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Nutraceutical Intervention with Ginger (*Zingiber officinale*) Modulates Age-Related Liver Changes in Mice

Interwencja nutraceutyczna z imbirem (*Zingiber officinale*) moduluje zmiany w wątrobie związane z wiekiem u myszy

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"Given my background and where I come from - crossing oceans as an immigrant, a person of a different color, a woman, ascending to build her career at this Institute was a challenging journey. I would love to see the most unusual, improbable people ascending to the top at the right time!"

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List of Abbreviations

DPPH- 2,2-diphenyl-1-picrylhydrazyl

MDA- Malondialdehyde

SOD- Superoxide dismutase

TAC- Total antioxidant capacity

TNF-α- Tumor necrosis factor-alpha

PI3K- Phosphatidylinositol 3-kinase

ROS- Reactive oxygen species

Nrf2- Nuclear factor erythroid 2-related factor 2

IL-6- Interleukin 6

NF-κB- Nuclear factor kappa-light-chain-enhancer of activated B cells

NLRP3- Nucleotide-binding oligomerization domain, leucine-rich repeat, and pyrin domain-containing protein 3

AMPK- AMP-activated protein kinase

SIRT1- Sirtuin 1, an NAD+-dependent deacetylase

BGRD- β-glucuronidase

ACP- Acid phosphatase

BGAL- β-galactosidase

AGLU- α-glucosidase

AGAL- α-galactosidase

HEX- β-N-acetylhexosaminidase

MAN- α-Mannosidase

ArgAP- Arginine aminopeptidase

AlaAP- Alanyl aminopeptidase

LeuAP- Leucyl aminopeptidase

IGF-signaling- Insulin-like growth factor signaling

TBARS- Thiobarbituric acid reactive substances

DTNB- 5,5′-dithiobis(2-nitrobenzoic acid)

CRP- C-reactive protein

LC3- Microtubule-associated protein 1 light chain 3

ATG- Autophagy-related gene/protein

pNP- p-nitrophenyl

List of publications constituting the doctoral dissertation

1. Maima Matin, Tanuj Joshi, Dongdong Wang, Nikolay T. Tzvetkov, Farhan Bin Matin, Agnieszka Wierzbicka, Artur Jóźwik, Jarosław Olav Horbańczuk, Atanas G. Atanasov. Effects of Ginger (*Zingiber officinale*) on the Hallmarks of Aging. Biomolecules, Volume 14, Issue 8, 2024

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2. Maima Matin, Kamil Wysocki, Jarosław Olav Horbańczuk, Luciana Rossi, Atanas G. Atanasov. Ginger (*Zingiber officinale*) dietary supplementation in mice regulates liver antioxidant defense systems in a dose- and age-dependent. Frontiers in Pharmacology, 2025

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Points of Ministry of Education and Science: 100

3. Maima Matin, Małgorzata Łysek-Gładysinska, Natalia Ksepka, Sara Frazzini, Anna Wieczorek, Artur Jozwik. Ginger Supplementation Supports Liver Health During Aging Through Regulation of Lysosomal Function and Molecular Markers. Animal Science Papers and Reports

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Summary

Aging is a complex biological process characterized by a gradual decline in physiological and metabolic functions, leading to increased susceptibility to degenerative and chronic diseases. Among the organs, the liver is vulnerable to age-related deterioration, which plays a central role in detoxification, metabolism, and homeostasis. As the global population ages, the exploration of natural compounds capable of mitigating the deleterious effects of aging has gained considerable attention. This PhD thesis is based on three monothematic publications that investigate the therapeutic potential of *Zingiber officinale*, commonly known as ginger, in counteracting key molecular, biochemical, structural, and physiological hallmarks of aging, with particular emphasis on liver health, using experimental mouse models.

The first publication (Publication 1) presents a comprehensive review of the evidence linking ginger and its bioactive constituents, including gingerols, shogaols, paradols, and zingerone, to the twelve established hallmarks of aging. This review integrates findings from *in vitro, in vivo*, and clinical studies, highlighting the anti-oxidative, anti-inflammatory, and pro-autophagic activities of ginger that are relevant to aging biology. Based on the information obtained in this publication, it was decided to conduct comprehensive studies using an animal model to further explore and validate the observed effects.

In the second publication (Publication 2), the main goal was to experimentally evaluate how dietary ginger supplementation affects the hepatic antioxidant system in a mouse model across three age groups (3, 6, and 12 months of age). Animals were fed a standard diet enriched with 0.6% or 1.8% dried ginger powder for three months and liver tissues were analyzed for antioxidant capacity, enzymatic activity, and oxidative stress markers. The findings from this study revealed age-dependent declines in key hepatic antioxidant parameters, including total antioxidant capacity (TAC), vitamin C levels, total phenolic contents, DPPH radical scavenging activity, and glutathione concentrations, in untreated control mice, confirming the progressive impairment of hepatic redox homeostasis with aging. Ginger supplementation modified these parameters in a dose-and age-dependent manner. While young mice exhibited reduced DPPH radical scavenging activity in response to ginger, both middle-aged and older mice showed significant improvements. Furthermore, the higher dose (1.8%) consistently enhanced glutathione concentration across all age groups, with the strongest effects observed in aged animals. Ginger reduced malondialdehyde (MDA), a key biomarker of lipid

peroxidation, particularly in young mice, while exerting modest non-significant reductions in older groups. Superoxide dismutase (SOD) activity remained largely unchanged by ginger in all cohorts, indicating that ginger's antioxidant effects may rely more heavily on non-enzymatic pathways and glutathione metabolism than on SOD modulation. Overall, this study established that ginger supports hepatic antioxidant defenses in both dose- and age-dependent manner, with pronounced protective effects in aging liver tissues.

The third publication (Publication 3) extended the investigation into deeper cellular and molecular aspects of liver aging by examining how ginger supplementation influences lysosomal function, inflammatory markers, phosphatidylinositol 3-kinase (PI3K) signaling, and the cell ultrastructural morphology. Using the same animal model and supplementation strategy, the study assessed lysosomal enzyme activity, gene expression of pro-inflammatory markers, and histological features of liver tissue. Aging was associated with increased activity of lysosomal enzymes such as β-galactosidase, acid phosphatase, and aminopeptidases, possibly reflecting modified autophagic efficiency and an accumulation of damaged proteins and organelles. Ultrastructural analyses revealed mitochondrial damage, hepatocellular hypertrophy, and lipofuscin accumulation, hallmarks of aging hepatocytes. Ginger supplementation improved several of these alterations. Particularly, it reduced β-galactosidase activity and preserved hepatocyte ultrastructure in young and middle-aged mice, suggesting improved autophagic regulation. Gene expression analysis demonstrated that ginger suppressed age-related upregulation of PI3K and modulated tumor necrosis factor-alpha (TNF-α) levels, implicating anti-inflammatory and metabolic regulatory pathways. High-dose supplementation in older mice showed mild subcellular stress, indicating the importance of dose optimization.

In conclusion, this PhD research provides foundational evidence for the hepatoprotective and anti-aging potential of *Zingiber officinale*. It positions ginger as a candidate for future translational studies in geriatric health, particularly for interventions targeting age-related liver dysfunction. The thesis highlights the significance of dietary phytochemicals in modulating biological aging and sets the stage for future investigations that may ultimately translate into clinical recommendations for aging populations.

Streszczenie

Starzenie się jest złożonym procesem biologicznym, charakteryzującym się stopniowym spadkiem funkcji fizjologicznych i metabolicznych, prowadzącym do zwiększonej podatności na choroby przewlekłe i zwyrodnieniowe. Wśród narządów najbardziej narażonych na związane z wiekiem uszkodzenia znajduje się wątroba, pełniąca kluczową rolę w detoksykacji, metabolizmie oraz utrzymaniu homeostazy organizmu. Wraz ze starzeniem się populacji światowej rośnie zainteresowanie naturalnymi związkami zdolnymi do łagodzenia niekorzystnych skutków starzenia. Ta rozprawa doktorska opiera się na trzech monotematycznych publikacjach, które badają potencjał terapeutyczny *Zingiber officinale*, powszechnie znanego jako imbir, w przeciwdziałaniu kluczowym molekularnym, biochemicznym, strukturalnym i fizjologicznym cechom starzenia się, ze szczególnym uwzględnieniem zdrowia wątroby, z wykorzystaniem eksperymentalnych modeli mysich.

W pierwszej publikacji przedstawiono kompleksowy przegląd badań łączących imbir i jego bioaktywne składniki, w tym gingerole, shogaole, paradole i zingiberon, z dwunastoma uznanymi cechami starzenia. Przegląd ten integruje wyniki badań in vitro, in vivo oraz badań klinicznych, podkreślając właściwości przeciwutleniające, przeciwzapalne i proautofagiczne imbiru, istotne z perspektywy biologii starzenia. Na podstawie informacji uzyskanych z różnych publikacji podjęto decyzję o przeprowadzeniu kompleksowych badań z wykorzystaniem modelu zwierzęcego w celu dalszego zbadania i potwierdzenia zaobserwowanych efektów.

W drugiej publikacji głównym celem było doświadczalne zbadanie, w jaki sposób suplementacja diety imbirem wpływa na wątrobowy układ antyoksydacyjny w modelu myszy w trzech grupach wiekowych (3, 6 i 12 miesięcy). Zwierzęta były karmione paszą standardową wzbogaconą o 0,6% lub 1,8% sproszkowanego imbiru przez okres trzech miesięcy, a następnie analizowano tkankę pobraną z wątroby pod kątem pojemności antyoksydacyjnej, aktywności enzymatycznej oraz markerów stresu oksydacyjnego. Wyniki wykazały zależne od wieku obniżenie kluczowych parametrów antyoksydacyjnych w wątrobie — całkowitej pojemności antyoksydacyjnej (TAC), stężenia witaminy C, zawartości związków fenolowych, aktywności zmiatania rodników DPPH oraz poziomu glutationu u niesuplementowanych zwierząt, co potwierdza postępujące zaburzenia równowagi redoks w procesie starzenia. Suplementacja imbirem modulowała te parametry w sposób zależny

zarówno od dawki, jak i wieku. Podczas gdy młode myszy wykazywały obniżoną aktywność DPPH w odpowiedzi na imbir, u myszy średniowiekowych i starszych odnotowano znaczące poprawy. Wyższa dawka (1,8%) konsekwentnie zwiększała stężenie glutationu we wszystkich grupach wiekowych, z najsilniejszym efektem u zwierząt starszych. Imbir redukował poziom dialdehydu malonowego (MDA), kluczowego markera peroksydacji lipidów, szczególnie u młodych myszy, podczas gdy u starszych obserwowano niewielkie, statystycznie nieistotne spadki. Aktywność dysmutazy ponadtlenkowej (SOD) pozostawała zasadniczo niezmieniona we wszystkich grupach, co sugeruje, że antyoksydacyjne działanie imbiru opiera się głównie na szlakach nieenzymatycznych oraz metabolizmie glutationu. Łącznie badanie to wykazało, że imbir wzmacnia mechanizmy antyoksydacyjne wątroby w sposób zależny od dawki i wieku, przy czym działanie ochronne było najbardziej wyraźne w wątrobie starzejącej się.

Trzecia publikacja rozszerzyła zakres badań o głębsze komórkowe i molekularne aspekty starzenia się wątroby, analizując, w jaki sposób suplementacja imbirem wpływa na funkcjonowanie lizosomów, markery zapalne, szlak sygnałowy fosfatydyloinozytolo-3-kinazy (PI3K) oraz ultrastrukturalną morfologię. Wykorzystując ten sam model zwierzęcy i schemat suplementacji, zbadano aktywność enzymów lizosomalnych, ekspresję genów prozapalnych oraz cechy histologiczne tkanek. Starzenie było związane ze zwiększoną aktywnością enzymów lizosomalnych, takich jak β-galaktozydaza, fosfataza kwaśna oraz aminopeptydazy, co może odzwierciedlać zmienioną efektywność autofagii oraz akumulację uszkodzonych białek i organelli. Analiza ultrastrukturalna ujawniła uszkodzenia mitochondriów, przerost hepatocytów oraz akumulację lipofuscyny — typowe markery starzenia komórkowego. Suplementacja imbirem łagodziła część tych zmian, szczególnie poprzez redukcję aktywności β-galaktozydazy i poprawę ultrastruktury hepatocytów u młodych myszy i tych w średnim wieku, co wskazuje na usprawnienie regulacji autofagii. Analiza ekspresji genów wykazała, że imbir hamował związane z wiekiem zwiększenie ekspresji PI3K oraz modulował stężenie czynnika martwicy nowotworów alfa (TNF-α), sugerując działanie przeciwzapalne i regulację procesów metabolicznych. Wysoka dawka imbiru u starszych myszy wywoływała łagodny stres subkomórkowy, co podkreśla znaczenie optymalizacji dawki.

Podsumowując, niniejsza rozprawa doktorska dostarcza podstawowych dowodów na hepatoprotekcyjne i przeciwstarzeniowe właściwości *Zingiber officinale*. Imbir jawi się jako obiecujący kandydat do przyszłych badań translacyjnych w geriatrii, szczególnie w kontekście interwencji ukierunkowanych na zaburzenia funkcji wątroby związane z wiekiem. Praca ta podkreśla znaczenie fitochemikaliów dietetycznych w modulowaniu procesów starzenia biologicznego i stanowi podstawę do dalszych badań, które w przyszłości mogą przełożyć się na praktyczne rekomendacje kliniczne dla starzejącej się populacji.

1 Introduction

1.1 Overview of the Aging Process and Hallmarks of Aging

Aging is a complex biological process characterized by the gradual decline of physiological function and homeostatic maintenance, leading to increased vulnerability to diseases and ultimately death. At the cellular and molecular levels, aging is defined by a set of well-established *hallmarks of aging*. Initially, nine core hallmarks were proposed in 2013(López-Otín et al., 2013a), and this framework was recently expanded to twelve hallmarks encompassing a broad range of degenerative processes. These hallmarks include genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, impaired autophagy (macroautophagy) and lysosomal function, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, altered intercellular communication, chronic inflammation, and gut microbiome dysbiosis(López-Otín et al., 2023). Each hallmark represents a biological process that contributes to age-associated functional decline, and interactions among hallmarks may drive cascading effects that accelerate the overall deterioration of organismal function (López-Otín et al., 2013b). For instance, genomic instability (DNA damage accumulation) can drive cellular senescence (Gorgoulis et al., 2019), while chronic inflammation can further damage tissues and promote stem cell exhaustion (Behrens et al., 2014), both of which accelerate aging processes. Targeting these interrelated mechanisms is viewed as a promising strategy to counteract aging and extend health span.

1.2 Antioxidant Defense, Inflammation, Autophagy, and Lysosomal Function in Liver Aging

The liver is a central metabolic organ with remarkable regenerative capacity, yet it undergoes aging-related changes. These changes have been linked to deficits in key protective and homeostatic mechanisms, among which oxidative stress, chronic inflammation, and impaired cellular clearance systems play central roles. An important framework that connects many of these processes is the Free Radical Theory of Aging, which suggests that aging results largely from the cumulative damage by free radicals, particularly reactive oxygen species (ROS), generated during normal cellular metabolism (Harman, 1992). As the liver is a highly metabolic organ responsible for detoxification, xenobiotic metabolism, and lipid and carbohydrate homeostasis, it is especially vulnerable to continuous oxidative challenge. Hepatocytes contain abundant mitochondria, which are the primary endogenous source of

ROS, and are also exposed to exogenous oxidants derived from drugs, environmental toxins, and dietary components. According to free radical theory, the lifelong imbalance between ROS production and antioxidant defense gradually leads to molecular damage, cellular dysfunction, and ultimately tissue aging (Mooli et al., 2022).

In cell, one important protective system affected during aging is the antioxidant defense system. Under physiological conditions, ROS such as superoxide anion, hydrogen peroxide, and hydroxyl radicals are neutralized by enzymatic antioxidants, including superoxide dismutase (SOD), catalase, and glutathione peroxidase, as well as non-enzymatic antioxidants such as glutathione and vitamin C (Liguori et al., 2018; Płóciniczak et al., 2025). With advancing age, however, the efficiency of these systems declines. Studies have shown that aging livers exhibit decreased levels and activities of glutathione, vitamin C, SOD, and total antioxidant capacity (TAC). Over time, this age-dependent weakening of antioxidant defenses leads to excessive ROS accumulation and promotes lipid peroxidation, protein oxidation, and oxidative DNA damage in hepatocytes (Matin et al., 2025b).

In line with the Free Radical Theory of Aging, oxidative stress in the aging liver is not only the consequence but a driving force of cellular decline (Harman, 2006). Although oxidative stress is not formally a single “hallmark” of aging, but it is closely tied to mitochondrial dysfunction and loss of protein homeostasis, and it exacerbates other hallmarks like inflammation and genomic instability (López-Otín et al., 2013a). In fact, oxidative stress and impaired antioxidant defenses are significant drivers of liver dysfunction in aging. Mitigating ROS and boosting antioxidant response (for instance via the Nrf2 pathway) is therefore considered vital for maintaining liver health in older organisms (Zhang et al., 2015).

A second major contributor to liver aging is chronic inflammation. Aging organisms exhibit a persistent low-grade inflammatory state commonly known as inflammaging, which is characterized by elevated circulating levels of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6) (Franceschi and Campisi, 2014). In the liver, this manifests as increased inflammatory signaling and immune cell infiltration that can promote tissue injury and impair regeneration (Tang et al., 2022). Thereby, aged mice have higher hepatic TNF-α expression associated with NF-κB pathway activation (García-García et al., 2021). Such pro-inflammatory changes not only damage hepatocytes but also interfere

with insulin signaling and metabolic homeostasis, linking inflammation to the nutrient-sensing hallmark of aging (Tilg and Moschen, 2008).

Importantly, oxidative stress and inflammation are closely interconnected. Excess ROS activates redox-sensitive transcription factors such as NF-κB, which induce the expression of pro-inflammatory genes (Morgan and Liu, 2011). In turn, inflammatory cells generate additional ROS, further increasing oxidative damage (García-García et al., 2021). Indeed, liver-specific studies have found that old animals show upregulation of NF-κB target genes and inflammasome components in the liver even in the absence of apparent disease, along with early fibrotic changes (García-García et al., 2021). This supports the concept that oxidative stress-driven inflammation is a central mechanism of hepatic aging.

Another important interconnected aspect of liver aging is the decline in autophagy and dysregulation of lysosomal function. Autophagy is a highly conserved cellular degradation and recycling process that plays a vital role in maintaining intracellular homeostasis, especially under stress conditions such as oxidative stress or aging (Mizushima and Komatsu, 2011).

At the cellular level, autophagy involves a tightly regulated sequence of events. The process begins with the initiation of a membrane structure known as phagophore which expands to engulf targeted components such as damaged mitochondria, misfolded proteins, or lipid droplets. This membrane eventually closes to form a double-membraned vesicle called the autophagosome which then fuses with lysosome, forming an autophagolysosome. The enclosed material is degraded by the lysosomal enzymes including proteases, lipases, nucleases, and glycosidases that are essential for breaking down cellular macromolecules. The resulting breakdown products are then recycled back into the cytoplasm for reuse in biosynthesis or energy production. This whole process allows the cell to remove dysfunctional components and also simultaneously supplying essential substrates during periods of metabolic stress (Feng et al., 2014).

In parallel, lysosomes also mediate the degradation of extracellular material through heterophagy**,** which encompasses endocytosis, pinocytosis, and phagocytosis (Uribe-Querol and Rosales, 2020). In heterophagic pathways, extracellular ligands, nutrients, lipoproteins, pathogens, and cell debris are internalized into endosomes or phagosomes that progressively

mature and fuse with lysosomes. The resulting endolysosomes or phagolysosomes serve as major degradative compartments where internalized material is enzymatically broken down and recycled (Huotari and Helenius, 2011; Saftig and Klumperman, 2009). In the liver, heterophagy is particularly important for hepatocyte nutrient uptake, lipoprotein turnover, and Kupffer cell–mediated clearance of apoptotic bodies, immune complexes, and microbes. Thus, lysosomes act as central hubs integrating autophagy and heterophagy, coordinating intracellular quality control with extracellular scavenging. Age-associated impairments in both processes contribute to the accumulation of damaged organelles, altered lipid handling, chronic inflammation, and metabolic dysfunction characteristic of the aging liver (Rubinsztein et al., 2011; Settembre et al., 2013).

Functionally, autophagy is indispensable for cellular survival and health (Mizushima and Komatsu, 2011). It protects against a wide range of diseases by limiting oxidative damage, preventing the accumulation of toxic protein aggregates, and removing damaged organelles (Rubinsztein et al., 2011). However, with advancing age, the autophagy process in liver tissue becomes markedly impaired. Numerous studies have reported an age-dependent decline in the expression of autophagy-related genes, including LC3, Beclin-1, and various ATG proteins, in aged hepatocytes (Palacios-Ramírez et al., 2025). This leads to reduced initiation of autophagosomes and diminished autophagic flux.

The consequences of impaired autophagy lead to the accumulation of dysfunctional mitochondria, which enhances the generation of ROS, further increasing oxidative stress and promoting cellular senescence (Green et al., 2011). Inefficient clearance of protein aggregates and other cellular debris leads to chronic inflammation and the activation of hepatic cells, ultimately resulting in fibrosis. Moreover, reduced lipophagy contributes to metabolic dysfunction in the elderly.

Thus, preserving autophagy and lysosomal function is thought to be crucial for mitigating liver aging. In summary, antioxidant defenses, inflammatory regulation, and degradation pathways form a set of protective mechanisms whose decline with aging drives hepatic functional loss. These processes are interrelated; impaired autophagy leads to oxidative stress, which in turn can trigger inflammatory pathways – suggesting that a multi-targeted intervention might be needed to effectively slow liver aging.

1.3 Relevance of Nutritional Interventions in Age-Related Liver Dysfunction

Given the contribution of metabolic and environmental factors to aging, there is increasing interest in nutritional strategies to counteract age-related liver dysfunction. Diet is a major modulator of hepatic metabolism and systemic aging processes. In the context of hepatic aging, regulatory nutrients and phytochemicals can serve as structural and energy-yielding functions that influence signaling pathways, gene expression and cellular homeostasis. Among these, polyphenols represent a diverse and promising class of bioactive compounds with strong antioxidant and anti-inflammatory properties (Vauzour et al., 2010). These include flavonoids, phenolic acids, stilbenes, and lignans, commonly found in fruits, vegetables, teas, and herbs (Hossain et al., 2025).

Dietary patterns rich in polyphenol-containing plant foods are associated with lower levels of hepatic lipid accumulation, improved insulin sensitivity, and reduced expression of pro-inflammatory cytokines (Han and Chen, 2025). These compounds often act on multiple molecular targets simultaneously, making them attractive as geroprotective agents that can address the multifactorial nature of liver aging (Davinelli et al., 2025). Studies have shown that several polyphenols exert beneficial effects in experimental models of liver aging. As demonstrated, resveratrol**,** a stilbene found in grapes and red wine, has been reported to activate nutrient-sensing pathways such as SIRT1 and AMPK, thereby supporting mitochondrial function, reducing oxidative damage, and attenuating inflammation in hepatocytes (Shang et al., 2008). The Zingiberaceae family comprises numerous bioactive plants, including Curcuma longa (turmeric), whose principal polyphenolic constituent curcumin, has been shown to confer cytoprotective effects by attenuating NF-κB-mediated inflammatory signaling and strengthening cellular antioxidant defenses (Liu et al., 2025).

The concept of functional foods and nutraceuticals has emerged as a promising area of research in nutrition and preventive medicine. Functional foods are defined as dietary components that offer additional physiological benefits beyond basic nutritional functions and contribute to the reduction of disease risk or enhancement of health (Marcìa-Fuentes et al., 2026). There is an increasing scientific interest in identifying natural dietary agents that can protect the liver from aging-related decline**.** Among these, ginger **(***Zingiber officinale***)** has attracted considerable attention as a bioactive-rich dietary ingredient with a functional properties and multi-targeted hepatoprotective potential (Huang, 2019)**.** Ginger contains a

complex array of phytochemicals, including gingerols, shogaols, paradols, and zingerone, which have been demonstrated to exert strong antioxidant**,** anti-inflammatory**,** and cytoprotective effects in various *in vitro* and *in vivo* models (Ayustaningwarno et al., 2024). As an example, 6-shogaol-rich extract from ginger, up-regulated antioxidant defenses through the induction of Nrf2 and HO-1 regulated by p38 MAPK and PI3k/Akt pathway *in vitro* and *in vivo* (Bak et al., 2012). Given its long history of use, favorable safety profile, and diverse bioactivity, ginger serves as a natural, non-pharmacological intervention**,** capable of modulating multiple cellular processes involved in liver aging.

1.4 Biological and Therapeutic Significance of Ginger (*Zingiber officinale*)

Ginger is the rhizome of the plant *Zingiber officinale*, and it has been valued for centuries both as a culinary spice and as a medicinal herb. In traditional medicine systems (such as Ayurveda, Traditional Chinese Medicine, and others), ginger has been used for various kinds of treatment. Biologically, ginger is known to exhibit a remarkable spectrum of pharmacological activities, making it a compelling candidate for health promotion in aging. However, beyond that, modern research has elucidated that ginger possesses strong antioxidant and anti-inflammatory properties, among other effects. These properties align closely with the processes implicated in aging. It has been shown that, ginger supplementation reduce markers of oxidative stress and enhance enzymatic antioxidants in various experimental models; animal studies report that gingerol-rich extracts increase activities of superoxide dismutase, catalase, glutathione peroxidase, and glutathione while lowering lipid peroxidation and H_2O_2 levels in rats and mice exposed to oxidative stressors (Shanmugam et al., 2011).

Ginger also modulates inflammatory pathways; for example, it can inhibit the activation of NF-κB and suppress pro-inflammatory cytokine production, while sometimes boosting levels of anti-inflammatory cytokines. An *in vivo* study showed that ginger extract (ethanol) inhibited NF-κB activity and lowered IL-1β levels in BALB/c female mice at the dose of 100 mg/kg (Ayustaningwarno et al., 2024). Such immunomodulatory activity may help to reduce the chronic inflammation associated with aging and age-related diseases.

Therapeutically, ginger has demonstrated benefits in a range of age-related or chronic conditions. Studies have reported its hepatoprotective effects in toxin-induced liver injury and in metabolic disease models. For instance, Shanmugam et al. (2011) showed that dietary

ginger protected rats from oxidative damage in multiple tissues under diabetic conditions, significantly improving hepatic antioxidant enzyme levels comparable to vitamin E (Shanmugam et al., 2011). Ginger has also shown anti-cancer, anti-obesity, and neuroprotective activities in preclinical studies (Ayustaningwarno et al., 2024).

Notably for metabolic and liver health, ginger supplementation in animal models of non-alcoholic fatty liver disease or high-fat diet intake has led to reduced steatosis, improved insulin sensitivity, and lower inflammatory liver damage (Liu et al., 2023).

1.5 Bioactive Compounds of Ginger

The broad pharmacological effects of *Zingiber officinale* are primarily attributed to its rich content of bioactive compounds, which include both volatile and non-volatile constituents. The volatile components, mainly essential oils, are composed of monoterpenoids and sesquiterpenes such as zingiberene, curcumene, β-bisabolene, cineole, citral (geranial and neral), borneol, and linalool. These molecules are responsible for ginger's characteristic aroma and contribute to its anti-cancer, anti-spasmodic, antimicrobial, and anti-nausea properties (Kamal et al., 2023; Mashhadi et al., 2013).

However, the non-volatile pungent compounds, particularly the phenolic constituents found in ginger oleoresin, are responsible for the majority of ginger's antioxidant, anti-inflammatory, and cytoprotective activities. The major phenolics include gingerols, shogaols, paradols, and zingerone. Among them, [6]-gingerol is the most abundant in fresh ginger and has been extensively studied for its strong radical scavenging capacity, modulation of redox-sensitive transcription factors, and inhibition of pro-inflammatory cytokines (Dugasani et al., 2010; Mao et al., 2019).

Upon drying or heating, [6]-gingerol undergoes dehydration to form [6]-shogaol, which is more prevalent in processed or dried ginger (Zagórska et al., 2022). Notably, [6]-shogaol often demonstrates greater antioxidant potency than gingerol in experimental assays, particularly in reducing lipid peroxidation and enhancing the activity of antioxidant enzymes like superoxide dismutase (SOD), catalase, and glutathione peroxidase in rodent models (Dugasani et al., 2010; Oğuz Cumaoğlu et al., 2022). Similarly, zingerone, formed during cooking, has been shown to ameliorate oxidative damage in diabetic and hepatotoxicity models by reducing malondialdehyde (MDA) levels and restoring glutathione levels (Ahmad et al., 2018).

Paradols, derived from hydrogenation of shogaols, also exhibit significant anti-inflammatory and antitumor properties, partly through the suppression of NF-κB signaling and inhibition of COX-2 expression in murine studies (Chung et al., 2001).

Collectively, these compounds demonstrate overlapping but distinct bioactivities, acting across multiple pathways related to oxidative stress, inflammation, and metabolic dysregulation. Their synergistic actions support the rationale for using whole ginger extract in anti-aging interventions, especially in liver aging, where multiple pathways converge in pathogenesis.

1.6 Rationale for Using Ginger as a Model Intervention

Considering the foregoing points, ginger emerges as an excellent model intervention to study in the context of aging and hepatic decline. The rationale is threefold: breadth of effect, relevance to hallmarks, and practical feasibility. First, ginger's pleiotropic biological profile supports the premise that it could counteract several mechanisms underlying aging simultaneously. As reviewed herein, preclinical studies indicate that ginger and its bioactive constituents may exert favorable effects spanning all twelve hallmarks of aging in at least some experimental models. A recent comprehensive review (Publication 1) critically evaluates the available literature on ginger and aging, thus formulating the research rationale and objectives. This publication concluded that preclinical evidence supports the beneficial effects of ginger on several hallmarks of aging, including nutrient dysregulation (e.g., improved metabolic profiles and insulin signaling), mitochondrial dysfunction (e.g., reduced oxidative damage), chronic inflammation (e.g., lower levels of inflammatory mediators), and microbiome dysbiosis (e.g., promoting gut microbiota homeostasis). This wide-ranging impact is rare among interventions and suggests ginger could address the multifactorial nature of aging. Notably, many of ginger's strongest effects in animal studies were on metabolic and inflammatory hallmarks – for example, multiple studies report ginger supplementation attenuating age-related increases in inflammatory cytokines and improving antioxidant status. If an intervention can hit multiple hallmarks, it stands a better chance of producing meaningful improvements in age-associated functional decline, as opposed to targeting just one mechanism of aging.

Second, ginger specifically targets processes highly relevant to liver aging. As elaborated above, oxidative stress, chronic inflammation, and autophagy defects are pivotal in liver aging – and these are precisely the domains where ginger shows activity. By boosting antioxidant defenses and reducing inflammation, ginger might mitigate two key drivers of hepatic aging. Its influence on autophagy and lysosomal enzymes could help maintain cellular cleanup in aging livers. Additionally, ginger's reported activation of the AMP-activated protein kinase (AMPK) pathway - a central regulator of energy balance and hepatic lipid metabolism, has been shown to reduce fat accumulation, enhance mitochondrial function, and improve metabolic homeostasis in experimental models of metabolic dysfunction, suggesting another mechanism by which ginger could counteract age-related metabolic derangements (Lee et al., 2021).

Third, from a practical standpoint, ginger is safe, inexpensive, and culturally accepted as a food, which is important when envisioning interventions for aging in humans. Any longevity or geroprotective strategy would likely require long-term administration, so safety and tolerability are essential. Ginger has a high margin of safety – it has been consumed by humans for millennia and is generally recognized as safe as a spice or supplement. Therefore, establishing scientific evidence for ginger's geroprotective effects could directly translate into a feasible approach for human health span extension. The existing gap, however, is that while preclinical studies are promising, human data on ginger's effects on aging hallmarks are limited. Apart from some trials showing ginger can improve specific outcomes (like markers of inflammation or glucose control), comprehensive clinical studies on aging parameters are lacking. This provides a strong reason for further research – including the present thesis work – to evaluate ginger in controlled settings and in depth. Ultimately, validating ginger as an intervention could pave the way for its use as a nutraceutical to support healthy aging, particularly for the liver and metabolic health.

1.7 Brief Introduction to the Three Included Studies and Their Relation to Aging Hallmarks

This doctoral thesis comprises three core works (Publications 1–3) that together explore ginger's effects on aging-related liver decline from complementary angles. Each work addresses specific hallmarks of aging and helps build a comprehensive picture of how ginger modulates age-associated changes in the liver.

Publication 1 is a systematic review entitled *"Effects of Ginger (Zingiber officinale) on the Hallmarks of Aging"* (Matin et al., 2024). In this publication, we surveyed and synthesized the existing literature on ginger and its bioactive components in the context of the twelve aging hallmarks. The review compiles evidence from cell culture, animal models, and human studies indicating that ginger or compounds like 6-gingerol and 6-shogaol can influence mechanisms such as DNA damage responses, degradation process through anti-aggregation and pro-autophagy effects), inflammatory pathways, mitochondrial function, and more. A key finding from this review was that ginger demonstrates beneficial effects across all major hallmarks of aging in preclinical models, with especially strong evidence for counteracting chronic inflammation, nutrient-sensing dysregulation, mitochondrial dysfunction, and gut dysbiosis. Previous research studies show ginger supplementation reduces markers of systemic inflammation and improves metabolic profiles (addressing the altered intercellular communication and deregulated nutrient-sensing hallmarks). This review thus establishes the broad therapeutic potential of ginger in aging and identifies gaps in knowledge, such as the need for more clinical research. It provides the conceptual foundation for following experimental studies, firmly rooting our experimental efforts in the hallmarks of aging framework.

Publication 2 is an original research study focused on how dietary ginger affects the aging liver's antioxidant defense systems (Matin et al., 2025b). In this study, mice of three different ages (young adult, middle-aged, and old) were fed diets either with or without ginger supplementation (at two doses: 0.6% and 1.8% ginger by weight) for 3 months. The study specifically examined a panel of hepatic antioxidant and oxidative stress markers: DPPH free radical scavenging activity, total antioxidant capacity (TAC), vitamin C content, total phenolic content, SOD activity, reduced glutathione (GSH), and malondialdehyde (MDA, an indicator of lipid peroxidation). The results provide important insights into how ginger supplementation may modulate oxidative balance in the aging liver, potentially offering a nutritional strategy to enhance antioxidant defenses and mitigate age-related hepatic decline.

Publication 3 is an original research study investigating ginger's impact on lysosomal function and inflammation in the aging liver (Matin et al., 2025a). This study used a similar mouse model and ginger supplementation doses but focused on different endpoints: activities of lysosomal enzymes (including glycosidases and aminopeptidases), expression of pro-

inflammatory and nutrient-sensing genes (TNF-α and PI3K), and histological and ultrastructural changes in liver tissue. Together, these findings suggest that ginger may help preserve lysosomal function and reduce inflammatory signaling in the aging liver, contributing to improved cellular homeostasis and tissue health.

In conclusion, these studies approach the problem of hepatic aging from complementary perspectives: a broad literature synthesis (hallmarks framework), a focused analysis of antioxidant defenses, and an in-depth look at lysosomal and inflammatory pathways. Collectively, the studies address distinct hallmark domains: antioxidant defenses are primarily linked to oxidative stress and mitochondrial dysfunction; cellular degradation and lysosomal pathways correspond to dysregulated autophagy and reduced protein turnover; and inflammatory signaling corresponds to altered intercellular communication, and is closely linked to nutrient-sensing pathways. By addressing these areas, the included studies collectively advance our understanding of how a natural intervention like ginger can target multiple hallmarks of aging. This comprehensive examination sets the stage for the subsequent chapters, where the methodologies and detailed findings are discussed, and it reinforces the central thesis that ginger supplementation exerts multi-faceted protective effects against aging-related liver decline. The general introduction provided here serves as a foundation, establishing why aging of the liver is a significant issue, why ginger is a promising countermeasure, and how each component of the research contributes to the primary objective of promoting healthy aging.

2. Aim of the study

The aim of this study was to investigate the therapeutic potential of *Zingiber officinale* (ginger) in mitigating age-related physiological, biochemical, and molecular alterations in the liver, using experimental mouse models. By focusing on key hallmarks of aging such as oxidative stress, chronic inflammation, impaired autophagy, and lysosomal dysfunction, this work was designed to evaluate the age- and dose-dependent effects of dietary ginger supplementation on hepatic redox balance, structural integrity, cellular homeostatic maintenance mechanisms or lysosome-dependent degradative pathways, and pro-aging signaling pathways. The goal was to assess whether ginger could serve as a safe and effective nutraceutical to promote healthy liver aging and counteract functional decline.

3. Hypothesis

- The ginger supplementation in diet increases hepatic antioxidant capacity and improves redox homeostasis in an age- and dose-dependent manner, as reflected by elevated TAC and glutathione levels and reduced oxidative damage markers.
- Dietary supplementation of ginger modulates lysosome-associated pathways in the liver in an age- and dose-dependent manner, leading to measurable changes in lysosomal enzyme activities
- Dietary ginger supplementation attenuates hepatic inflammatory signaling in an age- and dose-dependent manner, which is accompanied by improved liver morphology and ultrastructure of liver cell and reduced structural features of hepatocellular injury and/or steatosis.

4. Objectives

- To evaluate hepatic antioxidant defense systems including total antioxidant capacity, glutathione levels, vitamin C content, enzymatic antioxidants, and oxidative stress markers in order to determine how ginger supplementation influences age-related oxidative stress
- To characterize age- and dose-dependent morphological alterations in liver tissue, using histological and ultrastructural analyses, and to assess whether ginger supplementation preserves hepatocellular structure during aging

- To assess glycosidase and proteolytic enzyme activities as indicators of lysosomal system function and to determine whether ginger modulates age-associated disturbances in hepatic homeostasis
- To quantify expression levels of inflammatory and nutrient-sensing genes, particularly TNF-α and PI3K, in order to evaluate the impact of ginger on age-related inflammatory signaling and metabolic pathway regulation
- To integrate biochemical, molecular, and structural findings through correlation analyses to determine how ginger dose and age interact to influence hepatic aging pathways

5. Methodological Overview

5.1 Animal Model and Experimental Design

Two complementary *in vivo* investigations were conducted using male Swiss Webster mice to examine the effects of dietary ginger supplementation on aging-associated hepatic changes. Mice were stratified into three age groups (3, 6, and 12 months old) with n = 48 animals per age (total N = 144). Each age group was further divided into three dietary cohorts: a control group on standard diet, a low-ginger group receiving 0.6% ginger (w/w) in the diet, and a high-ginger group receiving 1.8% ginger (w/w) in the diet. The ginger powder (*Zingiber officinale* rhizome), standardized to contain 5% gingerols (supplied by Sabinsa Corporation, 20 Lake Drive, East Windsor NJ 08520, United States), was obtained from a commercial source and mixed freshly into the base feed twice weekly. Animals had access to food and water and were housed four per cage under standard laboratory conditions (22 °C, 12:12 h light/dark cycle) to minimize environmental variability. The feeding intervention lasted 3 months, (following American Veterinary Medical Association guidelines, 2020) and the livers were immediately collected for analysis. Figure 1 provides a schematic of the experimental design, illustrating the age groups, dietary treatments, and sample sizes in each cohort.

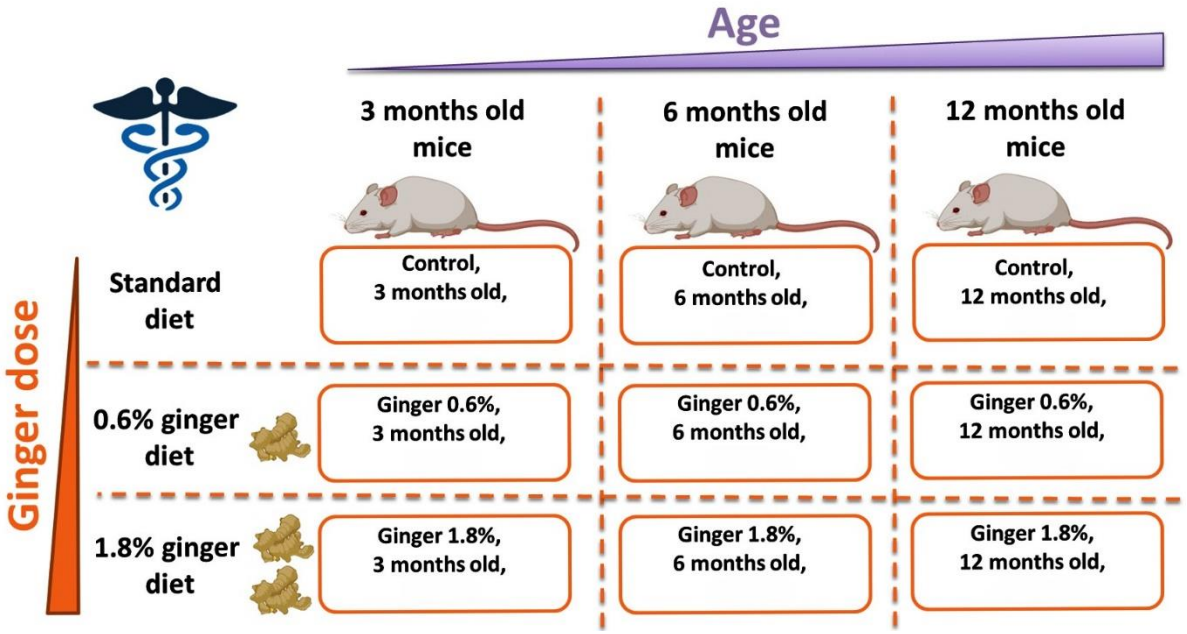

Figure 1. Schematic overview of the animal experiment design. Male Swiss Webster mice (3, 6, 12 months old; 48 per age) were assigned to control (0% ginger), low-dose (0.6% ginger), or high-dose (1.8% ginger) diet groups for 3 months. After the treatment period, liver tissues were collected for biochemical, molecular, and histological analyses.

