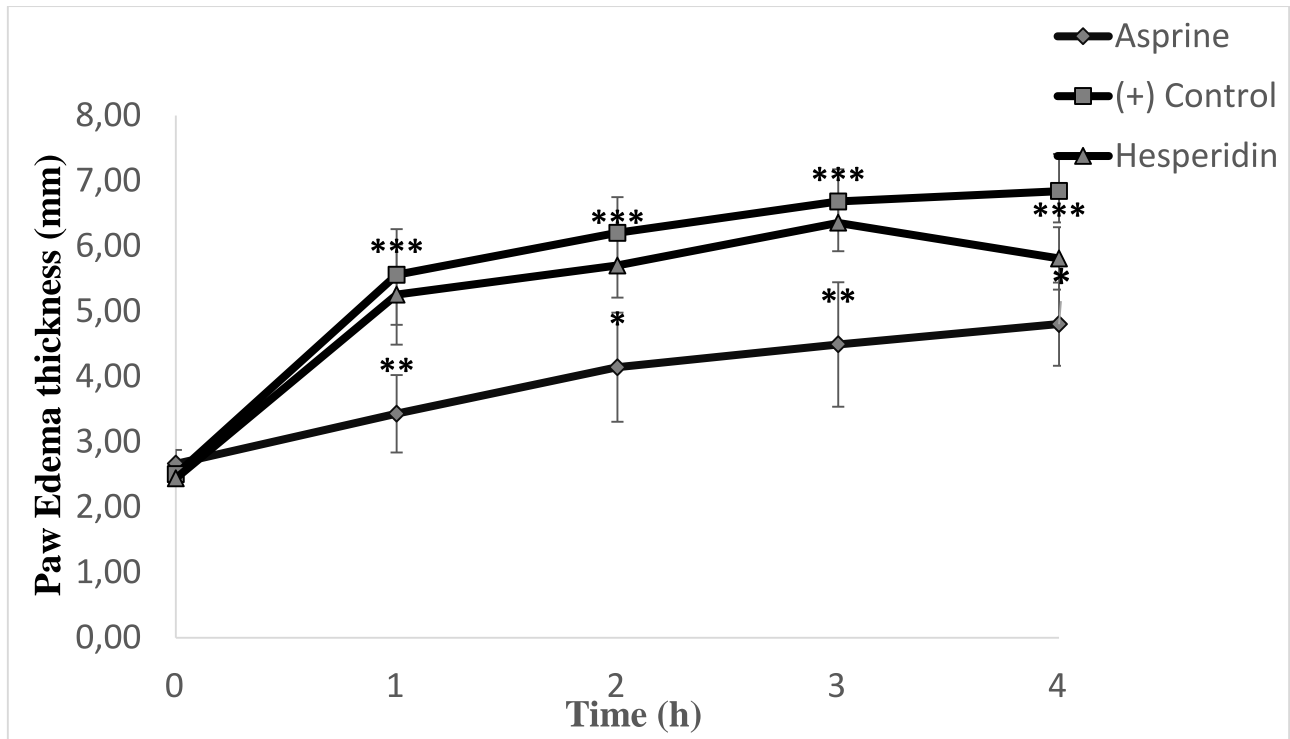


Figure 21. Evolution of paw thickness following the onset of carrageenan-induced edema after administration of Hesperidin and Aspirin over time. Results are presented as mean ± SEM for n = 6; ** if p < 0.01; * if p < 0.05; * if p< 0.001 compared to the positive control (Student's t-test).**

The Hesperidin-treated group also showed a reduction in edema progression compared to the untreated inflamed group. Paw thickness values rose from 2.44± 0.1 mm at T0 to 5.81 ±0.5 mm at T4 (Figue 19), with calculated inhibition percentages of 5% ($p < 0.05$) at T1, 8% at T2**,** 5% **(**$p < 0.01$) at T3 (Figure 20), and a more significant 15% ($p < 0.01$) at T4. These results indicate that Hesperidin was able to reduce paw inflammation over time, although its effect was less pronounced than Aspirin.

