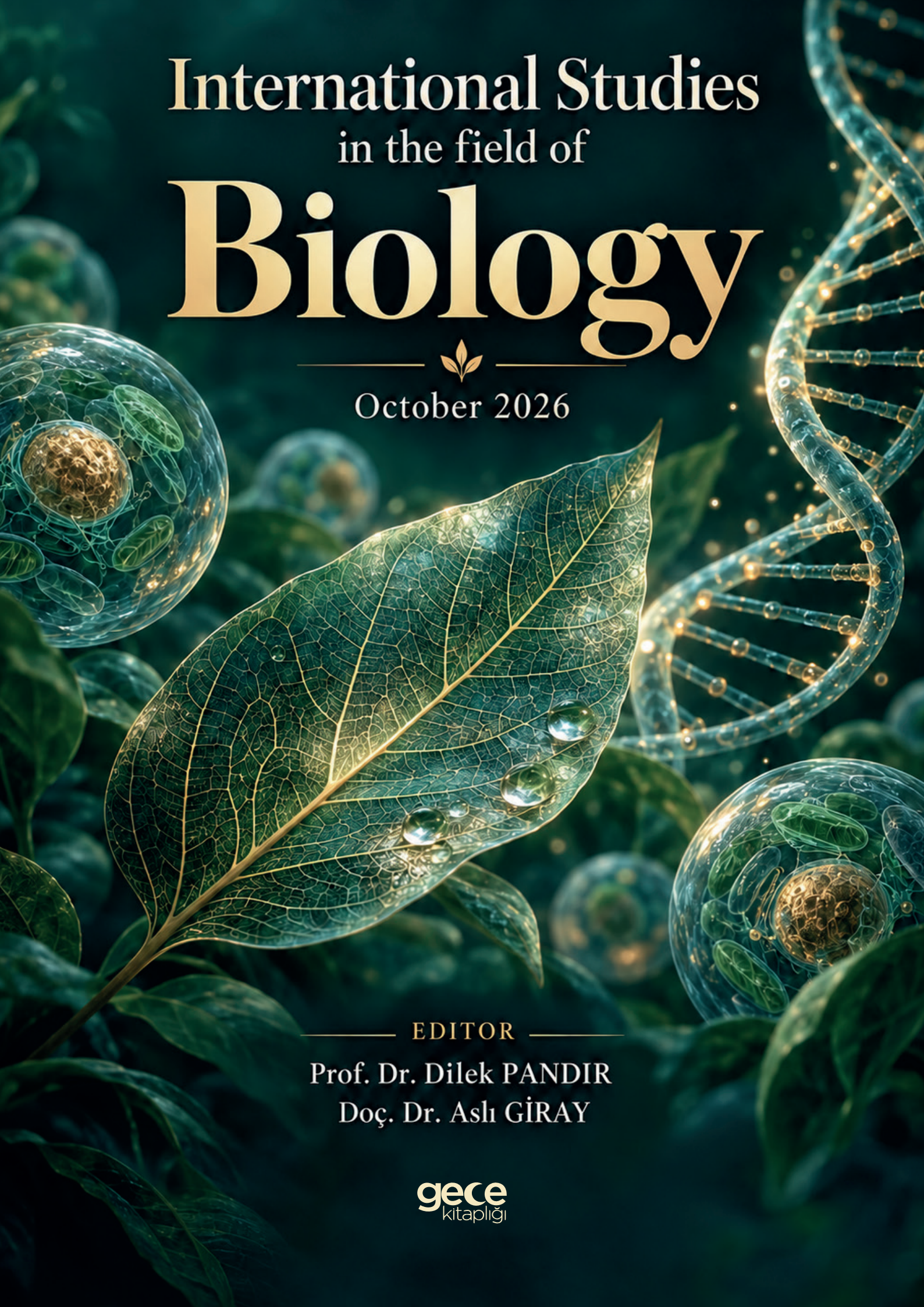




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October 2026

EDITOR

Prof. Dr. Dilek PANDIR
Doç. Dr. Aşlı GİRAY

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İÇİNDEKİLER

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CHAPTER 1

NITRIC OXIDE SIGNALING IN WOMEN'S HEALTH: MOLECULAR MECHANISMS IN REPRODUCTIVE PHYSIOLOGY, PREGNANCY, VASCULAR HOMEOSTASIS, MENOPAUSE, AND PELVIC HEALTH

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INTRODUCTION

The biological basis of women's health is shaped by complex interactions among hormonal regulation, vascular homeostasis, reproductive functions, inflammatory responses, and cellular redox balance. Intercellular signaling mechanisms play a central role in these processes, and nitric oxide (NO) is regarded as an important signaling molecule, particularly in the physiological regulation of the vascular and reproductive systems. The synthesis of NO by nitric oxide synthase (NOS) enzymes, particularly endothelial nitric oxide synthase (eNOS), followed by activation of soluble guanylate cyclase (sGC) and an increase in intracellular cyclic guanosine monophosphate (cGMP), constitutes one of the fundamental mechanisms involved in vascular smooth muscle relaxation and regulation of vascular tone (Furchgott and Zawadzki, 1980; Palmer et al., 1987; Ignarro et al., 1987; Moncada et al., 1991). However, the biological effects of NO extend beyond vasodilation. Through post-translational regulatory mechanisms such as protein S-nitrosylation and interactions with reactive oxygen species, NO can influence cellular functions at multiple levels (Hess et al., 2005; Pacher et al., 2007).

The identification of NO and NO-related signaling mechanisms within the female reproductive system has contributed to the understanding of their potential roles in processes such as ovulation, fertilization, uterine function, pregnancy, and uteroplacental circulation (Rosselli et al., 1998). During pregnancy, NO-mediated vasodilation has been investigated as an important component of the physiological adaptations occurring within the maternal vascular system, while alterations in NO bioavailability have also been examined in relation to placental vascular function and pregnancy-related hypertensive disorders. Similarly, the effects of estrogen on endothelial NO production and bioavailability have become an important area of research for understanding the biological relationship between hormonal status and vascular function in women (Förstermann and Sessa, 2012). The hormonal changes occurring during the transition from reproductive age to menopause further emphasize the need to consider endothelial function and NO signaling from a life-course perspective in women's health.

The NO system is not restricted to vascular function but represents a signaling network involved in physiological and pathophysiological processes in multiple tissues. Potential contributions of NO-mediated mechanisms have been investigated in female sexual function, particularly in relation to vascular responses of genital tissues, as well as in lower urinary tract function and pelvic tissue homeostasis. Nevertheless, a substantial proportion of the evidence in these fields is derived from experimental, physiological, or mechanistic studies rather than direct clinical outcomes. Therefore, when evaluating the relationship between NO and women's health,

biologically plausible mechanisms should be distinguished from evidence derived directly from clinical studies.

This chapter first addresses the molecular basis of NO biosynthesis and the characteristics of NOS enzymes, followed by an examination of NO–sGC–cGMP signaling and protein S-nitrosylation as major mechanisms underlying the cellular effects of NO. Subsequent sections evaluate the relationship between NO signaling and female reproductive physiology, pregnancy and uteroplacental circulation, placental function and preeclampsia, as well as estrogen–NO interactions and vascular changes during menopause. In addition, current biological and clinical evidence concerning NO signaling in female sexual function, the lower urinary tract, and pelvic health is discussed. Finally, the interaction between NO and reactive oxygen species, peroxynitrite formation, and the role of NO in oxidative and nitrosative stress are considered.

Accordingly, NO is considered not as an independent determinant of women's health, but as a multifunctional cellular signaling system positioned at the intersection of vascular homeostasis, hormonal regulation, reproductive functions, inflammation, and cellular redox balance. This perspective provides a framework for understanding both the physiological roles of NO in women's health and the molecular mechanisms through which disturbances in NO signaling may contribute to pathophysiological processes, while also highlighting the limitations of the current evidence.

1.1. Discovery and Biological Significance of Nitric Oxide

Nitric oxide (NO) is a short-lived signaling molecule capable of diffusing across biological membranes and participating in both intercellular and intracellular signal transduction. The importance of NO in vascular biology emerged from the demonstration that endothelial cells produce a factor capable of initiating relaxation of vascular smooth muscle. Furchgott and Zawadzki (1980) demonstrated that acetylcholine-induced vascular relaxation depends on the presence of an intact endothelium. Subsequently, Palmer et al. (1987) demonstrated that the biological activity of endothelium-derived relaxing factor could be attributed to NO release, while Ignarro et al. (1987) independently demonstrated that the endothelium-derived relaxing factor released from arteries and veins was NO. These landmark studies established the foundation for recognizing NO as a major regulator of vascular homeostasis.

A substantial proportion of the biological effects of NO is mediated through soluble guanylate cyclase (sGC)–cyclic guanosine monophosphate (cGMP) signaling. NO activates sGC, thereby increasing intracellular cGMP concentrations, while cGMP-mediated signaling contributes to

vascular smooth muscle relaxation (Moncada et al., 1991; Förstermann and Sessa, 2012). However, NO biology is not restricted to cGMP generation. NO and NO-derived reactive species can induce protein S-nitrosylation, a reversible post-translational modification that may alter protein function and modulate intracellular signaling pathways (Hess et al., 2005).

Accordingly, NO should not be regarded solely as a molecule responsible for vasodilation. Rather, it represents a multifunctional signaling molecule involved in a broad range of vascular, neurological, immunological, and reproductive processes (Moncada et al., 1991; Förstermann and Sessa, 2012).

1.2. Nitric Oxide Synthase Enzymes and NO Biosynthesis

The biosynthesis of NO is mediated by the nitric oxide synthase (NOS) enzyme family. Three major NOS isoforms have been identified in mammals: neuronal NOS (nNOS/NOS1), inducible NOS (iNOS/NOS2), and endothelial NOS (eNOS/NOS3). These enzymes catalyze the conversion of L-arginine into NO and L-citrulline (Förstermann and Sessa, 2012).

eNOS is predominantly expressed in vascular endothelial cells and plays a major role in the regulation of vascular tone. nNOS is primarily associated with neuronal tissues and certain peripheral tissues, whereas iNOS is particularly associated with NO production in cells responding to inflammatory stimuli (Förstermann and Sessa, 2012).

The activity of NOS enzymes is regulated by several mechanisms, including intracellular calcium concentrations, phosphorylation, protein–protein interactions, and the availability of essential cofactors. Impairment of eNOS activity has been associated with reduced endothelial NO bioavailability and altered vascular function (Förstermann and Sessa, 2012).

1.3. NO–sGC–cGMP Signaling

The best-characterized intracellular signaling pathway activated by NO is the sGC–cGMP pathway. NO binds to the heme moiety of sGC and increases its enzymatic activity, thereby stimulating the conversion of GTP to cGMP (Moncada et al., 1991; Förstermann and Sessa, 2012).

cGMP subsequently influences several intracellular targets, including protein kinase G, thereby contributing to the regulation of contractile mechanisms within smooth muscle cells. This pathway represents a fundamental mechanism through which NO promotes vascular smooth muscle relaxation (Förstermann and Sessa, 2012).

The potential significance of the NO–sGC–cGMP pathway in the female reproductive system has been investigated following the identification of NO signaling components in vascular and smooth muscle structures of the uterus and other genital tissues (Rosselli et al., 1998).

1.4. Protein S-Nitrosylation and Cellular NO Signaling

The cellular effects of NO are not limited to cGMP-dependent mechanisms. NO and NO-derived reactive species can induce S-nitrosylation of cysteine residues within proteins. S-nitrosylation is a reversible post-translational modification capable of altering protein activity, intracellular localization, and protein–protein interactions (Hess et al., 2005).

This mechanism demonstrates that NO can directly modify protein function. Therefore, comprehensive evaluation of NO signaling requires consideration of both the sGC–cGMP pathway and cGMP-independent mechanisms such as protein S-nitrosylation (Hess et al., 2005).

1.5. Nitric Oxide in the Female Reproductive System

Components of the NO system, including NOS enzymes, have been identified in the ovary, uterus, endometrium, and other reproductive tissues. Rosselli et al. (1998) comprehensively reviewed the potential roles of NO in female reproductive biology and reported that the NO system may be involved in ovulation, ovarian steroidogenesis, uterine function, tubal function, and vascular adaptations occurring during pregnancy.

The role of NO in the female reproductive system appears to involve both local vascular regulation and effects on smooth muscle and cellular signaling. However, female reproductive physiology depends on the coordinated interaction of numerous hormones and local mediators; therefore, NO should not be considered the sole determinant of these processes (Rosselli et al., 1998).

1.6. NO in Ovarian Function and Ovulation

Ovulation is a complex process accompanied by local vascular, inflammatory, and proteolytic changes within the follicular structure. The presence of NO within ovarian tissue and its association with local changes occurring during ovulation have suggested that the NO system may function as a potential regulatory mechanism in this process (Rosselli et al., 1998).

The potential effects of NO on ovulation should be considered in conjunction with gonadotropins, steroid hormones, prostaglandins, and other local mediators. Although available

evidence supports an association between NO and ovulatory processes, it does not indicate that ovulation is controlled exclusively by NO signaling (Rosselli et al., 1998).

1.7. NO in Endometrial and Uterine Physiology

The endometrium is a dynamic tissue that undergoes proliferation, differentiation, and tissue remodeling throughout the menstrual cycle under the influence of steroid hormones. The presence of NO and NOS components within endometrial tissue has led to investigations into the potential contribution of NO to local endometrial physiology (Rosselli et al., 1998).

Given the effects of NO on vascular smooth muscle, NO may contribute to the regulation of endometrial blood flow. However, complex processes such as implantation and endometrial receptivity cannot be explained by NO alone. Steroid hormones, adhesion molecules, cytokines, growth factors, and other local signaling systems collectively contribute to these processes.

1.8. NO and Maternal Vascular Adaptation During Pregnancy

Pregnancy is accompanied by substantial hemodynamic adaptations within the maternal circulation. Reductions in systemic vascular resistance and the increasing requirements of the uteroplacental circulation necessitate extensive adaptation of the maternal cardiovascular system. NO is among the vasodilatory systems that have been investigated as an important mediator of vascular adaptation and vasodilation during pregnancy (Conrad, 2011).

In addition to NO-mediated vasodilation, relaxin, prostacyclin, and other vasodilatory mechanisms contribute to the redistribution and adaptation of the maternal circulation during pregnancy (Conrad, 2011). Therefore, the role of NO during pregnancy should be interpreted as one component of a complex network of interacting vascular regulatory mechanisms.

1.9. Uteroplacental Circulation and Placental NO

Adequate uteroplacental circulation is essential for fetal growth and oxygenation. Because NO induces vascular smooth muscle relaxation, it has been suggested to contribute to the regulation of uteroplacental vascular tone (Rosselli et al., 1998; Conrad, 2011).

The identification of components of the NOS system in placental tissues has supported investigation of NO in placental vascular biology (Rosselli et al., 1998). However, placental circulation is not determined solely by NO. Trophoblast invasion, vascular remodeling, angiogenic signaling, and other local factors also contribute to the regulation of uteroplacental circulation.

1.10. NO, Endothelial Dysfunction, and Preeclampsia

Preeclampsia is a complex pregnancy disorder characterized by the interaction of maternal endothelial dysfunction and placental abnormalities. Powe et al. (2011) described preeclampsia as a systemic disorder in which maternal endothelial dysfunction represents an important component.

Alterations in NO bioavailability and oxidative stress are among the vascular mechanisms investigated in preeclampsia. The reaction of NO with superoxide can result in the formation of peroxynitrite, which may reduce NO bioavailability (Pacher et al., 2007).

Therefore, the NO system should not be considered an isolated cause of preeclampsia but should instead be evaluated in conjunction with endothelial dysfunction, oxidative stress, and placental pathophysiology (Powe et al., 2011).

1.11. The Estrogen–eNOS–NO Axis

A biological relationship exists between estrogen and the endothelial NO system. Estrogen has been shown to influence NO production in endothelial cells and vascular responses (Mendelsohn and Karas, 1999).

Some of the vascular effects of estrogen may be associated with alterations in endothelial NO production. However, the effects of estrogen on the vascular system cannot be explained solely by NO, as estrogen receptors also influence multiple endothelial and vascular signaling pathways (Mendelsohn and Karas, 1999).

Thus, the estrogen–eNOS–NO axis can be considered one of the molecular links between hormonal status and vascular function in women.

1.12. Menopause, NO Bioavailability, and Vascular Function

With menopause, ovarian estrogen production decreases substantially. Given the effects of estrogen on endothelial function, alterations in the vascular NO system after menopause have been investigated (Mendelsohn and Karas, 1999).

The reduction in NO bioavailability may result not only from hormonal changes but also from age-related oxidative stress and other cardiometabolic factors. The reaction of NO with superoxide is one of the mechanisms that can reduce NO bioavailability while increasing peroxynitrite formation (Pacher et al., 2007).

Therefore, endothelial changes during menopause should be examined by considering estrogen, NO, oxidative stress, and aging-related processes together.

1.13. NO in Female Sexual Function

The association of NO with vascular smooth muscle relaxation and blood flow to genital tissues has led to investigations of the NO system in female sexual physiology. The presence of NO and NOS components in the female genital system suggests that local NO production may be associated with genital vascular responses (Rosselli et al., 1998).

Nevertheless, female sexual function cannot be explained solely by vascular mechanisms. Sexual response is a multidimensional process influenced by hormonal, neurological, vascular, psychological, and interpersonal factors. Accordingly, NO should be considered one of the potential biological components contributing to female sexual function.

1.14. NO and the Lower Urinary Tract

The ability of NO to activate the sGC–cGMP pathway in smooth muscle cells has provided a basis for investigating nitrergic signaling in the lower urinary tract (Moncada et al., 1991; Förstermann and Sessa, 2012).

The potential role of NO in bladder and urethral function has been investigated particularly in relation to smooth muscle relaxation and neurotransmission. However, clinical conditions such as overactive bladder and urinary incontinence cannot be explained solely through the NO system. Multiple factors, including neural control, detrusor activity, urethral mechanisms, pelvic floor function, age, and hormonal status, contribute to the development of these conditions.

1.15. NO, Oxidative Stress, and Peroxynitrite

One of the most important characteristics of NO biology is its interaction with reactive oxygen species. NO rapidly reacts with the superoxide anion to form peroxynitrite (Pacher et al., 2007).

Peroxynitrite is a potent oxidant and nitrating agent. It can induce various molecular alterations, including protein nitration, lipid oxidation, and DNA damage (Pacher et al., 2007).

Therefore, the biological effects of NO depend not only on the amount of NO produced but also on the oxidative and redox status of the cell.

1.16. eNOS Uncoupling and Reduced NO Bioavailability

Appropriate levels of cofactors such as tetrahydrobiopterin (BH4) are required for the normal catalytic function of eNOS. Oxidative stress and disturbances in cofactor homeostasis may alter normal NO production by eNOS and promote a shift of the enzyme toward superoxide production. This phenomenon is referred to as eNOS uncoupling. NOS uncoupling is considered one of the molecular links between reduced NO bioavailability and oxidative stress (Förstermann and Sessa, 2012).

1.17. NO and Inflammatory Signaling

NO production is closely associated with inflammatory processes. In particular, inducible NOS (iNOS) can be induced in response to inflammatory stimuli and may contribute to the production of relatively high amounts of NO (Förstermann and Sessa, 2012).