All animal procedures were approved by the local Institutional Animal Care and Use Committee and performed in accordance with national and EU regulations for animal research. Specifically, the study protocol was authorized under permit No. 14186201 and complied with EU Directive 2010/63/EU as well as the Polish Act on the protection of animals used for scientific purposes. These ethical safeguards ensured that animal welfare considerations (housing conditions, minimization of pain/distress, humane endpoints) were integrated into the experimental design.

5.2 Biochemical Assays for Antioxidant Capacity

To evaluate hepatic antioxidant defenses and oxidative stress, we conducted biochemical assays on liver homogenates from the first animal study (Publication 2). Multiple antioxidant parameters were measured, including DPPH radical scavenging activity, total antioxidant capacity (TAC), vitamin C concentration, total phenolic content, superoxide dismutase (SOD) activity, malondialdehyde (MDA) levels, and reduced glutathione (GSH) levels. These endpoints collectively assess the liver's redox status and ability to counteract free radicals.

For DPPH scavenging activity, liver extracts were tested for their ability to scavenge the stable DPPH radical (1,1-diphenyl-2-picrylhydrazyl) according to the method of Brand-Williams et al. (1995) (Brand-Williams et al., 1995). The decrease in absorbance at 517 nm (after a 30 min incubation with DPPH in the dark) was measured spectrophotometrically, and results were expressed as the percentage of DPPH radicals scavenged by the sample. Total antioxidant capacity was assessed using a commercial kit (Cayman Chemical), which measures the cumulative ability of antioxidants in the liver homogenate to neutralize free radicals. This assay was performed in a 96-well format with Trolox as the standard; TAC values were calculated from a Trolox calibration curve and reported in millimolar Trolox equivalents.

Hepatic vitamin C content was quantified by a colorimetric assay based on Omaye's method as modified by Jóźwik *et al*., using acid extraction and reduction of Fe^{3+} to Fe^{2+} in the

presence of 2,2-dipyridyl (Jóźwik et al., 2012; Omaye et al., 1979). Absorbance was read at 525 nm, and vitamin C levels were expressed as mg per 100 g of tissue.

Total phenolic content was assessed by following the modified method by Škerget *et al* 2005, through spectrophotometric measurement of the colorimetric redox reaction upon application of the Folin - Ciocalteu assay (a measure of phenolic antioxidants), with results expressed in gallic acid equivalents per gram of liver (Škerget et al., 2005).

Key enzymatic and oxidative stress markers were also measured. SOD activity, reflecting an endogenous antioxidant enzyme, was measured with a tetrazolium salt-based kit (Cayman Chemical) that detects superoxide radical scavenging. The assay quantifies SOD function by the inhibition of superoxide-driven dye reduction, with absorbance read at 450 nm; SOD activity was standardized to units per mL of sample.

MDA levels, an index of lipid peroxidation, were determined via a thiobarbituric acid reactive substances (TBARS) assay kit. In this assay, MDA in the sample reacts with TBA under high temperature and acidic conditions to form a colored MDA–TBA adduct, which was measured at 532 nm and compared to an MDA (μM) standard curve. Finally, reduced GSH was assayed by a kinetic enzymatic method using 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) as a chromogen, based on the classic Beutler *et al.* protocol (Beutler et al., 1963). The sulfhydryl group of GSH reduces DTNB to 2-nitro-5-thiobenzoate, producing a yellow color (measured at 412 nm) proportional to GSH concentration. GSH values were calculated in micromoles per gram of tissue by reference to a standard and normalized for protein content.

All biochemical assays were performed in triplicate for each sample to ensure reliability, and appropriate blanks and calibration standards were included as per kit or literature protocols. These assays provided a comprehensive profile of the liver's antioxidant capacity and oxidative damage, forming the basis for assessing ginger's efficacy in counteracting age-related oxidative stress in the liver.

5.3 Lysosomal and Proteolytic Enzyme Activity Assays

The second experimental study (Publication 3) focused on measures of enzymatic activity in the aging liver. We measured a set of lysosomal hydrolase enzymes and aminopeptidases in

liver homogenates to investigate how ginger affects age-related changes in lysosomal function. The activities of acid phosphatase (ACP) (EC 3.1.3.2) and several glycosidases – including β-galactosidase (BGAL) (EC 3.2.1.23), β-glucuronidase (BGRD) (EC 3.2.1.31), α- and β-glucosidase (AGLU and BGLU) (EC 3.2.1.20 and EC 3.2.1.21), α-galactosidase (AGAL) (EC 3.2.1.22), β-N-acetylhexosaminidase (HEX) (EC 3.2.1.52), and α-mannosidase (MAN) (EC 3.2.1.24) – were measured using chromogenic *p*-nitrophenyl (pNP) substrate assays. In each assay, the specific pNP-linked substrate (e.g., pNP-β-D-glucuronide for BGRD, pNP-β-D-galactopyranoside for BGAL) was incubated with the liver extract at 37 °C. Enzymatic cleavage releases *p*-nitrophenol, which is quantified by its absorbance (typically around 405–420 nm). Enzyme activities were calculated from the absorption data using extinction coefficients and expressed as nanomoles of substrate hydrolyzed per mg protein per hour. Acid phosphatase activity was similarly determined by the hydrolysis of *p*-nitrophenyl phosphate to *p*-nitrophenol (absorbance at 420 nm), following the protocol of Barrett and Heath (1977) (Barrett A.J., 1977).

In addition, we measured the activities of key aminopeptidases involved in protein turnover, namely alanyl aminopeptidase (AlaAP) (EC 3.4.11.2), leucyl aminopeptidase (LeuAP) (EC 3.4.11.1), and arginyl aminopeptidase (ArgAP) (EC 3.4.11.6). Aminopeptidase assays were carried out using the method of McDonald and Barrett (1986) (Barrett A.J., 1977; McDonald J.K, 1986), which employs specific amino acid *p*-nitroanilide substrates in the presence of Fast Blue BB salt to detect released *p*-nitroaniline diazonium dye. The absorbance of the colored product was read at 540 nm, and activities were expressed in nmol/mg protein/hour. Notably, ArgAP activity was of particular interest as it tends to increase with age as a compensatory response to accumulated protein damage. By assessing these enzymatic activities, we aimed to determine whether ginger supplementation modulates lysosomal degradation pathways – either by enhancing clearance of damaged macromolecules in young animals or normalizing excessive lysosomal activity in old animals. All enzyme assays were performed under linear reaction conditions (with respect to time and protein concentration), and protein content in each sample was determined (e.g., by the Lowry assay) to allow normalization of enzyme specific activities (Lowry et al., 1951).

5.4 Gene Expression Analysis (qRT-PCR)

To interrogate molecular pathways related to inflammation and metabolic regulation, we analyzed hepatic gene expression of two representative markers: tumor necrosis factor-alpha (TNF-α) and phosphatidylinositol-3-kinase (PI3K). These were selected based on their relevance to aging – TNF-α as a proinflammatory cytokine often elevated in aging ("inflammaging"), and PI3K as a key component of insulin/IGF-1 nutrient-sensing and longevity pathways. We extracted total RNA from liver tissue (approximately 30 mg per sample) using a silica-membrane spin column kit (Qiagen RNeasy), following the manufacturer's protocol for animal tissues. RNA quality and concentration were verified by spectrophotometry (260/280 nm ratios ~2.0). cDNA synthesis was performed with a high-capacity reverse transcription kit (Bio-Rad iScript), using 1 μg of RNA per 20 μL reaction. The reverse transcription conditions (priming, extension, termination) were as recommended by the kit to yield comprehensive cDNA coverage of mRNA transcripts.

Quantitative real-time PCR (qRT-PCR) was then carried out on a Bio-Rad CFX Opus 96 thermocycler using SYBR Green chemistry for fluorescence detection. Each 20 μL PCR reaction contained 10 μL of 2× SYBR Green master mix, gene-specific forward and reverse primers (optimized at ~500 nM), and a fixed aliquot of the cDNA template (corresponding to ~10 ng of original RNA). We used gene-specific primer pairs for *Tnf* (coding TNF-α) and *Pik3c3* (coding a class of PI3K) that amplified ~100–200 bp fragments; primer sequences were designed to span intron–exon boundaries when possible to avoid genomic DNA amplification. The cycling program consisted of an initial 95 °C denaturation (30 s) followed by 40 cycles of 95 °C for 15 s and 60 °C for 30 s. Fluorescence was recorded in real-time, and melt-curve analysis was performed at the end of cycling to confirm the specificity of amplification (single peak for each primer set).

Each biological sample was run in triplicate PCR reactions to ensure reproducibility, and no-template controls (NTCs) were included to check for contamination. TNF-α and PI3K expression levels in ginger-fed versus control mice (across ages) thus provided insight into ginger's influence on pro-inflammatory signaling and insulin/IGF-related metabolic signaling, respectively. We anticipated, for example, that aging would elevate hepatic TNF expression (as reported by Kakino *et al.*, 2018) and reduce PI3K expression (Braccini *et al.*,

2015), and our analysis tested whether ginger could mitigate these age-associated transcriptional changes (Braccini et al., 2015; Kakino et al., 2018).

5.5 Histological and Ultrastructural Analysis

Morphological examinations of the liver were conducted to correlate biochemical and molecular findings with structural alterations in hepatic tissue architecture. Immediately after sacrifice, small pieces of liver (1–2 mm^3) were fixed for histology and transmission electron microscopy (TEM). Tissues were immersion-fixed in 2.5% glutaraldehyde (in 0.1 M cacodylate buffer, pH 7.2) at 4 °C for ~2 h. This primary fixation preserves ultrastructure by cross-linking proteins. Samples were then rinsed in buffer and post-fixed with 1% osmium tetroxide (OsO_4) in cacodylate buffer for 2 h. Osmium postfixation stabilizes membranes and imparts electron density. After thorough washing, tissues were dehydrated through an ascending ethanol series (30% to 100%) and cleared in propylene oxide. The specimens were infiltrated with epoxy resin and then embedded in fresh resin, polymerizing at 60 °C for 48 h to yield resin blocks containing the preserved tissue.

For light microscopy, semi-thin sections (~0.5–1 µm thick) were cut from the resin blocks using an ultramicrotome and collected on glass slides. These sections were stained with Sudan III and aniline blue (at 80 °C) to visualize general tissue architecture and lipid content. The stained slides were examined under a Nikon Eclipse light microscope, and digital photomicrographs were taken of representative fields. This histological evaluation allowed us to assess age-related changes such as hepatocyte size (e.g., hypertrophy), lipid droplet accumulation, and tissue organization, as well as any protective effects of ginger on these parameters.

For ultrastructural analysis, ultrathin sections (~50–60 nm) were cut from the same blocks with a diamond knife and mounted on copper grids. These sections were stained with saturated aqueous uranyl acetate followed by lead citrate. The contrast provided by these heavy metal stains highlights subcellular organelles when viewed with a transmission electron microscope (TESLA BS500 TEM). We captured TEM images using a digital CCD camera at various magnifications to examine organelle morphology. Key ultrastructural features of interest included mitochondrial shape and cristae integrity, endoplasmic reticulum appearance, presence of lipofuscin (age pigment) granules, and lysosomal structures. For example, aged hepatocytes are expected to exhibit enlarged, swollen mitochondria with

cristae disruption, proliferating secondary lysosomes, and accumulated lipofuscin. By comparing young control livers to aged control livers, and then determining how ginger-supplemented livers deviated from those, we assessed whether ginger mitigated age-associated cellular damage. The histology and TEM analyses were performed blinded to group to avoid observational bias. Our microscopy data thus provided qualitative and quantitative evidence of ginger's role in preserving liver cell morphology during aging – e.g., maintenance of normal hepatocyte structure in treated groups or reduction in the severity of aging lesions (like cellular inclusions or organelle degeneration).

5.6 Statistical Analysis

For both animal studies, data were analyzed using appropriate statistical tests. Group comparisons (e.g., ginger vs. control at each age, or effects of age and dose) were evaluated by analysis of variance (ANOVA), with post-hoc multiple comparison tests to pinpoint significant differences between specific groups. In Publication 2, a one-way or two-way ANOVA (factor(s): diet and/or age) was employed as needed, followed by Tukey's Honest Significant Difference (HSD) test for pairwise comparisons at $p < 0.05$. In Publication 3, a two-factor ANOVA (age × diet) examined interaction effects, and Duncan's multiple range test was used post-hoc for group comparisons. Fisher's test was applied to assess the variance among experimental groups. Pearson correlation analysis was conducted to determine associations between biochemical and molecular parameters. Additionally, linear regression models were used to evaluate age- and dose-dependent effects on hepatic outcomes. Results are presented as least squares means ± standard deviation (LSMean ± SD), and group differences are annotated using superscript letters indicating statistical significance ($*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$). The applied statistical approach provided rigorous assessment of differences across multiple endpoints (e.g., antioxidant status, enzyme activities, and gene expression), accounting for variability and supporting robust inference.

6 Results

6.1 Experimental Study 1 (Publication 2): Effects of Ginger Supplementation on Liver Antioxidant Defense Systems in Mice

This study investigated the impact of dietary ginger supplementation on key indicators of hepatic antioxidant status across three age groups of male Swiss Webster mice: 3 months, 6 months, and 12 months. Animals were assigned to one of three diet groups: control (standard feed), 0.6% ginger-supplemented feed, or 1.8% ginger-supplemented feed. The results revealed age-dependent deterioration in antioxidant defenses in control animals, and divergent age- and dose-dependent effects of ginger supplementation.

6.1.1 DPPH Radical Scavenging Activity

In the control group, DPPH radical scavenging activity in liver homogenates significantly declined with increasing age (as shown in Figure 2). The mean scavenging capacity decreased from 65.88% ± 5.69% in 3-month-old mice to 55.19% ± 7.87% and 59.00% ± 6.82% in 6- and 12-month-old animals, respectively. This reduction was statistically significant for both the 6-month-old ($p \leq 0.001$) and 12-month-old ($p \leq 0.05$) control groups when compared with the 3-month-olds. Ginger supplementation had opposing effects based on the age of the animals. In 3-month-old mice, both 0.6% and 1.8% ginger led to a significant reduction in DPPH scavenging capacity compared to controls ($p \leq 0.001$), suggesting a dose-dependent decrease. In contrast, 6-month-old and 12-month-old animals exhibited a significant increase in DPPH activity following ginger supplementation, with greater effects observed at the higher dose of 1.8% ($p \leq 0.001$).

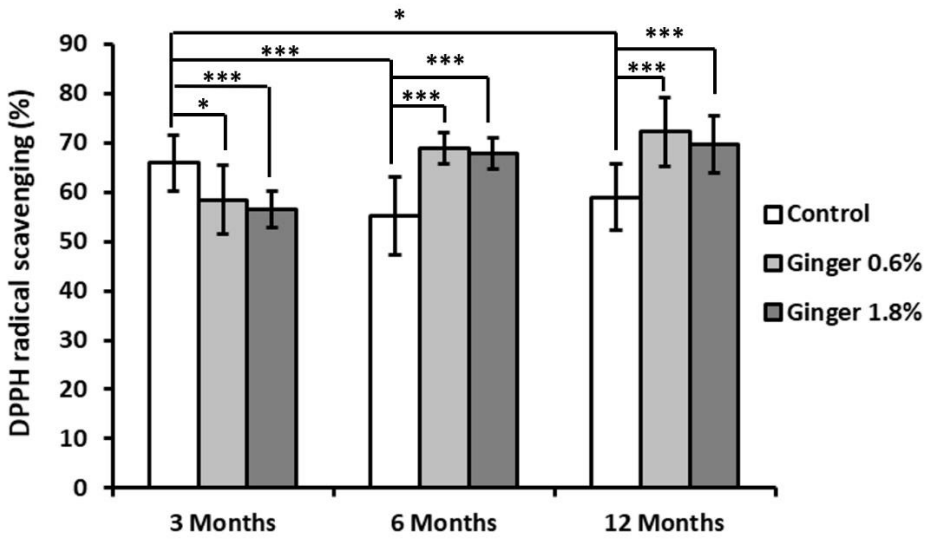

Figure 2: Liver DPPH radical scavenging activity. Data are presented as the percentage of DPPH radical scavenging across different age groups (3, 6, and 12 months) and dietary treatments (control, 0.6% ginger, 1.8% ginger). Values are shown as mean ± standard deviation (n = 16). Statistical significance between groups is indicated (ANOVA/Tukey HSD; *$p \leq 0.05$; ***$p \leq 0.001$)

6.1.2 Total Antioxidant Capacity

Age-associated reductions in total antioxidant capacity, expressed as Trolox equivalents, were observed in control animals. From Figure 3, it can be seen that values first slightly increased (non-significant) from 2.40 ± 0.40 in 3-month-olds to 2.79 ± 0.30 in 6-month-olds, and then sharply declined to 1.86 ± 0.66 in 12-month-olds. Ginger supplementation enhanced total antioxidant capacity across all age groups. The most pronounced increase was observed in the 12-month-old 1.8% ginger group, which reached 3.31 ± 0.33 Trolox equivalents, significantly higher than the age-matched control group ($p \leq 0.001$). The increase in total antioxidant capacity was less distinct in 3-month-old mice.

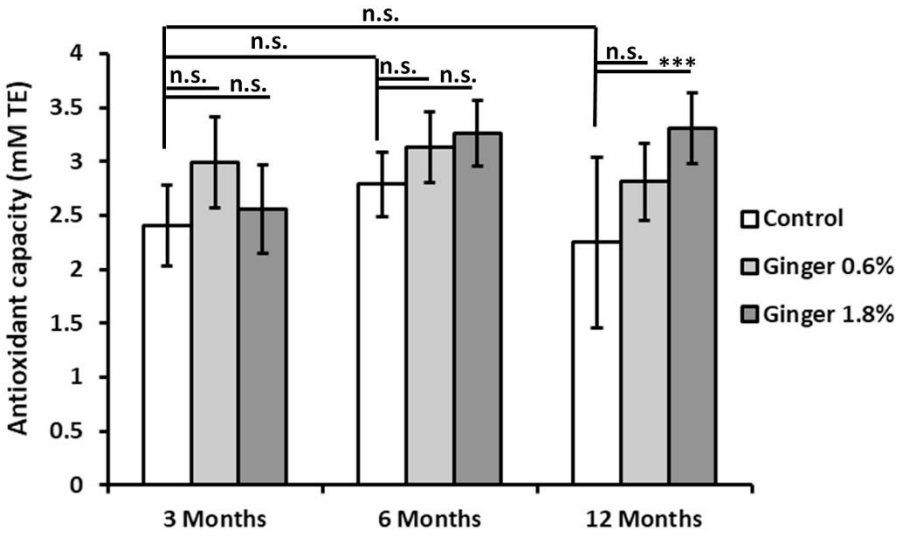

Figure 3 Liver total antioxidant capacity. Total antioxidant capacity of liver homogenates was determined as described in detail in the Methods section. The results are expressed in millimolar (mM) Trolox equivalents (TE). Data represent mean ± standard deviation (n = 8), with significance denoted by asterisks. (ANOVA/Tukey HSD; **$p \leq 0.01$; ***$p \leq 0.001$)

6.1.3 Vitamin C Concentration

Liver vitamin C concentrations in control animals decreased with age (as demonstrated in Figure 4), with values of 2.91 ± 0.36 mg/100 g in 3-month-old mice, 2.56 ± 0.64 mg/100 g in 6-month-olds, and 1.56 ± 0.15 mg/100 g in 12-month-olds. The reduction in the oldest group was statistically significant compared to the youngest ($p \leq 0.001$). Ginger supplementation increased vitamin C levels in all age groups relative to their respective controls. The most significant elevation occurred in the 6-month-old 1.8% group, which reached 3.46 ± 0.60 mg/100 g ($p \leq 0.01$). Supplemented 12-month-old mice also demonstrated modest increases compared to unsupplemented counterparts, although the absolute values remained lower than in younger animals.

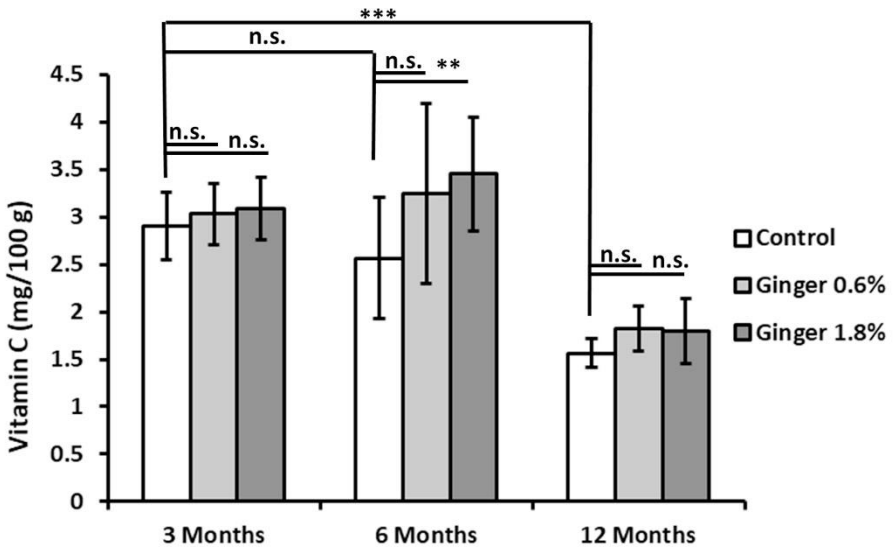

Figure 4 Liver vitamin C levels. Vitamin C concentrations in liver tissue were determined spectrophotometrically as described in the Methods section. The results are expressed as milligrams of vitamin C per Gram of tissue. Data represent mean ± standard deviation (n = 8). Statistical significance between groups is indicated (ANOVA/Tukey HSD; **$p \leq 0.01$; ***$p \leq 0.001$)

6.1.4 Total Phenolic Content

Total phenolic content declined with age in the control animals. Liver samples showed mean values of 6.47 ± 0.67 mg gallic acid equivalents (GAE)/g in 3-month-olds, 5.77 ± 0.38 mg GAE/g in 6-month-olds, and 5.04 ± 1.00 mg GAE/g in 12-month-olds. The decline was statistically significant between the youngest and oldest groups ($p \leq 0.001$). Ginger supplementation had varying effects depending on age. In 3-month-old mice, both ginger doses resulted in a significant reduction in phenolic content. Conversely, in 6-month-old animals, especially those receiving 1.8% ginger, phenolic content was significantly increased compared to controls (8.65 ± 0.89 mg GAE/g vs. 5.77 ± 0.38 mg GAE/g, $p \leq 0.001$). No notable differences were observed in 12-month-old mice in response to ginger supplementation.

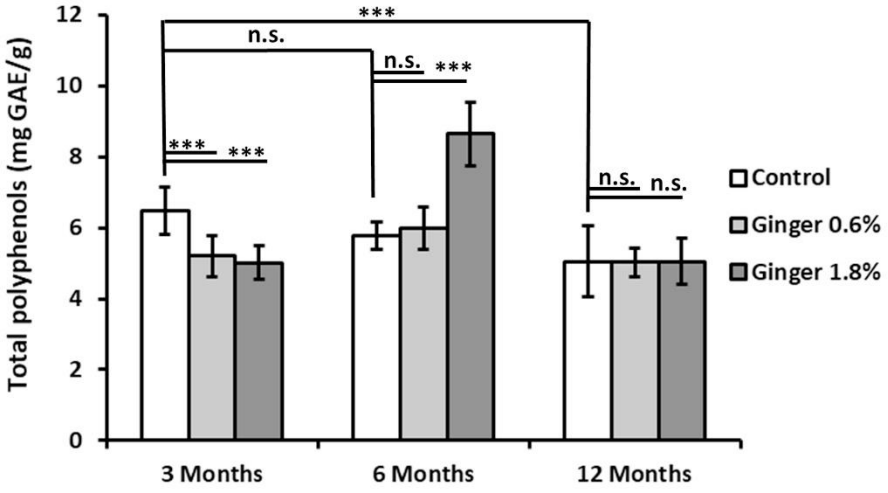

Figure 5 Liver total phenolic content. The total phenolic content in liver homogenates was assessed using the Folin-Ciocalteu reagent, as detailed in the Methods section. Results are expressed as milligrams of gallic acid equivalents (GAE) per Gram of tissue. Data are shown as mean ± standard deviation (n = 16). Statistical differences between groups are indicated (ANOVA/Tukey HSD; ***$p \leq 0.001$)

6.1.5 Superoxide Dismutase (SOD) Activity

SOD activity demonstrated a clear age-related decline in control groups (Figure 6). Mean activity values were 10.76 ± 1.15 U/mL in 3-month-olds, 6.93 ± 1.05 U/mL in 6-month-olds, and 7.04 ± 2.58 U/mL in 12-month-olds, with both older groups showing significantly lower activity than the youngest ($p \leq 0.001$). Ginger supplementation did not significantly influence SOD activity at any age, with values remaining comparable to respective controls across all treatment groups.

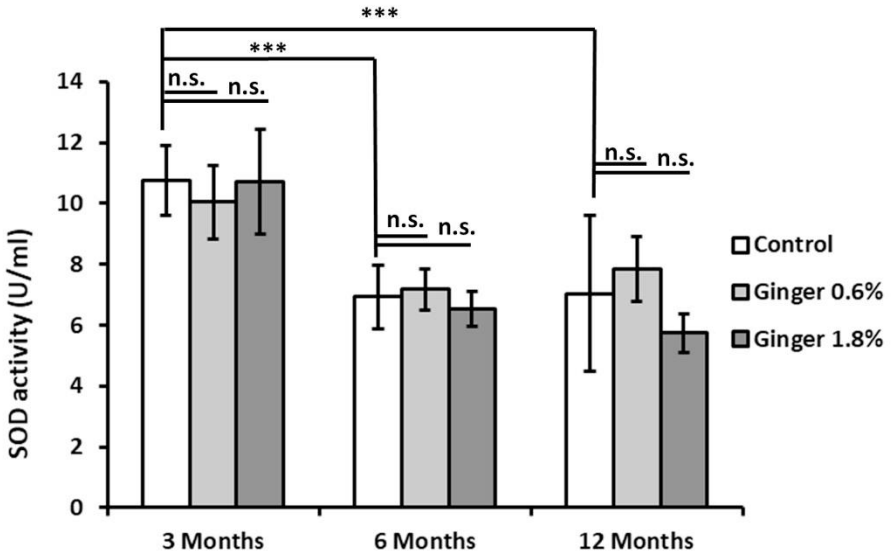

Figure 6 Liver superoxide dismutase (SOD) activity. Superoxide dismutase activity in liver homogenates was measured as described in the Methods section and is expressed in U/mL. Data are presented as mean ± standard deviation (n = 8). Statistical significance is indicated (ANOVA/Tukey HSD; ***$p \leq 0.001$).

6.1.6 Malondialdehyde (MDA) Levels

As shown in Figure 7, MDA levels, reflecting lipid peroxidation, were highest in the youngest control animals and decreased significantly with age. In control mice, levels were 81.08 ± 23.16 µM in 3-month-olds, 34.86 ± 4.40 µM in 6-month-olds, and 37.77 ± 5.89 µM in 12-month-olds ($p \leq 0.001$ for both older groups vs. 3-month-olds). Ginger supplementation at 1.8% significantly reduced MDA levels in 3-month-old mice ($p \leq 0.001$), with concentrations dropping to 45.44 ± 5.69 µM. In 6- and 12-month-old mice, both doses led to modest, non-significant reductions compared to controls.

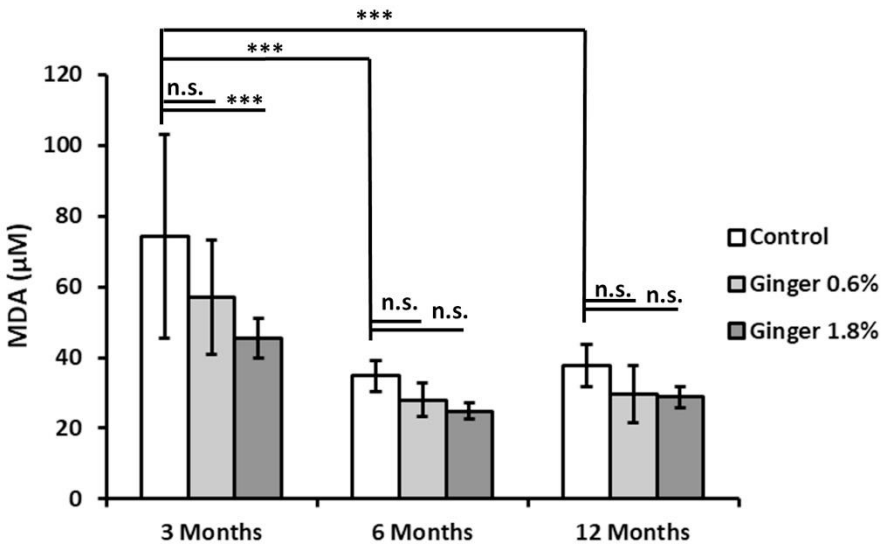

Figure 7 Liver malondialdehyde (MDA) levels. Malondialdehyde levels, an indicator of lipid peroxidation, were measured in liver tissue homogenates. Data are expressed as micromolar (µM) concentrations. Results are shown as mean ± standard deviation (n = 8). Statistical significance between groups is indicated (ANOVA/Tukey HSD; ***$p \leq 0.001$)

6.1.7 Reduced Glutathione (GSH) Concentration

GSH concentrations did not vary significantly with age in control groups (as seen in Figure 8). However, ginger supplementation markedly elevated GSH levels in all age groups, especially at the higher 1.8% dose. In 3-month-olds, GSH increased from 748.07 ± 104.25 µM (control) to 962.02 ± 204.59 µM ($p \leq 0.001$). Similar increases were observed in 6-month-olds (623.94 ± 88.30 vs. 791.75 ± 132.33 µM; $p \leq 0.05$) and 12-month-olds (634.57 ± 84.85 vs. 831.43 ± 83.73 µM; $p \leq 0.01$). These data indicate a robust and consistent effect of ginger on hepatic GSH levels, particularly with 1.8% supplementation.

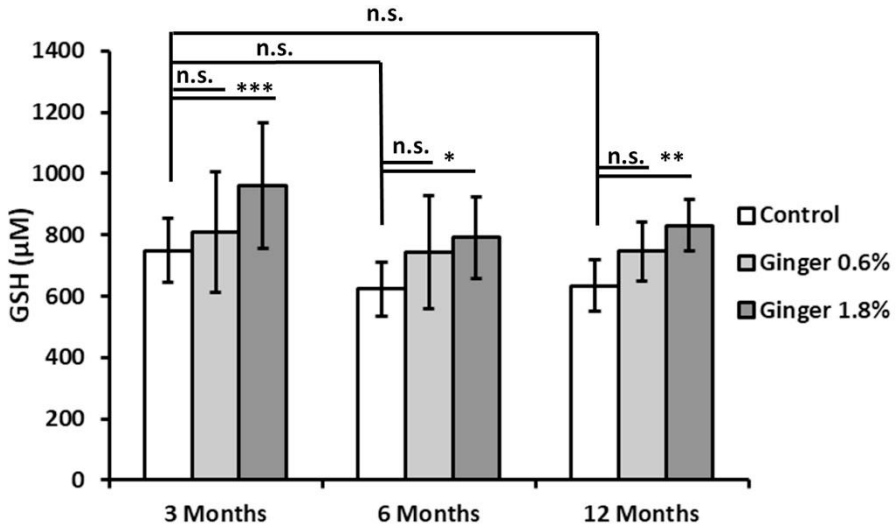

Figure 8 Liver reduced glutathione (GSH) levels. Reduced glutathione concentrations in liver homogenates were determined as described in the Methods section and expressed in micromolar (µM) concentrations. Data are shown as mean ± standard deviation (n = 16). Statistical significance is indicated (ANOVA/Tukey HSD; *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001).

6.2 Experimental Study 2 (Publication 3): Effects of Ginger Supplementation on Hepatic Morphology, Lysosomal Enzymes, and Gene Expression in Aging Mice

This study examined the morphological, enzymatic, and molecular consequences of ginger supplementation in aging male Swiss Webster mice. Liver samples were evaluated histologically and ultrastructurally, while enzyme activities and expression of PI3K and TNF-α were measured to assess lysosomal function, inflammation, and metabolic regulation.

6.2.1 Morphological and Ultrastructural Findings

Progressive age-related alterations in liver histology were observed in control groups (Figures 9 and 10). At 3 months, hepatocytes appeared structurally intact, with abundant cytoplasmic organelles, dense glycogen stores, and centrally located nuclei. At 6 months, hepatocytes began exhibiting mild cytoplasmic vacuolization and reduced organelle density. By 12 months, hepatocyte enlargement, lipid accumulation, and glycogen depletion were evident. Ultrastructural analysis revealed mitochondrial swelling, shortened cristae, diminished rough endoplasmic reticulum, and increased lysosome size and number in aged control mice.

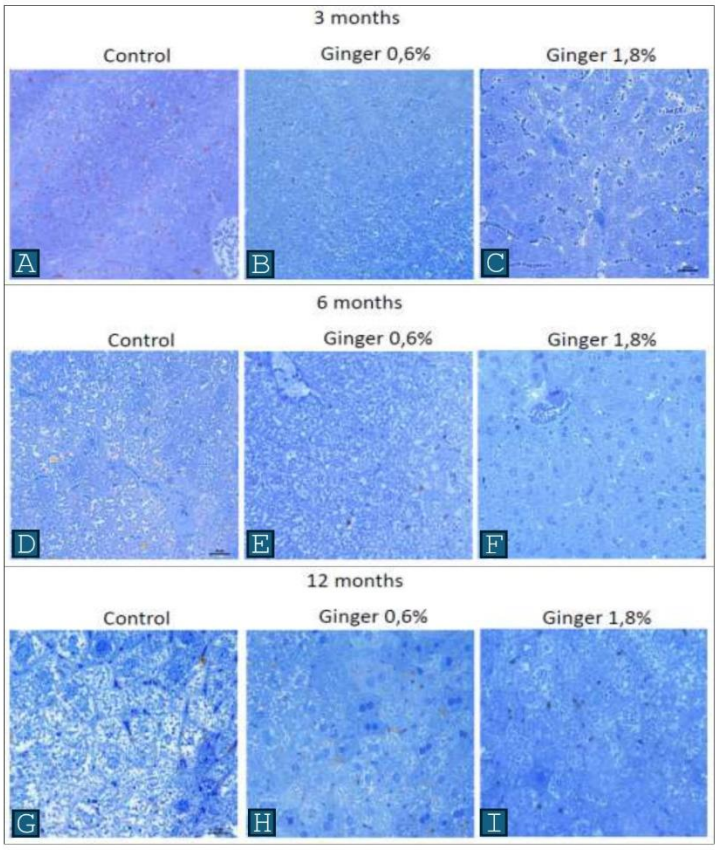

Figure 9 Histological evaluation of liver tissue architecture in mice across age and dietary groups. Representative light microscopy images of liver sections stained with Sudan III and aniline blue, highlighting morphological features across experimental conditions. The upper panel shows 3-month old mice: (A) control, (B) 0.6% ginger-supplemented, (C) 1.8% ginger-supplemented. The middle panel depicts 6-month-old mice: (D) control, (E) 0.6% ginger-supplemented, (F) 1.8% ginger-supplemented. The lower panel includes 12-month-old mice: (G) control, (H) 0.6% ginger-supplemented, (I) 1.8% ginger-supplemented. Notable features include hepatocyte morphology, lipid droplet accumulation, and sinusoidal organization. Scale bar = 20 μm

Ginger supplementation mitigated age-related structural changes. In 3-month-old mice, both 0.6% and 1.8% ginger preserved normal liver morphology. Mild mitochondrial enlargement and occasional RER depletion were noted only in high-dose animals. At 6 months, 0.6% ginger maintained hepatic architecture, while 1.8% supplementation was associated with greater cytoplasmic variability and reduced organelle density. In 12-month-old mice, 0.6% ginger resulted in modest ultrastructural abnormalities, whereas 1.8% ginger induced substantial mitochondrial swelling, organelle loss, and vacuolated cytoplasm, although without apparent tissue disruption.

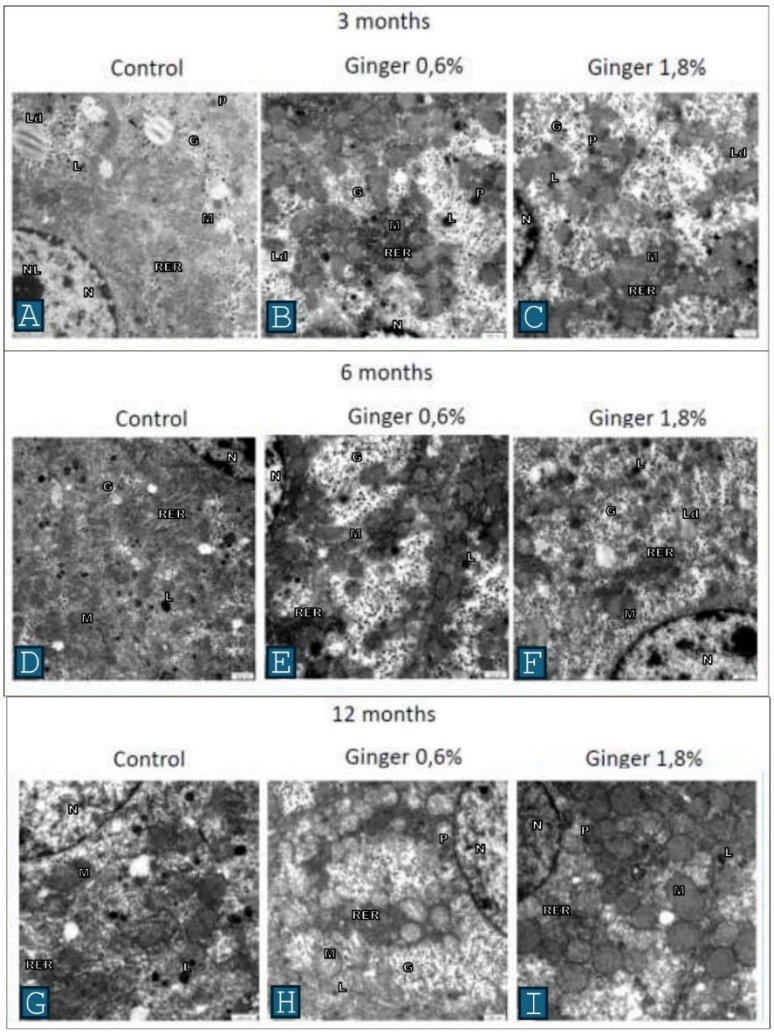

Figure 10 Transmission electron micrographs showing age- and diet-associated ultrastructural alterations in hepatocytes. Electron micrographs of liver sections from mice at different ages and treatment groups. The upper panel illustrates 3-month-old mice: (A) control, (B) 0.6% ginger-supplemented, (C) 1.8% ginger-supplemented. The middle panel shows 6-month-old mice: (D) control, (E) 0.6% ginger-supplemented, (F) 1.8% ginger-supplemented. The lower panel presents 12-month-old mice: (G) control, (H) 0.6% ginger-supplemented, (I) 1.8% ginger-supplemented. Labeled organelles include lipid droplets (Ld), lysosomes (L), mitochondria (M), damaged mitochondria (dM), nucleus (N), nucleolus (NL), peroxisomes (P), rough endoplasmic reticulum (RER), and vacuoles (V). Scale bar = 1000 nm

6.2.2 Lysosomal Enzyme Activity

Age-related increases in several lysosomal enzymes were evident. As detailed in Publication 3, (Table 2 of Publication 3), Arginyl aminopeptidase (ArgAP) displayed a progressive rise

from young to old control animals. Acid phosphatase (ACP) and α-galactosidase (AGAL) activities were also elevated with age. Leucyl aminopeptidase (LeuAP) remained unchanged across age groups. Moderate ginger supplementation (0.6%) enhanced ArgAP and AGAL activities in 3-month-old mice, while high-dose (1.8%) produced attenuated or unchanged responses.

In 6-month-old animals, 0.6% ginger increased ACP and AGAL while reducing ArgAP activity. The 1.8% dose produced similar but less pronounced effects. In 12-month-old mice, both ginger doses led to stable or reduced enzyme activities compared to controls, with slight reductions in ArgAP and ACP, suggesting a regulatory effect rather than stimulation.

6.2.3 PI3K and TNF-α Gene Expression

Age progression was associated with increased hepatic expression of PI3K and TNF-α in control mice (as shown in Table 2, Publication 3). Ginger supplementation significantly suppressed PI3K expression in a dose-dependent manner across all age groups. The 1.8% dose had the strongest suppressive effect. TNF-α expression was also reduced in ginger-treated animals, with specifically the 0.6% dose producing the strong reductions in 6- and 12-month-old mice.

