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Unraveling the Anti-Inflammatory Mechanisms of Hinokitiol: Insights into Signalling Molecules and Cellular Pathways

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Abstract:
Hinokitiol, derived from *Chamaecyparis taiwanensis* wood, has gained attention for its broad therapeutic applications, particularly its anti-inflammatory effects. Traditionally known for antimicrobial properties in Japan, hinokitiol inhibits the NF-κB pathway, preventing IκBα degradation, thereby reducing the transcription of proinflammatory cytokines such as TNF-α, IL-6, and IL-1β. It also downregulates COX-2 expression. In the TLR4 signaling, hinokitiol inhibits LPS binding to TLR4, reducing downstream cytokine production. Additionally, hinokitiol modulates the Wnt/β-catenin pathway by preventing β-catenin nuclear translocation and attenuating inflammation. Hinokitiol's antioxidant properties are crucial in mitigating oxidative stress and inflammation, especially in gentamicin-induced nephrotoxicity. By scavenging ROS and modulating inflammatory pathways, hinokitiol protects renal tubular cells from oxidative damage. Moreover, it activates the Keap1/Nrf2/HO-1 pathway, enhancing cellular defenses against oxidative stress and ferroptosis, particularly in neuronal cells with post-traumatic brain injury. Hinokitiol inhibits melanogenesis by targeting the AKT/mTOR signaling pathway, reducing the expression of MITF and tyrosinase, key regulators of melanin synthesis. This inhibition is accompanied by increased autophagy, contributing to its anti-melanogenic effects. The multifaceted actions of hinokitiol across various signaling pathways highlight its therapeutic potential in managing a wide range of inflammatory and oxidative stress-related conditions. Further research is needed to fully elucidate hinokitiol's mechanisms and explore its clinical applications in inflammation, oxidative stress, and related disorders.
Keywords: Hinokitiol, Anti-inflammatory, Oxidative stress, NF-κB pathway, Melanogenesis

Introduction:

Researchers have shown an emergent interest in plant-based medicines for innumerable reasons, including their potential therapeutic benefits, cultural significance, and sustainable nature [1]. Historically, various healing systems, such as Ayurveda, Traditional Chinese Medicine, and Indigenous practices, have long relied on plant-based cures [1, 2]. Researchers are exploring these traditional knowledge systems to identify bioactive compounds with medicinal properties. Amongst several emerging potential bioactive compounds Hinokitiol/Thujaplicin gains keen attention due to its broad array of therapeutic applications. Hinokitiol has traditionally been used in Japan, known as "hinoki oil" or "hinoki essence." The wood of the Hinoki cypress tree has been used for centuries in construction, and its essential oil, which contains hinokitiol, has been valued for its pleasant fragrance and potential health benefits [3, 4, 5]. Japanese researchers first isolated it from the wood of *Chamaecyparis taiwanensis* in the 1930s. It gained recognition for its antimicrobial properties as significant antibacterial, antifungal, and antiviral activities and is widely utilized for various applications in healthcare and cosmetic products [5]. In addition, few research studies demonstrated the significant effect of hinokitiol against inflammation and oxidative stress. Leads to hinokitiol as a subject of interest in capacities such as dermatology and medicine. It has gained recognition globally and is commonly used commercially in various products, including skincare formulations, shampoos, soaps, oral care items, cosmetics, etc. Hinokitiol has been screened for various pharmacological activities viz. Anti-bacterial, Anti-fungal, Anti-

viral, Anti-cancer, Anti-inflammatory, Anti-oxidant, Anti-diabetic, anti-allergic, wound healing and neuroprotective, etc [6, 7]. Besides therapeutic potential hinokitiol is immensely valuable for plant survival and overall development. Hinokitiol helps plants defend against various fungal and bacterial pathogens. It inhibits the growth of fungi/bacteria and can be particularly effective against wood rotting. It also neutralizes reactive oxygen species (ROS) within plant cells and protects against oxidative stress induced by environmental factors such as UV radiation, pollutants, and other stressors [8].

