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TITOLO

Health Benefits of Nuts Bioactive Compounds: Focus on Oxidative Stress and Inflammatory markers

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SUMMARY

Abstract:

Oxidative stress and inflammation are key contributors to the development of numerous inflammatory, metabolic, and neurodegenerative diseases. Increasing evidence highlights the role of diet, particularly the Mediterranean diet, in modulating these processes through the intake of bioactive compounds. Nuts represent an important source of polyphenols, unsaturated fatty acids, vitamins, and minerals; however, the biological potential of specific nut fractions and by-products remains only partially explored. This PhD thesis investigated the antioxidant, anti-inflammatory, and regenerative effects of bioactive compounds derived from almonds and cashews using validated experimental models of acute inflammation, wound healing, and neuroinflammation induced by a metabolic condition known as hyperhomocysteinemia. Almond skin extract was evaluated in a carrageenan-induced paw edema model, while the wound-healing properties of almond oil were assessed in a murine excisional wound model. In addition, the effects of cashew nut supplementation were examined in a methionine-induced hyperhomocysteinemia model, focusing on oxidative stress, neuroinflammation, and neuronal damage. The results demonstrate that nut-derived compounds significantly reduce oxidative stress and inflammatory markers, limit tissue damage, and promote repair processes. These protective effects are consistently associated with activation of the Nrf2/HO-1 antioxidant pathway and inhibition of NF-κB signaling. In the central nervous system, cashew supplementation attenuated glial activation, reduced apoptotic signaling, and preserved neuronal integrity. Overall, this work supports the potential of nuts and their by-products as sustainable sources of bioactive compounds with antioxidant, anti-inflammatory, and neuroprotective properties, suggesting their possible application in nutraceutical and preventive strategies against inflammation and oxidative stress related disorders.

INTRODUCTION

In recent years, the growing awareness of the role of nutrition on human health has led to a renewed interest in traditional dietary models, among which the Mediterranean diet is one of the most emblematic and studied examples. Nutritional research has highlighted the fundamental role of nutrition in preventing and treating various diseases (1). UNESCO recognizes the Mediterranean diet as a cultural heritage, which is characterized by the regular consumption of plant-based foods, including fruits, vegetables, whole grains, legumes, extra virgin olive oil, and nuts. This category includes almonds, walnuts, cashews, pistachios etc (2). In general, nuts play a fundamental role in human health and are the subject of numerous scientific studies due to their high content of bioactive compounds, in particular polyphenols, flavonoids, unsaturated fatty acids, phytosterols, fibres and vitamins, that give these foods important antioxidant, anti-inflammatory, cardio and neuroprotective properties, and in fact represent a real ally for human health (3). Regular intake of nuts and oilseeds has been associated with reduced cardiovascular risk, better control of lipid profile and systemic anti-inflammatory effect (3). However, most studies have focused on the overall nutritional composition, neglecting the therapeutic potential of the individual fractions of these fruits, such as the integument or the oil extracted. Interest in by-products of the agro-food industry is increasing, as they are rich in bioactive compounds with numerous beneficial effects (4). In addition, this interest is also driven by the need to adopt more accessible and sustainable economic strategies (5). The residues from industrial processing of dried fruit, such as the shell of almonds or the residues from cashew processing, are very rich sources of phytocompounds that are often discarded or underused. Reusing industrial by-products could help companies develop commercially viable products. By-products of the nuts industry, traditionally considered waste, are valuable sources of bioactive compounds with potential therapeutic application (4). Among them, polyphenols, flavonoids, tocopherols and unsaturated fatty acids have shown a marked ability to modulate cellular pathways crucial for the maintenance of redox homeostasis and inflammatory response (6, 7). In particular, several extracts from skins, shells and oils have shown an activation of the antioxidant **nuclear factor erythroid 2-related factor 2 (Nrf2)** pathway resulting in overregulation of cytoprotective enzymes such as **heme oxygenase-1 (HO-1)** and **superoxide dismutase (SOD)** (4, 8). Additionally, these components have been linked

to the inhibition of **nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)** activation and the reduction of pro-inflammatory mediator expression, such as **tumor necrosis factor-alfa (TNF-α)** and **interleukin-1 beta (IL-1β)** (4, 9). These combined effects not only help cells protect themselves against oxidative stress and chronic inflammation, but are also essential for the central nervous system, as they promote the downregulation of astrocyte and microglial activation markers like **glial fibrillary acidic protein** (**GFAP)** and **ionized calcium-binding adapter molecule 1 (Iba-1)** and the upregulation of proteins essential for neuronal maintenance like **microtubule associated protein 2 (MAP2)** (10). Furthermore, they also promote tissue regeneration mechanisms, positively influencing processes such as cell proliferation, extracellular matrix synthesis and neovascularization (11). Recent studies on experimental models of acute inflammation have shown that nut-derived extracts, such as those from almond seed coats, can significantly reduce phlogogen-induced paw edema. These effects are attributed to the modulation of oxidative stress and inflammatory response, mediated by the activation of the Nrf2 pathway and the inhibition of NF-κB, resulting in decreased production of cytokines and inflammatory mediators (4). Such findings suggest a potential therapeutic role of nut-derived components in controlling acute inflammatory states. In the context of wound healing (WH), almond oil, especially when cold-pressed and unrefined, has demonstrated notable regenerative properties. Thanks to its rich content of tocopherols and monounsaturated fatty acids, it promotes tissue repair by exerting emollient, moisturizing and antioxidant effects (12). These components assist in the remodeling of the extracellular matrix and stimulate cellular responses involved in regeneration, such as fibroblast proliferation and neovascularization. Moreover, recent investigations have reported a downregulation of pro-inflammatory mediators and an upregulation of matrix metalloproteinases, such as MMP-9, which are essential in the wound remodeling phase but require precise regulation to avoid tissue damage. The antioxidant and anti-inflammatory balance provided by almond oil helps regulate **matrix metalloproteinase (MMP) activity**, supporting proper healing dynamics (13, 14). These evidences support the potential use of nut by-products as sustainable resources for the development of new biomedical and nutraceutical approaches. *Almonds (Prunus dulcis)*, in particular, represent a food of high scientific interest. Their seed coat, commonly removed during processing, is rich in polyphenols,

flavonoids and phenolic acids, with documented antioxidant and anti-inflammatory properties (15, 16). Our recent study suggested that almond seed husk extract can modulate the inflammatory response in experimental models of phlogogen-induced paw edema in rodents, significantly reducing swelling and the production of pro-inflammatory mediators (4). In addition, almond oil, rich in monounsaturated fatty acids and tocopherols, has shown high efficacy in promoting skin regeneration and improving the WH process. Topical application of the oil has shown emollient, moisturizing and antioxidant effects that can contribute to tissue repair and the reduction of local oxidative stress (17). *Cashews (Anacardium occidentale)*, another widely consumed nut, are particularly rich in copper, magnesium and a wide variety of phenolic compounds. Recently, it has been observed that their intake can positively influence plasma levels of homocysteine, a sulfur-containing amino acid whose accumulation in the blood is associated with increased cardiovascular risk (18). Cashews, thanks to their content of B vitamins and minerals, seem to contribute to the conversion of homocysteine into methionine, thus promoting the maintenance of correct methyl metabolism (18, 19). During my master's thesis, we conducted a study using a rat model of induced hyperhomocysteinemia involving the blood, colon, liver, and kidneys, and we observed that cashew supplementation improved tissue inflammation and oxidative stress, probably through the regulation of the Nrf2 and NF-kB pathways, increasing the antioxidant capacity (18). More recently, the focus has shifted toward the neurological implications of hyperhomocysteinemia, which is known to promote oxidative and inflammatory damage at the central nervous system level (20). Cashew supplementation in these models has been associated with the attenuation of glial activation, reduction of oxidative stress markers, and preservation of neuronal integrity (21). These effects appear to be mediated by the same molecular mechanisms described above, including the activation of Nrf2 and suppression of NF-κB, thereby contributing to the modulation of neuroinflammatory pathways and maintenance of redox homeostasis in the brain (22). Therefore, this thesis aims to comprehensively study the physiological and therapeutic effects of nuts, focusing in particular on cashews and almonds, especially their skins and oils, using validated experimental models to understand the molecular mechanisms through which nuts produce their beneficial effects and support their potential functional and targeted use in clinical and nutraceutical fields.

CHAPTER 1: Oxidative stress and Inflammation

Oxidative stress and inflammation are interconnected biological processes that play a pivotal role in systemic diseases and central nervous system (CNS) disorders. Oxidative stress refers to an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses, and it plays a central role in tissue regeneration processes by promoting the infiltration of peripheral immune cells and facilitating the release of inflammatory mediators within the CNS (23). Excess ROS leads to structural damage to proteins and cellular membranes, thereby delaying healing in various inflammatory and metabolic conditions. Furthermore, **neuroinflammation**, an inflammatory response within the CNS, is a key factor in the pathogenesis of neurological diseases (24). This response is often sustained by **metabolic imbalances**, including **dysregulation of lipid metabolism**. For example, elevated **homocysteine** levels represent a significant metabolic disturbance known to promote a **pro-oxidant and pro-inflammatory environment**. Moreover, both systemic and neuroinflammation exacerbate oxidative damage by impairing **endothelial integrity** (25). This condition is strongly associated with **microglial activation** and increased release of **pro-inflammatory cytokines**, reinforcing chronic inflammation and contributing to neuronal injury (26). In particular, these phenomena frequently occur in the context of broader **metabolic dysregulation**, suggesting that **altered metabolic homeostasis** is a key driver of both systemic and CNS inflammation. Collectively, the convergence of oxidative stress, metabolic imbalance, and immune activation establishes a **self-perpetuating cycle** that underlies **edema formation**, **delayed tissue regeneration**, and **persistent neuroinflammation**. A comprehensive understanding of these interconnected pathways is essential for identifying **therapeutic targets** in diseases where metabolism and inflammation intersect.

1.1 Oxidative Stress: Definition, Sources, and Cellular Implications

Oxidative stress is a pathological condition resulting from an imbalance between the production of **ROS** and the capacity of endogenous antioxidant systems to neutralize or eliminate them effectively (27). Under physiological conditions, cells maintain a reduced intracellular environment through a finely tuned system that balances ROS generation and detoxification. However, when ROS production exceeds the antioxidant capacity of the cell, a chain of reactions is triggered, leading to structural and functional damage to essential macromolecules such as proteins, lipids, and nucleic acids (28). ROS comprise a wide variety of highly reactive molecules, including singlet oxygen ($^1O_2$), superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (•OH). The primary intracellular source of ROS is the electron transport chain (ETC) located in the inner mitochondrial membrane (29). In particular, during oxidative phosphorylation, a small percentage of molecular oxygen (~1–2%) is not fully reduced to H_2O but undergoes partial reduction to form superoxide anion, primarily at complexes I and III of the ETC. This superoxide is converted by **superoxide dismutase (SOD)** into H_2O_2, which, in the presence of transition metal ions (e.g., Fe^{2+}), can generate hydroxyl radicals via the Fenton reaction (30). In addition to ROS, **reactive nitrogen species (RNS)** play a crucial role in oxidative stress. These include nitric oxide (NO), peroxynitrite ($ONOO^-$), and nitrogen dioxide (NO_2). NO is synthesized from L-arginine by nitric oxide synthase (NOS), and acts either as a physiological signaling molecule (e.g., in vasodilation and neurotransmission) or as a contributor to oxidative damage when it combines with superoxide anion to form peroxynitrite, one of the most cytotoxic RNS. Damage induced by ROS and RNS occurs at multiple biological levels (31). **Proteins** may undergo irreversible oxidation at lysine, arginine, proline, and threonine residues, leading to carbonylation, loss of biological function, and, in severe cases, aggregation and intracellular accumulation (32). **DNA** is particularly vulnerable to oxidative modifications of nitrogenous bases (e.g., 8-oxoguanine), strand breaks, and protein-DNA cross-links. These events can alter gene expression, impair replication mechanisms, and, if not corrected by DNA repair systems, lead to permanent mutations (32). **Lipids**, particularly those containing polyunsaturated fatty acid chains, are highly susceptible to peroxidation. Hydroxyl radicals can initiate lipid peroxidation, compromising the integrity and fluidity of cellular membranes and

leading to the generation of toxic secondary metabolites such as **malondialdehyde (MDA)** and **4-hydroxynonenal (4-HNE)** (33, 34). Moreover, oxidative stress is closely associated with the **inflammatory response**, in which activated neutrophils and macrophages produce large quantities of ROS via enzymes such as NADPH oxidase and myeloperoxidase (35). This increased production contributes not only to tissue damage but also to edema development, as ROS enhance vascular permeability, promoting fluid and plasma protein leakage into the extracellular space (36). This effect is particularly evident in tissues affected by ischemia-reperfusion injury, infections, or trauma, where ROS accumulation also triggers endothelial alterations and activation of coagulation and thrombotic pathways (37, 38). Despite its harmful potential, oxidative stress also plays a crucial role in tissue regeneration. A moderate production of ROS is, in fact, essential for cell migration, fibroblast proliferation, extracellular matrix synthesis, and angiogenesis—all of which are crucial events in tissue repair. However, an excessive shift from physiological to pathological ROS levels may impair regeneration, promoting fibrosis, apoptosis, or necrosis in cells involved in tissue repair (36). Oxidative stress has been recognized as a central pathogenic factor in numerous clinical conditions, including **cardiovascular and neurodegenerative diseases**, diabetes mellitus, **premature aging**, **chronic inflammatory disorders**, and various forms of **cancer** (39, 40). Chronic ROS and RNS accumulation can trigger apoptotic or necrotic pathways, depending on the intensity and duration of the oxidative insult, thus representing not only a marker of cellular damage but also an active mediator in disease progression (41, 42) (Figure.1). In conclusion, oxidative stress reflects a breakdown in cellular redox homeostasis, with direct consequences on cellular viability and function. An in-depth analysis of the mechanisms underlying ROS and RNS production, as well as antioxidant defense systems (both enzymatic and non-enzymatic), forms a fundamental basis for understanding many pathologies and identifying potential antioxidant therapeutic strategies—especially in clinical contexts where tissue integrity must be preserved or restored.

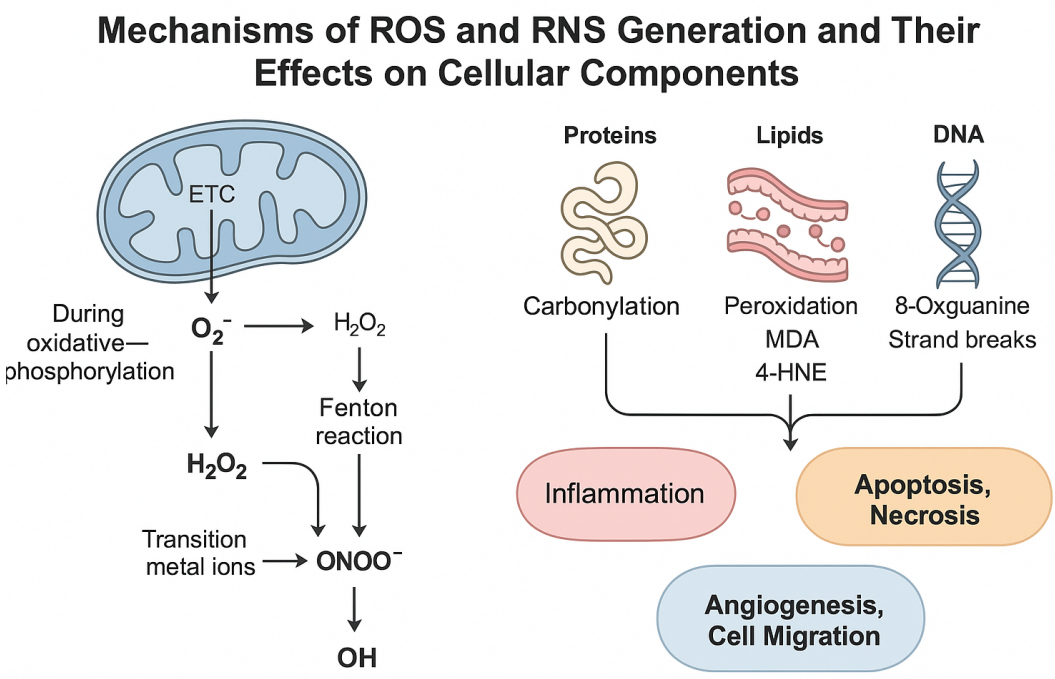

Figure 1. Mechanisms of ROS and RNS Generation and their effects on cellular components.

1.1.1. Endogenous Antioxidant Systems

To neutralize the excess of ROS and RNS, cells rely on a complex defense system composed of enzymatic and non-enzymatic antioxidants, which work synergistically to maintain intracellular redox homeostasis. Antioxidants perform their function by neutralizing reactive species through electron donation or catalyzing redox reactions, thus limiting damage to proteins, lipids, and nucleic acids. These systems are particularly effective thanks to their organization into an antioxidant network, in which each component supports or regenerates the others.

The main enzymatic antioxidants include (Tab.1):

- **SOD**, which catalyzes the dismutation of $O_2^{\bullet -}$ into H_2O_2 and molecular oxygen (O_2). Three isoforms exist: SOD1 (Cu/Zn-SOD) in the cytoplasm, SOD2 (Mn-SOD) in mitochondria, and SOD3 (EC-SOD) in the extracellular compartment (43).
- **Catalase (CAT)**, located in peroxisomes, rapidly converts H_2O_2 into water and oxygen (44).

- **Glutathione peroxidase (GPx)**, a selenium-dependent enzyme that uses reduced glutathione (GSH) to reduce H_2O_2 and organic hydroperoxides (ROOH) into less reactive molecules (45).
- **Thioredoxin (Trx)** and **HO-1** complement the antioxidant response by reducing oxidized proteins and detoxifying heme, respectively (46).

These systems are finely regulated by the transcription factor **Nrf2**, which activates genes containing the antioxidant response element (ARE) (47). In addition to promoting the expression of the aforementioned enzymes, Nrf2 enhances the synthesis of reducing cofactors such as GSH and NADPH, and chelating proteins, also contributing to the regulation of key cellular processes such as autophagy, apoptosis, inflammation, mitochondrial biogenesis, and cell regeneration (47, 48).

ENZYME	COFACTOR	MAIN LOCATION
SOD	Cu/Zn (SOD1, SOD3), Mn (SOD2)	Cytoplasm, mitochondria, extracellular space
CAT	Fe (heme group)	Peroxisomes
GPx	Selenium	Cytoplasm, mitochondria
Trx	NADPH	Cytoplasm, mitochondria
HO-1	NADPH, O_2, heme	Endoplasmic reticulum (RE)

Table 1. Main endogenous antioxidants and related cofactors.

1.2 Inflammation: definition and fundamental characteristics

Inflammation, also known as phlogosis, is a complex immune response coordinated by a network of molecular signals and immune cells, aimed at neutralizing harmful agents and initiating tissue repair processes (49). Although inflammation is a physiological process, many inflammatory responses can cause severe tissue damage, contributing to the development of chronic and acute inflammatory diseases. At the microvascular level, endothelial activation leads to increased capillary permeability and vasodilation, facilitating the migration of leukocytes (mainly neutrophils and macrophages) and plasma extravasation into the interstitial tissue, a phenomenon known as inflammatory edema (50). While this edema is essential for the transport of immune cells to the site of injury, it can also cause mechanical and functional tissue damage, particularly in space-limited organs such as the brain. Inflammation is a phenomenon found in all pathologies and affects all areas of the body. Its function is twofold: repair and defense. Defense, as its initial goal is to eliminate or contain the cause of the damage (e.g., an infection). Repair, as it stimulates and promotes repair processes following the damage (e.g., in the case of a wound). The process occurs

through overlapping phases: inflammation, cell proliferation, and tissue remodeling. In the inflammatory phase, immune cells release pro-inflammatory mediators such as cytokines (TNF-α, IL-1β, IL-6), chemokines, and ROS, which modulate the immune response and activate mesenchymal cells for the synthesis of the extracellular matrix (51). The proliferative phase involves neoangiogenesis, fibroblast proliferation, and collagen deposition, which are essential for tissue regeneration and the reconstruction of the extracellular matrix (52). The final remodeling phase ensures the maturation of the scar and the functional restoration of the tissue (Figure 2).

There are many types of pro-inflammatory stimuli perceived by innate immune cells. These include:

1. Pro-inflammatory cytokines (TNF-α, IL-1β, IL-6);
2. Chemokines;
3. Physical and chemical stimuli (UV rays, free radicals);
4. Pathogens

From a clinical point of view, there are two types of inflammatory processes:

ACUTE INFLAMMATION = (short-lived) from a few minutes to a few days and there is accumulation of fluid (edema) and neutrophils.

CHRONIC INFLAMMATION = (long-lasting) there is the formation of new blood vessels, tissue remodeling with fibrosis and accumulation predominantly of lymphocytes and macrophages.

Acute inflammation is characterized by the presence of the five cardinal signs.

- RUBOR (redness) = due to local vasodilation
- TUMOR (swelling) = linked to edema, i.e., the accumulation of fluid in the interstitial spaces
- of damaged tissue. This leads to an increase in the volume of the area
- CALOR (increased heat) = due to increased blood flow caused by
- vasodilation
- DOLOR (the area is painful) = has a defensive meaning to rest
- the damaged organ or anatomical part
- FUNCTIO LESA (loss of function of the affected area)

Chronic inflammation is a less definable process than acute inflammation because in most cases it is nothing more than a consequence of acute inflammation,

or its prolongation. It can be considered as the persistence of the inflammatory response for months or years. In chronic inflammation, processes of tissue damage and repair persist and coexist, whereas in acute inflammation they occur temporally and for a short period of time. Acute inflammation occurs through three phases:

A. Initiation phase: molecular recognition of the pathogen by innate immune cells and the synthesis of cytokines occurs;

B. Evolution phase: a series of hemodynamic changes in the microcirculation occur, such as vasodilation, active hyperemia, and passive hyperemia.

This phase also includes leukocyte diapedesis and exudate formation;

C. Resolution or chronicization phase: acute inflammation is a dynamic process whose outcomes can include NECROSIS (caused by cellular destruction due to the release of lysosomal enzymes), CHRONICITY (which occurs when the inflammatory reaction has not completely eliminated the damaging agent), or RESOLUTION (elimination of the damaging agent with progressive resolution of symptoms, reabsorption of the exudate, and leukocyte apoptosis).

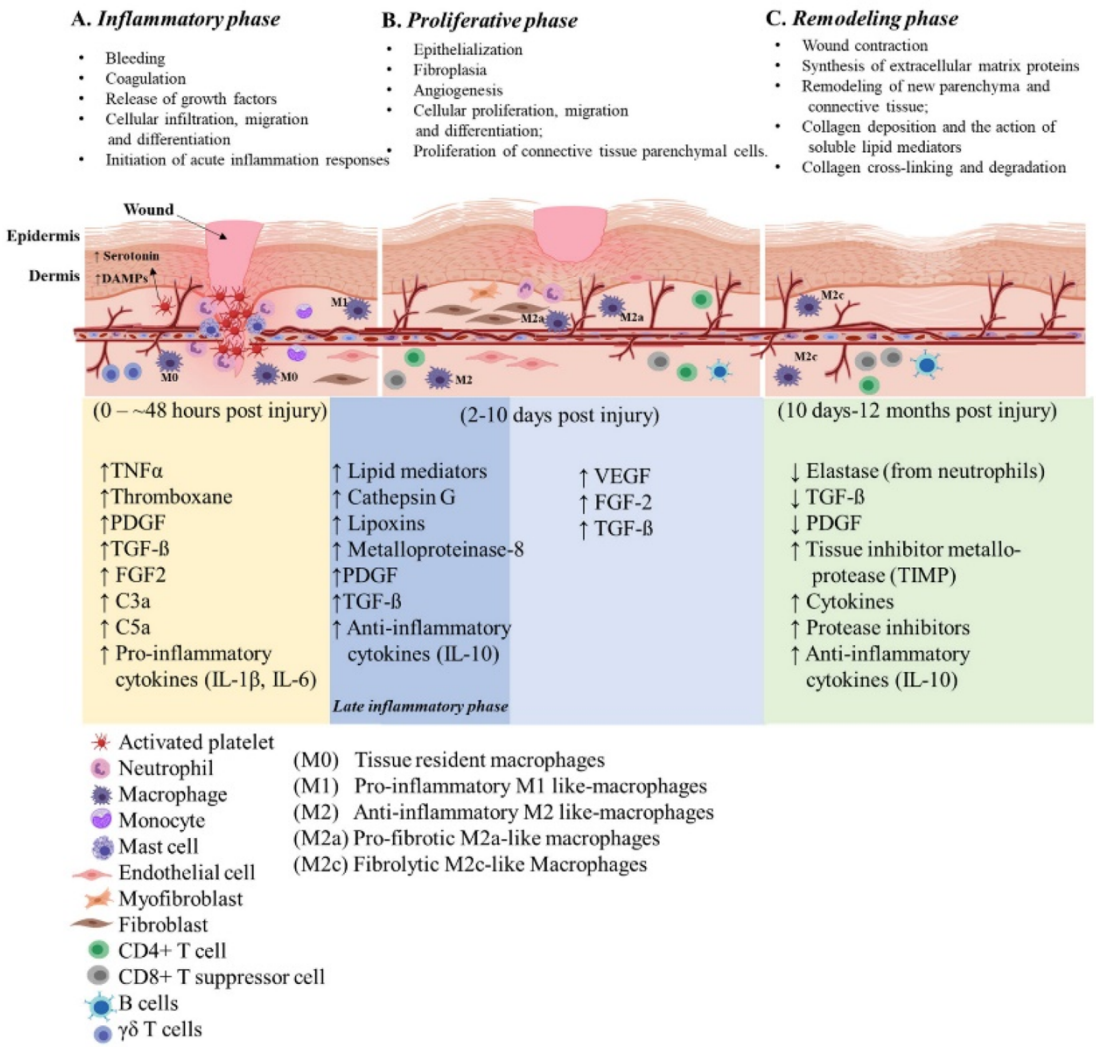

Figure 2. Schematic representation of the three overlapping phases of inflammation: inflammation, proliferation, and remodeling, including key cellular players and mediators involved in each phase.