6.2.4 Regression and Correlation Analyses

To explore potential relationships between lysosomal enzyme activities and molecular markers of inflammation and nutrient sensing, a Pearson correlation heatmap was generated using biochemical and gene expression data from liver samples (Figure 11). The heatmap visualizes pairwise correlation coefficients among key lysosomal enzymes (e.g., AlaAP, ArgAP, AGAL, BGAL, BGLU), acid phosphatase (ACP), hexosaminidase (HEX), and molecular markers including PI3K and TNF-α.

Overall, a high degree of positive correlation was observed among many of the lysosomal hydrolase enzymes, indicating possible co-regulation or convergent activity within the lysosomal degradation system. For instance, LeuAP, ArgAP, and AGAL exhibited strong correlations ($R > 0.70$) with each other, while BGLU, BGAL, and MAN also formed a highly interrelated cluster ($R = 0.59–0.68$), suggesting functionally synchronized lysosomal

processes. Similarly, ACP showed moderate positive correlations (R = 0.45–0.49) with several enzymes, including AlaAP and BGAL.

Importantly, PI3K gene expression was negatively correlated with several lysosomal enzymes, particularly HEX (R = –0.28) and, AGLU (R = –0.29) suggesting an inverse relationship between PI3K signaling and lysosomal activity. This pattern may reflect nutrient-sensing pathway suppression of lysosomal function, which is commonly seen in aging or metabolic dysregulation. A similar inverse trend was observed between PI3K and TNF-α (R= –0.05), though not statistically strong.

TNF-α expression, a marker of inflammatory signaling, showed generally weak or negative correlations with lysosomal enzymes. The strongest negative correlation was with BGRD (R= –0.36), suggesting that inflammatory activation may be associated with suppressed lysosomal repair or turnover capacity. Correlations between TNF-α and other enzymes were modestly negative (e.g., with AlaAP, R= –0.29; with BGLU, R = –0.25), indicating potential interplay between chronic inflammation and impaired degradative functions in the aging liver.

Collectively, these results highlight a coordinated regulation among lysosomal enzymes and suggest that elevated PI3K signaling and inflammatory tone may inversely affect lysosomal integrity and function. This network-level interaction provides insight into the molecular basis of age-related lysosomal decline and potential targets of ginger-mediated modulation.

Linear regression revealed strong associations between age and enzyme activities. ArgAP (R^2 = 0.70) and AGAL (R^2 = 0.60) showed the clearest age-related trends. ACP, HEX, and BGAL also correlated positively with age. TNF-α exhibited a weaker but significant positive age association ($R^2 \approx 0.13$). PI3K expression did not correlate significantly with age but decreased strongly with increasing ginger dose ($R^2 = 0.61$, $p < 0.001$).

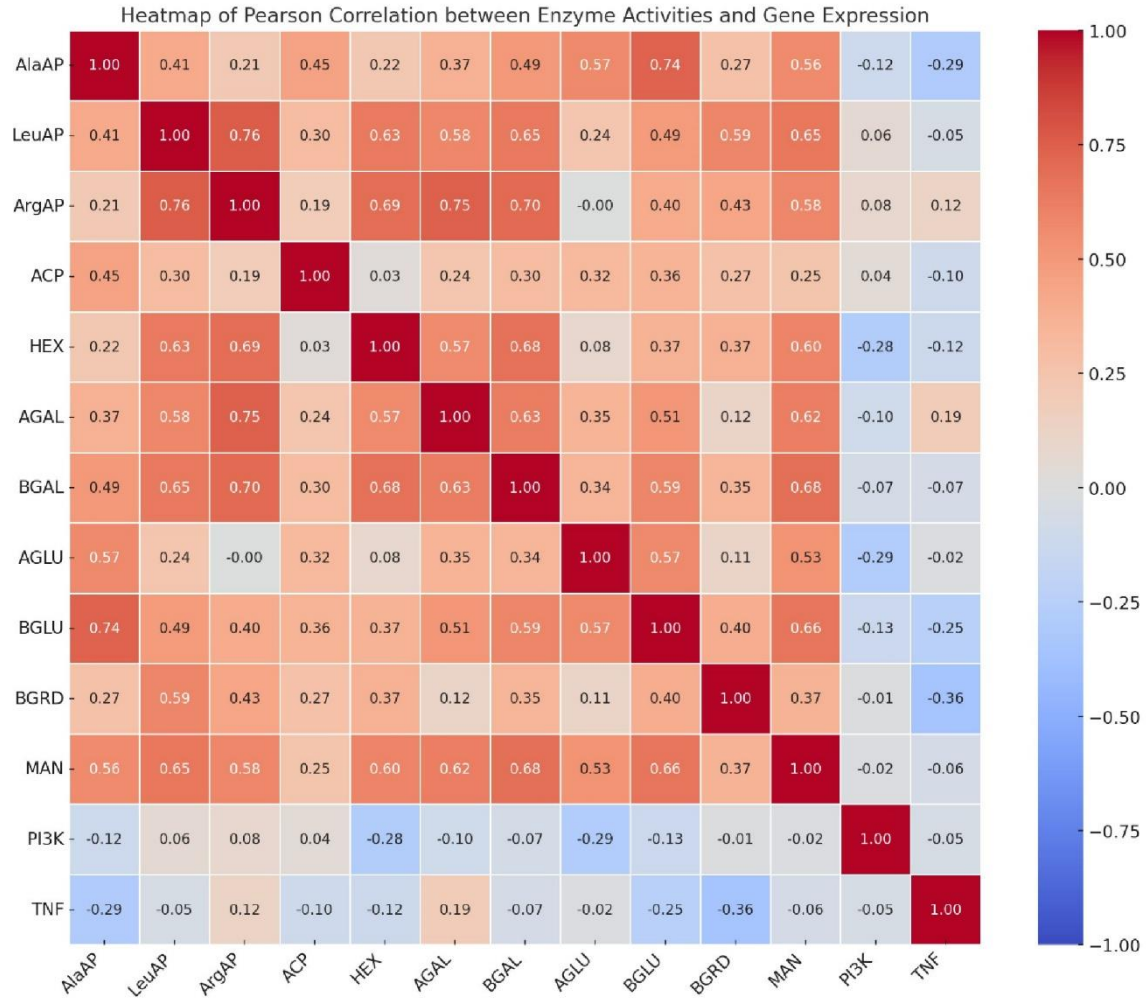

Figure 11 Correlation heatmap of biochemical and molecular markers in hepatic tissue. Heatmap illustrating Pearson correlation coefficients among key lysosomal enzymes (e.g., ArgAP, AGAL, ACP), inflammatory marker TNF-α, and PI3K gene expression. Positive correlations are depicted in red and negative correlations in blue, with intensity reflecting correlation strength. The map highlights co-regulated enzymatic activities and the inverse relationships between PI3K expression and both lysosomal and inflammatory markers, suggesting coordinated responses in aging and ginger-mediated modulation.

7 Discussion

Aging is a multifactorial process, and interventions that concurrently influence multiple aging mechanisms are thought to be especially promising for promoting healthspan (López-Otín et al., 2013a). Notably, ginger's effects were particularly well-substantiated in preclinical models for several key hallmarks: deregulated nutrient sensing, mitochondrial dysfunction, chronic inflammation, and dysbiosis. Taken together, the evidence from our experiments suggest that ginger addresses comprehensively diverse metabolic and molecular drivers of aging (Matin et al., 2025b, 2025a). In alignment with this, a study by Habib et al. (2008), demonstrated that ginger extract significantly reduced lipid peroxidation and enhanced hepatic glutathione levels in aged rats, indicating its potent role in oxidative stress mitigation: one of the major contributors to mitochondrial dysfunction and cellular aging (Habib et al., 2008). Notably, these prior studies align well with the results of our research (especially regarding the level of GSH and MDA) involving ginger supplementation in mice (Matin et al., 2025b). Furthermore, our literature review highlights that ginger exerts anti-inflammatory effects across numerous studies and models, which is pertinent because chronic low-grade inflammation (so-called "inflammaging") is a major contributor to age-related tissue damage (Matin et al., 2024).

Preclinical evidence from naturally aged rodent models of chronic inflammation supports the anti-inflammatory activity of ginger-derived constituents. In naturally aged animals, 6-shogaol, a major bioactive component of ginger, attenuated aging-associated inflammation and endoplasmic reticulum stress in 25-month-old mice, indicating a mitigation of inflammatory stress that accompanies physiological aging (Eo et al., 2025). Consistently, our own ultrastructural analyses provide morphological support for ginger-mediated modulation of age-associated cellular stress in the liver. Aging control mice exhibited typical ultrastructural alterations, including mitochondrial swelling, disorganization of the rough endoplasmic reticulum, cytoplasmic vacuolization, and increased lysosomal structures (Matin et al., 2025a). Ginger supplementation modified these features in an age- and dose-dependent manner, with moderate dosing better preserving hepatocyte ultrastructure, particularly in young and middle-aged animals. These observations indicate that ginger-associated attenuation of inflammatory stress is accompanied by measurable changes at the subcellular level (Matin et al., 2025a). In line with these findings, in a chronic inflammatory rat model, administration of ginger extract significantly reduced systemic and tissue inflammatory

signaling, including lower levels of TNF-α, IL-6 and IL-17, as well as decreased NF-κB–related signaling markers, thereby reinforcing the capacity of ginger preparations to modulate pro-inflammatory pathways implicated in inflammaging (Öz et al., 2021).

Another noteworthy aspect of ginger's biological activity is its capacity to ameliorate mitochondrial dysfunction, a key hallmark of cellular aging that is closely linked to oxidative stress and impaired cellular energy production (Li et al., 2019). Available evidence indicates that ginger and its bioactive constituents may help restore mitochondrial homeostasis by enhancing respiratory performance and ATP generation, stabilizing mitochondrial membrane potential, and decreasing mitochondrial reactive oxygen species (ROS) production, thereby supporting energy metabolism in aging cells (Deng et al., 2019). Experimental studies indicate that ginger and its phenolic constituents (including 6-gingerol, 6-shogaol, and related compounds) enhance antioxidant defenses and attenuate oxidative damage across diverse model systems (Bak et al., 2012). Proposed mechanisms include upregulation of endogenous antioxidant capacity (e.g., glutathione and antioxidant enzymes) together with direct or indirect reductions in reactive oxygen species (ROS), which may help preserve mitochondrial integrity under age-associated oxidative stress. In animal models, ginger supplementation has repeatedly been reported to lower indices of lipid peroxidation and markers of oxidative DNA damage (Mohd Sahardi and Makpol, 2019). In our study, a comparable antioxidant pattern was observed, however in an age-dependent manner (Matin et al., 2025b). Ginger supplementation significantly reduced lipid peroxidation, reflected by lower hepatic malondialdehyde levels, primarily in young adult mice, whereas in middle-aged and older animals lipid peroxidation was less responsive. In contrast, non-enzymatic antioxidant defenses—most notably glutathione—were consistently enhanced across age groups, indicating that in the aging liver ginger preferentially supports intracellular redox buffering rather than uniformly suppressing lipid oxidation (Matin et al., 2025b). By limiting macromolecular injury and preserving mitochondrial function, ginger-based interventions may sustain cellular energy metabolism and reduce the accumulation of oxidative lesions processes implicated in aging and the pathogenesis of degenerative diseases.

The cellular quality-control and protein/organelle turnover hallmark of aging is also potentially influenced by ginger. Emerging studies by others indicate that ginger compounds can activate lysosome-dependent degradation pathways and molecular systems involved in maintaining intracellular homeostasis (Matin et al., 2025a). For instance, 6-gingerol has been

reported to induce autophagy in human endothelial cells, protecting them from apoptosis (Wang et al., 2016). Enhanced autophagic clearance of damaged organelles and proteins would be beneficial in aging, since impaired autophagy is a known driver of cellular senescence and functional decline (Rubinsztein et al., 2011). Ginger's ability to modulate autophagy aligns with its observed effects on nutrient-sensing pathways like PI3K/Akt/mTOR (which ordinarily suppress autophagy when overactive) (Saxton and Sabatini, 2017). Taken together, these insights by others reinforce the idea that ginger can engage multiple protective processes, ranging from improving metabolic homeostasis to facilitating cellular clean-up of damage, thereby holistically supporting longevity. In line with these reports, our experimental data indicate that ginger supplementation modulates lysosome-associated processes in the aging liver in an age- and dose-dependent manner. Specifically, ginger altered the activity of lysosomal hydrolases and aminopeptidases that are involved in intracellular protein and organelle turnover, with moderate dosing tending to normalize age-associated elevations rather than uniformly stimulating degradation (Matin et al., 2025a). This pattern is consistent with a regulatory, rather than purely activating, effect on cellular quality-control systems, aligning our *in vivo* observations with prior reports of ginger-mediated modulation of autophagy-related pathways.

Ginger's broad spectrum of action parallels that of other geroprotective phytochemicals (such as resveratrol, curcumin, and green tea catechins), which likewise target overlapping aging pathways to help maintain healthy aging. A compelling aspect in support of this concept comes from rodent studies demonstrating that ginger supplementation modulates age-associated biological processes**.** In aged rats, it was demonstrated that ginger treatment counteracted age-related metabolic dysregulation**,** reversing alterations in pathways such as glutathione and nicotinamide metabolism that are commonly disrupted with aging (Ekeuku et al., 2024). Consistent with these observations, our experiments demonstrated that dietary ginger supplementation significantly enhanced hepatic glutathione levels across all age groups, with the most pronounced effects in older mice, while also improving overall antioxidant capacity (Matin et al., 2025b). These findings indicate that ginger can partially restore age-associated disturbances in redox-related metabolic pathways in the liver, supporting the relevance of glutathione-centered metabolic modulation observed in other aging models. From a biological standpoint, if an intervention consistently attenuates multiple aging-related processes in rodent models, such as oxidative stress, inflammatory burden, and

metabolic dysregulation. These results by us and others suggest that it could potentially contribute to delayed onset of age-related functional decline and improve healthspan in mammals.

A synthesis of the available literature indicates ginger's geroprotective potential; however, it also acknowledges important limitations and gaps that temper the implications. The beneficial effects of ginger on aging hallmarks have been demonstrated largely in cell culture and animal models; in contrast, clinical validation is limited (Matin et al., 2024). With the exception of a few endpoints – such as improved glucose control (nutrient sensing), reduced inflammation, and altered microbiota in small trials – there is lack of human studies directly assessing ginger's impact on aging-related outcomes. Moreover, the dosages and formulations of ginger vary widely across studies, which complicates interpretation. The literature on ginger encompasses a wide range of preparations, from whole ginger powder to isolated compounds, at doses ranging from dietary spice levels to concentrated extracts. This heterogeneity means that more work is needed to identify the optimal form and dose of ginger for geroprotection. Indeed, a theme that emerges is likely the dose-dependent nature of ginger's effects, possibly reflecting adaptive-stress response action. Low or moderate doses might stimulate adaptive stress responses (e.g. upregulating endogenous antioxidants or repair mechanisms), whereas excessive doses could paradoxically cause cellular stress or no added benefit (Calabrese and Kozumbo, 2021; Mattson, 2008). Specifically, the synthesis of available evidence indicated that ginger's pleiotropic bioactivities while potentially beneficial may not be uniformly advantageous across contexts and could require careful balancing to achieve an overall favorable effect on aging-related outcomes.

The key finding was that aging is associated with a decline in the liver's antioxidant capacity – evidenced by lower radical scavenging activity, total antioxidant capacity, vitamin C, phenolic content, and enzyme activities in older control mice – and that ginger supplementation can counteract some of these declines. However, the benefits of ginger were markedly more pronounced in older animals and at higher doses, whereas young mice showed minimal or even paradoxical reverse responses in certain parameters. This indicates that ginger acts as a context-dependent modulator of redox homeostasis. From a biological perspective, this aligns with the concept that antioxidant interventions yield greater benefits in aged or oxidatively stressed organisms, but might be redundant or disruptive in young,

healthy ones because manipulations that reduce oxidative stress tend to produce measurable effects on age-related pathology and healthspan primarily under chronic stress or pathological conditions rather than in optimal physiological states (Salmon et al., 2010)**.** Young mice (3 months old) naturally have robust antioxidant systems; introducing ginger at this life stage did not further boost some antioxidant metrics and in the case of DPPH free radical scavenging, actually led to a slight decrease (Matin et al., 2025b). This unexpected result (the decreased DPPH free radical scavenging in 3-months old mice) can be interpreted through the lens of adaptive stress response – a mild pro-oxidant challenge by ginger in youth could transiently lower measurable scavenging activity, potentially as a trade-off for triggering longer-term adaptive responses. By contrast, in middle-aged and older mice (6 and 12 months), ginger supplementation significantly elevated antioxidant capacities (e.g., higher DPPH scavenging and glutathione levels). These animals have accumulated oxidative damage and weaker baseline defenses, so ginger's rich antioxidant compounds and possible liver antioxidant pathways activation can provide a noticeable boost. This interpretation is supported by the consistent age-dependent differences observed in baseline antioxidant parameters in control animals, including reduced total antioxidant capacity and glutathione levels in older mice, together with the selective responsiveness of these parameters to ginger supplementation in the same age groups (Matin et al., 2025b). The absence of comparable improvements in young mice, despite identical dietary exposure, further indicates that ginger's antioxidant effects become apparent primarily under conditions of age-related redox imbalance. This finding has important implications: it suggests that age-personalized nutritional interventions could be optimal, wherein supplements like ginger might be reserved for or dosed higher in older individuals for whom such interventions may offer the greatest potential advantage.

Moreover, delving deeper into the mechanisms, the study points out that ginger's antioxidant effects were primarily observed in non-enzymatic antioxidant parameters. Notably, ginger at 1.8% of the diet raised hepatic reduced glutathione (GSH) content across all age groups, with the largest increases in older mice. GSH is a critical intracellular antioxidant, and its elevation signifies enhanced capacity to neutralize reactive oxygen species and detoxify peroxides. The increase in GSH could be due to ginger's bioactive phenolics triggering the Nrf2 regulatory pathway, which in turn upregulates glutathione synthesis and related phase II enzymes – a mechanism well documented for many polyphenols (Ayustaningwarno et al., 2024). Additionally, ginger contains vitamin C and polyphenolic compounds that could contribute to

the overall antioxidant capacity (Mustafa and Chin, 2023). The higher total phenolic content detected in the livers of ginger-fed mice may reflect bioavailability-driven tissue deposition and/or stimulation of endogenous phenolic pathways. Interestingly, ginger did not significantly change superoxide dismutase (SOD) activity in the liver, despite SOD being a key antioxidant enzyme that typically declines with age (as was seen in the control mice) (Matin et al., 2025b). The lack of SOD upregulation by ginger is not necessarily a negative finding; rather, it suggests that ginger's hepatoprotective action may rely more on boosting small-molecule antioxidants (GSH, vitamins, etc.) and possibly other enzymes like catalase or glutathione peroxidase, rather than on SOD itself (Ayustaningwarno et al., 2024). Another possibility is that ginger's effects on SOD are isoform-specific and were masked in the total SOD assay. For instance, some evidence indicates ginger might preferentially increase mitochondrial SOD2 activity, which a bulk assay might not detect if cytosolic SOD1 dominates (Matin et al., 2025b). Another explanation is that because ginger raised GSH and other antioxidants, the oxidative burden (especially superoxide levels) was reduced, so there was less need for SOD induction. Along this line, similar dissociation between enhanced glutathione-based redox buffering and unchanged SOD activity has been reported in other experimental studies involving polyphenol-rich interventions (Yokozawa et al., 2004). This interpretation portrays ginger as effectively rebalancing the redox network – enhancing certain antioxidant pathways that compensate for age-related losses, while alleviating the pressure on other pathways. Such insight is valuable because it cautions that measuring a single antioxidant enzyme in isolation may miss the broader redox adjustments happening in response to a nutraceutical.

A particularly thought-provoking outcome from this study was the effect of ginger on lipid peroxidation, measured via malondialdehyde (MDA) levels in the liver. High-dose ginger significantly reduced MDA levels in young adult mice, confirming ginger's ability to suppress lipid oxidative damage, consistent with prior reports in toxin-induced oxidative stress models (Moulima Maity et al., 2025; Nour El Deen et al., 2025). However, in middle-aged and older mice, ginger's effects on MDA were smaller and did not reach statistical significance. The latter finding might suggest diminishing returns of ginger's anti-lipid-peroxidation effect with advancing age. Surprisingly, baseline MDA levels were highest in 3-month-old control mice, and lower in 6- and 12-month controls. In young mice, at the peak of metabolic activity, generate a surge of reactive oxygen species that preferentially attack lipids – hence higher

MDA in young controls. As mice age further, their metabolism slows and their cellular composition changes; older tissues might display better correlation with protein and DNA oxidation rather than with lipid peroxidation (Sivoňová et al., 2007). This could explain why MDA did not rise with age, and why ginger's ability to lower MDA was most evident in the young group (where there was more lipid peroxidation to mitigate). The implication is that oxidative stress in aging is a moving target – different biomolecules become the primary victims of oxidation at different life stages (lipids earlier, proteins/DNA later) (Sivoňová et al., 2007). Therefore, an intervention like ginger might show age-specific efficacy depending on which aspect of oxidative damage is predominant. Nonetheless, the reduction in MDA levels following ginger treatment in young mice (Matin et al., 2025b) is biologically meaningful, as MDA is a direct end-product of lipid peroxidation and therefore a reliable marker of oxidative membrane damage (Oboh et al., 2012).The observed decrease in MDA indicates attenuated peroxidative chain reactions in membrane lipids, confirming a protective effect of ginger on cellular integrity.

Importantly, this reduction in lipid peroxidation is inversely related to the elevation of glutathione (GSH), a central intracellular antioxidant (Chuljerm et al., 2025; Mairuae et al., 2024) . GSH plays a dual protective role: it directly scavenges reactive oxygen species and, more critically, serves as a cofactor for glutathione peroxidase in the detoxification of lipid hydroperoxides before they decompose into secondary products such as MDA. Thus, increased GSH availability shifts the redox balance toward effective peroxide neutralization, thereby limiting MDA formation at its source.

In addition, ginger-derived chain-breaking antioxidants such as gingerols and shogaols can intercept lipid peroxyl radicals, further reducing oxidative stress and lipid peroxidation (Mashhadi et al., 2013). Together, the concurrent decrease in MDA and increase in GSH reflects a coordinated antioxidant response in which ginger enhances endogenous redox buffering capacity while simultaneously preventing membrane lipid damage. Over the long term, reducing lipid peroxidation could translate to less liver injury and fibrosis, given that oxidized lipids can propagate inflammatory damage.

It is further noted that ginger's antioxidant and hepatoprotective properties observed in mice are congruent with prior animal studies of ginger. For example, research by Ahmed *et al.* (2008) showed that dietary ginger protected rats from chemical-induced oxidative stress by

lowering MDA and boosting antioxidant enzymes, aligning well with the results of our study (Ahmed et al., 2008). Moreover, the dose-dependent efficacy observed (with 1.8% ginger yielding stronger effects than 0.6% in many measures) resonates with the idea that a threshold amount of bioactive compounds is needed to significantly impact systemic antioxidant status. This is a practical insight for translating to humans: the typical culinary intake of ginger might be too low to impart measurable anti-aging benefits, whereas a higher supplemental dose could be necessary. However, an important consideration discussed is the potential for adverse effects at very high doses. Previous studies indicate a dose-dependent adaptive response, whereby moderate intake appears to maximize antioxidant benefits while minimizing the risk of pro-oxidant or stress-related effects (Calabrese, 2014; Rattan, 2008). This ties back to the adaptive stress response paradigm: ginger's phenolics might act as mild stressors that upregulate defenses, but too large an amount could overwhelm adaptive capacity (Bondy, 2023; Mattson, 2008). From a gerontological standpoint, this means dosing of nutraceuticals should be carefully calibrated, especially for older individuals who may have reduced resilience to physiological stress.

The investigation to histological, ultrastructural, and molecular markers of liver aging, further provided a multidimensional view of how ginger supplementation influences hepatic aging (Matin et al., 2025a). A central observation was that normal aging in the control mice led to progressive degeneration of liver structure and function, evidenced by hepatocellular hypertrophy, fat accumulation, lipofuscin deposition, nuclear irregularities, and organelle deterioration (mitochondrial swelling, loss of rough ER) in older animals. These classic age-related changes, also reported by Terman and Brunk (2004) and Hunt *et al.* (2019), reflect declining autophagic clearance and metabolic dysregulation in senescent hepatocytes (Hunt et al., 2019; Terman and Brunk, 2004). Ginger supplementation markedly modulated these aging phenotypes. At a moderate dietary dose (0.6%), ginger effectively preserved youthful hepatic morphology in both young and middle-aged mice – their livers retained healthier cellular architecture with fewer aging defects compared to age-matched controls. This suggests that ginger can mitigate the onset of structural aging markers, in parallel with its experimentally observed effects on hepatic redox balance and inflammatory signaling, including reduced lipid peroxidation (MDA), improved total antioxidant capacity and glutathione levels, and attenuation of TNF-α expression (Matin et al., 2025b, 2025a). However, in the oldest mice, the higher ginger dose (1.8%) was associated with more

pronounced cellular changes (some mild hepatocyte ultrastructural stress) than the moderate dose. Rather than a straightforward harmful effect, this is interpreted as evidence of an adaptive stress response: a higher dose of ginger in already aged livers might induce a beneficial low-level stress response that pushes cells towards cleanup and renewal, manifesting as transient morphological alterations. Such dose-sensitive behavior is common in geroprotective nutraceutical research. In line with the research by Bondy (2023) and Caruso *et al.* (2024), many phytochemicals demonstrate biphasic effects – low-to-moderate doses confer benefits by engaging stress resistance pathways, whereas excessive doses can be counterproductive or toxic (Bondy, 2023; Caruso et al., 2024). In practical terms, this finding reinforces the need to determine an optimal ginger dose for aging interventions. This also resonates with our experimental findings demonstrating that moderate ginger supplementation was sufficient to improve key non-enzymatic antioxidant parameters, including total antioxidant capacity and glutathione levels, whereas higher dosing did not yield proportional additional benefits across all antioxidant readouts (Matin et al., 2025b). In the context of aging, an older organism's diminished stress resilience means a gentler intervention could be more efficacious than high dose.

On the cellular and molecular level, our work provides important insights into how ginger modulates aging pathways in the liver. One major focus was on the lysosomal enzyme activities (e.g., aminopeptidases, acid phosphatase, glycosidases) which are integral to intracellular degradation, protein turnover, and cellular quality control systems (Ajit Varki and Richard D. Cummings, n.d.; Kos et al., 2022). Aging is commonly associated with progressive lysosomal dysfunction. Early in this process, lysosomal enzyme activity may increase as a compensatory response to the rising burden of damaged macromolecules and organelles (e.g., lipofuscin accumulation and impaired autophagic flux) (Guerrero-Navarro et al., 2022; Nixon, 2020). With advancing age, however, lysosomal capacity can become compromised, leading to reduced hydrolase activity and/or lysosomal membrane permeabilization, which promotes aberrant release of cathepsins and other hydrolases into the cytosol (He et al., 2024). In the control mice, the study observed that older livers indeed had elevated activities of certain lysosomal enzymes, likely a compensatory response to heightened damage and impaired lysosome-dependent degradation pathways. Ginger's effects on these enzymes were age-specific: in young mice, ginger enhanced lysosomal enzyme activity above baseline, whereas in old mice, ginger limited the excessive lysosomal activity.

This bidirectional modulation is highly significant. In youth, a slight boost in lysosomal activity could improve the removal of damaged proteins and organelles, preventing their accumulation early on – essentially, ginger primes the cellular degradation and recycling machinery system when it's still relatively efficient. This finding parallels the idea that initiating pro-cleanup mechanisms earlier in life can delay aging manifestations. In the elderly livers, by contrast, lysosomal enzymes were already overactive (a sign of cellular distress), and ginger brought them to a more normalized level. Notably, this normalization of lysosomal enzyme activity in aged livers occurred alongside improvements in hepatic redox status, particularly increased glutathione levels and total antioxidant capacity, suggesting that reduced oxidative burden may contribute to stabilizing lysosome-dependent degradation processes (Matin et al., 2025b). Controlling excessive lysosomal activity is beneficial because hyperactive or engorged lysosomes in aged cells can lead to membrane permeabilization and release of hydrolases, exacerbating cell damage (Brunk and Terman, 2002). It's worth noting that β-galactosidase is also a common marker of cellular senescence (Dimri et al., 1995); the fact that ginger influenced its activity hints that ginger might reduce cellular senescence burden in the liver (Matin et al., 2025a). Nevertheless, these lysosomal enzyme activity results already place ginger as a modulator of the age-associated decline in intracellular clearance pathways**,** complementing the antioxidant findings and tying back to what our review (Matin et al., 2024) postulated about ginger's capacity to stimulate cellular degradation and recycling systems**.**

Another key molecular finding is ginger's effect on the PI3K/Akt/mTOR signaling pathway, a central regulator of aging and metabolism (Yeung et al., 2018). The study measured hepatic gene expression of phosphoinositide 3-kinase (PI3K), a kinase upstream of Akt and mTOR. In untreated aging, PI3K expression was found to increase with age in the liver (though some literature notes tissue-specific patterns, with age-related PI3K activity changes being complex). Consistent with pro-aging changes, higher PI3K/Akt signaling in older livers would promote anabolic processes and inhibit lysosome-dependent cellular clearance and recycling pathways, contributing to age-related metabolic dysfunction. In line with this concept, our experimental data show that ginger-mediated suppression of hepatic PI3K expression was accompanied by coordinated changes in lysosomal enzyme activities, including age-dependent modulation of aminopeptidases and glycosidases involved in intracellular protein turnover, consistent with a shift toward improved regulation of lysosome-dependent clearance

rather than excessive degradative activation (Matin et al., 2025a). Ginger supplementation caused a significant suppression of PI3K expression in older mice, particularly at the 1.8% dose. This aligns ginger with known longevity-promoting interventions: attenuating the PI3K/Akt/mTOR pathway is a well-established strategy to extend lifespan in diverse organisms. For instance, genetic or pharmacological inhibition of PI3K or mTOR can delay aging and diseases in mice (Abdellatif et al., 2022; Hedges et al., 2023). By downregulating PI3K, ginger likely alleviates the age-related hyperactivation of insulin/IGF-1 signaling, which could in turn enhance autophagy and stress resistance in liver cells. The discussion points out that, molecular mechanism underlying ginger's effects is supported by independent evidence on its bioactive constituents. Specifically, both 6-gingerol and 6-shogaol have been reported to attenuate PI3K/Akt signaling and to promote autophagy across multiple cellular models (Hung et al., 2009; Matin et al., 2024). For example, Pei *et al.* (2021) reported that 6-shogaol inhibited the PI3K/Akt/mTOR cascade in cancer cells, and Wang *et al.* (2016) found 6-gingerol induced autophagy to protect endothelial cells (Pei et al., 2021; Wang et al., 2016). Thus, the PI3K suppression by ginger in aged liver provides a molecular link between ginger intake and pro-longevity signaling pathways. It suggests that ginger can engage signaling patterns similar to other longevity-associated interventions, potentially fostering a metabolic state conducive to lifespan extension (lowered insulin signaling, enhanced cleanup via autophagy). It's particularly noteworthy that this effect was more pronounced at the higher dose – indicating dose responsiveness – but one must recall the earlier consideration: the high dose also introduced mild cellular stress in oldest mice. This implies that although high-dose ginger was associated with beneficial biochemical signaling changes, such as suppression of PI3K expression and modulation of lysosomal enzyme activities, these effects coincided with ultrastructural indicators of cellular stress in aged livers, including mitochondrial swelling, cytoplasmic vacuolization, and reduced organelle organization, suggesting an upper threshold beyond which structural integrity may be compromised (Matin et al., 2025a). Therefore, an intermediate dose might achieve PI3K/Akt pathway modulation with minimal side effects, which is a hypothesis future studies should test. In this context, inflammatory signaling followed a comparable dose-dependent pattern. Age-associated elevation of hepatic TNF-α expression in control mice was attenuated by ginger supplementation, with the most pronounced suppression observed at the intermediate (0.6%) dose in older animals. This parallels the dose range in which PI3K expression was reduced and lysosomal enzyme activities were more tightly regulated, suggesting that moderate ginger intake simultaneously

dampens pro-aging metabolic and inflammatory signaling while avoiding ultrastructural stress evident at higher doses (Matin et al., 2025a). Regardless, the clear outcome is that, ginger reduced age-associated inflammation in the liver. This is in line with ginger's well-documented anti-inflammatory properties: gingerols and related compounds inhibit NF-κB and COX-2 pathways (Grzanna et al., 2005), thereby lowering the production of cytokines like TNF-α and IL-6 (Mashhadi et al., 2013). In the context of the liver, reduced TNF signaling is highly relevant. Excess TNF-α is known to drive insulin resistance, hepatocyte injury, and activation of stellate cells that lead to fibrosis (Kakino et al., 2018). Therefore, ginger's attenuation of TNF-α suggests a protective effect against inflammatory liver damage and metabolic derangements. By lowering inflammatory stress, ginger helps maintain tissue integrity and could delay the progression of age-related liver diseases. Along this line, it is interesting to note that there was a lack of positive correlations between the tested genes and the activity of degradative enzymes. This anti-inflammatory action complements the antioxidant and effects on autophagy and lysosomal function, suggesting ginger as a versatile geroprotective agent for the liver. It also reflects a recurring theme: interventions that suppress chronic inflammation (whether through diet, drugs like aspirin, or supplements) have been associated with healthier aging profiles. Ginger falls into that category, and the findings by others also demonstrate inhibition of key inflammatory mediators *in vivo* (Shen et al., 2022) . Notably, these results echo human data that we summarized in our review article (Matin et al., 2024), pointing out that ginger supplementation lowered systemic inflammatory markers in older adults with metabolic conditions.

8 Summary and Conclusion

The obtained results, supported by a comprehensive literature analysis and complementary *in vivo* investigations to evaluate ginger (*Zingiber officinale*) as a nutraceutical intervention targeting age-associated liver alterations. The experimental findings demonstrate that hepatic aging in mice is characterized by progressive disturbances in redox balance, lysosomal enzyme regulation, inflammatory signaling, and ultrastructural integrity. Dietary ginger supplementation modulated these processes in a dose- and age-dependent manner, indicating that its biological effects are context-specific rather than uniform across the lifespan.

In relation to the stated aims and hypotheses, these findings demonstrate that ginger supplementation fulfills its intended role as an age- and dose-dependent modulator of hepatic redox homeostasis rather than a universal antioxidant enhancer. The data support the hypothesis that ginger is most effective under conditions of age-associated oxidative imbalance, where endogenous defenses are compromised, while providing limited or no benefit in young animals with intact antioxidant capacity. This context-dependent response underscores that ginger's action is not simply additive but adaptive, aligning with the objective of identifying nutraceutical interventions that selectively counteract aging-related functional decline rather than indiscriminately altering redox status across the lifespan.

Beyond redox modulation, the study further shows that ginger supplementation influences lysosome-associated enzyme activities and inflammatory signaling, including attenuation of age-related TNF-α expression and suppression of PI3K pathway activation. Importantly, these molecular and biochemical changes were accompanied by measurable alterations at the ultrastructural level, supporting the concept that ginger-mediated modulation of metabolic and inflammatory pathways is reflected in hepatocellular organization. The observed dose sensitivity, particularly the differential effects between moderate and higher supplementation levels, highlights the importance of dosage optimization when considering translational applications.

Collectively, this work provides experimental evidence that ginger can modulate key processes implicated in hepatic aging, including oxidative imbalance, dysregulated degradation pathways, and inflammatory activation. While extrapolation to humans requires cautious interpretation and further investigation, the findings support the concept that dietary

phytochemicals such as ginger may contribute to strategies aimed at preserving liver function during aging.

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10 Attachments

10.1 Percentage contribution of authors to each publication

I hereby give the percentage of each author's contribution to the publications:

Matin M, Joshi T, Wang D, Tzvetkov N T, Matin F B, Wierzbicka A, Jóźwik A, Horbańczuk J O, Atanasov A G. Effects of Ginger (*Zingiber officinale*) on the Hallmarks of Aging. *Biomolecules* 2024.

Lp.	**Imię i nazwisko** ***(name and surname)***	**Opis udziału autora** ***(Description of the author's participation)***	**% udział w opracowaniu publikacji** ***(participation in the publication in %)***	**Jednostka** ***(Institution)***
1.	Maima Matin	Conceptualization, Writing-Original Draft	84	Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences, Jastrzębiec, 05-552 Magdalenka, Poland
2.	Tanuj Joshi	Writing-Original Draft	2	Department of Pharmaceutical Sciences, Bhimtal, Kumaun University, Nainital 263002, India
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4.	Nikolay T. Tzvetkov	Writing-Original Draft	2	Department of Biochemical Pharmacology and Drug Design, Institute of Molecular Biology "Roumen Tsanev", Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria
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Matin M, Wysocki K, Horbańczuk J O, Rossi L, Atanasov A G. Ginger (*Zingiber officinale*) dietary supplementation in mice regulates liver antioxidant defense systems in a dose- and age- dependent. *Front. Pharmacol*. 2025

Lp.	**Imię i nazwisko** ***(name and surname)***	**Opis udziału autora** ***(Description of the author's participation)***	**% udział w opracowaniu publikacji** ***(participation in the publication in %)***	**Jednostka** ***(Institution)***
1.	Maima Matin	Writing-Original Draft, Investigation, Methodology, Conceptualization, Formal Analysis, Project Administration, Data curation	84	Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences, Jastrzębiec, 05-552 Magdalenka, Poland
2.	Kamil Wysocki	Writing-Review and Editing, Formal Analysis, Investigation	4	Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences, Jastrzębiec, 05-552 Magdalenka, Poland
3.	Jarosław Olav Horbańczuk	Conceptualization, Writing-Review and Editing	4	Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences, Jastrzębiec, 05-552 Magdalenka, Poland
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5.	Atanas G. Atanasov	Writing-Review and Editing, Conceptualization	4	Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences, Jastrzębiec, 05-552 Magdalenka, Poland Ludwig Boltzmann Institute Digital Health and Patient Safety, Medical University of Vienna, Vienna, Austria

Matin M, Gładysinka M L, Ksepka N, Frazzini S, Wieczorek A, Jóźwik A. Ginger supplementation supports liver health during aging through regulation of lysosomal function and molecular markers, *Animal Science Papers and Reports,* 2025

Lp.	**Imię i nazwisko** ***(name and surname)***	**Opis udziału autora** ***(Description of the author's participation)***	**% udział w opracowaniu publikacji** ***(participation in the publication in %)***	**Jednostka** ***(Institution)***
1.	Maima Matin	Writing-Original Draft, Investigation, Methodology, Conceptualization, Formal Analysis, Project Administration, Experimental Analysis	75	Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences, Jastrzębiec, 05-552 Magdalenka, Poland
2.	Małgorzata Łysek-Gładysinska	Writing-Review and Editing, Formal Analysis, Investigation	5	Division of Medical Biology, Institute of Biology, Jan Kochanowski University in Kielce, Uniwersytecka 7, 25-406 Kielce, Poland
3.	Natalia Ksepka	Writing- Review and Editing, Experimental Analysis, Investigation	5	Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences, Jastrzębiec, 05-552 Magdalenka, Poland
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10.2 Statement of co-authorship

3. Opis udziału autora w opracowaniu publikacji *(Description of the author's participation in preparation of the publication)*:

Lp.	Imię i nazwisko *(name and surname)*	Opis udziału autora *(Description of the author's participation)*	% udział w opracowaniu publikacji *(participation in the publication in %)*	Podpis autora[2] *(author's signature)*
1.	Maima Matin	Conceptualization, Writing – original draft	84	
2.	Tanuj Joshi	Writing – original draft	2	
3.	Dongdong Wang	Writing – original draft	2	
4.	Nikolay T. Tzvetkov	Writing – original draft	2	
5.	Farhan Bin Matin	Writing – original draft	2	
6.	Agnieszka Wierzbicka	Writing – review & editing	2	
7.	Artur Jóźwik	Writing – review & editing	2	
8.	Jarosław Olav Horbańczuk	Writing – review & editing	2	
9.	Atanas G. Atanasov	Conceptualization, Writing – review & editing	2	

[1] **Formularz musi zostać przygotowany i zaakceptowany przez Dyrekcję Instytutu przed wysłaniem publikacji do czasopisma /** ***The form must be prepared and approved by the Director before sending the publication to the journal.***

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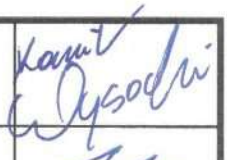
3. Opis udziału autora w opracowaniu publikacji *(Description of the author's participation in preparation of the publication)*:

Lp.	Imię i nazwisko *(name and surname)*	Opis udziału autora *(Description of the author's participation)*	% udział w opracowaniu publikacji *(participation in the publication in %)*	Podpis autora[2] *(author's signature)*
1.	Maima Matin	Conceptualization and planning, Experimental work, Data analysis, Writing – original draft	84	
2.	Kamil Wysocki	Experimental work, Writing – review & editing	4	
3.	Jarosław Olav Horbańczuk	Conceptualization and planning, Writing – review & editing	4	
4.	Luciana Rossi	Conceptualization and planning, Writing – review & editing	4	
5.	Atanas G. Atanasov	Conceptualization and planning, Writing – review & editing	4	

[1] **Formularz musi zostać przygotowany i zaakceptowany przez Dyrekcję Instytutu przed wysłaniem publikacji do czasopisma** */ The form must be prepared and approved by the Director before sending the publication to the journal.*

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3. Opis udziału autora w opracowaniu publikacji *(Description of the author's participation in preparation of the publication)*:

Lp.	Imię i nazwisko *(name and surname)*	Opis udziału autora *(Description of the author's participation)*	% udział w opracowaniu publikacji *(participation in the publication in %)*	Podpis autora[2] *(author's signature)*
1.	Maima Matin	Conceptualization and planning, Experimental work, Data analysis, Writing – original draft	75	
2.	Malgorzata Lysek-Gladysinska	Experimental work, Writing – review & editing	5	
3.	Natalia Ksepka	Experimental work, Writing – review & editing	5	
4.	Sara Frazzini	Experimental work, Writing – review & editing	5	
5.	Anna Wieczorek	Experimental work, Writing – review & editing	5	
6.	Artur Jóźwik	Conceptualization and planning, Data analysis, Writing – original draft	5	

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10.3 Publications constituting doctoral dissertation

biomolecules

MDPI

Review

Effects of Ginger (*Zingiber officinale*) on the Hallmarks of Aging

Maima Matin [1], Tanuj Joshi [2], Dongdong Wang [3], Nikolay T. Tzvetkov [4], Farhan Bin Matin [5], Agnieszka Wierzbicka [1], Artur Jóźwik [1], Jarosław Olav Horbańczuk [1] and Atanas G. Atanasov [1,6,7,*]

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Abstract: Ginger (*Zingiber officinale* Roscoe) is broadly used as a traditional remedy and food ingredient, and numerous preclinical and clinical studies have demonstrated health benefits in a range of age-related disorders. Moreover, longevity-promoting effects have been demonstrated in several (preclinical) research models. With this work, we aimed to comprehensively review the reported effects of ginger and its bioactive constituents on the twelve established hallmarks of aging, with the ultimate goal of gaining a deeper understanding of the potential for future interventions in the area of longevity-extension and counteracting of aging-related diseases. The reviewed literature supports the favorable effects of ginger and some of its constituents on all twelve hallmarks of aging, with a particularly high number of animal research studies indicating counteraction of nutrient-sensing dysregulations, mitochondrial dysfunction, chronic inflammation, and dysbiosis. On this background, validation in human clinical trials is still insufficient or is entirely missing, with the exception of some studies indicating positive effects on deregulated nutrient-sensing, chronic inflammation, and dysbiosis. Thus, the existing body of literature clearly supports the potential of ginger to be further studied in clinical trials as a supplement for the promotion of both lifespan and health span.

