Action and mechanisms of neferine in inflammatory diseases (Review)

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Abstract. Neferine is a bisbenzylisoquinoline alkaloid derived from the seed embryo of *Nelumbo nucifera*, a traditional Chinese medicine. It has been extensively studied for its therapeutic potential in various disease models. Extensive research has highlighted its diverse pharmacological activities, including antitumor, anti-inflammatory, anti-fibrosis, anti-oxidative stress, anti-platelet aggregation and anti-arrhythmic effects. The present review, however, focuses on the anti-inflammatory properties of neferine, emphasizing its fundamental mechanisms as demonstrated in both *in vivo* and *in vitro* studies. By critically evaluating its effect on inflammation and the underlying pathways, this review aims to provide a comprehensive understanding of the potential of neferine in the management of inflammatory diseases. Furthermore, it seeks to establish a foundational framework for the future development of neferine as a novel therapeutic agent for inflammatory conditions.

Contents

1. Introduction

Nelumbo nucifera, commonly known as the lotus, has been revered since ancient times; according to the Chinese proverb, it emerges untainted from the mud. This plant is widespread across Asia and Australia and is considered important in traditional Chinese medicine. Various parts of the lotus, including its seeds (Fig. 1A), rhizome, leaves and flowers, have been used for medicinal purposes. The rhizome is particularly effective in the treatment of hemorrhoids, dysentery, chronic dyspepsia and other digestive disorders, in addition to ameliorating diuresis and cholelithiasis. In addition, the leaves are used to treat conditions such as hematemesis, epistaxis, hemoptysis, hematuria, uterine bleeding and hyperlipidemia, and the flowers are known to treat diarrhea, cholera, fever and stomach ulcers. Furthermore, the lotus plumule has been found to beneficial for the treatment of conditions such as neurasthenia, insomnia, high fever with restlessness, and cardiovascular diseases, including hypertension and arrhythmia (1).

Advancements in medical chemistry and pharmacy have facilitated the isolation of numerous medicinal substances from different parts of the lotus, including alkaloids, terpenes, flavonoids, fatty acids, carbohydrates, minerals and proteins (2). These active compounds have received considerable interest from researchers worldwide, who have aimed to explore their chemical structures and pharmacological effects, thereby laying the groundwork for the development of novel drugs.

The lotus plumule (Fig. 1B), which comprises the radicle and young leaves of the mature lotus seed, is traditionally used in Chinese medicine. Lotus leaf, *Euryale ferox* Salisb., and lotus plumule are frequently used together as a three-component formulation. In traditional Chinese medicine, this formulation is considered to clear damp-heat, reduce excess and disperse evils (3). Similarly, Qinggong decoction (4), which comprises lotus plumule, is traditionally believed to be effective in clearing the heart, detoxifying, nourishing Yin and promoting fluid production, thereby treating fever, dizziness, insomnia and delirium (5). Key secondary metabolites in lotus plumule include alkaloids, such as dauricine, liensinine, isoliensinine, nuciferine, pronuciferine, roemerine, neferine and armepavine (2,6). Among these, neferine (Fig. 1C), a dibenzylisoquinoline alkaloid, has attracted considerable attention due to its wide range of pharmacological activities, including antitumor (7), anti-inflammatory, anti-fibrotic, anti-oxidative stress, anti-platelet aggregation and anti-arrhythmic properties (8,9). Inflammation is a crucial factor in the progression of various diseases, including cancer, cardiovascular diseases, diabetes, autoimmune diseases, obesity and ocular disorders (10). A number of studies have elucidated the anti-inflammatory

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potential of neferine, demonstrating that it exerts its biological effects via the inhibition of nuclear factor-kB (NF-kB), mitogen-activated protein kinases (MAPKs) (11), NOD-like receptor protein 3 (NLRP3), autophagy and the transforming growth factor-β (TGF-β)/Smad pathway, which mediate the production of various pro-inflammatory mediators. The present review aims to summarize the pharmacological effects and mechanisms of neferine in various disorders.