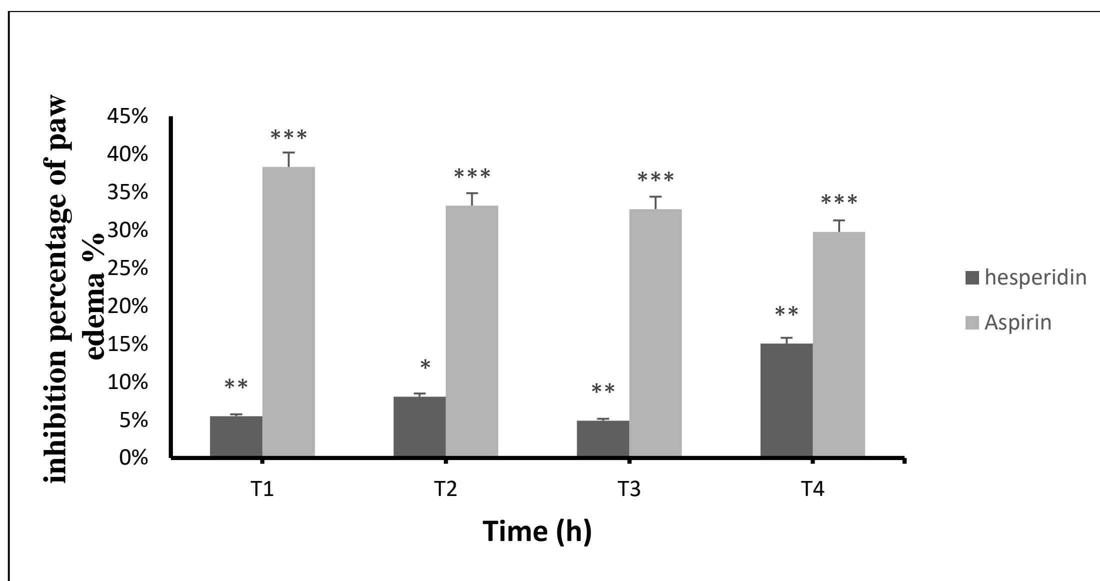


Figure 22. Inhibition percentage of paw edema induced by carrageenan following administration of hesperidin and Aspirin. Results are presented as mean ± SEM for n = 6; ** if p < 0.01; * if p < 0.05; * if p < 0.001 compared to the positive control group (Student's t-test).**

1.2.3. Effect of hesperidin on λ-carrageenan-induced peritonitis

As expected, the positive control group exhibited a significant increase in leukocyte count, reaching a mean of $333 \times 10^6 \pm 63$ cells/mL, confirming the strong inflammatory response induced by carrageenan. In contrast (Figure 23), the negative control group showed a markedly lower baseline leukocyte count ($9.23 \times 10^6 \pm 4$ cells/mL), indicative of normal physiological conditions without inflammation.

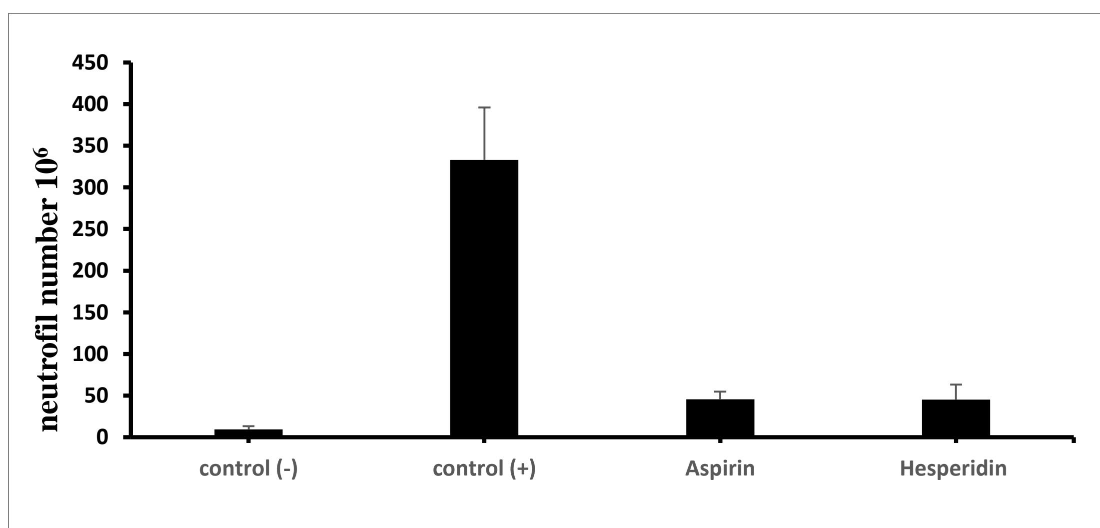


Figure 23. Leukocyte count in peritoneal exudate following inflammation induced by λ-carrageenan and treatment with Hesperidin and Aspirin. Results are expressed as mean ± SEM for n = 6; ** if p < 0.01; * if p < 0.05; * if p< 0.001 compared to the positive control group (Student t-test).**