Keywords: ginger; hallmarks of aging; longevity; life-extension; lifespan; health span; metabolism; inflammation

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1. Introduction

Aging is a complex multi-component process that is defined by the gradual decline of physiological functions and homeostasis maintenance, ultimately resulting in the death of the organism. The compromised physiological functions and homeostasis result in increased susceptibility to diverse age-related neoplastic and degenerative diseases. Twelve prominent hallmarks of the aging process have been outlined, which include genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, disabled macroautophagy, deregulated nutrient-sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, altered intercellular communication, chronic inflammation, and dysbiosis [1,2] (Figure 1). Counteracting aging by targeting mechanisms associated with its

hallmarks is of great interest, and modulation of diet and specific dietary ingredients have emerged as a powerful approach to improving health and expanding the lifespan [3–5].

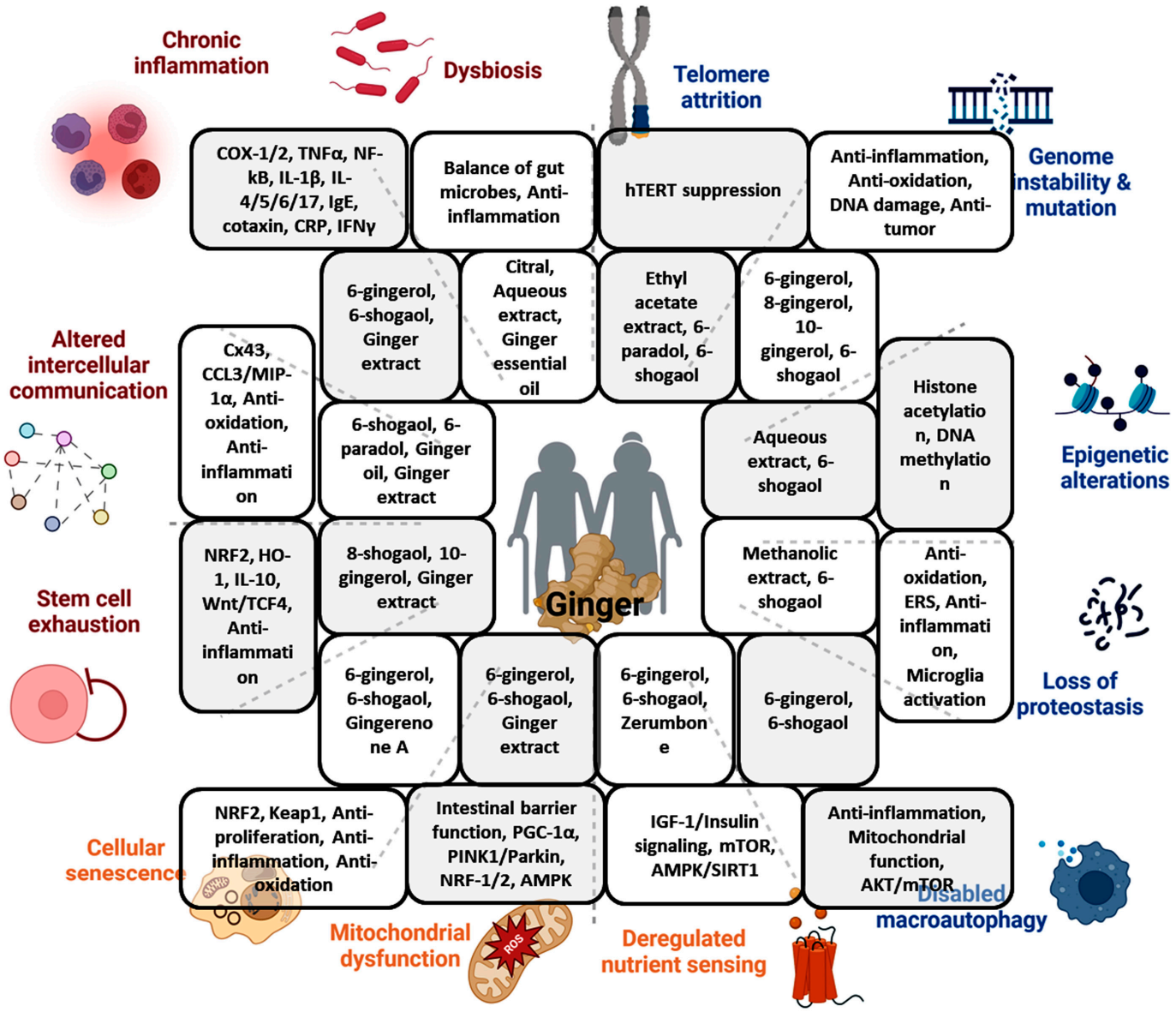

Figure 1. Summary of the effects of ginger and its constituents on the hallmarks of aging and the respective involved mechanisms (created with BioRender.com (accessed on 18 July 2024)).

Ginger (*Zingiber officinale* Roscoe) is broadly used in culinary as well as utilized as a versatile remedy in the traditional folk medicine of many countries [6,7]. The major bioactive constituents of ginger are nonvolatile and include gingerols, shogaols, paradols, and zingerone, while ginger volatile oil constituents that have been identified include sesquiterpenes (zingiberene, curcumene, and farnesene) and monoterpenes (cineole, linalool, borneol, geranial, and neral) (Figure 2) [8,9]. 6-gingerol specifically is considered to be a major pungent phytochemical found in fresh ginger, whereas, in dried rhizomes, 6-gingerol is dehydrated to 6-shogaol.

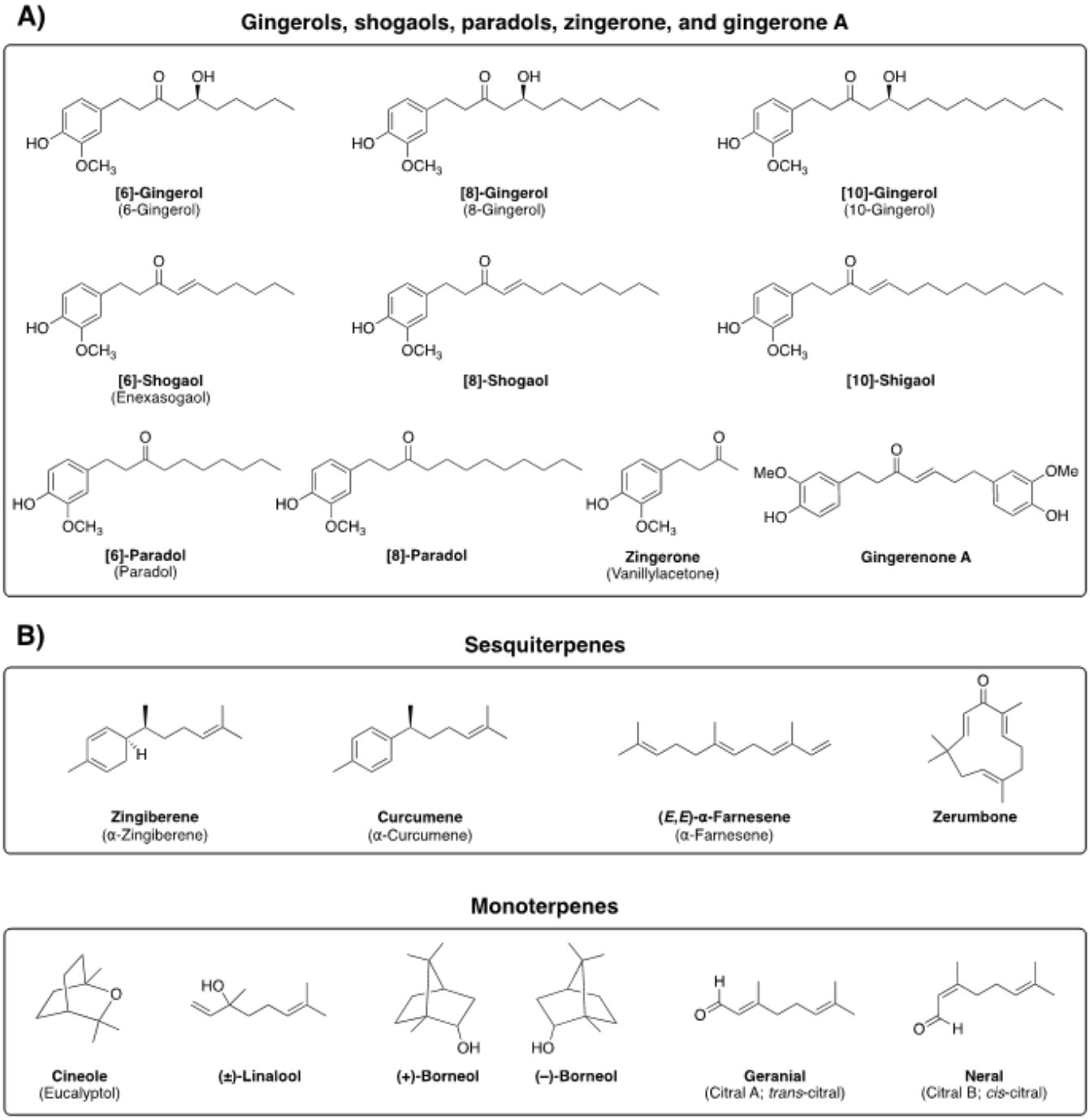

Figure 2. Chemical structures and common names of the major bioactive constituents of ginger, including nonvolatile (panel (**A**)) and volatile compounds (sesqui- and monoterpenes, panel (**B**)).

There has been a broad clinical interest in verifying in patients ginger bioactivities indicated by preclinical research and traditional folk medicine, and a recent systematic study of conducted randomized controlled trials indicated robust evidence for efficacy in counteracting metabolic syndrome, for improvement of nausea and vomiting in pregnancy, for anti-inflammatory activity, for digestive function support, and for positive impact on colorectal cancer's markers [10]. According to the European Medicines Agency's (EMA's) Committee on Herbal Medicinal Products (HMPC), the most established pharmacological properties of ginger rhizomes are its use in adults to prevent nausea and vomiting in motion sickness, to counteract mild symptoms of affecting the stomach or

gut (e.g., bloating and flatulence), and its application in children aged six and above to combat the symptoms of motion sickness (EMA website accessed on 11 of February 2024; https://www.ema.europa.eu/en/medicines/herbal/zingiberis-rhizoma). The efficacy of ginger and its ingredients for counteracting dementia and other age-related neurological disorders has also been broadly studied [11,12]. Moreover, longevity-promoting effectiveness has been demonstrated upon the application of both ginger extract [13] and 6-shogaol [14] in a *Caenorhabditis elegans* model. Similarly, the lifespan-promoting effect of ginger was also demonstrated in *Drosophila melanogaster* models [15–17].

Taking into consideration that ginger is a broadly consumed medicinal plant that has shown promising potential in modulating age-related disorders and promoting healthy aging, the aim of this work is to comprehensively review the impact of ginger on the hallmarks of aging, as inspired by the two influential reviews on the latter topic by López-Otín C et al. [1,2]. Evaluation of effects by single key constituents of ginger, such as 6-gingerol, 6-shogaol, and 6-paradol, on the recently recognized hallmarks of aging is also aimed. Special attention will be given to molecular mechanisms and affected cell signaling pathways underlying ginger's anti-aging effects, with the ultimate goal to gain a deeper understanding of the therapeutic potential of ginger and its constituents and inspire future research avenues in the area of longevity-extension interventions.

2. Potential of Ginger to Modulate Genomic Instability

Genomic instability represents an important contributor to aging, and it is characterized by diverse genetic lesions such as point mutations, single- and double-strand breaks, deletions, or translocations of DNA fragments, telomere shortening, and changes in chromosomal and nuclear architecture. These lesions are the result of exogenous agents (chemical, physical, or biological), as well as endogenous processes such as oxidative metabolic processes or DNA replication errors, among others. Genomic instability is counteracted by diverse mechanisms of repair and maintenance of both nuclear and mitochondrial DNA [18], and aging is associated with decreased efficiency of these protective repair mechanisms.

Several works examined in vitro or in vivo the effects of ginger or its constituents on genomic stability or parameters related to it. Ginger and some of its bioactive constituents have been shown to possess significant antioxidant and free radical scavenging activities [19], which can counteract DNA damage induced by oxidative stress. Direct comparison of the antioxidant potential of several of the major bioactive constituents of ginger, 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol, revealed the activity of all of the studied compounds, with 6-gingerol being the least potent and 6-shogaol being most potent in the scavenging of 1,1-diphenyl-2-picyrlhydrazyl (DPPH) radicals (IC50 of 8.05 μM), as well as superoxide (IC50 of 0.85 μM) and hydroxyl radicals (IC50 of 0.72 μM) [20]. In the same work, applied at 6 micromolar concentration, all four compounds also inhibited the oxidative burst induced by N-formyl-methionyl-leucyl-phenylalanine (f-MLP) in human polymorphonuclear neutrophils. In rats, ethanolic ginger extract applied orally (at 100, 200, 300 mg/kg body weight, applied daily for 21 days) exhibited marked protective effects against bromobenzene-induced oxidative stress by boosting antioxidant defense mechanisms (increasing hepatic glutathione levels, as well as the activities of glutathione reductase, glutathione S-transferase, glutathione peroxidase, and superoxide dismutase) [21]. Similarly, co-administration of dietary ginger (1%, *w*/*w*, in parallel with oral lindane administration of 30 mg/kg bw for 4 weeks) counteracted lindane-induced lipid peroxidation, along with changes of oxygen free radical scavenging enzymes such as superoxide dismutase and catalase, reduced glutathione, and the enzymes glutathione peroxidase, glutathione reductase, and glutathione-S-transferase [22]. Moreover, clinical studies in humans also supported the antioxidant effects of ginger. Thus, it was shown that newly diagnosed patients with solid tumors receiving moderate-to-high emetogenic potential chemotherapy, when compared to placebo, had a significant increase in antioxidant enzyme blood levels (superoxide dismutase, catalase, glutathione peroxidase) and the

levels of the nonenzymatic antioxidant glutathione, and also exhibited reduced oxidative marker levels (malondialdehyde and NO_2^-/NO_3^-) upon oral administration of standardized 6-gingerol (standardized 6-gingerol 20 mg/day) capsules of 5 mg twice daily, starting 3 days prior to the first cycle of chemotherapy until the fourth [23].

Anti-inflammatory properties have also been well-established for ginger, and some of its constituents, and this may also contribute to potential protective effects against genomic instability since chronic inflammation can induce DNA damage that can ultimately result in carcinogenesis [24]. In this context, aside from the mitigation of oxidative stress (that is known to be associated with chronic inflammation), the ability of ginger to counteract pro-inflammatory pathways could be of significance. In particular, research studies have indicated the inhibitory effects of ginger on pro-inflammatory enzymes such as lipoxygenase and cyclooxygenase-2 (COX-2), as well as the NF-κB pathway, among other inflammatory mediators [25].

In line with the postulated above potential of ginger to counteract DNA damage through its antioxidant and anti-inflammatory activities, a study in rats demonstrated that the pretreatment with the ginger constituent zingerone (150 mg/kg (p.o.) administrated 2 h before lipopolysaccharide challenge) abolished the increase of 8-Hydroxy-2′-deoxyguanosine (8-OHdG, DNA damage marker) levels that was associated with lipopolysaccharide-induced inflammation [26]. Similarly, the levels of the DNA damage marker 8-OHdG were suppressed upon oral aqueous ginger extract (500 mg/kg body weight, orally, for two months) application in a rat model of monosodium glutamate-induced neurotoxicity [27], upon co-application of hydro-alcoholic extract of ginger (50 mg/kg body weight, intragastrically by gavage, for six weeks) in a rat ethanol-induced kidney damage model [28], and upon hydro-alcoholic extract of ginger pretreatment (50 mg/kg body weight, intragastrically by gavage, for 10 days) in a rat model of radiation-induced kidney damage [29].

A summary of the effects of ginger and its constituents on genomic instability and the other hallmarks of aging with an outline of key supporting in vitro and animal research studies is presented in Tables 1 and 2.

Table 1. In vitro studies linked to the potential of ginger to modulate hallmarks of aging.

Ginger Ingredient	Hallmark of Aging	In Vitro Model and Reference	Dose and Time of Treatment	Outcomes
6-Shogaol	Epigenetic alterations	Rat primary cultured cardiac fibroblasts [30]	Preincubation with 0.3 μM and 1 μM 6-shogaol for 2 h	Blocked phenylephrine- and TGF-β-induced acetylation of histone H3K9
6-Shogaol	Epigenetic alterations	p300-Histone acetyltransferase assay [30]	30 min incubation with 6-shogaol (0.01–100 μM)	6-shogaol inhibits p300-histone acetyltransferase dose-dependently, with IC50 value of 6.77 μM
Water extract of ginger	Epigenetic alterations	Human breast cancer cell line MDA-MB-231 [31]	The extract was applied at a dose of 10, 30, 50, 80, and 100 μg for 24, 48, and 72 h	Restoration of the expression of tumor suppressive miRNAs, miR-200c, miR-30a, and miR-128, as well as a significant decrease of miR-200C promoter methylation and increased methylation status of LINE1

Table 1. *Cont.*

Ginger Ingredient	Hallmark of Aging	In Vitro Model and Reference	Dose and Time of Treatment	Outcomes
Metanolic extract of ginger	Loss of proteostasis	In vitro anti-glycating and anti-aggregation test with glucose-exposed bovine serum albumin [32]	The extract was applied in the concentration range of 100–600 μg/mL for 15 days	The extract exhibited anti-glycating and anti-aggregation properties
6-Gingerol	Disabled macroautophagy	Human umbilical vein endothelial cells exposed to hydrogen peroxide [33]	Pretreatment with 10–40 μM 6-gingerol for 24 h	Promoted autophagy, which protected the cells from apoptosis induction
Zerumbone	Deregulated nutrient-sensing	Mouse 3T3-L1 preadipocytes differentiated to adipocyte-like cells [34]	Treatment with 5 or 10 μM zerumbone for 48 h	Zerumbone inhibited adipogenesis in 3T3-L1 cells, reversed the dysregulation of miR-146b as well as attenuated decrease in SIRT1 expression
6-Gingerol	Deregulated nutrient-sensing	Mouse 3T3-L1 preadipocytes differentiated to adipocyte-like cells [35]	20 μM, for 8–9 days	Increased the expression levels of PGC-1α, PRDM16, and UCP1, promoted 3T3-L1 cells browning, mitochondrial biogenesis, p-AMPK and SIRT1 expression
6-Gingerol	Mitochondrial dysfunction	L6 myoblasts [36]	50–150 μM applied up to 24 h	Enhanced AMPK α-subunit phosphorylation, PGC-1α mRNA expression, and mitochondrial content
Ginger water extract	Mitochondrial dysfunction	CTLL-2 cells [37]	2.5–10 mg/mL for 24 h	Promoted cytotoxicity of CTLL-2 against cancer cells and increased mitochondrial mass, mtDNA copy number, and ATP production
Ginger water extract	Mitochondrial dysfunction	HepG2 cells [38]	Applied at 2.5 and 5 mg/mL for 3 days	Enhanced mitochondrial mass, mtDNA copy number, ATP production, and activities of mitochondrial respiratory chain complexes

Table 1. *Cont.*

Ginger Ingredient	Hallmark of Aging	In Vitro Model and Reference	Dose and Time of Treatment	Outcomes
Standardized ginger extracts (GE1 and GE2) enriched with 6-gingerol and 6-shogaol (GE1 contained 6-gingerol and 6-shogaol at concentrations of 289.531 µg/mL and 15.466 µg/mL, while GE2 contained 181.257 µg/mL and 63.425 µg/mL, respectively)	Cellular senescence	Senescent (population doubling = 21) primary human myoblasts [39]	Applied at 100 or 300 µg/mL for 24 h	The treatments led to morphological changes in the senescent myoblasts, resembling the morphology of young myoblasts, and reduced the expression of senescence-associated β-galactosidase, a marker of replicative senescence
Gingerenone A	Cellular senescence	WI-38 human fibroblasts rendered senescent by exposure to ionizing radiation [40]	Applied for 48 h at 10 or 20 µM	Gingerenone A is identified as a novel senolytic compound, effectively and selectively reducing the viability of senescent cells while enhancing the expression of cleaved caspase-3, decreasing the levels of Bcl-XL, and reducing secreted levels of pro-inflammatory factors IL-6, CCL2 (MCP-1), and interferon γ-induced protein 10 (IP-10) while increasing the anti-inflammatory cytokines IL-10 and IL-13
8-Paradol	Chronic inflammation	COX-1 inhibitor assay [41]	Tested for 10 min in several concentrations (0–100 µM) for IC50 determination	Among several ginger-derived compounds, 8-paradol exhibited the strongest COX-1 inhibitory activity, with an IC50 value of 4 µM
10-Gingerol, 8-shogaol and 10-shogaol	Chronic inflammation	COX-2 inhibitor assay [42]	Each compound was tested in 12 different concentrations for IC50 determination	The three compounds inhibited COX-2 with IC50 values of 32 µM, 17.5 µM, and 7.5 µM, respectively

Table 1. *Cont.*

Ginger Ingredient	Hallmark of Aging	In Vitro Model and Reference	Dose and Time of Treatment	Outcomes
Food-grade ginger powder suspended in water		Human tracheas with epithelia removed contracted with acetylcholine (a model for relaxation of the airway smooth muscle) [43]	The food-grade ginger powder (0.5 mg, 1 mg, 5 mg, 10 mg, 25 mg, 50 mg, and 100 mg, suspended in 1 mL water) was applied for 30 min	A dose-dependent relaxation of the airway smooth muscle was observed
Steamed ginger extract	Chronic inflammation	Helicobacter pylori-infected gastric (AGS) cells [44]	Applied at 1–200 μg/mL for 24 h	Inhibited the growth of Helicobacter pylori, but also reduced in the gastric cells Helicobacter pylori-induced inflammation markers, including IL-8, TNF-α, IFN-γ, IL-6, iNOS, and myeloperoxidase
6-Shogaol	Chronic inflammation	LPS-challenged HUVECs [45]	Applied 30 min before LPS challenge at 1–30 μM [45]	Exhibited inhibitory effects on angiogenesis and inflammation in LPS-activated HUVECs, inhibiting the activation of NF-κB, the expression of pro-inflammatory adhesion molecules (ICAM-1, VCAM-1, E-selectin), and the attachment of leukocytes
6-Shogaol	Chronic inflammation	Rats subjected to monoarthritis in the right knee by Freund's Adjuvant injection [46]	Applied daily via gavage at 6.2 mg/kg for 28 days	Decreased chronic inflammatory response and provided protection against damage to femoral cartilage, reflected in reduced swelling, preservation of the morphological integrity of the cartilage lining the femur, reduced leukocyte infiltration, and lower concentration of soluble 1 VCAM-1

3. Potential of Ginger to Modulate Telomere Attrition

Telomeres are nucleoprotein structures composed of repeated sequences of DNA that cap the end of chromosomes to maintain the function of genomic stability by protecting from degradation of each cell cycle. An enzyme called telomerase, responsible for the maintenance of telomere length, functions in the cell by adding TTAGGG repeats to the 3′ end of the sequence by retrotranscription. Each time the cell divides, telomeres become shorter by losing a small amount of DNA. Over time, as a result of the progressive shortening of telomeres, chromosomes become damaged, and the cells can no longer divide, resulting in cell apoptosis. However, the telomeres can be rebuilt by telomerase to restore cell division. The largest activities of telomerase are notably observed to function in stem cells, gametes, and tumor cells. During cellular replication in somatic cells, telomere length shortens gradually, giving rise to chromosomal instability and mutagenesis, resulting in tumorigenesis [47]. Short telomere length has been linked to many age-related diseases and noncommunicable diseases, which may reflect the effects of numerous behavioral, psychosocial, and environmental factors on health status [47]. Many factors linked to certain lifestyles, such as smoking, high consumption of unhealthy diet, lack of exercise, and obesity, can increase the chance of telomere shortening, leading to premature death [48]. Risk for various cancers, such as bladder, colorectal, breast, and gastric cancer, has been associated with accelerated telomere shortening [48].

Telomeres can also be highly sensitive to damage by oxidative stress due to plenty of guanines in the telomeric repeats. This oxidative stress can be the result of reactive oxygen species (ROS) or free radicals that are produced from ATP production of mitochondria and as a by-product of aerobic metabolism [49]. Due to oxidative stress, most telomere attrition occurs during the replication of DNA, resulting in single-stranded DNA damage [50]. Along this line, a study indicated that a diet containing antioxidant omega-3 fatty acids reduced the rate of telomere shortening, whereas a lack of these antioxidants increased the rate of telomere attrition [51]. Moreover, it was demonstrated that women who consumed a diet lacking antioxidants had shorter telomeres and had moderate chances of developing breast cancer, whereas consumption of a diet rich in antioxidants had longer telomeres and a lower risk of breast cancer [52]. Cytometry studies have also shown that patients with rheumatoid arthritis (chronic inflammatory disease associated with increased oxidative stress) have accelerated shortening of telomere lengths with the increase in age, resulting in premature cellular aging [53].

Diverse dietary phytochemicals have gained interest because of their potential for the prevention of cancer because of their therapeutic efficacy, bioavailability, and safety [54]. Telomerase enzymes are one of the attractive targets for cancer prevention because telomerase-specific inhibition causes cancer cells and cancer-initiating cells to undergo apoptosis and cell senescence without any effect on somatic cells. Several phytochemicals were found to inhibit telomerase in cancer cells, including curcumin, resveratrol, silibinin, pristimerin, EGCG, and sulforaphane, among others [55]. In recent years, scientists have revealed various chemical compounds found in ginger rhizomes, which exhibited anticancer properties in many experimental models [56]. In relation to telomerase in particular, a research study revealed that the crude ethyl acetate fraction of ginger extract exhibited inhibition of the expression of human telomerase reverse transcriptase (hTERT) and c-Myc in A549 lung cancer cells in a time-(up to 72 h) and concentration-dependent (up to 64 µg/mL) manner [57]. Moreover, the result showed that from the major bioactive compounds in ginger, only 6-paradol and 6-shogaol could suppress hTERT expression and telomerase activity in A549 lung cancer cells, whereas 6-gingerol could not [58].

Table 2. In vivo animal research studies linked to the potential of ginger to modulate hallmarks of aging.

Hallmark of Aging	In Vivo Model and Reference	Ginger Ingredient	Dose, Mode of Administration, and Time of Treatment	Outcomes
Genomic instability	Lipopolysaccharide-induced inflammation in rats [26]	Zingerone	150 mg/kg (p.o.) administrated 2 h before lipopolysaccharide challenge	Counteracted DNA damage, quantified by measuring the DNA damage marker 8-OHdG
	Monosodium glutamate-induced neurotoxicity in rats [27]	Aqueous extract of ginger	500 mg/kg body weight, orally, for two months	Counteracted DNA damage, quantified by measuring the DNA damage marker 8-OHdG
	Chronic ethanol administration in rats [28]	Hydro-alcoholic extract of ginger	50 mg/kg body weight, intragastrically by gavage, for six weeks	Counteracted DNA damage, quantified by measuring the DNA damage marker 8-OHdG
	Radiation exposed rats [29]	Hydro-alcoholic extract of ginger	50 mg/kg body weight, intragastrically by gavage, for 10 days	Counteracted DNA damage, quantified by measuring the DNA damage marker 8-OHdG
Epigenetic alterations	C57BL/6j male mice subjected to transverse aortic coarctation surgery (to create pressure overload) [30]	6-Shogaol	Daily oral administration of 0.2 mg/kg or 1 mg/kg of 6-shogaol for eight weeks	Increases in histone H3K9 acetylation due to the transverse aortic coarctation surgery were significantly suppressed by 6-shogaol
Loss of proteostasis	Alzheimer's disease induced in rats by using $AlCl_3$ [59]	Aqueous infusion of ginger	108 and 216 mg/kg b.wt/day applied orally for 12 weeks	Reduction in amyloid plaques and improvement in cognition, psychological state as well as in the locomotor activity, as determined by rewarded alternation T-Maze test, grid floor activity cage, and accelerating speed rotarod
	MPTP-induced model of Parkinson's disease in C57/BL mice [60]	6-Shogaol	10 mg·kg^{-1}·d^{-1}, orally, for 3 days	Reversal of the MPTP-induced changes in motor coordination and bradykinesia and reversal of dopaminergic neuronal loss in substantia nigra pars compacta and in stratum
	MPTP-induced model of Parkinson's disease in C57BL/6J mice [61]	6-shogaol and ginger extract	Ginger extract was applied at 30, 100, 300 mg/kg and 6-shogaol at 10 mg/kg, by gavage, for 15 days (including the period of the MPTP exposure)	Ginger and 6-shogaol protected intestinal tight junction proteins disrupted by MPTP in the mouse colon, inhibited the increase of inducible nitric oxide synthase, cyclooxygenase-2, TNF-α, and IL-1β produced by macrophages, and suppressed the MPTP-induced enteric dopaminergic neuronal damage

Table 2. *Cont.*

Hallmark of Aging	In Vivo Model and Reference	Ginger Ingredient	Dose, Mode of Administration, and Time of Treatment	Outcomes
Disabled macroautophagy	High-fat-diet + streptozotocin-induced diabetes in rats [62]	Gingerol-enriched ginger (18.7% 6-gingerol, 1.81% 8-gingerol, 2.86% 10-gingerol, 3.09% 6-shogoal, 0.39% 8-shogaol, and 0.41% 10-shogaol)	The gingerol-enriched ginger was applied as 0.75% wt/wt in the diet for 8 weeks	The ginger extract improved gastrointestinal health in part by improving mitochondrial function, including increased mitophagy (stimulating the removal of damaged mitochondria)
	Rats subjected to cerebral ischemia/reperfusion injury by middle cerebral artery occlusion (for 1 h, ischemia induction) followed by reperfusion for 24 h [63]	6-Gingerol	6-Gingerol was intraperitoneally injected (3 mg/kg or 6 mg/kg) 30 min before middle cerebral artery occlusion	6-Gingerol treatment induced autophagy, which counteracted the pathophysiological manifestations of the ischemia/reperfusion injury
Deregulated nutrient-sensing	Caenorhabditis elegans [13]	Ginger extract	Applied life-long at 60 µg/mL	Prolonged lifespan by 23.16% through the insulin/IGF-1 signaling pathway
	Mice with impaired insulin signaling as a result of sodium arsenite exposure [64]	6-Gingerol	50 mg/kg or 75 mg/kg body weight, daily supplied orally by gavage for 3 weeks	6-Gingerol treatment reduced elevated blood glucose levels, increased diminished plasma insulin levels, and counteracted the sodium arsenite-induced downregulation of GLUT4, IRS-1, IRS-2, PI3K, AKT, PPARγ at protein and mRNA levels
	Liquid fructose-induced adipose tissue insulin resistance in rats [65]	Alcoholic extract of ginger	50 mg/kg/day, by oral gavage, for five weeks	Attenuated hyperinsulinemia and insulin resistance (HOMA-IR) induced by liquid fructose, also reversed the increases in the Adipo-insulin resistance index and upregulated insulin receptor substrate (IRS)-1
	High-fat diet-induced obesity mouse model [66]	Steamed ginger extract (20,437.13 ± 228.75 mg/kg of 6-gingerol and 7356.67 ± 80.83 mg/kg of 6-shogaol)	40 mg/kg or 80 mg/kg daily, orally by gavage, for 8 weeks	Reduced body weight gain and fat mass accumulation, reduced liver steatosis, reversed the effect of the high-fat diet on the level of hepatic p-AMPK and SIRT1
	Obesity model induced by high-fat diet in C57BL/6N mice [34]	Zerumbone	Applied as 0.01% or 0.025% of the diet for 8 weeks	Decreased adiposity induced from the high-fat diet, increased the phosphorylation of AMPK in the white adipose tissues, and reversed the dysregulation of miR-146b as well as attenuated decrease in SIRT1 expression
	High-fat diet-fed rats [67]	6-Gingerol	200 mg/kg body weight, via oral gavage, for 2 weeks	Induced hypoglycemic effect, enhanced phosphorylated AMPK-a1, and suppressed P65 via upregulation of Sirt-6 and downregulation of resistin

Table 2. *Cont.*

Hallmark of Aging	In Vivo Model and Reference	Ginger Ingredient	Dose, Mode of Administration, and Time of Treatment	Outcomes
Mitochondrial dysfunction	High-fat-diet + streptozotocin-induced diabetes in rats [62]	Gingerol-enriched ginger (18.7% 6-gingerol, 1.81% 8-gingerol, 2.86% 10-gingerol, 3.09% 6-shogoal, 0.39% 8-shogaol, and 0.41% 10-shogaol)	The gingerol-enriched ginger was applied as 0.75% wt/wt in the diet for 8 weeks	The ginger extract improved gastrointestinal health in part by improving mitochondrial function, including increased mitophagy and influencing the balance between mitochondrial fusion and fission
	High-fat diet-fed rats [68]	Ginger water extract	Supplemented at 0.25% or 0.5% of the diet for 14 weeks	Reduced weight gain and increased mitochondrial size and mtDNA content in rat skeletal muscle, upregulating PGC1α, NRF1, and Tfam mRNA expression
	High-fat diet-induced obesity mouse model [66]	Steamed ginger extract (20,437.13 $\pm$ 228.75 mg/kg of 6-gingerol and 7356.67 $\pm$ 80.83 mg/kg of 6-shogaol)	40 mg/kg or 80 mg/kg daily, orally by gavage, for 8 weeks	Reduced body weight gain and fat mass accumulation, reduced liver steatosis, inhibited whitening of brown adipose tissue, prevented mitochondrial dysfunction, reversed the effect of the high-fat diet on the level of hepatic p-AMPK and SIRT1
	Balb/c mice [38]	Ginger water extract	2 g/kg administrated for 7 days in drinking water	Increased oxygen consumption, intrascapular temperature, and mitochondrial DNA copy number in muscle and liver, as well as increased the expression of proteins related to oxidative phosphorylation and proteins associated with the AMPK/PGC1α signaling pathway in muscle, liver, and brown adipose tissue
	Aging (22-month-old) rats [69]	6-Gingerol	At 0.05 or 0.2 mg/kg, once daily via oral gavage, for 7 weeks	Counteracted age-related hepatic steatosis, increased fat oxidation, decreased fat synthesis, and improved mitochondrial function in the liver, upregulating mitochondrial marker enzymes NOX, SDH, and SIRT3
Stem cell exhaustion	*plcg1*$^{-/-}$ mutant zebrafish embryos with hematopoiesis deficiency [70]	Ginger extract or 10-gingerol	Applied at 5 µg/mL and 2 µg/mL, respectively, from the from the late gastrulation (9–10 hpf) stage onward	Rescues the formation of hematopoietic stem/progenitor cells in hemogenic endothelium and caudal hematopoietic tissue
Chronic inflammation	Mouse model of ovalbumin-induced allergic asthma [71]	Ginger ethanol extract and aqueous extract	The ginger ethanol extract was applied at 500 mg/kg and the aqueous extract at 720 mg/kg, both for 7 days	Both extracts significantly inhibited Th2-mediated immune response, with reduction in goblet cell hyperplasia, infiltration of inflammatory cells in airways, edema with vascular congestion, and total and differential count of eosinophils and neutrophils in bronchoalveolar lavage fluid

Table 2. *Cont.*

Hallmark of Aging	In Vivo Model and Reference	Ginger Ingredient	Dose, Mode of Administration, and Time of Treatment	Outcomes
Chronic inflammation	Male A/J mice challenged with methacholine (a model to study airway resistance in the context of asthma) [43]	8-Gingerol	The compound (at 100 μM) was aerosolized and sprayed to the mice 15 min before challenge by methacholine	Attenuated changes in central airway resistance
	High-fat diet-fed rats [72]	Ginger standardized ethanoic extract	Applied daily at 400 mg/kg by oral gavage for 6 weeks	Suppressed hepatic NFκB activation and reduced the hepatic levels of several key pro-inflammatory cytokines, including IL-6 and TNFα
	Collagen-induced arthritis in mice [73]	Ginger water extract	Orally administered at 100 and 200 mg/kg body weight, once daily from day 22 to day 35 after the induction of arthritis	Exerted an anti-arthritic effect by inhibiting the production in the serum of the IL-17, IL-4, and IFN –γ, and the paw tissue expression of MMP-1, MMP-13, and MMP-3
	DSS-induced colitis mouse model [74]	6-Gingerol	Applied once a day by gavage at 250 mg/kg for 14 days	Ameliorated bowel damage and reduced incidence of weight loss, as well as suppressed the increased serum and bowel levels of cytokines such as IL-6 and IL-17 (indicating inhibition of inflammatory responses both systematically as well as locally), and affected the cell balance of Th17/Treg
Dysbiosis	High-fat diet-fed mice [75]	Dried ginger powder	Applied at 500 mg/kg body weight, once daily via gavage for 16 weeks	Induced a decrease in low-grade inflammation, liver steatosis, and body weight, improved insulin resistance, and produced modulation in the composition of gut microbiota with an increase in the species that belong to the genus Bifidobacterium and bacteria that produce SCFA. Importantly, through fecal microbiota transplantation, it was demonstrated that the anti-obesity and health-promoting effects of ginger were transferrable
	DSS-induced colitis in mice [76]	Ginger powder	Orally administered at 500 mg/kg daily for 7 days	Mice with colitis had lower species diversity and richness, a higher abundance of pathogenic bacteria, Proteobacteria and firmicutes, an increase in Lachnospiraceae_NK4A136_group, and an increase in the abundance of Lactobacillus_murinus, Lachnospiraceae_bacterium_615, and Ruminiclostridium_sp._KB18. These increased pathogenic bacteria were decreased by ginger intake. Similarly, the DSS-treated mice showed a lower abundance of Muribaculaceae, and ginger application reversed this trend

Table 2. *Cont.*

Hallmark of Aging	In Vivo Model and Reference	Ginger Ingredient	Dose, Mode of Administration, and Time of Treatment	Outcomes
Dysbiosis	Apo E mice made atherosclerotic through the administration of Gubra Amylin NASH (nonalcoholic steatohepatitis) diet with L-carnitine [77]	Ginger essential oil or citral	Ginger essential oil was applied at 50 mg/kg bw/day or 100 mg/kg bw/day via oral gavage for 16 weeks, and citral was applied at 20 mg/kg bw/day via oral gavage for 16 weeks	Citral and ginger essential oil produced improvement in resistance to insulin and plasma lipid profile; decreased levels of sugar in blood and trimethylamine-N-oxide levels; inhibited plasma levels of inflammatory cytokines like interleukin-1β, and most importantly, inhibited the formation of aortic atherosclerotic lesions in the animals used in the study, while also leading to an increase in the abundance of microbes that are beneficial and decreased the abundance of microbes that are involved in the pathogenesis of cardiovascular diseases

4. Potential of Ginger to Modulate Epigenetic Alterations

Epigenetics is concerned with functional and heritable changes in the regulation of gene activity and expression caused by non-genetic mechanisms without altering the primary DNA sequence, which is susceptible to environmental exposures [78]. A variety of epigenetic alterations can be cell- or tissue-specific and can persist throughout life and be passed on to multiple generations [79]. Epigenetic modification is attained by specific mechanisms, involving DNA methylation, miRNAs expression, and histone modifications, and the action of non-coding RNAs, which play an important role in maintaining the stability of the genome [80]. DNA methylation is an epigenetic process involved in many cellular functions that silences expression by the addition of a small chemical modification, in particular methyl group addition, to cytosine in DNA sequence. Histone modifications regulate the physical properties of chromatin and its corresponding transcriptional state, and non-coding RNA attaches the complementary sequence, resulting in silencing of the respective gene. To regulate the gene expression, these epigenetic modifications work in coordination with each other [81].