The effects of NO in inflammation vary according to the amount produced, its cellular source, and the accompanying oxidative conditions. Because the presence of NO together with reactive oxygen species may increase peroxynitrite formation, it is inappropriate to classify the role of NO in inflammation as exclusively protective or exclusively harmful (Pacher et al., 2007).

1.18. NO and Mitochondrial Function

NO can interact with the mitochondrial respiratory chain and, particularly through cytochrome c oxidase, modulate mitochondrial oxygen utilization (Moncada and Erusalimsky, 2002).

Conversely, high levels of NO and NO-derived reactive species may induce oxidative and nitrosative alterations in mitochondrial proteins (Pacher et al., 2007).

Therefore, the effects of NO on mitochondria may vary depending on NO concentration, exposure duration, and the cellular redox environment.

1.19. NO, Aging, and Women's Health

Endothelial function changes during the aging process, and reduced NO bioavailability has been associated with vascular aging (Förstermann and Sessa, 2012).

Oxidative stress is an important mechanism underlying alterations in NO bioavailability. Increased reaction between superoxide and NO may limit the vasodilatory effects of NO and increase peroxynitrite formation (Pacher et al., 2007).

Because aging and menopause occur within overlapping periods of the female life course, the effects of hormonal and age-related changes on the vascular NO system should be considered together.

1.20. NO and Pelvic Health: Current Evidence and Research Areas

Evidence regarding the role of NO in pelvic floor disorders is more limited than the evidence concerning NO biology in the vascular system and pregnancy. Therefore, the conclusion that NO is a direct cause of pelvic organ prolapse or urinary incontinence is not supported by the available general NO literature.

Nevertheless, the established effects of NO on vascular tone, smooth muscle function, redox homeostasis, and inflammatory signaling make investigation of the NO system in pelvic tissues a biologically relevant research area (Förstermann and Sessa, 2012; Pacher et al., 2007).

In particular, potential relationships among NO, oxidative stress, inflammation, and the extracellular matrix in pelvic organ prolapse should be investigated directly in human tissues. However, because the current evidence is insufficiently strong, these relationships should be presented as biological hypotheses requiring further investigation rather than as established mechanisms.

1.21. The Dietary Nitrate–Nitrite–NO Pathway

NO production does not occur exclusively through NOS enzymes. The conversion of dietary nitrate to nitrite by oral bacteria and the subsequent conversion of nitrite to NO under certain conditions constitute an alternative pathway for NO production (Lundberg et al., 2008).

Lundberg et al. (2008) demonstrated the importance of the nitrate–nitrite–NO pathway, particularly in vascular physiology. This pathway is considered an alternative component of NO biology in addition to classical NOS-dependent NO production.

Nevertheless, disease-specific clinical studies are required before concluding that dietary nitrate consumption provides a direct clinical benefit for the prevention or treatment of women-specific diseases.

1.22. Integrated Assessment of NO Signaling in Women's Health

The NO system in women's health can be considered through three interrelated biological axes.

The first axis is vascular regulation through the eNOS–NO–sGC–cGMP pathway. This pathway constitutes a fundamental mechanism involved in vascular smooth muscle relaxation and the regulation of vascular tone (Moncada et al., 1991; Förstermann and Sessa, 2012).

The second axis is the interaction among estrogen, endothelial function, and NO. The effects of estrogen on endothelial function and NO bioavailability represent an important component of the connection between hormonal status and vascular biology in women (Mendelsohn and Karas, 1999).

The third axis is the relationship among NO, oxidative stress, and peroxynitrite. The reaction of NO with superoxide can reduce NO bioavailability while increasing peroxynitrite formation (Pacher et al., 2007).

Considering these three axes together facilitates an understanding of the role of NO in women's health not only through vasodilation but also through interactions among reproduction, pregnancy, hormonal changes, aging, and cellular redox homeostasis.

2. FUTURE RESEARCH

Future research on NO in women's health should move beyond the measurement of serum NO metabolites alone and consider multiple components of the NO system simultaneously.

Combined assessment of parameters such as eNOS and iNOS expression, eNOS activation, BH4 bioavailability, NO metabolites, cGMP, oxidative stress, and peroxynitrite may contribute to a more detailed understanding of NO biology (Förstermann and Sessa, 2012; Pacher et al., 2007).

In particular, the relationship between NO and oxidative stress during pregnancy, estrogen–eNOS interactions during menopause, and the local functions of NO in the female reproductive system represent important areas for future investigation (Rosselli et al., 1998; Mendelsohn and Karas, 1999; Conrad, 2009).

With respect to pelvic floor disorders, the current evidence remains limited. Therefore, studies involving human tissues and clinical specimens are needed to determine the potential role of NO in pelvic organ prolapse and urinary incontinence.

3. CONCLUSION

Nitric oxide is a multifunctional signaling molecule associated with vascular homeostasis, the reproductive system, and pregnancy physiology in women's health. Its biological effects are mediated, particularly in the vascular system, through the regulation of smooth muscle relaxation via the NO–sGC–cGMP pathway (Moncada et al., 1991; Förstermann and Sessa, 2012).

The NOS/NO system has been identified in the ovaries, uterus, and other reproductive tissues, and it has been reported to be associated with ovulation, steroidogenesis, uterine function, and vascular adaptation during pregnancy (Rosselli et al., 1998).

During pregnancy, NO is one of the important vasodilatory systems investigated in relation to maternal vascular adaptation and the regulation of uteroplacental circulation (Conrad, 2009). In preeclampsia, the relationship among NO bioavailability, endothelial dysfunction, and oxidative stress has also been investigated (Powe et al., 2011; Pacher et al., 2007).

The relationship between estrogen and the endothelial NO system represents an important molecular link between hormonal status and vascular function in women (Mendelsohn and Karas, 1999).

The biological effects of NO, however, are not limited to physiological vasodilation. The ability of NO to react with superoxide and generate peroxynitrite demonstrates the close relationship between NO biology and oxidative and nitrosative stress (Pacher et al., 2007).

Accordingly, the assessment of NO in women's health should not rely on a simple dichotomous model of whether NO is "beneficial or harmful." Instead, NOS isoforms, the amount of NO produced, NO bioavailability, cGMP signaling, protein S-nitrosylation, and the cellular redox environment should be considered together (Hess et al., 2005; Förstermann and Sessa, 2012).

Although evidence concerning NO signaling is relatively stronger in female reproductive physiology, pregnancy, menopause, and vascular aging, direct evidence regarding pelvic organ prolapse and other pelvic floor disorders remains more limited. This distinction is important for evaluating the role of NO biology in women's health according to the available level of scientific evidence.

REFERENCES

- Conrad KP. Maternal vasodilation in pregnancy: the emerging role of relaxin. *Am J Physiol Regul Integr Comp Physiol.* 2011 Aug;301(2):R267-75. doi: 10.1152/ajpregu.00156.2011.
- Förstermann U, Sessa WC. Nitric oxide synthases: regulation and function. *Eur Heart J.* 2012 Apr;33(7):829-37, 837a-837d. doi: 10.1093/eurheartj/ehr304.
- Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature.* 1980 Nov 27;288(5789):373-6. doi: 10.1038/288373a0.
- Hess DT, Matsumoto A, Kim SO, Marshall HE, Stamler JS. Protein S-nitrosylation: purview and parameters. *Nat Rev Mol Cell Biol.* 2005 Feb;6(2):150-66. doi: 10.1038/nrm1569.
- Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc Natl Acad Sci U S A.* 1987 Dec;84(24):9265-9. doi: 10.1073/pnas.84.24.9265.
- Kuhn M. Molecular physiology of natriuretic peptide signalling. *Basic Res Cardiol.* 2004 Mar;99(2):76-82. doi: 10.1007/s00395-004-0460-0.
- Lundberg JO, Weitzberg E, Gladwin MT. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. *Nat Rev Drug Discov.* 2008 Feb;7(2):156-67. doi: 10.1038/nrd2466.
- Mendelsohn ME. Protective effects of estrogen on the cardiovascular system. *Am J Cardiol.* 2002 Jun 20;89(12A):12E-17E; discussion 17E-18E. doi: 10.1016/s0002-9149(02)02405-0.
- Moncada S, Higgs EA. The discovery of nitric oxide and its role in vascular biology. *Br J Pharmacol.* 2006 Jan;147 Suppl 1(Suppl 1):S193-201. doi: 10.1038/sj.bjp.0706458.
- Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev.* 1991 Jun;43(2):109-42.
- Moncada S, Erusalimsky JD. Does nitric oxide modulate mitochondrial energy generation and apoptosis? *Nat Rev Mol Cell Biol.* 2002 Mar;3(3):214-20. doi: 10.1038/nrm762.
- Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature.* 1987 Jun 11-17;327(6122):524-526. DOI: 10.1038/327524a0.

Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. *Physiol Rev.* 2007 Jan;87(1):315-424. doi: 10.1152/physrev.00029.2006.

Powe CE, Levine RJ, Karumanchi SA. Preeclampsia, a disease of the maternal endothelium: the role of antiangiogenic factors and implications for later cardiovascular disease. *Circulation.* 2011 Jun 21;123(24):2856-69. doi: 10.1161/CIRCULATIONAHA.109.853127.

Rosselli M, Keller PJ, Dubey RK. Role of nitric oxide in the biology, physiology and pathophysiology of reproduction. *Hum Reprod Update.* 1998 Jan-Feb;4(1):3-24. doi: 10.1093/humupd/4.1.3.

CHAPTER 2

NEURODEGENERATIVE DISORDERS BIOMARKERS AND DIAGNOSIS MATERIALS

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1. Neurological Disorders

Neurodegenerative disorders (ND) manifest through the progressive dysfunction of neurons, glia, synapses, and neural networks [1]. A hallmark characteristic of these disorders is the aggregation and deposition of various physiological protein variants within the central nervous system (CNS). Both neurons and glia possess the capability to accumulate these pathological variants[2]. The prevalence of ND is anticipated to escalate due to the increasing life expectancy. Each neurodegenerative disease (ND) exhibits a certain degree of heterogeneity, reflected in differences in the distribution of pathological changes, the nature and extent of neuroinflammation, and the severity and progression of neurodegeneration. Consequently, distinct patient subgroups, potentially identifiable through specific biomarkers, may exhibit differential responses to particular therapeutic interventions. Furthermore, disease-modifying therapies may have distinct therapeutic windows during the course of disease, within which their efficacy is maximized [3].

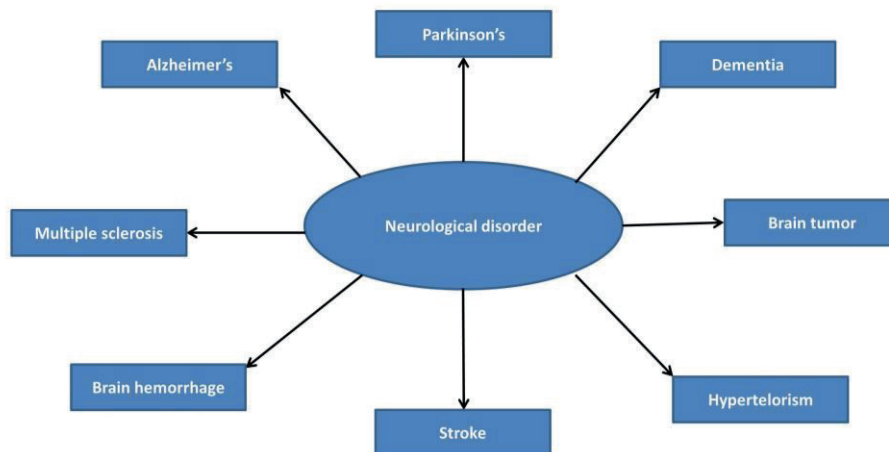


Figure 1. Common neurological disorders.

1.1. Prognostic Biomarkers in Neurological Disorders

1.1.1. Neurofilament proteins (NfPs)

Neuronal injury is a prevalent occurrence in diseases affecting both the central and peripheral nervous systems, underscoring the need for biomarkers to comprehend and address neurologic disorders effectively. Neurofilament, identified as a blood biomarker associated with neuronal injury, offers a pivotal tool for disease understanding and treatment evaluation across various

neurologic conditions [4]. Neurofilament proteins (NfPs), belonging to the type IV class of intermediate filaments (IFs) specific to neurons, have emerged as promising indicators of neuroaxonal integrity or damage. Their significance lies in being major neuron-specific constituents crucial for structural integrity, rendering them sensitive indicators of neurodegeneration and neuronal injury in diverse neurologic ailments [5]. Under physiological conditions spanning normal brain development, maturation, and aging, NfPs are consistently released from neurons into the extracellular milieu, eventually reaching cerebrospinal fluid (CSF) and blood. Elevated levels of NfPs in CSF and blood signify neuronal injury and neurodegeneration, irrespective of the underlying cause [6].

Furthermore, NfPs play pivotal roles in the pathophysiology of various nervous system diseases, reflecting alterations in structural integrity and abnormal accumulation or distribution of these proteins. Experimental evidence underscores the significance of NfPs in maintaining sensorimotor and cognitive functions, with their absence or overexpression resulting in neuropathological manifestations in animal models [5, 7]. The absence of NfL from neurons leads to sensorimotor and cognitive impairments in quails [8]. Meanwhile, the overexpression of certain NfPs in animals can induce neuropathology associated with motor neuron diseases [9]. Clinical research underscores the vital role of intact NfPs in human health, emphasizing their presence, normal structure, and organized network. Loss-of-function mutations in NfL, observed in cases of human neuropathy, result in significantly reduced NfL protein levels, leading to diminished axon diameters and consequent sensorimotor and cognitive impairments in humans [10, 11]. NfL serves as a biologically valid marker of axonal damage, reflecting the severity of neurological diseases across various conditions, from degenerative to inflammatory central nervous system disorders. It demonstrates clear predictive value, correlating with the severity of clinical manifestations across multiple functional domains, ranging from motor function to cognitive performance [12, 13]. Multiple sclerosis (MS), the most prevalent chronic inflammatory disease of the central nervous system (CNS), is a progressive disorder that typically manifests in early adulthood and follows a protracted clinical course. Elevated serum NfL levels have been linked to a heightened risk of subsequent MS development, shedding light on its potential as a prognostic indicator [14].

1.1.2. Amyloid Beta Protein

Amyloid precursor protein (APP) is a ubiquitously expressed type I single-pass transmembrane protein consisting of a large extracellular domain, a hydrophobic transmembrane domain, and

a short intracellular domain [15]. In the human brain, amyloid- β ($A\beta$) exists in multiple molecular and structural forms, ranging from insoluble fibrillar deposits to soluble monomers, dimers, and higher-order oligomeric assemblies [16]. Vascular $A\beta$ deposition, particularly in the context of cerebral amyloid angiopathy, can compromise vascular integrity and contribute to impaired cerebral blood flow and intracerebral hemorrhage [17]. In contrast, the precise contribution of parenchymal $A\beta$ deposits, including neuritic plaques, to neuronal dysfunction and clinical disease remains incompletely understood. Importantly, the relationship between fibrillar amyloid deposition and clinical dementia is not invariably concordant. Rare cases of genetically determined Alzheimer's disease have been described in which pathogenic mutations are associated with clinical dementia despite minimal or absent amyloid-PET-detectable fibrillar plaque burden, suggesting that soluble or non-fibrillar $A\beta$ species and other downstream pathological mechanisms may contribute substantially to disease expression [18]. Mounting evidence suggests that elevated levels of amyloid- β could serve as one of the triggers initiating Alzheimer's disease, instigating a cascade of events, including the accumulation of toxic forms of tau, ultimately culminating in downstream neurodegeneration and dementia [19]. However, the mechanisms underlying the transition from rising amyloid- β levels to tau deposition and the considerable delay between them remain unclear. In the early stages of Alzheimer's disease, as amyloid- β levels increase, plaques begin to deposit and progressively enlarge, impairing glutamatergic transmission and causing damage to nearby synapses [20, 21]. This initial synaptic damage near amyloid- β plaques triggers local network dysfunction, prompting a response from microglia, which eliminate damaged synapses to forestall further harm or significant network disruption [25]. Yet, with the continued accumulation of amyloid- β plaques, especially across multiple sites, synaptic damage intensifies and spreads, leading to tau phosphorylation [22]. As the accumulation of amyloid- β plaques persists, especially across multiple locations, the extent of synaptic damage intensifies and extends, leading to the phosphorylation of tau [23, 24].

1.1.3. Tau protein

Tau protein, together with β -amyloid ($A\beta$), represents a key biomarker in the diagnostic evaluation and pathophysiological characterization of Alzheimer's disease (AD) [25]. However, in recent years, evidence has emerged, particularly from cerebrospinal fluid studies in individuals with multiple sclerosis, exploring its potential as a diagnostic and prognostic biomarker [26]. Tau protein, primarily located on neuronal axonal tracts and to a lesser extent in glial cells such as oligodendrocytes or astrocytes, plays a pivotal role in cellular structure

[27]. Functionally, it is a microtubule-associated protein crucial for microtubule assembly and stability, with its activity predominantly regulated by phosphorylation [28-30]. Upon hyperphosphorylation, tau protein aggregates into neurofibrillary tangles (NFTs) marking a characteristic feature of Alzheimer's disease pathology [31]. This aggregation is accompanied by excessive activation of glial cells, abnormal accumulation of hyperphosphorylated tau (p-tau) in the brain, the formation of neurofibrillary tangles, and subsequent neuronal dysfunction and apoptosis [32, 33]. Both tau protein and amyloid β -protein ($A\beta$) are pivotal in determining the onset and progression of neurodegenerative diseases, with $A\beta$ generated through the cleavage of amyloid precursor proteins in the brain [34]. Notably, compared with $A\beta$ accumulation, p-tau aggregation is more closely associated with early cognitive decline and the clinical progression of Alzheimer's disease (AD) [35].

1.1.4. Alpha-synuclein

Alpha-synuclein (α -syn) is a central protein implicated in the pathophysiology of Parkinson's disease (PD). It is detectable in a variety of biological fluids and cellular compartments, including cerebrospinal fluid (CSF), plasma, serum, erythrocytes, and platelets, making it a promising candidate for the development of minimally invasive biomarkers for PD. In vivo assessment of α -syn levels may therefore provide valuable information for the diagnosis and biological characterization of PD. Previous meta-analyses have reported significantly lower CSF α -syn concentrations in patients with PD than in healthy controls, supporting its potential utility as a diagnostic biomarker [36, 37]. Given its established involvement in PD pathophysiology and its detectability in CSF, blood, and other biological fluids, α -synuclein has been extensively investigated as a potential biomarker for PD and other synucleinopathies. Nevertheless, α -synuclein is expressed by various tissues, and its movement between blood and the brain complicates the interpretation of its levels in body fluids as indicative of PD pathology in the brain [38]. Moreover, α -synuclein can exist in different forms, including monomeric, oligomeric, and post-translationally modified forms, further complicating its measurement. Although many studies have reported significantly lower CSF total α -synuclein levels in patients with PD compared with age-matched healthy controls or individuals with other neurological disorders, the diagnostic performance of this biomarker remains inconsistent across studies [39-43]. However, most research, systematic reviews, and meta-analyses investigating the potential of CSF α -synuclein as a biomarker for PD have emphasized its reliability in distinguishing PD patients from control subjects based on decreased levels [36, 40, 44-46].