Treatment with Aspirin resulted in a substantial reduction in leukocyte migration ($45.5 \times 10^6 \pm 9.16$ cells/mL) ($p< 0.001$) , representing a clear anti-inflammatory effect consistent with its known action as a cyclooxygenase inhibitor. Similarly, Hesperidin administration led to a decrease in leukocyte infiltration ($45.03 \times 10^6 \pm 18$ cells/mL) ($p< 0.001$)(Figure 23), nearly equivalent to that of Aspirin .

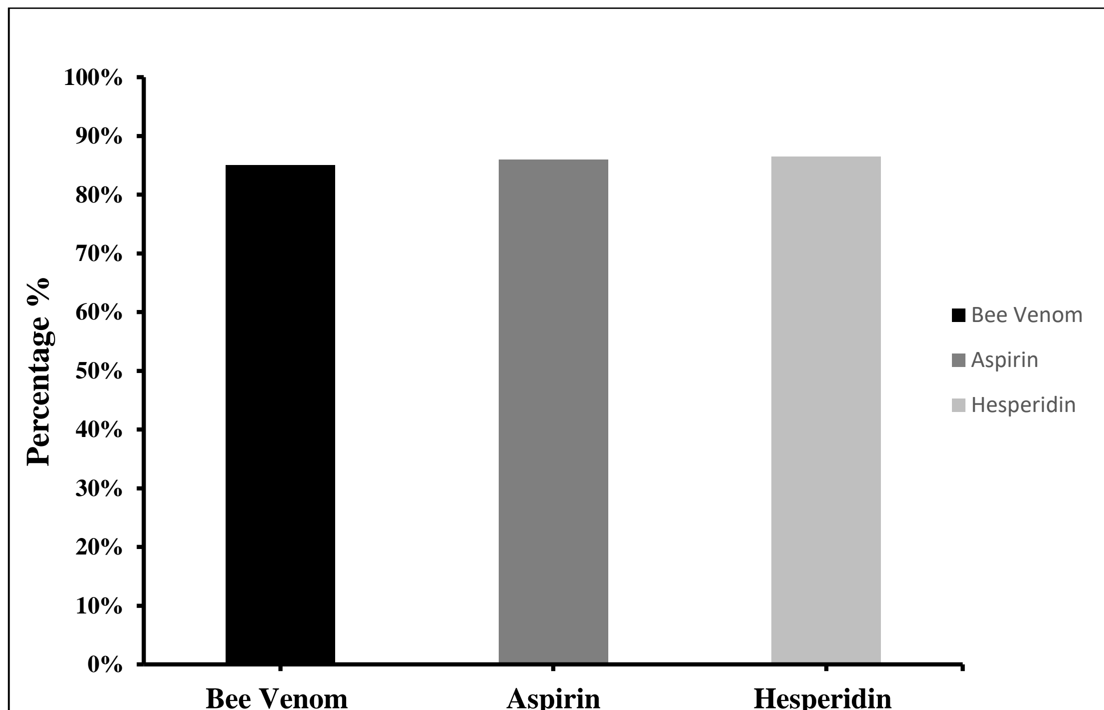


Figure 24. Percentages of inhibition of peritonitis by aspirin (reference) and bee venom, the results are presented as mean ±SEM for n=6; * if p<0.001, ** if p<0.01 and *if p<0.05 compared to the control + (student test).**

2. Discussion

This study aimed to evaluate the anti-inflammatory properties of two natural products: bee venom and hesperidin. Bee venom is a complex natural secretion, it has demonstrated significant anti-inflammatory effects, and the modulation of immune responses. Hesperidin, a flavonoid glycoside, exerts anti-inflammatory activity. In this study, both natural products were tested using three experimental models of acute inflammation in rats: carrageenan-induced paw edema, xylene-induced ear edema, and carrageenan-induced peritonitis.

2.1. Discussion of the results of Bee Venom

The results obtained from three experimental models of acute inflammation in rats: carrageenan-induced paw edema, xylene-induced ear edema, and carrageenan-induced peritonitis highlight the powerful anti-inflammatory activity of bee venom, which has been shown, in several cases, to be comparable to that of the reference drug, aspirin.