1.2.1. Focus on Neuroinflammation

Within the neuroinflammatory context, **microglia** act as the primary immune sentinels of the CNS. In response to injury or infection signals, microglia activate and adopt a **pro-inflammatory phenotype** (commonly referred to as M1), characterized by the production of **pro-inflammatory cytokines** (TNF-α, IL-1β, IL-6), chemokines, nitric oxide, and ROS. If this state persists, it can lead to neurotoxicity and neuronal damage (53, 54). The protein **Iba1**, a marker of microglial activation, is closely associated with cell motility and phagocytic capacity, representing a reliable indicator of ongoing inflammation (54). In parallel, **astrocytes** play a critical role by increasing **GFAP expression**, which is associated with extracellular environment modulation, **blood-brain barrier (BBB)** regulation, and the release of inflammatory

and neurotrophic mediators. **Astrocyte hyperplasia**, or **astrogliosis**, is often linked to the formation of a **glial scar**, which can contain damage spread but may also hinder neuronal regeneration (55, 56). At the molecular level, the **NF-κB signaling pathway** acts as a central hub regulating the expression of major pro-inflammatory cytokines, oxidative mediators, and cell adhesion molecules (57). Persistent activation of NF-κB in glial cells perpetuates inflammation, contributing to **synaptic dysfunction**, **neuronal death**, and the progression of neuroinflammatory diseases (58). At the same time, **anti-inflammatory factors** and **resolution mechanisms**, including the secretion of **interleukin-10 (IL-10)** and activation of **pro-regenerative signaling pathways**, aim to restore homeostasis and promote neural plasticity (59). In summary, **neuroinflammation** represents a delicate balance between immune defense and tissue damage, where **glial activation** and **molecular regulation** are key determinants of the functional outcome of nervous tissue. Neuroinflammation is closely linked to apoptotic processes, involving a finely tuned balance between pro- and anti-apoptotic proteins, particularly members of the **Bcl-2 family**. **Bcl-2** proteins exert cytoprotective functions by inhibiting apoptosis, whereas **Bax** promotes cell death by increasing mitochondrial membrane permeability and facilitating the release of pro-apoptotic factors such as **cytochrome c** (60, 61). Under conditions of **chronic inflammatory stress** in the CNS, activation of microglia and astrocytes results in the release of **pro-inflammatory cytokines** and **ROS**, which can disrupt the Bcl-2/Bax balance in favor of apoptosis (55, 62) (Figure 3). This process contributes to tissue damage and worsening of neurocognitive functions, underscoring the importance of **modulating neuroinflammation** to preserve neuronal integrity and support tissue regeneration. Targeting the **Bcl-2/Bax-mediated apoptotic pathway** represents a crucial therapeutic strategy in the management of **neurodegenerative diseases** and **inflammation-related brain injury** (62).

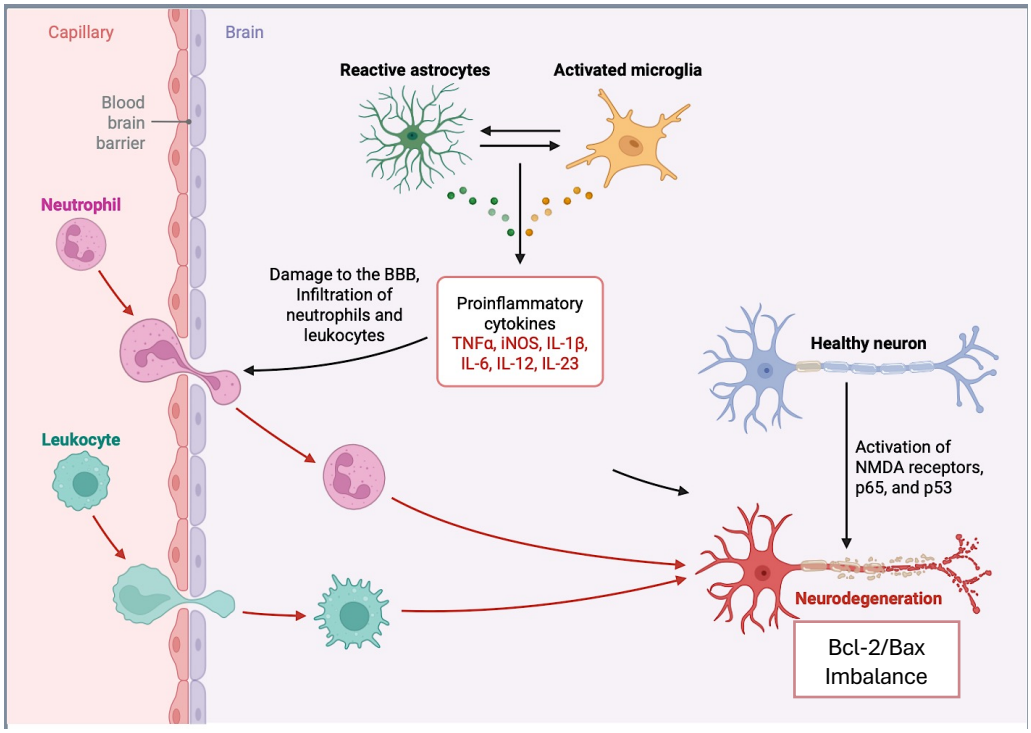

Figure 3. Cellular and molecular mechanisms of neuroinflammation. Activation of microglia and astrocytes in response to inflammatory stimuli leads to the production of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-12, IL-23) and reactive oxygen species (ROS). Damage to the BBB allows infiltration of neutrophils and leukocytes into brain tissue, amplifying the inflammatory process. This cascade results in activation of neuronal apoptotic pathways—particularly involving NMDA receptors, p65, and p53—disruption of the Bcl-2/Bax balance, and ultimately neuronal degeneration.

1.3 Experimental models

Experimental models have been widely used to study the mechanisms of inflammation due to their genetic and physiological relevance to humans. Experimental models represent fundamental tools for evaluating the efficacy of bioactive compounds, safety of new drugs, discovering the pathophysiological mechanisms and molecular pathways, the complex cellular and biochemical processes of wound repair and possible therapeutic strategies in the biomedical field. In this study, we focused on different animal models of inflammation, neuroinflammation, wound healing and metabolic conditions respectively: Carrageenan induced paw edema, wound healing and methionine induced Hyperhomocysteinemia (HHcy).

1.3.1. Pathophysiology of Edema and Carrageenan-Induced Paw Edema Model

Edema is one of the cardinal manifestations of the acute inflammatory process and is closely linked to vascular changes occurring at the site of the lesion. Its onset is determined by the accumulation of fluid in the interstitial space, a direct consequence of increased vascular permeability and plasma leakage from the capillaries (63). The main causes include:

(i) increased hydrostatic pressure (e.g., heart failure);
(ii) reduced oncotic pressure (e.g., hypoproteinemia);
(iii) increased vascular permeability (e.g., inflammation);
(iv) lymphatic obstruction (e.g., lymphedema) (64).

The sequence of events that leads to edema formation begins with arteriolar **vasodilation**, mediated by vasoactive substances such as **histamine**, **bradykinin**, **nitric oxide**, and **prostaglandins**, which cause an increase in local blood flow (hyperemia) (65). At the same time, endothelial cells undergo structural and functional changes that increase **vascular permeability**, allowing the passage of plasma proteins (e.g., fibrinogen, immunoglobulins) and fluid into the extravascular space. This process leads to the formation of **exudate**, a fluid rich in proteins and inflammatory cells, distinct from the transudate observed in non-inflammatory states (66). In the early stages, edema is, therefore, primarily due to hemodynamic and biochemical phenomena, while in a later phase, leukocyte infiltration, especially neutrophils, also contributes (67). These infiltrates amplify endothelial damage and maintain high vascular permeability through the release of proteolytic enzymes and reactive oxygen species. The presence of fibrin and other plasma proteins in the exudate also promotes the formation of a temporary matrix that hinders the spread of pathogens and facilitates cell recruitment. From a functional standpoint, edema, therefore, represents a defensive mechanism, as it allows the dilution of toxic substances, the transport of antibodies and complement factors, and the migration of immune cells toward the inflammatory focus. However, if excessive or prolonged, it can compromise the oxygenation and functionality of the tissue, contributing to the chronicity of the inflammatory process and secondary tissue damage (68). To reproduce these mechanisms in a controlled manner and study their temporal evolution, the carrageenan-induced paw edema model is widely used (69). The injection of

carrageenan (generally at 1%) into the subplantar region of the rodent's hind paw triggers an acute inflammatory response that mirrors the typical phases of inflammatory edema (70).

- **Early phase (0–2 h):** dominated by vasoactive mediators such as histamine, serotonin, and bradykinin, which induce vasodilation and increase endothelial permeability, leading to plasma and protein extravasation.
- **Intermediate phase (2–6 h):** characterized by the involvement of prostaglandins and other eicosanoids, which sustain swelling and promote the onset of local hyperalgesia.
- **Late phase (>6 h):** marked by the recruitment of neutrophils and macrophages, accompanied by the production of ROS and pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), which amplify endothelial damage and sustain the inflammatory response (71).

The measurement of paw volume or thickness over time provides a quantitative index of edema severity and allows the evaluation of the effectiveness of anti-inflammatory compounds (72). Thanks to its reproducibility and sensitivity, this experimental model represents a well-established reference for the study of acute inflammation, as it directly links the pathophysiological mechanisms of edema—vasodilation, vascular permeability, and leukocyte infiltration—with potential therapeutic strategies aimed at modulating them.

1.3.2. Wound Pathophysiology and Excisional Wound Model

The critical processes underlying wound healing have been initially described using animal models (73, 74). Wound healing is a complex and dynamic process involving various cellular and molecular mechanisms, coordinated sequentially. This process can be divided into four main phases: **hemostasis, inflammation**, **proliferation**, and **remodeling (75)**.

- **Hemostasis Phase**: the hemostatic phase occurs immediately after injury. Interruption of vascular continuity leads to platelet activation and the formation of a fibrin clot, which stops bleeding and acts as a temporary matrix. Platelets also release numerous growth factors, such as PDGF, TGF-β, and VEGF,

which are essential for the recruitment of inflammatory cells and the initiation of the angiogenic process (76).

- **Inflammatory Phase**: the inflammatory phase, which occurs in the first 96 hours, is characterized by the release of chemical mediators such as histamine, bradykinin, and prostaglandins (77). These molecules cause vasodilation and increased vascular permeability, facilitating cell migration. Neutrophils are the first cells to reach the injured site: they phagocytose microorganisms and cellular debris and release lytic enzymes and ROS. Subsequently, macrophages take over with a dual role: they complete the removal of necrotic debris and orchestrate the transition to the proliferative phase through the secretion of cytokines (IL-1, IL-6, TNF-α) and growth factors (78).
- **Proliferative Phase**: the proliferative phase, which extends approximately from day 3 to day 21, is dominated by the activity of fibroblasts, responsible for the deposition of extracellular matrix (collagen and glycosaminoglycans) (76). At the same time, angiogenesis is activated, ensuring the proper supply of oxygen and nutrients. During this phase, granulation tissue forms, characterized by a high density of fibroblasts, immune cells, and capillaries. Keratinocytes migrate from the wound edges, thus initiating the re-epithelialization process.
- **Remodeling or Maturation Phase**: the final phase is characterized by the replacement of the initially deposited type III collagen with the more resistant and stable type I collagen. Regression of newly formed capillaries and reorganization of the extracellular matrix are observed, resulting in a reduction in the cellular component. The process culminates with the formation of a scar, which exhibits significantly increased mechanical resistance compared to newly formed tissue, but does not match that of the original tissue (79).

The clinical progression of healing depends on the type of wound and the presence of comorbidities such as diabetes or infections. An alteration in one or more of the phases described can lead to clinical complications. Chronic inflammation, for example, can prevent proper progression to the proliferative phase, causing the formation of chronic wounds, such as diabetic ulcers or bedsores. Excessive collagen deposition can lead to the formation of hypertrophic scars or keloids, while a disorganization of the repair processes can culminate in fibrosis, with loss of functionality of the affected tissue. To

systematically reproduce and analyze these repair dynamics, several experimental models of cutaneous injury have been developed. Among them, the **full-thickness excisional wound model** is one of the most widely employed. In this approach, a circular or square excision is created on the dorsal skin of the animal, allowing researchers to monitor in a reproducible way the sequential phases of healing: clot formation and early inflammation, cellular proliferation with extracellular matrix deposition, angiogenesis, and finally tissue remodeling (80). Macroscopic observation of wound closure rate, combined with histological examination, provides information on crucial parameters such as re-epithelialization, collagen deposition, neovascularization, and the degree of inflammatory infiltration (81). Moreover, immunohistochemical and biochemical analyses allow the quantification of key mediators, including MMP-9, TGF-β, and VEGF, as well as markers of oxidative stress and inflammation (82). Thanks to its reproducibility and sensitivity, the excisional wound model represents a reference tool for studying the molecular mechanisms of wound healing and for evaluating the efficacy of pharmacological or nutraceutical compounds in promoting tissue repair and reducing complications associated with chronic inflammation or redox imbalance.

1.3.3. Pathophysiology of Hyperhomocysteinemia and Experimental Models

Several animal models of HHcy have been developed to examine the pathophysiology of altered homocysteine metabolism. Animal models have been used to investigate mechanisms of thrombosis and vascular or brain dysfunction and to explore the effects of specific genetic or dietary interventions. HHcy is a metabolic condition characterized by elevated levels of homocysteine (Hcy), a sulfur-containing non-proteinogenic amino acid and key intermediate in methionine metabolism. The pathophysiological effects of HHcy involve multiple interconnected mechanisms that can be conceptually divided into metabolic dysregulation, oxidative stress, endothelial dysfunction, thrombogenicity, and mitochondrial impairment. Hcy is produced from methionine via the methionine cycle and metabolized through two main pathways: remethylation to methionine, which requires folate and vitamin B12, and trans-sulfuration to cysteine, which requires vitamin B6. Genetic mutations such as CBS or MTHFR variants, nutritional deficiencies, renal impairment, aging, and certain medications can disrupt these pathways, leading to elevated plasma Hcy levels (83-

86). Excess hcy undergoes autoxidation, generating ROS that damage lipids, proteins, and DNA, while depleting glutathione and impairing endogenous antioxidant defenses. These processes create a cytotoxic environment that can compromise cellular function across multiple organ systems (87). HHcy also reduces nitric oxide bioavailability, impairing vascular tone and promoting vasoconstriction. It upregulates adhesion molecules such as ICAM-1, VCAM-1, and E-selectin, facilitates leukocyte recruitment, and induces platelet activation, tissue factor upregulation, and downregulation of natural anticoagulants such as thrombomodulin, increasing the risk of arterial and venous thrombotic events (88, 89). Furthermore, homocysteine impairs mitochondrial respiration and oxidative phosphorylation by inhibiting electron transport chain complexes (I, IV, V) in multiple tissues, leading to ATP depletion, energetic failure, and predisposition to cell death (90, 91). To systematically reproduce and analyze these pathophysiological processes, several experimental models of HHcy have been developed. These include dietary methionine supplementation, vitamin B6/B12/folate deficiency models, and genetic models targeting CBS or MTHFR enzymes (92). In the methionine supplementation model, HHcy is induced in rodents by adding L-methionine to the drinking water (typically 1–2% w/v) or to the chow (1.5–2% w/w) for 4–12 weeks, a regimen that overwhelms the remethylation and trans-sulfuration pathways and leads to sustained elevations of plasma Hcy often reaching the moderate to intermediate range (30–80 μmol/L) (93-95). Rodent models, in particular, allow precise monitoring of plasma Hcy levels and sequential assessment of oxidative stress, endothelial function, mitochondrial activity, and thrombogenic potential (96). Researchers typically combine behavioral tests, histology, immunohistochemistry, and biochemical assays to evaluate cellular damage, ROS production, endothelial markers, and coagulation parameters. The reproducibility and sensitivity of these models make them essential for investigating HHcy mechanisms and for testing pharmacological or nutraceutical interventions aimed at restoring homocysteine homeostasis. From a clinical perspective, HHcy is classified based on plasma Hcy concentrations as mild (16–30 μmol/L), moderate (31–60 μmol/L), intermediate (61–100 μmol/L), and severe (>100 μmol/L, often associated with classical homocystinuria). Elevated homocysteine is a recognized risk factor for cardiovascular disease, cerebrovascular events, and neurodegenerative disorders, emphasizing its systemic impact beyond metabolism (97, 98). Importantly, the

metabolic, oxidative, endothelial, and mitochondrial disturbances induced by HHcy create a proinflammatory environment in the central nervous system, predisposing to neuroinflammation and neuronal dysfunction, which are discussed in the following section.

1.3.4. Role of Hyperhomocysteinemia in Neuroinflammation

Based on previous evidence linking metabolic stress to neuroinflammation and neurodegeneration, HHcy has emerged as a potent neuropathological factor (99). While HHcy influences multiple neurodegenerative pathways, increasing attention has been directed toward its pivotal role in **inducing and sustaining neuroinflammation**, which acts as both a driver and amplifier of neuronal damage. Homocysteine promotes the activation of **microglia and astrocytes**, leading to a chronic proinflammatory environment within the CNS. It acts as an excitotoxin by interacting with NMDA receptors and promoting the entry of intracellular Ca^{2+}, resulting in neuronal damage, apoptosis, and the activation of neuroinflammatory processes (100). These effects are particularly detrimental in the **CSN**, where neurons are highly dependent on aerobic metabolism for function and survival. In addition to its redox-related toxicity, HCY may influence **neurodegenerative processes** by impairing neuronal metabolism, modifying DNA and protein methylation patterns, and enhancing **glutamate excitotoxicity (101)**. Several in vivo studies have indicated that prolonged HHcy leads to **synaptic dysfunction, cognitive decline**, and **alterations in emotional behavior**, supporting a potential causative role in diseases such as **Alzheimer's disease, vascular dementia, and depression** (101). These conditions are accompanied by elevated expression of glial markers such as **Iba1** and **GFAP**, along with increased production of **proinflammatory cytokines** including **IL-1β**, TNF-**α**, and **IL-6**. Mechanistically, HHcy triggers **Toll-like receptor (TLR)** signaling and activates the **NF-κB** pathway, a central transcriptional regulator of the inflammatory response (102). The activation of NF-κB promotes transcription of genes involved in cytokine release, nitric oxide synthesis, and ROS generation, further exacerbating oxidative and nitrosative stress. Simultaneously, HHcy inhibits the **Nrf2/ARE antioxidant pathway**, impairing the cellular defense against oxidative damage and shifting the redox balance toward a proinflammatory state (103). Moreover, HHcy-induced **endothelial dysfunction** and **BBB disruption** allow peripheral immune

mediators to infiltrate the brain parenchyma, reinforcing the neuroinflammatory response and contributing to sustained glial activation. These alterations are not limited to transient responses to cellular stress but have profound and chronic implications for cognitive function, particularly in the context of neurodegenerative diseases such as Alzheimer's disease (20). Clinical and experimental data demonstrate that HHcy is an independent risk factor for cognitive decline and progression to dementia, both in healthy older adults and in those with mild cognitive impairment (MCI) (20). In addition to impairing hippocampal plasticity and synaptic function, HHcy-driven neuroinflammation has been implicated in the enhancement of **amyloid-beta deposition**, **tau hyperphosphorylation**, and **neuronal apoptosis**, thereby reinforcing a pathogenic cascade. Additional molecular pathways have implicated HHcy in GABAergic dysregulation, as homocysteine can act as an excitatory neurotransmitter, antagonizing GABA-A/B receptor activity, resulting in increased neuronal excitability and redox imbalance (20). This mechanism also plays a role in the degradation of extracellular matrix components through the activation of metalloproteinases, further compromising the neurovascular unit. Overall, these findings support the view that HHcy acts as a neuroinflammatory amplifier, operating at the intersection of **oxidative stress**, **glial reactivity**, **immune activation**, and **neuronal degeneration**, culminating in the structural and functional deterioration of neural circuits. Overall, these data highlight how HHcy represents a significant amplifier of neuroinflammation, acting through redox imbalance, glial activation, and alteration of neurovascular homeostasis. Given this complex pathogenetic role, growing attention is being paid to identifying therapeutic strategies capable of counteracting HHcy-induced damage. In addition to conventional vitamin therapy, new evidence is emerging regarding the use of nutraceutical approaches, including supplementation with nuts, particularly cashews, which may offer complementary benefits by modulating redox and inflammatory pathways. Furthermore, they are useful for studying the link between homocysteine and neurodegeneration and for evaluating the efficacy of targeted pharmacological or nutraceutical interventions.

CHAPTER 2: Cytoprotective Mechanisms and Regulation Pathways of Inflammation

The ability of cells to cope with oxidative stress and inflammatory responses is essential to maintain tissue homeostasis and prevent chronic cellular damage. In this context, the transcription factor **Nrf2** represents a key mediator in regulating antioxidant and anti-inflammatory defenses, coordinating the expression of numerous protective genes (104). Concurrently, the **NF-κB** signaling pathway modulates the inflammatory response by inducing the production of pro-inflammatory cytokines and effector molecules (105). The interaction between these two pathways finely tunes the balance between cellular protection and inflammation, with important implications in tissue repair processes, edema, and neuroinflammation, also under metabolic stress conditions such as HHcy. The following sections will delve into the molecular and functional mechanisms of Nrf2, HO-1, NF-κB, and antioxidant enzymes.

2.1 Cytoprotective Effect of Nrf2 and HO-1

Nrf2 is a cytoprotective factor that regulates the expression of genes encoding antioxidant, anti-inflammatory, and detoxifying proteins. Some Nrf2-dependent genes and proteins, such as HO-1, play a protective role against oxidative stress and aging by exerting beneficial effects through the regulation of apoptosis, modulation of inflammation, and control of angiogenesis. Nrf2 is a transcription factor that regulates cellular redox balance and protective responses mediated by antioxidant enzymes. Genes involved in the synthesis of thioredoxin and heme oxygenase-1 are regulated through the binding of Nrf2 to the Antioxidant Response Element (ARE) (106). This results in a cascade of events leading to the regulation of the cellular redox state, providing excellent protection against ROS. Nrf2 consists of six Neh functional domains, including the N-terminal Neh2 domain, which, when bound to the protein Keap1, promotes the degradation of Nrf2, which has a half-life of about twenty minutes (107, 108). Under cellular stress conditions, Nrf2 is released from Keap1 and translocates to the nucleus, where it binds to the ARE sequence, leading to the transcription of cytoprotective genes (Tab.2) (Figure 4).

Gene	Major Function
Ferritin (Fn)	Sequesters free iron
Glucose-6-phosphate dehydrogenase (G6PD)	Provides NADPH to glutathione reductase
GPx	Detoxifies peroxides and hydroperoxides
Glutathione S-transferases (GSTs)	Catalyze the conjugation of the reduced form of GSH to xenobiotic substrates
Glutathione reductase	Catalyzes the reduction of glutathione disulfide (GSSG) to the sulfhydryl form of GSH
γ-Glutamylcysteine ligase (GCL)	Catalyzes the rate-limiting step in the cellular GSH biosynthesis pathway
HO-1	Degrades heme and generates the antioxidant molecules, biliverdin and carbon monoxide (CO)
NAD(P)H:quinone dehydrogenase 1(NQO1)	FAD-binding protein, reduces quinones to hydroquinones
Sulfotransferases (SULTs)	Catalyze sulfation of many xenobiotics
SOD	Catalyzes the dismutation of the superoxide radical (O_2^-) into molecular oxygen (O_2) or hydrogen peroxide (H_2O_2)
Trx	Reduces thioredoxin
UDP-glucose dehydrogenase (UGDH)	Converts UDP-glucose to UDP-glucuronate

Table 2. Nrf2-Regulated Genes

These include genes for enzymes such as SOD, CAT, GPx, and HO-1, which contribute to maintaining the cellular redox balance and neutralizing free radicals (106). HO-1 is a highly inducible protein whose expression increases in response to stimuli such as lipid peroxidation, nitric oxide, heavy metals, growth factors, and cytokines. It catalyzes the degradation of heme into iron ions, biliverdin, and carbon monoxide (CO) (Figure 8), products that play a protective role against oxidative stress and inflammation. Biliverdin, converted into bilirubin by biliverdin reductase, represents a potent antioxidant that modulates cellular processes such as apoptosis and inflammatory responses. This mechanism is particularly important following tissue damage including in the central nervous system, where neuroinflammation can be aggravated by metabolic alterations such as HHcy (109, 110).

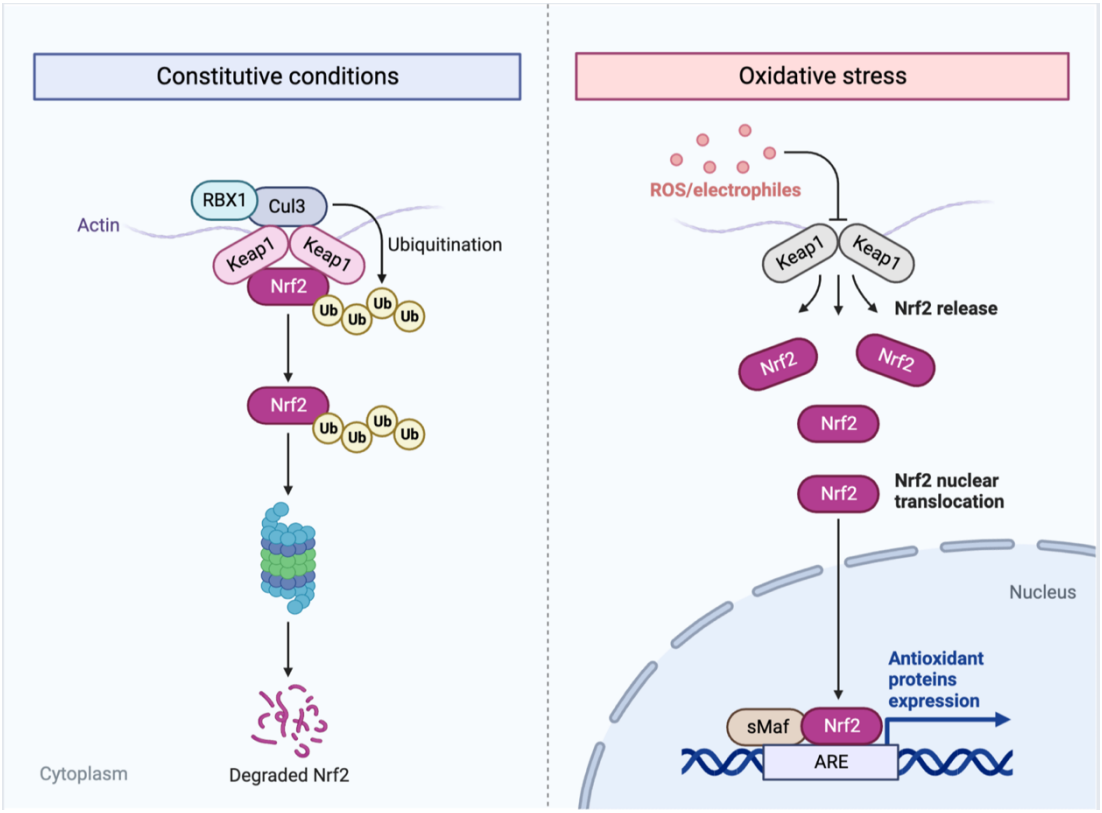

Figure 4. Nrf2 regulation under constitutive and oxidative stress conditions. Under constitutive conditions, Nrf2 binds to Keap1, which promotes its ubiquitination and subsequent degradation. Under oxidative stress, ROS and electrophiles inhibit Keap1 activity, allowing Nrf2 to accumulate and translocate into the nucleus, where it binds to the ARE and promotes the transcription of antioxidant and cytoprotective genes such as HO-1, SOD, and GPx. This pathway plays a key role in cellular defense against oxidative damage.

2.1.1. NF-κB and Cross-talk with Nrf2

Nrf2 and NF-κB are two fundamental signaling pathways that regulate redox balance and inflammatory response. Their interaction is modulated by transcriptional and post-transcriptional pathways, which vary depending on cell type and environmental stimuli. The absence of Nrf2 is associated with increased oxidative and nitrosative stress, favoring the activation of the transcription factor NF-κB, which in turn induces the production of numerous pro-inflammatory cytokines (Figure 5) (47, 111).