1.1.5. Neurogranin

Neurogranin (Ng) is a neuron-specific postsynaptic protein known for its role in enhancing synaptic function and promoting [47]. It is perceived as a marker for synaptic degeneration and a predictor of cognitive decline in Alzheimer's disease (AD) [48, 49]. Neurogranin (Ng) is predominantly expressed in the brain and is enriched in the dendritic spines of postsynaptic neurons, where it interacts with calmodulin and contributes to synaptic plasticity, long-term potentiation, and memory formation [50, 51]. Notably, CSF neurogranin levels are significantly elevated in patients with Alzheimer's disease (AD) compared with healthy controls, reflecting synaptic dysfunction and neuronal injury. Consequently, neurogranin has emerged as a promising CSF biomarker for assessing synaptic damage in AD [52, 53].

The significance of postsynaptic proteins in Alzheimer's disease (AD) has been reinforced by research indicating that elevated levels of neurogranin in cerebrospinal fluid (CSF) could serve as an early indicator of cognitive decline. Studies have demonstrated that higher CSF neurogranin levels in individuals with mild cognitive impairment (MCI) are associated with subsequent progression to Alzheimer's disease (AD), compared with individuals whose cognitive status remains stable over time [54-56]. Additionally, there is clinical evidence suggesting that levels of neurogranin in both CSF and blood exosomes could serve as cognitive biomarkers for AD and MCI-AD [57]. Notably, the elevation of CSF neurogranin levels appears to be specific to AD [58]. One possible explanation for this specificity is that while synaptic degeneration is a common feature in various neurodegenerative conditions, neurogranin is predominantly expressed in brain regions implicated in AD pathology, such as the neocortex, and hippocampus, amygdala [58, 59].

2. Neurological Disorders Diagnosis Materials

2.1. Natural Compounds

2.1.1. Alkaloids

Neurodegenerative diseases (NDs) are characterized by the progressive and irreversible loss of neuronal structure and function, frequently accompanied by the accumulation of misfolded or otherwise pathologically altered proteins in the brain [60]. Alkaloids, a diverse group of naturally occurring compounds found predominantly in flowering plants, have garnered interest

for their potential therapeutic effects in these conditions [61, 62]. Alkaloids typically contain oxygen, hydrogen, carbon, and nitrogen molecules, with certain structures facilitating hydrogen bond formation with enzymes, receptors, and proteins [63, 64]. Various alkaloid compounds have shown promise in the management of NDs such as Alzheimer's disease (AD), Parkinson's disease (PD), schizophrenia (S), Huntington's disease (HD), epilepsy (E), and stroke. These compounds include derivatives of β -carboline, vinca, isoquinoline, pyridine, aporphine, indole, pyrroloindole, oxindole, piperidine, and methylxanthene. Their mechanisms of action encompass a wide range of effects, including agonism of muscarinic and adenosine receptors, inhibition of amyloid aggregation, inhibition of cholinesterase enzymes, modulation of dopaminergic and nicotine receptors, and antagonism of N-methyl-D-aspartate receptors. Alkaloids have the potential to exert positive effects on neuronal health and function [65-67].

2.1.2. Phenolic Compounds

2.1.2.1. Phenolic acids

Phenolic acids are classified into two groups: derivatives of cinnamic acid and derivatives of benzoic acid. While the hydroxybenzoic acid content of edible plants is generally low, hydroxycinnamic acids are relatively common, consisting mainly of ferulic, sinapic acids, p-coumaric, and caffeic [68-71]. From a general perspective, phenolic acids may have several neuroprotective and cognition-enhancing properties related to their anti-aggregant activity and anti-amyloidogenic [72]. Moreover, they have been shown to inhibit the aggregation of pathological proteins implicated in the pathogenesis of several neurodegenerative diseases (NDs) associated with cognitive decline, including dementia with Lewy bodies (DLB), Parkinson's disease (PD), and Alzheimer's disease (AD) [73-76]. Caffeic acid, in particular, demonstrates neuroprotective effects by eliminate oxidative stress and neuroinflammation. [77]. Additionally, this phenolic acid has been observed to decrease the levels of the TNF- α in serum and malondialdehyde [78].

2.1.2.2. Flavonoids

Flavonoids exhibit various pharmacological effects, including antioxidative, anti-inflammatory, anti-carcinogenic, and neuroprotective properties, making them integral components of herbal supplements, pharmaceuticals, medicinal products, and cosmetics. Extensive literature supports the neuroprotective effects of flavonoids, with recent attention focusing on their efficacy in NDs such as AD and PD [79]. Flavonoids can modulate kinase

signaling pathways, gene/protein expression, and transcription factors involved in memory and learning processes within the hippocampus [80]. Specific flavonoids like luteolin and apigenin have been shown to protect dopaminergic neurons by reducing oxidative stress [81]. Studies have reported that apigenin significantly ameliorates behavioral abnormalities induced by chronic unpredictable mild stress (CUMS) by inhibiting IL-1 β and caspase-1 through disrupting the assembly of the NLRP3 inflammasome. Apigenin achieves this by upregulating peroxisome proliferator-activated receptor-gamma (PPAR γ) [82]. Nrf2, a key regulator of neuroprotection, plays a crucial role in eliminating reactive oxygen species (ROS) and regulating peripheral inflammatory responses [83]. Several flavonoids, including flavones, flavonols, dihydroflavones, dihydroflavonols, chalcones, dihydrochalcones, and isoflavones, have demonstrated Nrf2-induced activity, as evidenced by the NAD(P)H [84].

2.1.2.3. Lignans

Lignans are naturally occurring secondary metabolites widely distributed across plant species and constitute a structurally diverse class of phenylpropanoid-derived compounds. They are generally formed through the oxidative coupling of two monolignol units, typically involving the C8 positions of their propyl side chains, and consequently exhibit a characteristic C6–C3–C3–C6 carbon framework. Based on their structural features and biosynthetic characteristics, lignans can be classified into five major groups: lignans, hybrid lignans, norlignans, neolignans, and oligomeric lignans [85] [86]. These compounds possess diverse pharmacological activities, including anti-Alzheimer's, antioxidant, immunosuppressive, antibacterial, antiasthmatic, antitumor, and immunomodulatory properties [87-91]. For instance, the lignan sesamin, derived from *Asiasari radix*, has demonstrated neuroprotective activities against 6-OHDA-induced cytotoxicity. It achieves this by transiently phosphorylating extracellular signal-regulated kinase 1/2 and Bad while inhibiting the sustained phosphorylation of p38 mitogen-activated protein and c-Jun N-terminal kinases [92]. Moreover, chronic treatment with the lignan 7-hydroxymatairesinol at high doses has shown efficacy in neuroinflammatory processes [93].

2.1.2.4. Stilbenes

Plants produce polyphenolic compounds known as stilbenes as a defense mechanism against pathogens, bacterial and fungal growth, and the harmful effects of UV light, making them phytoalexins. Stilbenes are characterized by a carbon skeleton of 1,2-diphenylethylene (C6–C2–C6), comprising an ethylene moiety sandwiched between two benzene rings [94, 95].

2.1.2.4.1. Resveratrol: Resveratrol is the most extensively studied stilbene, renowned for its anticancer, antioxidant, cardioprotective, antidiabetic, antiangiogenic, and neuroprotective properties. It has shown promise in reducing depressive behaviors in animal models [96, 97].

2.1.2.4.2. Piceatannol, Piceatannol, a derivative of resveratrol, enhances motor and memory function by increasing cerebral blood flow. It achieves this by inhibiting c-Jun N-terminal kinase 3 (JNK3) signaling, thereby disrupting the mitochondrial apoptotic pathway and exerting a neuroprotective effect[98].

2.1.2.4.3. Pterostilbene: Pterostilbene may modulate the Nrf2 signaling pathway, exerting an antioxidant effect.[99]. It reduces the protein levels of Keap1 and promotes Nrf2 translocation into the nucleus, leading to the transcriptional activation of downstream antioxidant stress genes [100].

2.1.2.4.4. Polydatin: Polydatin, a herbal medicine, exhibits biological protective properties against inflammation and oxidative stress by inhibiting the ROS generation and mitigating inflammatory damage [101]. In cases of traumatic brain injury, where SOD2 downregulation and SOD2 acetylation upregulation occur, leading to NLRP3 inflammasome activation, polydatin plays a protective role by inhibiting SOD2 acetylation and reducing NLRP3 inflammasome activation, thereby mitigating secondary brain injury [102].

2.2. Enzymes

Numerous enzymes have been implicated in neurodegenerative diseases. Oxidative stress, characterized by disruptions in redox homeostasis, is a key feature in the progression of these conditions. In neurodegeneration, a decline in antioxidant enzyme levels, coupled with elevated levels of ROS and RNS in certain brain regions, can lead to damage to crucial macromolecules such as DNA, proteins, and lipids. This oxidative damage contributes to the pathogenesis of neurodegenerative diseases [103].

2.2.1. Acetylcholinesterase (AChE) is a crucial enzyme involved in neurotransmission, primarily located in synaptic regions within both the central and peripheral nervous systems. [104, 105] Acetylcholinesterase (AChE) is indeed the enzyme responsible for breaking down the neurotransmitter acetylcholine. This process is essential for terminating the action of

acetylcholine at synapses and neuromuscular junctions, thereby regulating neurotransmission. AChE plays a vital role in the human nervous system by ensuring precise control over neuronal signaling and facilitating proper functioning of various physiological processes [106, 107]. AChE breaks down acetylcholine into choline and acetate [108-110]. This enzymatic process ensures the precise termination of acetylcholine's action, facilitating discrete neurotransmission between axons [107]. AChE has been associated with cell development and is thought to aid in nerve regeneration and neuronal maturation [111]. Inhibition of this enzyme results in the accumulation of acetylcholine in nerve synapses, leading to prolonged and excessive neurotransmission. AChE, primarily distributed throughout nervous tissue, rapidly disrupts the hydrolysis of acetylcholine, thereby halting nerve impulse transmission and ensuring normal physiological function in the body. AChE plays a significant role in the nervous system, so any impairment in its efficiency may result in neurotoxicity [112-114].

2.2.2. Butyrylcholinesterase (BChE) is a serine hydrolase found in various tissues, with particularly high concentrations in plasma and the liver [115]. BChE has been recognized as a significant enzyme in the development of several neurological disorders, notably its association with β -amyloid plaques in Alzheimer's disease (AD) [116]. Butyrylcholinesterase (BChE) is widely distributed throughout the human body and contributes to the metabolism of various endogenous and exogenous compounds, including acetylcholine (ACh). In the central nervous system (CNS), BChE is present in glial cells and white matter and, together with acetylcholinesterase (AChE), contributes to the regulation of cholinergic neurotransmission [117]. Unlike AChE, which is predominantly localized in neurons, BChE is expressed mainly in non-neuronal cells, particularly astrocytes. Nevertheless, BChE is also detectable in specific neuronal populations, with notable expression in brain regions such as the hippocampus, thalamus, and amygdala [118]. Astrocytes and microglia represent important sources of BChE in the CNS and also express nicotinic acetylcholine (ACh) receptors. This co-localization suggests that BChE-mediated hydrolysis of ACh may contribute to the modulation of the functional activity of these glial cells [119, 120].

2.2.3. Peroxiredoxins (PRDXs): They belong to a family of thiol-dependent peroxidases responsible for reducing hydrogen peroxide, peroxynitrite, and alkyl hydroperoxides [121, 122]. Peroxiredoxins (PRDXs) function as antioxidant enzymes, facilitating the reduction of peroxides to regulate cellular H_2O_2 levels, crucial for cellular signaling. They serve as pivotal regulators of redox signaling [123]. Peroxiredoxins (PRDXs) exhibit distinct expression patterns across different brain regions and cell types, depending on the specific subfamily.

PRDX1 and PRDX6 are predominantly expressed in glial cells, whereas PRDX2, PRDX3, PRDX4, and PRDX5 are primarily localized in neurons [124]. The changes in PRDX expression seem to primarily stem from cellular responses to oxidative damage, considering its involvement in the pathogenesis of neurodegenerative diseases. Numerous studies have proposed associations between PRDXs and the development of neurodegenerative diseases, as well as their potential neuroprotective effects [125]. PRDX2 plays a vital neuroprotective role in specific neurological conditions, such as multiple sclerosis, amyotrophic lateral sclerosis, and aging [126]. Multiple sclerosis (MS) is characterized by chronic inflammatory demyelination within the central nervous system (CNS) and involves complex interactions between oxidative stress and neuroinflammation. Studies suggest that upregulation of peroxiredoxin 2 (PRDX2) may contribute to the reduction of reactive oxygen species (ROS) levels in brain tissue, thereby potentially protecting neurons from oxidative stress associated with MS [127].

2.3. Hormones

Hormones are categorized based on their chemical structures into steroid, protein, peptide, and polypeptide hormones. Examples of peptide and polypeptide hormones include oxytocin, leptin, ghrelin, corticotropin-releasing hormone, gonadotropin-releasing hormone, vasopressin, and cholecystikinin. The pharmaceutical industry has invested significant efforts in developing complex peptide hormone products. Additionally, research has focused on exploring various uptake pathways of peptide hormones in psychiatric diseases [128].

2.3.1. Dopamin: The dopaminergic system is a key neuromodulatory network involved in the regulation of diverse physiological and behavioral processes, including motor control, motivation, reward processing, cognitive function, and reproductive behavior. Dopamine, synthesized in both the central and peripheral nervous systems, mediates its effects predominantly through dopamine receptors belonging to the G protein-coupled receptor (GPCR) family [129]. The dopaminergic system regulates various critical functions in the central nervous system, such as movement control, reward behaviors, and cognition. Changes in dopaminergic signaling are implicated in the development of neurodegenerative and psychiatric disorders, particularly Parkinson's disease, which is linked with a nuanced and chronic inflammatory response [130]. Studies on Parkinson's disease have demonstrated that levodopa can decrease tremor-related activity in the ventrolateral thalamus, particularly in patients who exhibit a clinical response to dopamine [131] levodopa administration has been

shown to reduce thalamo-cortical connectivity [132]. These findings highlight the crucial involvement of the dopaminergic system in Parkinson's disease resting tremor [133].

2.3.2. Irisin: Irisin has demonstrated a protective effect on the central nervous system. Its secretion triggers an elevation in brain-derived neurotrophic factor levels within the hippocampus, which contributes to the improvement of cognitive impairments. Additionally, irisin plays a crucial role in the survival, differentiation, development, and growth of neurons [134]. Studies have shown that deletion of the *Fndc5*/irisin gene impairs cognitive function in individuals with AD. Moreover, peripheral administration of irisin in two different AD mouse models demonstrated that irisin can cross the blood-brain barrier. This peripheral irisin administration was found to mitigate cognitive decline associated with pathological changes in these models [135]. Furthermore, studies have reported that irisin avoid cognitive deficits by modulating the JAK/STAT and transcription 3 (STAT3) pathways. Significant associations were discovered between irisin and episodic memory as well as global cognition in individuals at risk of dementia [136].

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References

1. Kovacs, G.G., 2016. Molecular pathological classification of neurodegenerative diseases: turning towards precision medicine. *International journal of molecular sciences*. **17**, 189.
2. Kovacs, G.G., *Concepts and classification of neurodegenerative diseases*, in *Handbook of clinical neurology*. 2018, Elsevier. p. 301-307.
3. Hansson, O., 2021. Biomarkers for neurodegenerative diseases. *Nature medicine*. **27**, 954-963.
4. Barro, C., T. Chitnis, and H.L. Weiner. 2020. Blood neurofilament light: a critical review of its application to neurologic disease. *Annals of clinical and translational neurology*. **7**, 2508-2523.
5. Yuan, A., M.V. Rao, A. Kumar, J.-P. Julien, and R.A. Nixon. 2003. Neurofilament transport in vivo minimally requires hetero-oligomer formation. *Journal of Neuroscience*. **23**, 9452-9458.
6. Yuan, A. and R.A. Nixon. 2021. Neurofilament proteins as biomarkers to monitor neurological diseases and the efficacy of therapies. *Frontiers in neuroscience*. **15**, 689938.
7. Hamberger, A., Y.-L. Huang, H. Zhu, F. Bao, M. Ding, K. Blennow, A. Olsson, H.-A. Hansson, D. Viano, and K.G. Haglid. 2003. Redistribution of neurofilaments and accumulation of β -amyloid protein after brain injury by rotational acceleration of the head. *Journal of neurotrauma*. **20**, 169-178.
8. Yamasaki, H., C. Itakura, and M. Mizutani. 1991. Hereditary hypotrophic axonopathy with neurofilament deficiency in a mutant strain of the Japanese quail. *Acta neuropathologica*. **82**, 427-434.
9. Côté, F., J.-F. Collard, and J.-P. Julien. 1993. Progressive neuronopathy in transgenic mice expressing the human neurofilament heavy gene: a mouse model of amyotrophic lateral sclerosis. *Cell*. **73**, 35-46.
10. Yum, S.W., J. Zhang, K. Mo, J. Li, and S.S. Scherer. 2009. A novel recessive NEFL mutation causes a severe, early-onset axonal neuropathy. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society*. **66**, 759-770.
11. Sainio, M.T., E. Ylikallio, L. Mäenpää, J. Lahtela, P. Mattila, M. Auranen, J. Palmio, and H. Tyynismaa. 2018. Absence of NEFL in patient-specific neurons in early-onset Charcot-Marie-Tooth neuropathy. *Neurology: Genetics*. **4**, e244.
12. Gaetani, L., L. Parnetti, P. Calabresi, and M. Di Filippo. 2021. Tracing neurological diseases in the presymptomatic phase: insights from neurofilament light chain. *Frontiers in Neuroscience*. **15**, 672954.
13. Gaetani, L., K. Blennow, P. Calabresi, M. Di Filippo, L. Parnetti, and H. Zetterberg. 2019. Neurofilament light chain as a biomarker in neurological disorders. *Journal of Neurology, Neurosurgery & Psychiatry*. **90**, 870-881.
14. Bjornevik, K., K.L. Munger, M. Cortese, C. Barro, B.C. Healy, D.W. Niebuhr, A.I. Scher, J. Kuhle, and A. Ascherio. 2020. Serum neurofilament light chain levels in patients with presymptomatic multiple sclerosis. *JAMA neurology*. **77**, 58-64.
15. Kang, J., H.-G. Lemaire, A. Unterbeck, J.M. Salbaum, C.L. Masters, K.-H. Grzeschik, G. Multhaup, K. Beyreuther, and B. Müller-Hill. 1987. The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature*. **325**, 733-736.
16. Dickson, D.W., 1997. The pathogenesis of senile plaques. *Journal of Neuropathology & Experimental Neurology*. **56**, 321-339.