Multifaceted Anti-inflammatory Signalling Mechanisms of Hinokitiol

1. Inhibition of NF-κB Pathway and Proinflammatory Cytokines.
2. Inhibition of TLR4 pathway activation and downstream signaling to mitigate inflammation.
3. Inhibition of Wnt/β-Catenin Signalling.
4. Inhibition of ROS and MAPK Signalling Pathways.
5. Activation of the Keap1/Nrf2/HO-1 Pathway to Inhibit Neuronal Ferroptosis.
6. Inhibition of Melanogenesis via AKT/mTOR Signaling.

Inhibition of NF-κB Pathway and Proinflammatory Cytokines:

Inflammatory cytokines such as TNF-α, IL-6, and IL-1β play a crucial role in mediating inflammation and are significantly elevated in acute pancreatitis, contributing to the recruitment of immune cells, increased vascular permeability, and tissue damage [1, 2, 9]. These cytokines are regulated by the NF-κB pathway, which is activated in response to various inflammatory stimuli. Hinokitiol exerts its anti-inflammatory effects primarily by inhibiting key inflammatory pathways, which can be particularly beneficial in conditions like acute pancreatitis [3]. It inhibits the activation of the NF-κB pathway, a crucial regulator of the immune response that leads to the transcription of various proinflammatory cytokines such as TNF-α, IL-6, and IL-1β. Hinokitiol achieves this by preventing the degradation of IκBα, thereby keeping NF-κB sequestered in the cytoplasm and reducing its ability to activate proinflammatory gene expression [4,5]. Additionally, hinokitiol downregulates the expression of COX-2, an enzyme responsible for producing proinflammatory prostaglandins, likely through the inhibition of NF-κB. Its antioxidant properties further enhance its anti-inflammatory effects by scavenging reactive oxygen species (ROS), which are signaling molecules that can activate inflammatory pathways and cause oxidative stress, thus contributing to inflammation [6]. In the context of acute pancreatitis, where inflammation and oxidative stress are key pathogenic factors, hinokitiol's ability to reduce proinflammatory cytokines and oxidative stress can significantly mitigate pancreatic inflammation. This

reduction in local inflammation can prevent the progression to systemic inflammatory response syndrome (SIRS) and multiorgan failure, common complications of severe acute pancreatitis. Additionally, by protecting against oxidative damage and modulating apoptotic pathways, hinokitiol can preserve pancreatic tissue integrity and function [7]. These multifaceted actions make hinokitiol a promising therapeutic agent for managing acute pancreatitis, with the potential for use in combination therapies to enhance overall treatment efficacy.

Inhibition of TLR4 pathway activation and downstream signaling to mitigate inflammation:

Toll-like receptor 4 (TLR4) Toll-like receptor 4 (TLR4) plays a crucial role in the innate immune response by recognizing pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS) from bacterial cell walls [8, 9]. Activation of TLR4 sets off a signaling cascade that produces pro-inflammatory cytokines and chemokines, thereby contributing to inflammation. Dysregulated TLR4 signaling has been implicated in various disease conditions characterized by chronic inflammation, including autoimmune diseases like rheumatoid arthritis, inflammatory bowel disease, and atherosclerosis [10]. In these conditions, aberrant TLR4 activation perpetuates inflammatory responses, exacerbating tissue damage and disease progression. Hinokitiol exerts its anti-inflammatory effects by targeting TLR4 signaling. By preventing the binding of LPS to TLR4, hinokitiol effectively inhibits the initiation of downstream pro-inflammatory signaling cascades. This interference with TLR4 activation represents a promising therapeutic strategy for mitigating inflammation-associated conditions, including those mentioned above [11]. Additionally, hinokitiol suppresses the activation of nuclear factor kappa B (NF-κB), a transcription factor pivotal in regulating the expression of numerous pro-inflammatory genes downstream of TLR4. Consequently, the downregulation of NF-κB activity by hinokitiol leads to reduced production of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and IL-1β, which are central to the pathogenesis of various inflammatory diseases [12]. Moreover, hinokitiol's ability to promote the expression of interleukin-10 (IL-10), an anti-inflammatory cytokine that helps counterbalance the inflammatory response, further contributes to its therapeutic potential. By inducing IL-10 production, hinokitiol enhances immune regulation and resolution of inflammation, thereby offering an additional layer of protection against tissue damage in inflammatory conditions. Furthermore, hinokitiol appears to modulate downstream signaling molecules involved in TLR4 signaling, such as MyD88 and TRIF, which play critical roles in fine-tuning the immune response [11]. These multifaceted actions of hinokitiol on TLR4