2.1.1. Effect of Bee Venom on Xylene-Induced Ear Edema

Acute inflammation manifests with characteristic signs such as redness, warmth, swelling, and pain. Topical application of xylene to the ear causes immediate irritation, local vasodilation, and increased vascular permeability, leading to interstitial fluid accumulation, which is responsible for the observed swelling **(Okoli *et al.,* 2007).** This method is widely used to evaluate the effects of topical anti-inflammatory agents.

Xylene-induced ear edema is recognized as a robust model for assessing anti-inflammatory activity, enabling comparisons across studies. For example, Lim et al. illustrated the efficacy of this model in evaluating different substances, highlighting its reliability **(Lim *et al.*, 2020)**. **Zhang *et al.* (2018)** also reported significant inhibition of edema using xylene-induced models, further corroborating this methodology.

The anti-inflammatory effects of bee venom (BV), dosed at 0.8 mg/kg, have been shown to result in a 40% inhibition of xylene-induced ear edema in rats at the 1st hour mark. This finding is supported by various studies utilizing xylene-induced ear edema models, demonstrating both methodological similarities and differences, as well as potential pharmacological mechanisms involved. In fact, **Kim *et al.* (2020)** demonstrated that BV reduced inflammatory cytokine levels in various models, validating its role as an anti-inflammatory agent **(Jeong *et al.,* 2017 ; Kim *et al*., 2020).** Specifically, **Kim *et al.* (2020)** highlighted BV's potential in dampening inflammatory responses, akin to our observed effects on edema inhibition.

The pharmacological mechanisms underlying BV's anti-inflammatory action primarily involve its active components, especially melittin and phospholipase A2. These ingredients significantly impact inflammatory pathways by inhibiting pro-inflammatory cytokine. At low doses, melittin inhibits the production of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, and blocks the activation of NF-κB and MAPK signaling pathways involved in the inflammatory response **(Wehbe *et al.,* 2019).** These pharmacological mechanisms can be responsible for the anti-inflammatory effects obtained in our study as previously reported discussed **(Sadeghi *et al.,* 2014; Liang *et al.,* 2017).** Moreover, the suppression of nuclear factor kappa B (NF-κB) signaling by active components in BV illustrates a fundamental mechanism in downregulating inflammation **(Jeong *et al.,* 2017 ; Hanafi *et al.,* 2018).** This mechanism may help to explain the observed inhibition in our study, as similar pathways were activated in research involving other agents with notable efficacy against edema.

Quantitative comparisons reveal that other studies have also measured similar effects. For instance, **Zhao *et al.* (2019)** reported a 35% reduction in xylene-induced edema with a different compound, indicating that our results of bee venom's anti-inflammatory effects are superior in comparison to established treatments. **Ajawobu *et al*. (2023)** noted a 21.21% inhibition with a distinct

extract, reinforcing the efficacy of BV within this model despite variations in specific compounds used. These data underline bee venom's established efficacy alongside other anti-inflammatory agents, suggesting its potential for therapeutic applications.

Previous studies have shown that melittin inhibits COX-2 and reduces ICAM-1 expression, thereby attenuating leukocyte migration to inflamed tissues **(Park *et al.*, 2004).** This inhibition of the vascular and cellular response may explain the significant reduction of the inflammation observed in our model.

Thus, the results obtained confirm the local anti-inflammatory activity of bee venom, suggesting its potential efficacy for inflammatory skin conditions.

2.1.2. Effect of bee venom on λ-carrageenan-induced paw edema

λ-carrageenan-induced paw edema is a classic model of acute inflammation, characterized by two distinct phases: a first phase (0–2 h) mediated by the release of histamine, serotonin, and bradykinin, followed by a second phase (3–6 h) dominated by the production of prostaglandins and pro-inflammatory cytokines **(Vinegar *et al.*, 1969 ; Di Rosa *et al.*, 1971 ; Niu *et al.*, 2012).**

Our study shows that bee venom significantly reduced carrageenan induced paw edema. In a similar context, **Park & Im, 2021** reported substantial reductions in paw edema, utilizing various doses of bee venom ranging from 10 to 50 mg/kg at comparable time intervals**.** The methodologies employed by these studies reinforce the relevance of bee venom's observed efficacy, despite our study utilizing a notably lower dose.