17. Charidimou, A., Q. Gang, and D.J. Werring. 2012. Sporadic cerebral amyloid angiopathy revisited: recent insights into pathophysiology and clinical spectrum. *Journal of Neurology, Neurosurgery & Psychiatry*. **83**, 124-137.
18. Schöll, M., A. Wall, S. Thordardottir, D. Ferreira, N. Bogdanovic, B. Långström, O. Almkvist, C. Graff, and A. Nordberg. 2012. Low PiB PET retention in presence of pathologic CSF biomarkers in Arctic APP mutation carriers. *Neurology*. **79**, 229-236.
19. Jack, C.R., D.S. Knopman, W.J. Jagust, L.M. Shaw, P.S. Aisen, M.W. Weiner, R.C. Petersen, and J.Q. Trojanowski. 2010. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *The Lancet Neurology*. **9**, 119-128.
20. Wu, H.-Y., E. Hudry, T. Hashimoto, K. Kuchibhotla, A. Rozkalne, Z. Fan, T. Spires-Jones, H. Xie, M. Arbel-Ornath, and C.L. Grosskreutz. 2010. Amyloid β induces the morphological neurodegenerative triad of spine loss, dendritic simplification, and neuritic dystrophies through calcineurin activation. *Journal of Neuroscience*. **30**, 2636-2649.
21. Burgold, S., T. Bittner, M.M. Dorostkar, D. Kieser, M. Fuhrmann, G. Mitteregger, H. Kretschmar, B. Schmidt, and J. Herms. 2011. In vivo multiphoton imaging reveals gradual growth of newborn amyloid plaques over weeks. *Acta neuropathologica*. **121**, 327-335.
22. Cummings, D.M., T.A. Benway, H. Ho, A. Tedoldi, M.M. Fernandes Freitas, L. Shahab, C.E. Murray, A. Richard-Loendt, S. Brandner, and T. Lashley. 2017. Neuronal and peripheral pentraxins modify glutamate release and may interact in blood-brain barrier failure. *Cerebral Cortex*. **27**, 3437-3448.
23. Howlett, D.R., K. Bowler, P. Soden, D. Riddell, J. Davis, J. Richardson, S. Burbidge, M. Gonzalez, E. Irving, and A. Lawman. 2008. AB deposition and related pathology in an APP x PS1 transgenic mouse model of Alzheimer's disease. *Histology and histopathology*.
24. Hong, S., V.F. Beja-Glasser, B.M. Nfonoyim, A. Frouin, S. Li, S. Ramakrishnan, K.M. Merry, Q. Shi, A. Rosenthal, and B.A. Barres. 2016. Complement and microglia mediate early synapse loss in Alzheimer mouse models. *Science*. **352**, 712-716.
25. Dubois, B., H.H. Feldman, C. Jacova, H. Hampel, J.L. Molinuevo, K. Blennow, S.T. DeKosky, S. Gauthier, D. Selkoe, and R. Bateman. 2014. Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. *The Lancet Neurology*. **13**, 614-629.
26. Virgilio, E., F. De Marchi, E. Contaldi, U. Dianzani, R. Cantello, L. Mazzini, and C. Comi. 2022. The role of Tau beyond Alzheimer's disease: a narrative review. *Biomedicines*. **10**, 760.
27. Gentile, A., F. Mori, S. Bernardini, and D. Centonze. 2015. Role of amyloid- β CSF levels in cognitive deficit in MS. *Clinica Chimica Acta*. **449**, 23-30.
28. Iqbal, K., A.d.C. Alonso, S. Chen, M.O. Chohan, E. El-Akkad, C.-X. Gong, S. Khatoon, B. Li, F. Liu, and A. Rahman. 2005. Tau pathology in Alzheimer disease and other tauopathies. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*. **1739**, 198-210.
29. Polydoro, M., C.M. Acker, K. Duff, P.E. Castillo, and P. Davies. 2009. Age-dependent impairment of cognitive and synaptic function in the htau mouse model of tau pathology. *Journal of Neuroscience*. **29**, 10741-10749.
30. Chen, Z., S. Wang, Z. Meng, Y. Ye, G. Shan, X. Wang, X. Zhao, and Y. Jin. 2023. Tau protein plays a role in the mechanism of cognitive disorders induced by anesthetic drugs. *Frontiers in Neuroscience*. **17**, 1145318.
31. Grundke-Iqbal, I., K. Iqbal, Y.-C. Tung, M. Quinlan, H.M. Wisniewski, and L.I. Binder. 1986. Abnormal phosphorylation of the microtubule-associated protein tau

- (tau) in Alzheimer cytoskeletal pathology. *Proceedings of the National Academy of Sciences*. **83**, 4913-4917.
32. Wang, J., B.J. Gu, C.L. Masters, and Y.-J. Wang. 2017. A systemic view of Alzheimer disease—insights from amyloid- β metabolism beyond the brain. *Nature reviews neurology*. **13**, 612-623.
 33. Chen, X.-Q. and W.C. Mobley. 2019. Alzheimer disease pathogenesis: insights from molecular and cellular biology studies of oligomeric A β and tau species. *Frontiers in Neuroscience*. **13**, 464423.
 34. Scarano, S., S. Lisi, C. Ravelet, E. Peyrin, and M. Minunni. 2016. Detecting Alzheimer's disease biomarkers: From antibodies to new bio-mimetic receptors and their application to established and emerging bioanalytical platforms—A critical review. *Analytica Chimica Acta*. **940**, 21-37.
 35. Spires-Jones, T.L. and B.T. Hyman. 2014. The intersection of amyloid beta and tau at synapses in Alzheimer's disease. *Neuron*. **82**, 756-771.
 36. Gao, L., H. Tang, K. Nie, L. Wang, J. Zhao, R. Gan, J. Huang, R. Zhu, S. Feng, and Z. Duan. 2015. Cerebrospinal fluid alpha-synuclein as a biomarker for Parkinson's disease diagnosis: a systematic review and meta-analysis. *International journal of neuroscience*. **125**, 645-654.
 37. Zubelzu, M., T. Morera-Herreras, G. Irastorza, J.C. Gómez-Esteban, and A. Murueta-Goyena. 2022. Plasma and serum alpha-synuclein as a biomarker in Parkinson's disease: a meta-analysis. *Parkinsonism & related disorders*. **99**, 107-115.
 38. Sui, Y.-T., K.M. Bullock, M.A. Erickson, J. Zhang, and W. Banks. 2014. Alpha synuclein is transported into and out of the brain by the blood–brain barrier. *Peptides*. **62**, 197-202.
 39. Tokuda, T., S.A. Salem, D. Allsop, T. Mizuno, M. Nakagawa, M.M. Qureshi, J.J. Locascio, M.G. Schlossmacher, and O.M. El-Agnaf. 2006. Decreased α -synuclein in cerebrospinal fluid of aged individuals and subjects with Parkinson's disease. *Biochemical and biophysical research communications*. **349**, 162-166.
 40. Mollenhauer, B., E. Trautmann, P. Taylor, P. Manninger, F. Sixel-Döring, J. Ebentheuer, C. Trenkwalder, and M.G. Schlossmacher. 2013. Total CSF α -synuclein is lower in de novo Parkinson patients than in healthy subjects. *Neuroscience letters*. **532**, 44-48.
 41. Öhrfelt, A., P. Grognet, N. Andreasen, A. Wallin, E. Vanmechelen, K. Blennow, and H. Zetterberg. 2009. Cerebrospinal fluid α -synuclein in neurodegenerative disorders—a marker of synapse loss? *Neuroscience letters*. **450**, 332-335.
 42. Park, M.J., S.-M. Cheon, H.-R. Bae, S.-H. Kim, and J.W. Kim. 2011. Elevated levels of α -synuclein oligomer in the cerebrospinal fluid of drug-naïve patients with Parkinson's disease. *Journal of clinical neurology (Seoul, Korea)*. **7**, 215.
 43. Shi, M., J. Bradner, A.M. Hancock, K.A. Chung, J.F. Quinn, E.R. Peskind, D. Galasko, J. Jankovic, C.P. Zabetian, and H.M. Kim. 2011. Cerebrospinal fluid biomarkers for Parkinson disease diagnosis and progression. *Annals of neurology*. **69**, 570-580.
 44. Aerts, M., R. Esselink, W. Abdo, B. Bloem, and M. Verbeek. 2012. CSF α -synuclein does not differentiate between parkinsonian disorders. *Neurobiology of aging*. **33**, 430.e1-430.e3.
 45. Zhou, B., M. Wen, W.-F. Yu, C.-L. Zhang, and L. Jiao. 2015. The diagnostic and differential diagnosis utility of cerebrospinal fluid α -synuclein levels in Parkinson's disease: a meta-analysis. *Parkinson's Disease*. **2015**,
 46. Chahine, L.M., T.G. Beach, M.C. Brumm, C.H. Adler, C.S. Coffey, S. Mosovsky, C. Caspell-Garcia, G.E. Serrano, D.G. Munoz, and C.L. White III. 2020. In vivo

- distribution of α -synuclein in multiple tissues and biofluids in Parkinson disease. *Neurology*. **95**, e1267-e1284.
47. Pak, J.H., F.L. Huang, J. Li, D. Balschun, K.G. Reymann, C. Chiang, H. Westphal, and K.-P. Huang. 2000. Involvement of neurogranin in the modulation of calcium/calmodulin-dependent protein kinase II, synaptic plasticity, and spatial learning: a study with knockout mice. *Proceedings of the National Academy of Sciences*. **97**, 11232-11237.
48. Kaleka, K.S. and N.Z. Gerges. 2016. Neurogranin restores amyloid β -mediated synaptic transmission and long-term potentiation deficits. *Experimental neurology*. **277**, 115-123.
49. Xue, M., F.-R. Sun, Y.-N. Ou, X.-N. Shen, H.-Q. Li, Y.-Y. Huang, Q. Dong, L. Tan, J.-T. Yu, and A.s.D.N. Initiative. 2020. Association of cerebrospinal fluid neurogranin levels with cognition and neurodegeneration in Alzheimer's disease. *Aging (Albany NY)*. **12**, 9365.
50. Díez-Guerra, F.J., 2010. Neurogranin, a link between calcium/calmodulin and protein kinase C signaling in synaptic plasticity. *IUBMB life*. **62**, 597-606.
51. Shinohara, M., S. Fujioka, M.E. Murray, A. Wojtas, M. Baker, A. Rovelet-Lecrux, R. Rademakers, P. Das, J.E. Parisi, and N.R. Graff-Radford. 2014. Regional distribution of synaptic markers and APP correlate with distinct clinicopathological features in sporadic and familial Alzheimer's disease. *Brain*. **137**, 1533-1549.
52. Galasko, D., M. Xiao, D. Xu, D. Smirnov, D.P. Salmon, N. Dewit, J. Vanbrabant, D. Jacobs, H. Vanderstichele, and E. Vanmechelen. 2019. Synaptic biomarkers in CSF aid in diagnosis, correlate with cognition and predict progression in MCI and Alzheimer's disease. *Alzheimer's & Dementia: Translational Research & Clinical Interventions*. **5**, 871-882.
53. Janelidze, S., J. Hertze, H. Zetterberg, M. Landqvist Waldö, A. Santillo, K. Blennow, and O. Hansson. 2016. Cerebrospinal fluid neurogranin and YKL-40 as biomarkers of Alzheimer's disease. *Annals of clinical and translational neurology*. **3**, 12-20.
54. Kvartsberg, H., F.H. Duits, M. Ingelsson, N. Andreasen, A. Öhrfelt, K. Andersson, G. Brinkmalm, L. Lannfelt, L. Minthon, and O. Hansson. 2015. Cerebrospinal fluid levels of the synaptic protein neurogranin correlates with cognitive decline in prodromal Alzheimer's disease. *Alzheimer's & Dementia*. **11**, 1180-1190.
55. Portelius, E., H. Zetterberg, T. Skillbäck, U. Törnqvist, U. Andreasson, J.Q. Trojanowski, M.W. Weiner, L.M. Shaw, N. Mattsson, and K. Blennow. 2015. Cerebrospinal fluid neurogranin: relation to cognition and neurodegeneration in Alzheimer's disease. *Brain*. **138**, 3373-3385.
56. Mattsson, N., P.S. Insel, S. Palmqvist, E. Portelius, H. Zetterberg, M. Weiner, K. Blennow, O. Hansson, and A.s.D.N. Initiative. 2016. Cerebrospinal fluid tau, neurogranin, and neurofilament light in Alzheimer's disease. *EMBO molecular medicine*. **8**, 1184-1196.
57. Liu, W., H. Lin, X. He, L. Chen, Y. Dai, W. Jia, X. Xue, J. Tao, and L. Chen. 2020. Neurogranin as a cognitive biomarker in cerebrospinal fluid and blood exosomes for Alzheimer's disease and mild cognitive impairment. *Translational psychiatry*. **10**, 125.
58. Agnello, L., C.M. Gambino, B. Lo Sasso, G. Bivona, S. Milano, A.M. Ciaccio, T. Piccoli, V. La Bella, and M. Ciaccio. 2021. Neurogranin as a novel biomarker in Alzheimer's disease. *Laboratory Medicine*. **52**, 188-196.
59. Yilmaz, A., D. Fuchs, R.W. Price, S. Spudich, K. Blennow, H. Zetterberg, and M. Gisslén. 2019. Cerebrospinal fluid concentrations of the synaptic marker neurogranin in neuro-HIV and other neurological disorders. *Current HIV/AIDS Reports*. **16**, 76-81.

60. Poddar, M.K., A. Chakraborty, and S. Banerjee, *Neurodegeneration: Diagnosis, prevention, and therapy*. 2021: IntechOpen London, UK.
61. Bayazeid, O., E. Nemutlu, C.C. Eylem, M. İlhan, E. Küpeli-Akkol, H. Karahan, P. Kelicen-Uğur, T. Ersoz, and F.N. Yalçın. 2021. Neuroactivity of the naturally occurring aporphine alkaloid, roemerine. *Natural product research*. **35**, 6147-6152.
62. Girdhar, S., A. Girdhar, S.K. Verma, V. Lather, and D. Pandita. 2015. Plant derived alkaloids in major neurodegenerative diseases: from animal models to clinical trials. *Journal of Ayurvedic and Herbal Medicine*. **1**, 91-100.
63. Casciaro, B., L. Mangiardi, F. Cappiello, I. Romeo, M.R. Loffredo, A. Iazzetti, A. Calcaterra, A. Goggiamani, F. Ghirga, and M.L. Mangoni. 2020. Naturally-occurring alkaloids of plant origin as potential antimicrobials against antibiotic-resistant infections. *Molecules*. **25**, 3619.
64. Aryal, B., B. Raut, S. Bhattarai, S. Bhandari, P. Tandan, K. Gyawali, K. Sharma, D. Ranabhat, R. Thapa, and D. Aryal, *Potential therapeutic applications of plant-derived alkaloids against inflammatory and neurodegenerative diseases. Evidence-Based Complement Altern Med* 2022: 1–18. 2022.
65. Ng, Y.P., T.C.T. Or, and N.Y. Ip. 2015. Plant alkaloids as drug leads for Alzheimer's disease. *Neurochemistry international*. **89**, 260-270.
66. Hussain, G., A. Rasul, H. Anwar, N. Aziz, A. Razzaq, W. Wei, M. Ali, J. Li, and X. Li. 2018. Role of plant derived alkaloids and their mechanism in neurodegenerative disorders. *International journal of biological sciences*. **14**, 341.
67. Küpeli Akkol, E., I. Tatlı Çankaya, G. Şeker Karatoprak, E. Carpar, E. Sobarzo-Sánchez, and R. Capasso. 2021. Natural compounds as medical strategies in the prevention and treatment of psychiatric disorders seen in neurological diseases. *Frontiers in Pharmacology*. **12**, 669638.
68. Caccetta, R.A.-A., K.D. Croft, L.J. Beilin, and I.B. Puddey. 2000. Ingestion of red wine significantly increases plasma phenolic acid concentrations but does not acutely affect ex vivo lipoprotein oxidizability. *The American journal of clinical nutrition*. **71**, 67-74.
69. Shahidi, F. and M. Naczk, *Food phenolics*. 1995: Technomic Pub. Co.
70. Pandey, K.B. and S.I. Rizvi. 2009. Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative medicine and cellular longevity*. **2**, 270-278.
71. ARAS, A. and İ. DEMİRTAŞ. A LOOK FOR PLANT POLYPHENOLS.
72. Caruso, G., S.A. Torrisi, M.P. Mogavero, W. Currenti, S. Castellano, J. Godos, R. Ferri, F. Galvano, G.M. Leggio, and G. Grosso. 2022. Polyphenols and neuroprotection: Therapeutic implications for cognitive decline. *Pharmacology & Therapeutics*. **232**, 108013.
73. Hamaguchi, T., K. Ono, A. Murase, and M. Yamada. 2009. Phenolic compounds prevent Alzheimer's pathology through different effects on the amyloid- β aggregation pathway. *The American journal of pathology*. **175**, 2557-2565.
74. Ardah, M.T., K.E. Paleologou, G. Lv, S.B. Abul Khair, A.S. Kazim, S.T. Minhas, T.H. Al-Tel, A.A. Al-Hayani, M.E. Haque, and D. Eliezer. 2014. Structure activity relationship of phenolic acid inhibitors of α -synuclein fibril formation and toxicity. *Frontiers in aging neuroscience*. **6**, 197.
75. Freyssin, A., G. Page, B. Fauconneau, and A.R. Bilan. 2018. Natural polyphenols effects on protein aggregates in Alzheimer's and Parkinson's prion-like diseases. *Neural regeneration research*. **13**, 955-961.
76. Caruso, G., J. Godos, A. Privitera, G. Lanza, S. Castellano, A. Chillemi, O. Bruni, R. Ferri, F. Caraci, and G. Grosso. 2022. Phenolic acids and prevention of cognitive

- decline: Polyphenols with a neuroprotective role in cognitive disorders and Alzheimer's disease. *Nutrients*. **14**, 819.
77. Mallik, S.B., J. Mudgal, M. Nampoothiri, S. Hall, S. Anoopkumar-Dukie, G. Grant, C.M. Rao, and D. Arora. 2016. Caffeic acid attenuates lipopolysaccharide-induced sickness behaviour and neuroinflammation in mice. *Neuroscience letters*. **632**, 218-223.
78. Caruso, G., C.G. Fresta, M. Grasso, R. Santangelo, G. Lazzarino, S.M. Lunte, and F. Caraci. 2020. Inflammation as the common biological link between depression and cardiovascular diseases: can carnosine exert a protective role? *Current Medicinal Chemistry*. **27**, 1782-1800.
79. Maan, G., B. Sikdar, A. Kumar, R. Shukla, and A. Mishra. 2020. Role of flavonoids in neurodegenerative diseases: limitations and future perspectives. *Current Topics in Medicinal Chemistry*. **20**, 1169-1194.
80. Rendeiro, C., J.S. Rhodes, and J.P. Spencer. 2015. The mechanisms of action of flavonoids in the brain: Direct versus indirect effects. *Neurochemistry international*. **89**, 126-139.
81. Patil, S.P., P.D. Jain, J.S. Sancheti, P.J. Ghumatkar, R. Tambe, and S. Sathaye, *RETRACTED: Neuroprotective and neurotrophic effects of Apigenin and Luteolin in MPTP induced parkinsonism in mice*. 2014, Elsevier.
82. Li, R., X. Wang, T. Qin, R. Qu, and S. Ma. 2016. Apigenin ameliorates chronic mild stress-induced depressive behavior by inhibiting interleukin-1 β production and NLRP3 inflammasome activation in the rat brain. *Behavioural brain research*. **296**, 318-325.
83. Shen, Y., X. Liu, J. Shi, and X. Wu. 2019. Involvement of Nrf2 in myocardial ischemia and reperfusion injury. *International journal of biological macromolecules*. **125**, 496-502.
84. Thimmulappa, R.K., H. Lee, T. Rangasamy, S.P. Reddy, M. Yamamoto, T.W. Kensler, and S. Biswal. 2016. Nrf2 is a critical regulator of the innate immune response and survival during experimental sepsis. *The Journal of clinical investigation*. **116**, 984-995.
85. Cui, Q., R. Du, M. Liu, and L. Rong. 2020. Lignans and their derivatives from plants as antivirals. *Molecules*. **25**, 183.
86. Isla, M.I., M.A. Moreno, M. de los Ángeles Álvarez, and I.C. Zampini. 2021. Some plants of the Monte region from Argentina: Phytochemistry and its use in health care. *Studies in Natural Products Chemistry*. **69**, 349-369.
87. Lu, H. and G.-T. Liu. 1992. Anti-oxidant activity of dibenzocyclooctene lignans isolated from Schisandraceae. *Planta Medica*. **58**, 311-313.
88. Hirano, T., A. Wakasugi, M. Oohara, K. Oka, and Y. Sashida. 1991. Suppression of mitogen-induced proliferation of human peripheral blood lymphocytes by plant lignans. *Planta medica*. **57**, 331-334.
89. Kawazoe, K., A. Yutani, K. Tamemoto, S. Yuasa, H. Shibata, T. Higuti, and Y. Takaishi. 2001. Phenyl-naphthalene compounds from the subterranean part of *Vitex rotundifolia* and their antibacterial activity against methicillin-resistant *Staphylococcus aureus*. *Journal of Natural Products*. **64**, 588-591.
90. Iwasaki, T., K. Kondo, T. Kuroda, Y. Moritani, S. Yamagata, M. Sugiura, H. Kikkawa, O. Kaminuma, and K. Ikezawa. 1996. Novel selective PDE IV inhibitors as antiasthmatic agents. Synthesis and biological activities of a series of 1-aryl-2, 3-bis (hydroxymethyl) naphthalene lignans. *Journal of medicinal chemistry*. **39**, 2696-2704.
91. Korkina, L., 2007. Phenylpropanoids as naturally occurring antioxidants: from plant defense to human health. *Cellular and molecular biology*. **53**, 15-25.