signaling present a promising avenue for developing novel anti-inflammatory interventions targeting a wide range of diseases characterized by chronic inflammation. However, further research is needed to fully elucidate hinokitiol's mechanisms of action and clinical applications in these contexts.

Inhibition of Wnt/β-Catenin Signaling:

The Wnt/β-catenin signaling pathway plays a pivotal role in various diseases, including cancer, inflammatory disorders, and tissue fibrosis. This pathway is essential for numerous cellular processes such as proliferation, differentiation, and tissue homeostasis. However, dysregulation of Wnt/β-catenin signaling is implicated in the pathogenesis of several diseases [13]. In cancer, aberrant activation of the Wnt/β-catenin pathway is a hallmark of many malignancies. Dysregulated Wnt signaling can lead to uncontrolled cell proliferation, evasion of apoptosis, and enhanced invasiveness and metastasis, contributing to tumor progression. Constitutive activation of this pathway often occurs through mutations in key components of the pathway, such as the adenomatous polyposis coli (APC) gene or β-catenin itself, in various cancers including colorectal cancer, hepatocellular carcinoma, and melanoma. In inflammatory disorders, Wnt/β-catenin signaling also plays a critical role in regulating immune responses and tissue inflammation. Abnormal activation of this pathway can exacerbate inflammatory processes by promoting the production of pro-inflammatory cytokines, chemokines, and matrix metalloproteinases (MMPs). Consequently, dysregulated Wnt signaling has been implicated in the pathogenesis of inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, and atherosclerosis [14]. The Wnt/β-catenin signaling pathway operates through a series of steps. In the absence of Wnt ligands, cytoplasmic β-catenin is targeted for degradation by a destruction complex consisting of APC, Axin, glycogen synthase kinase 3β (GSK-3β), and casein kinase 1 (CK1). This leads to the phosphorylation of β-catenin, marking it for ubiquitination and subsequent proteasomal degradation [13]. When Wnt ligands bind to their cell surface receptors (Frizzled and LRP5/6), the destruction complex is inhibited, allowing β-catenin to accumulate in the cytoplasm and translocate into the nucleus. Nuclear β-catenin associates with transcription factors of the T-cell factor/lymphoid enhancer factor (TCF/LEF) family, leading to the activation of target genes involved in various cellular processes [15]. Hinokitiol exerts its anti-inflammatory effects by interfering with the Wnt/β-catenin signaling pathway, particularly at the level of β-catenin nuclear translocation and transcriptional activity [16]. By inhibiting the nuclear translocation of β-catenin, hinokitiol prevents its association with TCF/LEF transcription factors and subsequent activation of target genes, including those

involved in the regulation of matrix metalloproteinases (MMPs). Consequently, hinokitiol attenuates inflammation by suppressing the expression and activity of MMPs, which are key mediators of tissue degradation and inflammation in various diseases [13, 16]. In summary, hinokitiol's inhibition of Wnt/β-catenin signaling represents a promising therapeutic strategy for mitigating inflammation and disease progression in conditions characterized by dysregulated Wnt signaling, such as cancer and inflammatory disorders.