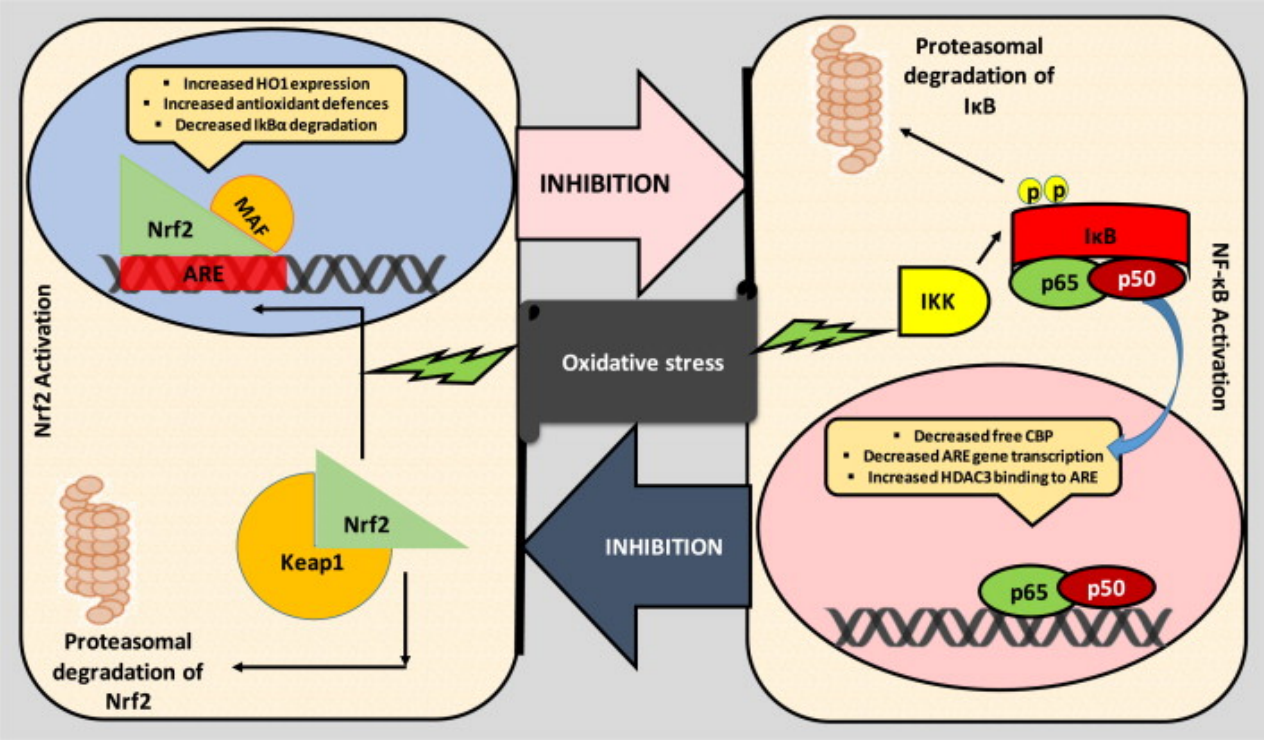

Figure 5. Crosstalk Between Nrf2 and NF-κB Pathways Under Oxidative Stress Conditions. Schematic illustration of the reciprocal inhibition between the Nrf2 and NF-κB signaling pathways during oxidative stress. On the left, Nrf2 is released from Keap1 and translocates into the nucleus, where it binds to ARE, leading to increased expression of antioxidant genes (e.g., HO-1) and suppression of NF-κB signaling. On the right, activation of NF-κB via IKK-mediated phosphorylation and degradation of IκB allows nuclear translocation of p65/p50, which promotes inflammation and inhibits Nrf2-dependent gene expression. This image highlights how oxidative stress shifts the balance between inflammation and antioxidant defense.

NF-κB is a protein complex composed of different subunits (p50, p65/RelA, RelB, c-Rel, p52) that, upon activation, translocates to the nucleus and promotes the transcription of genes involved in the inflammatory response, such as the cytokines IL-1β, IL-6, TNF-α, chemokines, cell adhesion molecules, and acute phase proteins. Its activity is tightly regulated by the inhibitor IκBα, which, when phosphorylated, is degraded, allowing NF-κB to translocate to the nucleus and exert its function (Figure 6) (57, 112-114).

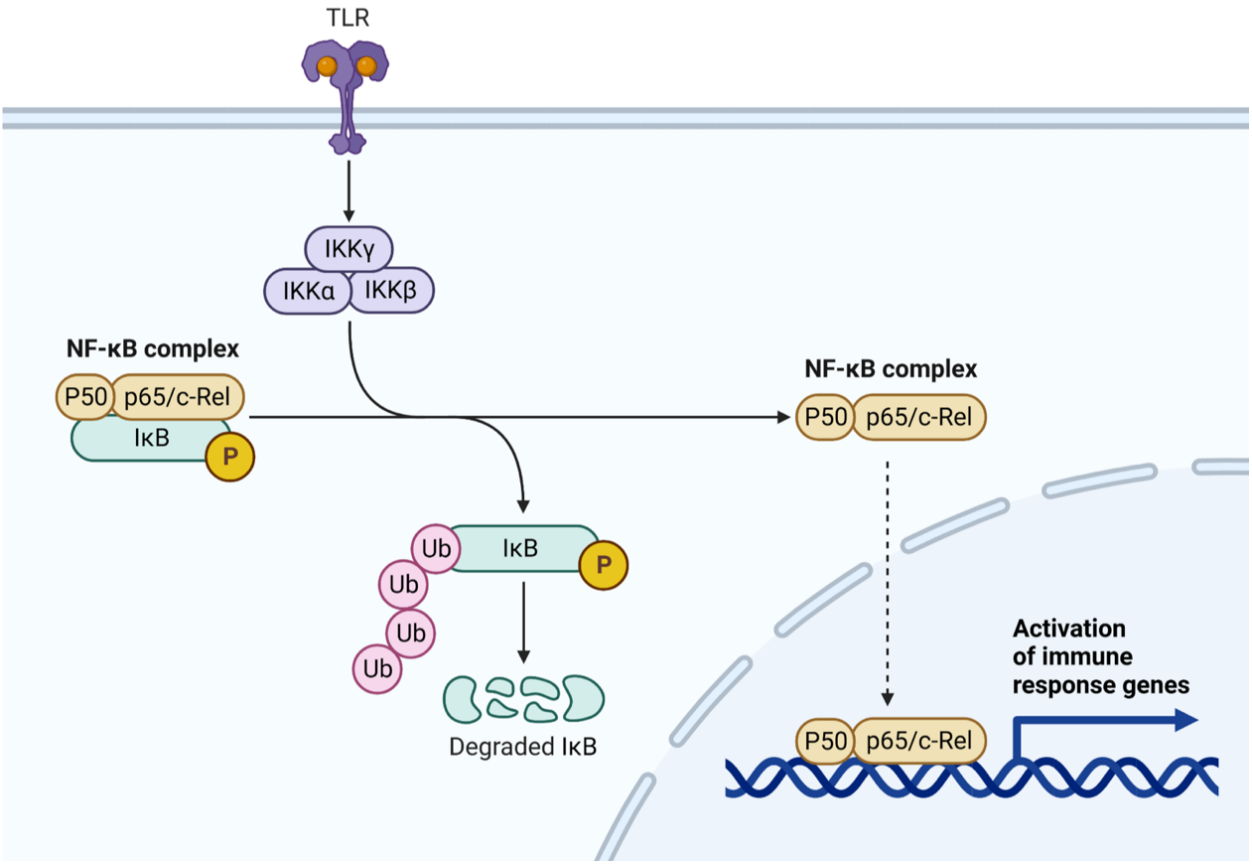

Figure 6. Canonical NF-κB Signaling Pathway Activation. Schematic representation of the canonical NF-κB signaling pathway. Upon stimulation, toll-like receptors (TLRs) activate the IKK complex (IKKα, IKKβ, and IKKγ), which phosphorylates the inhibitor IκB. Phosphorylated IκB is ubiquitinated and degraded, releasing the NF-κB complex (p50/p65 or p50/c-Rel). The activated complex translocates to the nucleus, where it binds DNA and induces the transcription of genes involved in inflammation and immune responses.

NF-κB activation mainly occurs via two pathways: the canonical, rapid and transient pathway, and the non-canonical, slower and specific for cell differentiation functions. NF-κB activation also increases mitochondrial and NADPH oxidase activity, the main sources of endogenous free radicals, further increasing oxidative stress. The result is the synthesis of various molecules such as: cytokines (IL-1, IL-2, IL-6, IL-12, TNF-α, LT-α, LT-β and GM-CSF), chemokines (IL-8, MIP-1, MCP1, RANTES and eotaxin), adhesion molecules (ICAM, VCAM and E-selectin), acute phase proteins (serum amyloid A; SAA) and inducible effector enzymes (inducible nitric oxide synthase; iNOS and cyclooxygenase-2; COX-2), which regulate the inflammatory process. NF-κB inducers are highly variable and include bacterial lipopolysaccharides, ionizing radiation, ROS, cytokines such as TNF-α, IL-1β, viral DNA and RNA (115). These

mechanisms are particularly relevant in neuroinflammation, where glial response, edema, and WH processes are modulated by the balance between pro-inflammatory and antioxidant pathways.

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CHAPTER 3: The Mediterranean Diet and the Functional Role of Nuts – Focus on Almonds and Cashews

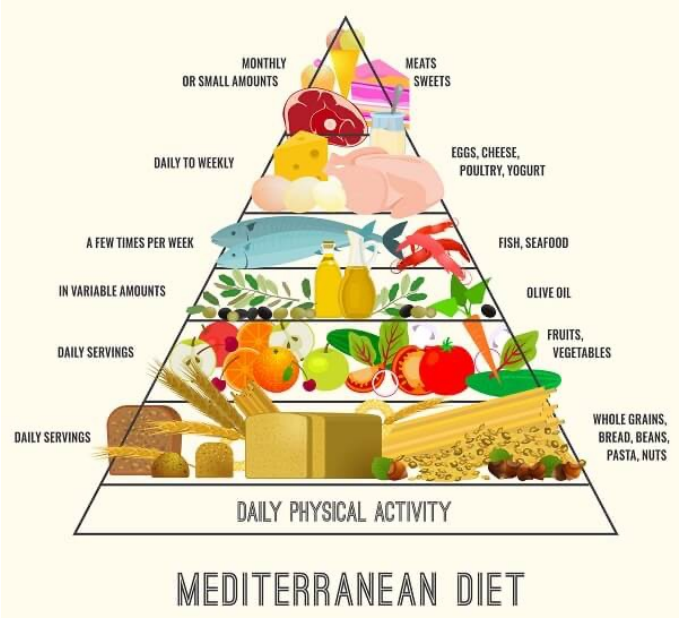
The Mediterranean Diet is one of the most extensively studied and globally recognized nutritional models for its protective effects against non-communicable chronic diseases, including cardiovascular, neurodegenerative, metabolic, and inflammatory disorders (116). In addition to its high nutritional density, this dietary pattern is distinguished by its balanced macronutrient composition and abundant supply of bioactive compounds such as polyphenols, dietary fiber, unsaturated fatty acids, and phytonutrients. Within the Mediterranean Diet, tree nuts play a physiologically relevant role. Nuts—including almonds, cashews, pistachios, and walnuts—are concentrated sources of polyunsaturated fatty acids, natural antioxidants (such as vitamin E and polyphenols), essential minerals, and bioactive amino acids like L-arginine (117). A growing body of clinical and preclinical evidence has demonstrated that regular nut consumption is associated with significant improvements in lipid profile, reductions in systemic inflammation, and enhanced endothelial function (118-120). At the molecular level, these effects appear to be mediated by intracellular pathways involved in antioxidant responses (e.g., Nrf2-HO-1) and inflammatory regulation (e.g., NF-κB), contributing to the maintenance of redox homeostasis and the prevention of oxidative cellular damage (121). Beyond their overall nutritional profile, increasing scientific interest is being directed toward the study of individual nut components and by-products—traditionally considered waste by the agro-food industry—now re-evaluated for their therapeutic potential (122, 123). In this context, particular attention has been given to the almond skin (from *Prunus dulcis*) and almond oil (*California Grown Almonds*), as well as cashews (*Anacardium occidentale*), due to their high concentration of bioactive molecules with immunomodulatory, cytoprotective, and regenerative properties. Almond skin, rich in flavonoids, phenolic acids, and tannins, has demonstrated strong antioxidant and anti-inflammatory activity in experimental models (4). Almond oil, due to its content of monounsaturated fatty acids and tocopherols, has shown efficacy in promoting tissue regeneration and enhancing the WH process (124). Cashews, in addition to being a source of copper, magnesium, and B vitamins, have been linked to reduced plasma homocysteine levels—a known cardiovascular risk factor—by supporting methylation

metabolism (18). Recent studies suggest that extracts from nut processing by-products (e.g., shells and skins) may contribute to modulating inflammatory responses and antioxidant capacity in various tissues through the regulation of Nrf2/NF-κB signaling pathways (125). This chapter aims to outline the scientific rationale supporting the inclusion of tree nuts in the Mediterranean Diet, with a focus on the molecular mechanisms underlying their health-promoting properties. Special emphasis will be placed on almonds and cashews, examining not only the chemical composition of their functional fractions but also experimental and clinical evidence regarding their application in nutraceutical and therapeutic contexts. These findings will be discussed in light of sustainable valorization strategies for agro-industrial waste, with the goal of highlighting the potential use of nut by-products in the prevention and management of conditions associated with oxidative stress, chronic inflammation, and metabolic dysregulation.

3.1 Mediterranean Diet and the Role of Nuts

In recent years, the scientific literature has provided substantial evidence supporting the beneficial effects of the MedDiet in counteracting oxidative stress and inflammation. The MedDiet is a traditional nutritional model characterized by a high intake of plant-based foods (fruits, vegetables, legumes, whole grains), unsaturated fats—particularly monounsaturated fatty acids derived from extra virgin olive oil—and a low intake of saturated fats, red meats, and dairy products (126, 127). Within this framework, numerous clinical trials and meta-analyses have confirmed a strong association between adherence to the MedDiet and a reduced risk of chronic degenerative diseases, including cardiovascular diseases, type 2 diabetes, metabolic syndrome, cognitive decline, and neuroinflammatory processes (3, 117, 128). These effects are largely attributed to the high content of bioactive compounds in these foods, such as polyphenols, flavonoids, and essential fatty acids, conferring the MedDiet a protective potential against oxidative stress and systemic inflammation. A crucial component of a balanced Mediterranean dietary pattern is the inclusion of tree nuts, which are well known for their multiple health-promoting effects. Nuts are particularly rich in polyunsaturated fatty acids (PUFAs), especially alpha-linolenic acid (ALA), a precursor of long-chain omega-3 fatty acids, known for its anti-inflammatory and cardioprotective properties. Additionally, nuts provide significant amounts of vitamin

E, melatonin, polyphenols (particularly ellagitannins), phytosterols, selenium, and L-arginine—molecules that act synergistically to exert antioxidant and vasoprotective effects. Several randomized controlled trials have demonstrated that regular consumption of nuts, as part of a Mediterranean dietary pattern, is associated with a significant reduction in low-density lipoprotein (LDL) cholesterol, improved endothelial function, and a decrease in systemic inflammatory biomarkers such as C-reactive protein (CRP) and interleukin-6 (IL-6) (117, 129, 130). Moreover, daily supplementation with nuts has been shown to improve the lipid profile and reduce the risk of cardiovascular events. These benefits are not solely attributable to lipid metabolism modulation but also to epigenetic regulation and protection against plasma lipoprotein oxidation (129, 131). Therefore, the inclusion of tree nuts within the Mediterranean diet not only enhances the overall nutritional profile of this dietary model but also represents an effective and practical strategy for the prevention of HHcy-related conditions and age-associated diseases, through modulation of oxidative stress, inflammation, and overall cardiovascular health (Figure 7) (127, 129, 130).



↓ LDL
↓ CRP / IL-6
↑ Antioxidant defenses
↓ Oxidative stress
Cardioprotective effects
↓ Cognitive decline

Figure 7. The Mediterranean Diet and its protective role against Inflammation and Oxidative Stress.

3.2 Exogenous Antioxidants and the Role of Nuts in Modulating Oxidative Stress and Inflammation

Exogenous antioxidants, dietary bioactive agents, and functional foods are essential nutritional tools in counteracting oxidative stress—a condition characterized by an imbalance between ROS production and the organism's antioxidant defense capacity (132). This imbalance plays a key role in the onset and progression of numerous chronic diseases, including cardiovascular and neurodegenerative disorders, metabolic syndromes, and cancer. Among the most potent natural antioxidants are **polyphenols**, a heterogeneous group of phytochemicals widely distributed in plant-based foods, recognized for their radical-scavenging, anti-inflammatory, and cytoprotective properties (133) . The principal classes of polyphenols include **phenolic acids**, **stilbenes, flavonoids**, and **lignans** (Figure 8).

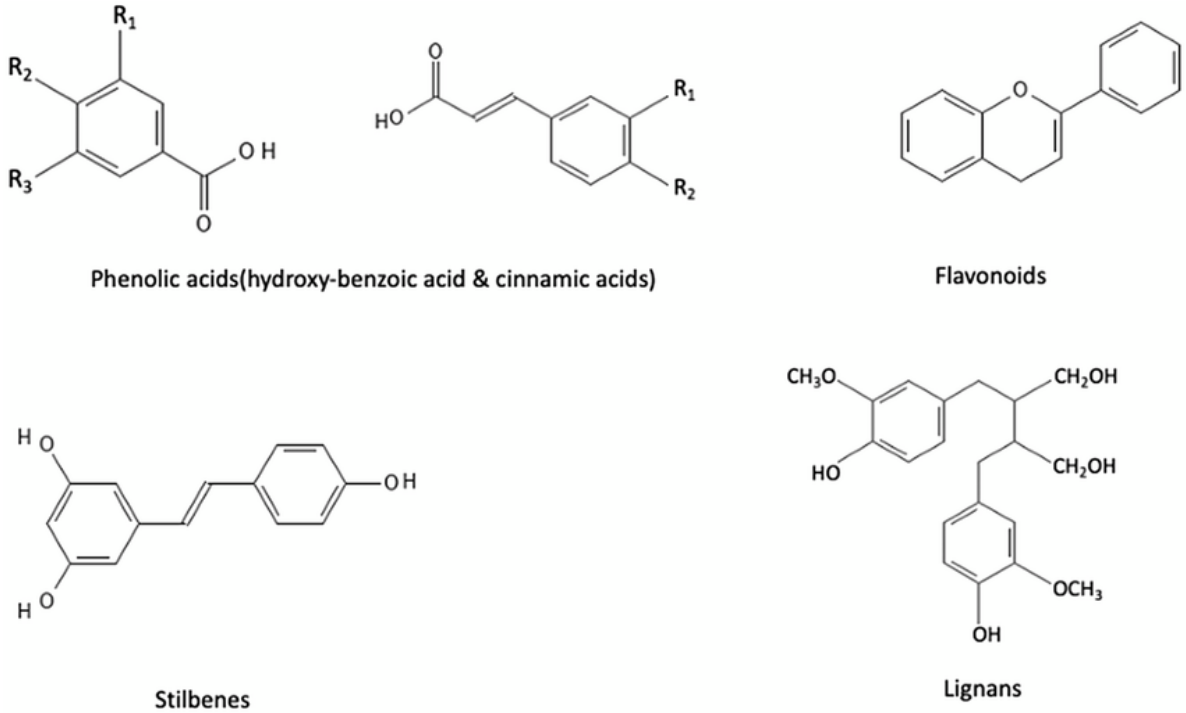


Figure 8. Principal classes of polyphenols.

Nuts represent a particularly rich dietary source of exogenous antioxidants, including polyphenols, α-tocopherol (vitamin E), selenium, and unsaturated fatty acids. Regular consumption of nuts—such as almonds, hazelnuts, pistachios, and cashews—has been consistently associated with reduced systemic markers of oxidative stress and inflammation, thereby contributing to cardiovascular, metabolic, and neurological protection (3, 134, 135). Almonds, for example, are especially high in vitamin E and flavonoids, which help to protect lipid membranes from peroxidation and oxidative

damage. Cashews, in contrast, are notable for their content of copper, magnesium, and phenolic compounds that modulate redox-sensitive pathways and enhance the enzymatic activity of endogenous antioxidants, including SOD and GPx (136). Moreover, several non-enzymatic antioxidant molecules obtained through the diet play a synergistic role in protecting cells from ROS-mediated damage. These include GSH, ascorbic acid (vitamin C), α-tocopherol (vitamin E), ubiquinone (CoQ10), and uric acid. These molecules act in various cellular compartments—both aqueous and lipidic—providing broad-spectrum protection against oxidative stress, as summarized in the following table 3.

Antioxidant	Biological Function	Primary Cellular Location
GSH	Scavenges ROS, cofactor for GPx	Cytoplasm, mitochondria, nucleus
Ascorbic acid (Vitamin C)	Regenerates vitamin E; scavenges ROS in aqueous compartments	Plasma, cytosol
α-Tocopherol (Vitamin E)	Inhibits lipid peroxidation; protects cell membranes	Lipid membranes
Ubiquinone (CoQ10)	Electron carrier; antioxidant in mitochondrial membranes	Mitochondria
Uric acid	Scavenges singlet oxygen and reactive radicals	Plasma, extracellular fluids

Table 3. Major Exogenous (Non-Enzymatic) Antioxidants and Their Mechanisms of Action

These dietary antioxidants not only provide direct protection against free radical damage, but also enhance endogenous defense systems by modulating signaling pathways. In particular, many of these compounds activate the **Nrf2/ARE pathway**, which regulates the transcription of key antioxidant enzymes such as SOD, CAT, HO-1, and GPx. Through these mechanisms, regular nut consumption may potentiate endogenous antioxidant systems and reinforce cellular resilience to oxidative stress. In both experimental models and clinical studies, these effects translate into improved oxidative stress resilience, more efficient tissue regeneration, and a lower risk of chronic diseases associated with oxidative damage, including type 2 diabetes, atherosclerosis, neurodegenerative diseases, and cancer (137, 138).

3.2.1. Flavonoids and the Role of Nuts in Oxidative Stress and Inflammation

Flavonoids, which are widely present in various plant-based foods, exhibit strong free radical scavenging activity in addition to their metal-chelating properties against pro-oxidant ions. Epidemiological studies suggest that an adequate intake of flavonoids is

associated with a reduction in cardiovascular mortality and a lower incidence of myocardial infarction (139). Furthermore, in animal models, polyphenols have been linked to increased intracellular concentrations of **GSH** and enhanced enzymatic activity, particularly that of **GPx** (140). These compounds also exert their effects by regulating the activity of key enzymes involved in oxidative and inflammatory responses, including **telomerase**, **lipoxygenase**, **xanthine oxidase**, and **protein kinases**. Notably, they activate the redox-sensitive **Nrf2/ARE** signaling pathway, which is essential in the cellular cytoprotective response. **Resveratrol**, one of the most extensively studied stilbenes, has shown potent vasoprotective, anti-platelet, and anti-inflammatory effects. It inhibits cyclooxygenase (COX-1) activity, promotes vasodilation through **NO** release, and modulates the activity of transcription factors such as **NF-κB**, **Bcl-2**, and **MMP-9 (141)**. These mechanisms contribute to **neuroprotective** effects and the attenuation of **neuroinflammation**. In this context, tree nuts represent a highly functional dietary component for preventing oxidative stress and inflammation. Nuts are particularly rich in polyunsaturated fatty acids, including alpha-linolenic acid (ALA)—a precursor of long-chain omega-3 fatty acids—known for their anti-inflammatory and neuroprotective properties. Additionally, nuts provide a broad range of antioxidant compounds, including polyphenols (ellagitannins, flavonoids, phenolic acids), vitamin E, melatonin, selenium, L-arginine, and phytosterols, which act synergistically to modulate redox and inflammatory pathways (3). Numerous clinical trials and meta-analyses have shown that daily nut consumption (20–60 g/day) is associated with a significant reduction in inflammatory biomarkers (e.g., CRP, TNF-α, IL-6), improved plasma antioxidant capacity, and modulation of neuroinflammation—an important contributor in neurodegenerative conditions such as Alzheimer's and Parkinson's disease (142, 143). The beneficial effects also extend to neurological health, where improvements in cognitive parameters have been observed, likely due to the reduction of oxidative stress and neuroinflammatory processes within the central nervous system (144). Due to their high content of fiber and essential micronutrients, tree nuts also promote gut microbiota health, thereby contributing to both systemic and neurological homeostasis. Within this food category, particular attention has been given to almonds (*Prunus dulcis*) and cashews (*Anacardium occidentale*), two widely consumed nut types, both as whole foods and in the form of oils or functional derivatives. Almonds, rich in oleic

acid, tocopherols, and flavonoids, exhibit high antioxidant capacity—especially in their skin, which is one of the richest polyphenol sources among tree nuts. Recent studies suggest that almonds may support tissue regeneration through their ability to modulate inflammatory processes and enhance cellular responses to oxidative damage. Cashews, on the other hand, have a distinctive mineral and lipid profile, being particularly rich in copper, magnesium, and tryptophan. In pathological conditions characterized by redox imbalance, chronic inflammation, and mitochondrial dysfunction—such as HHcy, as well as neurodegenerative and inflammatory syndromes—these foods may help mitigate molecular and cellular damage by strengthening endogenous defenses and modulating inflammatory pathways, including those involved in neuroinflammation (145).

3.3 Therapeutic Potential of nuts

Tree nuts, including almonds, walnuts, and cashews, are widely recognized for their beneficial role in the prevention and therapeutic support of numerous chronic pathological conditions (3). The Mediterranean diet, one of the most extensively studied dietary models in clinical research, regularly incorporates these foods due to their nutraceutical properties and high density of functional nutrients. The growing interest in integrative and personalized approaches to health has further encouraged the use of tree nuts as adjuvant treatments in cardiovascular, metabolic, neurodegenerative, and gastrointestinal diseases (146). In this context, the bioactive compounds found in almonds and cashews—not only in the edible seed, but also in their skins and extracted oils—have been employed in experimental models and preclinical studies, demonstrating effectiveness in controlling inflammatory and oxidative processes, as well as in promoting tissue protection (Tab.4) (4, 15, 16, 18).

Nuts	Biochemical Characteristics	Main Biological Effects
Tree nuts	Rich in unsaturated fatty acids (oleic, linoleic, α-linolenic acid), fiber, phytosterols, polyphenols, vitamin E, magnesium, selenium	Antioxidant and anti-inflammatory action; support for gut microbiota; improvement of lipid profile and cognitive function
Whole Almonds	High content of oleic acid, α-tocopherol, flavonoids; high phenolic content in the skin	Reduction of oxidized LDL; vascular and mitochondrial protection; neuroprotective and anti-aging support
Almond skin	Rich in flavonoids, phenolic acids (catechin, epicatechin, p-coumaric acid), tannins	Radical scavenging activity; inhibition of lipid peroxidation; synergistic effects with dietary fiber
Almond oil	Rich in oleic acid (>60%), phytosterols, tocopherols	Emollient and antioxidant effects; also beneficial for topical and cosmetic applications
Cashews	Source of copper, magnesium, tryptophan, monounsaturated fats, polyphenols	Immunoregulatory effects; neurotransmission support (via serotonin pathway); systemic anti-inflammatory properties

Tab 4. Biochemical composition and main biological effects of almonds, cashews, and their derivatives.

3.3.1. Anti-inflammatory Activity

The anti-inflammatory effects of tree nuts are well documented and largely mediated by inhibition of the NF-κB signaling pathway. Phenolic extracts from cashews and almonds have been shown, in both in vitro and in vivo models, to significantly reduce leukocyte infiltration, cytokine production, and tissue damage (4, 18).