The potential of ginger or ginger-derived compounds to impact chromatin in a cell's nucleus and regulate epigenetic mechanisms is under investigation, whereby there are indications for effects on histone acetylation. Along this line, one research study showed that both phenylephrine and transforming growth factor-beta (TGF-β) induced histone H3K9 acetylation pathways were blocked in primary cultured cardiomyocytes and cardiac fibroblasts in rats when treated with 6-shogaol (0.3 or 1 μM pretreatment for 2 h), followed by stimulation with 30 μM phenylephrine for 48 h or TGF-β 10 ng/mL for 6 h, respectively [30]. Furthermore, upon oral administration in mice, 6-shogaol (daily oral administration of 0.2 mg/kg or 1 mg/kg for eight weeks) inhibited transverse aortic constriction-induced left ventricular hypertrophy and progression to left ventricular systolic dysfunction. All these results demonstrated the potential of 6-shogaol as a therapy for heart failure. Another in vitro histone acetyltransferase (HAT) assay was also carried out next to investigate whether 6-shogaol directly diminishes p300-HAT activity, and the results revealed that p300 HAT activity was suppressed by 6-shogaol in a dose-dependent manner (upon 30 min incubation with 6-shogaol at 0.01–100 μM) [30]. In another work, an aqueous extract of ginger (applied at a dose of 10, 30, 50, 80, and 100 μg for 24, 48, and 72 h) was demonstrated to regulate breast cancer stem cells associated-miRNAs in human breast cancer cell line MDA-MB-231, restoring of the expression of tumor suppressive miRNAs,

miR-200c, miR-30a, and miR-128, as well as significantly decreasing miR-200C promoter methylation, whereby the methylation of LINE1 sequences, which correlates with global genomic DNA methylation, was increased upon ginger extract treatment, predicting an increase in genomic stability [31].

5. Potential of Ginger to Modulate the Loss of Proteostasis

Aging itself, as well as some age-related pathologies, such as Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, and cataracts, are linked to impaired protein homeostasis (proteostasis), resulting in the accumulation of oxidized, glycated, misfolded, or ubiquitinylated proteins that readily aggregate as extracellular plaques or intracellular inclusion bodies [1]. Along this line, aging represents a major risk factor for the development of a broader range of impactful diseases, such as cancer, inflammation-associated disorders, diabetes, Alzheimer's, Parkinson's, or Huntington's disease, which are associated with diverse dysfunctions in protein homeostasis (proteostasis) leading to the accumulation of intracellular damage [82]. Proteostasis is a process that regulates the protein metabolism within the cell for maintaining the cellular proteome and the organism itself, through a vast network of biochemical pathways. This requires regulated control of protein folding, post-translational modification, and protein degradation [83]. In healthy cells, a complex proteostasis network consisting of molecular chaperones and proteolytic machinery helps to maintain the proteostasis [82]. Highly complex interactions and intersections of proteostasis pathways might result in stress on the level of the cells and organelles, leading to the disruption of the entire network [83]. Alzheimer's disease is an example of a complex multifactorial disease associated with protein degradation and aggregation, the pathogenesis of which involves the intersection of many biochemical pathways, and the exact mechanisms triggering the disease are still being debated [84]. In the context of this disorder, one study investigated the possible prophylactic and curative effects of an aqueous infusion of ginger (108 and 216 mg/kg b.wt/day, applied orally for 12 weeks) to assess behavioral effects on rats with induced Alzheimer's disease [59]. The result showed that both doses exhibited a significant improvement (cognition, psychological state as well as in locomotor activity, as determined by rewarded alternation T-Maze test, grid floor activity cage, and accelerating speed rotarod, respectively) on Alzheimer's disease features by increasing acetylcholine and decreasing acetylcholinesterase in the brain. However, it also showed the high dose of ginger (216 mg/kg) exhibited a better effect than the low dose (108 mg/kg). Histopathological findings in brain cells showed closer to the control group phenotype, and the amyloid plaques disappeared [59]. The second most common neurodegenerative disorder after Alzheimer's disease is Parkinson's disease. Its pathogenesis is mainly characterized by the progressive loss of dopaminergic neurons of the substantia nigra pars compacta of the midbrain and the abnormal accumulation and aggregation of the protein alpha-synuclein in the form of Lewy bodies and Lewy neurites. Thereby, the protein misfolds to give rise to beta-sheet-rich amyloid fibrils. This phenomenon leads to a deficiency of dopamine with associated symptoms such as posture rigidity, bradykinesia or slow movement, instability, and resting tremors [85]. Current treatment of this disease includes levodopa and dopamine agonists which provide systematic relief accompanied with severe side effects. In contrast, natural phytochemicals have obtained promising attention as potential candidates for the therapeutic and preventative effect of Parkinson's disease, given their multitarget pharmaceutical mechanism of actions and good safety profile; ginger and its bioactive compounds, in particular, have shown a beneficial effect in multiple Parkinson's disease models [86–88]. One study, for example, demonstrated that the pungent compound of ginger, 6-shogaol (10 $mg \cdot kg^{-1} \cdot d^{-1}$, orally, for 3 days), protected against MPTP- (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) or MPP^+- (metabolite of MPTP) induced movement impairment and neuronal damage (reversal of dopaminergic neuronal loss in substantia nigra pars compacta and in stratum) by regulating microglial activation and downstream pro-inflammatory factors in a mouse (C57/BL) model of Parkinson's disorder [60]. Another similar work showed that ginger and 6-shogaol (ginger extract

was applied at 30, 100, 300 mg/kg and 6-shogaol at 10 mg/kg, by gavage, for 15 days, including the period of the MPTP exposure) restored the disruption of intestinal barrier and enteric dopaminergic neurons in an MPTP-injected mouse Parkinson's disease model by impeding the processes of inflammation and apoptosis, suggesting that they may have potential application to attenuate the gastrointestinal dysfunction in patients with Parkinson's disorder [61].

In general, different lines of evidence have pointed out the correlation between aging and the accumulation of oxidatively damaged proteins, lipids, and nucleic acids. Oxidatively modified proteins increase along with age, as well as the manifestation of age-related diseases associated with oxidatively modified proteins also increases [89,90]. The accumulation of these oxidized proteins during aging randomly damages the DNA responsible for protein synthesis, which also controls the generation of reactive oxygen species, proteolytic activities to degrade oxidized proteins, and antioxidant defense mechanisms [91]. Protein oxidation serves as a useful marker for assessing oxidative stress in vivo. Proteins are oxidized when there is an imbalance of reactive oxygen species and antioxidant defenses. One research study investigated the effect of ginger on the occurrence of oxidative stress in the small intestine of streptozotocin-induced diabetic rats. The results revealed that consumption of ginger (applied as a ginger powder as 5% of the daily food for 6 weeks), l, a potent oxidant, improved diabetes-induced oxidative stress in diabetic rats with attenuation of lipid peroxidation and protein oxidation and increased catalase activity [92]. Patients suffering from diabetic conditions produce oxidative stress, and various tissues are damaged [93,94]. Ginger showed hypoglycemic effects and helped to delay the development of diabetes mellitus in several studies, highlighting its antidiabetic potential [95]. One of the relevant studies was undertaken to see the dose-response effect of ginger in the inhibition of oxidative stress and clastogenicity in streptozotocin-induced diabetic rats. The results of this study in vivo showed that feeding the rats with ginger at different concentrations, such as 0.5%, 1%, and 5% of the total diet, significantly lowered the glucose, cholesterol, and triglycerides in streptozotocin (30 mg/kg)-induced diabetic rats in a dose-dependent manner. It was noted that serum glucose levels did not reach up to normal levels when ginger was fed to diabetic rats, but a reduction was observed in a dose-dependent manner [96]. Similar results were also reported in other studies in streptozotocin-induced diabetic rats, with ginger administration leading to a significant decrease in fasting glucose levels [97] and delay of diabetic cataracts [98]. In another in vitro study, the therapeutic potential of methanolic extract from ginger was investigated against glycation and oxidative stress, along with several other methods of anti-glycating and anti-aggregation test of glucose-exposed bovine serum albumin in the presence and absence of the ginger extract [32]. Taking into consideration the known pro-inflammatory action of advanced glycation end-products [99], the results of the latter study indicated that the ability of the ginger extract to inhibit glycation and heat-induced protein denaturation could be a possible contributing factor to its anti-inflammatory activity. In more detail, in the discussed work, the ginger extract (prepared by soaking of hundred grams of ginger powder in 1 L of 97% methanol) was effective in vitro against heat-induced albumin denaturation, and the percent of inhibition increased along with the increased concentration of the ginger extract that was applied in the concentration range 100–600 μg/mL [32]. A similar result was reported in another study, which showed that ginger extracts (at 125–500 μg/mL) possessed high anti-inflammatory activity by inhibiting the heat-induced albumin denaturation [100]. The highest percentage of inhibition (66%) was exerted by 50% of ethanolic extract, followed by 70% and 90% of ethanol extracts (65% and 63%, respectively), and moderate inhibition activity was observed with 80% ethanol extract (58%) [100].

While the research described above indicates that ginger counteracts protein modification and denaturation in the context of conditions such as diabetes and inflammation, another work indicated that activation of endoplasmic reticulum (ER) stress, associated with accumulation of unfolded or misfolded proteins in the ER lumen and unfolded protein response, underlies the mode of the killing of cancer cells exposed to ginger extract [101].

ER is, in general, responsible for the biosynthesis, folding, maturation, and stabilization of proteins and represents an organelle of major importance for proteostasis maintenance. A variety of pathophysiological conditions disrupt the protein folding capacity, resulting in the accumulation of unfolded, misfolded or toxic proteins in this organelle, with the process being termed "ER stress" [102]. Previous research has demonstrated that ER dilation is a characteristic response to ER stress, which develops as a result of the buildup of misfolded proteins in the ER lumen. To overcome the imbalance of ER protein folding capacity, cells have a signal transduction pathway called unfolded protein response (UPR) that becomes triggered and integrates ER stress signals to relieve the ER stress resulting in the re-establishment of the proteostasis balance. The UPR is controlled by three ER-transmembrane receptors, namely inositol-requiring enzyme 1α (IRE1α), pancreatic endoplasmic reticulum kinase (PERK), and activating transcription factor 6 (ATF6) [103]. When these ER transmembrane receptors detect the onset of ER stress, it initiates the unfolded protein response, whereby the prolongation of the stress causes apoptotic death of the cell [102]. In the above-mentioned study with ginger extract-exposed cancer cells [101], expressions of important ER stress markers such as polyubiquitinated proteins, Bip and CHOP were probed, and it was found that the ER stress proteins were increased when treated with increasing ginger extract concentrations. Moreover, this work indicated that ginger extract induced the paraptosis mode of death in cancer cells [101].

6. Potential of Ginger to Modulate Disabled Macroautophagy

Macroautophagy represents a process in which cytoplasmic content is incorporated in double-membrane vesicles, autophagosomes, which fuse with lysosomes and mediate degradative processes that are of essential importance for cellular homeostasis and adaptation to stress conditions such as energy- or nutrient-starvation. Autophagy declines with age advancement, and impaired autophagy is involved in the pathogenesis of a number of age-related diseases, while interventions stimulating autophagy have been shown to promote longevity [104].

In the context of autophagy modulation, one research work examined the effect of gingerol-enriched ginger extract in diabetic rats [62]. In this study, dietary application of gingerol-enriched ginger (at 0.75% of the total diet for 8 weeks) induced improved glucose tolerance and increased pancreatic insulin content in the diabetic animals, which was in part explained by improved intestinal integrity and mitochondrial dysfunction [62]. Along this line, the gingerol-enriched ginger supplementation was associated with an increase in the intestinal tissue expression of tight junction (Claudin-3) and antioxidant capacity (SOD1) genes in the intestine, and diminished expression of genes associated with mitochondrial fusion (MFN1), fission (FIS1), biogenesis (PGC-1α, TFAM), mitophagy (the removal of dysfunctional mitochondria through autophagy; LC3B, P62, PINK1), and inflammation (NF-κB) [62]. In another work, 6-gingerol (upon pretreatment with 10–40 μM 6-gingerol for 24 h) was also shown to induce autophagy in human umbilical vein endothelial cells, resulting in their improved survival from apoptosis induced by hydrogen peroxide exposure, with the findings suggesting that the compound may be beneficial in the prevention of atherosclerosis (a pathological process promoted by endothelial apoptosis) [33]. Moreover, 6-gingerol also displayed potential for the treatment of ischemia-reperfusion injury by a mechanism involving inhibition of NLRP3 inflammasome and apoptosis through TRPV1/FAF1 complex dissociation-mediated autophagy [63].

Several compounds from ginger also displayed anticancer cell-specific effects mediated through autophagy induction. Thus, 6-shogaol was shown to induce autophagy associated with inhibiting the AKT/mTOR pathway in the human non-small cell lung cancer A549 cells and in cervical carcinoma HeLa and SiHa cells [105,106]. 6-shogaol was also shown to enhance the anticancer effects of several chemotherapeutics via increase of apoptosis and autophagy in colon cancer cells under hypoxic/aglycemic conditions [107], and to suppress breast cancer cells and stem cell-like spheroids by modulation of the Notch pathway and autophagic cell death [108]. Similarly, anticancer cell-specific effects mediated through autophagy induction were reported for other ginger constituents. Thus, 6-gingerol induced

caspase-3 dependent apoptosis and autophagy in cancer HeLa cells [109], zingiberene suppressed in vivo (xenografted tumors, zingiberene applied at 10, 20, and 40 mg/kg) and in vitro (growth inhibition IC_{50} of 20 μM) human colon cancer HT-29 cell growth via autophagy induction with inhibition of PI3K/AKT/mTOR pathway and caspase-2 [110], and zerumbone activated apoptosis and autophagy in human hormone-refractory prostate cancers [111].

7. Potential of Ginger to Modulate Deregulated Nutrient-Sensing

Nutrients are simple organic chemical compounds in food that are involved in biochemical reactions producing energy for the maintenance of health and the ability to grow, move, and reproduce [112]. Nutrition has scientifically been proved by various studies as one of the key factors for healthy aging. Imbalance in diet can cause deficiency- or excess-related diseases such as blindness, anemia, scurvy, rickets, and goiter, as well as common chronic systemic diseases associated with aging such as cardiovascular disease, diabetes, and osteoporosis [113]. It has been demonstrated that caloric restriction can be a successful dietary approach to increase the lifespan in a healthy manner [114]. Alterations in nutrient sensing pathways have gained increased attention in the field of aging as they can be regulated both pharmacologically and with dietary interventions [115]. Nutrient sensing pathways become deregulated (a hallmark of cellular aging) and lose their effectiveness along with age. Metabolic processes are causing stress to cells. Vigorous metabolic activity and changes in nutrient composition can cause cells to age faster. Metabolism and its by-products over time damage the cells through oxidative stress, ER stress, mitochondrial dysfunction, and calcium signaling [116]. Some of the key protein mediators/pathway hubs that regulate some of the nutrient-sensing pathways in the context of aging are the insulin-like growth factor 1 (IGF-1), mammalian target of rapamycin (mTOR), AMP-activated protein kinase (AMPK), and sirtuins [115,117]. Nutrient-sensing pathways play an important role in the regulation of protein synthesis, cell cycle, DNA replication, autophagy, stress response, and glucose homeostasis [115,118].

One of the most discussed topics in gerontology is the role of IGF-1/insulin signaling in the regulation of longevity. Accumulating evidence suggests that downregulated IGF-1 significantly prolonged the lifespan in vertebrate and invertebrate models [119]. Several in vitro and in vivo studies demonstrated favorable modulation of IGF-1/insulin signaling by ginger or some of its bioactive ingredients. Thus, a longevity-promoting effect of ginger (lifespan extension of 23.16% upon application of 60 μg/mL ginger extract) via the insulin/IGF-1 signaling pathway was demonstrated in a *Caenorhabditis elegans* model [13]. Suppression of IGF-1 was also observed in humans, in particular in obese women diagnosed with breast cancer, who received orally four capsules containing 750 mg of ginger flour for 6 weeks [120]. Multiple studies also demonstrated the beneficial effects of ginger on insulin signaling in the context of diabetes. Thus, ginger extract enhanced the differentiation of mouse 3T3-L1 preadipocytes (in vitro model for anti-diabetic action), and the isolated active constituent 6-gingerol upregulated insulin-sensitive glucose uptake [121]. Moreover, 6-gingerol (50 mg/kg or 75 mg/kg body weight, daily supplied orally by gavage for 3 weeks) was also shown to display anti-hyperglycemic activity and to attenuate sodium arsenite-induced impairment of insulin signaling in mice [64]. Along the same line, orally applied ginger extract (50 mg/kg/day, for five weeks) attenuated hyperinsulinemia and insulin resistance induced by liquid fructose supplementation in rats [65], and human trials (Table 3) have consistently demonstrated improvement of blood glucose levels (key target of insulin action) in type 2 diabetes patients supplemented with ginger [122]. As one example, Arablou et al. performed a double-blind, placebo-controlled clinical trial with 70 type 2 diabetes patients who were allocated randomly to receive a placebo or 1600 mg ginger (equaling two capsules) for 12 weeks, and the ginger-receiving group displayed both improved metabolic parameters (reduction of fasting glucose, HbA_{1C}, insulin, HOMA, triglycerides, and total cholesterol) as well as reduction in the level of the inflammatory markers CRP and PGE_2 [123]. Similarly, a randomized, placebo-controlled clinical trial with 103 individuals demonstrated that daily intake of 1.2 g of ginger (two capsules, 600 mg ginger powder in each) for 90 days resulted in a greater reduction in the blood glucose and total cholesterol in comparison to the placebo group [124].

Table 3. Human research studies linked to the potential of ginger to modulate hallmarks of aging.

Hallmark of Aging	Study Design and Reference	Ginger Ingredient	Dose and Time of Treatment	Outcomes
Deregulated nutrient-sensing	A double-blind, randomized, placebo-controlled trial with 70 type 2 diabetes patients [123]	Ginger capsules	1600 mg ginger (equaling two capsules) daily for 12 weeks	The ginger-receiving group displayed both significantly improved metabolic parameters (reduction of fasting glucose, HbA_{1C}, insulin, HOMA, triglycerides, and total cholesterol) as well as a reduction in the level of the inflammatory markers CRP and PGE_2
	A double-blind, randomized, placebo-controlled clinical trial with 103 type 2 diabetes patients [124]	Ginger capsules	Daily intake of 1.2 g of ginger (two capsules, 600 mg ginger powder in each) for 90 days	A greater reduction in the blood glucose and total cholesterol in comparison to the placebo group
Chronic inflammation	Randomized, double-blind, placebo-controlled trial with 64 type 2 diabetes patients [125]	Ginger tablets	2 g per day (equaling two tablets) for 2 months	The ginger supplemented group displayed significantly lower serum TNF-α and hs-CRP levels
	A double-blind, randomized, placebo-controlled trial with 70 type 2 diabetes patients [123]	Ginger capsules	1600 mg ginger (equaling two capsules) daily for 12 weeks	The ginger-receiving group displayed not just significantly improved metabolic parameters (reduction of fasting glucose, HbA_{1C}, insulin, HOMA, triglycerides, and total cholesterol) but also a reduction in the level of the inflammatory markers CRP and PGE_2
Dysbiosis	Crossover intervention study with 123 healthy subjects, of which 63 men and 60 women [126]	Freshly squeezed ginger juice	20 mL freshly squeezed ginger juice per day for 7 days	The species number of the microbial flora of intestines was increased, and also the ratio of Firmicutes-to-Bacteroidetes; levels of anti-inflammatory Faecalibacterium; and levels of Proteobacteria, while there was a decrease in relative abundance in the ratio of Prevotella-to-Bacteroides and levels of pro-inflammatory Ruminococcus_1 and Ruminococcus_2

mTOR is a key coordinator of eukaryotic cell growth and metabolism with input from environmental signals, including growth factors and nutrients (notably, being activated by high amino acid concentrations) [127]. Suppression of mTOR activity is one of the most established interventions resulting in extended lifespan in a wide range of different species [128]. Several research works reported the modulation of mTOR by ginger and some of its bioactive constituents. Thus, supplementation with steamed ginger extract (40 mg/kg or 80 mg/kg daily, orally by oral gavage, for 8 weeks) was demonstrated to inhibit mTOR activation and fat accumulation induced by high-fat diet in mice [66]. Several studies also reported that 6-shogaol inhibited mTOR and induced cell death in non-small cell lung cancer and cervical carcinoma cells [105,106]. Similarly, zingerone (applied at 15 and 20 μM for 24 h) and zerumbone induced apoptosis and inhibition of the mTOR signaling in human prostate cancer PC-3 cells [129] and oral squamous cell carcinoma cells [130], respectively. 6-Gingerol was also reported to act as an mTOR inhibitor, and the compound was shown to counteract neuroinflammation and ischemic brain injuries through suppression of Akt/mTOR/STAT3 signaling in microglia cells [131].

AMPK senses low energy status in cells by detecting high levels of adenosine monophosphate (AMP), which is a product of the energy production happening through ATP hydrolysis. The activation of AMPK by high AMP levels activates catabolic processes aiming to restore energy homeostasis (to generate more ATP) and, among other effects, increases cellular NAD^+ levels, which in turn leads to the activation of sirtuins, NAD^+-dependent family of deacetylases, the activation of which is also viewed as a key anti-aging mechanism [132,133], along with the suppression of mTOR and IGF-1/insulin signaling [117]. Similarly to modulating mTOR and the IGF-1/insulin signaling (as discussed above), many research works have also studied the effects of ginger and some of its compounds on AMPK and sirtuins. Thus, it was demonstrated that steamed ginger extract supplementation reduces liver steatosis and adipocyte metabolic dysfunction through AMPK-SIRT-1 activation in a high-fat diet (HFD)-induced obesity mouse model [66]. Similarly, zerumbone, a ginger sesquiterpene (applied as 0.01% or 0.025% of the diet for 8 weeks), counteracted the obesity induced by high-fat diet in C57BL/6N mice through activation of AMPK and sirtuins (SIRT1) [34] and 6-gingerol (20 μM, for 8–9 days) promoted browning (favorable metabolic phenotype) in mouse 3T3-L1 adipocytes with the upregulation of SIRT1 and p-AMPK/AMPK together with several other biomarkers [35], as well as it exhibited hypoglycemic effect in high-fat diet-fed rats that was associated with increased phosphorylated (activated) AMPK-α1 and sirtuin-6 [67].

8. Potential of Ginger to Modulate Mitochondrial Dysfunction

Mitochondria exhibit the capability to produce ROS alongside their electron transport process. While ROS at low concentrations function as physiological signals, their excessive generation in response to stress exerts a toxic influence, leading to detrimental effects on mitochondrial components and gradual impairment of mitochondrial function. To counterbalance this potential harm, a range of endogenous antioxidant defense mechanisms come into play. These encompass antioxidant proteins like glutathione peroxidase 1, superoxide dismutase 2, and peroxiredoxin 3/5, which are regulated by transcription factors such as Nuclear factor (erythroid 2-like)-2 (Nrf2) [134]. Additionally, small molecules including coenzyme Q and glutathione contribute to mitigating the oxidative stress within mitochondria [135]. As individuals age, there is a decrease in the mitochondrial membrane potential, resulting in diminished ATP generation. This decline is further associated with the activation of molecules associated with inflammation and ROS, ultimately culminating in cellular demise [136]. A notable hallmark of aging mitochondria is the surge in somatic point mutations and large deletions in mtDNA, leading to dysfunction. These mtDNA mutations, often arising due to oxidative damage in proximity to ROS sources, play a central role in mitochondrial dysfunction. The Mitochondrial Free Radical Theory of Aging (MFRTA) posits a cycle where oxidative damage to mtDNA initiates respiratory chain protein dysfunction, elevating ROS production, thereby perpetuating mitochondrial dysfunction [137]. Mitochondrial dynamics, involving fusion, fission, and mitophagy, are

crucial for cellular functions. One recent study indicated that Gingerol Enriched Ginger (GEG, 18.7% 6-gingerol, 1.81% 8-gingerol, 2.86% 10-gingerol, 3.09% 6-shogoal, 0.39% 8-shogaol, and 0.41% 10-shogaol) applied as 0.75% wt/wt in the diet for 8 weeks could enhance insulin production, improve intestinal integrity, and counteract mitochondrial dysfunction in high-fat-diet and streptozotocin-induced diabetes in rats [62]. GEG supplementation boosted intestinal barrier function by increasing Claudin-3 expression and curbing mitochondrial oxidative stress and inflammation. Along this line, GEG influenced the balance between mitochondrial fusion and fission, triggering mitophagy through the PINK1/Parkin pathway, and regulating PGC-1α and TFAM expression [62]. The findings suggest that GEG has the potential to alleviate type 2 diabetes-induced intestinal dysfunction, enhance insulin production, and boost beta cell number (as evidenced by the observed increase in insulin-producing beta cells detected through insulin-immunohistochemistry staining) [62].

Mitochondrial dysfunction in skeletal muscle is linked to various transcription regulators involved in mitochondrial biogenesis. Peroxisome proliferator-activated receptor gamma coactivator-1-a (PGC-1α) increases nuclear respiratory factor (NRF)-1 and NRF-2, leading to mitochondrial DNA transcription [138]. A study showed that ginger ethanol extract (applied at 100 mg/kg or 200 mg/kg daily by oral gavage for 10 weeks) improves insulin resistance by enhancing AMPK-α1 expression in skeletal muscle in high-fat, high-carbohydrate diet-fed rats [36]. Additionally, 6-gingerol (50–150 μM, applied up to 24 h) promotes AMPK-α1 activity, PGC-1α mRNA expression, and mitochondrial biogenesis in L6 myotubes [36]. One study also sheds light on the putative anti-tumor effects of 6-gingerol, focusing on the promotion of mitochondrial biogenesis in tumor-infiltrating $CD8^+$ T cells and their cytotoxic effect [37]. The latter study indicates that 6-gingerol (applied at 25–50 mg/kg, i.p., for 16 days) inhibits tumor growth by 33–37% in a Lewis lung carcinoma xenograft mouse model. The ginger extract increased mitochondrial mass in $CD8^+$ T cells in vivo as well as in vitro in the CTLL-2 cells model (upon application at 5 and 10 mg/mL for 24 h), enhancing their cytotoxicity against cancer cells [37]. Further analysis with the active ingredient 6-gingerol showed that it promotes mitochondrial function and biogenesis, potentially contributing to the $CD8^+$ T cells' anti-tumor effects. These findings offer a deeper understanding of the mechanisms behind ginger's anti-tumor properties and its potential application in cancer management [37].

Muscle mitochondrial dysfunction is associated with obesity-related metabolic disorders [139]. Ginger water extract supplementation (at 0.25% or 0.5% of the diet for 14 weeks) in high-fat diet-fed rats reduced weight gain and increased mitochondrial size and mtDNA content in rat skeletal muscle, upregulating PGC1α, NRF1, and Tfam mRNA expression [68]. The ginger extract supplementation also raises serum HDL-C levels and contains compounds like 6-gingerol, known for antioxidant, anti-inflammatory, and anti-obesity effects [140]. Another research study shows that the addition of Steamed Ginger Extract (SGE) (applied at 40 mg/kg or 80 mg/kg daily, via gavage, for 8 weeks) inhibited the whitening of brown adipose tissue in supplemented mice groups by preventing increased lipid deposition and mitochondrial dysfunction [66]. This resulted in the restoration of reduced mitochondrial DNA, enzyme activities, and thermogenesis genes like UCP1. In liver and adipose tissues, SGE controlled ROS amplification and mitochondrial dysfunction induced by hyper-nutrient conditions through the mTOR-SREBP1-ER stress pathway. AMPK-SIRT1 activation by SGE was found to regulate cellular pathological signaling for liver and adipocyte dysmetabolism, mitigating liver steatosis and adipocyte metabolic issues while addressing ER and mitochondrial redox-linked disorders [66].

Another relevant study showed that ginger water extract, particularly at a dose of 2 g/kg administrated for 7 days, increased oxygen consumption, intrascapular temperature, and mitochondrial DNA copy number in muscle and liver in Balb/c mice [38]. This outcome was accompanied by an increase in the expression of proteins related to oxidative phosphorylation and proteins associated with the AMPK/PGC1α signaling pathway in muscle, liver, and brown adipose tissue. In HepG2 cells, the ginger extract (applied

at 2.5 and 5 mg/mL for 3 days) enhanced mitochondrial mass, mtDNA copy number, ATP production, and activities of mitochondrial respiratory chain complexes. The study suggests that the extract promotes mitochondrial biogenesis and function through the AMPK-PGC1α signaling pathway, with 6-gingerol being the likely active component, while 6-shogaol showed inhibitory effects on cell viability [38]. It is also demonstrated that 6-gingerol (applied at 2 μM) acts as a PPARδ ligand, enhancing PGC-1α expression and muscle mitochondrial biogenesis [141].

Hepatic fat accumulation is controlled by factors like lipid processing and oxidative stress. 6-gingerol showed promise in reducing liver fat in models of non-alcoholic fatty liver disease. Along this line, one study showed that in aging (22-month-old) rats, 6-gingerol treatment (0.05 or 0.2 mg/kg, once daily oral gavage for 7 weeks) counteracted age-related hepatic steatosis, increased fat oxidation, decreased fat synthesis, and improved mitochondrial function in the liver, upregulating mitochondrial marker enzymes such as NOX, SDH, and SIRT3 [69]. These effects were achieved by affecting specific proteins and microRNAs related to fat metabolism. The results suggested that 6-gingerol application could be a valuable approach to combat age-related hepatic steatosis, potentially aiding in treating metabolic disorders [69].

9. Potential of Ginger to Modulate Cellular Senescence

Cellular senescence is characterized by a permanent cell cycle arrest state where cells are unable to divide and no longer proliferate. Senescent cells exhibit distinct features such as alterations in appearance and metabolism, modifications in chromatin structure, and shifts in gene expression patterns [142,143]. Senescence plays a crucial role in the age-related decline of tissue regenerative potential. This notion is supported, for example, by evidence from BubR1 progeroid mice, where progenitor cell populations in skeletal muscle and fat tissues show a high susceptibility to cellular senescence [144]. Apart from inducing a persistent growth arrest in stem cells, senescence can also disrupt the local stem-cell niche through the secretion of a group of pro-inflammatory and pro-growth factors known as the Senescence Associated Secretory Phenotype (SASP) [145] or Senescence Messaging Secretome [146]. Moreover, additional SASP-related mechanisms may contribute to tissue dysfunction. As an example, senescent cells release proteases that can continuously affect the structure and organization of tissues by breaking down membrane-bound receptors, proteins, or surrounding components within the tissue environment [147]. In Blagosklonny's theory of 'hyperfunction,' aging is conceptualized as a quasi-program resulting from the intricate processes that take place during early life's development and growth [148]. For instance, when growth arrest happens, certain nutrient-sensing pathways, such as mTOR, continue to operate. However, instead of promoting cell proliferation and growth as they did before, these pathways now trigger cellular senescence [148]. As human lifespans increase, age-related diseases become more prevalent in the aging population. Some of these diseases, such as sarcopenia [149], osteoarthritis, idiopathic pulmonary fibrosis, atherosclerosis, and Alzheimer's disease exhibit features related to the activation of senescence [150].

Ginger might hold promise as a potential dietary additive to counteract muscle aging due to its antioxidant properties. Such effects of ginger can be attributed to 6-gingerol and 6-shogaol, which both were shown to exert antioxidant properties through the Nrf2 signaling pathway [151]. Nrf2 dissociates from its inhibitory protein partner, Kelch-like ECH-associated protein 1 (Keap1), leading to Nrf2 translocation into the nucleus. Once in the nucleus, Nrf2 binds to the antioxidant response element (ARE) and induces the transcription of antioxidant genes [152]. One research study investigated the effects of 6-gingerol and 6-shogaol, present in two different standardized ginger extracts (GE1 and GE2), on myoblast cells in culture. The composition of these extracts differed significantly in their content of 6-gingerol and 6-shogaol, likely attributed to variations in the extraction conditions (GE1 contained 6-gingerol and 6-shogaol at concentrations of 289.531 μg/mL and 15.466 μg/mL, while GE2 contained 181.257 μg/mL and 63.425 μg/mL, respectively). The

findings revealed that treatment with 6-gingerol and 6-shogaol enriched extracts (at 100 or 300 μg/mL, for 24 h) led to morphological changes in senescent (population doubling = 21) primary human myoblasts, resembling the morphology of young myoblasts [39]. These extracts exhibited an aging-reversal effect on myoblast cells by reducing the expression of senescence-associated β-galactosidase, a marker of replicative senescence. The study further demonstrated that both 6-gingerol and 6-shogaol, despite their distinct chemical structures, produced similar effects on myoblasts when used in combination with the standardized ginger extracts. Moreover, treatment with 6-gingerol and 6-shogaol enriched extracts influenced the progression of the cell cycle in myoblast cells, with cell cycle arrest observed during the G0/G1 phase. The study suggested that the expression of the Ki67 protein, crucial for cell proliferation, was lower during the G0/G1 phase, indicating a reduction in proliferative capacity in the myoblast cells. Taken together, these findings demonstrate the potential of 6-gingerol and 6-shogaol, present in ginger extracts, to protect against cellular senescence and promote myoblast differentiation.

Replicative senescence, caused by telomere shortening, is a tumor-suppressor mechanism. However, cancer cells can evade this process by reactivating telomerase. As already mentioned in the chapter "Potential of ginger to modulate telomere attrition", a study by Kaewtunjai et al. demonstrated that ginger extract suppressed hTERT expression, inhibited telomerase, and induced cellular senescence in A549 lung cancer cells [58]. Further investigations confirmed that prolonged treatment of A549 cancer cells with sub-cytotoxic ginger extract doses (5 and 10 μg/mL) resulted in decreased telomere length, induced cellular senescence, and reduced clonogenicity. Additionally, the active compounds within the ginger extract, 6-paradol, and 6-shogaol, showed a favorable safety profile in rats and exhibited an ancillary chemoprotective effect against the formation of liver micronuclei induced by diethylnitrosamine (DEN) [58]. The latter finding is of relevance in the context of aging since micronuclei (generally linked to chromosome instability, mutagenesis, and genome rearrangements) are frequently observed during cellular senescence [153].

Senescence is a complex phenomenon with both beneficial and harmful effects depending on the physiological context and the age of the organism. In young organisms, senescence plays a positive role in processes like wound healing, tissue remodeling, embryonic development, and tumor suppression [154]. However, in aging tissues, the accumulation of senescent cells can lead to premature aging and age-related disorders, including chronic inflammation, tumorigenesis, liver fibrosis, atherosclerosis, insulin resistance, and neurodegenerative disorders [150]. To address this issue, senotherapies, such as senolytics and senomorphics, have emerged, aiming to selectively target and eliminate senescent cells to mitigate their adverse impact. In this context, a ginger ingredient, gingerenone A (applied for 48 h at 10 or 20 μM), is identified as a senolytic compound as it effectively and selectively reduced the viability of senescent cells (WI-38 human fibroblasts rendered senescent by exposure to ionizing radiation), enhanced the expression of cleaved caspase-3 (a marker of apoptosis), and decreased levels of the anti-apoptotic protein Bcl-XL [40]. Additionally, gingerenone A treatment led to a reduction in secreted levels of pro-inflammatory factors IL-6, CCL2 (MCP-1), and interferon γ-induced protein 10 (IP-10) while increasing anti-inflammatory cytokines IL-10 and IL-13. Interestingly, treatment with 72.4 nM of 6-shogaol resulted in a notable increase in the number of proliferating cells, indicating its ability to promote cell proliferation at that application dose [40].

According to global cancer statistics from 2020, breast cancer is the most frequently diagnosed cancer in women and the leading cause of cancer-related deaths [155]. It accounts for 11.7% of all cancer cases. Various markers, including estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2 (HER2), and Ki67, are used for its diagnosis [156]. One study demonstrated that gingerenone A had a higher cytotoxic impact (IC50 values ranging from 50 to 76 μM) on breast cancer cells compared to normal breast cells [157]. Gingerenone A also demonstrated a dose-dependent inhibition of cell viability in various breast cancer cell lines (SKBR3, MCF7, and MDA-MB-231). The study also revealed that gingerenone A upregulated senescence-associated gene expressions and

oxidative stress were identified to play a crucial role in the antiproliferative effects of the compound on breast cancer cells [157].

10. Potential of Ginger to Modulate Stem Cell Exhaustion

Stem cell exhaustion represents a qualitative and quantitative decline of stem cells, that is characteristic to senescent tissues and organs and is regarded as one of the driving forces of aging in general [1]. Several works explored the effects of ginger and some of its bioactive compounds on stem cells. One study investigated exosome-like nanoparticles from four edible plants, including ginger, demonstrating that upon oral delivery (1 mg per mouse via gavage for 6 h), these nanoparticles are taken up by intestinal macrophages and stem cells [158]. Importantly, the ginger nanoparticles activated Nrf2, enhanced the expression of the antioxidant gene heme oxygenase-1 and the anti-inflammatory cytokine IL-10, as well as enhanced the activity of the Wnt/TCF4 pathway, which is known to be of critical importance for stem cell maintenance [159].

Another study investigated the effect of 8-shogaol on fibroblast-like synoviocytes (sometimes referred to in the literature as "synovium-derived mesenchymal stem cells" [160]), a cell type with a crucial role in rheumatoid arthritis development [161]. In this work, 8-shogaol application resulted in significant inhibition of TNF-α-, IL-1β-, and IL-17-mediated inflammation and migration of fibroblast-like synoviocytes derived from rheumatoid arthritis patients (applied at 5 μM and 10 μM) as well as in 3D synovial culture system. Further study of the associated mechanism of action revealed that the compound suppressed IKK, Akt, and MAPK signaling pathways [161] via direct inhibition of transforming growth factor-β (TGF-β)-activated kinase 1 (TAK1), signaling protein of key importance for both inflammatory responses and mesenchymal stem cell proliferation [162,163]. Reflecting these molecular events, 8-shogaol treatment reduced paw thickness and improved walking performance in the adjuvant-induced arthritic rat model.

It was also demonstrated that ginger extract or 10-gingerol treatment (applied at 5 μg/mL and 2 μg/mL, respectively, from the late gastrulation (9–10 hpf) stage onward) could rescue the formation of hematopoietic stem/progenitor cells in hemogenic endothelium and caudal hematopoietic tissue in *plcg1*$^{-/-}$ mutant zebrafish embryos with hematopoiesis deficiency [70]. The latter research suggests that ginger may act towards the promotion of hematopoiesis.

11. Potential of Ginger to Modulate Altered Intercellular Communication

Effective intercellular communication is a fundamental requirement for maintaining the balanced functioning of cells, tissues, and organs within a healthy organism. When this communication breaks down, it has been linked to the aging process and the emergence of various age-related disorders. This disrupted communication is considered a key aspect of aging, along with other factors like cellular senescence, mitochondrial dysfunction, and genomic instability [2]. A notable example of communication disruption during aging is "inflammaging", a persistent low-grade inflammatory state observed in older individuals. This state arises due to continuous stimulation of the immune system by various signals, including misdirected molecules and the presence of senescent cells. Intercellular communication mediated by various mechanisms, including gap junctions, tunneling nanotubes (TNTs), and extracellular vesicles, plays a critical role in maintaining tissue homeostasis and coordinating cellular functions. Gap junctions are channels connecting adjacent cells, allowing the exchange of ions, small molecules, and regulatory RNAs. They are essential for processes like electrical signaling in the heart and metabolic coupling. However, during aging, there is a decline in gap junction function due to changes in protein levels and distribution, contributing to age-related disorders in various organs [164]. A decline in the levels of Connexin 43 (Cx43), a protein involved in gap junction formation, leads to impaired intercellular communication in various organs. For example, in the heart, reduced Cx43 levels and disrupted gap junctions result in slower electrical impulse propagation and cardiac dysfunction [165]. One study using obese rats fed with a high-fat diet showed that

ginger (applied orally at 500 mg/kg body weight/day for 40 days) markedly reduced triacylglycerol, total cholesterol, LDL-C, MDA, collagen I, and MMP-2, potentially mitigating tissue damage and the progression of cardiovascular complications [166]. In bone tissue, reduced Cx43 levels contribute to increased susceptibility to oxidative stress-induced cell death and decreased bone strength. Notably, manipulating Cx43 expression can mitigate these age-related changes, indicating its significance in protecting against age-induced alterations [167]. Notably, TNF-α expression-inhibitory effects were also demonstrated in other works, for example, upon application of ginger extract in human synoviocyte cultures [168], and upon ginger extract (100 mg/Kg b.wt) oral application before gamma radiation exposure in Swiss albino mice [169].