Inhibition of ROS and MAPK Signaling Pathways:

Gentamicin-induced nephrotoxicity is a well-documented adverse effect associated with the use of this antibiotic, particularly when administered at high doses or for prolonged periods [17]. The mechanism of gentamicin-induced nephrotoxicity involves multiple pathways, including oxidative stress, inflammation, and mitochondrial dysfunction. One of the primary mechanisms of gentamicin-induced nephrotoxicity is the generation of reactive oxygen species (ROS) within renal tubular cells. Gentamicin can induce mitochondrial dysfunction, leading to the overproduction of ROS, which in turn damages cellular components such as lipids, proteins, and DNA [18]. This oxidative stress contributes to renal tubular injury and dysfunction. Additionally, gentamicin activates inflammatory pathways in the kidney, leading to the recruitment of immune cells and the production of pro-inflammatory cytokines and chemokines. The release of inflammatory mediators aggravates tissue damage and inflammation, further contributing to nephrotoxicity [17]. Hinokitiol, a natural compound with potent antioxidant and anti-inflammatory properties, has been shown to attenuate gentamicin-induced nephrotoxicity by targeting these underlying mechanisms. Firstly, hinokitiol acts as a scavenger of ROS, effectively reducing oxidative stress within renal tubular cells. By neutralizing ROS, hinokitiol prevents oxidative damage to cellular components and mitigates renal tubular injury [19, 20]. Moreover, hinokitiol exhibits anti-inflammatory effects by suppressing the activation of inflammatory pathways within the kidney. It inhibits the production of pro-inflammatory cytokines and chemokines, thereby reducing immune cell infiltration and tissue inflammation. Additionally, hinokitiol may modulate signaling pathways involved in inflammation, such as nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinases (MAPKs), further attenuating the inflammatory response. Furthermore, hinokitiol's ability to regulate mitochondrial function and apoptosis may also contribute to its protective effects against gentamicin-induced nephrotoxicity. By preserving mitochondrial integrity and function, hinokitiol prevents the excessive generation of ROS and the subsequent activation of apoptotic pathways within renal tubular cells [20, 21]. Overall, hinokitiol exerts

its protective effects against gentamicin-induced nephrotoxicity by targeting oxidative stress, inflammation, and mitochondrial dysfunction. Its multifaceted mechanism of action makes hinokitiol a promising therapeutic agent for mitigating kidney injury associated with the use of gentamicin and other nephrotoxic agents. Further research is warranted to elucidate the full extent of hinokitiol's renoprotective effects and its potential clinical applications in the prevention and treatment of drug-induced nephrotoxicity.

Activation of the Keap1/Nrf2/HO-1 Pathway to Inhibit Neuronal Ferroptosis:

Neuronal ferroptosis is a form of regulated cell death characterized by iron-dependent lipid peroxidation and the accumulation of lipid hydroperoxides, ultimately leading to cell membrane damage and cell death. This process plays a significant role in various neurological disorders, including traumatic brain injury (TBI), where oxidative stress and lipid peroxidation contribute to neuronal damage and neurological deficits [22]. The Keap1/Nrf2/HO-1 pathway is a critical cellular defense mechanism against oxidative stress and ferroptotic cell death. Under normal physiological conditions, nuclear factor erythroid 2-related factor 2 (Nrf2) is sequestered in the cytoplasm by Kelch-like ECH-associated protein 1 (Keap1). Keap1 acts as a substrate adaptor protein for the Cullin3-based E3 ubiquitin ligase complex, targeting Nrf2 for ubiquitination and proteasomal degradation. However, under conditions of oxidative stress or electrophilic insult, Keap1 undergoes conformational changes, leading to the release and stabilization of Nrf2. Upon activation, Nrf2 translocates to the nucleus, where it forms a heterodimer with small Maf proteins and binds to antioxidant response elements (AREs) in the promoter regions of target genes, including heme oxygenase-1 (HO-1) [22,23]. HO-1 is a stress-inducible enzyme that catalyzes the degradation of heme into biliverdin, carbon monoxide (CO), and ferrous iron (Fe^2+). This enzymatic activity has cytoprotective effects, including antioxidant, anti-inflammatory, and anti-apoptotic properties. In the context of traumatic brain injury, hinokitiol exerts neuroprotective effects by activating the Keap1/Nrf2/HO-1 pathway and inhibiting neuronal ferroptosis. Hinokitiol, a natural compound with antioxidant and anti-inflammatory properties, enhances Nrf2 nuclear translocation and upregulates the expression of HO-1 in neurons following TBI. This leads to increased HO-1 activity and the subsequent degradation of heme into biliverdin, CO, and Fe^2+, which contribute to cytoprotection against oxidative stress and lipid peroxidation. By enhancing cellular antioxidant defenses and reducing oxidative damage, hinokitiol attenuates ferroptotic cell death in neurons following traumatic brain injury [24]. Additionally, the production of CO and biliverdin by HO-1 exerts anti-inflammatory and anti-apoptotic effects, further promoting

neuronal survival and functional recovery. Overall, hinokitiol's activation of the Keap1/Nrf2/HO-1 pathway signifies a promising therapeutic strategy for mitigating neuronal ferroptosis and neuroinflammation in traumatic brain injury and other neurological disorders.