Cashew has attenuated myeloperoxidase activity and lipid peroxidation in murine models of colitis and osteoarthritis (63, 147), while almond oil reduced systemic inflammation in diabetic models by modulating cytokine profiles and inhibiting NF-κB (148). Almond skin, rich in flavonoids and tannins, exhibited in vivo anti-inflammatory activity comparable to reference drugs, supporting its potential as a natural therapeutic agent (4, 9).

3.3.2. Antioxidant Activity

Tree nuts are a major dietary source of antioxidant compounds, effective in counteracting oxidative stress and restoring redox homeostasis. A key mechanism involves activation of the Nrf2/ARE pathway, leading to the expression of endogenous antioxidant enzymes. Cashews, rich in α-tocopherol and unsaturated fatty acids, have shown protective effects in murine models of intestinal ischemia and pancreatitis (8, 22). Almond skins, in turn, reduced ROS production and activated the Nrf2 pathway in both cellular (U937) and animal models (carrageenan-induced edema), confirming efficacy in acute and chronic oxidative stress (4). Almond oil further enhanced redox

regulation in diabetic models by increasing antioxidant enzyme expression and reducing systemic oxidative damage (148, 149).

3.4 Properties and Use of By-Products

Growing scientific and industrial interest is increasingly oriented toward the **valorization of by-products from tree nuts**, particularly **almond skins** and **residual cold-pressed oils**, as sustainable sources of **bioactive compounds** with high **nutraceutical and therapeutic potential**. These secondary materials—traditionally treated as waste—are now being re-evaluated through the lens of **functional food systems and circular economy principles**, aligning health innovation with environmental and economic sustainability (150, 151). In particular, **almond skins**, which are commonly discarded during the industrial blanching process, have been shown to retain more than **70% of the nut's total phenolic content**, including a wide spectrum of **flavonoids, tannins, and phenolic acids** (150). These molecules exert **strong antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory properties**, making almond skins promising candidates for use in **nutraceutical formulations**, **gastroprotective agents**, and **dermocosmetic applications** targeting oxidative stress and inflammation (152). Likewise, **cold-pressed almond oil**, rich in **monounsaturated fatty acids**, **tocopherols**, and **phytosterols**, offers demonstrated **vascular protection**, **anti-peroxidative activity**, and support for **tissue regeneration**—attributes that make it an effective ingredient in both **topical therapeutic formulations** and **functional supplements** (123). While this thesis focuses primarily on almond-derived by-products, it is important to note that similar **valorization strategies are being explored for other nuts**, such as **cashews, pistachios, and walnuts**. For instance, **cashew shells**, which are a major agro-industrial residue in tropical regions, contain **anacardic acids and cardanols**—bioactive compounds with recognized **antibacterial, antioxidant, and anticancer properties** (153, 154). However, their application is often limited by intrinsic toxicity and requires **advanced extraction and detoxification techniques**. Still, these efforts demonstrate the broader interest in exploiting the **therapeutic value** of nut-processing waste across species and contexts. The **recovery and refunctionalization of nut by-products** reflect an integrated approach that delivers **multiple benefits**:

- **Environmental**, by reducing organic waste, minimizing pollution, and promoting resource circularity;
- **Industrial**, by enhancing the profitability and efficiency of agri-food chains through the creation of **high-value secondary products**;
- **Social**, by fostering **local development**, especially in rural and nut-producing regions, and promoting **employment in green innovation sectors**;
- **Economic**, by lowering raw material costs and encouraging the production of **accessible natural alternatives** to synthetic compounds in the pharmaceutical, cosmetic, and nutraceutical industries.

In this perspective, the **use of almond skins and oils** as explored in this thesis exemplifies how agro-industrial residues can be transformed from waste into **functional ingredients** capable of **modulating biological pathways**—such as **Nrf2** and **NF-κB** - relevant to oxidative stress, inflammation, and tissue repair (123, 152). This paradigm not only supports **scientific and clinical progress**, but also contributes to the development of **sustainable and inclusive innovation ecosystems**, in line with global health and environmental priorities.

3.5 Cashew (Anacardium occidentale L.): Nutritional Profile and Therapeutic Potential

Among the various types of tree nuts, cashews (*Anacardium occidentale* L.) stand out for their interesting nutritional and functional profile, making them a particularly promising food in the prevention and modulation of pathophysiological conditions associated with oxidative stress and chronic inflammation, including cardiovascular, metabolic, and neurodegenerative diseases (Figure 9) (8, 18, 22, 147).

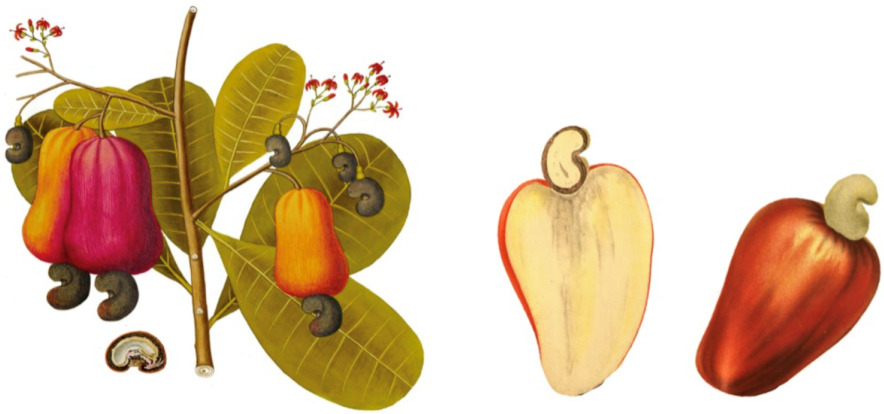


Figure 9. Cashew nuts.

A balanced daily intake of tree nuts is important for health, as they provide proteins, beneficial fatty acids, and essential nutrients (154). In particular, foods rich in polyphenols, such as fresh fruit, vegetables, and nuts, may help neutralize the oxidative effects of ROS. Cashews, which are cholesterol-free and rich in monounsaturated (mainly oleic acid) and polyunsaturated fatty acids (linoleic acid), also contain high levels of vitamin E (α-tocopherol), polyphenols, flavonoids, tannins, fiber, and micronutrients such as magnesium, copper, zinc, phosphorus, selenium, potassium, as well as B-complex vitamins and vitamin K (Figure 10). The typical nutritional composition of cashews includes approximately 40–50% lipids, 20–25% proteins, and 23–25% carbohydrates (63).

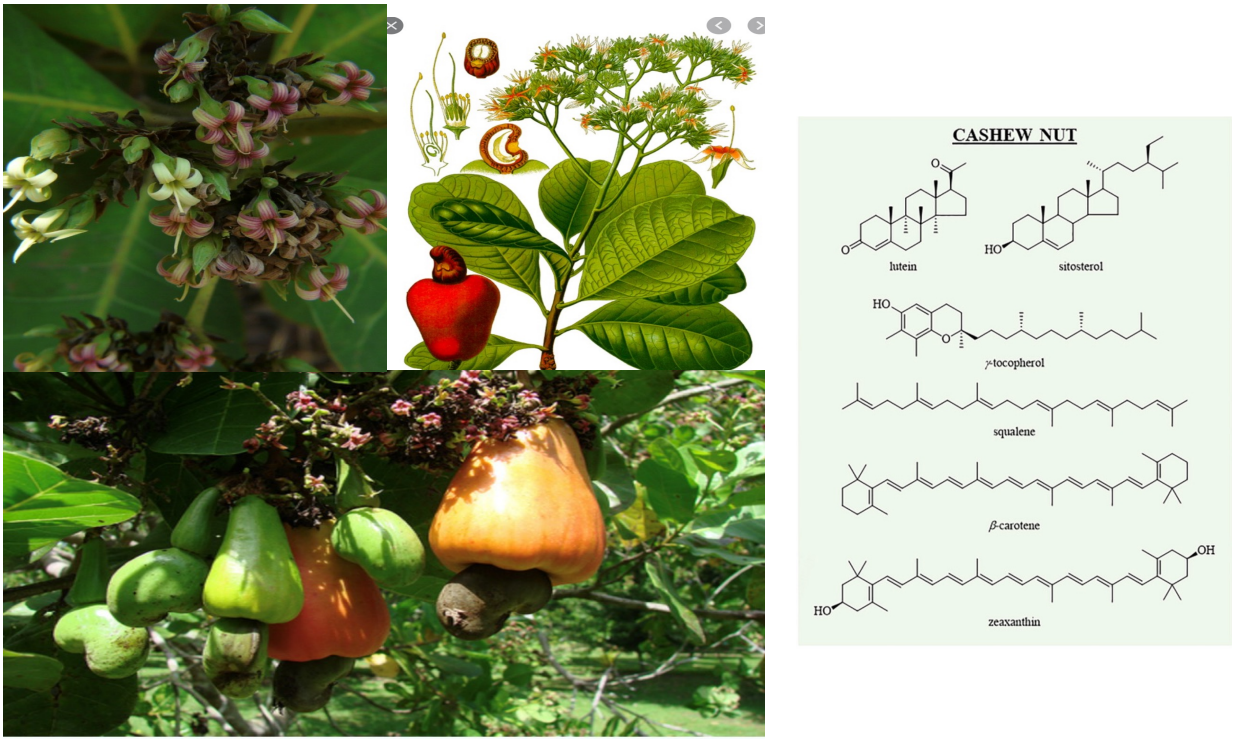


Figure 10. The main components of cashews.

Among their proteins, cashews are a good source of 13S globulin, composed of two main polypeptides with different molecular weights (155). α-Tocopherol, the main active form of vitamin E, is a fat-soluble molecule with strong antioxidant activity, capable of neutralizing ROS and interrupting lipid peroxidation. After performing its antioxidant function, it can be regenerated through interactions with molecules such as coenzyme Q10 and glutathione, maintaining its biological activity (156). Numerous studies have shown that vitamin E plays a role in maintaining an efficient immune response, in part through the regulation of NF-κB, a protein complex involved in the transcription of pro-inflammatory genes such as TNF-α, IL-6, and COX-2 (156). The beneficial effects of cashews have been documented in various animal models, including colitis, intestinal ischemia, pancreatitis, osteoarthritis, and paw edema (8, 18, 22, 147). These effects are mainly mediated by activation of the antioxidant Nrf2/ARE pathway, which promotes the expression of endogenous protective enzymes, and inhibition of the inflammatory NF-κB pathway, contributing to reduced immune cell recruitment and lower production of pro-inflammatory cytokines (22). In addition, the combination of cashew nut consumption with a balanced diet has shown protective effects for the treatment of inflammatory conditions associated with HHcy (18). From an ethnopharmacological perspective, different parts of the *A.*

occidentale plant (seeds, seed coat, oil, leaves, and bark) have been used for therapeutic purposes. The cashew seed coat, for instance, is a rich source of phenolic compounds, flavonoids, and tannins with strong antioxidant and anti-inflammatory properties. The oil extracted from the seeds by cold-pressing has also been studied for its ability to inhibit oxidative and inflammatory pathways while promoting cellular redox balance (157). Cashews also contain phytosterols, phenylalanine, and compounds with immunomodulatory activity. Their near-total absence of purines and cholesterol makes them suitable for both preventive and therapeutic dietary regimens (158). The preparation of cashew extracts varies depending on the part used: extracts can be obtained through maceration in polar or hydroalcoholic solvents, or through cold-pressing (for the oil) (159). Once purified, these extracts have been tested in experimental models and have shown the ability to reduce MPO activity, lipid oxidative damage, and leukocyte infiltration, indicating protective effects on both intestinal and joint tissues (160). In conclusion, the inclusion of cashews in a healthy and balanced diet may contribute to the prevention of oxidative damage and chronic inflammation. Thanks to their rich nutritional composition and high content of bioactive compounds, cashews offer a wide range of biological activities—antioxidant, anti-inflammatory, and antibacterial—that could play a role in preventing and managing several chronic diseases (Tab.5) (161).

Type of Study	Experimental Model	Results	References
In vivo	Animal model of acute pancreatitis	Modulation of Nrf2 and NLRP3 pathways	(162)
In vivo	Mild HHcy in Rats	Reduction of inflammation and tissue injury via Nrf2/ARE activation and NF-κB inhibition	(18)
In vivo	Rat model of ischemia/reperfusion (I/R)	Modulation of NRF2 and NF-kB pathways and reduction of plasma levels of cytokines, nitrotyrosine and PARP and adhesion molecules	(22)
In vivo	Rat model of osteoarthritis	Reduction of pain intensity, restoration of pro-oxidant/antioxidant balance and limitation of joint inflammation and reduction of tissue damage.	(147)

Table 5. Scientific Evidence on the Effects of Cashew (Anacardium occidentale)

3.5.1 Therapeutic Role of Cashews in Hyperhomocysteinemia: Preclinical Evidence

In addition to the established use of vitamin therapy—particularly folic acid, vitamin B12 (pyridoxine), and vitamin B13—as a key component in managing HHcy, growing attention has been focused on the role of specific dietary components with intrinsic antioxidant and anti-inflammatory properties. Among these, nuts have shown promising nutraceutical potential. In particular, recent preclinical evidence highlights the therapeutic efficacy of cashews (*Anacardium occidentale L.*) in experimental models of HHcy (18, 158). Cashew nut supplementation has been shown to significantly reduce plasma homocysteine concentrations, ameliorate oxidative damage, and attenuate inflammatory responses by modulating the Nrf2 and NF-κB signaling pathways, leading to the restoration of endogenous antioxidant defenses (e.g., SOD, CAT, GPx) and the downregulation of pro-inflammatory cytokines such as TNF-α and IL-1β (163). These findings provide a compelling rationale for considering cashew nuts as a complementary dietary approach in the management of HHcy. Specifically, their studies demonstrated that cashew nut administration (100 mg/kg/day) in rodent models not only normalized redox homeostasis but also mitigated nitrosative stress and mitochondrial dysfunction, key pathological mechanisms implicated in HHcy-induced neurovascular and systemic damage (18). While traditional vitamin supplementation remains essential—reducing plasma homocysteine levels by up to 43% with folic acid, 5–12% with vitamin B12, and up to 12% with vitamin B12, supplementation with functional foods such as cashews may enhance therapeutic outcomes through non-redundant molecular pathways (164-167). While specific human data on cashew consumption and homocysteine metabolism are currently limited, preclinical findings strongly justify future translational studies and randomized clinical trials to validate these effects in clinical populations.

3.6 Almonds: Nutritional Composition and Role in Modulating Redox-Inflammatory Pathways

Almonds (Prunus dulcis), belonging to the Rosaceae family, represent one of the main plant sources of functional nutrients and bioactive compounds, whose regular consumption is associated with numerous benefits for human health (Figure 11).

Figure 11. Almond (Rosaceae family).

The nutritional profile of almonds is particularly rich and balanced: they contain about **50–55% lipids**, **20–25% proteins**, and **15–20% carbohydrates**, as well as a significant amount of **dietary fiber** (**10–12%**). The lipid component is dominated by monounsaturated fatty acids, particularly oleic acid, and a significant portion of linoleic acid, with favorable effects on the lipid profile and systemic inflammation. Almonds are also an excellent source of **vitamin E** (especially in the form of **α-tocopherol**), **phytosterols**, **polyphenols**, **flavonoids**, **phenolic compounds** (including **gallic acid**, **ellagic acid**), and minerals such as **magnesium**, **calcium**, **zinc**, **copper**, and **manganese**. **B vitamins** (B1, B2, B3, B6, folates) are also well represented (168). From an antioxidant standpoint, almonds exert a marked protective action against **oxidative stress** thanks to the synergy between **α-tocopherol**, **phenolic compounds**, and **unsaturated fatty acids**. In particular, **antioxidant** and **anti-inflammatory** activity has been demonstrated through activation of the **Nrf2/ARE pathway** and inhibition of the **NF-κB pathwa**y (4). Polyphenols and tocopherols, in particular, have been associated with a reduction in the expression of endothelial adhesion molecules and leukocyte recruitment in tissues, suggesting a key role in regulating chronic inflammation. The almond skin, often removed in industrial products but rich in bioactive components, represents a particularly interesting fraction

from a nutraceutical perspective (6). Phytochemical studies have shown that the skin concentrates over 70% of the total polyphenol content of the almond, including flavanols (catechin, epicatechin), flavonols (quercetin, isorhamnetin), phenolic acids, and condensed tannins. These compounds exhibit marked antioxidant properties, with a high capacity for free radical scavenging, and documented anti-inflammatory activity in cellular and animal models through modulation of cytokines and inflammatory mediators (160). Almond oil, extracted by cold pressing from shelled seeds, also constitutes a product of high functional value (17). Its composition is dominated by oleic and linoleic acids, accompanied by tocopherols, squalene, and phytosterols. Clinical studies have shown that almond oil contributes to improved lipid profiles and LDL oxidation resistance. Protective effects against lipid oxidative damage and inflammatory conditions have also been observed in experimental models, suggesting potential benefits for skin, gastrointestinal, and cognitive health (169). Moreover, it has been observed that almond oil can induce the expression of antioxidant genes and reduce markers of systemic inflammation in experimental models of diabetes and obesity. Its emollient and antioxidant properties also make it of interest in dermocosmetic and therapeutic fields (170). From a technological perspective, almond derivatives can be obtained through different extraction methods, including maceration in polar solvents (ethanol, methanol) to recover polyphenols from the skin, and mechanical pressing for oil production. These extracts have been successfully employed in experimental studies to evaluate their biological activity, showing significant reductions in oxidative stress, lipid peroxidation, and MPO activity, as well as positive modulation of the gut microbiota (171, 172). Overall, almonds, thanks to the synergy among the bioactive components of the seed, skin, and oil, represent a functional food with high nutraceutical potential, useful in the prevention and therapeutic support of chronic diseases based on inflammatory and oxidative processes (Tab.6).

Type of Study	Experimental Model	Results	References
In vitro/ In vivo	Human monocytic U937 cells / Paw edema model in rats	Activation of Nrf2/ARE and inhibition of NF-κB pathways; reduction of systemic inflammation and oxidative stress	(4)
In vivo	Rat model of colitis (IBD)	Reduction of oxidative stress, TNF-α, iNOS, COX-2 expression; protection of colonic mucosa	(160)
In vitro	Skin and seed oil in oxidative models	High free radical scavenging capacity; protection from oxidative damage and modulation of inflammatory mediators	(170)
Clinical trial	Randomized crossover study in healthy adults	Improved plasma lipid profile, reduced LDL oxidizability after almond oil or whole almond consumption	(169)
Clinical study	Healthy human volunteers (topical application, induced skin erythema)	Nanoemulsions with almond or neem oil delivered vitamin E; neem oil NE reduced chemically induced skin erythema	(170)
Systematic Review / Meta-analysis	Randomized Controlled Trials in patients with type 2 diabetes	Almond consumption improved glycemic control, reduced systemic inflammation, and modulated gut microbiota	(172)

Table 6. Scientific Evidence on the Effects of Almond.

CHAPTER 4: AIM

As discussed in previous chapters, oxidative stress and inflammation represent fundamental pathological mechanisms implicated in a wide range of chronic diseases, including cardiovascular, metabolic, and neurodegenerative disorders. At the molecular level, these processes are largely governed by two key signaling pathways: Nrf2 and NF-κB. Nrf2 is a key regulator of the cellular antioxidant response. In contrast, NF-κB is a key transcription factor involved in the regulation of inflammation, apoptosis, and immune responses. In light of these biological processes, the objective of this thesis is to explore the functional and therapeutic potential of bioactive compounds derived from almonds (Prunus Dulcis), with particular attention to the polyphenol-rich skin and cold-pressed oil, and cashews (Anacardium occidentale), known for their antioxidant and methylation-supporting properties. To this end, three different experimental models were used: carrageenan-induced paw edema to mimic acute inflammation and evaluate anti-inflammatory efficacy; a full-thickness excisional wound model to evaluate the regenerative and remodeling capacity of almond oil; and a methionine-induced HHcy model to study the neuroinflammatory and oxidative consequences in the brain and the neuroprotective effects of cashew supplementation. These models were selected to simulate distinct yet interconnected pathological conditions in which oxidative stress and inflammation interact, thus providing a comprehensive preclinical evaluation. The research focuses specifically on the modulation of the Nrf2 and NF-κB signaling axes, which were assessed using immunohistochemistry, Western blot analysis, and quantification of relevant biomarkers (e.g., MDA, MPO, cytokines). By studying the effects of nut-derived treatments on these molecular pathways, the thesis aims to identify new nutraceuticals capable of supporting redox balance, limiting inflammation, and promoting tissue repair. The results obtained suggest that nuts and their byproducts, such as almond skins, often considered agro-industrial waste, may represent a sustainable and rich source of bioactives for the development of therapeutic strategies against oxidative and inflammatory diseases. This approach is in line with current trends in both translational medicine and the circular bioeconomy, promoting not only healthcare innovation but also environmental sustainability.

CHAPTER 5: Antioxidant activity of a Sicilian Almond Skin Extract using *in vivo* model

5.1 Materials and Methods

5.1.1. Almond skin extract: sample preparation

Natural almond skin (NS) was obtained and extracted from almonds (Prunus dulcis (Mill.) D.A. Webb) of the Sicilian Cultivar "Fascionello" (173). A manually stripping process, by repeated cycles of freeze in liquid nitrogen and thawing at RT (174). The obtained NS was milled with a stainless-steel blade analytical mill (IKA® A11, IKA®-Werke GmbH & Co. KG, Staufen, Germany) with liquid nitrogen. Powdered NS (10 g) was defatted three times with n-hexane (20 mL) for 6 h under constant agitation in order to remove the lipid fraction and to obtain a more selective polyphenol extract. After filtration on Whatman filter paper no.1, the residue was mixed with methanol/0.1% HCl (v/v, 100 mL) and sonicated for 15 min (Ultrasonic Cleaner USC300TH, VWR International, Radnor, PA, USA). The sample was centrifuged (5000 × g, 10 min, 4 °C), and the extraction procedure repeated two more times. The methanol fractions were combined and concentrated to dryness by a rotary evaporator (Büchi R-205, Cornaredo, Italy); the residue was dissolved in MilliQ water (20 mL) and extracted four times with ethyl acetate (20 mL). The combined organic phases were dried on anydrous sodium sulphate for 20 min and then concentrated to dryness by a rotary evaporator. The extraction yield was 1.86%.

5.1.2. Qualitative and quantitative analysis of polyphenols

Polyphenol characterization was carried out by reverse-phase high-performance liquid chromatography coupled with diode array, electrospray ionization and mass spectrometry (RP-HPLC-DAD-ESI-MS) (175). A fully porous silica column Luna Omega 5u PS C18 100A, 150 x 2.1 mm (Phenomenex, Torrance, CA, USA) was used. Elution was carried out with a mobile phase consisting of 0.1% formic acid (Solvent A) and methanol (Solvent B) according to the following program: 0–3 min, 0% B; 3–9 min, 3% B; 9–24 min, 12% B; 24–30 min, 20% B; 30–33 min, 20% B; 33–43 min, 30% B; 43–63 min, 50% B; 63–66 min, 50% B; 66–76 min, 60% B; 76–81 min, 60% B; 81–86 min, 0% B and equilibrated 4 min. The injection volume was 5 µL. The UV–Vis spectra were recorded ranging from 190 to 600 nm, and acquisition was done at

different wavelengths (260, 292, 330, and 370 nm) in order to identify all polyphenol classes. The experimental parameters of the mass spectrometer (ion trap, model 6320, Agilent Technologies, Santa Clara, CA, USA) operating in the negative (ESI-) and positive (ESI+) ionization mode were set as follows: 3.5 kV capillary voltage, 40 psi nebulizer (N2) pressure, 350 °C drying gas temperature, 9 L/min drying gas flow. and 40 V skimmer voltage. Acquisition was carried out in full-scan mode (90–1000 m/z). Data were acquired by Agilent ChemStation software version B.01.03 and Agilent ion trap control software version 6.2. Quantification was done by building calibration curves of each compound identified using HPLC-grade (purity ≥ 95%) reference standards (Extrasynthase, Geney, France).

5.2. In Vivo Studies

5.2.1. Animals

This study used male Sprague-Dawley rats (200-230 g, Envigo, Milan, Italy). The University of Messina's Review Board for Animal Care (OPBA) approved the study. All of the animal studies were in accordance with the current Italian legislation (D. Lgs 2014/26) and the EU regulations (EU Directive 2010/63).

5.2.2. CAR-Induced Paw Edema

Rats were given a subplantar injection of CAR (0.1 mL/rat of a 1% solution in saline) using a 27-gauge needle into the right hind paw following anesthesia with 5.0% isoflurane in 100% oxygen, as previously described by Morris and Britti (176, 177). After the CAR injection, the animals were sacrificed by isoflurane overdose six hours later. All analyses were conducted using blinded experimental groups (178).

5.2.3. Experimental Groups

Rats were randomly divided into the following groups n=6 for each:

- CAR + vehicle (saline): rats were subjected to CAR-induced paw edema;
- CAR + ASE (100 mg/kg): rats were subjected to CAR-induced paw edema and almond skin extract (100 mg/kg) was administered 30 min before CAR;

- The sham-operated group underwent the same surgical procedures as the CAR group, except that saline or compound were administered instead of CAR.

The measured dose was determined by experiments performed in our facilities. Following sacrifice, paw tissue and blood were taken for histological and biochemical examination.

5.2.4. Assessment of CAR-Induced Paw Edema

Edema was assessed as previously described (176). A plethysmometer (Ugo Basile, Comerio, Italy) was used to quantify paw volume before and after CAR injection at 6-hour intervals. Each animal's edema was measured as an increase in paw volume (mL) from the pre-injection value following CAR injection.

5.2.5. Behavioral Analysis

We employed the plantar and Von Frey tests to assess ASE's analgesic effects. We used a Basile Plantar Test (Ugo Basile, Varese, Italy) with a latency limit of 20 seconds to avoid tissue damage, and we studied the hyperalgesic reaction to heat at various durations (179-181). Furthermore, the Von Frey test (BIO-EVF4, Bioseb, Vitrolles, France) was performed in accordance with the previous descriptions (179, 182-184).

5.2.6. MPO Activity

MPO activity, an indication of PMN accumulation, was measured spectrophotometrically at 650 nm (185-187).

5.2.7. MDA levels

Lipid peroxidation was assessed as previously described (188-194).

5.2.8. Evaluation of Cytokines

TNF-α and IL-1β levels were measured using a colorimetric commercial ELISA kit from R&D Systems in Minneapolis, MN, USA (195, 196). Furthermore, blood levels of SOD, GSH, and CAT were measured in line with the manufacturer's recommendations (Cusabio Biotech Co., Ltd, Wuhan, Hubei, China) (197-202).

5.2.9. Histological Examination of the CAR-Inflamed Hind Paw

Hematoxylin and eosin (H/E) staining was performed for histological analysis, and the observers were unaware of the treatment schedule. Paw biopsies were taken 6 hours following intraplantar administration of carrageenan. Using a knife, tissue from the back paw pads was removed. The tissue slices were dehydrated using a graduated series of ethanol solutions, embedded in Paraplast (Sherwood Medical), and fixed in Dietrick's solution (14.25% ethanol, 1.85% formaldehyde, and 1% acetic acid) for one week at room temperature. The sections immersed in Paraplast were stained with H/E and then cut into 7 μm pieces for examination under a microscope (Leica DM7, Milan, Italy). The degree of inflammation was assessed using a five-point scale with the following points: none, mild, mild/moderate, moderate, moderate/severe, and severe inflammation, respectively (203, 204).