The inhibitory effect of bee venom observed in our study was particularly during the second phase, suggesting inhibition of the prostaglandin cascade, particularly PGE_2, via downregulation of the COX-2 enzyme. This is consistent with previous works who demonstrated that melittin inhibits COX-2 expression in activated macrophages **(Son et al., 2007)** and suppresses the expression of pro-inflammatory cytokines through modulation of key signaling pathways such as MAPK and NF-κB **(Kim & Seo, 2024).**

Furthermore, melittin interferes with Toll-like receptors (particularly TLR4), thereby reducing the expression of inflammatory mediators induced by LPS or carrageenan **(Han *et al.*, 2013).** Blocking these pathways reduces leukocyte infiltration and the production of mediators such as NO, TNF-α, and IL-6, all involved in amplifying the inflammatory response.

In support, **Huang *et al.* (2012)** illustrated that various anti-inflammatory agents diminish paw edema through inhibition of these signaling pathways, indicating a common mechanism among different compounds. Therefore, the pharmacological action of bee venom aligns with that of other anti-inflammatory treatments by modulating cytokine production and signaling pathways.

These results confirm the systemic anti-inflammatory activity of bee venom and suggest that its

bioactive compounds may act both upstream and downstream of the inflammatory cascade.

2.1.3. Effect of bee venom on λ-carrageenan-induced peritonitis

Carrageenan-induced peritonitis is a relevant model for studying cell migration, particularly the infiltration of polymorphonuclear leukocytes into the peritoneal cavity. This response is initiated by a massive production of cytokines (such as TNF-α, IL-1β, IL-6) and chemokines that promote immune cell adhesion and transmigration **(Sadeghi *et al.*, 2014 ; Barth *et al.*, 2016)**

In our study, rats treated with bee venom exhibited a significant decrease in total leukocyte count, indicating inhibition of inflammatory migration. This result is consistent with data published by **Choi *et al.* (2018)**, who showed that melittin inhibits neutrophil infiltration and cytokine secretion in a model of induced peritonitis.

Melittin may act by modulating the expression of adhesion molecules (such as ICAM-1 and VCAM-1) and inhibiting the activation of the transcription factor NF-κB, which plays a central role in orchestrating the acute inflammatory response **(Park *et al.*, 2004 ; Wehbe *et al.*, 2019).**

Therefore, bee venom exhibits a significant systemic anti-inflammatory effect in this model, likely through a mechanism combining inhibition of chemotaxis, suppression of pro-inflammatory signaling, and blockade of endothelial activation.

2.2. Discussion of the results of Hesperidin

The results obtained from three experimental models of acute inflammation in rats: carrageenan-induced paw edema, xylene-induced ear edema, and carrageenan-induced peritonitis highlight the powerful anti-inflammatory activity of hesperidin, which has been shown, in several cases, to be comparable to that of the reference drug, aspirin**.**

2.2.1. Effect of hesperidin on xylene-induced ear edema

The xylene-induced ear edema test is a well-established model for evaluating acute topical inflammation and the effectiveness of anti-inflammatory agents. As expected, the positive control group developed rapid and pronounced edema, confirming the effectiveness of the inflammatory stimulus **(Huang *et al.,* 2021 ; Martínez-Rizo *et al.*, 2023).**

Aspirin, used as a reference anti-inflammatory drug, effectively reduced edema formation in the early phase, particularly at T1 (23%) and T2 (21%), before tapering off by T4. Interestingly, Hesperidin, administered one hour prior to xylene exposure, showed a progressive and sustained inhibitory effect, with inhibition reaching 30% at T3 and remaining high at 32% at T4. This profile suggests that Hesperidin may have a slower onset but longer-lasting anti-inflammatory effect**,** which is consistent with its pharmacological profile as a flavonoid compound **(Shahraki *et al.,* 2021 ; Muhammad *et al.*, 2022).**

The exploration of Hesperidin's anti-inflammatory properties has garnered significant attention, particularly in the context of its ability to modulate inflammation induced by irritants such as xylene.