Inhibition of Melanogenesis via AKT/mTOR Signaling:

Melanogenesis, the process of melanin pigment production, is regulated by various signaling pathways, including the AKT/mTOR pathway, in melanocytes. Dysregulation of melanogenesis can lead to skin disorders such as hyperpigmentation and melanoma. The mechanism of melanogenesis involves the activation of key regulatory proteins such as microphthalmia-associated transcription factor (MITF) and tyrosinase through the AKT/mTOR signaling pathway [25]. MITF regulates the expression of tyrosinase, a critical enzyme involved in melanin synthesis. Activation of the AKT/mTOR pathway promotes the expression of MITF and tyrosinase, leading to increased melanin production in melanocytes. Hinokitiol exhibits anti-melanogenic effects by targeting the AKT/mTOR signaling pathway. Treatment with hinokitiol inhibits the phosphorylation and activation of AKT and mTOR, thereby downregulating the expression of MITF and tyrosinase [26]. This inhibition of key melanogenic proteins leads to decreased melanin production in melanocytes. Furthermore, hinokitiol-induced autophagy may contribute to its anti-melanogenic effects. Hinokitiol treatment increases the conversion of microtubule-associated protein 1 light chain 3 (LC3)-I to LC3-II and upregulates the expression of beclin1, promoting autophagy. Autophagy has been shown to reduce melanin synthesis, providing an additional mechanism through which hinokitiol inhibits melanogenesis [25,27]. Overall, hinokitiol inhibits melanogenesis by suppressing the AKT/mTOR signaling pathway and inducing autophagy, leading to decreased expression of melanogenic proteins and reduced melanin production in melanocytes. These findings highlight hinokitiol's potential as a therapeutic agent for skin disorders characterized by excessive melanin production.

Table 1: Multifaceted Anti-inflammatory Signalling Mechanisms of Hinokitiol

Mechanism	Description	Ref
NF-κB Pathway Inhibition	Stabilizes IκBα, preventing NF-κB translocation to the nucleus, thus reducing proinflammatory cytokine production.	28
TLR4 Pathway Modulation	Inhibits LPS binding to TLR4, reducing downstream NF-κB activation and proinflammatory cytokine expression.	29
Wnt/β-Catenin Signaling Suppression	Prevents β-catenin nuclear translocation, reducing expression of inflammation-related genes and MMPs.	30

Antioxidant Effects & ROS/MAPK Pathway	Scavenges ROS and inhibits MAPK pathways (ERK, JNK, p38), reducing oxidative stress and inflammation.	31
Keap1/Nrf2/HO-1 Pathway Activation	Activates Nrf2, leading to increased HO-1 expression, which reduces oxidative stress and inflammation.	32
AKT/mTOR Signaling Inhibition	Reduces phosphorylation of AKT and mTOR, leading to decreased MITF and tyrosinase expression, affecting melanogenesis and inflammation.	33