92. Park, H.J., T.T. Zhao, K.S. Lee, S.H. Lee, K.S. Shin, K.H. Park, H.S. Choi, and M.K. Lee. 2015. Effects of (-)-sesamin on 6-hydroxydopamine-induced neurotoxicity in PC12 cells and dopaminergic neuronal cells of Parkinson's disease rat models. *Neurochemistry International*. **83**, 19-27.
93. Giuliano, C., F. Siani, L. Mus, C. Ghezzi, S. Cerri, B. Pacchetti, C. Bigogno, and F. Blandini. 2020. Neuroprotective effects of lignan 7-hydroxymatairesinol (HMR/lignan) in a rodent model of Parkinson's disease. *Nutrition*. **69**, 110494.
94. Ansari, N. and F. Khodagholi. 2013. Natural products as promising drug candidates for the treatment of Alzheimer's disease: molecular mechanism aspect. *Current neuropharmacology*. **11**, 414-429.
95. Nagumo, M., M. Ninomiya, N. Oshima, T. Itoh, K. Tanaka, A. Nishina, and M. Koketsu. 2019. Comparative analysis of stilbene and benzofuran neolignan derivatives as acetylcholinesterase inhibitors with neuroprotective and anti-inflammatory activities. *Bioorganic & Medicinal Chemistry Letters*. **29**, 2475-2479.
96. Nabavi, S.M., M. Daglia, N. Braidy, and S.F. Nabavi. 2017. Natural products, micronutrients, and nutraceuticals for the treatment of depression: A short review. *Nutritional neuroscience*. **20**, 180-194.
97. Socała, K., E. Żmudzka, K. Lustyk, M. Zagaja, V. Brighenti, A.M. Costa, M. Andres-Mach, K. Pytko, I. Martinelli, and J. Mandrioli. 2024. Therapeutic potential of stilbenes in neuropsychiatric and neurological disorders: A comprehensive review of preclinical and clinical evidence. *Phytotherapy Research*.
98. Rajan, R.K., R.P. Kumar, and M. Ramanathan. 2024. Piceatannol improved cerebral blood flow and attenuated JNK3 and mitochondrial apoptotic pathway in a global ischemic model to produce neuroprotection. *Naunyn-Schmiedeberg's Archives of Pharmacology*. **397**, 479-496.
99. Benlloch, M., E. Obrador, S.L. Valles, M.L. Rodriguez, J.A. Sirerol, J. Alcácer, J.A. Pellicer, R. Salvador, C. Cerdá, and G.T. Sáez. 2016. Pterostilbene decreases the antioxidant defenses of aggressive cancer cells in vivo: a physiological glucocorticoids-and Nrf2-dependent mechanism. *Antioxidants & redox signaling*. **24**, 974-990.
100. Xu, J., J. Liu, Q. Li, Y. Mi, D. Zhou, Q. Meng, G. Chen, N. Li, and Y. Hou. 2021. Pterostilbene alleviates Aβ1-42-induced cognitive dysfunction via inhibition of oxidative stress by activating Nrf2 signaling pathway. *Molecular nutrition & food research*. **65**, 2000711.
101. Gu, Z., L. Li, Q. Li, H. Tan, Z. Zou, X. Chen, Z. Zhang, Y. Zhou, D. Wei, and C. Liu. 2021. Polydatin alleviates severe traumatic brain injury induced acute lung injury by inhibiting S100B mediated NETs formation. *International Immunopharmacology*. **98**, 107699.
102. Li, Q., P. Zhao, Y. Wen, Z. Zou, X. Qin, H. Tan, J. Gong, Q. Wu, C. Zheng, and K. Zhang. 2023. POLYDATIN AMELIORATES TRAUMATIC BRAIN INJURY–INDUCED SECONDARY BRAIN INJURY BY INHIBITING NLRP3-INDUCED NEUROINFLAMMATION ASSOCIATED WITH SOD2 ACETYLATION. *Shock*. **59**, 460-468.
103. Good, P.F., A. Hsu, P. Werner, D.P. Perl, and C.W. Olanow. 1998. Protein nitration in Parkinson's disease. *Journal of Neuropathology & Experimental Neurology*. **57**, 338-342.
104. Buldurun, K., N. Turan, E. Bursal, A. Aras, A. Mantarcı, N. Çolak, F. Türkan, and İ. Gülçin. 2021. Synthesis, characterization, powder X-ray diffraction analysis, thermal stability, antioxidant properties and enzyme inhibitions of M (II)-Schiff base ligand complexes. *Journal of Biomolecular Structure and Dynamics*. **39**, 6480-6487.

105. Bursal, E., F. Turkan, K. Buldurun, N. Turan, A. Aras, N. Çolak, M. Murahari, and M.C. Yergeri. 2021. Transition metal complexes of a multidentate Schiff base ligand containing pyridine: synthesis, characterization, enzyme inhibitions, antioxidant properties, and molecular docking studies. *Biometals*. **34**, 393-406.
106. Richardson, J.R., V. Fitsanakis, R.H. Westerink, and A.G. Kanthasamy. 2019. Neurotoxicity of pesticides. *Acta neuropathologica*. **138**, 343-362.
107. Miao, Y., N. He, and J.-J. Zhu. 2010. History and new developments of assays for cholinesterase activity and inhibition. *Chemical reviews*. **110**, 5216-5234.
108. Hofer, P. and U. Fringeli. 1981. Acetylcholinesterase kinetics. *Biophysics of structure and mechanism*. **8**, 45-59.
109. Türkan, F., M.N. Atalar, A. Aras, İ. Gülçin, and E. Bursal. 2020. ICP-MS and HPLC analyses, enzyme inhibition and antioxidant potential of *Achillea schischkinii* Sosn. *Bioorganic chemistry*. **94**, 103333.
110. Aras, A., F. Türkan, U. Yildiko, M.N. Atalar, Ö. Kılıç, M.H. Alma, and E. Bursal. 2021. Biochemical constituent, enzyme inhibitory activity, and molecular docking analysis of an endemic plant species, *Thymus migricus*. *Chemical Papers*. **75**, 1133-1146.
111. Zhang, C., D. Chen, X. Liu, and I. Du. 2016. Role of brominated diphenyl ether-209 in the proliferation and apoptosis of rat cultured neural stem cells in vitro. *Molecular & Cellular Toxicology*. **12**, 45-52.
112. Mrdaković, M., L. Ilijin, M. Vlahović, D. Matić, A. Gavrilović, A. Mrkonja, and V. Perić-Mataruga. 2016. Acetylcholinesterase (AChE) and heat shock proteins (Hsp70) of gypsy moth (*Lymantria dispar* L.) larvae in response to long-term fluoranthene exposure. *Chemosphere*. **159**, 565-569.
113. Bursal, E., A. Aras, Ö. Kılıç, P. Taslimi, A.C. Gören, and İ. Gülçin. 2019. Phytochemical content, antioxidant activity, and enzyme inhibition effect of *Salvia eriophora* Boiss. & Kotschy against acetylcholinesterase, α -amylase, butyrylcholinesterase, and α -glycosidase enzymes. *Journal of food biochemistry*. **43**, e12776.
114. Taslimi, P., E. Köksal, A.C. Gören, E. Bursal, A. Aras, Ö. Kılıç, S. Alwasel, and İ. Gülçin. 2020. Anti-Alzheimer, antidiabetic and antioxidant potential of *Satureja cuneifolia* and analysis of its phenolic contents by LC-MS/MS. *Arabian journal of chemistry*. **13**, 4528-4537.
115. Lockridge, O., 2015. Review of human butyrylcholinesterase structure, function, genetic variants, history of use in the clinic, and potential therapeutic uses. *Pharmacology & therapeutics*. **148**, 34-46.
116. Guillozet, A., M.M. Mesulam, J. Smiley, and D. Mash. 1997. Butyrylcholinesterase in the life cycle of amyloid plaques. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society*. **42**, 909-918.
117. Darvesh, S., D.A. Hopkins, and C. Geula. 2003. Neurobiology of butyrylcholinesterase. *Nature Reviews Neuroscience*. **4**, 131-138.
118. Darvesh, S. and D.A. Hopkins. 2003. Differential distribution of butyrylcholinesterase and acetylcholinesterase in the human thalamus. *Journal of Comparative Neurology*. **463**, 25-43.
119. Darreh-Shori, T., A. Forsberg, N. Modiri, N. Andreasen, K. Blennow, C. Kamil, H. Ahmed, O. Almkvist, B. Långström, and A. Nordberg. 2011. Differential levels of apolipoprotein E and butyrylcholinesterase show strong association with pathological signs of Alzheimer's disease in the brain in vivo. *Neurobiology of aging*. **32**, 2320.e15-2320.e32.

120. Jasiecki, J., M. Targońska, and B. Wasąg. 2021. The role of butyrylcholinesterase and iron in the regulation of cholinergic network and cognitive dysfunction in Alzheimer's disease pathogenesis. *International Journal of Molecular Sciences*. **22**, 2033.
121. Rhee, S.G., H.Z. Chae, and K. Kim. 2005. Peroxiredoxins: a historical overview and speculative preview of novel mechanisms and emerging concepts in cell signaling. *Free radical biology and Medicine*. **38**, 1543-1552.
122. Szeliga, M., 2020. Peroxiredoxins in neurodegenerative diseases. *Antioxidants*. **9**, 1203.
123. Park, M.H., M. Jo, Y.R. Kim, C.-K. Lee, and J.T. Hong. 2016. Roles of peroxiredoxins in cancer, neurodegenerative diseases and inflammatory diseases. *Pharmacology & Therapeutics*. **163**, 1-23.
124. Hanschmann, E.-M., J.R. Godoy, C. Berndt, C. Hudemann, and C.H. Lillig. 2013. Thioredoxins, glutaredoxins, and peroxiredoxins—molecular mechanisms and health significance: from cofactors to antioxidants to redox signaling. *Antioxidants & redox signaling*. **19**, 1539-1605.
125. Perez-Pinzon, M.A., R.A. Stetler, and G. Fiskum. 2012. Novel mitochondrial targets for neuroprotection. *Journal of Cerebral Blood Flow & Metabolism*. **32**, 1362-1376.
126. Liu, J., G. Su, J. Gao, Y. Tian, X. Liu, and Z. Zhang. 2020. Effects of peroxiredoxin 2 in neurological disorders: a review of its molecular mechanisms. *Neurochemical Research*. **45**, 720-730.
127. Voigt, D., U. Scheidt, T. Derfuss, W. Brück, and A. Junker. 2017. Expression of the antioxidative enzyme peroxiredoxin 2 in multiple sclerosis lesions in relation to inflammation. *International journal of molecular sciences*. **18**, 760.
128. Yayla, M.A., B. Arda, Ö. Çağlar, and O. Erbaş. 2021. Peptide hormones and neurodegenerative diseases. *Journal of Experimental and Basic Medical Sciences*. **2**, 062-075.
129. Klein, M.O., D.S. Battagello, A.R. Cardoso, D.N. Hauser, J.C. Bittencourt, and R.G. Correa. 2019. Dopamine: functions, signaling, and association with neurological diseases. *Cellular and molecular neurobiology*. **39**, 31-59.
130. Broome, S.T., K. Louangaphay, K.A. Keay, G.M. Leggio, G. Musumeci, and A. Castorina. 2020. Dopamine: an immune transmitter. *Neural regeneration research*. **15**, 2173-2185.
131. Dirkx, M.F., H.E. den Ouden, E. Aarts, M.H. Timmer, B.R. Bloem, I. Toni, and R.C. Helmich. 2017. Dopamine controls Parkinson's tremor by inhibiting the cerebellar thalamus. *Brain*. **140**, 721-734.
132. Pollok, B., H. Makhoulfi, M. Butz, J. Gross, L. Timmermann, L. Wojtecki, and A. Schnitzler. 2009. Levodopa affects functional brain networks in Parkinsonian resting tremor. *Movement disorders*. **24**, 91-98.
133. Zach, H., M.F. Dirkx, D. Roth, J.W. Pasma, B.R. Bloem, and R.C. Helmich. 2020. Dopamine-responsive and dopamine-resistant resting tremor in Parkinson disease. *Neurology*. **95**, e1461-e1470.
134. Qi, J.-y., L.-k. Yang, X.-s. Wang, M. Wang, X.-b. Li, B. Feng, Y.-m. Wu, K. Zhang, and S.-b. Liu. 2022. Irisin: a promising treatment for neurodegenerative diseases. *Neuroscience*. **498**, 289-299.
135. Islam, M.R., S. Valaris, M.F. Young, E.B. Haley, R. Luo, S.F. Bond, S. Mazuera, R.R. Kitchen, B.J. Caldarone, and L.E. Bettio. 2021. Exercise hormone irisin is a critical regulator of cognitive function. *Nature Metabolism*. **3**, 1058-1070.
136. Küster, O.C., D. Laptinskaya, P. Fissler, C. Schnack, M. Zügel, V. Nold, F. Thurm, S. Pleiner, A. Karabatsiakis, and B. von Einem. 2017. Novel blood-based biomarkers of cognition, stress, and physical or cognitive training in older adults at risk of dementia:

preliminary evidence for a role of BDNF, irisin, and the kynurenine pathway. *Journal of Alzheimer's disease*. **59**, 1097-1111.

CHAPTER 3

ACOUSTIC STIMULI IN PLANTS: BIOCHEMICAL, MOLECULAR, NUTRITIONAL, AND STRUCTURAL RESPONSES

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Introduction

Sound is a mechanical wave generated by a vibrating source and propagating through air, water, or solid media as pressure fluctuations and particle motion. From the perspective of plants, sound should therefore be considered not as a stimulus perceived directly through an auditory organ, but as an environmental signal capable of generating mechanical stress on plant surfaces, the cell wall–plasma membrane interface, and mechanosensitive perception systems. This approach requires the effects of sound in plants to be addressed not merely as an “auditory” phenomenon, but within the framework of mechanoperception and mechanotransduction (Demey, Mishra, & Van Der Straeten, 2023; Hassanien, Hou, Li, & Li, 2014; Kinsler, Frey, Coppens, & Sanders, 2000; Pagano & Del Prete, 2024).

From an acoustic perspective, sound is a mechanical wave phenomenon that can be described by parameters such as frequency, period, amplitude, sound pressure, energy density, and acoustic intensity. Frequency refers to the number of repetitions of a vibration or wave per unit time; period denotes the time required to complete one full oscillation; amplitude indicates the magnitude of the vibration; sound pressure is the measurable deviation produced by an acoustic wave in the ambient pressure of the medium; energy density refers to the amount of sound energy per unit volume; and acoustic intensity denotes the flow of this energy through a given area. As these definitions indicate, amplitude, sound pressure, energy density, and acoustic intensity are related to one another, but they are not identical physical quantities (Kinsler et al., 2000). Erbe, Duncan, Hawkins, Terhune, and Thomas (2022) also emphasize that terms such as sound pressure, sound exposure, particle velocity, sound speed, frequency modulation, amplitude modulation, frequency weighting, power spectral density, and one-third-octave band level should be systematically defined in bioacoustic and environmental acoustic studies.

The nature of the vibrational effect produced by sound in plants depends on these physical properties of the applied sound. While frequency determines how often the mechanical oscillation generated in plant tissues is repeated, amplitude- and sound pressure-related level properties may determine the magnitude of this oscillation and the strength of the resulting mechanical effect. By contrast, the decibel (dB) is not frequency, amplitude, or sound pressure itself, but a logarithmic scale used to express the level of such acoustic quantities. Robin and Plante (2022) clearly demonstrate that the dB is used in acoustics primarily to express ratios and level differences. Therefore, although a high dB value often indicates a stronger acoustic stimulus, it does not by itself fully explain the physical character of the resulting vibration.

Moreover, the vibration pattern that emerges in a plant is shaped not only by frequency and level, but also by the waveform, spectral composition, continuity of the sound, and its mode of transmission through air, water, or substrate. Thus, plant responses should be considered in relation not only to the applied sound parameters, but also to the specific vibration regime generated by these parameters within the tissue.

These distinctions are particularly important for the characterization of naturally derived complex sounds. Structurally, sounds can be considered as simple/pure or complex. Simple or pure sounds ideally consist of a single frequency component and exhibit a periodic waveform. Complex sounds, by contrast, consist of a temporally varying combination of different frequency and amplitude components. Most natural environmental sounds and animal sounds have a complex structure. In the acoustic characterization of such sounds, dominant frequencies, frequency bands, amplitude pattern, continuity/intermittency, modulation properties, and spectral energy distribution should be evaluated together. Therefore, in environmental and biological sound studies, acoustic parameters such as sound pressure, sound exposure, particle velocity, sound speed, frequency/amplitude modulation, frequency weighting, power spectral density, and one-third-octave band level should be clearly defined and reported (Erbe et al., 2022).