Hesperidin has shown promise in reducing edema formation in experimental models, demonstrating a noteworthy capability to inhibit the chronic phase of inflammation. Comparatively, traditional anti-inflammatory medications like Aspirin are effective primarily in the early stages of inflammation **(Hassan *et al.,* 2022).** It is essential to note that some studies suggest Hesperidin may have sustained anti-inflammatory effects, although exact metrics can vary based on the model employed **(Hassan *et al.,* 2022).**

Previous research indicates that Hesperidin has significant anti-inflammatory effects, contributing to a reduction in chronic inflammatory markers such as C-reactive protein (hs-CRP) and interleukin-6 (IL-6) (**Homayouni *et al.,* 2018**). Moreover, Hesperidin's mechanism of action appears to involve the down regulation of NF-κB and the suppression of pro-inflammatory cytokines, supporting the findings of Xiao et al. that Hesperidin affects NF-κB signaling in various models of inflammation **(Xiao *et al*., 2018).** This evidence underscores Hesperidin's potential as a natural anti-inflammatory agent.

The mechanisms underlying Hesperidin's efficacy are often associated with its antioxidant properties and its ability to stabilize cell membranes under inflammatory conditions, similar to traditional non-steroidal anti-inflammatory drugs (NSAIDs). **Ashry et al.** (**2023)** reported that Hesperidin induces the inhibition of specific signaling pathways, including p38 MAPK, which are crucial in mediating inflammatory responses. Such inhibition may not only mitigate acute inflammatory responses but also interfere with the progression of chronic inflammatory processes.

Contrasting Hesperidin's effects against other anti-inflammatory treatments reveals a potential advantage. While Aspirin provides rapid relief in acute inflammatory conditions, Hesperidin's delayed yet sustained effect may be more beneficial in circumstances requiring prolonged anti-inflammatory intervention **(Zhang et al., 2021).**

2.2.2. Effect of hesperidin on λ-carrageenan-induced paw edema

The results obtained from the carrageenan-induced paw edema model demonstrate that Hesperidin exhibits a measurable anti-inflammatory effect *in vivo*. The positive control group, which received carrageenan without any treatment, developed a marked and progressive edema, confirming the reliability of the model for inducing acute inflammation. Aspirin, used as a reference anti-inflammatory drug, produced a strong and consistent inhibitory effect on edema formation across all time points, validating the responsiveness of the model.

Hesperidin, administered orally produced a moderate but clear reduction in paw swelling, particularly evident at T4, where it reached 15% inhibition**.** Although its activity was significantly lower than that of Aspirin, the data show a delayed anti-inflammatory response, suggesting that Hesperidin

may act more gradually or may require a longer period to exert its full effect due to absorption and metabolism dynamics.

In assessing the anti-inflammatory effects of Hesperidin in carrageenan-induced paw edema, our findings illustrate a distinctive response compared to traditional non-steroidal anti-inflammatory drugs (NSAIDs) such as Aspirin. Carrageenan, a well-established model for inducing acute inflammation, exhibits a biphasic inflammatory response that can be effectively monitored through variations in paw thickness over time (**Zhang *et al*., 2008; Mathew *et al.,* 2014 ; Li *et al*., 2020**). In our study, we demonstrated that the positive control group, receiving carrageenan alone, experienced a substantial increase in paw thickness, corroborating other literature that emphasizes the model's reliability under acute inflammatory conditions **(Zhang *et al.,* 2008; Mathew *et al*., 2014; Onoja *et al.,* 2017).**

Aspirin showcased a significant early anti-inflammatory effect, inhibiting edema by 38% at T1, which aligns with known characteristics of NSAIDs that typically exert a rapid onset with waning effects over time **(Oyekunle *et al.,* 2010; Belay & Makonnen, 2020).** This was corroborated by the literature, which documents similar patterns of NSAID action within acute inflammation models, particularly their initial potency that diminishes subsequently, leaving a degree of inflammation unresolved **(Luo *et al.,* 2010; Belay & Makonnen, 2020).** However, it's imperative to consider that while Aspirin exhibits quickly diminishing effects, it may have less potential to manage prolonged inflammation phases effectively.

Conversely, Hesperidin revealed a unique inhibition profile. Initially, its effect was less pronounced, with only 5% and 8% inhibition at T1 and T2 respectively; nevertheless, its inhibitory action progressively intensified and became significantly more pronounced from T3 to T4, culminating in a 15% reduction by T4 **(Emim *et al.,* 1994).** This delayed yet sustained anti-inflammatory response may be attributed to Hesperidin's flavonoid nature, which suggests mechanisms involving antioxidant activity, stabilization of cellular membranes, and modulation of various inflammatory mediators **(Emim *et al*., 1994 ; Manthey & Bendele, 2008 ; Hà *et al*., 2022).**

Similar studies have noted the importance of Hesperidin's action in inflammatory contexts. For instance, a study on various flavonoids indicated their role in inhibiting cyclooxygenase activity and promoting reductions in inflammatory responses **(Emim *et al.,* 1994; Manthey & Bendele, 2008).**

Moreover, other research has demonstrated comparable anti-inflammatory effects by Hesperidin through the inhibition of mediators such as prostaglandins, which aligns with our findings that underscore the compound's gradual yet robust therapeutic advantages in managing carrageenan-induced inflammation (**Emim *et al*., 1994; Hà *et al*., 2022**).