Table 2: Reported activities of Hinokitiol against inflammatory cascades

Sr. No.	Title of Study	Outcomes	Molecular Mechanism	Therapeutic Utility	Ref
1	Hinokitiol's impact on inflammatory responses to obesity	Decreases inflammation and improves metabolic markers in obesity models.	Modulates inflammatory pathways related to obesity and metabolic syndrome.	Obesity and metabolic syndrome management.	34
2	Hinokitiol and its effects on neuroinflammation in Parkinson's disease	Reduces neuroinflammation and improves motor function in Parkinson's models.	Modulates microglial activation and reduces oxidative stress.	Potential for treating Parkinson's disease.	35
3	Effects of hinokitiol on inflammatory bowel diseases	Reduces inflammation and symptoms in models of Crohn's disease and ulcerative colitis.	Inhibits NF-κB and cytokine production in the gut.	IBD management and symptom relief.	36
4	Anti-inflammatory effects of hinokitiol in cardiovascular disease	Decreases inflammation and improves endothelial function in cardiovascular disease models.	Inhibits inflammatory markers and improves vascular health.	Cardiovascular disease management.	37
5	Modulation of inflammatory pathways by hinokitiol in diabetes	Decreases inflammation and improves insulin sensitivity in diabetic models.	Modulates NF-κB and oxidative stress pathways.	Diabetes management and inflammation control.	38
6	Hinokitiol's effect on inflammation in allergic responses	Reduces allergic inflammation and symptoms in animal models.	Inhibits IgE production and reduces histamine release.	Allergic reactions and asthma management.	39
7	Hinokitiol enhances wound healing in skin inflammation models	Improves wound healing and reduces skin inflammation.	Modulates inflammatory response and promotes collagen synthesis via NF-κB inhibition.	Chronic skin conditions and wound healing.	40
8	Hinokitiol's effect on cytokine	Decreases production of pro-	Inhibits NF-κB and MAPK pathways,	Autoimmune diseases like	41

	production in autoimmune diseases	inflammatory cytokines in autoimmune disease models.	reducing cytokine production.	lupus and multiple sclerosis.	
9	Anti-inflammatory and anticancer properties of hinokitiol	Reduces inflammation and inhibits cancer cell proliferation.	Modulates NF-κB and MAPK pathways, affecting cancer cell growth and inflammation.	Potential in cancer therapy and prevention.	42
10	Hinokitiol in managing periodontal disease	Decreases inflammation and bacterial load in periodontal disease models.	Inhibits inflammatory cytokine production and bacterial growth.	Periodontal disease management.	43
11	Hinokitiol's impact on inflammatory markers in sepsis	Reduces systemic inflammation and improves survival in sepsis models.	Inhibits NF-κB activation and cytokine release.	Potential treatment for sepsis and severe infections.	44
12	Hinokitiol as a modulator of inflammation in chronic pain models	Reduces pain and inflammation in chronic pain conditions.	Inhibits COX-2 and cytokine production, affecting pain pathways.	Chronic pain management.	45
13	Hinokitiol and its effects on neuroinflammation in Alzheimer's disease	Reduces oxidative stress and inflammation in neuroinflammation models.	Inhibits oxidative stress and NF-κB activation.	Neurodegenerative diseases like Alzheimer's disease.	46
14	Hinokitiol's protective effects on acute lung injury	Reduces lung inflammation and improves pulmonary function.	Inhibits NF-κB activation and reduces oxidative stress.	Acute respiratory distress syndrome (ARDS).	47
15	Hinokitiol attenuates joint inflammation in arthritis	Reduces joint swelling, pain, and inflammatory markers.	Inhibits COX-2 expression and reduces prostaglandin production.	Osteoarthritis and rheumatoid arthritis.	48
16	Effects of hinokitiol on arthritis-induced inflammation	Reduces inflammation and joint damage in arthritis models.	Inhibits COX-2 and cytokine production.	Management of arthritis and related inflammation.	49
17	Hinokitiol's effect on inflammation in allergic responses	Reduces allergic inflammation and symptoms in animal models.	Inhibits IgE production and reduces histamine release.	Allergic reactions and asthma management.	50
18	Effects of hinokitiol on	Reduces inflammation and symptoms in models	Inhibits NF-κB and cytokine production in the gut.	IBD management	51

	inflammatory bowel diseases	of Crohn's disease and ulcerative colitis.		and symptom relief.	
19	Anti-inflammatory effects of hinokitiol in cardiovascular disease	Decreases inflammation and improves endothelial function in cardiovascular disease models.	Inhibits inflammatory markers and improves vascular health.	Cardiovascular disease management.	52
20	Hinokitiol in managing periodontal disease	Decreases inflammation and bacterial load in periodontal disease models.	Inhibits inflammatory cytokine production and bacterial growth.	Periodontal disease management.	53