The fact that sound is a mechanical wave requires it to be considered in living systems not only as an auditory phenomenon, but also as a potential mechanical stimulus. Since plants can respond to mechanical environmental signals such as touch, wind, rain, and similar stimuli, sound vibrations may also function as signals that can be perceived by plant structures and affect physiological processes (Ali, Tyagi, Park, & Bae, 2024; Chowdhury, Lim, & Bae, 2014). Studies in this field indicate that sound applications may be associated with various biological responses, including enzyme activities and gene expression (Chowdhury et al., 2014; Hassanien et al., 2014).

The effects of sound may vary depending on plant species, developmental stage, sound level and frequency, sound type (simple/pure sound, complex sound, music, noise, or biologically derived natural sound), the mode of stimulus transmission (airborne sound waves or substrate/organ vibrations), and exposure duration (Chowdhury et al., 2014; Collins & Foreman, 2001; Hassanien et al., 2014; Son, Jang, Mathevon, & Ryu, 2024; Veits et al., 2019). For the response categories considered in this chapter, this variability makes it necessary to evaluate biochemical, molecular, nutritional, and tissue-level findings together with the physical characteristics of the applied stimulus and the existing experimental limitations.

Within this framework, this chapter evaluates current evidence on the effects of acoustic stimuli on oxidative stress and antioxidant responses, defense responses, secondary metabolism, genetic and molecular responses, nutrient uptake and mineral element accumulation, and anatomical or tissue-level traits in plants. Methodological limitations related to acoustic characterization, exposure conditions, transmission mode, experimental controls, and the interpretation of biological indicators are also considered. Finally, current research gaps and future perspectives are evaluated with particular attention to the characterization and dissection of complex acoustic stimuli, unresolved causal links between sound-induced mechanical signaling and downstream biochemical and molecular responses, quantitative tissue-level analyses, mineral nutrition, and the transferability of controlled experimental findings to greenhouse and field conditions.

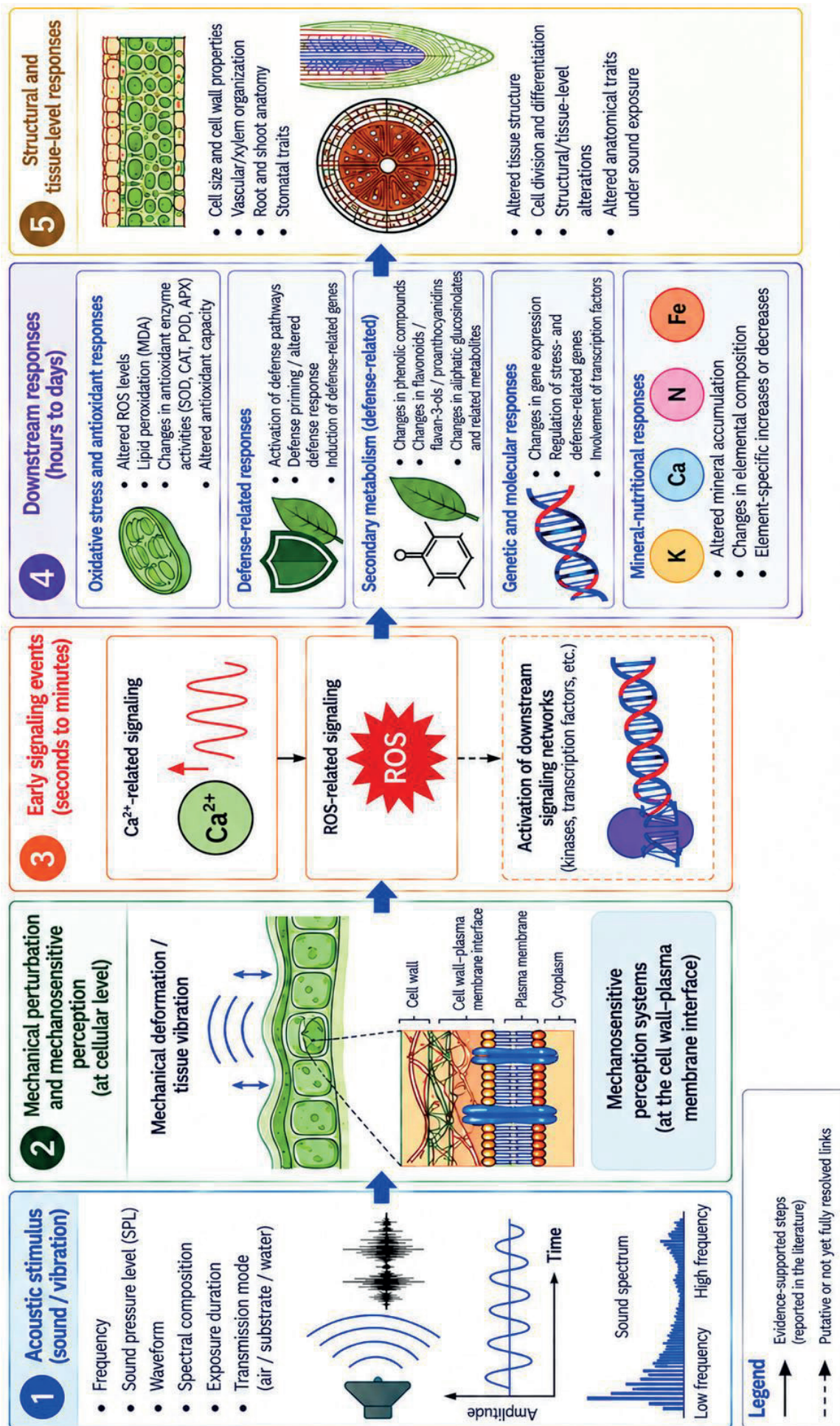


Fig. 1. Conceptual model of biochemical, molecular, nutritional, and structural responses to acoustic stimuli in plants. Sound and vibration stimuli differing in frequency, sound pressure level, waveform, spectral composition, exposure duration, and transmission mode may generate mechanical deformation and tissue vibration and interact with mechanosensitive systems at the cell wall–plasma membrane interface. Mechanically initiated responses may involve Ca^{2+} - and ROS-related early signaling and downstream signaling networks, providing a potential link between the physical stimulus and subsequent biological responses. These responses include alterations in oxidative stress and antioxidant systems, defense-related processes and secondary metabolism, genetic and molecular regulation, mineral accumulation and elemental composition, and anatomical or tissue-level traits. Defense-related responses may include pathway activation, defense priming, and changes in defense-related gene expression, whereas mineral-nutritional responses may involve element-specific increases or decreases in tissue mineral accumulation. Structural responses may include changes in cell size and cell wall properties, vascular/xylem organization, root and shoot anatomy, stomatal traits, cell division and differentiation, and other tissue-level alterations. Solid arrows represent evidence-supported steps reported in the literature, whereas dashed arrows indicate putative or incompletely resolved relationships. The causal sequence linking mechanically initiated early signaling to downstream biochemical, molecular, nutritional, and structural responses remains incompletely resolved, and not all connections shown have been demonstrated directly within the same experimental system. The mechanistic framework was developed as a conceptual synthesis based on current evidence and established models of plant mechanosignaling and sound responses (de Melo, 2023; Demey et al., 2023; Sparke & Wünsche, 2020).

Abbreviations: APX, ascorbate peroxidase; Ca, calcium; Ca^{2+} , calcium ion; CAT, catalase; Fe, iron; K, potassium; MDA, malondialdehyde; N, nitrogen; POD, peroxidase; ROS, reactive oxygen species; SOD, superoxide dismutase; SPL, sound pressure level.

Effects of Sound on Stress Biochemistry, Defense Responses, and Secondary Metabolism

The effects of sound applications on stress biochemistry, defense responses, and secondary metabolism have been evaluated through ROS accumulation, lipid peroxidation, antioxidant enzyme activities, early signaling responses, defense-related metabolites, and phenolic compounds. A substantial proportion of the available studies are based on controlled single-frequency or sinusoidal sound-wave applications, whereas fewer studies have evaluated complex or biotic stimuli such as music, environmental noise, or herbivore chewing vibrations.

Controlled frequency or sinusoidal sound-wave applications show that the antioxidant system can respond differently depending on sound level, exposure duration, organ, and tissue context. Li et al. (2008) examined the effect of 1000 Hz and 100 dB sound application on oxidative balance in *Dendrobium candidum* plantlets over a time series at leaf, stem, and root levels. SOD, CAT, POD, and APX activities generally increased at the initial stage and then partially declined; however, the peak points of these enzymes differed according to organ and measurement time. The initial increase in MDA content, followed by a decrease and subsequent increase, suggests that sound does not induce a fixed antioxidant increase, but rather creates a dynamic, organ- and time-dependent oxidative stress–defense balance. Similarly, Xiujuan et al. (2003) reported that 1000 Hz and 100 dB sound application increased CAT, POD, and SOD activities in *Chrysanthemum* seedlings depending on exposure duration; although no new bands

appeared in the POD isoenzyme analysis, the staining intensity of existing bands increased, which may be related to increased activity or expression intensity of existing isoenzymes rather than the formation of new isoforms. Heidari, Kafi, Kalatejari, Shafaghathian, and Mollakarimi (2020) compared 1000 Hz sound application at 90, 100, and 110 dB in *Salvia splendens* seedlings and reported that protein content as well as CAT, POD, and APX activities increased with increasing sound level. However, MDA content also reached its highest level under the 110 dB treatment, where the highest enzyme activities were observed. This result indicates that sound can activate antioxidant defense, but especially at high sound levels it may also produce oxidative pressure together with lipid peroxidation.

Similarly, Sardari, Ghanati, Mobasheri, and Hajnorouzi (2024) showed that the effects of sound-wave treatments on stress biochemistry should be evaluated together with membrane integrity and cell wall composition. In suspension-cultured *Nicotiana tabacum* cells, responses to a 1000 Hz sound wave differed according to sound level and exposure duration. The 90 dB treatment, particularly under longer exposure durations, was associated with increased H₂O₂ and MDA levels, reduced total antioxidant capacity and radical scavenging capacity, decreased cell viability, and lower dry weight. By contrast, under 60 and 75 dB treatments, cell viability and biomass did not change markedly, whereas radical scavenging capacity, antioxidant capacity, and cell wall polysaccharide content increased. The reported changes in pectin, hemicellulose, cellulose, lignin, wall-bound phenolic acids, PAL, peroxidase, EGase, and cellulase activities further indicate that sound may affect not only oxidative responses, but also cell wall metabolism and wall rigidity. These findings suggest that low to moderate sound levels may induce an adaptive/protective response associated with antioxidant capacity and cell wall polysaccharide accumulation, whereas high-sound-level exposure may exceed or suppress this defense capacity and become a mechanical/oxidative stress input associated with membrane damage, cell wall stiffening, and reduced biomass.

Calderón, Almagro, Martínez-Calderón, and Ferrer (2024), in contrast, showed that the effects of 100, 500, and 1000 Hz sound vibrations on phenolic metabolism and defense enzymes in tomato were not unidirectional. Under the 500 and 1000 Hz treatments in particular, total phenolic content, hydroxycinnamic acids, flavonoids, leucocyanidins, and anthocyanidins decreased, whereas flavan-3-ols and proanthocyanidins increased. In addition, PAL activity did not change significantly, PPO activity increased at all frequencies on day 3, and PRX activity decreased particularly under 1000 Hz. SOD and CAT activities showed time-dependent changes, decreasing under 500 and 1000 Hz on day 1 but increasing above the control under 1000 Hz on day 3. Therefore, this study should not be interpreted as showing that sound

generally enhances phenolic metabolism, but rather as indicating that sound can suppress some phenolic classes while reorganizing the accumulation of flavan-3-ols/proanthocyanidins and the activities of specific defense enzymes.

Sound-level-dependent secondary metabolic responses have also been reported for fruit quality parameters. Ozkurt and Altuntas (2018) exposed *Fragaria* × *ananassa* cv. Festival plants to a constant frequency of 1000 Hz at 95, 100, and 105 dB for 1 h per day over 30 days and evaluated root and shoot fresh/dry weights together with fruit pH, total soluble solids, titratable acidity, total acid, total sugar, total phenolic content, and ascorbic acid. In that study, differences in pH, total soluble solids, titratable acidity, and total acid were limited, whereas total sugar, total phenolic content, and ascorbic acid increased depending on sound level. In particular, the 100 and 105 dB treatments produced the highest values for total sugar and ascorbic acid; under 105 dB, total sugar, total phenolic content, and ascorbic acid increased by approximately 9%, 4.5%, and 19%, respectively, relative to the control. However, the same study also reported reductions in root and shoot weights under sound treatments, with the weight loss being more pronounced at 105 dB. Therefore, the strawberry example should be interpreted cautiously, as it indicates that high-sound-level exposure may increase some fruit quality- and defense-related metabolites, but that this increase does not necessarily represent a directly positive effect on growth or yield.

Music and environmental/anthropogenic complex sounds can produce divergent responses in secondary metabolism depending on the structural properties of sound. Azgomi, Iranbakhsh, Majd, Ebadi, and Oraghi Ardebili (2023) compared the effects of Iranian music, rock music, and urban traffic noise on secondary metabolism in *Satureja hortensis*. PAL activity, total phenolic content, and DPPH radical scavenging capacity increased under all treatments, with the strongest increases mostly observed under Iranian music. By contrast, flavonoid levels decreased slightly under music treatments but increased by 53.97% under urban traffic noise. This divergence indicates that complex sounds should be evaluated not only as “music” or “noise”, but also in terms of the metabolic redirection they induce in different branches of the phenylpropanoid pathway.

Among defense studies based on natural biotic vibrations, Appel and Coccoft (2014) occupy a methodologically distinct position. The researchers recorded real chewing vibrations produced by *Pieris rapae* caterpillars feeding on *Arabidopsis* leaves and applied these vibrations to other *Arabidopsis* plants through piezoelectric actuators. Plants previously exposed to chewing vibrations had higher aliphatic glucosinolate levels after subsequent real herbivory. In the extended experiment, chewing vibrations, wind vibrations, leafhopper

vibrational signals, and silent control were compared; the anthocyanin response was shown to be strengthened not directly by vibration alone, but as priming against subsequent herbivory in plants previously exposed to chewing vibrations. This study is particularly important because it shows that plants can distinguish herbivory-related vibrations from other environmental vibrations and selectively prepare defense responses.

Overall, sound applications can affect ROS balance, antioxidant enzyme activities, lipid peroxidation, membrane integrity, cell wall metabolism, defense-related metabolites, and secondary metabolite profiles in plants. However, this effect is not unidirectional. Antioxidant enzyme activities and certain secondary metabolite responses increase in some applications, whereas MDA accumulation or decreases in specific phenolic classes may occur in others. The direction and magnitude of the response vary depending on the organ evaluated, time, frequency, sound level, exposure duration, and stimulus type. Therefore, the effect of sound on stress biochemistry and defense responses should not be interpreted as directly beneficial or harmful, but rather as a reprogramming of oxidative balance and defense responses that varies according to application conditions and the biological system.

Genetic and Molecular Effects of Sound in Plants

The genetic and molecular effects of sound applications in plants have been evaluated through promoter activity, gene expression, transcriptomic changes, RNA and protein accumulation, early signaling components, and molecular responses related to metabolism and defense. Studies on mechanical vibration allow sound- and vibration-induced molecular responses to be interpreted within the broader framework of mechanotransduction. Johnson, Sistrunk, Polisensky, and Braam (1998) showed that low-frequency 50 Hz vibration delivered through a speaker-platform system induced *TCH* gene expression in 14-day-old *A. thaliana* seedlings and increased hypocotyl elongation in etiolated seedlings. The persistence of these responses in the ethylene perception/signaling mutants *etr1-3* and *ein2-1* suggests that vibration-induced molecular and developmental responses at the seedling stage are not obligatorily dependent on ETR1/EIN2-mediated ethylene signaling. By contrast, Uchida and Yamamoto (2002) reported that the germination-accelerating effect of controlled sinusoidal vibration in *Arabidopsis* seeds disappeared in the ethylene-insensitive *etr1-3* mutant and in the presence of the ethylene biosynthesis inhibitor AVG. Taken together, these two studies indicate that vibration-induced responses may be shaped through ethylene-dependent or ethylene-

independent signaling pathways depending on developmental stage and the biological output evaluated.

Kim, Lee, Kim, and Jeong (2021) provide a complementary example in which sound-induced root growth was associated with molecular changes related to cell division and hormonal regulation. In *A. thaliana*, 100 Hz and 100 Hz + 9 kHz dual-frequency treatments increased the expression of the cell division-related genes *CYCB1;1* and *CYCD3;3*, together with upregulation of genes associated with auxin transport and biosynthesis and suppression of cytokinin and ethylene signaling. These findings indicate that sound-induced root responses may involve coordinated regulation of cell division and phytohormone-related molecular pathways.

Jeong et al. (2008) constructed a subtractive cDNA library to identify sound-responsive genes in rice and showed that transcripts of *ald*, *rbcS*, DNA-J-like protein, and calreticulin increased after sound application. Analyses showed that the *ald* gene responded in a frequency- and time-dependent manner; *ald* mRNA levels increased under 125 Hz, 250 Hz, and 1 kHz treatments, whereas they decreased under 50 Hz. The strongest methodological aspect of this study was the use of transgenic rice lines carrying an *ald* promoter–GUS fusion. The increase in GUS mRNA levels under 250 Hz sound and the decrease under 50 Hz in three independent transgenic lines demonstrate that sound can regulate gene expression in a frequency-sensitive manner at the promoter level, rather than only through indirect physiological responses.

The genetic effect of sound may also emerge at the level of RNA and protein synthesis without changes in DNA content. Xiujuan et al. (2003) showed that 1000 Hz and 100 dB sound application in *Chrysanthemum* seedlings did not cause a marked change in DNA content, whereas it increased RNA and soluble protein contents; both parameters reached their highest levels after 9 days of treatment. The high correlation between RNA and soluble protein content suggests that sound stimulation may increase transcriptional activity, leading to mRNA accumulation and subsequent protein synthesis. This finding indicates that the molecular effect of sound may arise mainly at regulatory steps related to gene expression and translation rather than through changes at the heritable DNA level.

One of the important studies showing that sound may induce molecular priming related to defense and pathogen responses is that of Choi et al. (2017). The researchers demonstrated that sound vibration pretreatment in *A. thaliana* affected molecular defense responses against *Botrytis cinerea* infection. While 1000 Hz sound pretreatment reduced disease severity after infection, it directly induced several genes before infection, including *TCH4*, *LTP*, *MDAR3*, and *GRX480*. After infection, stronger expression was observed for *PR1*, members of the *WRKY*

family, *PBS3*, *FMO1*, *AZ11*, *NIMIN1*, and other defense-related genes. Hormonal and transcriptional data suggest that sound pretreatment strengthened SA-dominant defense responses while maintaining the JA response at a lower and more stable level. In addition, the increase in DAMP-related *PROPEP2* and *PROPEP3* genes indicates that sound vibration may initiate early defense signaling as a mechanical stimulus perceived at the cell wall–plasma membrane interface. The fact that some fungal virulence genes were also affected is important because it suggests that sound may reshape plant–pathogen interactions bilaterally through molecular priming.