5.2.10. Immunohistochemistry for iNOS and COX-2

The studies of and COX-2 immunohistochemistry were performed, as previously described (205-207). Slices were treated with anti-iNOS and anti-COX-2 mouse monoclonal antibodies overnight (1:100 in PBS, v/v; all from Santa Cruz Biotechnology) (208, 209). Samples were washed with PBS before being exposed to secondary antibodies for incubation (205). The particular labeling was identified using a biotin-conjugated goat anti-rabbit IgG and an avidin-biotin peroxidase combination (Vector Laboratories, Burlingame, California, USA) (63). The stained sections were examined using a Leica DM6 microscope (Leica Microsystems S.p.A., Milan, Italy) according to usual practice (210, 211).

5.2.11. Western Blots Analysis

The cytosolic fraction of paw tissue was prepared for Western blot examination, as previously described (212). To standardize the technique, membranes were treated with anti-NF-κB (1:100) (213), anti-IkB-α (1:100), anti-Nrf2 (1:100), anti-HO-1 (1:100), β-actin (1:500), and β-laminin (1:500) (all from Santa Cruz Biotechnology, Heidelberg, Germany) (214-216). Using the BIORAD ChemiDocTM XRS+ software and the enhanced chemiluminescence (ECL) detection system reagent, signals were detected, and the relative expression of the protein bands was assessed (217). An

picture of the blot signals was entered into the analysis tool (picture Quant TL, v2003) (218).

5.2.12. Reagents

All other components were obtained from Sigma-Aldrich Co. Stock solutions were made using nonpyrogenic saline (0.9% NaCl, Baxter Healthcare Ltd., Thetford, Norfolk, UK).

5.2.13. Data Analysis

All values are expressed as mean ± standard error of the mean (N observations). For in vivo investigations, N denotes the number of animals. For histology experiments, the photographs provided represent at least three tests carried out on distinct experimental days on tissue sections obtained from all animals in each group. When investigating the effect of the treatment in time-dependent mode, the data were analyzed using two-way ANOVA, or one-way ANOVA when analyzing the means of two or more samples. A Bonferroni post-hoc test for multiple comparisons was used after each analysis. All statistical investigations were conducted using GraphPad Software Prism 8 (La Jolla, CA, USA). A p-value of <0.05 was judged significant. #p < 0.05 vs CAR; ##p < 0.01 vs CAR; **p < 0.01 vs sham; ***p < 0.001 vs sham.

5.3. Results

5.3.1. Results of the phytochemical characterization of ASE

Table 7 summarizes the phytochemical characterisation performed using RP-HPLC-DAD-ESI-MS. Twenty-one (21) polyphenols from various classes were found, including flavanols (51.38%), flavonols (35.88%), phenolic acids (8.82%), and flavanones (3.32%). The most abundant chemical was isorhamnetin-3-O-glucoside, followed by catechin, epicatechin, naringenin-7-O-glucoside, and protocatechuic acid. The NS polyphenolic profile described here differs from that previously reported by Smeriglio et al. (2016) for NS cv. Pizzuta (173). Although the same polyphenol classes were previously found, the distribution percentages were drastically different, with flavonols (65.09) being the most prevalent components, followed by flavanones (24.70%), phenolic acids (9.54%), and flavanols (0.67%). The most prevalent chemicals were kaempferol and its derivatives, followed by naringenin, chlorogenic, and vanillic acids (173). On the other hand, an isorhamnetin derivative (isorhamnetin-

3-*O*-rutinoside) was the most abundant compound in Californian natural almond skin (174), followed by kaempferol derivatives and naringenin. Making meaningful comparisons is challenging due to a multitude of factors, including variety, production procedures, ambient conditions, and extraction processes. These discrepancies could be related to the diverse cultivars tested, but, above all, to alterations in pedo-climatic conditions (219). It is well-known that, although the production area of the Avola almond is concentrated within the provinces of Syracuse and Ragusa, the pedo-climatic features of the territory as well as seasonality and climatic changes over the last few years could be responsible for the significantly variability recorded in the polyphenolic profile (219).

Polyphenols	**RT[a] (min)**	**λ_{max} (nm)**	**$[M-H]^-$**	**mg/100 g DE[b]**
Hydroxybenzoic acids				
Protocatechuic acid	7.04	258;293	153	38.01 ± 0.55
4-Hydroxybenzoic acid	12.00	253	137	0.29 ± 0.01
Vanillic acid	16.00	262;291	167	30.88 ± 0.42
Hydroxycinnamic acids				
Chlorogenic acid	20.50	291;319	353	28.28 ± 0.28
trans-*p*-Cumaric acid	22.80	309	163	-
Flavanones				
Eriodictyol-7-*O*-glucoside	29.71	283	449	3.90 ± 0.08
Naringenin-7-*O*-glucoside	32.43	282	433	39.36 ± 0.67
Eriodictyol	35.66	287	287	0.01 ± 0.00
Naringenin	40.26	289	271	0.02 ± 0.00
Flavonols				
Quercetin-3-*O*-galactoside	32.36	253;354	463	0.52 ± 0.01
Quercetin-3-*O*-rutinoside	32.41	254;354	609	0.08 ± 0.00
Quercetin-3-*O*-glucoside	32.64	254;354	463	2.81 ± 0.02
Kaempferol-3-*O*-rutinoside	33.96	265;348	593	9.20 ± 0.03
Kaempferol-3-*O*-glucoside	34.34	264;347	447	3.33 ± 0.02
Quercetin-3-*O*-rhamnoside	34.36	257;358	447	2.04 ± 0.01
Isorhamnetin-3-*O*-glucoside	34.88	254;353	477	377.29 ± 1.55
Quercetin	39.39	255;370	301	0.09 ± 0.00
Kaempferol	43.66	264;365	285	1.02 ± 0.02
Isorhamnetin	44.47	253;368	315	0.11 ± 0.00
Flavanols				
Catechin	18.65	279	289	388.91 ± 2.21
Epicatechin	**23.64**	**279**	**289**	**178.92 ± 1.44**

[a]RT, retention time; [b]DE, dry extract.

Table 7. Qualitative and quantitative characterization of polyphenols in the natural skin (NS) extract by RP-HPLC-DAD-ESI-MS analysis. Data, which are the mean ± the standard deviation of three independent experiments in triplicate (*n*=3), are expressed as mg/100 g of NS dry extract (DE).

5.4 In vivo studies: Effect of ASE on CAR-Induced Inflammation and Pain

One of the first signs of intraplantar CAR injection was a time-dependent increase in paw volume (Figure 12A), which was measured at several timepoints ranging from 0 (when the experiment began) to 6 hours (when the experiment concluded). The increase in paw volume causes pain, which was determined by the development of heat hyperalgesia (plantar test) and mechanical allodynia (Von Frey test) (Figure 12B). In our work, we discovered that CAR injection exacerbated heat and mechanical hyperalgesia, whereas oral therapy with ASE at a dose of 100 mg/kg given 30 minutes before CAR was able to considerably lower the volume of the rat paw at 6 hours post-CAR while also decreasing pain.

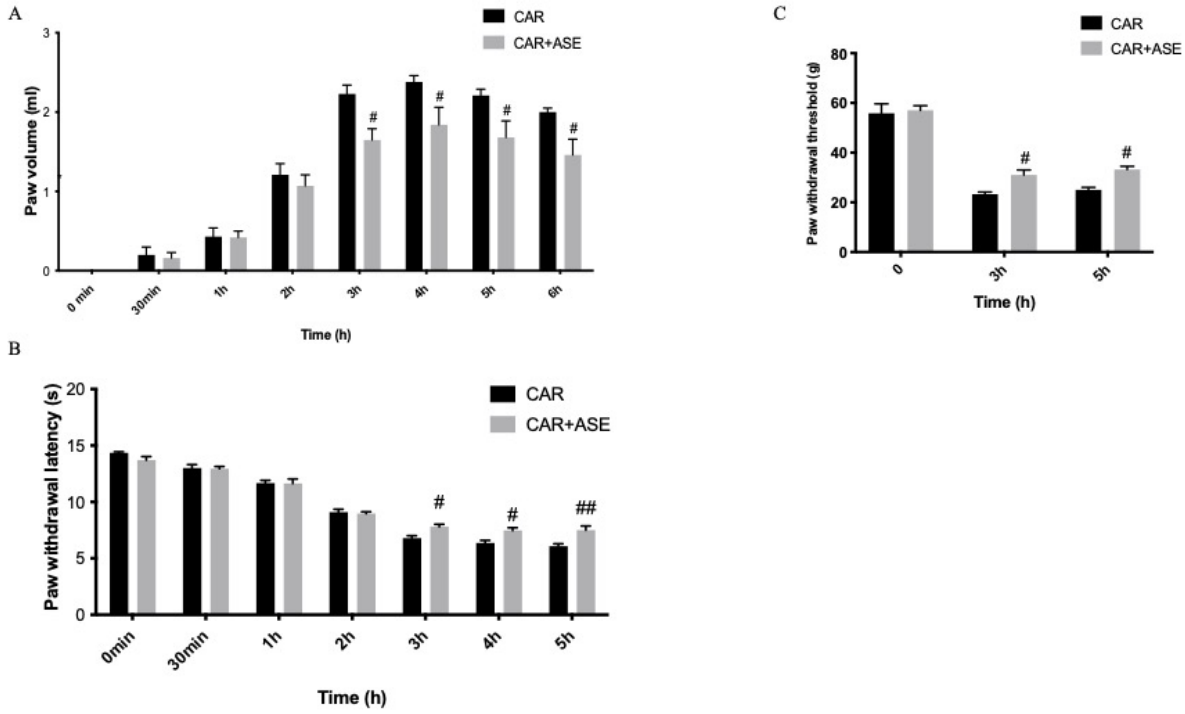


Figure 12. Evaluation of the effects of ASE on CAR-induced inflammation and pain. Paw volume in millilitres (**A**), plantar test (**B**), and von Frey test (**C**). ASE delivery resulted in considerable improvements in the management of inflammation and pain. Data are presented as means ± SEM of six animals per group. #P<0,05 vs CAR ## P<0,01 vs CAR.

5.4.1 Effects of ASE on Histological Alteration after CAR Injection

At the conclusion of the experiment, paw tissue underwent histological investigation using H/E. A microscopic examination of the CAR group's paw samples revealed edema formation and cellular diffuse infiltration, as well as significant changes in tissue architecture (Figure 13B, see histological score D). ASE treatment at a dose of 100 mg/kg reduced histological harm in rats' paw tissues (Figure 13C, see histological

grade D), counteracting both cellular infiltration and edema development. Sham animals demonstrated normal paw tissue architecture (Figure 13A).Histological damage was associated with a considerable increase in MPO activity (Figure 13E). In our investigation, we discovered that administering ASE 30 minutes before CAR injection reduced the activity of MPO.

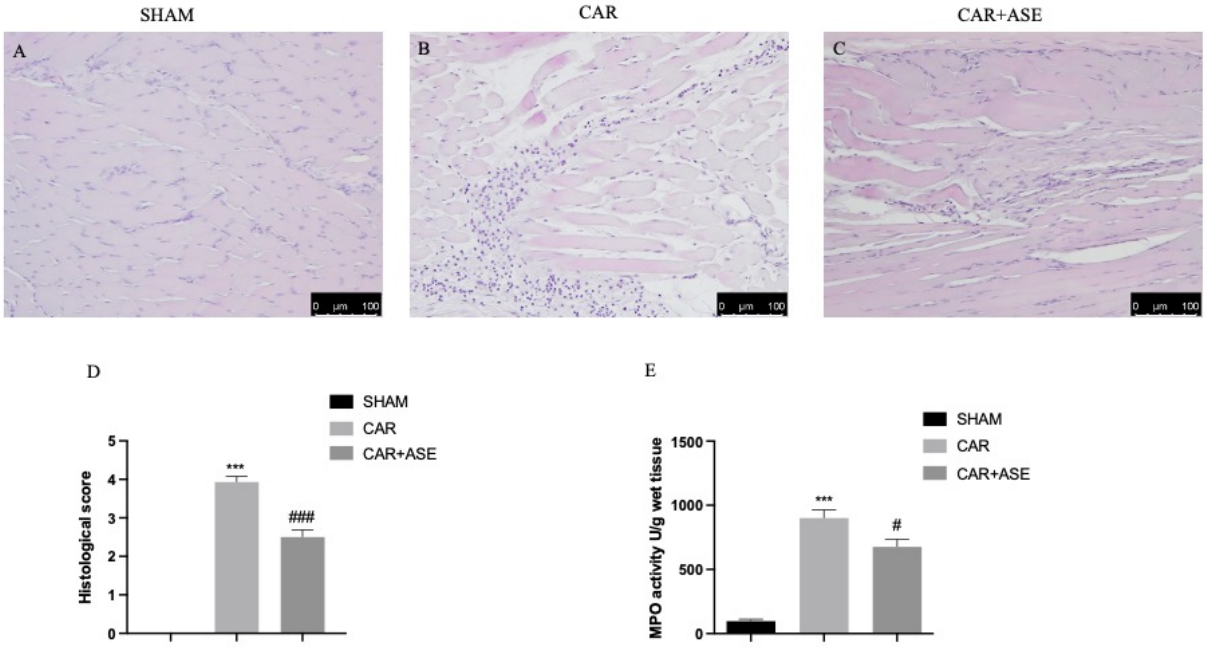


Figure 13. Histological evaluation of paw tissue and neutrophil infiltration after ASE treatment following CAR-injection. Investigation of tissue injury of the animals subjected to CAR was made by H/E staining. CAR group showed a loss of the physiological architecture compared to sham. ASE administered 30 min before CAR-injection showed reduction in infiltrating cells and edema formation compared to vehicle. Sham **(A)**; CAR **(B)**; CAR+ASE **(C)**. Histological score **(D)** As a consequence of neutrophils infiltration MPO was assessed. CAR induced a significant increase of MPO. Oral treatment with ASE at the dose of 100 mg/kg significantly reduced MPO. MPO (E). Data are expressed as means ± SEM of 6 animals for each group. ### P<0,001 vs CAR. ***P<0,001 vs sham; # P<0,05 vs CAR.

5.4.2 Effect of ASE on iNOS and COX-2 expression

Considering iNOS and COX-2 enzymes play a critical role in inflammation development, we used immunohistochemistry to analyze their expression. iNOS expression was high in CAR-treated animals (Figure 14B), whereas treatment with ASE reduced its expression and muscle fibers restoration occurred (Figure 14C); similarly, COX-2 expression was elevated in CAR-treated animals (Figure 14F), but decreased in ASE-treated animals (Figure 14G).

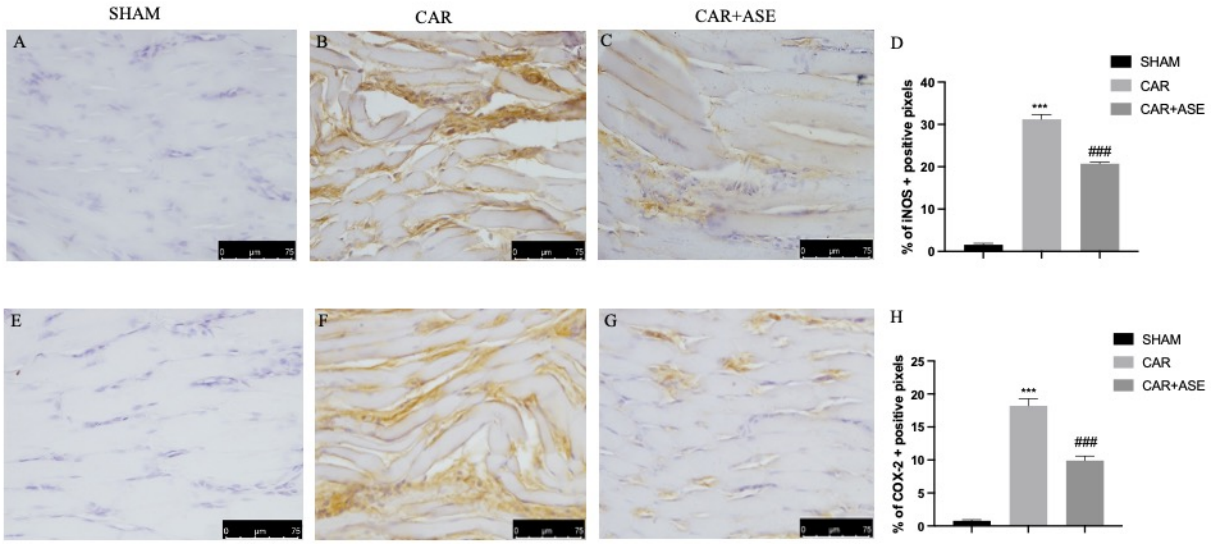


Figure 14. Administration of ASE reduces the expression of iNOS and COX-2. Immunohistochemical analysis of iNOS and COX-2: sham **(A, E)**, vehicle **(B, F)**, ASE treatment **(C, G)**. The results are expressed as % of positive pixels **(D, H)**. Figures are representative of at least three independent experiments. Values are means ± SEM of 6 animals for each group. Scale bar: 75 µm ***P<0.001 vs sham ### P<0.001 vs CAR.

5.4.3. Effects of ASE on Cytokines Production

As is widely known, cytokines exert essential effects during inflammatory processes; hence, they can be utilized as biomarkers in identifying or monitoring inflammation and its progression (220). In our study, we found a significant increase compared to sham animals in serum pro-inflammatory cytokine levels of TNF-α and IL-1β in the group subjected to CAR (Figure 15A–B). ASE administration given 30 min before CAR-injection at the dose of 100 mg/kg was able to significantly decrease pro-inflammatory cytokines production.

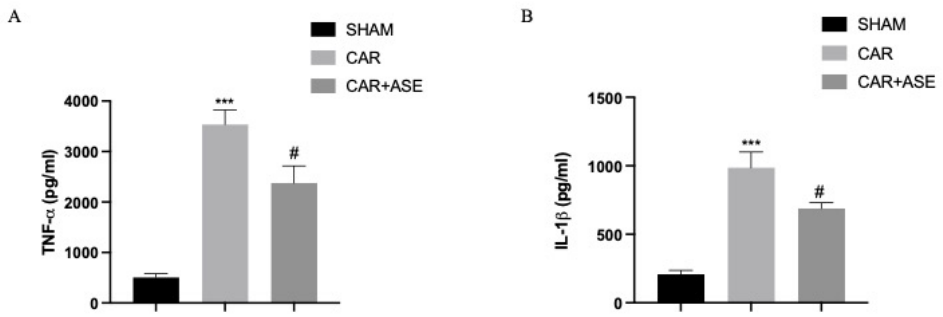


Figure 15. Effects of ASE on cytokines production CAR-induced. During CAR-caused inflammation, we observed a significant increase in TNF-α, IL-1β levels. ASE administration 30 min before CAR-injection at the dose of 100 mg/kg significantly reduced pro-inflammatory cytokines expression. TNF-α (**A**), IL-1β (**B**). Values are means ± SEM of 6 animals for each group. ***P<0,001 vs sham; # P<0,05 vs CAR. # $p < 0.05$ vs. CAR; ## $p < 0.01$ vs. CAR; *** $p < 0.001$ vs. sham.

5.4.4. Effect of ASE on CAR-Induced Oxidative Stress

We investigated SOD (Figure 16A), GSH (Figure 16B), and CAT (Figure 16C) in light of the various oxidants and free radicals implicated in the pathogenesis of inflammatory processes, as well as the possibility that dietary components may contribute to antioxidant defense, either by providing redox active compounds that can directly scavenge or neutralize free radicals. As expected, after CAR injection, we saw a reduction in SOD (Figure 16A), GSH (Figure 16B), and CAT (Figure 16C) activity relative to sham mice. Following ASE therapy, oxidative stress decreased while SOD, GSH, and CAT activities increased. In addition, after CAR-injection, MDA levels increased, indicating lipid peroxidation (Figure 16D). ASE therapy dramatically decreased MDA levels.

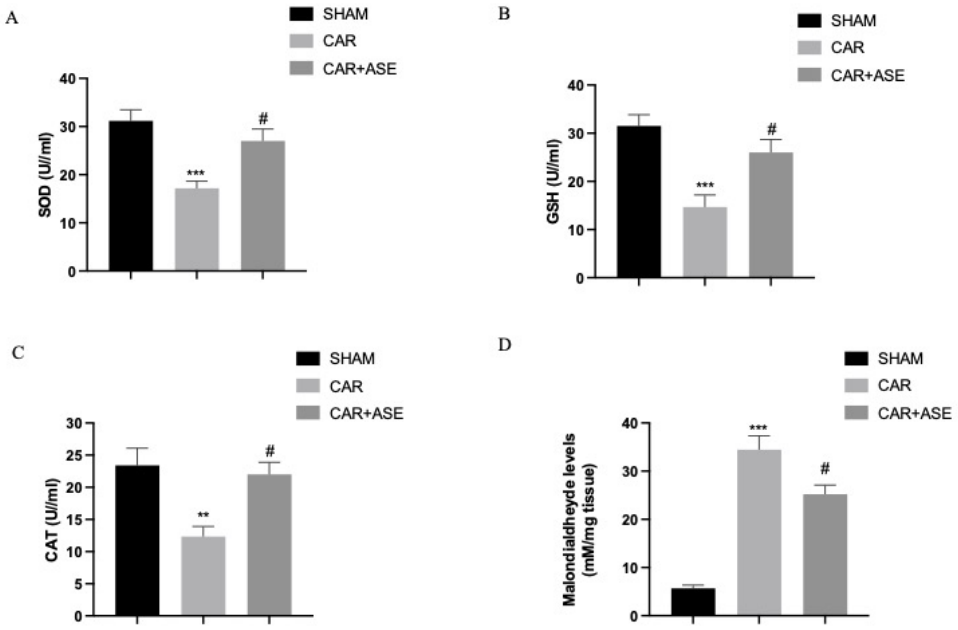


Figure 16. Anti-oxidant effects of ASE after CAR-induction. First line of defense against free radical production is represented by SOD **(A)**, GSH **(B)**, and CAT (**C**). Administration of ASE at the dose of 100 mg/kg significantly increased the activity of the anti-oxidant enzymes SOD, CAT, and GSH which had been significantly reduced by CAR-injection. As a consequence of lipid peroxidation, MDA was assessed. CAR induces a significant increase of MDA **(D)**. Oral treatment with ASE at the dose of 100 mg/kg significantly reduced MDA (**D**). Values are means ± SEM of 6 animals for each group. ** $p <$ 0.01 vs. sham; *** $p < 0.001$ vs. sham # P<0,05 vs CAR, # $p < 0.05$ vs. CAR; ## $p < 0.01$ vs. CAR.

5.4.5. Effect of ASE on CAR-Induced NF-κB, Nrf2, HO-1, and IkB-α expression

Western blots were performed on paw tissues to determine if ASE interacts with signaling pathways such NF-κB, Nrf2/HO-1, and IkB-α. Induction with CAR led to increased nuclear translocation of NF-κB (Figure 17A) and decreased expression of its inhibitor IκB-α (Figure 17B; see densitometric analysis Figure 17B1). Treatment with ASE reduced NF-κB expression levels (Figure 17A, densitometric analysis Figure 17A1) and restored IκB-α expression (Figure 17B, see Figure 17B1). Following CAR induction, Nrf2 expression was reduced relative to sham mice (Figure 17C, C1). ASE increased Nrf2 expression relative to the CAR vehicle group (Figure 17A, A1, C, and C1). At the same time, Western blot analysis revealed that ASE therapy greatly enhanced the CAR-induced decrease in HO-1 protein expression (Figure 17D, D1).

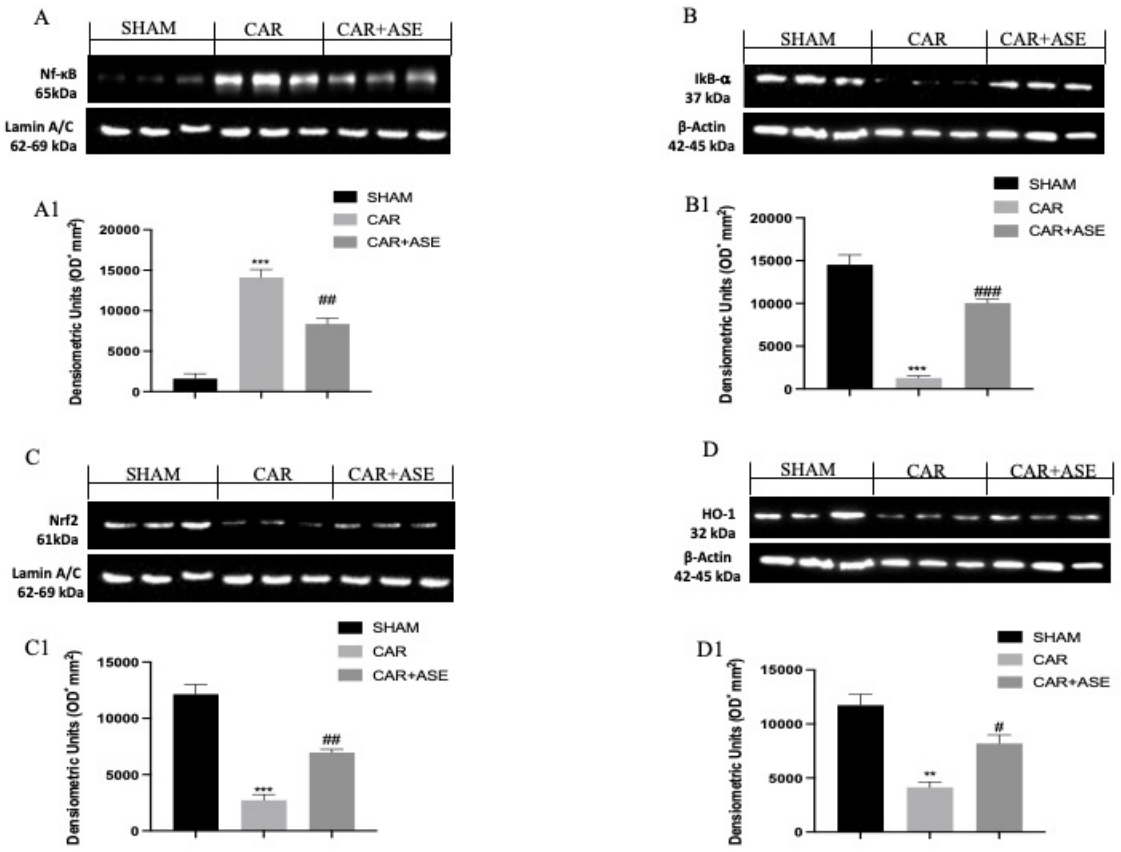


Figure 17. Effects of ASE on the expressions of NF-κB, Nrf2/HO-1 and IkB-α. Representative Western blots were performed for NF-κB, Nrf2, IkB-α and HO-1 **(A-D)**. We found a significant increase in NF-κB after CAR injection, compared with sham, and a significant reduction in IkB-α Nrf2, and HO-1 **(A-A1, B-B1, C-C1, D-D1)**. ASE was able to reduce the expression of NF-κB and restore the expression of its inhibitor IkB-α **(A-A1, B-B1)**, Nrf2 and HO-1 **(C-C1, D-D1)**. Exposed is a blot of lysates (6 animals/group) with a densitometric analysis for all animals. $^{###}$ $p < 0.001$ vs. CAR. $^{##}$ $p < 0.01$ vs. CAR, # $p < 0.05$ vs. CAR; *** $p < 0.001$ vs. sham; ** $p < 0.01$ vs. sham.