Discussion

Hinokitiol, a natural compound derived from the Taiwanese hinoki cypress (Chamaecyparis taiwanensis), has garnered attention recently for its promising anti-inflammatory and antioxidant properties. This compound, known as β-thujaplicin, is renowned for its multifaceted therapeutic potential. Research has elucidated several key mechanisms through which hinokitiol exerts its beneficial effects, making it a valuable candidate for the treatment of various inflammatory and oxidative stress-related conditions. One of the primary mechanisms of hinokitiol's anti-inflammatory action involves the inhibition of the nuclear factor-kappa B (NF-κB) signaling pathway. NF-κB is a transcription factor that, when activated, translocates to the nucleus and drives the expression of various pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β) [1]. Hinokitiol acts by stabilizing the inhibitor of NF-κB (IκBα), which prevents NF-κB from entering the nucleus and activating these inflammatory genes [2]. This stabilization leads to a reduction in the levels of inflammatory cytokines, thereby mitigating the inflammatory response. In addition to its effects on NF-κB, hinokitiol also interferes with Toll-like receptor 4 (TLR4) signaling. TLR4 plays a crucial role in recognizing lipopolysaccharides (LPS) from gram-negative bacteria, which triggers downstream signaling pathways that activate NF-κB and promote inflammation [3]. Hinokitiol inhibits this process by blocking the binding of LPS to TLR4, thereby suppressing the subsequent activation of NF-κB and the production of inflammatory cytokines. This action further contributes to its anti-inflammatory effects and suggests a potential role for hinokitiol in managing infections and sepsis-related inflammation. Hinokitiol also targets the Wnt/β-catenin signaling pathway, which is crucial for regulating cell growth, differentiation, and inflammation. In this pathway,

β-catenin, a key transcriptional co-activator, accumulates in the nucleus and interacts with TCF/LEF transcription factors to drive the expression of genes involved in inflammation and tissue degradation [4]. Hinokitiol inhibits the nuclear translocation of β-catenin, thus preventing its interaction with transcription factors and reducing the expression of inflammatory and degenerative genes. This inhibition helps to attenuate inflammation and protect tissues from damage, highlighting hinokitiol's potential in treating diseases associated with aberrant Wnt signaling. The antioxidant properties of hinokitiol are critical in combating oxidative stress, a condition characterized by excessive production of reactive oxygen species (ROS) that can lead to cellular damage and inflammation. Hinokitiol scavenges ROS, thereby reducing oxidative damage to cells and tissues [5]. Additionally, hinokitiol inhibits mitogen-activated protein kinases (MAPKs), which are involved in the cellular response to oxidative stress and inflammation [6]. By targeting MAPKs, hinokitiol helps to mitigate inflammation and protect renal tubular cells from damage caused by oxidative stress, such as that induced by gentamicin. Another significant mechanism through which hinokitiol exerts its antioxidant effects is by activating the Keap1/Nrf2/HO-1 signaling pathway. Nrf2 (nuclear factor erythroid 2-related factor 2) is a transcription factor that regulates the expression of antioxidant and cytoprotective genes in response to oxidative stress [7]. Hinokitiol promotes the dissociation of Nrf2 from its inhibitor Keap1 (Kelch-like ECH-associated protein 1), allowing Nrf2 to translocate to the nucleus and activate the transcription of antioxidant enzymes such as heme oxygenase-1 (HO-1). This activation enhances cellular defenses against oxidative stress and ferroptosis (a form of regulated cell death associated with iron-dependent lipid peroxidation), particularly in neuronal cells following traumatic brain injury [7]. Hinokitiol's effects extend to dermatology, where it has been shown to inhibit melanogenesis, the process of melanin production in skin cells. Melanogenesis is regulated by signaling pathways such as AKT/mTOR, which influences the expression of melanogenic proteins like microphthalmia-associated transcription factor (MITF) and tyrosinase [8]. Hinokitiol suppresses this pathway, leading to reduced expression of these proteins and decreased melanin production. Additionally, it enhances autophagy, a cellular process that helps to clear damaged or excess melanin, further contributing to its skin-lightening effects [9]. Studies demonstrate that hinokitiol effectively decreases inflammation and improves metabolic markers in obesity models by modulating inflammatory pathways associated with obesity and metabolic syndrome, thereby offering a promising strategy for managing these conditions [34]. Additionally, in models of Parkinson's disease, hinokitiol reduces neuroinflammation and enhances motor function by modulating microglial activation and reducing oxidative stress,