2.2.3. Effect of hesperidin on λ-carrageenan-induced peritonitis

The findings from our study on the anti-inflammatory effects of hesperidin, particularly its influence on leukocyte migration in the carrageenan-induced peritonitis model, align with our previous results obtained with the two previous tests and with a growing body of literature highlighting the therapeutic potential of flavonoids in managing inflammation. The notable decrease in leukocyte counts in the peritoneal exudate upon hesperidin treatment, comparable to that observed with aspirin, underscores its efficacy in modulating acute inflammatory responses **(Homayouni *et al.,* 2018; Ashry *et al.,* 2023).**

Emerging studies further corroborate our findings. For example, Jain and Parmar reported similar anti-inflammatory properties of hesperidin in a rat air pouch model, emphasizing its capability to diminish leukocyte infiltration and enhance tissue antioxidant defenses through the reduction of oxidative stress markers such as lipid peroxidation **(Jain & Parmar, 2010)**. Such studies suggest that hesperidin exerts anti-inflammatory effects not only by inhibiting cell migration but also by modulating oxidative stress, which critically influences inflammatory pathways.

Moreover, the work of **Ashry et al.** (**2023)** supports our assertion that hesperidin inhibits the production of pro-inflammatory cytokines like IL-1β and TNF-α, contributing to its systemic anti-inflammatory effects. These cytokines play a pivotal role in leukocyte recruitment during inflammation, indicating that hesperidin may exert its effects through multiple pathways that regulate immune cell activity. The consistency of leukocyte response across studies substantiates the hypothesis that hesperidin can serve as a viable therapeutic agent against inflammation-associated conditions.

Our results obtained in this test contribut significantly to the existing literature, proposing that hesperidin's pharmacological applications extend beyond its antioxidant capacity to include direct modulation of leukocyte behavior in inflammatory states. Future studies could explore the dose-response relationship of hesperidin and its long-term effects on immune function and inflammation to further delineate its therapeutic potential.

V.CONCLUSION and PERSPECTIVES

CONCLUSION and PERSPECTIVES

In this study we have evaluated the anti-inflammatory effects of bee venom and hesperidin using three models of *in vivo* experimental inflammation : xylene-induced ear edema, **λ**-carrageenan-induced paw edema, and **λ**-carrageenan-induced peritonitis.

The results obtained confirm the significant efficacy of these two substances. In the ear edema model, they provided almost complete inhibition of inflammation, with an efficacy comparable to that of aspirin, used as a reference.

Regarding paw edema, hesperidin demonstrated a significant, albeit delayed, anti-inflammatory effect, suggesting a progressive action. In contrast, bee venom exerted rapid and sustained inhibition of inflammation, equivalent to that of aspirin.

In the peritonitis model, both substances also demonstrated a strong ability to inhibit leukocyte recruitment, achieving levels of efficacy close to those of aspirin.

Overall, these results indicate that bee venom and hesperidin have promising therapeutic potential as natural anti-inflammatory agents and could be considered as an adjuvant treatment in chronic inflammatory diseases. Their complementary action profile paves the way for future in-depth research into their mechanisms of action and their potential use as alternatives or adjuvants to conventional anti-inflammatory treatments, particularly in inflammatory conditions resistant to current treatments.

Despite its promising effects, bee venom remains a biologically active agent with potential allergens. Hypersensitivity reactions, ranging from simple redness to anaphylactic shock, have been reported. This highlights the need for dose standardization, purification of active compounds, and rigorous tolerance testing before any large-scale clinical application.

The emerging profile of hesperidin illustrates its multifaceted therapeutic potential across various domains of health, particularly in inflammation-related diseases, reproductive toxicity management, and cancer treatment. Its anti-inflammatory potential establishes it as a promising natural compound with significant medical applications. Further research into its mechanisms of action and clinical efficacy will be essential in harnessing hesperidin's full therapeutic potential.

VI. BIBLIOGRAPHY

BIBLIOGRAPHY

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