CHAPTER 6: Assessment of the Wound-Healing Properties of Almond Oil in a Murine Excisional Wound Model

6.1 Materials and Methods

6.1.1. Animals

Male CD1 mice (25-30 g, Envigo, Milan, Italy) were housed in a controlled environment and given free access to food and water. The University of Messina's Review Board for Animal Care (OPBA) approved the study. We respected the legislation for the protection of laboratory animals (D.Lgs 2014/26 and EU Directive 2010/63- n° 1141/2024-PR)

6.1.2. Nutrition Facts of Fresh Vintage Farms Almond Oil

Almond Oil was kindly provided by freshvintagefarms.com from Almond Board of California. In short, almond oil is cold-pressed, resulting in artisanal and more nutritious oils. Nutritional information per 12.68 mL serving: 135 calories, 130 calories from fat, total fat 15g (19%*), saturated fat 1g (6%), trans fat 0g, polyunsaturated fat 3g, monounsaturated fat 10g, cholesterol 0mg (0%), sodium 0mg (0%), total carbohydrate 0g (0%), protein 0g (0%).

6.1.3. Experimental wound model

Mice received anesthesia with isoflurane before cutting their back hair, and the dorsum was prepared for wounding by applying a betadine scrub and 70% ethanol alternately three times. A sterile biopsy punch (KAI Corporation, Tokyo, Japan) was used to make two circular, full-thickness skin wounds on either side of the median line on the dorsum, each measuring 5 mm in depth (221, 222). Mice were sacrificed after 7 days, and the wounds were divided into two halves. The first was used for histology, while the second was for molecular analysis.

6.1.4. Experimental Groups.

The animals were randomly distributed into the following groups (*n=6* for each group):

- Sham group: mice were subjected to all procedures described above, full-thickness excision wounds were not applied.

- WH + vehicle: mice were subjected to full-thickness excisional wounds as described above.
- WH + Almond oil: animals were subjected to full-thickness excisional wounds as described above, and wounds were treated once a day for 7 days with a topical application of 400 µl of almond oil with a micropipette.

The dose and route of administration of Almond oil was chosen based on tests conducted in our laboratory.

6.1.5. Histological Analysis

Sections of the wound area were stained with Hematoxylin/Eosin (H/E) and using a Leica DM6 microscope associated with Leica LAS X Navigator software (Leica Microsystems SpA, Milan, Italy) (223, 224). The histological score on a point scale from 0 to 4 was determined as previously described (221). Briefly, the scores were 0: absence of epithelial proliferation in >70% of the tissue; 1: poor epidermal organization in >60% of the tissue; 2: incomplete epidermal organization in >40% of the tissue; 3: moderate epithelial proliferation in >60% of the tissue; 4: complete epidermal remodeling in >80% of the tissue.

6.1.6. Masson's trichrome staining

Sections were also stained by Masson's trichrome method for collagen detection (221). The relative density of blue-stained collagen after Masson's trichrome staining was quantified in the wound area using ImageJ.

6.1.7. Immunohistochemical for Nitrotyrosine and Poly (ADP-Ribose Polymerase) (PARP)

Immunohistochemical analysis was performed as previously described (224). Sections of wound area were incubated with the primary antibodies: anti-nitrotyrosine antibody (Millipore, 1:200 in PBS, v/v), anti-poly (ADP)-ribose (PAR) antibody (Santa Cruz Biotechnology, 1:500 in PBS, v/v). Samples were washed with PBS before being exposed to secondary antibodies for incubation (205). A biotin-conjugated goat anti-rabbit IgG and an avidin-biotin peroxidase complex were used to identify the specific labeling (Vector Laboratories, Burlingame, CA, USA) (63). Using a Leica DM6

microscope (Leica Microsystems S.p.A., Milan, Italy), the stained sections were examined as per standard practice (210, 211). The histogram profile is related to the positive pixel intensity value obtained.

6.1.8. Evaluation of Cytokines

A colorimetric commercial ELISA kit was used to measure the levels of IL-1β, TNF-α and IL-6 (R&D Systems, Minneapolis, MN, USA).

6.1.9. Western Blot Analysis

Western blot analysis was performed on skin samples as previously described (225). The following primary antibodies were used: anti-MMP-9 (1:500; SCB, sc-393859); anti-TGF-β (1:1000; Abcam, ab-9758) ; anti-VEGF (1:1000; SCB, sc-57496); anti-Nrf2 (1:100; SCB, sc-365949), anti-HO-1 (1:100; SCB, sc-136960); anti-SOD-2 (1:100, SCB, sc-137254); anti-NF-κB p65 (1:100; SCB, sc-841465), anti-IkB-α (1:500; SCB, sc-1643), in phosphate-buffered saline, 5% *w*/*v* non-fat dried milk, and 0.1% Tween-20 at 4 °C overnight. Membranes were incubated with peroxidase-conjugated bovine anti-mouse IgG secondary antibody or peroxidase-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch, West Grove, PA, USA; 1:2000) for 1 h at room temperature. Anti-β-actin (1:500; SCB, sc-8432) or anti-lamin A/C (1:500) antibodies were used as controls. Protein expression was quantified by densitometry with BIORAD ChemiDocTM XRS+ software and normalized to housekeeping genes β-actin and lamin A/C as previously reported (226).

6.1.10. Materials

Unless otherwise stated, all compounds were purchased from Sigma-Aldrich. All solutions used for in vivo infusions were prepared using nonpyro-genic saline (0.9% NaCl; Baxter Healthcare Ltd., Thetford, Norfolk, UK).

6.1.11. Statistical Evaluation

All values are expressed as mean ± standard error of the mean (SEM) of N observations. The images shown are representative of the least 3 experiments performed on diverse experimental days on tissue sections collected from all animals in each group. For in vivo studies, N represents the number of animals used. The results

were analyzed by one-way ANOVA followed by a Bonferroni post-hoc test for multiple comparisons. A P value less than 0.05 was considered significant.

6.2. Results

6.2.1 Effects of topical application of Almond Oil on wound closure and histological tissue damage

As shown in Figure 19A, on day 0, we created full-thickness excisional wounds with a 5 mm biopsy punch. Almond oil was administered topically (400 μL) to the wound site for 7 days to test its potential therapeutic effects on the healing process. As shown by the wound images and the wound closure percentage graph (Figures 18A, B), we observed that, compared to the control, daily almond oil treatment significantly improved the WH process from day 3 to day 7 in terms of the speed and percentage of wound closure. Furthermore, to observe the effect of almond oil on WH-induced tissue changes, hematoxylin and eosin (H&E) staining was performed (Figure 18C, D, F, and relative histological analysis (Figure 18G). H&E staining showed a compromised wound characterized by extensive tissue damage, a large number of neurophils and fibroblasts in edematous tissue, and limited cutaneous and epidermal organization in the WH+ vehicle (Figure 18D, and relative histological analysis, Figure 18G), compared to tissue from the sham group (Figure 18C, and relative histological analysis, Figure 18G). Topical administration of almond oil (Figure 18F, and relative histological analysis, Figure 18G) resulted in improvements in wound closure, granulation, and re-epithelialization, all of which contributed to near-complete restoration of the epidermis in this group.

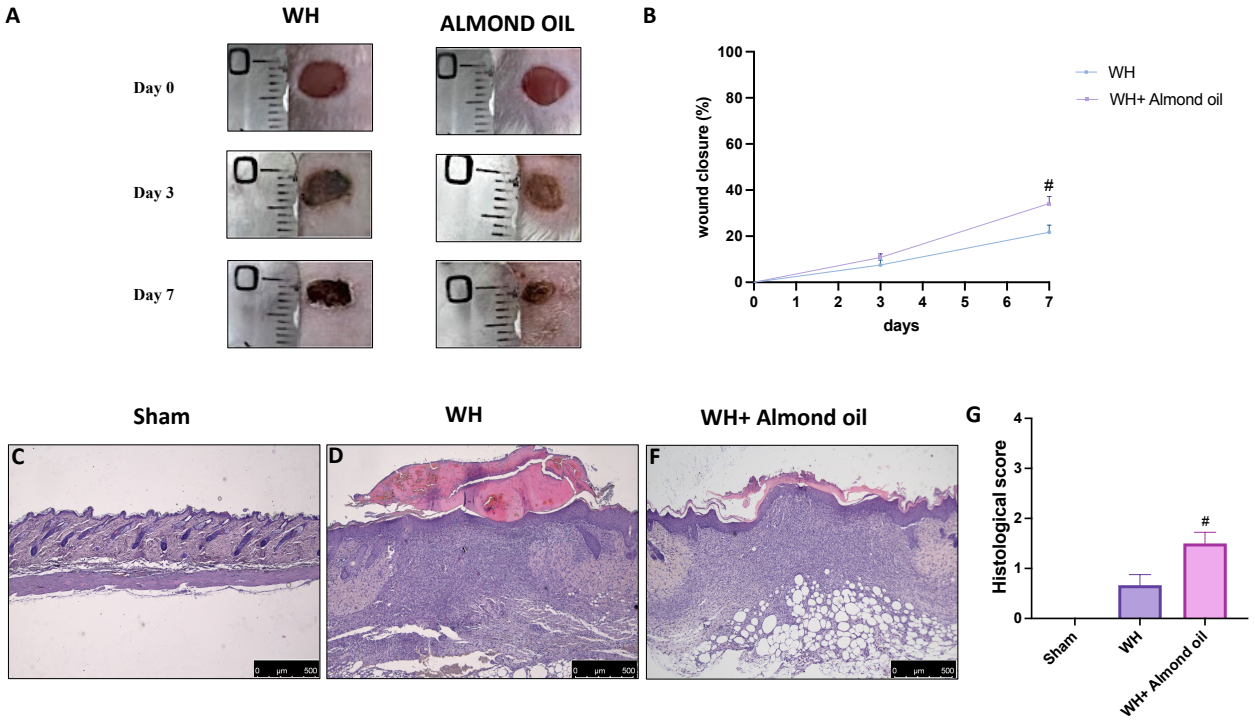


Figure 18. Effect of topical application of almond oil on WH rate and histological damage in a murine full-thickness excisional wound model. **(A)** Representative picture of wounds at the different time points. **(B)** The graph shows the results expressed as the percentage of original wound size over time. **(C-G)** H&E staining of wound granulation tissue at 7 days after induction and relative histological score graph. Data are presented as means ± SEM of 6 mice for each group. # $p < 0.05$ vs. WH.

6.2.2 Effects of topical application of Amond Oil on collagen fibers and the expression of MMP-9, TGF-b, and VEGF in wounded skin

To evaluate extracellular matrix remodeling in the dermis, Masson's trichrome staining was performed. The staining revealed the presence and organization of both fine and coarse collagen fibers in the wounded skin tissue (Figure 19A–C; graph D). In the control group, normal collagen deposition was observed (Figure 19A), whereas the WH + vehicle group showed a marked reduction in collagen content seven days after wound induction (Figure 19B). Significantly, the WH + Almond Oil group exhibited enhanced collagen fiber synthesis and improved organization around the wound area (Figure 19C), indicating a stimulatory effect of topical almond oil treatment. WH is closely associated with fibrosis and neovascularization, both of which are regulated by various signaling molecules. Among them, matrix metalloproteinases (MMPs) play a critical role in tissue remodeling. To further investigate the molecular mechanisms involved, we analyzed the expression of MMP-9, TGF-β, and VEGF by Western blot. As shown in Figure 19E (densitometric analysis in Figure 19E1), the expression of

MMP-9 was significantly elevated in wounded tissue but was notably reduced following treatment with almond oil, suggesting its inhibitory effect on wound-induced MMP-9 upregulation. Regarding TGF-β expression (Figure 19F; densitometric analysis in Figure 19F1), a high level was detected in the Sham group, while it was reduced in the WH group. Topical almond oil treatment significantly restored TGF-β expression to levels comparable to the control. Finally, VEGF expression (Figure 19G; densitometric analysis in Figure 19G1) was significantly increased in the WH + vehicle group and further upregulated in the WH + Almond Oil group, confirming the pro-angiogenic effect of the treatment.

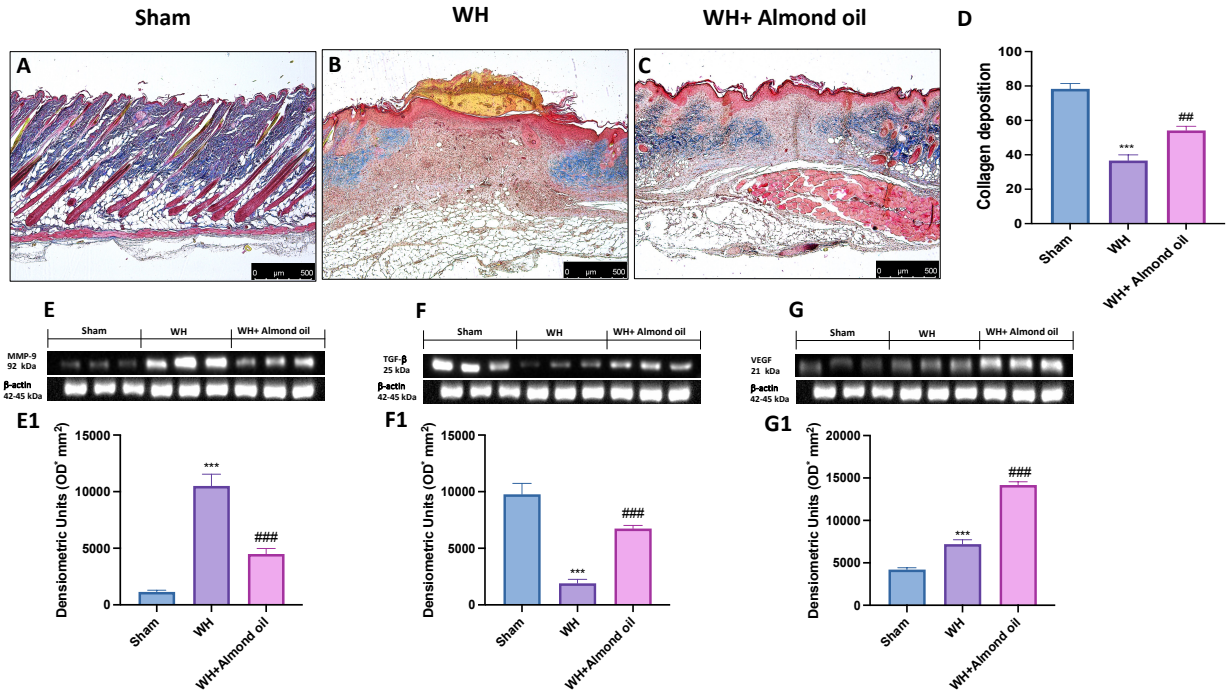


Figure 19. Effect of topical Almond Oil treatment on collagen deposition (Masson's trichrome staining) and expression levels of MMP-9, TGF-β, and VEGF, seven days after injury. (**A–C** and relative collagen quantification in graph **D**): Masson's trichrome staining shows collagen fibers in the control group (A), reduced fibers in the WH + vehicle group (**B**), and enhanced deposition in the WH + Almond Oil group (**C**). (**E–G**): Western blot analysis of MMP-9 (**E**), TGF-β (**F**), and VEGF (**G**) expression levels. (**E1–G1**): Corresponding densitometric analyses. Exposed is a blot of lysates (6 animals/group) with a densitometric analysis for all animals. Data are expressed as mean ± SEM of six animals for each group. *** $p < 0.001$ versus Sham. ## $p < 0.01$ vs. WH. ### $p < 0.001$ vs. WH.

6.2.3. Effect of topical application of Almond Oil on Nitrotyrosine and PARP expressions

The expression of nitrotyrosine, a specific marker of nitrosative stress, and PARP, a marker of DNA degradation, was analyzed by immunohistochemical staining. Skin

tissue sections from sham-operated mice showed no evidence of nitrotyrosine staining (Figure 20A, A1), whereas sections from WH+vehicle mice showed robust positive staining for nitrotyrosine (Figure 20B, B1). Furthermore, increased positive staining for PARP was also observed in tissues from WH+vehicle mice (Figure 21B, B1) compared to the sham group (Figure 21A, A1). Topical treatment with 400 µL of almond oil significantly reduced positive staining for nitrotyrosine and PARP in all tissues (Figure 20C, C1 and Figure 21C, C1, and see related graphs in Figure 20D and Figure 21D).

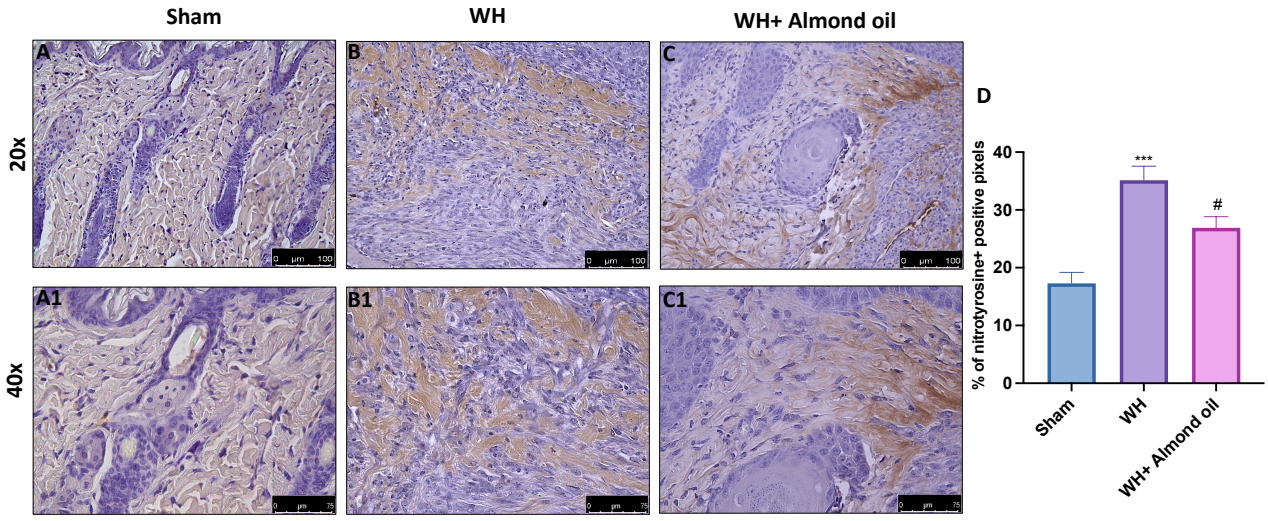


Figure 20. Effects of topical almond oil treatment on nitrotyrosine in skin 7 days after wounding. Immunohistochemical analysis showed positive staining for nitrotyrosine in the WH group compared to the sham group (**B, B1, A, A1**). Reduced nitrotyrosine expression was observed in skin from the WH + Almond Oil group 7 days after wounding (**C, C1**). The results are expressed as % of positive pixels (**D**). Data are presented as means ± SEM of 6 mice for each group. *** $p < 0.001$ vs. sham. # $p < 0.05$ vs. WH.

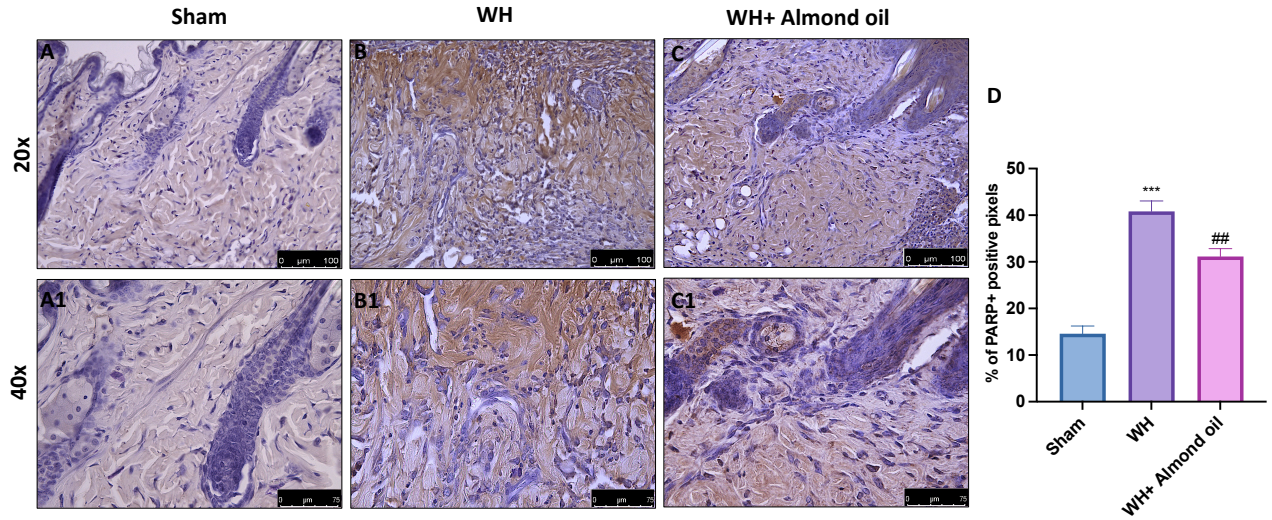


Figure 21. Effects of topical almond oil treatment on PARP in skin 7 days after wounding. Immunohistochemical analysis showed positive staining for PARP in the WH group compared to the sham group (**B, B1, A, A1**). Reduced PARP expression was observed in skin from the WH + Almond Oil group 7 days after wounding (**C, C1**). The results are expressed as % of positive pixels (**D**). Data are presented as means ± SEM of 6 mice for each group. *** $p < 0.001$ vs. Sham. ## $p < 0.01$ vs. WH.

6.2.4. Effect of Topical Almond Oil Application on Inflammation Induced by Full-Thickness Excisional Wounds

To evaluate the anti-inflammatory activity of topical application of almond oil to the wound, we analyzed the levels of IL-1β (Figure 22A), TNF-α (Figure 22B), and IL-6 (Figure 22C). Levels of these cytokines were significantly increased in the WH group compared to sham-operated mice. In contrast, cytokine release was significantly reduced in mice treated with Almond Oil. An increase in NF-κB nuclear translocation was found after wound induction (Figure 22E) along with a reduced expression of its inhibitor IκB-α (Figure 22D, see densitometric analysis Figure 22E1, D1). Treatment with Almond oil was able to reduce NF-κB expression levels (Figure 22E and densitometric analysis Figure 22E1) and restore IκB-α expression (Figure 22D, see Figure 22D1).

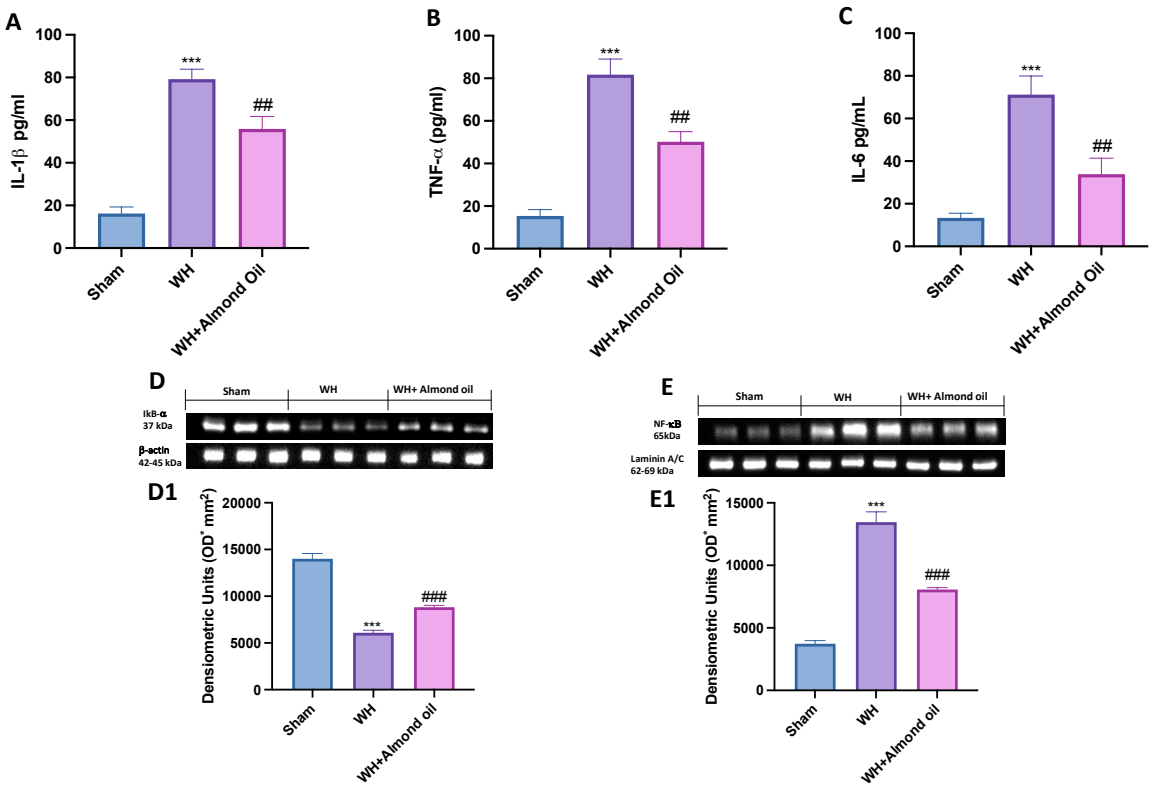


Figure 22. Effects of topical application of almond oil on the levels of the cytokines and on inflammation. Measurement of IL-1b (A), TNF-α (B), and IL-6 (C). Representative Western blots showing the effects of almond oil on: (D) Ikb-α expression, (E) NF-κB expression at 7 days post-injury. Almond oil treatment increased Ikb-α levels (D) and reduced NF-κB expression (E) in the WH+Almond Oil group compared to the WH+vehicle group. Exposed is a blot of lysates (6 animals/group) with a densitometric analysis for all animals. Data are expressed as mean ± SEM of six animals for each group. *** $p < 0.001$ vs. Sham. ## $p < 0.01$ vs. WH, ### $p < 0.001$ vs. WH.

6.2.5. Effect of topical application of almond oil on oxidative stress

To further investigate whether almond oil may act by interacting with signaling pathways such as Nrf2/HO-1, Western blot analyses for the Nrf2/HO-1 and SOD-2 were also performed on skin tissues following wound induction. A reduction in nuclear translocation of Nrf-2 was found in the WH group, compared to the Sham group after wound induction (Figure 23A, see densitometric analysis Figure 23A1). While treatment with almond oil increased the expression of Nrf2 compared to the WH+vehicle group (Figure 23A, see densitometric analysis Figure 23A1). At the same time, Western blot analysis showed that after treatment with almond oil, the expression of HO-1 protein and SOD-2 significantly increased compared to the WH group (Figure 23B and C, see densitometric analysis Figure 23B1 and C1), indicating an increase in the antioxidant activity of the bioactive molecules present in almond oil.