Calderón et al. (2024) examined how 100, 500, and 1000 Hz sound vibrations affected the transcriptional response of tomato to *Pseudomonas syringae* pv. *tomato* DC3000. In plants treated with 1000 Hz alone, without infection, 157 differentially expressed genes were detected on day 1, but this response decreased to only 12 specific DEGs on day 3. By contrast, when the pathogen was considered, 1065 DEGs were identified in the pathogen-only group at 1 dpi, whereas 1430 DEGs were identified in the 1000 Hz + pathogen group, with many genes specific to the sound + pathogen combination. Among these genes, defense response, cell communication, recognition processes, cell wall reinforcement, phenylpropanoid metabolism, PR proteins, proteinase inhibitors, PPO, PAL, and especially *CA3* were prominent. The upregulation of *CNGC18* under both sound treatments suggests that Ca^{2+} signaling may be part of this response. These findings indicate that sound alone can induce a transient transcriptomic response, whereas upon pathogen encounter it can more extensively reorganize defense-related gene expression.

In postharvest fruit tissue, molecular responses to sound also emerge through the regulation of genes associated with ethylene biosynthesis and ripening. Kim et al. (2015) applied a 1 kHz sound wave to harvested tomato fruit for 6 h and showed that ethylene production decreased relative to the control during storage. This reduction was accompanied by the suppression of ethylene biosynthesis and ethylene-related genes such as *ACS2*, *ACS4*, *ACO1*, *E4*, and *E8*, as well as ripening-regulatory genes such as *RIN*, *TAGL1*, *HB-1*, *NOR*, and *CNR*. These findings show that sound-associated delayed ripening was accompanied by broad suppression of ethylene biosynthesis-, signaling-, and ripening-related gene expression.

A separate study by Kim et al. (2016) examined this postharvest response at the promoter and transcription-factor level. The authors applied a 1 kHz, 80 dB sound wave for 6 h to tomato fruit at the mature green stage. After sound treatment, *ACO4* expression decreased particularly on days 12–14 of storage. The study also tested *RIN*- and *HB-1*-mediated regulation of the *ACS2*, *ACS4*, *ACO1*, *ACO3*, and *ACO4* promoters using transient expression systems

established in *Arabidopsis* protoplasts and *Nicotiana tabacum* leaves. The marked decrease in some *ACS/ACO* promoter activities under sound application in the presence of RIN indicates that sound does not merely directly suppress ethylene biosynthesis genes, but can also reorganize the ripening process through transcription factors and promoter activity. Therefore, the study by Kim et al. (2016) provides a distinct promoter-level extension of the gene-expression changes associated with sound-induced suppression of ethylene biosynthesis and ripening.

The molecular effects of sound may also differ according to the structural properties of the acoustic stimulus used. Azgomi et al. (2023) compared the effects of Iranian music, rock music, and urban traffic noise on the expression of *bZIP*, *WRKY1*, *TPS5*, and *GTS* genes in *Satureja hortensis*. *bZIP* expression increased very strongly, particularly in the urban traffic noise group; it also increased markedly under Iranian music, whereas the change under rock music was more limited. *WRKY1* decreased under all sound applications, while *TPS5* was upregulated under all acoustic treatments. *GTS* was strongly suppressed, particularly under Iranian music and rock music. The fact that music and traffic noise produced different gene expression responses even at the same reported sound level and within partially similar frequency ranges suggests that the effect of sound on gene expression may depend not only on frequency or sound level, but also on rhythmic, spectral, and temporal structure.

Tyagi, Ali, Park, and Bae (2023) proposed that sound vibration may affect genes associated with neurotransmitter-like molecules at an early stage. In *A. thaliana* seedlings, 500 Hz and 100 dB sound vibration was applied for 30 and 60 min, and the experimental response was confirmed by the induction of the positive control genes *TCH4* and *CML38*. Sound application increased transcripts of *ASMT* and *GCRI*, associated with melatonin biosynthesis, *ACT*, associated with an acetylcholine receptor, and *DOMON*, associated with dopamine biosynthesis; by contrast, suppression was reported for some genes associated with serotonin and GABA. However, the concept of neurotransmitter-like signaling in plants should not be equated with synaptic transmission in animals. Therefore, this study provides a distinctive example showing that sound may also affect some alternative signaling components beyond classical stress/defense pathways.

Overall, the genetic and molecular effects of sound applications in plants are not limited to a single gene or pathway. Promoter activity, RNA and protein accumulation, transcriptomic responses, defense priming, genes associated with ethylene biosynthesis and ripening, secondary metabolism genes, and early signaling components have been found to be sensitive to sound/vibration applications in different studies. However, these responses vary according

to the frequency, sound level, duration, and structural properties of sound, as well as plant species, developmental stage, and the organ or tissue evaluated. Current evidence indicates that sound is an environmental stimulus capable of inducing molecular reprogramming in plants. Therefore, the molecular effects of sound should be interpreted not only in terms of the presence of changes in gene expression, but also by considering the time elapsed after treatment, the biological material evaluated, and the physiological state.

Effects of Sound on Nutrient Uptake and Mineral Element Accumulation

The effects of sound applications on nutrient uptake and mineral element accumulation have been evaluated through macro- and microelement contents, leaf mineral levels, nutritional responses occurring together with fertilizer applications, and the relationship between mineral nutrition and growth/yield outputs. These studies show that mineral responses may vary depending on plant species, frequency, sound level, and whether the application is performed alone or together with additional inputs such as fertilizer.

Hou and Mooneyham (1999) examined yield and quality responses in spinach and lettuce under Agri-wave technology, which included special-frequency sound waves and micronutrient fertilizer. In spinach, mineral analyses were performed between the Agri-wave treatment and the control group; 29 of 33 elements increased in the Agri-wave group, with particularly higher K, P, Ca, Mg, Fe, and Se contents, whereas Na, Co, V, and Zn levels decreased. However, because sound alone and fertilizer alone groups were not separately reported for mineral element results in spinach, the element increases should be interpreted not as the effect of sound alone, but as the result of the Agri-wave application containing sound + micronutrient fertilizer.

Controlled frequency and PAFT applications show that mineral responses can change selectively according to frequency, element type, and sound level. Ozkurt and Altuntas (2016) reported that 600, 1240, and 1600 Hz applications at a constant sound level of 90 dB produced different effects on leaf mineral contents in *Sansevieria trifasciata*. N, P, and K contents increased relative to the control at 600 Hz, P and K decreased markedly at 1240 Hz, and they rose above the control again at 1600 Hz. By contrast, the highest values for Ca, Mg, and Zn were obtained at 1240 Hz, whereas Fe content remained below the control under all frequency treatments. Zakariya, Rivai, and Aini (2017) reported that a 2000 Hz square-wave PAFT application at 80–100 dB increased leaf N and K contents in *Brassica rapa* var. *parachinensis*; the authors related this increase to enhanced stomatal aperture and facilitated foliar nutrient

entry. This interpretation indicates that mineral uptake should be evaluated not only in terms of root-level absorption, but also together with stomatal behavior, transpiration, and transport processes associated with the leaf surface. By contrast, Altuntas and Ozkurt (2020) applied sound to *Mentha piperita* plants at 1000 Hz and 95–105 dB and showed that N, K, and Fe contents were higher in the control group, whereas sound applications generally reduced these elements. Although higher values in favor of the control were also observed for P, Mg, and Zn, the differences were not pronounced. These findings show that sound does not alter mineral element accumulation in the same direction for all elements; the response should be evaluated separately in terms of frequency, sound level, element type, and possible transport pathway.

Overall, the available findings indicate that sound applications can affect mineral element contents and growth/yield outputs related to nutrition; however, it cannot be clearly distinguished in every study whether this effect arises from direct mineral uptake, transport processes associated with stomatal aperture and transpiration, or the contribution of simultaneous fertilizer application. Mineral responses are element-specific; the same application may increase macroelements such as N, P, or K while suppressing microelements such as Fe. Therefore, the role of sound in nutrient uptake should be investigated by jointly evaluating controlled nutrient media, element-specific analyses, mineral accumulation at root, stem, and leaf levels, gene expression related to transporter proteins, and physiological indicators associated with transpiration.

Effects of Sound on Plant Anatomy and Tissue-Level Traits

The effects of sound on plant anatomy and tissue-level traits have been addressed only to a very limited extent in the literature. Among the available studies, Kim et al. (2021) focused on the effects of sound on cellular/developmental indicators such as root apical meristem length and the number of meristematic cells, whereas Azgomi et al. (2023) examined the anatomical and histological effects of different acoustic stimuli. Therefore, the evaluation in this section is based on a narrower body of evidence compared with the physiological and biochemical topics discussed above.

Apart from classical histological tissue analyses, one of the important findings on the structural effects of sound at the cellular/developmental level was reported by Kim et al. (2021) in the root system of *Arabidopsis thaliana*. The authors reported that 100 Hz and 100 Hz + 9 kHz sound applications increased not only primary root length, but also root tip length, root apical meristem length, and the number of cells in the meristematic zone. These findings

suggest that sound application may affect not only root elongation at the organ level, but also structural indicators associated with cell division and meristematic zone organization in the root apical meristem. However, because the study did not comprehensively evaluate the anatomical characteristics of differentiated tissues such as xylem, phloem, cortex, or epidermis, it should be considered not as direct evidence for the effects of sound on classical anatomical tissue structures, but as cellular/developmental evidence at the level of the meristematic growth zone.

Regarding stomatal traits, Jusoh et al. (2023) showed that sound applications (350–380 Hz; 60–78 dB) may affect stomatal pore length and pore area in rice (*Oryza sativa* L.) leaves. These findings indicate that the leaf-level effects of sound are not limited to gas exchange and photosynthetic performance, but may also be reflected in morphological traits associated with stomatal aperture.

Azgomi et al. (2023) compared the anatomical and histological effects of rhythmic-harmonic Iranian music, rhythmic but non-harmonic rock music, and non-harmonic/non-rhythmic urban traffic noise in *Satureja hortensis* seedlings. Iranian music was applied at approximately 800–2000 Hz, rock music at 1100–7000 Hz, and urban traffic noise at approximately 800–2000 Hz, all at 80 dB for 1 h three times daily over 2 weeks. Microscopic observations indicated that shoot apex development was more pronounced, procambium formation was expanded, and axillary buds at the basal part of the shoot apex became more conspicuous, particularly under Iranian music and rock music. In stem cross-sections, an increase in diameter was observed in sound-treated groups, and this change was associated with an increase in pith parenchyma. At the level of vascular tissue, especially the xylem region in the stem showed more pronounced differentiation under Iranian music and rock music, whereas xylem differentiation remained weaker in seedlings exposed to urban traffic noise. In root cross-sections, increased xylem differentiation and cell size were observed under rock music and urban traffic noise; however, a reduction in root diameter and irregularity in metaxylem organization were detected in the urban traffic noise group. In addition, morphological abnormalities such as leaf deformation and altered phyllotaxis were reported in seedlings exposed to urban traffic noise. These findings suggest that complex acoustic stimuli may affect not only growth and metabolic responses, but also structural traits such as shoot apex organization, vascular tissue prominence, root xylem organization, leaf deformation, and altered phyllotaxis.

The changes in cell wall polysaccharides, lignin, and wall-bound phenolic acids reported by Sardari et al. (2024) may also provide a mechanistic intermediate step for possible tissue-

level effects of sound. However, because that study was conducted in suspension-cultured cells, its findings should not be interpreted as direct evidence for root, stem, or leaf anatomy.

Nevertheless, a substantial part of the anatomical interpretations in the study by Azgomi et al. (2023) is based on qualitative observations. Expressions such as “more pronounced differentiation,” “more conspicuous shoot apex development,” or “weaker xylem differentiation” indicate possible tissue-level effects of sound, but are not sufficiently supported by quantitative anatomical parameters such as xylem area, phloem area, tracheary element diameter, metaxylem number, cortex/epidermis thickness, or vascular bundle measurements. The authors’ explanations based on auxin, lignin, and flavonoid metabolism are also not confirmed directly by lignin staining, lignin quantification, auxin distribution, or markers of vascular differentiation. Therefore, although the available data indicate that sound may induce anatomical and tissue-level effects in plants, the evidence in this area remains limited. To reliably determine the effects of sound on plant anatomy, future studies should include quantitative, organ-based, and comparable anatomical analyses covering epidermis, cortex, mesophyll, xylem, phloem, tracheary elements, stomatal traits, cell dimensions, and vascular bundle characteristics.

Methodological Limitations and Evaluation of Experimental Setups

The interpretation of biochemical, molecular, nutritional, and structural responses to acoustic stimulation is complicated by the physical heterogeneity of the stimuli grouped under the general term “sound.” Studies in this field have employed pure or single-frequency tones, sinusoidal and mechanically transmitted vibrations, PAFT systems, music, white noise, traffic noise, rain-related acoustic stimuli, bee sounds, and herbivore chewing vibrations. These exposures differ in spectral organization, temporal structure, transmission pathway, and the mechanical effects they may generate in plant tissues. Consequently, similar changes in ROS accumulation, gene expression, mineral content, or tissue-level traits cannot automatically be assumed to arise from equivalent acoustic exposure (Appel & Cocroft, 2014; Azgomi et al., 2023; Chowdhury et al., 2014; Hassanien et al., 2014; Veits et al., 2019).

A major source of uncertainty is that frequency and sound level alone do not fully characterize the stimulus received by the plant. For complex sounds, information on dominant frequencies, frequency bands, power spectral density, waveform, amplitude variation over time, modulation, and spectral composition is often incomplete. The interpretation of reported dB values is further limited when the weighting procedure, measurement distance, or sound level

at the plant surface is not specified. The spatial distribution of the acoustic field can also be altered by speaker placement, background noise, insulation, reverberation, standing waves, reflections within the exposure chamber, and the presence or absence of sound-absorbing surfaces. Thus, nominally identical frequency and dB values do not necessarily indicate equivalent mechanical/acoustic exposure between experiments (Erbe et al., 2022; Kinsler et al., 2000; Makris & Navarro, 2026; Robin & Plante, 2022).

The physical pathway through which the stimulus reaches the plant should likewise be distinguished from the nominal acoustic treatment. Airborne sound pressure, vibration transmitted through a platform or substrate, acoustic propagation through water, and direct mechanical excitation of plant tissue represent different exposure conditions. Mechanical energy produced by a loudspeaker may also reach the plant indirectly through shelves, pots, culture media, or supporting structures. In experiments based on controlled vibration, displacement, acceleration, amplitude, or particle motion may therefore be more informative than sound pressure level alone. Sound pressure level should consequently not be treated as a direct measure of the mechanical displacement or acceleration experienced by plant tissues (Erbe et al., 2022; Kinsler et al., 2000; Makris & Navarro, 2026; Uchida & Yamamoto, 2002).

Experimental hardware introduces an additional layer of potential confounding. Loudspeakers, amplifiers, PAFT devices, ultrasonic sources, and piezoelectric actuators can generate effects other than the intended acoustic stimulus, including heat, air movement, electromagnetic fields, structural vibration, and device-generated noise. A silent untreated group may therefore be insufficient to isolate the acoustic component of the treatment. Depending on the experimental system, stronger controls may require sham operation of the device without acoustic delivery to the plant, isolation of actuator- or speaker-derived mechanical vibration, background-noise controls, and monitoring of temperature, humidity, and air movement. The controlled transmission approach used by Appel and Cocroft (2014) illustrates the value of separating the intended vibrational input from other physical effects, whereas such separation is less clearly documented in several speaker- or PAFT-based studies (Appel & Cocroft, 2014; Meng, Zhou, Zheng, & Gao, 2012; Qi, Teng, Hou, Zhu, & Liu, 2010; Zakariya et al., 2017).

Interpretation is further affected by the biological material and the timing of measurement. Sound-related responses have been examined in seedlings, differentiated vegetative tissues, callus and suspension cultures, postharvest fruit, and pathogen-challenged plants, each of which represents a different physiological state. Moreover, biochemical and molecular responses can change rapidly after treatment and may not persist in the same

direction over time. Time-dependent patterns reported for antioxidant responses, ROS-related signaling, and transcriptional reprogramming show that measurements taken at a single time point may capture only one phase of a dynamic response (Ali, Park, Tyagi, & Bae, 2026; Calderón et al., 2024; Li et al., 2008). Exposure duration, measurement time, developmental stage, organ or tissue type, and the presence of an additional biological challenge should therefore be considered when comparing results across studies.

The measured endpoint also determines how strongly a biological interpretation can be made. Increased antioxidant enzyme activity may reflect activation of protective capacity, but it may occur simultaneously with increased oxidative load or lipid peroxidation and therefore should not be interpreted automatically as a beneficial response. Likewise, changes in transcript abundance do not by themselves establish corresponding changes in protein abundance, metabolite concentration, or stable phenotype. In structural studies, qualitative observations of stronger or weaker differentiation provide useful preliminary evidence, but conclusions about anatomical organization remain limited without quantitative measurements of traits such as xylem or phloem dimensions, cortex and epidermis characteristics, tracheary elements, vascular bundles, or stomatal parameters. The interpretation of studies such as Ghosh et al. (2016), Kim et al. (2021), and Azgomi et al. (2023) therefore depends not only on the direction of the reported response, but also on how directly the measured variable represents the biological process being inferred.

These methodological issues do not negate the evidence that acoustic and vibrational stimuli can modify plant biochemistry, molecular regulation, mineral-related responses, and tissue-level traits. They instead define the limits within which such findings can be compared and interpreted. Reliable evaluation requires the physical stimulus, transmission pathway, exposure field, device-related controls, biological material, measurement timing, and evidential strength of the selected endpoint to be considered together. Without this information, differences among studies may reflect not only biological variation, but also differences in the acoustic and experimental conditions under which the responses were generated.

Research Gaps and Future Perspectives

Current evidence indicates that acoustic and vibrational stimuli can alter stress biochemistry, defense responses, secondary metabolism, gene expression, mineral nutrition, and tissue-level traits in plants. However, the physical components responsible for these

responses and the causal sequence linking sound-induced mechanical signaling to downstream biochemical, molecular, nutritional, and structural outcomes remain incompletely resolved.

The dissection of natural and complex sounds remains a priority research area. Bee sounds, insect sounds, rain-related acoustic stimuli, flowing water sounds, herbivore chewing vibrations, traffic noise, and music contain spectral and temporal structures that cannot be represented adequately by a single nominal frequency. Future experiments should therefore compare whole recordings with dominant frequencies, filtered frequency bands, and synthetic pure tones while evaluating whether different acoustic components produce distinct biochemical, defense, molecular, or structural responses (Appel & Cocroft, 2014; Azgomi et al., 2023; Makris & Navarro, 2026; Veits et al., 2019). Such designs would help distinguish responses associated with specific frequency components from those arising from the broader temporal and spectral organization of complex stimuli.

A major unresolved issue concerns the sequence by which mechanically initiated signaling is translated into downstream biological responses. Sound treatments have been associated with changes in Ca^{2+} and ROS signaling, hormone-related pathways, gene expression, energy metabolism, and antioxidant systems, but these events are rarely resolved within the same time course. It therefore remains unclear when ROS functions primarily as an early signaling component, when it reflects oxidative load, and when it contributes to subsequent defense adjustment; similarly, changes in gene expression cannot yet be assumed to lead directly to stable biochemical or phenotypic outcomes. Multi-level experimental designs combining time-series measurements, mutant and inhibitor approaches, omics analyses, and direct biochemical and physiological measurements are required to establish causal relationships among these response levels (Ali et al., 2026; de Melo, 2023; Demey et al., 2023; Ghosh et al., 2016).