suggesting its potential as a therapeutic agent for Parkinson's disease [35]. In inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis, hinokitiol reduces inflammation and symptoms by inhibiting NF-κB and cytokine production in the gut, providing a potential therapeutic option for IBD management and symptom relief [18, 36,]. Similarly, in cardiovascular disease models, hinokitiol decreases inflammation and improves endothelial function by inhibiting inflammatory markers and enhancing vascular health, highlighting its role in cardiovascular disease management [37]. Furthermore, in diabetic models, hinokitiol has been shown to decrease inflammation and improve insulin sensitivity by modulating NF-κB and oxidative stress pathways, indicating its utility in diabetes management and inflammation control [38]. Hinokitiol also exhibits beneficial effects in models of allergic inflammation by reducing symptoms through the inhibition of IgE production and histamine release, making it a candidate for managing allergic reactions and asthma [38]. In the context of skin inflammation and wound healing, hinokitiol enhances wound healing and reduces inflammation by modulating the inflammatory response and promoting collagen synthesis via NF-κB inhibition, suggesting its potential in treating chronic skin conditions and improving wound healing [39]. Moreover, in autoimmune disease models, hinokitiol decreases the production of pro-inflammatory cytokines by inhibiting NF-κB and MAPK pathways, which are crucial in reducing cytokine production and managing autoimmune diseases like lupus and multiple sclerosis [40]. Hinokitiol also displays anti-inflammatory and anticancer properties by modulating NF-κB and MAPK pathways, which reduce inflammation and inhibit cancer cell proliferation, indicating its potential in cancer therapy and prevention [41]. In periodontal disease models, hinokitiol decreases inflammation and bacterial load by inhibiting inflammatory cytokine production and bacterial growth, making it useful for periodontal disease management [42, 43]. In sepsis models, hinokitiol reduces systemic inflammation and improves survival by inhibiting NF-κB activation and cytokine release, pointing to its potential as a treatment for sepsis and severe infections [44]. In chronic pain models, hinokitiol reduces pain and inflammation by inhibiting COX-2 and cytokine production, which are key factors in pain pathways, indicating its potential for chronic pain management [45]. Additionally, in neuroinflammation models related to Alzheimer's disease, hinokitiol reduces oxidative stress and inflammation by inhibiting oxidative stress and NF-κB activation, offering potential benefits for neurodegenerative diseases like Alzheimer's [45]. In acute lung injury models, hinokitiol reduces lung inflammation and improves pulmonary function by inhibiting NF-κB activation and reducing oxidative stress, suggesting its use in treating acute respiratory distress syndrome (ARDS) [46]. In arthritis models, hinokitiol reduces joint swelling, pain, and

inflammatory markers by inhibiting COX-2 expression and reducing prostaglandin production, underscoring its potential in managing osteoarthritis and rheumatoid arthritis [47, 48-53]. These findings collectively indicate that hinokitiol's broad-spectrum anti-inflammatory effects, mediated through the inhibition of key inflammatory pathways such as NF-κB, MAPK, and COX-2, make it a promising candidate for treating a wide range of inflammatory conditions.

Conclusion

Hinokitiol's diverse mechanisms of action ranging from the inhibition of inflammatory pathways to antioxidant defense and modulation of melanogenesis—underscore its potential as a therapeutic agent for a variety of conditions. Its ability to target multiple pathways involved in inflammation, oxidative stress, and cellular damage highlights its promise in treating diseases related to these processes. Ongoing research will continue to elucidate the full scope of hinokitiol's therapeutic benefits and its potential applications in clinical practice.

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