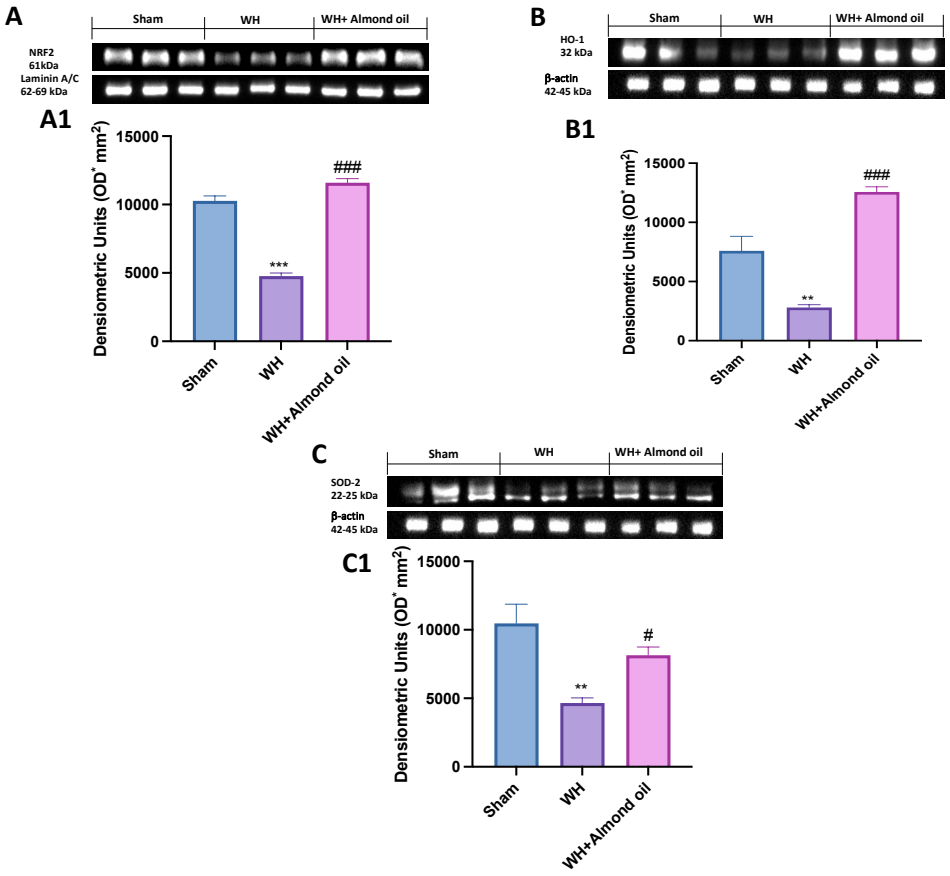


Figure 23. Effects of topical application of almond oil on oxidative stress. Representative Western blots showing the effects of almond oil on: (**A**) Nrf-2 expression, (**B**) HO-1 expression, and (**C**) SOD-2 expression at 7 days post-injury. Almond oil treatment increased Nrf-2 (**A**), HO-1 (**B**) and SOD-2 expression (**C**) in the WH+Almond Oil group compared to the WH+vehicle group. Exposed is a blot of lysates (6 animals/group) with a densitometric analysis for all animals. Data are expressed as mean ± SEM of six animals for each group. *** $p < 0.001$ vs. Sham **$p<0.01$ vs. Sham. # $p < 0.05$ vs. WH. ### $p < 0.001$ vs. WH.

CHAPTER 7: Consumption of Cashew nuts on oxidative stress and neuroinflammation markers in a hyperhomocysteinemic condition

7.1 Materials and Methods

7.1.1. Animals

Sprague Dawley rats (male, 250 g, Envigo, Milan, Italy) were housed in a well-organized environment and fed normal rodent food and water. The animals were acclimated to these circumstances for a week. The Animal Welfare Review Board at Messina University gave its approval to the study. All animal research complies with EU rules (EU Directive 2010/63) and new Italian law (D.Lgs 2014/26).

7.1.2. Cashew Nuts' Nutritional Composition

The cashew kernel samples (*Anacardium occidentale* L.) were obtained from the Ivory Coast; per 100 g, they contained 5.40 g moisture, 22.46 g protein, 44.19 g total lipids, 4.48 g total dietary fiber, 30.95 g total sugars, 2.68 g ash, and 80.01 mg total phenols. The nutritional composition was analyzed according to the Association of Official Analytical Chemists (AOAC) Official Method, as previously reported (227). The total content of folate in cashew nuts is 25 µg/100 mg (https://fdc.nal.usda.gov/fdc-app.html#/food-details/170162/nutrients, accessed on 5 March 2022).

7.1.3. Cashew Nuts Nutritional Composition

As previously stated, the Association of Official Analytical Chemists (AOAC) Official Method was used to test the nutritional composition of cashew (8, 18).

7.1.4. HHcy induction and experimental groups

Male rats were given oral methionine (Meth) (1 g/kg) dissolved in drinking water for 30 days in order to induce HHcy (18, 228).

The animals were divided up into groups at random:

- Sham controls: rats received only normal saline;
- HHcy+vehicle: rats received Meth as above, for 30 days and were treated with saline;
- HHcy+cashew nuts: rats were subjected daily to methionine and received cashew nuts (100 mg/kg, oral) for 30 days.

Cashew nut dosages were selected using data from earlier research (63, 229).

All data about the sham+vehicle groups was shown because there was no discernible difference between the sham groups. The animals were slaughtered after the 30-day experiment was over. All of the animals' blood and brain tissues were taken.

7.1.5. Biochemical analyses

Serum levels of Hcy were evaluated with a commercially available kit for HPLC assays (Bio-Rad, Milan, Italy), in accordance with the manufacturer's guidelines (18).

7.1.6. Antioxidant levels

The concentrations of glutathione (GSH) in blood were assessed following the manufacturer's guidelines (Cusabio Biotech Co., Ltd, China) (230). The concentrations of GSH in brain tissues were quantified using the previously described method, with absorbance assessed spectrophotometrically at 412 nm (231).

7.1.7. Malondialdehyde (MDA) levels

As indicated, plasma MDA levels were assessed as an indication of lipid peroxidation (18, 232). MDA levels were measured in brain tissue, as previously described (233).

7.1.8. Western blots for NRF-2 and HO-1, Bax and Bcl-2

The cytosolic and nuclear extracts were produced as previously reported. (234, 235). The primary antibodies employed were anti-NRF-2 (sc-365949, SCB), anti-HO-1 (sc-136960, 1:1000 SCB), anti-Bcl-2 (SCB, sc-7382), and anti-Bax (SCB, sc-7480), in phosphate-buffered saline, 5% w/v non-fat dried milk, and 0.1% Tween-20 at 4°C overnight. Membranes were incubated with peroxidase-conjugated bovine anti-mouse IgG secondary antibody or peroxidase-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch, West Grove, PA, USA; 1:2,000) at room temperature for one hour. Antibodies against β-actin and lamin A/C were employed as controls. The expression of protein bands was detected using a previously established technique (236).

7.1.9. Immunohistochemical analysis

The immunohistochemical analysis was performed as previously described (233, 237). The sections were treated overnight with primary antibodies against GFAP (sc-51908, Santa Cruz Biotechnology (SCB)), Iba-1 (sc-SCB), and MAP-2 (sc-74421, SCB). Images were taken using a Leica DM6 microscope (Leica Microsystems SpA, Milan,

Italy) in accordance with standard procedures. The histogram profile corresponds to the positive pixel intensity value obtained.

7.1.10. Terminal Deoxynucleotidyl Nick-End Labeling (TUNEL) Assay

A TUNEL experiment was performed using a cell death detection kit to determine apoptosis. TUNEL staining for apoptotic cell nuclei was performed according to the previously stated methodology (238).

7.1.11. Materials

Unless otherwise specified, all chemicals were bought from Sigma-Aldrich.

7.1.12. Statistical evaluation

All values are presented as mean ± standard error of the mean (SEM) for N observations. The images shown are representative of at least three tests conducted over multiple experimental days, with tissue sections collected from each animal in each group. In vivo experiments, N denotes the number of animals utilized. The findings were analyzed using one-way ANOVA with a Bonferroni post-hoc test for multiple comparisons. P-values < 0.05 were considered significant.

7.2. Results

7.2.1 Valuation of serum Hcy, plasma MDA and plasma GSH levels after HHcy

Initially, we examined Hcy levels in the blood to determine whether daily Meth administration effectively generated an HHcy condition. Oral Meth delivery at a dose of 1 gr/kg oral for 30 days resulted in a higher level of Hcy than the control group (Figure 24A). However, treatment with Cashew nuts (100 mg/kg) did not significantly reduce increased blood Hcy levels (Figure 24A), implying that the probable protective effect of cashews could be attributable to the control of oxidative stress caused by an HHcy condition. To validate this, we found higher plasma levels of MDA (a marker of lipid peroxidation) and an important decrease in plasma GSH levels in HHcy rats compared to sham animals (Figure 24C). In contrast, cashew nuts significantly reduced MDA levels and elevated GSH levels in plasma (Figure 24B and 24C, respectively), regulating oxidative stress.

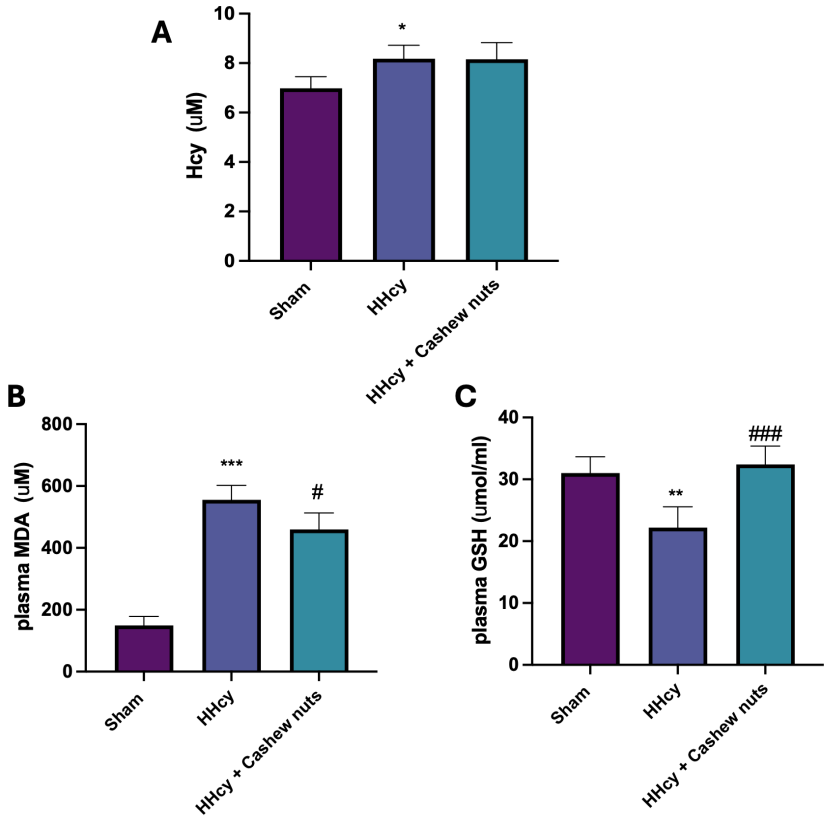


Figure 24. Evaluation of Hcy, MDA and GSH levels. Serum levels of Hcy (**A**). Plasma levels of MDA (**B**) and GSH (**C**). Values are means ± SEM of 5 animals for each group. *p<0.05 vs sham; **p<0.01 vs sham; ***p<0.001 vs sham; #p<0.05 vs HHcy; ### p<0.001 vs HHcy.

7.2.2 Valuation of oxidative stress in brain induced by HHcy

Furthermore, because the brain is one of the organs that are altered by HHcy, we investigated into the role of oxidative stress in the brain. Our findings revealed an increase in MDA levels and an important decrease in GSH levels in brain samples from the HHcy group compared to the control group (Figure 25 A-B). Similarly, we detected a modification of the Nrf2/HO-1 pathway, as evidenced by lower expression of Nrf2 and HO-1 in HHcy mice (Figure 25 C-D). Daily ingestion of cashew nuts lowered MDA levels in the brain while increasing GSH levels, Nrf2 and HO-1

expressions, demonstrating their antioxidant capabilities.

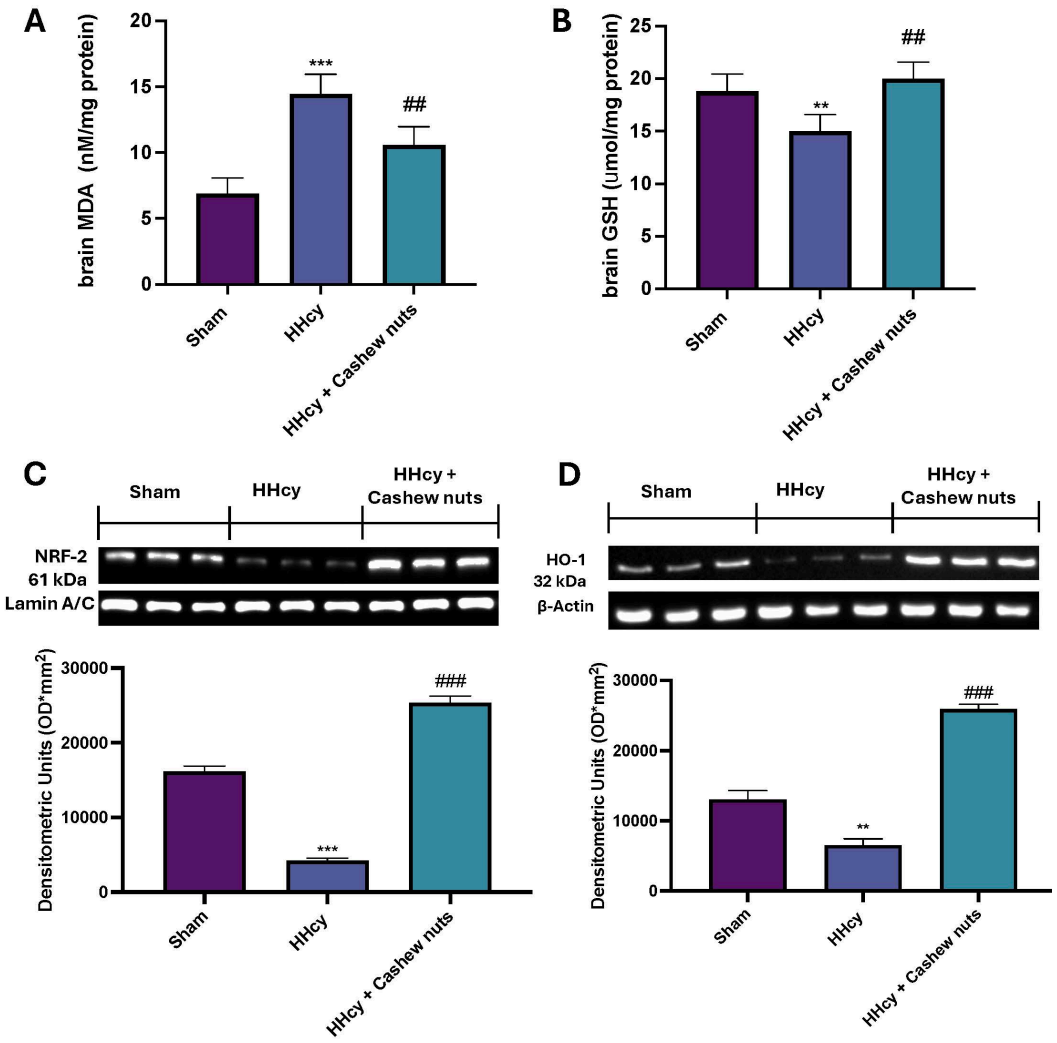


Figure 25. Evaluation of oxidative stress in brain. MDA (**A**) and GSH (**B**) levels in brain tissue. Western blot and relative densitometric analysis: Nrf2 (**C**) and HO-1 (**D**). Exposed is a blot of lysates (5 animals/group) with a densitometric analysis for all animals. Values are means ± SEM of 5 animals for each group. **p<0.01 vs sham; ***p<0.001 vs sham; ##p<0.01 vs HHcy; ### p<0.001 vs HHcy.

7.2.3 Valuation of markers related to neuroinflammation induced by HHcy

To measure neuroinflammation, we used immunohistochemical studies to look at the expression of GFAP, an astrocyte marker, and Iba-1, a microglia activation marker. Our findings revealed a significant increase in GFAP expression in both the cortex and the CA1 area of the hippocampus in the HHcy group (Figure 26B, D, and 26F, H, respectively) as compared to sham animals (Figure 26A, D, and 26E, H). Similarly, we found considerable positive staining for Iba-1 in the cortex and CA1 area of the hippocampus in HHcy rats (Figures 27B, D, and 27F, H, respectively) compared to the control group (Figures 27A, D, and 27E, H). Oral consumption of cashew nuts

significantly reduced both neuroinflammation markers (Figure 27C, D, and 27G, H for GFAP and 27C, D, and 27G, H for Iba-1).

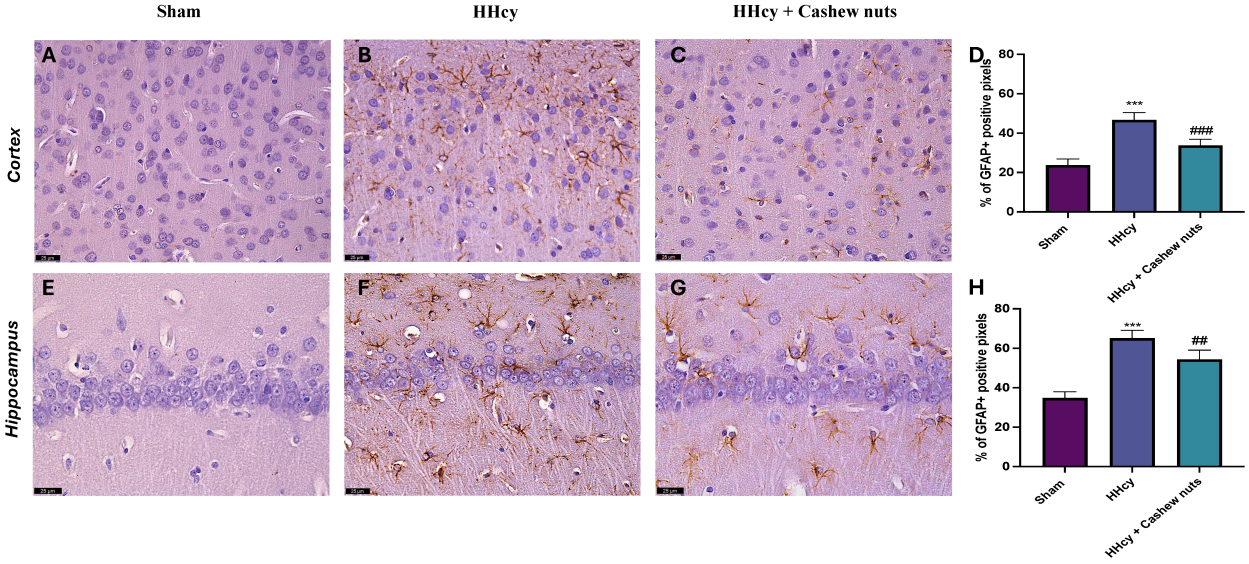


Figure 26. Evaluation of GFAP expression in brain section**.** Immunohistochemical analysis for GFAP in cortex area: Sham (**A**), HHcy (**B**), HHcy + Cashew nuts (**C**), graphical quantification (**D**). Immunohistochemical analysis for GFAP in CA1 hippocampus area: Sham (**E**), HHcy (**F**), HHcy + Cashew nuts (**G**), graphical quantification (**H**). A 40X magnification is shown (25-µm scale bar). Values are means ± SEM of 5 animals for each group. ***p<0.001 vs sham; ##p<0.01 vs HHcy; ### p<0.001 vs HHcy.

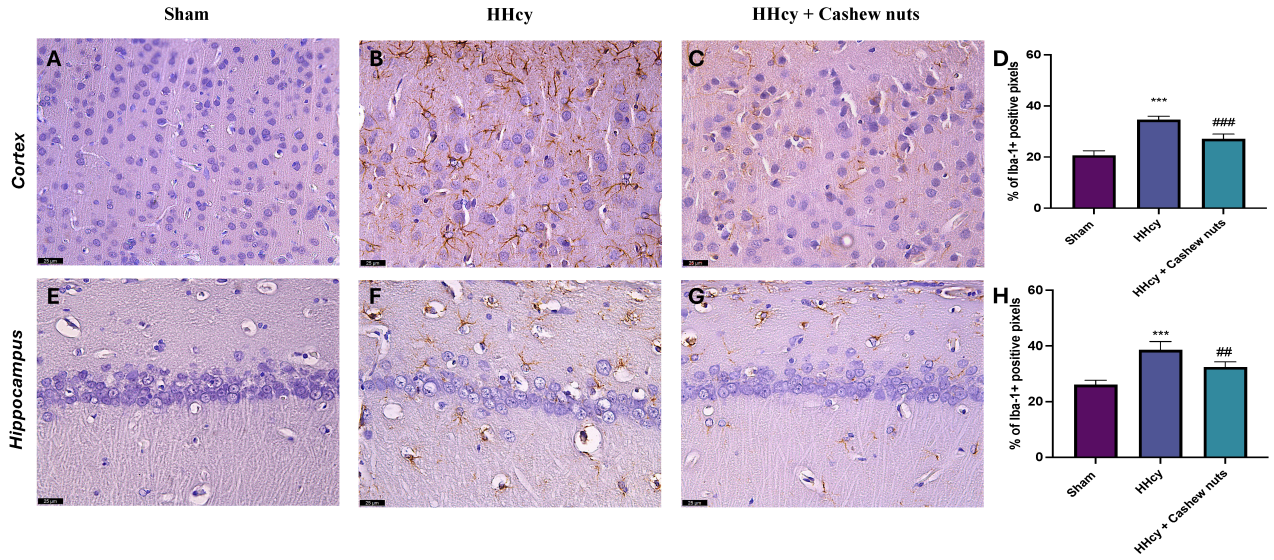


Figure 27. Evaluation of Iba-1 expression in brain section. Immunohistochemical analysis for Iba-1 in cortex area: Sham (**A**), HHcy (**B**), HHcy + Cashew nuts (**C**), graphical quantification (**D**). Immunohistochemical analysis for Iba-1 in CA1 hippocampus area: Sham (**E**), HHcy (**F**), HHcy + Cashew nuts (**G**), graphical quantification (**H**). A 40X magnification is shown (25-µm scale bar). Values are means ± SEM of 5 animals for each group. ***p<0.001 vs sham; ##p<0.01 vs HHcy; ### p<0.001 vs HHcy.

7.2.4 Valuation of MAP2 expression, a neuronal marker, after HHcy

MAP2 is the major cytoskeletal regulator within neuronal dendrites, required to sustain neuroarchitecture.(239). Consistent with the literature, sections from the sham group showed significant positive MAP2 immunostaining in both the cortex and the CA1 area of the hippocampus (Figure 28A, D, and 28E, H, respectively); however, MAP2 expression was significantly reduced after Meth administration in both the cortex and the CA1 area of the hippocampus (Figure 28B, D, and 28F, H). Cashew nuts were shown to partially restore MAP2 expression (Figure 28C, D, and 28G, H, respectively).

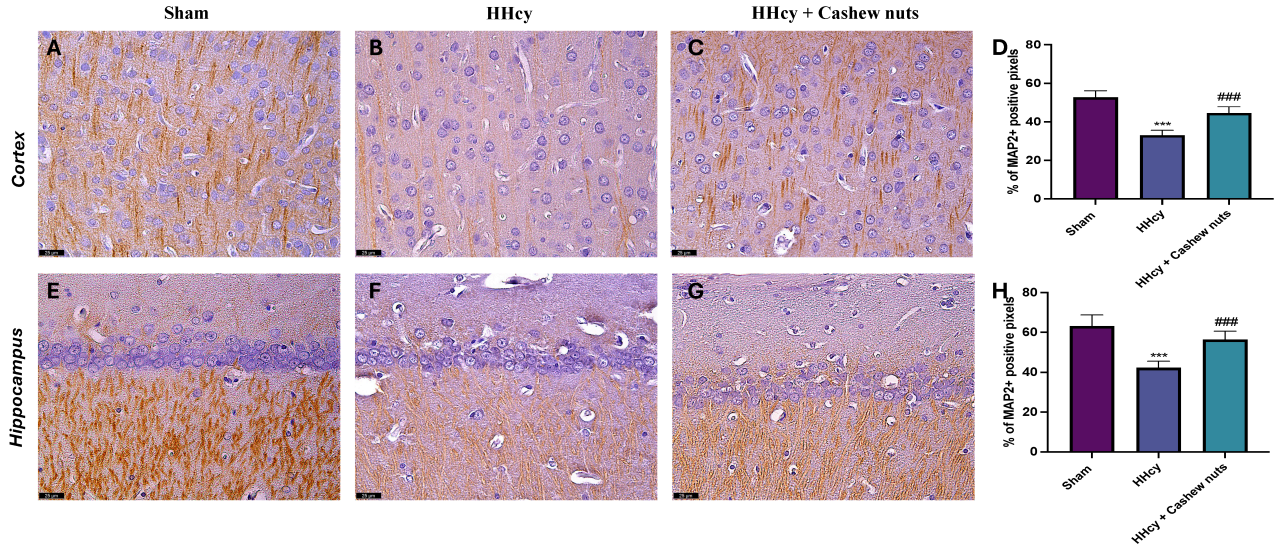


Figure 28. Evaluation of MAP2 expression in brain section. Immunohistochemical analysis for MAP2 in cortex area: Sham (**A**), HHcy (**B**), HHcy + Cashew nuts (**C**), graphical quantification (**D**). Immunohistochemical analysis for MAP2 in CA1 hippocampus area: Sham (**E**), HHcy (**F**), HHcy + Cashew nuts (**G**), graphical quantification (**H**). A 40X magnification is shown (25-µm scale bar). Values are means ± SEM of 5 animals for each group. ***p<0.001 vs sham; ### p<0.001 vs HHcy.

7.2.5 Effect of Cashew nuts on neuronal death induced by HHcy

To determine if the apoptotic pathway is implicated in Meth-induced HHcy damage, we used the TUNEL test on brain sections. In comparison to the sham group (Figure 29A, D, and 29E, H, respectively), our data showed that HHcy rats had a considerably larger number of TUNEL-positive cells in the cortex and CA1 area of the hippocampus. The administration of cashew nuts significantly reduced the number of apoptotic cells (Figures 29C, D, and 29G, H, respectively). We confirmed this by examining the levels of anti-apoptotic Bcl-2 and pro-apoptotic Bax proteins in brain tissues. Bcl-2 levels were much lower in the HHcy group (Figure 29I), but Bax

expression increased after meth consumption (Figure 29J). Following cashew nut treatments, both indicators rebounded to levels similar to those reported in the sham group.