Tunneling nanotubes are thin, dynamic membrane structures that facilitate the transfer of cellular components between distant cells. While they can promote beneficial signals and rescue cells from damage, they can also propagate harmful molecules and cellular stress. These nanotubes are increasingly recognized for their role in various physiological processes and diseases, including cancer, development, and immune responses [170]. During aging, the formation of TNTs may increase due to elevated oxidative stress and inflammation, contributing to age-related changes and disease progression. Extracellular vesicles (EVs), such as exosomes and microvesicles, are small membrane-bound particles that carry various biomolecules and can influence neighboring cells. EVs derived from senescent cells are known to be part of the senescence-associated secretory phenotype (SASP) and contribute to age-related changes. They can trigger cellular responses and spread senescence-related signals, impacting inflammation, immune responses, and tissue regeneration. The composition of EVs changes with aging, affecting their functional effects on recipient cells [164]. One research finding specifically indicates that ginger-derived exosome-like nanoparticles (EPDENs) exert notable effects on cellular behavior. When ginger EPDENs (applied at 1.0 μg/mL for 24 h) were introduced to RAW 264.7 macrophages, they distinctly induced the expression of two significant elements: antioxidation gene heme oxygenase-1 and the anti-inflammatory cytokine IL-10. This differential impact of ginger EPDENs showcases their potential to modulate key genetic factors associated with antioxidation and anti-inflammatory responses within macrophages. This insight into ginger EPDENs' ability to influence specific gene expression highlights their unique properties and underscores their potential therapeutic value for enhancing antioxidative and anti-inflammatory cellular pathways [158].

The process of aging is characterized by deficiencies in various neural, neuroendocrine, and hormonal signaling pathways [171]. One study focused on assessing the anti-inflammatory and neuroprotective effects of 6-shogaol, a key active compound in ginger, across molecular, cellular, and in vivo contexts. The investigation centered on its potential to regulate neuroinflammation by inhibiting microglial activation in the brain. The study found that 6-shogaol exhibited anti-inflammatory properties, downregulated microglial activation, and demonstrated significant neuroprotective effects in a model of ischemia (applied at 1, 3, and 6 mg/kg). The results highlighted the potential of 6-shogaol in combating neurodegenerative diseases associated with inflammation and its possible utility as a therapeutic agent [172]. Another study showcased Fermented Ginger's potential anti-amnesic effects against Aβ1–42 plaque toxicity by safeguarding against neuronal cell loss and synaptic disturbance. The presence of 6-paradol, stemming from the fermentation process, was identified as a potential mediator of memory improvement. The research suggests that fermented ginger prepared using *Schizosaccharomyces pombe* fermentation could offer relief from memory dysfunction and neuronal degradation associated with conditions like Alzheimer's disease [173]. Through heterochronic parabiosis experiments and detailed single-cell transcriptomic analysis, it has been confirmed that young blood can rejuvenate various tissues [174]. On the level of molecular mediators, for example, the chemokine CCL3/MIP-1a rejuvenates hematopoietic stem and progenitor cells, the metalloproteinase inhibitor TIMP2 is implicated in hippocampal rejuvenation, and the anti-inflammatory interleukin IL-37 enhances endurance exercise and metabolic function in aged mice [175].

On this background, numerous studies demonstrated brain-specific benefits in numerous works [11]. Thus, one study showed that 6-shogaol showed improved brain function and reduced brain damage caused by ischemia, partly by impacting oxidative stress, neuroinflammation, and cell signaling pathways. The compound also protects against hippocampal cell damage [11]. 6-Paradol had a similar effect, reducing brain damage and neurological deficits. Zingerone alleviated behavioral and histological issues linked to brain ischemia by countering oxidative stress and apoptotic markers [11]. The compounds' potential benefits were proposed to be related to their structural similarity to curcumin, which has shown protective effects in endothelial function decline and vascular atherosclerosis [176]. Taken together, while ginger and some of its bioactive compounds have demonstrated numerous presumably beneficial effects specific to the nervous system (being an important part of the body's intercellular communication), the underlying molecular modes of action merit further research.

12. Potential of Ginger to Modulate Chronic Inflammation

The anti-inflammatory actions of ginger have been proven by several studies [25,177]. Inflammation has been implicated in several impactful diseases like arthritis, autoimmune diseases, cardiovascular diseases, and neurodegenerative diseases. For many centuries people have used ginger against conditions suggesting potential anti-inflammatory effects [178]. It was confirmed decades ago by researchers that ginger has an inhibitory effect on prostaglandin synthesis, and its anti-inflammatory properties resemble that of nonsteroidal anti-inflammatory drugs (NSAIDs). Along this line, ginger has an inhibitory effect on cyclooxygenase 1 and 2, and thereby, it produces an inhibitory effect on prostaglandin synthesis [41,42]. Along this line, among several ginger-derived compounds tested for COX-1 inhibition 8-paradol exhibited the strongest inhibitory activity, with an IC50 value of 4 μM [41], and among several ginger-derived compounds tested for COX-2 inhibition, the most potent was 10-shogaol, with an IC50 value of 7.5 μM [42]. Besides the above inhibitory action, ginger also suppresses 5-lipoxygenase and inhibits the synthesis of leukotrienes [177,179,180]. Thus, gingerdione, in particular, was shown to inhibit in human leukocytes 5-HETE produced by 5-lipoxygenase with an IC50 of 15 μM [179]. Chronic inflammation is an important cause of various diseases like cancer, atherosclerosis, diabetes, and rheumatoid arthritis [177,178,180,181]. Ginger and its various phytoconstituents can play a potential role in reducing inflammation. In Asian traditional medicinal preparations, ginger rhizomes have been used for the treatment of mild forms of rheumatoid arthritis and fever, and in line with such effect, ginger phenylpropanoids (gingerols, shogaols) were found to target phospholipases A2, inhibiting IL-1beta and prostanoid secretion and disrupting arachidonate-phospholipid remodeling [181].

In a study carried out on ginger extract in rats, it was found that ginger extract possessed both anticancer and anti-inflammatory properties [182]. This effect was observed by inducing hepatoma in the rats by ethionine. It was found in the study that ginger extract (100 mg/kg body weight) suppressed the increased levels of tumor necrosis factor α (TNFα) and nuclear factor kappa B (NFκB) in rats that had liver cancer thus expressing both anticancer and anti-inflammatory potential [182]. Ginger also exhibited good potential in the treatment of inflammatory bowel diseases like ulcerative colitis (UC) and Crohn's disease (CD) [183]. Ginger and its constituents have also been shown to be effective in the context of airway inflammation, ameliorating ovalbumin-induced Th2 responses and enhancing Th1 response resulting in decreased airway inflammation in mice [71,184,185]. In the work of Khan et al. specifically, both ethanol extract (500 mg/kg) and aqueous extract (720 mg/kg) of ginger were tested (applied for 7 days) in a mouse model of ovalbumin-induced allergic asthma, and both extracts significantly inhibited Th2-mediated immune response, with reduction in goblet cell hyperplasia, infiltration of inflammatory cells in airways, edema with vascular congestion, and total and differential count of eosinophils and neutrophils in bronchoalveolar lavage fluid [71]. Also, levels of interleukin (IL)4, IL5, immunoglobulin E (IgE), and eotaxin have been found to be decreased by ginger, mediating

the reduction of airway inflammation [184,186]. Ginger also regulates the functions of calcium ion channels, and by virtue of this effect, it is able to relax smooth muscles present in the airways to provide relief in symptoms of asthmatic patients [43]. In more detail, when food-grade ginger powder (0.5 mg, 1 mg, 5 mg, 10 mg, 25 mg, 50 mg, and 100 mg, suspended in 1 mL water) was applied for 30 min to human tracheas with epithelia removed and contracted with acetylcholine, a dose-dependent relaxation of the airway smooth muscle was observed [43]. Further study of the bioactive compounds of ginger identified 8-gingerol as one of the key ingredients, and when the compound (at 100 μM) was aerosolized and sprayed to naively hyperresponsive male A/J mice 15 min before challenge by methacholine it attenuated changes in central airway resistance [43]. With respect to its actions on the liver, ginger has been shown to decrease levels of interferon-gamma (INFγ) and IL6 and inhibit inflammation while also inducing improvements in pathological changes in the liver. In this context, through inhibition of NFκB activation ginger (standardized ethanoic extract, applied daily at 400 mg/kg by oral gavage, for 6 weeks) reduced the hepatic levels of several key pro-inflammatory cytokines, including IL-6, TNFα, and blunted liver pro-inflammatory responses in rats fed a high-fat diet [72]. The anti-inflammatory action of ginger is also beneficial in the case of patients suffering from diabetes. It has been found that ginger consumption shows improvements in diabetes patients by decreasing inflammatory factors like C-reactive protein (CRP), TNFα, and IL6 [123,125,184]. Along this line, Mahluji et al. conducted a randomized, double-blind, placebo-controlled trial with 64 type 2 diabetes patients randomly assigned to receive ginger (2 g per day) or placebo tablets for 2 months, and the ginger supplemented group displayed significantly lower serum TNF-α and hs-CRP levels [125]. Similarly, Arablou et al. performed a double-blinded, randomized, placebo-controlled clinical trial with 70 type 2 diabetes patients who were allocated to receive placebo or 1600 mg ginger (equaling 2 capsules) for 12 weeks, and the ginger-receiving group displayed not just significantly improved metabolic parameters (reduction of fasting glucose, HbA_{1C}, insulin, HOMA, triglycerides, and total cholesterol) but also reduction in the level of the inflammatory markers CRP and PGE_2 [123]. Through its antioxidant and anti-inflammatory effects ginger might also provide protection against arthritis, musculoskeletal disorders, and joint inflammation [180,187]. In the latter context, a study examining the effects of ginger water extract on collagen-induced arthritis (CIA) in mice and IL-1β-induced inflammation in human synovial fibroblasts [73]. The results of the study showed that ginger water extract (orally administered at 100 and 200 mg/kg body weight, once daily from day 22 to day 35 after the induction of arthritis) exerted an anti-arthritic effect by inhibiting the production in the serum of the IL-17, IL-4, and IFN –γ (interferon gamma) and the paw tissue expression of MMP (matrix metalloproteinase)-1, MMP-13, and MMP-3 in CIA mice and IL-1β-activated synovial cells were decreased [73]. Another study investigated the anti-inflammatory effect of steamed ginger extract on *Helicobacter pylori*-infected gastric (AGS) cells, that was mediated by inhibition of NF-κB [44]. The results of the study showed that the ginger extract used in the study (applied at 1–200 μg/mL for 24 h) not only inhibited the growth of *Helicobacter pylori* but also reduced in the gastric cells *Helicobacter pylori* induced inflammation markers including IL-8, TNF-α, IFN-γ, IL-6, iNOS, and myeloperoxidase [44].

Several specific phytochemicals present in ginger were demonstrated to play a role in its anti-inflammatory actions. The inhibitory effects of 6-shogaol on cell functions related to angiogenesis and inflammation were evaluated in primary endothelial cells of human origin (HUVEC) [45]. The results of the study showed that 6-shogaol (applied 30 min before LPS challenge at 1–30 μM) showed inhibitory effects on angiogenesis and inflammation in LPS-activated HUVECs, inhibiting the activation of NF-κB, the expression of pro-inflammatory adhesion molecules (ICAM-1, VCAM-1, E-selectin), and the attachment of leukocytes [45]. Another study also concluded that 6-shogaol (applied daily via gavage at 6.2 mg/kg for 28 days) was effective in decreasing chronic inflammatory response, and provided protection against damage to femoral cartilage (reflected in reduced swelling, preservation of the morphological integrity of the cartilage lining the femur, reduced leuko-

cyte infiltration and lower concentration of soluble 1 VCAM-1) in rats who were subjected to monoarthritis in the right knee (by Freund's Adjuvant injection) [46]. Agents that can inhibit microglial activation have the potential to counteract neurodegenerative diseases, and in this context, one study examined the effects of 6-shogaol (applied at 10 μM for up to 24 h) on microglia activation in BV-2 and primary microglial cell cultures [172]. The results of the study demonstrated that 6-shogaol proved to be an effective candidate for treating neurodegenerative diseases since it inhibited microglial activation and showed neuroprotective and anti-neuroinflammatory actions [172]. Another study showed that 6-shogaol (10 $mg \cdot kg^{-1} \cdot d^{-1}$, orally, for 3 days) in a mouse (C57/BL) model of Parkinson's disease provided anti-neuroinflammatory action and neuroprotective effects to dopaminergic neurons (including reversal of the MPTP-induced changes in motor coordination and bradykinesia, and reversal of dopaminergic neuronal loss in substantia nigra pars compacta and in stratum) [60]. Anti-inflammatory activities have been also demonstrated for 6-gingerol. Thus, one study found that 6-gingerol (given once a day by gavage at 250 mg/kg for 14 days) showed beneficial effects on ulcerative colitis mice model (dextran sulfate sodium (DSS)-induced colitis) by ameliorating bowel damage and reducing incidence of weight loss, as well as suppressing the increased serum and bowel levels of cytokines such as IL-6 and IL-17 (indicating inhibition of inflammatory responses both systematically as well as locally), and affecting the cell balance of Th17/Treg [74].

13. Potential of Ginger to Modulate Dysbiosis

Dysbiosis can be regarded as an alteration in the composition or balance of gut microbes that has an impact on the health of a person, and new research is indicating an increasing number of diverse diseases associated with gut dysbiosis [188,189]. In this context, newer medicines that can produce a positive effect to restore the proper balance of intestinal microbes and cure dysbiosis are urgently required, and natural products can be good candidates with potential effectiveness and proven safety [190,191]. Several studies have assessed the impact of ginger on dysbiosis. In one study, the effect of a combined aqueous extract of olive leaves and ginger rhizome was assessed on gut microbiota in healthy rats as well as rats that were made diabetic with alloxan [192]. The results of the study showed that as a result of the application of the extracts (given separately or in a combination at a dose of 500 mg/kg per day, with or without insulin, for 7 days) there was a general enhancement in the bacterial diversity and an increase in the Firmicutes/Bacteroidetes ratio observed in both healthy and diabetic rats upon the administration of the combined aqueous extract. In insulin treated diabetic rats, the combined extract decreased the levels of the unfavorable *Clostridium*. The results in healthy rats showed that the administration of the combined extract caused an increase in the relative abundance of the considered as favorable *Lactobacillus* and *Prevotella* whereas there was a decrease in the relative abundance of the considered as unfavorable *Clostridium* and *Bacteroides* [192].

Research was also carried out to evaluate the effect of dried ginger rhizome powder (DGRP) to reduce obesity by modulation of gastrointestinal microbiota in mice [75]. In this study, two groups of mice were fed a high-fat diet (HFD), and two groups of mice were fed a normal chow diet (NCD). Among the HFD groups, one group received HFD only and one group received HFD along with DGRP. On the other hand, in the NCD groups, one group received only NCD, and the other group received NCD along with DGRP. Also, mice that had depleted microbiota were transplanted with the fecal microbiota of mice administered a HFD or mice administered a HFD and ginger supplementation [75]. Different parameters were studied in this work, and it was found that in mice who received HFD along with DGRP treatment (applied at 500 mg/kg body weight ginger once daily via gavage for 16 weeks), there was a decrease in low-grade inflammation, liver steatosis, and body weight. Also, improvements with respect to insulin resistance were observed in these mice. It was found that ginger supplementation produced modulation in the composition of gut microbiota with an increase in the species which belong to the genus *Bifidobacterium* and bacteria that produce short-chain fatty acids (SCFAs). Fecal concentration of SCFAs was also found

to increase [75]. *Bifidobacterium* bacteria play an important role in improving metabolic health. This group of microorganisms is considered to provide protection to the body against obesity, insulin resistance, and low-grade inflammation [193,194]. SCFA-producing bacteria are also considered beneficial for health in general, with a negative correlation existing between these bacteria and obesity, inflammation, and insulin resistance [195–197]. Importantly, through fecal microbiota transplantation (FMT), it was demonstrated that the anti-obesity and health-promoting effects of ginger were transferrable [75].

Another study was conducted on healthy humans (crossover intervention study with 123 healthy subjects, of which 63 men and 60 women) to study the effect of a short-term intake of fresh ginger juice (20 mL freshly squeezed ginger juice per day for 7 days) on the variation of gut microbiota and its relevance to human health [126]. The results of the study demonstrated that the species number of the microbial flora of intestines was increased. An increase in the ratio of Firmicutes-to-Bacteroidetes, levels of anti-inflammatory *Faecalibacterium*, and levels of Proteobacteria was also observed [126]. Also, a decrease in relative abundance in the ratio of Prevotella-to-Bacteroides and levels of pro-inflammatory *Ruminococcus_1* and *Ruminococcus_2* was observed in the same study. Overall, this work provides evidence that ginger juice had a strong effect on the function and composition of gut microbiota in people who were healthy [126].

In the context of dysbiosis, a study was also carried out in mice (BALB/c mice) administered dextran sulfate sodium to induce ulcerative colitis [76]. In this study, the effect of ginger powder on reducing the severity of ulcerative colitis by improving the function and diversity of gut microbiota was assessed. The results of the study highlighted the fact that ginger powder (orally administered at 500 mg/kg daily for 7 days) produced a beneficial effect on ulcerative colitis by positively influencing the microbial population of the intestine [76]. Mice with colitis had lower species diversity and richness, a higher abundance of pathogenic bacteria, *Proteobacteria* and *firmicutes*, an increase in *Lachnospiraceae_NK4A136_group*, and an increase in the abundance of *Lactobacillus_murinus*, *Lachnospiraceae_bacterium_615*, and *Ruminiclostridium_sp._KB18*. These increased pathogenic bacteria were decreased by ginger intake. Similarly, the DSS-treated mice showed a lower abundance of *Muribaculaceae*, and ginger application reversed this trend [76].

The role of gut microbiota with respect to cardiovascular diseases is gaining increasing interest in modern medical science [188]. The effect of ginger essential oil (50 mg/kg bw/day or 100 mg/kg bw/day, via oral gavage, for 16 weeks) and one of its bioactive compounds, citral (20 mg/kg bw/day, via oral gavage, for 16 weeks), on atherosclerosis was evaluated in Apo E mice made atherosclerotic through administration of Gubra Amylin NASH (non-alcoholic steatohepatitis) diet with L-carnitine [77]. During the metabolism of L-carnitine, trimethylamine-N-oxide is formed which is involved in the formation of atherosclerotic plaques leading to thrombosis. The conducted study showed that citral and ginger essential oil produced improvement in resistance to insulin and plasma lipid profile; decreased levels of sugar in blood and trimethylamine-N-oxide levels; inhibited plasma levels of inflammatory cytokines like interleukin-1β, and most importantly, inhibited the formation of aortic atherosclerotic lesions in the animals used in the study [77]. Treatment with citral and the essential oils of ginger also produced a modulating effect on the function and diversity of gut microbiota. Both treatments lead to an increase in the abundance of microbes that are beneficial and a decrease in the abundance of microbes that are involved in the production of cardiovascular diseases. Thus, by positively influencing the gut microbiota, citral and ginger essential oils demonstrated a good potential recommending them as beneficial supplements to counteract cardiovascular diseases [77].

Since dysbiosis has been suggested to be implicated in a variety of diseases and ginger supplementation displayed benefits in restoring a healthy microbiota composition, the study of the potential of ginger-based supplements to combat such diseases can prove to be a highly interesting topic for researchers in the coming years.

14. Conclusions

Preclinical research has shown that ginger extracts and bioactive compounds are able to exhibit some favorable effects on all twelve hallmarks of aging. Particularly, numerous preclinical animal research studies have indicated positive impacts on deregulated nutrient-sensing, mitochondrial dysfunction, chronic inflammation, and dysbiosis. The reviewed research suggests that ginger might have the potential to act as a multi-targeting nutraceutical, simultaneously affecting multiple hallmarks of aging through several of its bioactive constituents and a range of diverse mechanisms. However, the present work also reveals that validation in human clinical trials is still insufficient or entirely missing, with the exception of some studies indicating benefits on deregulated nutrient-sensing, chronic inflammation, and dysbiosis. Thus, the accumulated compelling body of research evidence warrants further clinical validation studies of ginger and some of its constituents as interventions for lifespan extension and healthy aging.

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Ginger (*Zingiber officinale*) dietary supplementation in mice regulates liver antioxidant defense systems in a dose- and age-dependent

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Introduction: Oxidative stress and impaired antioxidant defenses contribute significantly to liver dysfunction, particularly with aging. This study evaluated the dose- and age-dependent effects of dietary ginger (*Zingiber officinale*) supplementation on liver antioxidant defense systems in mice.

Methods: Male Swiss Webster mice aged 3, 6, and 12 months (n = 48 per age group) received standard feed or feed supplemented with either 0.6% or 1.8% dried ginger powder for 3 months. Liver tissue was analyzed for multiple antioxidant parameters, including DPPH radical scavenging activity, total antioxidant capacity, vitamin C levels, total phenolic content, superoxide dismutase (SOD) activity, malondialdehyde (MDA) levels, and reduced glutathione (GSH) concentrations.

Results: The results demonstrated significant age-dependent declines in several antioxidant parameters in control animals, including DPPH scavenging activity, total antioxidant capacity, vitamin C levels, total phenolic content, and SOD activity. Ginger supplementation produced differential effects based on both dose and age. While 3-month-old mice showed decreased DPPH radical scavenging with ginger supplementation, both 6- and 12-month-old mice exhibited significantly increased activity. Higher-dose (1.8%) ginger supplementation enhanced GSH levels across all age groups, with effects being most pronounced in older mice. SOD activity remained unaffected by ginger supplementation across all groups. MDA levels were significantly reduced by 1.8% ginger supplementation in 3-month-old mice, with smaller, dose-dependent but non-significant reductions in older groups.

Discussion: These findings demonstrate that ginger's effects on liver antioxidant systems are both dose- and age-dependent, with generally stronger beneficial effects observed at higher doses and in older animals. The observed dose- and age-dependent variations emphasize the importance of personalized supplementation strategies and provide a foundation for future research into the molecular mechanisms underlying ginger's antioxidant effects.

KEYWORDS

bioactivity, antioxidant, hepatoprotection, ginger, liver, aging, metabolism

1 Introduction

Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the body's ability to detoxify these reactive intermediates or repair the resultant damage (Burton and Jauniaux, 2011; Afzal et al., 2023). The liver represents a key target that is often attacked by these reactive species (Sanchez-Valle et al., 2012). Many external factors such as drinking alcohol, overusing drugs, exposure to toxins, viruses, and smoking, and internal factors like obesity and insulin resistance can increase ROS production in the liver (Manna and Jain, 2015; Muriel and Gordillo, 2016). To counteract this oxidative damage, the liver, which is the central metabolic organ for detoxification, has a sophisticated defense system. Specifically, it produces more antioxidants under stress conditions through both enzymatic and non-enzymatic mechanisms (Caetano et al., 2013; Li et al., 2015). Enzymatic antioxidants, such as superoxide dismutase (SOD), alongside non-enzymatic antioxidants like vitamin C, among others, are important in neutralizing the adverse effects of ROS and maintaining hepatic function (Cichoz-Lach and Michalak, 2014). Aside from external stressors, age is another key factor for the redox balance of the body, with the activities of key antioxidant enzymes such as SOD tending to decline with age, which is associated with increased oxidative damage due to reduced ability to neutralize ROS (Sohal et al., 1990; Tian et al., 1998). When antioxidant defenses get overwhelmed, the imbalance caused due to oxidative stress can disrupt cellular homeostasis, leading to inflammation, fibrosis, and metabolic dysfunction (Cahill et al., 2006; Kattoor et al., 2017; Masenga et al., 2023).

Dietary supplements with antioxidant properties are often used to help manage liver diseases caused by oxidative stress (Li et al., 2015). Examples include silymarin, curcumin, fenugreek seeds, vitamin C, vitamin E, and selenium, among others (Almeida et al., 2011; Tewari et al., 2020; Ahamed et al., 2022; Mohammadi et al., 2024). These supplements work by neutralizing excessive ROS and reactive nitrogen species (RNS), which, if unchecked, can severely damage cellular lipids, proteins, and DNA. By doing so, they not only preserve cellular integrity but also support critical regulatory pathways involved in maintaining the oxidative-reductive balance (Kurutas, 2016). Among natural supplements with antioxidant properties, several prior works have demonstrated that ginger (*Zingiber officinale*) exhibits promising effects in enhancing the body's antioxidant defenses and protecting against oxidative damage (Ballester et al., 2023; Mustafa and Chin, 2023; Ayustaningwarno et al., 2024). Recent works have also emphasized that ginger constituents possess potent antioxidant, anti-inflammatory, and anticancer properties, which are critical in regulating oxidative stress and protecting liver health, especially in age-related liver dysfunction (Bekkouch et al., 2022; Ozkur et al., 2022). The antioxidant potential of ginger is largely attributed to its major bioactive phenolic compounds, including [6]-gingerol, [8]-gingerol, [10]-gingerol, [6]-shogaol, and zingerone, which are abundant in fresh or dried rhizome extracts (Mao et al., 2019; Wu et al., 2023). These compounds have been shown to exert antioxidant activity by both direct radical scavenging (e.g., against DPPH, superoxide, hydroxyl, and nitric oxide radicals) and indirect modulation of cellular defense pathways (Peng et al., 2015). In particular, [6]-gingerol and [6]-shogaol can activate the Nrf2–ARE pathway, promoting transcription of cytoprotective genes like HO-1, NQO1, and GCLC, which help regulate glutathione metabolism and maintain redox homeostasis (Kim and Jang, 2016; Hong et al., 2020). Moreover, these compounds can inhibit NF-κB signaling, thereby reducing oxidative damage linked to chronic inflammation. These pleiotropic effects on redox-sensitive transcription factors make ginger a compelling candidate for dietary antioxidant intervention, particularly in age-associated liver dysfunction where these pathways are often dysregulated. Additionally, research has shown that ginger extracts can effectively scavenge superoxide, hydroxyl, and nitric oxide radicals in a dose-dependent manner (Jagetia et al., 2004). Such activity may support minimizing cellular damage and maintaining redox balance. In human cell studies, such as those conducted on C28/I2 chondrocytes, pretreatment with ginger extract reduced ROS levels, inhibited lipid peroxidation, and enhanced the expression of antioxidant enzymes, showing its role at a cellular level (Hosseinzadeh et al., 2017). Although ROS are deeply involved in liver injury, leading to the loss of its structure and function, the potential of ginger in counteracting such oxidative effects through various pathways is worth exploring (Banerjee et al., 2023). Building on *in vitro* findings, animal studies have further highlighted ginger's therapeutic potential in diverse conditions associated with oxidative stress, for example, in the context of nephrotoxicity caused by agents like gentamicin and cadmium (Hegazy et al., 2016; Gabr et al., 2019) and in Polycystic Ovary Syndrome (PCOS), among other disorders (Novakovic et al., 2024). While numerous studies have shown that ginger has strong antioxidant properties, limited research has explored dose-dependent *in vivo* effects. The significance of such studies, is for example, highlighted in a work conducted on STZ-induced diabetic rats, demonstrated that ginger not only reduces oxidative stress markers such as malondialdehyde (MDA) but also reduces genetic damage caused by oxidative stress in a dose-dependent manner (Kota et al., 2012). Moreover, recent clinical research showed that ginger supplementation improved the levels of liver stress-associated biomarkers (most consistently ALT) in patients (Rahimlou et al., 2016; Rafie et al., 2020; Matin et al., 2024b). Additionally, taking in consideration the age-dependency of the regulation of liver antioxidant defense systems it is of interest to examine how ginger's effects on liver redox balance may vary with age. While there is still a lack of conclusive research on this specific aspect, previous works have explored diverse ginger bioactivities that support putative general anti-aging action (Matin et al., 2024a).

On the background of the outlined prior research, the goal of the present study is to explore how ginger (Zingiber officinale) affects the liver's antioxidant defense system in mice, focusing on the impact of different doses and ages. While planning this research work, we hypothesized that ginger would improve the liver's antioxidant defenses at moderate to high doses, and that the effects will be stronger in older mice.

2 Materials and methods

2.1 Experimental setup

The study was carried out with 144 Swiss Webster male mice, that were 3 months (n = 48), 6 months (n = 48), or 12 months (n =

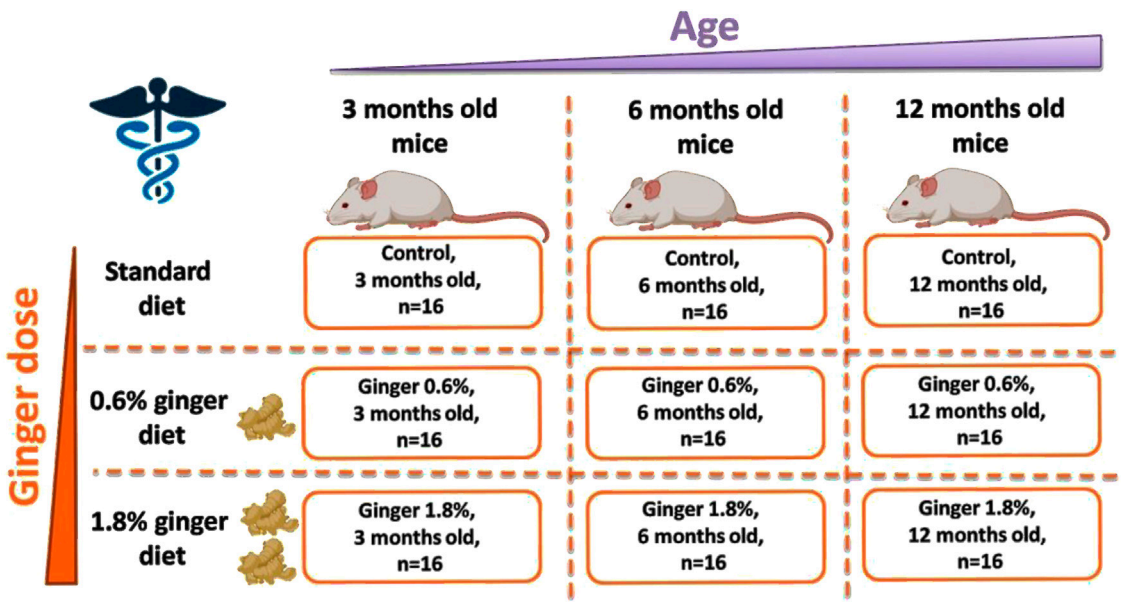

FIGURE 1
Scheme of the conducted ginger supplementation experiment.

48) old at the start of the supplementation (Figure 1). The Swiss Webster mouse strain was selected due to its widespread use in nutritional, toxicological, and pharmacological studies (Meek and Olson, 2007; Abd El-Salam and Hassan, 2017; Glavas et al., 2019). This outbred strain exhibits robust hepatic function and age-dependent redox alterations that make it suitable for modeling liver oxidative stress and assessing dietary antioxidant interventions. Furthermore, Swiss Webster mice are genetically heterogeneous, which allows for broader applicability of the findings and reduces the risk of strain-specific bias in response to dietary supplementation.

Only male Swiss Webster mice were used in this study to reduce variability associated with the estrous cycle, which can influence hormonal regulation of antioxidant enzyme systems and confound interpretation of redox-related endpoints. This approach allows for more consistent assessment of age and dose-related effects of ginger supplementation. However, it is acknowledged that sex-based differences in antioxidant capacity exist, and future studies should aim to include female mice to assess the potential for sex-specific responses to ginger supplementation.

The animals were maintained in standard cages (with four mice per cage), at the temperature of 22°C under standard conditions with 12 h of daylight and 12 h of darkness, with access to food and water. Both food and water were provided *ad libitum* throughout the entire study period, ensuring unrestricted access to standard or ginger-supplemented diets and hydration. For 3 months of supplementation period, the animals were fed with standard mouse feed (Control) or standard diet supplemented with 0.6% (Ginger 0.6%) or 1.8% (Ginger 1.8%) dried ginger (*Z. officinale*) powder (5% gingerols, supplied by Sabinsa Corporation, 20 Lake Drive, East Windsor NJ 08520, United States). Ginger powder was stored at room temperature in a foil-lined, airtight zip bag, protected from light to prevent degradation and oxidation. The ginger-supplemented fodder was prepared freshly by mixing standard feed with the respective amounts of ginger powder (0.6% or 1.8%, corresponding to 0.6 g or 1.8 g per 100 g of fodder) twice a week. After 3 months of supplementation, all animals were anesthetized with diethyl ether and decapitated. Immediately after decapitation, the liver was isolated and further processed for subjection to the described analytical procedures.

The total number of animals (144 mice) was determined based on allocating n = 16 mice per group, with three dietary treatments (control, 0.6% ginger, 1.8% ginger) across three age groups (3, 6, and 12 months). This sample size was chosen to ensure sufficient statistical power to detect medium-to-large effect sizes (Cohen's $f \geq 0.25$) using one-way and two-way ANOVA with multiple comparisons. While a formal *a priori* power analysis was not performed during the initial design phase, assuming an alpha of 0.05 and a power (1-β) of 0.80, the required sample size per group for medium effect detection using ANOVA would typically fall within the range of 12–16 animals per group (Faul et al., 2007). Future studies may refine group sizes using precise *a priori* calculations based on pilot effect sizes.

The selected ginger concentrations (0.6% and 1.8% w/w in the diet) were based on previous rodent studies that demonstrated antioxidant and hepatoprotective effects at similar levels. For instance, Ahmed et al. and Mallikarjuna et al. reported that 1% ginger in rat diets significantly improved oxidative stress biomarkers and liver function (Ahmed et al., 2008; Mallikarjuna et al., 2008). Moreover, dose-dependent antioxidant responses have been observed with 1% and 2% dietary ginger in rats (Shanmugam et al., 2011). In our study, 0.6% served as a moderate dose comparable to those previously shown to be effective, while 1.8% was selected to probe enhanced biological activity without exceeding known safe dietary limits in rodents. These levels also correspond to human-equivalent doses of approximately 3–10 g of ginger daily for a 70-kg adult, which aligns with the range used in multiple clinical supplementation studies (Anh et al., 2020).

The work was performed in the frame of animal handling permission No. 14186201 in accordance with national regulations (Act on the Protection of Animals Used for Scientific or Educational Purposes of 15 January 2015).

2.2 DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay

The potential of liver homogenates to scavenge the free synthetic DPPH radical was determined according to the procedure described by Brand-Williams et al. (1995). A 150 mg portion of liver tissue was cut and mixed with 1.5 mL of ultrapure methanol containing 1% acetic acid. The sample was then homogenized, and incubated in a water bath at 40°C for 2 h. Following incubation, the tubes were removed, allowed to cool briefly, and then centrifuged at 4,000 RPM for 10 min. Next, 0.5 mL of supernatant was mixed with the same volume of ethanolic solution of 0.5 mM DPPH, which was previously diluted to yield an absorbance of 0.9 when measured at a wavelength of λ = 517 nm. Next, the obtained mixture was thoroughly mixed and incubated in a dark and cool place for 30 min for stabilization of the color. Finally, Cary 50 Bio UV-VIS spectrophotometer was used for extinction measurements at a wavelength of λ = 517 nm with the use of Cary WinUV software.

2.3 Antioxidant capacity

The liver samples were handled according to the instructions of the Antioxidant Assay Kit by Cayman Chemical (Ann Arbor, MI, United States), according to the manufacturer's recommendations. The liver tissue (50 mg) was homogenized in 1 mL of Assay Buffer (1X), centrifuged at 10,000 × *g* for 10 min (at 4°C), and the supernatant was promptly used for the test. According to the manufacturer's instructions, the assay was further conducted with the use of 96-well plates in a 210 µL of total volume per well for 5 min, with the inclusion of 10 µL of Trolox standards in each handled plate. After the end of the incubation period, absorbance was read at 750 nm. A Trolox standard curve was plotted, and the antioxidant capacity of the samples was calculated and expressed as millimolar (mM) Trolox equivalents.

2.4 Determination of vitamin C

Vitamin C levels in livers of the experimental animals was determined with a LambdaBio-20 spectrophotometer (Perkin Elmer, Waltham, MA, United States) according to the method described by Omaye et al. (1979) with modification by Jóźwik et al. (2012). For sample preparation, 300 mg portion of liver tissue was weighed and placed into a 5 mL Eppendorf tube. A total of 1.5 mL of phosphate buffer (pH 7.0) was added to the liver tissue. The sample was homogenized thoroughly to ensure uniform consistency. For deproteination and supernatant preparation, 500 µL aliquot of the homogenized sample was transferred into a test tube. An equal volume (500 µL) of tri-chloro-acetic acid (TCA) was added, and the mixture was vortexed thoroughly. The sample was centrifuged at 3,000 RPM for 10 min. A 500 µL aliquot of the resulting supernatant was carefully collected into a clean Eppendorf tube. For colorimetric reaction development, 200 µL of phosphoric acid (H_3PO_4) was added to the supernatant, followed by vortexing. Next, 200 µL of 2,2-dipyridyl was added and the solution vortexed. Lastly, 100 µL of ferric chloride ($FeCl_3$) was added and the solution was vortexed. Two blank samples using 500 µL of TCA were prepared for reference. The reaction mixture was incubated at 37°C for 1 h. Following incubation, the absorbance of the samples was measured at 525 nm using a spectrophotometer.

2.5 Determination of total phenolic content

The total phenolics content of liver homogenates was assessed following the modified method by Škerget et al. (2005) through spectrophotometric measurement of the colorimetric redox reaction upon application of the Folin-Ciocalteu reagent. Preparation of supernatant was the same as the procedure of DPPH described above. 0.5 mL of supernatant were transferred to 6 mL tubes and mixed with 2.5 mL of Folin-Ciocalteu reagent that was diluted 10-fold with demineralized water. Next, the samples were thoroughly mixed and left for 6 min, then 2 mL of saturated sodium carbonate solution was added. The following step was an incubation for 30 min at 40°C (to allow the development of a stable blue color). Again centrifugation was done for 5 min at the speed of 3000 RPM. Finally, the absorbance of the samples was measured at 765 nm wavelength in comparison to a blank sample of double-distilled H_2O (0.5 mL). Evaluation of the results was performed with a calibration curve based on the absorbance of Gallic acid standard solutions in the range 0–0.5 mg/mL. The final results were expressed as milligrams of Gallic acid equivalents (GAE) per Gram of tissue.

2.6 Superoxide dismutase activity assay

The liver tissue samples were perfused with phosphate-buffered saline (PBS; pH 7.4), and homogenized in chilled HEPES buffer (20 mM, pH 7.2, containing 1 mM EDTA, 70 mM sucrose, and 210 mM mannitol). The chilled homogenates were then centrifuged for 5 min at 1,500 x *g* (4°C). The samples were then promptly tested for SOD activity with the Cayman Chemical Superoxide Dismutase Assay Kit (Ann Arbor, MI, United States) according to the manufacturer's manual. The measurement setup included 96-well plates and a total volume of 230 µL of total volume per well, and SOD standard solutions were included on each assayed plate. The absorbance was determined at 450 nm wavelength using Synergy4 microplate reader (Biotek; Winooski, VT, United States). A SOD standard curve was plotted, and the SOD activity of the test samples was calculated and expressed in U/mL.

2.7 Determination of malondialdehyde levels

MDA levels in liver tissue were determined using the Thiobarbituric Acid Reactive Substances (TBARS) Assay Kit (Cayman, Ann Arbor, MI, United States) following the manufacturer's instructions. Briefly, 25 mg of liver tissue was weighed into a 1.5 mL test tube, and 250 µL of RIPA buffer was

added. The mixture was homogenized while being kept on ice. The homogenized samples were centrifuged at 1,600 × g for 10 min at 4°C, and the resulting supernatants were stored on ice. The tissue homogenates did not require dilution before the assay. For the colorimetric assay, 100 µL of each sample or standard was added to appropriately labeled microcentrifuge vials, followed by the addition of 100 µL of 10% trichloroacetic acid (TCA) assay reagent. The mixture was swirled to ensure thorough mixing before the addition of 800 µL of Color Reagent, after which the vials were vortexed. The vials were then incubated in a boiling water bath for 1 h. After incubation, the vials were transferred to an ice bath for 10 min to stop the reaction, followed by centrifugation at 1,600 × g at 4°C. From each vial, 200 µL of the supernatant was transferred (in duplicate) to a clear plate, and the absorbance was measured at 530–540 nm using a Cary Varian 50Bio spectrophotometer (Santa Clara, CA, United States). The MDA concentration was calculated using a calibration curve generated from the MDA standard provided by the manufacturer, with a concentration range of 0–50 µM.

2.8 Determination of reduced glutathione concentration

Reduced glutathione (GSH) concentrations were determined according to the method of Beutler et al. (1963), with minor modifications, using DTNB as the chromogenic reagent and absorbance measured at 412 nm (Beutler et al., 1963). The liver tissue sample was homogenized at a ratio of 50 mg per 250 µL of 0.1 M of phosphoric buffer containing 0.01 M of EDTA (pH = 7.4). The homogenized sample was centrifuged for 10 min at 3000 RPM. For deproteinization of the sample, a mixture was prepared by adding 100 µL of 10% TCA, 100 µL of the supernatant, and 100 µL of 10 mM EDTA and vortexed. The mixture was then left to stand for 10 min. Following this, it was centrifuged again at 3000 RPM for 10 min. For the preparation of the sample and blank solutions, 230 µL of water was added to the plates. In the sample wells, 30 µL of 3.2 M Tris-HCl (pH 8.1), 10 µL of 10 mM EDTA, 25 µL of the obtained supernatant, and 10 µL of 3 mM DTNB were added. For the blank, 30 µL of 3.2 M Tris-HCl (pH 8.1), 20 µL of 10 mM of EDTA, 10 µL of TCA and 10 µL of 3 mM DTNB were added. Plate was placed on the shaker and incubated at room temperature for 5 min. Finally, the absorbance was measured at 412 nm. Bio-Tek Synergy4 microplate reader (Winooski, VT, United States) was used to determine absorbance at 412 nm and data on reaction kinetics. The obtained results were evaluated with the Gen5 program (BioTek), and ultimately the glutathione levels were calculated as µM concentration values.