Anatomical and tissue-level responses remain among the least studied aspects of plant responses to acoustic stimulation. The available evidence suggesting effects on root apical meristem organization, shoot apex development, vascular tissue differentiation, root xylem organization, stomatal traits, leaf deformation, and phyllotaxis is derived from only a small number of studies. Future work should quantitatively and comparably assess epidermis, cortex, mesophyll, xylem, phloem, tracheary element diameter, vascular bundle area, stomatal density and index, epidermal cell size, and organ-specific root, stem, and leaf anatomy. In addition, stomatal morphology, cell wall composition, membrane integrity, and classical organ anatomy have rarely been examined together. Consequently, the relationships among epidermal or stomatal responses, cell wall remodeling, meristematic activity, and broader root–stem–leaf

structural changes remain unresolved (Azgomi et al., 2023; Jusoh et al., 2023; Kim et al., 2021; Sardari et al., 2024).

Mineral nutrition represents another major research gap. Current studies do not clearly establish the relative contributions of root uptake, root-to-shoot transport, stomatal behavior, transpiration, and simultaneous fertilizer application to changes in tissue mineral concentrations. Controlled nutrient-solution experiments are needed in which mineral accumulation is measured separately in roots, stems, and leaves and evaluated together with transporter-related gene expression and physiological indicators such as transpiration and stomatal conductance (Altuntas & Ozkurt, 2020; Hou & Mooneyham, 1999; Ozkurt & Altuntas, 2016; Zakariya et al., 2017). Such designs would help distinguish genuine changes in uptake from changes in transport, redistribution, or concentration within specific tissues.

The thresholds separating adaptive or potentially beneficial responses from stress-related effects also remain poorly defined for biochemical, metabolic, and structural endpoints. In some studies, antioxidant capacity or particular secondary metabolites increase under moderate acoustic exposure, whereas higher sound levels or longer treatments are accompanied by increased lipid peroxidation, reduced viability, biomass loss, or unfavorable mineral responses. Thus, apparently positive biochemical changes should not automatically be interpreted as evidence of an overall beneficial effect. Future studies should systematically vary frequency, sound level, and exposure duration and should include short- and long-term measurements, recovery periods, and reversibility analyses to identify conditions under which adaptive responses shift toward oxidative or metabolic stress (Altuntas & Ozkurt, 2020; Heidari et al., 2020; Ozkurt & Altuntas, 2018; Sardari et al., 2024).

The transferability of biochemical, molecular, nutritional, and structural responses observed under controlled laboratory conditions to greenhouse and field environments also remains uncertain. Future studies should determine whether molecular or metabolic responses detected under precisely controlled acoustic exposure persist under variable environmental conditions and whether they translate into agronomically meaningful outcomes such as stress tolerance, disease resistance, nutritional status, or product quality. Experiments linking controlled laboratory systems with semi-controlled greenhouse and field conditions will therefore be essential for distinguishing reproducible biological effects from responses that depend strongly on the experimental environment (Hou & Mooneyham, 1999; Minh & Bui, 2026).

Overall, future research should move beyond demonstrating that acoustic stimulation can alter individual biochemical or molecular indicators and instead determine how defined

acoustic exposures are connected to coordinated downstream responses across multiple biological levels. This will require rigorous acoustic characterization, dissection of complex sounds, time-resolved causal experiments, quantitative anatomical analyses, controlled mineral-nutrition systems, and validation under greenhouse and field conditions. Such an approach would provide a stronger basis for determining when acoustic stimulation produces adaptive, stress-related, or structurally meaningful responses in plants.

Conclusion

The evidence reviewed in this chapter shows that acoustic and vibrational stimuli can modify multiple downstream biological processes in plants, including oxidative balance, antioxidant responses, defense-related metabolism, secondary metabolite profiles, gene expression, mineral element accumulation, and structural or tissue-level traits. However, the strength of evidence is uneven among these response categories. Molecular and biochemical responses have been documented more extensively, whereas mineral-nutritional and quantitative anatomical effects remain comparatively less resolved. Taken together, current findings support the view that acoustic stimulation can produce measurable biological responses beyond growth-related effects, although their interpretation depends strongly on the physical characteristics of the stimulus and the experimental system used (Chowdhury et al., 2014; Demey et al., 2023; Ghosh et al., 2016; Hassanien et al., 2014).

A central feature of the available evidence is its conditionality. Increased antioxidant activity, accumulation of particular secondary metabolites, or induction of defense-related genes does not necessarily indicate an overall beneficial effect, because such responses may occur together with lipid peroxidation, reduced viability, altered growth, or other indicators of stress. Likewise, music, environmental noise, controlled tones, and biologically generated vibrations cannot be treated as equivalent stimuli simply because all are described as “sound.” Responses may change with frequency, sound level, exposure duration, spectral and temporal structure, organ or tissue type, and developmental or physiological state (Appel & Cocroft, 2014; Azgomi et al., 2023; Calderón et al., 2024; Heidari et al., 2020; Kafash, Khoramnejadian, Ghotbi-Ravandi, & Dehghan, 2022; Sardari et al., 2024).

The major unresolved question is therefore not only whether plants respond to acoustic stimulation, but how the physical vibration regime generated in plant tissues is translated into specific downstream biochemical, molecular, nutritional, and structural outcomes. Ca^{2+} and ROS signaling, hormone-related pathways, transcriptional regulation, energy metabolism,

membrane and cell wall responses, and defense networks provide plausible interacting components, but their temporal and causal relationships remain incompletely established (Ali et al., 2026; de Melo, 2023; Demey et al., 2023; Ghosh et al., 2016; Mishra, Ghosh, & Bae, 2016; Son et al., 2024). Establishing these relationships will require experiments that connect precisely characterized acoustic exposure with time-resolved molecular and biochemical measurements, quantitative anatomical analyses, and controlled assessments of mineral uptake and transport.

Accordingly, future progress in this field will depend on moving beyond demonstrations of isolated sound-responsive indicators toward experimental designs that determine which acoustic conditions generate which downstream responses, through which biological pathways, and with what degree of reproducibility. Rigorous acoustic characterization, appropriate sham and physical controls, multi-level response measurements, quantitative tissue analysis, controlled nutrient systems, and validation under greenhouse and field conditions will be essential for distinguishing reproducible acoustic effects from responses that arise from experimental or environmental confounding. Such evidence is necessary before controlled acoustic stimulation can be evaluated reliably for biological or agricultural applications.

References

- Ali, S., Park, S., Tyagi, A., & Bae, H. (2026). Decoding the interplay of ROS and hormonal reprogramming in *Arabidopsis* after sound vibration treatments. *Physiologia Plantarum*, 178(1), e70800. <https://doi.org/10.1111/ppl.70800>
- Ali, S., Tyagi, A., Park, S., & Bae, H. (2024). Understanding the mechanobiology of phytoacoustics through molecular lens: mechanisms and future perspectives. *Journal of Advanced Research*, 65, 47–72. <https://doi.org/10.1016/j.jare.2023.12.011>
- Altuntas, O., & Ozkurt, H. (2020). Effects of different dB sound levels on plant growth, nutrient elements uptake and essential oil yield of *Mentha piperita*. *Journal of Essential Oil Bearing Plants*, 23(6), 1345–1355. <https://doi.org/10.1080/0972060X.2020.1842254>
- Appel, H. M., & Cocroft, R. B. (2014). Plants respond to leaf vibrations caused by insect herbivore chewing. *Oecologia*, 175(4), 1257–1266. <https://doi.org/10.1007/s00442-014-2995-6>
- Azgomi, S., Iranbakhsh, A., Majd, A., Ebadi, M., & Oraghi Ardebili, Z. (2023). The importance of sound rhythm: music and noise elicit different biological responses in *Satureja hortensis* L. *Theoretical and Experimental Plant Physiology*, 35(3), 215–232. <https://doi.org/10.1007/s40626-023-00277-1>
- Calderón, A. A., Almagro, L., Martínez-Calderón, A., & Ferrer, M. A. (2024). Transcriptional reprogramming in sound-treated Micro-Tom plants inoculated with *Pseudomonas syringae* pv. tomato DC3000. *Physiologia Plantarum*, 176(3), e14335. <https://doi.org/10.1111/ppl.14335>
- Choi, B., Ghosh, R., Gururani, M. A., Shanmugam, G., Jeon, J., Kim, J., ... Bae, H. (2017). Positive regulatory role of sound vibration treatment in *Arabidopsis thaliana* against *Botrytis cinerea* infection. *Scientific Reports*, 7, 2527. <https://doi.org/10.1038/s41598-017-02556-9>
- Chowdhury, M. E. K., Lim, H. S., & Bae, H. (2014). Update on the effects of sound wave on plants. *Research in Plant Disease*, 20(1), 1–7. <https://doi.org/10.5423/RPD.2014.20.1.001>
- Collins, M. E., & Foreman, J. E. K. (2001). The effect of sound on the growth of plants. *Canadian Acoustics*, 29(2), 3–8.
- de Melo, H. C. (2023). Plants detect and respond to sounds. *Planta*, 257(3), 55. <https://doi.org/10.1007/s00425-023-04088-1>
- Demey, M. L., Mishra, R. C., & Van Der Straeten, D. (2023). Sound perception in plants: from ecological significance to molecular understanding. *Trends in Plant Science*, 28(7), 825–840. <https://doi.org/10.1016/j.tplants.2023.03.003>
- Erbe, C., Duncan, A., Hawkins, L., Terhune, J. M., & Thomas, J. A. (2022). Introduction to acoustic terminology and signal processing. In C. Erbe & J. A. Thomas (Eds.), *Exploring animal behavior through sound: Vol. 1. Methods* (pp. 111–152). Cham: Springer. https://doi.org/10.1007/978-3-030-97540-1_4
- Ghosh, R., Mishra, R. C., Choi, B., Kwon, Y. S., Bae, D. W., Park, S. C., ... Bae, H. (2016). Exposure to sound vibrations lead to transcriptomic, proteomic and hormonal changes in *Arabidopsis*. *Scientific Reports*, 6, 33370. <https://doi.org/10.1038/srep33370>
- Hassanien, R. H. E., Hou, T. Z., Li, Y. F., & Li, B. M. (2014). Advances in effects of sound waves on plants. *Journal of Integrative Agriculture*, 13(2), 335–348. [https://doi.org/10.1016/S2095-3119\(13\)60492-X](https://doi.org/10.1016/S2095-3119(13)60492-X)
- Heidari, S., Kafi, M., Kalatejari, S., Shafaghatian, M., & Mollakarimi, N. (2020). Effects of sound stimulation on physiological and biochemical responses of *Salvia splendens*. *Journal of Ornamental Plants*, 10, 59–68.
- Hou, T. Z., & Mooneyham, R. E. (1999). Applied studies of the plant meridian system II: Agri-wave technology increases the yield and quality of spinach and lettuce and enhances the disease-

- resistant properties of spinach. *The American Journal of Chinese Medicine*, 27(2), 131–141. <https://doi.org/10.1142/S0192415X99000173>
- Jeong, M. J., Shim, C. K., Lee, J. O., Kwon, H. B., Kim, Y. H., Lee, S. K., ... Park, S. C. (2008). Plant gene responses to frequency-specific sound signals. *Molecular Breeding*, 21(2), 217–226. <https://doi.org/10.1007/s11032-007-9122-x>
- Johnson, K. A., Sistrunk, M. L., Polisensky, D. H., & Braam, J. (1998). *Arabidopsis thaliana* responses to mechanical stimulation do not require ETR1 or EIN2. *Plant Physiology*, 116(2), 643–649. <https://doi.org/10.1104/pp.116.2.643>
- Jusoh, M., Ramlee, S. I., Pydi, F. N. I., Mazlan, N. A., Berahim, Z., Mujab, A. A. M., ... Yaapar, M. N. (2023). Specific sound frequency improves intrinsic water efficiency in rice leaf by imparting changes in stomatal dimensions. *Pertanika Journal of Tropical Agricultural Science*, 46(2), 439–457. <https://doi.org/10.47836/pjtas.46.2.05>
- Kafash, Z. H., Khoramnejadian, S., Ghotbi-Ravandi, A. A., & Dehghan, S. F. (2022). Traffic noise induces oxidative stress and phytohormone imbalance in two urban plant species. *Basic and Applied Ecology*, 60, 1–12. <https://doi.org/10.1016/j.baae.2022.01.010>
- Kim, J. Y., Ahn, H. R., Kim, S. T., Min, C. W., Lee, S. I., Kim, J. A., ... Jeong, M. J. (2016). Sound wave affects the expression of ethylene biosynthesis-related genes through control of transcription factors RIN and HB-1. *Plant Biotechnology Reports*, 10(6), 437–445. <https://doi.org/10.1007/s11816-016-0419-2>
- Kim, J. Y., Lee, H. J., Kim, J. A., & Jeong, M. J. (2021). Sound waves promote *Arabidopsis thaliana* root growth by regulating root phytohormone content. *International Journal of Molecular Sciences*, 22(11), 5739. <https://doi.org/10.3390/ijms22115739>
- Kim, J. Y., Lee, J. S., Kwon, T. R., Lee, S. I., Kim, J. A., Lee, G. M., ... Bae, H. (2015). Sound waves delay tomato fruit ripening by negatively regulating ethylene biosynthesis and signaling genes. *Postharvest Biology and Technology*, 110, 43–50. <https://doi.org/10.1016/j.postharvbio.2015.07.015>
- Kinsler, L. E., Frey, A. R., Coppers, A. B., & Sanders, J. V. (2000). *Fundamentals of acoustics* (4th ed.). New York: Wiley.
- Li, B., Wei, J., Wei, X., Tang, K., Liang, Y., Shu, K., & Wang, B. (2008). Effect of sound wave stress on antioxidant enzyme activities and lipid peroxidation of *Dendrobium candidum*. *Colloids and Surfaces B: Biointerfaces*, 63(2), 269–275. <https://doi.org/10.1016/j.colsurfb.2007.12.012>
- Makris, N. C., & Navarro, C. (2026). Seeds accelerate germination at beneficial planting depths by sensing the sound of rain. *Scientific Reports*, 16, 11248. <https://doi.org/10.1038/s41598-026-44444-1>
- Meng, Q., Zhou, Q., Zheng, S., & Gao, Y. (2012). Responses on photosynthesis and variable chlorophyll fluorescence of *Fragaria ananassa* under sound wave. *Energy Procedia*, 16, 346–352. <https://doi.org/10.1016/j.egypro.2012.01.057>
- Minh, T. T., & Bui, D. N. (2026). Preharvest sound waves enhance yield and nutritional quality of greenhouse vegetables in a frequency-dependent manner. *Russian Journal of Plant Physiology*, 73(2), 88. <https://doi.org/10.1134/S1021443725607608>
- Mishra, R. C., Ghosh, R., & Bae, H. (2016). Plant acoustics: in the search of a sound mechanism for sound signaling in plants. *Journal of Experimental Botany*, 67(15), 4483–4494. <https://doi.org/10.1093/jxb/erw235>
- Ozkurt, H., & Altuntas, O. (2016). The effect of sound waves at different frequencies upon the plant element nutritional uptake of snake plant (*Sansevieria trifasciata*) plants. *Indian Journal of Science and Technology*, 9(48), 1–5. <https://doi.org/10.17485/ijst/2016/v9i48/54716>

- Ozkurt, H., & Altuntas, O. (2018). Quality parameter levels of strawberry fruit in response to different sound waves at 1000 Hz with different dB values (95, 100, 105 dB). *Agronomy*, 8(7), 127. <https://doi.org/10.3390/agronomy8070127>
- Pagano, M., & Del Prete, S. (2024). Symphonies of growth: unveiling the impact of sound waves on plant physiology and productivity. *Biology*, 13(5), 326. <https://doi.org/10.3390/biology13050326>
- Qi, L., Teng, G., Hou, T., Zhu, B., & Liu, X. (2010). Influence of sound wave stimulation on the growth of strawberry in sunlight greenhouse. In *International Conference on Computer and Computing Technologies in Agriculture* (pp. 449–454). Berlin, Heidelberg: Springer. https://doi.org/10.1007/978-3-642-12220-0_65
- Robin, O., & Plante, C. (2022). An illustrated tutorial for logarithmic scales and decibels in acoustics. *The Journal of the Acoustical Society of America*, 152(5), 2880–2892. <https://doi.org/10.1121/10.0015144>
- Sardari, M., Ghanati, F., Mobasheri, H., & Hajnorouzi, A. (2024). Sound waves alter the viability of tobacco cells via changes in cytosolic calcium, membrane integrity, and cell wall composition. *PLOS ONE*, 19(3), e0299055. <https://doi.org/10.1371/journal.pone.0299055>
- Son, J. S., Jang, S., Mathevon, N., & Ryu, C. M. (2024). Is plant acoustic communication fact or fiction? *New Phytologist*, 242(5), 1876–1880. <https://doi.org/10.1111/nph.19648>
- Sparke, M. A., & Wünsche, J. N. (2020). Mechanosensing of plants. *Horticultural Reviews*, 47, 43–83. <https://doi.org/10.1002/9781119625407.ch2>
- Tyagi, A., Ali, S., Park, S., & Bae, H. (2023). Assessing the effect of sound vibrations on plant neurotransmitters in *Arabidopsis*. *Journal of Plant Growth Regulation*, 42(8), 5216–5223. <https://doi.org/10.1007/s00344-023-10918-z>
- Uchida, A., & Yamamoto, K. T. (2002). Effects of mechanical vibration on seed germination of *Arabidopsis thaliana* (L.) Heynh. *Plant and Cell Physiology*, 43(6), 647–651. <https://doi.org/10.1093/pcp/pcf079>
- Veits, M., Khait, I., Obolski, U., Zinger, E., Boonman, A., Goldshtein, A., ... Hadany, L. (2019). Flowers respond to pollinator sound within minutes by increasing nectar sugar concentration. *Ecology Letters*, 22(9), 1483–1492. <https://doi.org/10.1111/ele.13331>
- Xiujuan, W., Bochu, W., Yi, J., Defang, L., Chuanren, D., Xiaocheng, Y., & Sakanishi, A. (2003). Effects of sound stimulation on protective enzyme activities and peroxidase isoenzymes of chrysanthemum. *Colloids and Surfaces B: Biointerfaces*, 27(1), 59–63. [https://doi.org/10.1016/S0927-7765\(02\)00038-3](https://doi.org/10.1016/S0927-7765(02)00038-3)
- Zakariya, F. H., Rivai, M., & Aini, N. (2017). Effect of automatic plant acoustic frequency technology (PAFT) on mustard pakcoy (*Brassica rapa* var. *parachinensis*) plant using temperature and humidity parameters. In *2017 International Seminar on Intelligent Technology and Its Application (ISITIA)* (pp. 334–339). Surabaya, Indonesia. <https://doi.org/10.1109/ISITIA.2017.8124105>