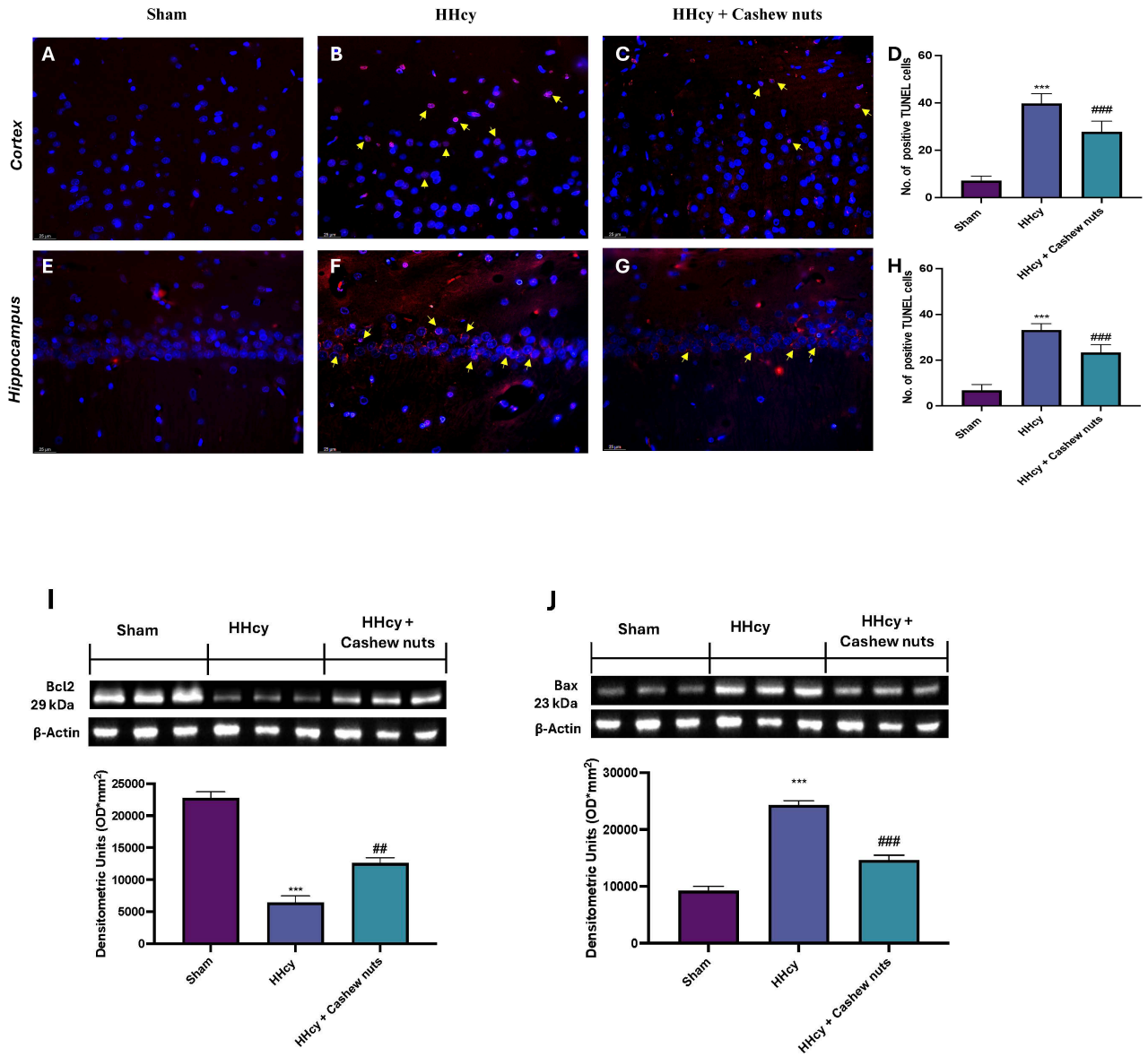


Figure 29. Evaluation of apoptotic process. TUNEL assay in cortex area: Sham (**A**), HHcy (**B**), HHcy + Cashew nuts (**C**), number of positive cells per high-power field (**D**). TUNEL assay in CA1 hippocampus area: Sham (**E**), HHcy (**F**), HHcy + Cashew nuts (**G**), number of positive cells per high-power field (**H**). A 40X magnification is shown (25-µm scale bar). Western blot and relative densitometric analysis: Bcl-2 (**I**) and Bax (**J**). Exposed is a blot of lysates (5 animals/group) with a densitometric analysis for all animals. Values are means ± SEM of 5 animals for each group. ***p<0.001 vs sham; ##p<0.01 vs HHcy; ### p<0.001 vs HHcy.

CHAPTER 8: DISCUSSION

The Mediterranean diet is universally recognized as one of the healthiest eating patterns, characterized by a high consumption of fruit, vegetables, whole grains, legumes, fish, extra virgin olive oil, and nuts (240). The latter, in particular, plays a fundamental role thanks to its richness in unsaturated fatty acids, fiber, vitamins, minerals, and phytochemical compounds with strong antioxidant and anti-inflammatory properties (3). Numerous studies have shown that the regular inclusion of dried fruit in the diet helps improve lipid profiles, reduce oxidative stress and decrease the incidence of cardiovascular and metabolic diseases (241). Among the various types of dried fruit, **almonds** (Prunus dulcis) and **cashews** (Anacardium occidentale) have compositional peculiarities that make them of great interest in both the nutraceutical and pharmaceutical fields (8, 15). Almonds are not only appreciated for their edible part, but also for the by-products of industrial processing, such as the **skin** and the **oil extracted by cold pressing**. The skin, often considered a waste product destined for animal feed, is actually a rich source of polyphenols, flavonoids, and fiber, with potential applications as a functional ingredient. **Almond oil**, extracted from the kernel by cold pressing, preserves intact essential fatty acids, fat-soluble vitamins and bioactive compounds, which justify its traditional use as a skin soothing and regenerating agent. At the same time, **cashews** represent an important nutritional resource, containing good quality proteins, minerals and phenolic compounds. Recent evidence suggests their use in improving homocysteine metabolism and protecting vascular function. In a context where anti-inflammatory drugs can have side effects, the search for natural molecules with antioxidant and anti-inflammatory activity, such as those present in functional foods and nutraceuticals, has become increasingly popular (242). This study explored, in an integrated and multidisciplinary manner, the physiological and therapeutic potential of almonds and cashews, with a particular focus on their antioxidant, anti-inflammatory, and neuroprotective effects. Through phytochemical analyses and experimental studies on in vivo models, we observed how the bioactive compounds present in these foods and their agro-industrial byproducts (**ASE**) are able to modulate molecular pathways central to maintaining redox homeostasis and regulating inflammation, particularly the **Nrf2/HO-1** and **NF-κB** pathways. Regarding **ASE**, several in vitro studies have highlighted its ability to reduce ROS production and protect cells from apoptosis induced by oxidative stress.

Preliminary in vitro results have demonstrated that in human monocytic U937 cells, H_2O_2-induced ROS production was inhibited in cells pretreated with ASE, even at the lowest concentration, and ASE was able to make cells resistant to apoptosis, supporting the hypothesis that it may constitute an important antioxidant defense (4) (243). In our study, a carrageenan-induced paw edema (CAR) model was used, a very sensitive and reproducible model to study the anti-inflammatory activity of novel molecules (244). Induction of an inflammatory state with CAR causes a local acute inflammatory response, characterized by edema, increased ROS and depletion of antioxidant activity (245). ROS cause severe cellular damage, leading to lipid peroxidation with the formation of **MDA** (246). Following CAR-induced inflammation, the **Nrf2** pathway is altered, with a decrease in the expression of cytoprotective genes such as **HO-1**, while the **NF-κB** pathway shows an increased response, leading to the expression of pro-inflammatory genes and the release of cytokines such as **TNF-α** and **IL-1β** (247) (248, 249). Almond (*Prunus dulcis*) is a source of essential nutrients and a widely studied food. Characterization of its macro- and micronutrients has shown that the peel possesses many anti-inflammatory and antioxidant activities. Our in vivo work demonstrated that the oral intake of the ASE, at a dose of 100 mg/kg in an experimental model of CAR-induced paw edema, exhibits a protective action on inflammation and oxidative stress, accentuating the interest in waste products, such as the almond skin. CAR-induced inflammation causes edema and hind paw pain in animals, leading to impaired motility and problems in hind paw use (244, 250). Notably, in our study, we observed that the oral administration of ASE reduced the histological damage and inflammatory cell infiltration measured through the MPO assay, restoring the tissue architecture of the paw as a result. These results were in accordance with those of Mandalari et al., in which ASE was able to reduce neutrophilic infiltration into tissues (251). Furthermore, the induction of paw edema with CAR caused the sensitization of primary sensory neurons. In this work, ASE administration was able to significantly reduce the thermal hyperalgesia and mechanical allodynia caused by the inflammatory process. The inflammatory response is caused by the activation of the NF-κB pathway, which, upon inactivation of its inhibitor IkB-α, translocates to the nuclear level, leading to the transcription of pro-inflammatory genes. Consequently, the inflammatory cascade induces the production of pro-inflammatory cytokines, such as TNF-α and IL-1β, or inflammatory enzymes,

such as iNOS and COX-2 (252-254). Our results showed that the oral administration of ASE reduced the degradation of IkB-α and the translocation of NF-κB into the nucleus. Here, as a consequence, there was a reduction in the production of the pro-inflammatory cytokines, including TNF-α and IL-1β. These results are in agreement with those of a previous study, in which treatment with the almond skin was observed to exhibit beneficial effects in IBD by modulating the NF-κB pathway (251). A previous study also reported that ASE administration reduced the release of pro-inflammatory cytokines (255). Furthermore, in our experimental model, following induction with CAR, there was an inflammatory response that resulted in the formation of immune-positive cells to COX-2 and iNOS. Both the amplification of the inflammatory response and oxidative stress were aided by the expression of COX-2 and iNOS. An in vitro study on activated macrophages also reported that the protein fraction of almonds reduced the production of pro-inflammatory cytokines, such as TNF-α and IL-1β, and also reduced the expression levels of the inflammatory enzyme indicators iNOS and COX-2 (256). Therefore, in our study, we assessed the finding that following the administration of ASE there was a reduction in the level of immune-positive cells to COX-2 and iNOS. In fact, according to the study of Lauro et al., ASE leads to a significant reduction in these inflammatory enzymes. One of the most important and dangerous consequences of CAR injection is ROS induced damage (255, 257). Several studies reported the antioxidant effects of almonds, suggesting that the intake of almonds may have a beneficial role in strengthening antioxidant defenses (258-260). In a study using yeast models, ASE was observed to significantly reduce the presence of ROS (261). This led to a strengthening cambia of the antioxidant defenses under the conditions of oxidative stress. These mechanisms may be due to the presence of the polyphenols present in ASE and their antioxidant activity. Free radicals are difficult to quantify directly in vivo; hence, it is typical to quantify a variety of molecules that can interact with these free radicals, such as lipids. In our study, we evaluated the levels of lipid peroxidation caused by oxidative stress with the MDA assay. MDA is a by-product and is typically used as a marker of cell membrane damage (262). In our study, we found how the oral administration of ASE at a dose of 100 mg/kg was able to significantly reduce lipid peroxidation. In agreement with the studies of Van-Long et al., we assumed that ASE enhanced antioxidant activity due to the activation of the Nrf2 pathway (263). Nrf2 encompasses important activities

against oxidative stress as its translocation to the nuclear level causes the transcription of cytoprotective genes, such as HO-1 (264, 265). It has been observed that the almond skin can induce the activation of the phase II detoxifying/antioxidant enzymes mediated by Nrf2 and can therefore have antioxidant and hepatoprotective effects (263). Experimental models on diabetes-related erectile dysfunction revealed that the intake of almonds increased Nrf2 activity due to their polyphenolic profiles (266). Following CAR administration, in our work, we observed that upon inflammation there was a reduction in the Nrf2 pathway. Therefore, our study highlighted the fact that the oral administration of ASE can lead to increased Nfr2 and HO-1. In agreement with other studies, almonds and their constituents demonstrated a considerable reducing power, enhancing the activity of antioxidant enzymes, including SOD, GSH, GPx, and CAT (6). In our study, following CAR induction, we detected reduced SOD, GSH, GPx, and CAT levels, while ASE treatment increased endogenous antioxidant activity. According to previous studies, these observations are also due to the activation of Nrf2 by ASE, which is involved in the antioxidant system (267). In parallel, given the remarkable anti-inflammatory and antioxidant properties of different almond components, we aimed to evaluate the activity of **almond oil applied topically** to full-thickness excisional wounds in a mouse model. The skin is essential for maintaining physiological homeostasis, and the wound healing process is divided into four phases: hemostasis, inflammation, proliferation, and remodeling (268). Furthermore, these phases include contraction, granulation, epithelialization, and collagenation (269). WH involves a complex and tightly regulated series of molecular events streamlined by a variety of cell types recruited to the site of injury, including keratinocytes, fibroblasts, neutrophils, and macrophages (270-272). Furthermore, contraction, granulation, epithelialization, and collagenation occur during these phases (270). Compromised WH is a major concern for hospitalization, particularly in conditions where wounds do not heal properly and remain at the site of injury. For this reason, in the present study we evaluated the effects of topical application of almond oil, obtained by cold-pressing the seed, on wounds. To assess the impairment of WH, the delay in granulation tissue formation, and the reduction in collagen content, we performed histological analysis and Masson's trichrome staining. Our results showed that almond oil was able to accelerate the healing process in mice as early as 7 days after injury, compared to animals in the untreated WH group. Furthermore, collagen

synthesis was observed as early as the seventh day. Furthermore, histological analysis confirmed a reduction in tissue damage by almond oil and an increase in re-epithelialization and granulation tissue formation. These results were supported by the modulation of molecular markers: MMP-9, TGF-β, and VEGF. MMPs are a family of zinc-dependent endopeptidases that degrade approximately all proteins of the extracellular matrix and basement membrane at neutral pH. MMPs play an important role in numerous pathological and biological processes, with their involvement in WH first revealed in guinea pigs (82). Their most important functions are degradation, by elimination of damaged extracellular matrix during the inflammatory phase, disruption of the capillary basement membrane for angiogenesis and cell migration during the proliferation phase, and tissue contraction and remodeling during the remodeling phase. Our results clarified that increased MMP-9 expression may be a factor leading to WH impairment, while topical treatment with almond oil prevented MMP-9 overexpression while promoting WH in mice 7 days after injury. As previously mentioned, TGF-β is another important molecular marker, involved in all phases of WH: inflammation, angiogenesis, fibroblast proliferation, collagen synthesis and deposition, as well as the restoration of new extracellular matrix (82). After tissue damage, rupture of blood vessels and subsequent exposure of platelets to subendothelial collagen leads to platelet aggregation, degranulation, and activation of the coagulation cascade. Our results, using Western blot analysis, demonstrate that, after injury, TGF-β levels are low in mice at 7 days after wound induction, while almond oil treatment led in both cases to a significant increase in TGF-β expression, highlighting its important role in the WH process in mice. Regarding VEGF, it plays a role in angiogenic activity during the proliferative phase of WH, and TGF is known to recruit VEGF-generating hematopoietic effector cells to stimulate angiogenesis (273, 274). Vascular endothelial growth factor plays a central role in inducing angiogenesis based on its ability to stimulate the expression of proteases that digest extracellular matrix components that block angiogenesis, stimulate endothelial cell proliferation, and prevent their apoptosis (275). Our study showed that seven days after injury, almond oil stimulated endothelial cell proliferation and the secretion of angiogenic cytokines and growth factors, such as VEGF, thereby inducing the formation of new blood vessels in the injured area. These data suggest that almond oil not only accelerates wound closure but also promotes angiogenesis and matrix

remodeling, essential components of the regenerative phase. To assess oxidative and nitrosative stress, we measured the expression of nitrotyrosine and PARP. ROS are centrally involved in all wound healing processes. Excessive ROS production or impaired ROS detoxification causes oxidative damage. Nitrotyrosine is a biomarker of oxidative stress due to the nitration of protein-bound and free tyrosine residues by reactive peroxynitrite molecules (276). Immunohistochemical analysis of nitrotyrosine expression showed an increase in its level in the WH group compared to the sham group, while topical treatment with almond oil was able to restore normal levels of this biomarker. Another immunohistochemical analysis was performed to assess Parp activity; it is involved in DNA damage recovery (277). In the WH group, there was an overexpression of this enzyme, while the almond oil group demonstrated a normal level of expression. Furthermore, to explore anti-inflammatory properties, we assessed the expression of pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-6. The inflammatory process, associated with wound healing, leads to increased levels of cytokines such as IL-6, IL-1β, and TNF-α (273, 278, 279). Specifically, our analysis showed higher levels of expression of these cytokines in the WH group, while almond oil treatment demonstrated anti-inflammatory activity, reducing their levels in the treated group compared to the WH group. The nuclear factor NF-κB is an important factor in the regulation of a wide range of genes and pathways in the state of inflammation (273, 280); for this reason, we performed Western blot analysis to evaluate the activity of almond oil and its anti-inflammatory activity on the NF-κB pathway. Specifically, we demonstrated that almond oil reduced the cytosolic degradation of IκB-α and the nuclear expression of NF-κB, regulating inflammation associated with wound healing. Finally, to investigate and confirm the antioxidant activity of almond oil, we analyzed the Nrf2/HO-1 and SOD-2. Indeed, almond oil enhanced the nuclear translocation of Nrf2 and upregulated the expression of HO-1 and SOD-2, suggesting that it exerts antioxidant activity by activating endogenous protective pathways. Overall, our results demonstrate that topical application of almond oil accelerates wound healing through a multifaceted mechanism involving anti-inflammatory action via inhibition of NF-κB, reduction of oxidative and nitrosative stress via activation of Nrf2/HO-1/SOD-2, promotion of collagen deposition and tissue regeneration via regulation of MMP-9 and TGF-β, and stimulation of angiogenesis via increased VEGF expression. Nuts have been

extensively studied for their beneficial effects on metabolism, with documented improvements in lipid profile, inflammation and insulin resistance (281). These properties are particularly important in relation to metabolic disorders, such as HHcy, which can also have negative consequences on brain function (282, 283). In this context, interest in cashews has grown in light of evidence suggesting a potential neuroprotective role, attributable to their content of bioactive compounds with antioxidant and metabolic process-modulating activity. In this context, interest in cashews has grown in light of evidence suggesting a potential neuroprotective role, attributable to their content of bioactive compounds with antioxidant and metabolic process-modulating activity (284). For this reason, we examined their potential in light of evidence suggesting a neuroprotective role, attributable to their content of bioactive compounds with antioxidant and metabolic process-modulating activity, particularly in a condition of HHcy. HHcy occurs when blood levels of Hcy are above normal limits due to an alteration in its metabolism (285). The main causes are nutritional deficiencies of vitamins B6, B12, choline and folate, or genetic defects in enzymes that metabolize Hcy (286). Hcy has a pro-oxidant activity, so HHcy-induced oxidative stress may result from reduced activity of antioxidant enzymes and increased **ROS formation** (287). HHcy has been documented to stimulate the generation of hydroxyl radicals, which initiate lipid peroxidation with the formation of **MDA** (288). Elevated Hcy levels may also suppress the production of antioxidant enzymes such as **GPx** (289, 290). Recent evidence suggests that mild HHcy impairs endothelial cell function, producing ROS and inflammation, which increases the risk of stroke and cerebrovascular problems (291-294). The oxidative effect can be counteracted by consuming polyphenol-rich products, such as cashews. Cashew (*Anacardium Occidentale L.*) is one of the most well-known nuts, used in traditional medicine as a nutritious food with a high content of minerals, anthocyanins, carotenoids, flavonoids and other polyphenols (230). In our work, we demonstrated that daily ingestion of cashew nuts (100 mg/kg) reduced neuroinflammation and oxidative stress in rats with methionine-induced HHcy. Our findings are consistent with previous research showing that Meth supplementation decreased the activity of antioxidant enzymes and increased plasma Hcy levels (18, 295-297). Conversely, oral administration of cashew nuts reduced lipid peroxidation and up-regulated blood GSH levels, confirming its antioxidant properties. This HHcy model is widely used to study the neurotoxic

consequences of elevated Hcy and replicates, in part, the conditions of moderate hyperhomocysteinemia found in the adult population (298). Meth-rich diets have been shown to alter brain activity and trigger neurotoxic effects leading to increased neuroinflammation and cognitive deficits (299, 300). Furthermore, clinical research shows that HHcy causes apoptosis, synaptic remodeling, memory loss, dementia, and cerebrovascular microhemorrhages, and is linked to Alzheimer's disease (301, 302) (303, 304). Although the precise toxic process is not yet fully understood, studies have shown that high levels of Hcy lead to alterations in brain tissue in the cortex and hippocampus (305-307). The hippocampus, and in particular the CA1 region, is extremely vulnerable to insults, making it one of the most studied brain regions for neurodegenerative processes (301, 308, 309). Our work is in line with previous research, as observed by an increased activation of astrocytes and microglia by immunohistochemistry technique with a significant increase in positive staining for GFAP and Iba-1 in the Meth-treated group, while daily treatment with cashews was able to modulate astrocyte and micro-glial activation. At the same time, MAP2 expression was significantly reduced after Meth intake for 4 weeks. MAP-2 is a static, structural protein, that is essential for maintaining neuroarchitecture, along with other cytoskeletal proteins. Due to its exceptional sensitivity to a wide range of stimuli, MAP-2 has been shown to play dynamic roles in the development, differentiation, and plasticity of neurons. These functions include important involvement in the responses of neurons to growth factors, neuro-transmitters, synaptic activity, and neurotoxins (239, 310, 311). Our findings are consistent with previous research, as evidenced by immunohistochemical analysis and the restoration of MAP2 expression following cashew nut treatment. This neuroprotective effect of cashews probably resulted from a significant modulation of oxidative stress, not only at a systemic level but also at a brain level, as demonstrated by a reduction in MDA levels and significant upregulation of Nrf2, HO-1 and GSH in brain collected from cashew-treated rats. Additionally, experiments with on Meth intake on mice or rats demonstrated an increased number of TUNEL+ cells with the increase in apoptosis rates, DNA damage and caspase activity (301, 312). Consistently, our results detected increased neuronal cell death stained with TUNEL assay specifically in the CA 1 region of the hippocampus and cortex, in the Meth group compared to control animals. Moreover, HHcy also induced several alterations of apoptotic markers, such as Bax and Bcl-2. Here we demonstrated

that treatment with cashews is able to reduce the apoptotic process, suggesting a protective action due to cashew intake. The main scientific contribution of this work lies in the experimental demonstration of the efficacy of almond peel extract in reducing acute inflammation and oxidative stress, thus providing a rationale for its use in nutraceutical or phytotherapeutic formulations. This is complemented by preclinical evidence of the neuroprotective action of cashews in the context of HHcy, with potential implications for the prevention of neurodegenerative diseases. A distinctive element is also the integration of the concept of circular economy into the nutraceutical field, valorizing by-products of the agri-food supply chain as functional resources. From a biological perspective, the findings reinforce the concept that activation of the **Nrf2** pathway and inhibition of **NF-κB** represent strategic targets in the management of conditions characterized by oxidative stress and chronic inflammation. Clinically, the possibility of using almond and cashew derivatives as nutritional or topical interventions opens up interesting prospects in chronic skin diseases and slow healing processes, metabolically based systemic inflammatory conditions, and in the prevention of cognitive decline and HHcy-related neurodegenerative diseases. A distinctive element of this thesis is the focus on recovering and valorizing agro-industrial byproducts. Almond peel, usually discarded, represents a concentrated source of phenolic compounds, while cashews can be appropriately processed to obtain extracts with high biological value. This perspective fits within a paradigm of **sustainable innovation**, in which human health and the circular economy converge, reducing environmental impact and creating new market opportunities. Standardizing extracts, in terms of composition and dosage, represents a challenge to ensure reproducibility and comparability of results. Finally, the bioavailability of bioactive compounds and potential interactions with drugs or nutrients require further investigation. Based on the results obtained, several lines of research are emerging: clinical studies on patients with HHcy and neuroinflammatory conditions to assess the effects of cashew nut and derivative supplementation; the development of nutraceutical or topical formulations based on standardized almond peel extract; the analysis of the synergy between bioactive compounds of different origins and their impact on complex molecular pathways; and the implementation of industrial processes for the sustainable extraction and purification of byproducts.

Limitations and Future Perspectives

Although this study provides solid experimental evidence of the antioxidant and anti-inflammatory effects of bioactive compounds derived from dried fruit, certain limitations must be acknowledged. The experimental research was conducted exclusively on validated preclinical animal models, which are essential tools for mechanistic and research studies but cannot fully reproduce the complexity of human physiology. Consequently, although the results strongly support the biological relevance of compounds derived from dried fruit, further clinical and translational studies will be necessary to confirm their efficacy, safety, and dose-response relationships in humans. Another limitation of the present work concerns the characterization of the metabolic fate of polyphenols derived from nuts after ingestion. Although phytochemical analyses provided quantitative information on the bioactive composition of almond skin extracts and cashew supplementation, the biotransformation of these compounds has not been directly studied. It is known that polyphenols undergo extensive metabolism, mediated in particular by the gut microbiota, leading to the formation of circulating and excreted metabolites that may represent the actual bioactive forms responsible for systemic effects. Future studies integrating mass spectrometry-based approaches would therefore be useful to clarify the bioavailability, metabolic pathways, and tissue distribution of these compounds. Finally, expanding the current experimental framework to include additional disease models and combined nutraceutical strategies could further strengthen the translational relevance of the findings. The study of potential synergistic effects between bioactive compounds and conventional therapeutic approaches could contribute to a more comprehensive understanding of their role in the prevention and management of oxidative stress and inflammation-related disorders.

CONCLUSIONS

Inflammation is known to be related to oxidative processes, primarily because these processes share several common pathways, such as the cross-talk between NF-κB and Nrf2/HO-1. Since oxidative stress is common to several degenerative diseases, it has been hypothesized that dietary antioxidants may explain a highly significant protective effect. Almonds and cashews are a major source of antioxidants in diets worldwide. In this thesis, all three experiments evaluated and demonstrated in vivo that ASE, a polyphenol-rich byproduct of almonds, almond oil, and cashews, has great potential as a nutraceutical due to its ability to reduce the negative effects of oxidation and inflammation. Specifically, ASE was able to reduce inflammation by modulating the NF-κB pathway and increased antioxidant activity by upregulating the Nrf2/HO-1 pathway. Almond oil, on the other hand, can modulate and accelerate skin WH in mice by inducing the expression of numerous factors involved in the different phases of the healing process. Specifically, topical administration of almond oil improved tissue damage, reduced cellular infiltration, and improved tissue quality. Furthermore, it modulated the activity of growth factors, such as TGF-β and VEGF, which are essential for wound healing. In this study, almond oil also demonstrated significant anti-inflammatory and antioxidant activity, reducing the activation of the NF-κB signaling pathway and the expression of nitrotyrosine and PARP-1, and increasing the expression of Nrf2/HO-1. This study highlights the potential of cashews in modulating HHcy and consequently neuroinflammation, a finding that opens up exciting prospects for future research. Our research demonstrates that elevated Hcy levels cause ROS generation, morphological changes in the cortex and hippocampus, and neuronal death, all of which contribute to cellular dysfunction and tissue damage. Current results indicate that cashews significantly reduced neuroinflammation and apoptosis in hyperhomocysteinemic rats. The neuroprotective effects of cashews are likely due to their antioxidant properties, which reduce lipid peroxidation and increase the activity of antioxidant enzymes such as GSH in plasma. The results of this study confirm the significant health-promoting potential of almonds and cashews, both as functional foods and as sources of high-value biological derivatives. Almond skin extract demonstrated effective anti-inflammatory and antioxidant activity, supporting its potential use in nutraceutical and phytotherapeutic formulations, while almond oil showed regenerative and protective properties in the healing of skin wounds.

Similarly, cashew supplementation proved capable of reducing oxidative stress, modulating neurovascular inflammation, and counteracting apoptotic processes associated with hyperhomocysteinemia, with possible implications for the prevention of neurodegenerative diseases. This thesis highlights the valorisation of agro-industrial by-products within the circular economy framework, contributing to sustainable development goals while reducing environmental impact and promoting innovative market opportunities. Overall, Nrf2 activation and NF-κB inhibition emerge as central mechanisms in maintaining redox homeostasis and protecting tissues, highlighting the translational potential of these findings. Based on these results, future clinical studies and standardisation of formulations are essential to fully exploit the therapeutic benefits of almond and cashew-derived products.

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