2.9 Statistical analysis

The data are presented as mean ± standard deviation (SD). Data analysis was performed using the Real Statistics Resource Pack software (Release 8.9.1; copyright 2013–2023; Charles Zaiontz; www.real-statistics.com). Analysis of Variance (ANOVA) at α = 0.05, with Bonferroni correction for multiple comparisons, was used to determine statistical significance. Tukey's Honest Significant Difference (HSD) test was conducted as a follow-up to ANOVA. In the generated graphs, statistically significant differences ($p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$) were indicated by one, two, or three asterisks, respectively.

3 Results

The effects of dietary ginger supplementation on liver antioxidant defense systems were evaluated across different age groups of mice through multiple biochemical and enzymatic tests.

DPPH radical scavenging activity in liver tissue demonstrated both age- and dose-dependent responses to ginger supplementation (Figure 2). In the control group animals, there was a decrease in the DPPH scavenging activity with advancement of age (65.88% ± 5.69%, 55.19% ± 7.87%, and 59.00% ± 6.82%, for the 3-month-old, 6-month-old, and the 12-month-old animals, respectively; with a significance of $p \leq 0.001$ in the 6-month-old control group animals and $p \leq 0.05$ in the 12-month-old control animals, both in comparison to the 3-month-old control group). In the 3-month-old mice, both 0.6% and 1.8% ginger supplementation significantly decreased DPPH radical scavenging activity compared to control (65.88% ± 5.69% for the control, 58.46% ± 6.91% for the 0.6% ginger supplemented animals, and 56.52% ± 3.70% for the 1.8% ginger supplemented animals). This effect was more pronounced in the 1.8% ginger supplemented group ($p \leq 0.001$). The 6-month-old mice and the 12-month-old mice groups showed an inverse pattern, with significant increase ($p \leq 0.001$) in the DPPH radical scavenging in response to ginger supplementation, thus exhibiting ginger supplementation-induced reversal of the age-dependent decrease observed for the control animal groups.

Total antioxidant capacity, measured as Trolox equivalents, declined in the control animals (2.40 ± 0.40, 2.79 ± 0.30, and 1.86 ± 0.66 Trolox equivalents for the 3-month-old, 6-month-old, and the 12-month-old animals, respectively) with significant decrease especially in the 12-month-old control group ($p \leq 0.01$, Figure 3). Ginger supplementation overall displayed a pattern of enhanced total antioxidant capacity across all age groups, with the effect being most pronounced in the 1.8% supplemented 12-month-old group (1.86 ± 0.66 Trolox equivalents in the control group versus 3.31 ± 0.33 Trolox equivalents in the supplemented animals; with $p \leq 0.001$, in comparison to the 12-month-old control group).

Liver vitamin C levels showed an age-dependent decline in control animals (2.91 mg ± 0.36 mg, 2.56 mg ± 0.64 mg, and 1.56 mg ± 0.15 mg, for the 3-month-old, 6-month-old, and the 12-month-old animals, respectively; Figure 4), with the 12-month-old control group exhibiting approximately twice lower vitamin c levels compared to the 3-month-old control group (1.56 ± 0.15 versus 2.91 ± 0.36 mg/100 g; mean ± SD; $p \leq 0.001$). Ginger supplementation overall displayed a pattern of increased vitamin C levels in comparison to the respective controls in all age groups, with a significant effect in the 6-month-old group supplemented with 1.8% ginger (2.56 mg ± 0.64 mg versus 3.46 mg ± 0.60 mg; $p \leq 0.01$ compared to respective age-matched control).

Total phenolic content showed a tendency of age-related decline in control animals (6.47 ± 0.67, 5.77 ± 0.38, and 5.04 ± 1.00 mg GAE/g for the 3-month-old, 6-month-old, and the 12-month-old animals,

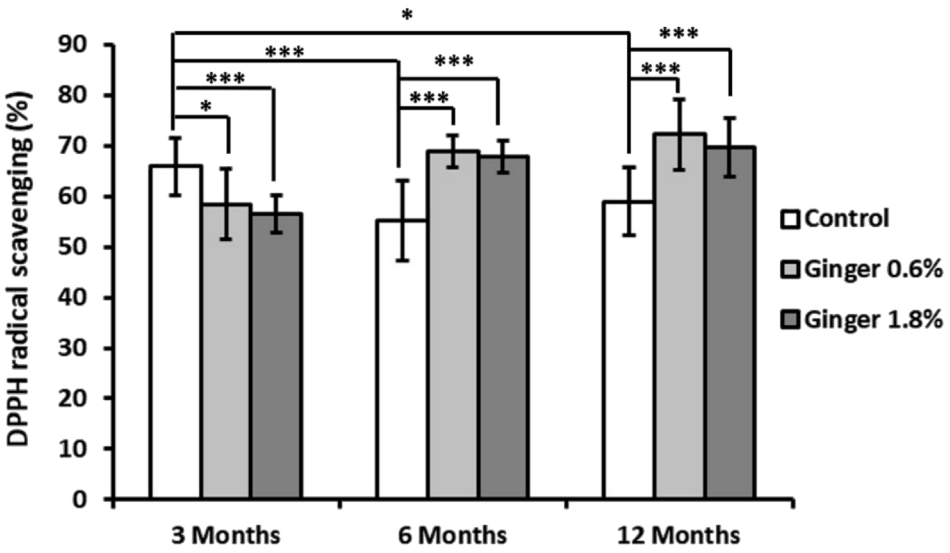

FIGURE 2
Liver DPPH radical scavenging activity. Data are presented as the percentage of DPPH radical scavenging across different age groups (3, 6, and 12 months) and dietary treatments (control, 0.6% ginger, 1.8% ginger). Values are shown as mean ± standard deviation (n = 16). Statistical significance between groups is indicated (ANOVA/Tukey HSD; *p ≤ 0.05; ***p ≤ 0.001).

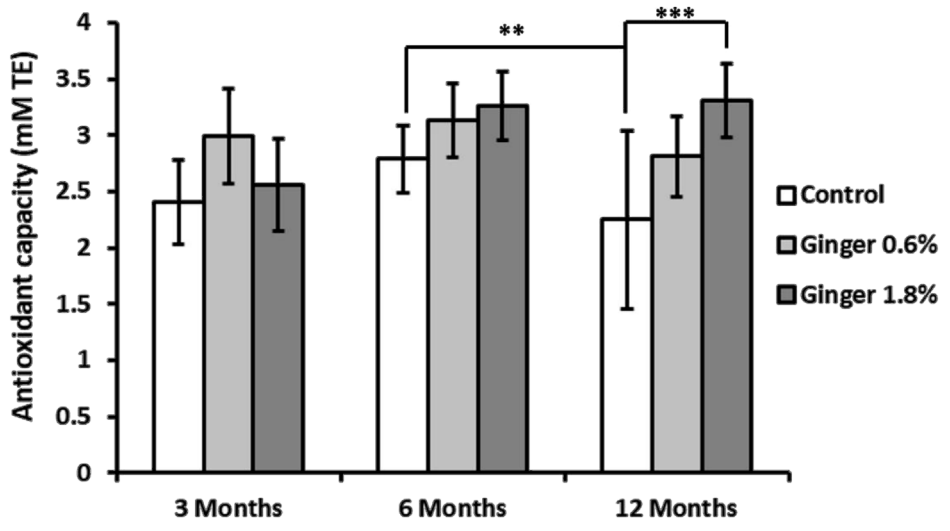

FIGURE 3
Liver total antioxidant capacity. Total antioxidant capacity of liver homogenates was determined as described in detail in the Methods section. The results are expressed in millimolar (mM) Trolox equivalents (TE). Data represent mean ± standard deviation (n = 8), with significance denoted by asterisks. (ANOVA/Tukey HSD; **p ≤ 0.01; ***p ≤ 0.001).

respectively; Figure 5), with the 12-month-old control group showing significantly lower levels compared to the 3-month-old control group (5.04 ± 1.00 versus 6.47 ± 0.67 mg GAE/g tissue; mean ± SD; $p \leq 0.001$). Ginger supplementation led to differential effects in the different age groups, with decrease of phenolic content levels in the 3-month-old animals, increase in the 6-month-old animals (especially the 1.8% supplemented group, 5.77 ± 0.38 versus 8.65 ± 0.89 mg GAE/g tissue; $p \leq 0.001$ in comparison to the age-matched control), and no effect in the 12-months-old animal groups.

Superoxide dismutase (SOD) activity showed significant age-related decline in control animals (10.76 ± 1.15, 6.93 ± 1.05. and 7.04 ± 2.58 U/mL for the 3-month-old, 6-month-old, and the 12-month-old animals, respectively; Figure 6), with both the 6-month-old and the 12-month-old control group exhibiting markedly lower activity compared to the 3-month-old control group (6.93 ± 1.05 and 7.04 ± 2.58 versus 10.76 ± 1.15 U/mL; mean ± SD; $p \leq 0.001$). Ginger supplementation did not produce significantly different effects on SOD activity in comparison to the respective age-matched control groups.

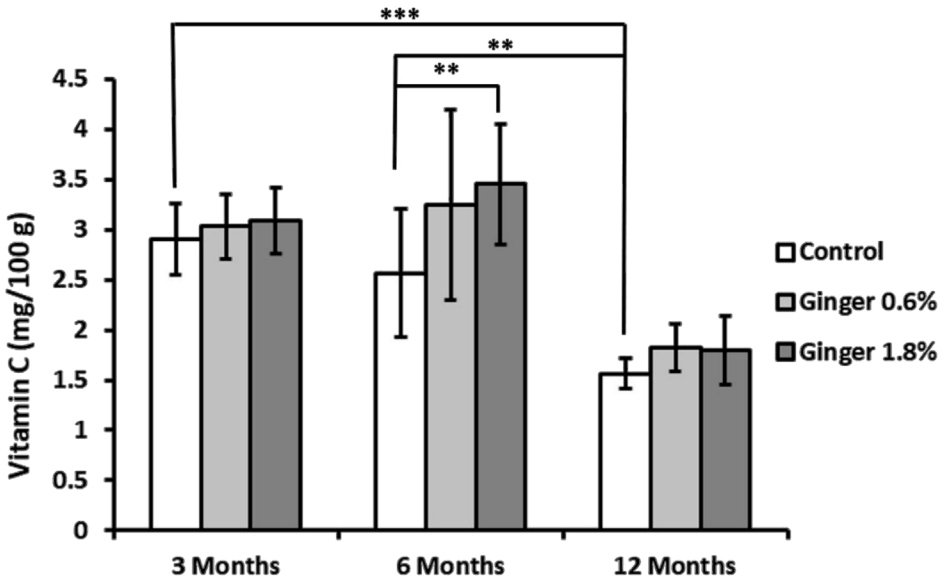

FIGURE 4
Liver vitamin C levels. Vitamin C concentrations in liver tissue were determined spectrophotometrically as described in the Methods section. The results are expressed as milligrams of vitamin C per Gram of tissue. Data represent mean ± standard deviation (n = 8). Statistical significance between groups is indicated (ANOVA/Tukey HSD; **p ≤ 0.01; ***p ≤ 0.001).

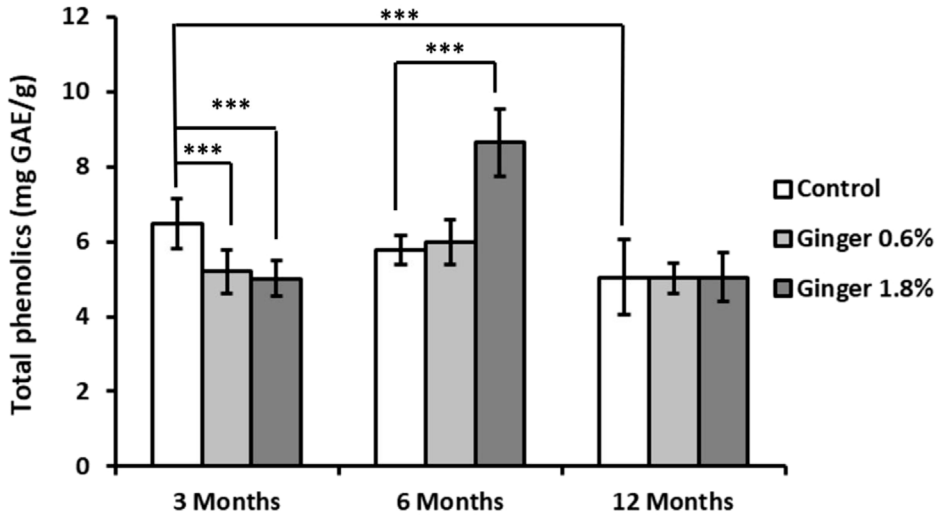

FIGURE 5
Liver total phenolic content. The total phenolic content in liver homogenates was assessed using the Folin-Ciocalteu reagent, as detailed in the Methods section. Results are expressed as milligrams of gallic acid equivalents (GAE) per Gram of tissue. Data are shown as mean ± standard deviation (n = 16). Statistical differences between groups are indicated (ANOVA/Tukey HSD; ***p ≤ 0.001).

Malondialdehyde (MDA) levels demonstrated a significant age-related decrease in control animals (81.08 ± 23.16, 34.86 ± 4.40, and 37.77 ± 5.89 μM for the 3-month-old, 6-month-old, and the 12-month-old animals, respectively; Figure 7), with both the 6-month-old and 12-month-old control groups exhibiting substantially lower levels compared to the 3-month-old control group (34.86 ± 4.40 and 37.77 ± 5.89 versus 81.08 ± 23.16 μM; mean ± SD; $p \leq 0.001$). In 6-month-old and 12-month-old animals, ginger supplementation at both concentrations resulted in moderate decreases in MDA levels compared to age-matched controls, which did not reach statistical significance. The most pronounced effect was observed in 3-month-old animals supplemented with 1.8% ginger, where MDA levels were significantly ($p \leq 0.001$; 81.08 ± 23.16 versus 45.44 ± 5.69 μM) reduced compared to the age-matched control group.

Reduced glutathione (GSH) levels did not display statistically significant age-related variations in control groups animals (Figure 8). Ginger supplementation produced enhancing effects

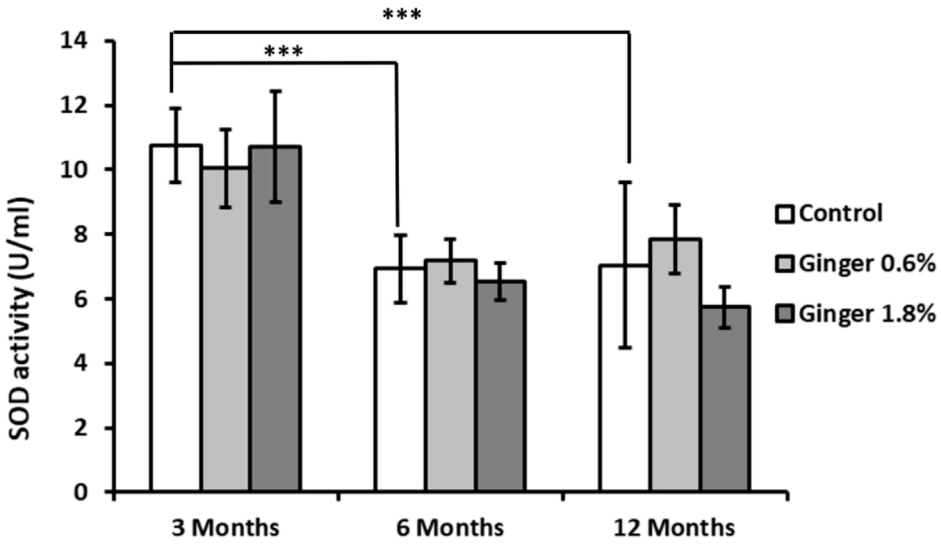

FIGURE 6
Liver superoxide dismutase (SOD) activity. Superoxide dismutase activity in liver homogenates was measured as described in the Methods section and is expressed in U/mL. Data are presented as mean ± standard deviation (n = 8). Statistical significance is indicated (ANOVA/Tukey HSD; ***p ≤ 0.001).

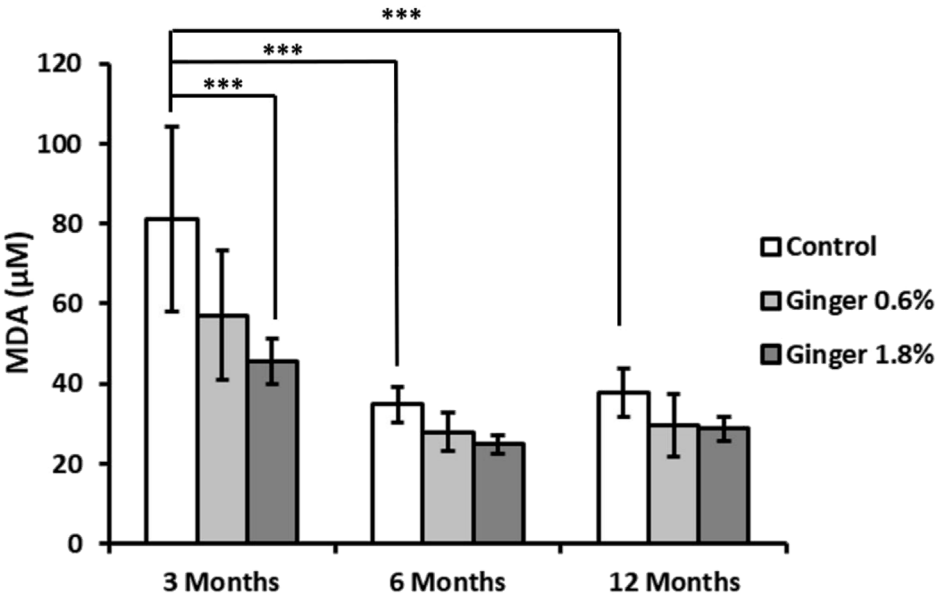

FIGURE 7
Liver malondialdehyde (MDA) levels. Malondialdehyde levels, an indicator of lipid peroxidation, were measured in liver tissue homogenates. Data are expressed as micromolar (μM) concentrations. Results are shown as mean ± standard deviation (n = 8). Statistical significance between groups is indicated (ANOVA/Tukey HSD; ***p ≤ 0.001).

in comparison to the age-matched controls in all age groups, with the 1.8% concentration being more effective (inducing statistically significant effects with $p \leq 0.001$, $p \leq 0.05$, and $p \leq 0.01$ in the 3-month-old, 6-month-old, and 12-month-old animal groups, with 748.07 ± 104.25 versus 962.02 ± 204.59, 623.94 ± 88.30 versus 791.75 ± 132.33, and 634.57 ± 84.85 versus 831.43 ± 83.73 μM, respectively).

4 Discussion

This study provides novel insights into how ginger dietary supplementation affects liver antioxidant defense systems across different age groups in mice. The results demonstrate complex age- and dose-dependent effects of ginger supplementation on various markers of oxidative stress and antioxidant capacity in liver tissue.

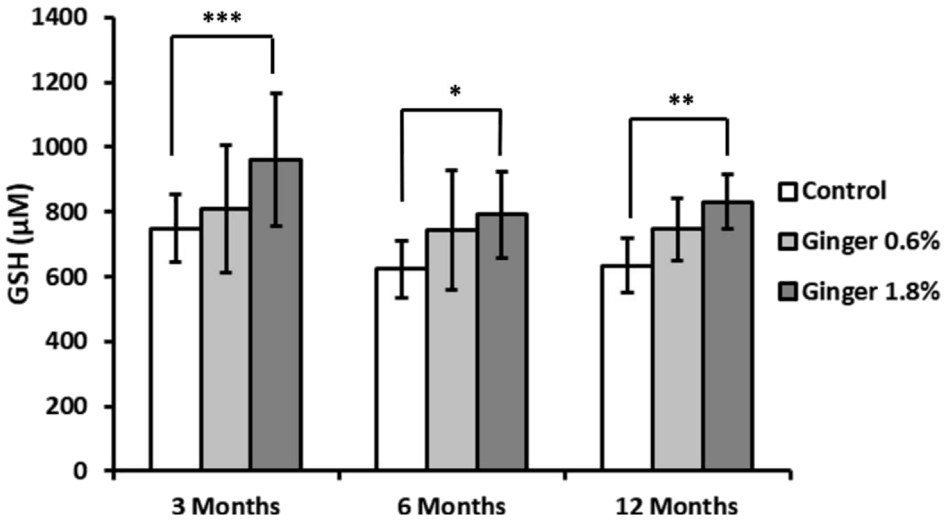

FIGURE 8
Liver reduced glutathione (GSH) levels. Reduced glutathione concentrations in liver homogenates were determined as described in the Methods section and expressed in micromolar (µM) concentrations. Data are shown as mean ± standard deviation (n = 16). Statistical significance is indicated (ANOVA/Tukey HSD; *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001).

Our results confirm the age-dependent decline in several key antioxidant parameters, including DPPH radical scavenging activity, total antioxidant capacity, vitamin C levels, total phenolic content, and SOD activity. This aligns with previous research suggesting that aging is associated with diminished antioxidant defenses and increased oxidative stress, which contribute to liver dysfunction (Shih and Yen, 2007; Castro Mdel et al., 2012; Lozada-Delgado et al., 2020). Our findings support this paradigm and extend it by demonstrating the differential response of antioxidant defenses to ginger supplementation at various life stages. Moreover, the observed decline in parameters such as SOD activity and vitamin C levels in the control animal groups emphasizes the importance of age-specific interventions to counteract these deficits.

The dose-dependent effects of ginger supplementation were evident across most of the studied biochemical markers. At higher doses (1.8%) and most consistently in older mice, ginger supplementation increased DPPH radical scavenging activity, enhanced total antioxidant capacity, as evidenced by increased Trolox equivalents, and increased reduced GSH levels. These findings align with prior studies suggesting that ginger's antioxidant properties are mediated through effects such as scavenging of reactive oxygen species and enhancement of endogenous antioxidant systems (Stoilova et al., 2007; Lee et al., 2011; Sueishi et al., 2019).

From a mechanistic standpoint, the antioxidant effects of ginger are likely mediated by both direct and indirect pathways. Ginger's bioactive constituents—particularly gingerols, shogaols, paradols, and zingerone—are known to exert free radical scavenging activity, lipid peroxidation inhibition, and upregulation of endogenous antioxidant defenses. These actions are partially attributed to modulation of the Nrf2 (nuclear factor erythroid 2–related factor 2) signaling pathway, which regulates the expression of antioxidant response element (ARE)-dependent genes such as those encoding glutathione S-transferase, heme oxygenase-1 (HO-1), and NAD(P)H:quinone oxidoreductase 1 (NQO1) (Peng et al., 2015). Furthermore, ginger has been shown to inhibit inflammatory signaling cascades like NF-κB, which are often intertwined with oxidative stress responses. In aged organisms, these pathways may become dysregulated or exhibit reduced responsiveness. This could explain the enhanced efficacy of ginger observed in older animals in the present study—where the supplementation may help restore diminished redox regulation by reactivating Nrf2 signaling or replenishing depleted non-enzymatic antioxidants such as GSH and vitamin C. Moreover, pharmacokinetic alterations with age—such as slower metabolism and longer tissue retention of ginger compounds—may enhance their local effectiveness in older livers.

Although the total antioxidant capacity (TAC) appeared numerically higher in the 6-month-old control group than in the 3-month-old control group, and lower in the 1.8% ginger-supplemented group at 3 months compared to the corresponding control, these differences did not reach statistical significance. Therefore, these observations should be interpreted with caution. However, minor fluctuations in TAC between age groups could reflect transitional physiological states in antioxidant regulation during early adulthood. Some studies suggest that compensatory upregulation of endogenous antioxidant systems may transiently occur during midlife before the onset of age-related decline (Benzi et al., 1989; Shih and Yen, 2007), potentially contributing to slightly elevated TAC at 6 months. Regarding the numerically lower TAC in the high-dose ginger group at 3 months, it is possible that high-dose antioxidant supplementation in physiologically young and redox-balanced animals may trigger negative feedback regulation or subtle pro-oxidant shifts, as previously proposed in models of hormesis and antioxidant overcompensation (Calabrese and Baldwin, 2003). Although these effects were not statistically significant in our study, they highlight the importance of considering age and baseline redox status when evaluating the impact of dietary antioxidant interventions. Future studies with larger sample sizes or targeted molecular profiling could help determine whether these trends are biologically meaningful.

A key finding was the age-dependent pattern observed in DPPH radical scavenging activity. While younger (3-month-old) mice showed decreased DPPH radical scavenging with ginger supplementation, both middle-aged (6-month-old) and older (12-month-old) mice exhibited significantly increased activity. This differential response suggests that the impact of ginger on free radical scavenging mechanisms varies with age, potentially due to age-related changes in baseline antioxidant capacity as well as age-related changes of in the rate of metabolizing exogenously supplied bioactive compounds including ginger phytochemicals with antioxidant action (Kim et al., 2002; Lee et al., 2008). Meanwhile, the enhanced DPPH radical scavenging observed in older mice aligns with previous research showing ginger's ability to combat oxidative stress that is increased with aging (Peng et al., 2015; Hosseinzadeh et al., 2017; Al Hroob et al., 2018).

The observed decrease in DPPH radical scavenging activity in 3-month-old mice supplemented with ginger, particularly at the 1.8% dose, may be explained by several interconnected mechanisms. First, young mice generally possess robust endogenous antioxidant systems, with high baseline levels of enzymatic and non-enzymatic antioxidants. Introducing additional exogenous antioxidants like ginger may lead to transient pro-oxidant effects or feedback inhibition of endogenous antioxidant responses—a phenomenon often observed in hormetic responses to phytochemicals. This biphasic behavior is supported by the concept that mild oxidative stress can activate protective mechanisms, whereas excessive antioxidant input in already-balanced systems may dampen radical signaling or paradoxically impair redox balance (Calabrese and Baldwin, 2003). Additionally, certain phenolic compounds in ginger may exhibit dual roles—acting as antioxidants at low or moderate ROS levels but exhibiting pro-oxidant effects under high-reducing or low-ROS cellular states (Maliar et al., 2023). This could explain the reduced DPPH scavenging in young mice where oxidative burden is minimal. Moreover, the higher metabolic activity and detoxification efficiency in younger animals may lead to faster metabolism and clearance of ginger phytochemicals, limiting their local accumulation and effect in liver tissue. Collectively, these age-dependent pharmacodynamic and redox-regulatory differences help explain the attenuated response in younger animals and underscore the importance of physiological context in interpreting antioxidant interventions.

The age-related differences in antioxidant response to ginger supplementation observed in this study highlight a complex interplay between baseline redox status and metabolic responsiveness. Younger mice (3 months old) displayed higher endogenous antioxidant levels in control groups, which may have limited the observable benefits of supplementation or even led to paradoxical effects (e.g., reduced DPPH scavenging and phenolic content). This may be due to a "ceiling effect," where antioxidant systems are already well-regulated and additional stimulation is unnecessary or counterproductive. In contrast, middle-aged and older mice (6 and 12 months) exhibited significant age-associated declines in DPPH scavenging, vitamin C levels, and SOD activity—creating a physiological state of redox vulnerability. Ginger supplementation in these groups likely restored redox homeostasis by bolstering antioxidant reserves, particularly through the replenishment of GSH and enhancement of total antioxidant capacity. This aligns with prior findings that antioxidant interventions have greater benefit in aged or oxidatively stressed organisms compared to healthy young counterparts (Shih and Yen, 2007; Castro Mdel et al., 2012). These observations support the concept of age-personalized antioxidant supplementation, where physiological context determines the efficacy of dietary interventions.

The significant reduction MDA levels, particularly in younger mice receiving high-dose ginger supplementation, reflects the previously reported ability of dietary ginger to counter lipid peroxidation (Ahmed et al., 2000; Ahmed et al., 2008). However, the less pronounced effects in older animals might indicate an age-related resistance to modulation of lipid peroxidation pathways. These findings warrant further investigation into the molecular mechanisms underpinning these differences.

Interestingly, MDA levels in control animals were significantly higher at 3 months compared to 6 and 12 months, which contrasts with the typical expectation of age-related increases in lipid peroxidation. However, several factors may explain this finding. In early adulthood, mice exhibit higher metabolic rates, elevated mitochondrial respiration, and increased membrane lipid turnover, all of which can transiently elevate ROS production and enhance susceptibility to lipid peroxidation (Sohal et al., 1990). Moreover, older tissues may shift toward protein and DNA oxidation markers, with relatively less lipid peroxidation occurring at baseline (Cakatay et al., 2003; Ma et al., 2013). Therefore, MDA should not be viewed in isolation as a direct surrogate of total oxidative burden across ages. These findings highlight that age-related shifts in oxidative stress profiles can vary depending on the molecular target (lipid, protein, DNA), metabolic context, and tissue-specific vulnerability.

Superoxide dismutase activity demonstrated significant age-related decline, confirming previous findings about the deterioration of liver enzymatic antioxidant defenses with age (Reiss and Gershon, 1976; Santa Maria et al., 1996). The lack of significant changes with ginger supplementation suggests that ginger's antioxidant effects might primarily operate through non-enzymatic pathways, rather than by modulating SOD activity directly.

The lack of a measurable effect of ginger supplementation on total SOD activity, despite improvements in other antioxidant parameters, may stem from several factors. First, the assay used in this study measured total SOD activity, without discriminating between isoforms such as SOD1 (cytosolic) and SOD2 (mitochondrial). Prior studies have shown that ginger may preferentially affect mitochondrial oxidative stress pathways and upregulate SOD2 (Homa and Wentz-Hunter, 2018), changes that could remain undetected in whole-tissue homogenates where SOD1 predominates. Second, it is possible that the antioxidant benefits of ginger in this model were mediated more through non-enzymatic antioxidants (e.g., GSH, vitamin C) or via modulation of other enzymatic systems (e.g., catalase, glutathione peroxidase), thereby limiting the need for enhanced SOD activity. Third, redox homeostasis involves complex compensatory networks. Increased glutathione or phenolic content may reduce superoxide burden, alleviating the need for further SOD induction. Finally, assay sensitivity and technical variability may also obscure subtle isoform-specific changes. Further studies examining specific SOD isoforms at the transcriptional and protein level, or

subcellular localization of antioxidant effects, could help clarify these findings.

Although ginger supplementation did not produce statistically significant changes in SOD activity in any group, it is worth noting that SOD activity values in the 1.8% ginger groups at 6 and 12 months were numerically lower than those in the 0.6% ginger groups. This observation, while not statistically significant, may reflect a dose-dependent regulatory ceiling or feedback suppression of enzymatic antioxidants when exogenous antioxidant input is high. At elevated doses, ginger-derived phenolics may strongly increase non-enzymatic antioxidant reserves—such as GSH and vitamin C—which could reduce the cellular demand for SOD activity, leading to compensatory downregulation. This type of adaptive response has been reported in other antioxidant supplementation studies, where excessive exogenous antioxidant availability dampened the expression of genes of endogenous antioxidant defense proteins (Halliwell, 2000; Calabrese and Baldwin, 2003). Another possibility is that higher doses of ginger exert differential effects on antioxidant enzyme regulation depending on cellular redox state and age, which may not follow a linear dose–response pattern. However, due to the lack of statistical significance, these interpretations remain speculative and should be explored in future studies involving isoform-specific enzyme measurements and redox-sensitive gene expression profiling.

Glutathione levels showed a consistent positive response to ginger supplementation across all age groups, with the higher dose (1.8%) being more effective. This enhancement of GSH levels is particularly noteworthy given glutathione's central role in cellular redox regulation and antioxidant defense (Zhang and Forman, 2012). The ability of ginger to boost GSH levels across different age groups suggests it could have broad applications in supporting liver antioxidant defenses.

The findings of the present study collectively support our initial hypothesis regarding ginger's beneficial effects on liver antioxidant systems, particularly at higher doses. However, the age-dependent variations in response highlight the complexity of antioxidant system regulation and suggest that ginger's effects may be modulated by age-related factors. The stronger responses observed in older mice for several parameters (particularly DPPH radical scavenging and total antioxidant capacity) align with our hypothesis about enhanced effects in older animals.

The results also provide insights into potential mechanisms of ginger's antioxidant effects. The simultaneous enhancement of non-enzymatic antioxidants (vitamin C, GSH) and total antioxidant capacity, coupled with reduced lipid peroxidation (MDA), suggests multiple pathways of action. This multi-target effect is consistent with previous studies showing ginger's diverse bioactive properties (Mao et al., 2019; Wu et al., 2023).

These findings have several potential implications for therapeutic applications. The age-dependent effects observed suggest that ginger supplementation might be particularly beneficial in supporting liver antioxidant defenses in older individuals, where natural antioxidant systems may be compromised. This aligns with prior research showing ginger's potential versatile benefits in supporting liver health (Gao et al., 2012; AuthorAnonymous et al., 2017; Hamza et al., 2021; Guo et al., 2022). The study outcomes also hint to potential broader anti-aging benefits of ginger supplementation, in line with its previously characterized versatile bioactivity profile simultaneously contracting different hallmarks of aging (Ozkur et al., 2022; Matin et al., 2024a). Additionally, the dose-dependent effects observed in the present work support the importance of appropriate dosing in ginger supplementation strategies.

Future research could focus on elucidating the molecular mechanisms underlying the age-specific effects observed, particularly the differential responses in young versus older animals. Investigation of changes in antioxidant gene expression and protein levels could provide additional insights into how ginger modulates these systems. Additionally, longer-term studies could help understand the sustainability of these effects and potential adaptations over time.

5 Limitations

This study has several limitations that should be considered when interpreting the findings. While we evaluated total superoxide dismutase (SOD) activity, the assay did not distinguish between SOD isoforms (e.g., SOD1 and SOD2), nor did it assess other antioxidant enzymes such as catalase or glutathione peroxidase. Thus, potential isoform-specific or compensatory effects may have been masked in total activity measurements.

A limitation of this study is the exclusive use of male animals, which, while controlling for hormonal variability, does not account for sex-specific differences in antioxidant defense systems. Estrogen, for instance, has been shown to modulate redox signaling and influence expression of key antioxidant enzymes such as SOD and GPx. Therefore, future research should include both sexes to determine whether the antioxidant effects of ginger exhibit sex-specific patterns, particularly in the context of aging.

Further, although we observed several trends across age and dose groups, some differences—particularly those in DPPH scavenging, total antioxidant capacity, and SOD activity—did not reach statistical significance. These non-significant patterns should be interpreted with caution and may benefit from further validation with larger sample sizes or targeted molecular analyses. In addition, we did not perform transcriptomic or proteomic analyses to directly assess the molecular pathways modulated by ginger, such as Nrf2 or NF-κB signaling, which are known to regulate antioxidant defenses. These mechanistic insights remain speculative and should be explored in future investigations.

Finally, the study was conducted over a 3-month period of supplementation, which may not fully capture the long-term effects of dietary ginger supplementation on liver function and redox balance. Longer-term studies and broader biomarker panels would strengthen understanding of the sustained impact and safety profile of ginger in aging models.

6 Conclusion

This study demonstrates that ginger dietary supplementation exerts significant dose- and age-dependent effects on liver antioxidant defense systems in mice. Our findings confirm the initial hypotheses regarding both the dose-dependency of ginger's effects and their more pronounced effects in older animals for

several key parameters. Ginger supplementation enhanced key markers of antioxidant capacity, including DPPH radical scavenging activity, reduced glutathione (GSH) levels, and total antioxidant capacity, particularly at higher doses and in older animals. These findings highlight ginger's potential to mitigate age-associated oxidative stress and support hepatic antioxidant defenses.

The differential impacts of ginger supplementation observed in this study underscore the importance of tailoring dietary interventions based on age and dosage. The demonstrated dose-dependent effects emphasize the importance of appropriate dosing strategies, while age-specific responses highlight the need for personalized antioxidant supplementation strategies. Future research should focus on elucidating the molecular mechanisms underlying these age-specific effects and investigating the long-term implications of ginger supplementation on liver health across different life stages.

Overall, this study establishes ginger as a promising dietary supplement for supporting liver antioxidant defenses, particularly in aging populations, while also highlighting the complexity of its biological effects across different age groups. These findings contribute to our understanding of natural antioxidant interventions and their potential role in promoting healthy liver aging.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was approved by the animal handling permission No. 14186201 in accordance with national regulations (Act on the Protection of Animals Used for Scientific or Educational Purposes of 15 January 2015). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

MM: Writing – original draft, Investigation, Methodology, Conceptualization, Formal Analysis, Project administration, Data curation. KW: Writing – review and editing, Formal Analysis, Investigation. JH: Conceptualization, Writing – review and editing. LR: Writing – review and editing, Conceptualization. AA: Writing – review and editing, Conceptualization.

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Ginger supplementation supports liver health during aging through regulation of lysosomal function and molecular markers

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Aging is marked by hepatic structural deterioration, inflammation, and impaired autophagy. This study evaluated the effects of dietary ginger (*Zingiber officinale*) on liver aging in Swiss Webster mice across three age groups (3, 6, and 12 months) supplemented with 0.6% or 1.8% ginger powder for three months. Liver samples were assessed for lysosomal enzyme activity, expression of PI3K and TNF-α, histology, and ultrastructure of hepatocytes.

Aging induced hepatocellular hypertrophy, mitochondrial damage, and increased lysosomal enzyme activity, particularly arginyl aminopeptidase and acid phosphatase, and β-galactosidase - a widely recognized marker of aging, particularly cellular senescence. Ginger supplementation preserved liver morphology in young and middle-aged mice, while high doses showed mild subcellular stress in older mice. Ginger also suppressed age-related PI3K upregulation and influenced TNF-α expression, indicating potential anti-inflammatory effects.

These findings suggest that ginger modulates hepatic aging through lysosomal, inflammatory, and metabolic pathways in an age- and dose-dependent manner. Moderate intake appears most beneficial, supporting its potential as a nutraceutical for liver health during aging.

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Aging is a complex biological process characterized by the progressive loss of physiological integrity, leading to organ dysfunction with an increased vulnerability to chronic diseases, including metabolic, neurodegenerative, and inflammatory conditions [López-Otín *et al*. 2013]. At the cellular level, aging is defined by various well-established hallmarks, including genomic stability, cellular senescence, altered intercellular communication, mitochondrial dysfunction, impaired autophagy, and loss of proteostasis [Kennedy *et al*. 2014]. Among these, chronic inflammation and impaired autophagic-lysosomal function play central roles in hepatic aging by deteriorating the turnover of damaged organelles and promoting oxidative stress [Rubinsztein *et al*. 2011]. The liver, as a primary metabolic and detoxifying organ, is highly susceptible to age-related changes, was selected in this study for comprehensive analysis due to its central role in systemic aging and metabolic regulation. Thus, exploring therapeutic strategies that can modulate age-related pathways could lead to promising interventions for promoting healthy aging.

In recent years, the use of natural compounds with antioxidant and anti-inflammatory properties has shown a growing interest as potential agents to counteract the effects of aging [Bensz *et al*. 2025, Cătană *et al*. 2018]. Among these, ginger *Zingiber officinale* stands out as a widely consumed medicinal plant with a wide range of bioactive compounds such as gingerols, shogaols, and paradols, demonstrating anti-inflammatory, antioxidant, and hepatoprotective effects in various experimental models [Mao *et al*. 2019, Matin *et al*. 2024b, Shanmugam *et al*. 2011]. We previously demonstrated in a mouse model that dietary ginger (0.6 and 1.8%) modulates hepatic antioxidant defenses in a clear dose- and age-dependent manner, showing increased Glutathione level (GSH) and total antioxidant capacity, and lowered Malondialdehyde level (MDA), with the strongest benefits in older animals [Matin *et al*. 2025]. Emerging studies also suggest that the ginger compounds can modulate autophagy, reduce oxidative damage, and impede pro-senescent signaling cascades [Matin *et al*. 2024a]. However, the potential effect of ginger supplementation to influence age-associated cellular and molecular hallmarks, particularly on lysosomal dysfunction, gene expression, and ultrastructure in the context of aging mice in liver tissue, remains underexplored. Given the rising interest in nutraceuticals for healthy aging, it is critical to understand how dietary ginger might possess a key influence on the age-related pathways in a tissue-specific and age-dependent manner.

To address this gap, the present study was designed to investigate the potential effect of ginger supplementation to modulate the hallmarks of aging in the liver of mice across different age groups. This age-stratified approach allows for the evaluation of age- and dose-dependent effects of ginger on the physiology of the liver and molecular signaling. Specifically, an investigation on the activity of lysosomal enzymes (aminopeptidases and glycosidases) was undertaken to determine how ginger affects the lysosomal system across different age groups. Furthermore, gene expression analyses of tumor necrosis factor-alpha TNF-α (a pro-inflammatory cytokine linked

to age-related inflammation and immune dysfunction) and phosphoinositide 3-kinase PI3K (a critical node in the insulin/IGF-1 signaling pathway, implicated in longevity and metabolic homeostasis) were conducted to explore the impact of ginger on key inflammatory and metabolic pathways. Of relevance, a prior study by Kakino et al. (2018) reported increased hepatic TNF-α mRNA expression in aged mice, which was associated with enhanced NF-κB activation and inflammatory gene expression [Kakino *et al*. 2018]. Furthermore, aging is associated with decreased PI3K activity in liver tissues, leading to impaired downstream signaling and glucose metabolism [Braccini *et al*. 2015].

In addition to biochemical and molecular analyses, histological and ultrastructural studies on mice liver were performed in our study to visualize the morphological changes associated with aging and their modulation by ginger. Ultrastructure analyses have previously revealed that aging hepatocytes exhibit significant morphological alterations, including mitochondrial swelling, increased lipofuscin accumulation, and disrupted endoplasmic reticulum [Łysek-Gładysińska *et al*. 2021]. Thus, a study by Terman and Brunk [2004] highlighted such changes suggesting the impaired autophagy contributes to the accumulation of damaged organelles in aged liver [Terman and Brunk 2004]. By correlating these microscopic findings with biochemical and molecular markers, our study aims to investigate age- and dose-dependent effects of ginger influencing hepatic aging at multiple biological levels.

Material and methods

Experimental setup

In this study, 144 male Swiss Webster mice were used, categorized into three age groups (Tab. 1).

Table 1. Experimental animal groups

Ginger dose/age	3-month-old mice	6-month-old mice	12-month-old mice
Standard diet	control 3 months old, n = 16	control 6 months old, n = 16	control 12 months old, n = 16
0.6% ginger diet	ginger 0.6%, 3 months old, n = 16	ginger 0.6%, 6 months old, n = 16	ginger 0.6%, 12 months old, n = 16
1.8% ginger diet	ginger 1.8%, 3 months old, n = 16	ginger 1.8%, 6 months old, n = 16	ginger 1.8%, 12 months old, n = 16

Only male mice were used to avoid variations in liver physiology and enzyme activities associated with hormonal changes throughout the female estrous cycle. This strategy was intended to reduce biological variability and support clearer interpretations of the age- and dose-dependent outcomes of ginger supplementation.

Animals were housed in groups of four per standard cage under controlled environmental conditions (22°C, 12-hour light/dark cycles), with food and water

available ad libitum. Over the course of the 3-month supplementation, animals were fed either a standard diet (Control group) or the same base diet enhanced with 0.6 or 1.8% dried ginger powder. The ginger powder, standardized to contain 5% gingerols, was sourced from Sabinsa Corporation (20 Lake Drive, East Windsor, NJ 08520, USA). Fresh supplemented feed was prepared twice weekly. Upon completion of the supplementation period, fresh liver tissues of mice were taken following the American Veterinary Medical Association (AVMA) Guidelines (2020) and processed for further investigation of biochemical and analytical procedures.

All procedures involving animals were conducted in the frame of animal handling permission No. 14186201 in accordance with the relevant institutional and national regulations (including the EU Directive 2010/63/EU for animal experiments and National Research Council's Guide for the Care and Use of Laboratory Animals). The chosen dietary inclusion rates of ginger (0.6 and 1.8%) were informed by earlier rodent studies demonstrating the efficacy of ginger in improving liver function. Prior research has reported relevant bioactivities with dietary ginger concentrations ranging from 1 to 2% [Ahmed *et al*. 2008, Mallikarjuna *et al*. 2008, Shanmugam *et al*. 2011].

Tissue preparation for morphological examination

Liver fragments were promptly excised from mice upon the feeding experiment termination. The samples were finely chopped and immersed in a fixative consisting of 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for two hours at 4°C. Following primary fixation, the tissue fragments were rinsed in the same buffer, followed by post-fixation in a 1% osmium tetroxide solution prepared in 0.1 M cacodylate buffer for two hours at ambient temperature, protected from light. Subsequently, the specimens were washed with buffer and dehydrated using a graded ethanol series, with each step lasting 10 minutes. Dehydrated tissues were infiltrated and embedded in epoxy resin (Agar 100) using propylene oxide as an intermediary solvent, following standard protocols. The embedded samples were polymerized in silicone molds at 60°C for 48 hours, resulting in hardened resin blocks containing the preserved tissue.

Sectioning and staining for light and electron microscopy

Embedded liver samples were first trimmed into semithin sections to survey broader tissue architecture under a light microscope, followed by the preparation of ultrathin sections for detailed ultrastructural analysis using transmission electron microscopy (TEM). Semithin sections, ranging from 0.5 to 1 μm in thickness, and ultrathin sections of approximately 50-60 nm were obtained using a Reichert-Jung ultramicrotome equipped with either dry or wet glass knives.

Semithin slices were floated onto water droplets on glass slides and then dried by placing the slides on a hot plate at 60°C to facilitate adhesion. These sections were stained at 80°C with Sudan III and aniline blue. After staining, slides were thoroughly rinsed under running water, air-dried, mounted with Histokitt, and examined under a

light microscope. Selected fields from each section were photographed using a Nikon Eclipse 80i microscope equipped with Nikon NIS Elements D 3.10 imaging software (Nikon Instruments, Inc., NY, USA).

Ultrathin sections, characterized by their grey-to-silver interference color and 50-60 nm thickness, were transferred to 300-mesh copper grids. These grids were then double-stained with saturated aqueous uranyl acetate and lead citrate. The stained samples were observed using a TESLA BS500 transmission electron microscope. High-resolution images were captured with a Variospeed 1K-SSCCD slow scan camera (TRS, Moorenweis, Germany) and processed using the associated image acquisition software.

Determination of enzyme activities related to carbohydrate metabolism, aminopeptidases, and acid phosphatase

The activities of enzymes involved in carbohydrate metabolism, lysosomal function, and protein degradation were analyzed using specific substrates obtained from Sigma-Aldrich (St. Louis, MO, USA). Lysosomal enzyme activity measurements were performed following the methodology established by Barrett and Heath (1977). Specifically, assays were conducted for β-glucuronidase (BGRD; EC 3.2.1.31), α-galactosidase (AGAL; EC 3.2.1.22), β-glucosidase (BGLU; EC 3.2.1.21), α-glucosidase (AGLU; EC 3.2.1.20), β-galactosidase (BGAL; EC 3.2.1.23), β-N-acetylhexosaminidase (HEX; EC 3.2.1.52), and mannosidase (MAN; EC 3.2.1.24). Each enzyme was incubated with its corresponding chromogenic substrate at 37°C: p-nitrophenyl-β-D-glucuronide, p-nitrophenyl-α-D-galactopyranoside, p-nitrophenyl-β-D-glucopyranoside, p-nitrophenyl-β-D-galactopyranoside, p-nitrophenyl-N-acetyl-β-D-glucosaminide, p-nitrophenyl-β-D-mannopyranoside, and p-nitrophenyl-α-D-glucopyranoside [Barrett and Heath 1977]. Acid phosphatase (ACP; EC 3.1.3.2) activity was evaluated by monitoring the conversion of a 4-nitrophenyl phosphate derivative. The absorbance was measured at 420 nm using a Cary 50 Bio UV-VIS spectrophotometer (Varian, part of Agilent Technologies, Mulgrave, VA, Australia), following the same protocol described by Barrett and Heath [Barrett and Heath 1977].

The activities of three aminopeptidases - alanyl aminopeptidase (AlaAP; EC 3.4.11.2), leucyl aminopeptidase (LeuAP; EC 3.4.11.1), and arginyl aminopeptidase (ArgAP; EC 3.4.11.6) were quantified in the prepared supernatants using the method reported by McDonald and Barrett [McDonald and Barrett 1986]. These assays employed Fast Blue BB salt (4-benzoylamino-2,5-diethoxybenzene-diazonium chloride) as the chromogenic reagent, and absorbance was measured at 540 nm with the same Cary 50 Bio spectrophotometer. All enzyme activities were standardized and reported as nanomoles per milligram of total protein per hour (nmol/mg protein/h).

RNA extraction, cDNA synthesis, and quantitative Real-Time PCR (qRT-PCR)

Liver tissue samples were processed to extract total RNA using the RNeasy Mini Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's guidelines. The RNA concentration and purity were assessed using a NanoDrop™ 2000

Spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA) by measuring absorbance at 230, 260, and 280 nm. Isolated RNA samples were stored at -80°C until further processing. For cDNA synthesis, 1 µg of total RNA from each sample was reverse transcribed using the iScript™ cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA, USA) following the manufacturer's instructions. The reverse transcription reaction (20 µL total volume) was conducted with the following thermal profile: 5 minutes at 25°C, 20 minutes at 46°C, and 1 minute at 95°C.

Quantitative real-time PCR (qRT-PCR) was carried out using the CFX Opus 96 thermal cycler (Bio-Rad, Richmond, CA, USA). Each 20 µL PCR reaction contained 10 µL of 2X SsoAdvanced Universal SYBR Green Supermix (Bio-Rad), 500 nM of primers specific to phosphoinositide 3-kinase (PI3K) or tumor necrosis factor alpha (TNF), and 10 ng of cDNA template. The qPCR cycling conditions were initial denaturation at 95°C for 30 seconds, followed by 40 cycles consisting of 95°C for 15 seconds and 60°C for 30 seconds for annealing and extension. A Ct value greater than 37 was considered indicative of a negative result for target gene expression.

A negative control from a healthy mouse in the 3-month control group was designated as the calibrator. Gene expression levels were normalized to the housekeeping gene GAPDH and calculated using the 2-ΔΔCT method as described by Ruiz-Villalba *et al*. [2017] and He *et al*. [2024]. Data were normalized and analyzed using CFX Maestro V2.2 software (Bio-Rad). Each gene expression measurement was conducted in technical triplicate, and biological replicates were derived from independent cell cultures.

Statistical analysis

All statistical analyses were conducted using GraphPad Prism version 9.0.0 (GraphPad Software, Boston, MA, USA). A significance level of $p \leq 0.05$ was considered statistically significant throughout all the analyses. Multi-factorial ANOVA (multiplate ANOVA) was used to evaluate the effects of age and ginger dosage on enzyme activity and gene expression. When significant differences were identified, Duncan's multiple range test was used for post hoc comparisons.

Fisher's test was applied to assess the variance among experimental groups. Pearson correlation analysis was conducted to determine associations between biochemical and molecular parameters. Additionally, linear regression models were used to evaluate age- and dose-dependent effects on hepatic outcomes. Results are presented as least squares means±standard deviation (LSM±SD), and group differences are annotated using superscript letters indicating statistical significance.

Results and discussion

Morphological analysis

As evident from Figure 1 and 2 histological and ultrastructural analysis of liver tissue revealed progressive age-dependent changes in hepatocyte morphology and subcellular organization. In 3-month-old (3M) control mice, hepatocytes appeared

uniformly polygonal with centrally located nuclei, abundant cytoplasmic organelles, and small lipid droplets. Sinusoids were patent, and hepatic architecture was well-preserved. Transmission electron microscopy (TEM) (as shown in Fig. 2), confirmed the presence of nuclei with normal distribution of eu- and heterochromatin, with an active nucleolus and correct nuclear envelope, numerous oval mitochondria with normal matrix density, abundant rough endoplasmic reticulum (RER), numerous peroxisomes, primary lysosomes, and glycogen granules, indicating robust metabolic activity.

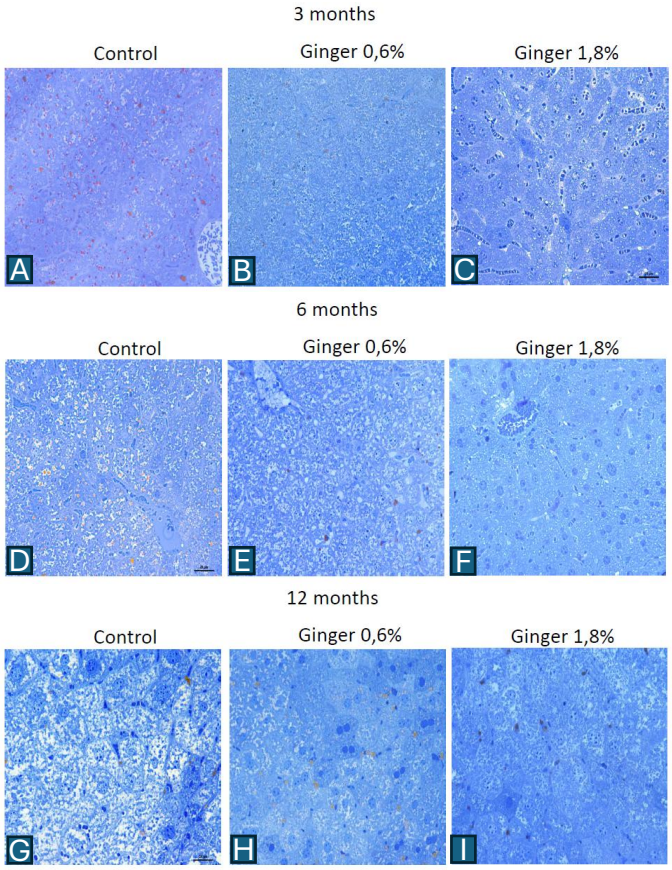

Fig. 1. Histological evaluation of liver tissue architecture in mice across age and dietary groups. Representative light microscopy images of liver sections stained with Sudan III and aniline blue, highlighting morphological features across experimental conditions. The upper panel shows 3-month-old mice: (A) control, (B) 0.6% ginger-supplemented, (C) 1.8% ginger-supplemented. The middle panel depicts 6-month-old mice: (D) control, (E) 0.6% ginger-supplemented, (F) 1.8% ginger-supplemented. The lower panel includes 12-month-old mice: (G) control, (H) 0.6% ginger-supplemented, (I) 1.8% ginger-supplemented. Notable features include hepatocyte morphology, lipid droplet accumulation, and sinusoidal organization. Scale bar = 20 μm.

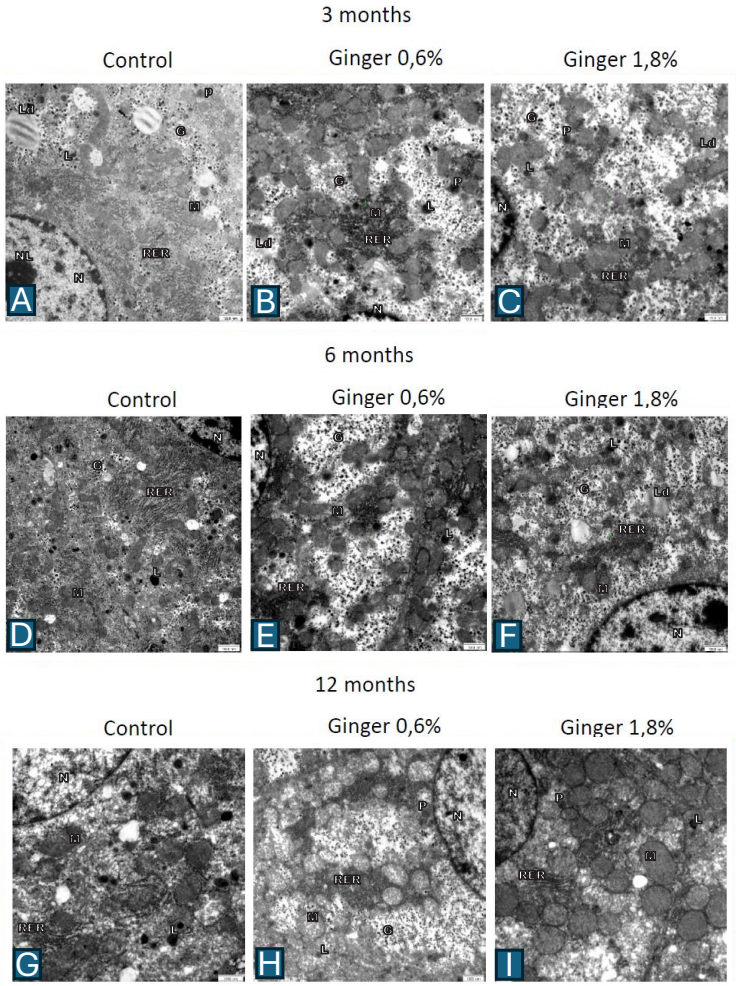

Fig. 2. Transmission electron micrographs showing age- and diet-associated ultrastructural alterations in hepatocytes. Electron micrographs of liver sections from mice at different ages and treatment groups. The upper panel illustrates 3-month-old mice: (A) control, (B) 0.6% ginger-supplemented, (C) 1.8% ginger-supplemented. The middle panel shows 6-month-old mice: (D) control, (E) 0.6% ginger-supplemented, (F) 1.8% ginger-supplemented. The lower panel presents 12-month-old mice: (G) control, (H) 0.6% ginger-supplemented, (I) 1.8% ginger-supplemented. Labeled organelles include lipid droplets (Ld), lysosomes (L), mitochondria (M), damaged mitochondria (dM), nucleus (N), nucleolus (NL), peroxisomes (P), rough endoplasmic reticulum (RER), and vacuoles (V). Scale bar = 1000 nm.

By 6 months (6M), the control livers maintained their general structure, but early signs of cellular aging were evident. Some hepatocytes displayed lightly transparent cytoplasm with fewer visible organelles, though mitochondria and RER remained structurally intact. At 12 months (12M), age-associated changes became

more pronounced. Hepatocytes were often enlarged, with variable nuclear sizes and increased binucleation. Lipid droplets were more numerous, especially visible in Ito cells, and glycogen reserves were reduced. Ultrastructural analysis revealed mitochondrial enlargement and swelling, shortened cristae, and reduced RER, all signs of declining biosynthetic and energy-related functions. Primary lysosomes exhibit an increase in both number and size compared to those of younger mice.

Ginger supplementation influenced these age-related patterns in an age- and dose-dependent manner. In young mice, both 0.6 and 1.8% ginger diets preserved normal hepatocyte morphology, with minimal ultrastructural deviations. Occasional cells in the high-dose group displayed mildly enlarged mitochondria and slightly reduced RER content, but overall organelle integrity was maintained. At 6M, low-dose ginger maintained cellular architecture similar to controls, while high-dose ginger resulted in greater variability in hepatocyte size and cytoplasmic clarity. Ultrastructurally, RER was slightly diminished, and glycogen accumulation was more localized.

In aged mice (12M), the 0.6% ginger diet was associated with moderate ultrastructural alterations, including mitochondrial enlargement and swelling and RER depletion, although lipid droplet accumulation was not exacerbated. The 1.8% ginger group exhibited more substantial changes: mitochondria appeared greatly enlarged, swollen and vacuolated, RER was scarce, and cytoplasmic areas devoid of organelles were common, filled mainly with residual glycogen. The number of primary lysosomes was reduced compared to a control. Despite these subcellular changes, overall tissue architecture remained intact in all groups, suggesting that while high-dose ginger may intensify age-related cellular stress, it does not induce overt hepatocellular damage.

Enzyme activity

Lysosomal enzyme activity was significantly influenced by both age and diet (Tab. 2). Aging alone resulted in elevated activity of several key lysosomal enzymes. Among them, arginyl aminopeptidase (ArgAP) showed a marked and consistent increase with age, particularly evident in 12M controls. Acid phosphatase (ACP), a general marker of lysosomal hydrolysis, and α-galactosidase (AGAL), involved in glycoside metabolism, also exhibited clear age-dependent increases. These patterns suggest an upregulation of degradative lysosomal function as a compensatory response to accumulating macromolecular waste in aging cells.

Not all enzymes changed with age. Leucyl aminopeptidase (LeuAP), for instance, remained stable across time points, indicating that age effects are enzyme-specific. Other glycosidases, such as β-galactosidase (BGAL) and β-glucosidase (BGLU) exhibited increases by 12M, though less consistently across groups.

Ginger supplementation modulated enzyme activity in complex ways that depended on both dose and age. At 3M, 0.6% ginger significantly increased the activity of enzymes such as ArgAP and AGAL compared to controls. This suggests that moderate ginger intake may stimulate lysosomal turnover and enhance protein and glycoside catabolism early in life. However, increasing the dose to 1.8% did not

Table 2. Impact of ginger supplementation on the liver enzyme activities (nmol/mg of protein/hour) and gene expression across the different age groups

Factors		3M			6M			12M			F-test	p-value
		control	EX1	Ex2	control	EX1	Ex2	control	EX1	Ex2		
AlaAP	LSM	308.92^{A}	483.39^{B}	258.18^{A}	$223.15^{A}c$	404.00^{Bd}	267.72^{Ac}	364.16^{BEd}	337.97^{ABEcd}	313.66^{ABEcd}	18.754	0.000
	SD	60.03	100.20	53.28	34.51	32.56	39.52	51.85	26.88	30.93		
LeuAP	LSM	407.97^{A}	397.39^{A}	340.38^{A}	293.37^{A}	386.30A	343.37^{A}	529.65^{b}	478.26^{Abc}	419.66^{Abc}	6.759	0.039
	SD	106.39	55.13	44.90	36.30	67.37	70.85	122.29	99.29	58.93		
ArgAP	LSM	259.03^{A}	253.88^{A}	225.25^{A}	204.43^{A}	239.97	219.12^{A}	500.29^{B}	442.08^{BC}	410.11^{BCD}	68.818	0.003
	SD	39.70	29.84	33.81	21.17	14.90	24.64	46.05	75.05	23.23		
ACP	LSM	2825.75^{A}	3443.68^{A}	3005.52^{A}	2271.12^{AB}	3606.84^{A}	2932.32^{AB}	3561.16^{AB}	2780.34^{AB}	2710.24^{AB}	4.388	0.000
	SD	323.12	613.73	312.20	135.93	1393.14	599.19	352.63	394.23	237.77		
HEX	LSM	710.72^{A}	703.30^{A}	702.15^{A}	594.91^{A}	749.23^{A}	613.30^{A}	820.35^{AD}	1001.58^{B}	917.71^{ABCD}	4.388	0.000
	SD	47.67	76.04	57.66	117.98	210.32	81.11	93.42	44.72	86.02		
AGAL	LSM	70.85^{a}	92.23^{b}	70.95^{a}	76.57^{ab}	119.76^{C}	89.33^{ab}	130.79^{CD}	122.14^{CDE}	129.41^{CDEF}	32.49	0.000
	SD	5.17	11.20	6.27	10.49	11.85	7.58	13.68	19.84	18.31		
BGAL	LSM	258.41^{A}	308.67^{A}	229.97^{E}	198.10^{Eb}	288.78^{A}	238.42^{AEFb}	349.06^{AC}	329.27^{ACD}	306.51^{ACD}	18.186	0.000
	SD	20.53	31.82	43.22	22.57	45.54	37.94	29.57	38.29	17.99		
AGLU	LSM	211.90^{a}	267.05^{b}	199.29^{a}	175.21^{a}	269.36^{bc}	230.10^{abc}	211.40^{abc}	197.89^{abc}	229.30^{abc}	7.202	0.000
	SD	27.45	33.06	34.84	30.34	40.50	41.35	12.21	47.21	12.10		
BGLU	LSM	59.77^{A}	78.63^{B}	52.86^{A}	47.71^{Ac}	64.92^{d}	49.71^{Ac}	67.76^{ABd}	64.65^{ABde}	65.26^{ABdef}	15.237	0.000
	SD	9.14	5.86	5.24	6.55	6.83	8.10	5.13	8.30	7.86		
BGRD	LSM	223.69^{A}	197.92^{A}	196.44^{A}	143.00^{B}	168.82^{ABC}	155.21^{ABCD}	209.35^{A}	193.03^{ABC}	177.17^{ABCDE}	13.691	0.002
	SD	30.98	19.94	15.12	13.15	13.03	9.78	22.20	31.42	7.98		
MAN	LSM	257.16^{A}	299.42^{A}	208.11^{b}	206.50^{bc}	$291.27A^{bc}$	208.83^{Abcd}	337.65^{Abc}	295.46^{Abc}	314.13^{Abc}	7.068	0.006
	SD	48.49	48.41	55.92	69.56	78.84	66.83	30.48	29.03	25.76		
PI3K	LSM	0.976^{A}	0.954^{A}	1.014^{A}	2.179^{B}	0.869^{AC}	0.684^{D}	2.409^{E}	0.606^{DF}	0.191^{G}	1363.694	0.000
	SD	0.028	0.019	0.021	0.095	0.026	0.019	0.118	0.047	0.008		
TNF	LSM	1.02^{A}	1.06^{A}	1.01^{A}	1.70^{B}	0.96^{A}	3.42^{C}	2.13^{D}	0.95^{A}	2.68^{E}	420.202	0.000
	SD	0.040	0.020	0.110	0.130	0.020	0.170	0.110	0.050	0.250		

LSM – least squares mean; SD – standard deviation; control – control group; EX1 – experimental group 1 (0.6%); EX2 – experimental group 2 (1.8%); F-test – Fisher test; p-value – statistically significant value.

$^{Aa...}$Statistically significant differences: small letters – $p \leq 0.05$; capitals –$p \leq 0.01$; groups that share the same letter (e.g., both marked A or a) are not significantly different from one another.

further enhance these enzyme activities, and in some cases, levels returned closer to control values indicative of a threshold or adaptive response.

At 6M, low-dose ginger increased ACP and AGAL activities while reducing ArgAP, implying a selective enhancement of glycosidase over protease activity. The 1.8% dose elicited similar but less pronounced changes, possibly reflecting metabolic compensation or saturation. In older mice (12M), enzyme activities in ginger-fed animals were generally lower or equal to those in age-matched controls. For example, ArgAP and ACP were slightly reduced in both ginger groups compared to 12M controls. This may reflect ginger's potential to normalize or moderate overactive lysosomal function in aged tissue, potentially by reducing inflammatory or oxidative stress signals that drive enzyme upregulation. Increased activity of specific lysosomal enzymes enhances some autophagy, which plays a protective role by preventing the onset of inflammation and oxidative stress [Butler and Bahr, 2006].

Taken together, the data indicate that ginger exerts both stimulatory and regulatory effects on hepatic lysosomal enzymes. These effects are more pronounced in younger animals and tend to stabilize or reduce enzyme activity in older mice, especially at the higher dose. The 0.6% dose often had a more favorable impact, supporting the idea that moderate ginger intake may help maintain lysosomal function without over activation.

Gene expression

Gene expression analyses of PI3K and TNF-**α** (Tab. 2) provided insight into the molecular pathways affected by aging and ginger supplementation. In control mice, PI3K mRNA levels increased in with the advancement of age in the control animals, and this effect was counteracted in a dose-dependent manner from ginger supplementation. TNF-**α** expression, a marker of inflammatory signaling, increased by 12 months, consistent with a low-grade, chronic inflammatory state in aging livers.

Ginger supplementation induced more distinct changes. PI3K expression was significantly suppressed in a dose-dependent manner, especially in mice receiving the 1.8% diet. This suppression was consistent across ages, indicating a robust dietary effect on this key metabolic regulator. Although the physiological implications remain to be fully elucidated, reduced PI3K signaling could reflect altered autophagic or insulin-responsive pathways.

In contrast, TNF-α expression was consistently reduced in ginger-fed mice, with the most significant suppression observed in the 6M and 12M groups receiving the lower dose. These findings support ginger's known anti-inflammatory effects and suggest a potential role in attenuating age-related inflammatory signaling in hepatic tissue.

The aging process is influenced by an increase in PI3K gene expression, which may be linked to liver injury and the decrease in function of the liver cell [Dostert *et al*. 2012]. While PI3K activity supports cellular survival mechanisms, its over activation can lead to detrimental effects, including apoptosis of the cell and damage of the organelles. Research suggests that reducing PI3K activity can promote longevity and potentially delay aging-related processes [Hedges *et al*. 2023].

Regression analysis

Linear regression confirmed aging as a major driver of changes in lysosomal enzyme activity. In Table 3 ArgAP and AGAL showed the strongest correlations with age (R^2 values of 0.70 and 0.60, respectively), consistent with their steady increase across the lifespan. ACP, HEX, and BGAL also demonstrated moderate positive associations with age. TNF-α had a weaker but significant correlation with age (R^2 ~0.13), while PI3K expression showed no statistically significant age-related trend.

Table 3. Linear Regression (influences of aging)

Factor	R_squared	p_value	Slope	Intercept
AlaAP	0.0	0.961	-0.142	330.013
LeuAP	0.206	0.0	12.158	314.488
ArgAP	0.7	0.0	24.971	131.222
ACP	0.001	0.818	-5.17	3051.409
HEX	0.375	0.0	26.0	575.023
AGAL	0.595	0.0	5.476	61.893
BGAL	0.28	0.0	8.021	222.427
AGLU	0.018	0.258	-1.545	232.092
BGLU	0.025	0.184	0.484	57.865
BGRD	0.002	0.739	-0.329	187.263
MAN	0.175	0.0	7.705	214.789
PI3K	0.001	0.851	0.004	1.069
TNF	0.128	0.002	0.082	1.086

Regression with ginger dose as the predictor (Tab. 4) revealed that PI3K expression decreased significantly with increasing ginger concentration ($R^2 = 0.61$, $p<0.001$), making it the most diet-sensitive parameter. AGLU activity also rose modestly with dose, while other enzymes showed no consistent linear dose response, reflecting the previously observed non-linear or age-specific effects of ginger. These results confirm that aging is a broad modulator of lysosomal and inflammatory markers, while ginger exerts more targeted, pathway-specific effects.

Table 4. Linear Regression (influences of diet)

Factor	R_squared	p_value	Slope	Intercept
AlaAP	0.057	0.044	45.41	298.744
LeuAP	0.006	0.527	-16.105	410.329
ArgAP	0.009	0.42	-22.85	321.249
ACP	0.017	0.274	193.813	2886.01
HEX	0.046	0.069	72.554	708.656
AGAL	0.04	0.093	11.235	92.735
BGAL	0.016	0.294	15.078	268.524
AGLU	0.129	0.002	32.657	199.506
BGLU	0.031	0.14	4.259	58.413
BGRD	0.026	0.174	-10.585	192.016
MAN	0.0	0.89	2.433	267.103
PI3K	0.607	0.0	-1.135	1.855
TNF	0.001	0.776	0.062	1.618

Correlation analysis

As evident from Figure 3 correlation mapping revealed networks of co-regulated biochemical markers. Lysosomal enzymes such as ArgAP, AGAL, and ACP were positively correlated with one another, forming a cluster indicative of synchronized age-related upregulation. TNF also showed moderate positive correlations with this cluster (with the exception of ACP), suggesting that inflammatory signaling may accompany lysosomal activation in the aging liver.

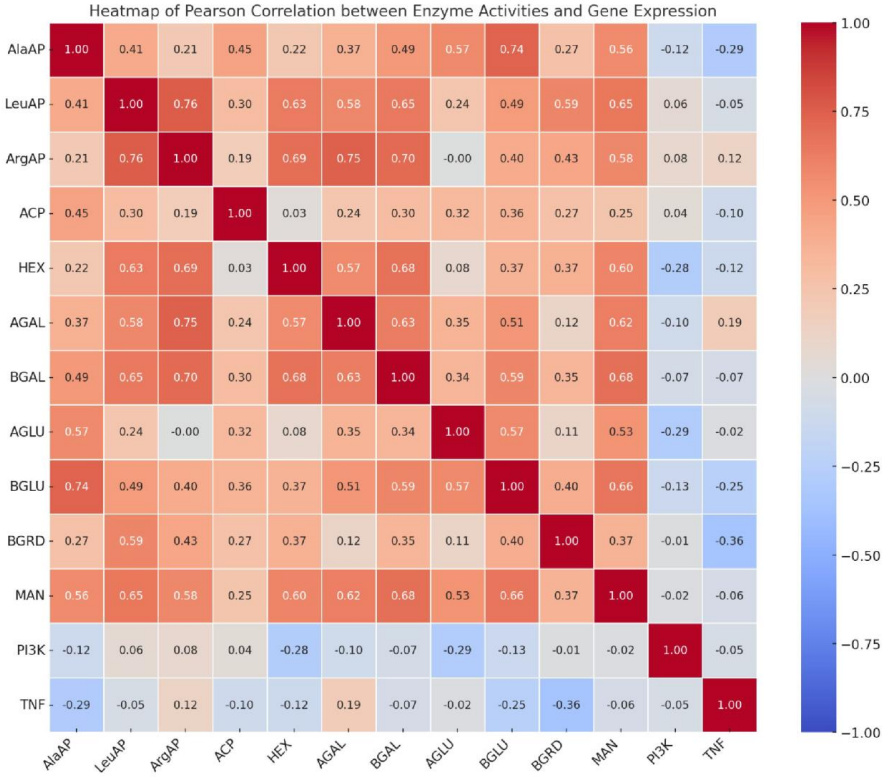

Fig. 3. Correlation heatmap of biochemical and molecular markers in hepatic tissue. Heatmap illustrating Pearson correlation coefficients among key lysosomal enzymes (e.g., ArgAP, AGAL, ACP), inflammatory marker TNF-α, and PI3K gene expression. Positive correlations are depicted in red and negative correlations in blue, with intensity reflecting correlation strength. The map highlights co-regulated enzymatic activities and the inverse relationships between PI3K expression and both lysosomal and inflammatory markers, suggesting coordinated responses in aging and ginger-mediated modulation.

PI3K expression, in contrast, negatively correlated with many enzymes and with TNF, highlighting an opposing regulatory pattern. This suggests that high PI3K levels are associated with lower inflammatory and catabolic activity, and that ginger's suppression of PI3K aligns with increased lysosomal turnover and reduced

inflammation. These correlation patterns reinforce the broader view that aging enhances a network of inflammatory and degradative functions, while ginger modulates this system, suppressing PI3K and TNF-**α** and stabilizing lysosomal activity in a dose- and age-dependent fashion.

Hepatic aging is a complex, multifactorial process marked by various morphological and cellular changes in hepatocytes [Hunt *et al*. 2019]. Our present study offers novel insights into the effects of ginger supplementation on age-related alterations in hepatic structure and function. By employing a stratified approach across three different age groups, we investigated the influence of two doses of ginger on lysosomal enzyme activity, inflammatory and metabolic gene expression, and histological features of liver tissue. The present study confirms that aging is associated with progressive histological changes, including hepatocellular hypertrophy, increased nuclear pleomorphism, enhanced lipid droplet accumulation, and reduction in glycogen storage. At the subcellular level, transmission electron microscopy revealed mitochondrial enlargement and swelling, cristae shortening, and reduction in rough endoplasmic reticulum (RER), which were particularly prominent, indicating impaired energy metabolism and protein biosynthesis capacity in senescent liver cells. These observations are consistent with prior studies reporting similar features of hepatocellular aging [Terman and Brunk, 2004]. Our findings provide substantial evidence that ginger exerts age- and dose-dependent regulatory effects on liver aging processes, primarily through modulating autophagy, inflammation, and metabolic signaling.

Interestingly, ginger supplementation demonstrated a dose- and age-dependent ability to partially modulate these age-related hepatic changes. Moderate ginger supplementation (0.6%) effectively preserved hepatocellular morphology in both young and middle-aged mice, while the older animals receiving a higher dose (1.8%) led to greater morphological changes, suggesting a potential adaptive effect, where higher doses may induce mild cellular stress. Such dose-sensitive behavior is increasingly recognized in nutraceutical research, emphasizing the need to identify optimal intake levels for long-term benefit [Bondy 2023, Caruso *et al*. 2024].

Lysosomal enzyme activity, which plays an important role in autophagic degradation and turnover of macromolecules, was significantly influenced by both age and ginger supplementation. In younger mice, ginger enhanced the activity of enzymes such as ArgAP and AGAL. These findings align with the concept that aged cells increase the function of lysosomal enzymes as a compensatory response to macromolecular damage and impaired autophagy [Cuervo and Dice 2000]. This suggests that ginger stimulates degradative capacity early in life, potentially preventing the buildup of damaged proteins and organelles. In contrast, aged mice exhibited naturally elevated lysosomal enzyme activity likely as a compensatory response to cellular damage and ginger moderated this increase, potentially stabilizing lysosomal function. Such nuanced regulation is beneficial, excessive lysosomal activation in aging can be deleterious, leading to leakage and further stress, whereas targeted

enhancement or normalization helps maintain cellular homeostasis [Gómez-Sintes *et al*. 2016, Tan and Finkel 2023]. In our study, the activity of lysosomal enzymes generally corresponds to the number of primary lysosomes present in the hepatocytes of the mice studied groups.

At the molecular level, ginger influenced key regulators of metabolic and inflammatory signaling. Expression of phosphoinositide 3-kinase (PI3K), a critical mediator in insulin/IGF and mTOR pathways [Harrington *et al*. 2005], increased with age in control livers but was significantly suppressed by ginger, especially at higher doses. This downregulation may enhance autophagy and reduce metabolic stress. Dysregulation of PI3K/Akt/mTOR is a hallmark of aging, and inhibition of this pathway has been linked to lifespan extension in various models [Abdellatif *et al*. 2022, Castillo-Quan *et al*. 2019]. Our findings are consistent with studies reporting that ginger and its active constituents, such as 6-gingerol and 6-shogaol, suppress PI3K/Akt activity and promote autophagy [Pei *et al*. 2021, Wang *et al*. 2016].

The anti-inflammatory effects of ginger were corroborated by the significant reduction in TNF-α expression across ginger-supplemented groups, particularly at the lower dose (0.6%) in older mice (both 6-and 12-month groups). Tumor necrosis factor-alpha (TNF-α), is a key pro-inflammatory cytokine that is often elevated with aging (a phenomenon known as inflammaging) [Muhammad 2020] and is known to disrupt insulin signaling and promote oxidative stress. Interestingly, the most notable effects observed in the 6- and 12-month-old mice at the lower dose, aligns with ginger's known anti-inflammatory properties through suppression of NF-κB and COX-2 signaling [Kim *et al*. 2004]. Reducing chronic hepatic inflammation is critical for maintaining tissue integrity and preventing age-associated liver pathologies such as fibrosis or steatosis.

Together, these data demonstrate that ginger targets multiple hallmarks of hepatic aging. Its effects include preservation of ultrastructure, modulation of lysosomal activity, and suppression of inflammatory and metabolic signaling. This multifaceted action profile is characteristic of other phytochemicals known to promote healthy aging, such as resveratrol, curcumin, and green tea catechins, all of which act through overlapping pathways like Nrf2 activation, mTOR inhibition, or AMPK signaling [Chestnut *et al*. 2021].

While these findings are encouraging, further research is needed to determine the long-term functional outcomes of ginger supplementation. Future studies should assess whether these molecular and histological changes translate into improved liver performance, resistance to disease, or extended health span. Additionally, direct measurement of autophagy markers (e.g., LC3, p62, Beclin-1) and profiling of hepatic immune cells would clarify the underlying mechanisms.

Importantly, our study reveals a dose-sensitive response to ginger. While 0.6% ginger frequently exhibited beneficial effects across all parameters, the 1.8% dose, though effective in gene expression modulation, showed signs of subcellular stress in older animals. This aligns with the concept of hormesis, where low-to-moderate

doses of bioactive compounds elicit adaptive beneficial responses, while higher doses may impose stress [Mattson 2008]. Therefore, optimizing ginger dosage is crucial for therapeutic efficacy in aging interventions. From a translational perspective, the observed dose-dependent effects highlight the importance of defining optimal intake levels. Although ginger is widely consumed and generally safe, excessive doses could pose risks, especially in older individuals with reduced stress resilience. Clinical studies are warranted to test ginger's efficacy in mitigating liver aging in humans, particularly in the context of metabolic disorders or age-associated hepatic decline.

From our study, the linear regression and correlation analyses provided further insight into the relationship between age, ginger dose, and molecular changes. Aging was a strong predictor of increased lysosomal enzyme activity and TNF expression, whereas ginger dose was more tightly associated with PI3K suppression. These findings emphasize that while aging globally impacts cellular catabolism and inflammation, ginger exerts targeted effects on specific pathways, particularly autophagy and inflammatory regulation.

Nevertheless, the study has limitations. The effects of ginger were only evaluated in male mice; sex-specific responses should be addressed in future research. Additionally, long-term studies are needed to evaluate whether the observed molecular and structural changes translate into improved liver function or lifespan extension.

Conclusion

In conclusion, this study demonstrates that dietary ginger supplementation exerts beneficial effects on key biological processes associated with liver aging in a dose- and age-dependent manner. Ginger significantly exerted protective effects on key hallmarks of hepatic aging, including stabilized lysosomal enzyme activity, inflammation, and metabolic signaling pathways in moderate doses. Moderate supplementation (0.6%) effectively preserved hepatocellular ultrastructure and improved lysosomal function, particularly in young and middle-aged mice, while also suppressing age-related increases in PI3K and TNF-α expression. Although high-dose ginger (1.8%) also influenced gene expression, it was associated with mild subcellular stress in aged mice, suggesting an adaptive response and underscoring the importance of dose optimization. These findings contribute to the growing body of evidence supporting the potential of ginger as a safe and natural nutraceutical for promoting healthy liver aging and warrant further investigation in clinical settings.

Data availability statement

The authors declare that all data and materials supporting the findings of this article are available from the corresponding authors upon request.

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Ethics declaration

All procedures involving animals were conducted in the frame of animal handling permission No. 14186201 in accordance with the relevant institutional and national regulations (including the EU Directive 2010/63/EU for animal experiments and National Research Council's Guide for the Care and Use of Laboratory Animals).

Declaration of competing statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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