2. Anti-inflammatory action of neferine

Various factors, including pathogens, autoimmune diseases, malignancies, metabolic disorders and certain therapeutic interventions, provoke systemic inflammatory effects. Inflammation is a complex, multi-stage process involving various cell types and signaling mediators, and can be detrimental when prolonged or chronic. A hallmark of inflammation is the excessive release of cytokines due to the overactivation of immune cells (12), which plays a crucial role in the progression of the inflammatory response. Thus, the inhibition of pro-inflammatory cytokines is a key strategy for combating inflammation. Tumor necrosis factor (TNF) is a critical cytokine in inflammatory responses, promoting inflammation by directly inducing inflammatory gene expression and indirectly causing cell death (13).

Numerous studies have demonstrated the anti-inflammatory properties of neferine, and its ability to inhibit inflammatory mediators. For example, in one study, neferine reduced TNF-α levels and enhanced insulin sensitivity in insulin-resistant rats (14). In another study, neferine modulated the hypoxia-induced inflammatory response in human peripheral blood mononuclear cells by inhibiting the release of TNF-α, interleukin (IL)-6 and IL-8 under hypoxic conditions (15). Similarly, neferine exhibited anti-inflammatory effects in a dextran sulfate sodium-induced ulcerative colitis mouse model (16). Furthermore, neferine promoted wound healing in diabetic rats, potentially via the downregulation of inflammatory mediators, including NF-κB, TNF-α, IL-1β, IL-8, nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), and the upregulation of growth factors (17).

Lipopolysaccharide (LPS) and inflammation. LPS is a component of gram-negative bacterial endotoxin, which elicits strong inflammatory responses in the host (18). Neferine has been shown to significantly reduce the production of LPS-induced inflammatory mediators in RAW 264.7 macrophages (19), human endothelial cells (20-23), NRK-52E cells (24) and BV-2 microglial cells (25). These mediators include nitric oxide (NO), TNF-α, COX-2, iNOS, IL-1b, IL-6 and IL-10. Furthermore, neferine has been shown to upregulate B-cell lymphoma 2 expression and suppress cleaved caspase-3 activity in LPS-induced mouse heart tissue and H9c2 cells (26), highlighting its protective effects against LPS-induced damage.

Fibrosis. Pulmonary fibrosis, a type of interstitial lung disease, can be triggered by bacteria, viruses, smoking, autoimmune diseases, drugs and gastroesophageal reflux. Current treatments for pulmonary fibrosis mainly involve anti-inflammatory and immunosuppressive therapies; however, drugs such as prednisone and pirfenidone have limited efficacy and numerous side effects (27). Studies have shown that neferine can mitigate the experimental pulmonary fibrosis induced by bleomycin (28) and amiodarone (29). In the bleomycin-induced pulmonary fibrosis model, the beneficial effects neferine were attributed to its anti-inflammatory, antioxidant and cytokine-inhibitory properties. Similarly, in the amiodarone-induced pulmonary fibrosis model, neferine demonstrated efficacy by exerting anti-inflammatory effects, inhibiting surfactant protein D and modulating the T helper (Th)1/Th2 imbalance via the suppression of Th2 responses. In addition, neferine exhibited anti-inflammatory and antioxidant activity in a carbon tetrachloride (CCl_4)-induced liver fibrosis model, indicating its antifibrotic effects may be mediated by the inhibition of inflammation (30).

Oxidative stress. Oxidative stress, defined as an imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses, modulates the NF-κB pathway and the expression of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1, all of which are critical mediators of the inflammatory process (31). Lowering oxidant levels inhibits NF-κB activity, whereas oxidants activate NF-κB, leading to increased levels of proinflammatory cytokines such as TNF-α and IL-6 (32). The interplay between inflammation and oxidative stress is a key contributor to the pathogenesis of numerous chronic diseases.

Neferine has demonstrated potent antioxidant properties in numerous studies. For example Lalitha *et al* (33) reported that neferine protected rats against isoproterenol-induced oxidative stress and myocardial infarction. In addition, other studies showed that neferine pretreatment reduced ROS levels and mitigated changes in superoxide dismutase (SOD) and malondialdehyde levels in high glucose-treated human umbilical vein endothelial cells (34) and in UV-A-induced skin photoaging (35,36). Furthermore, Priya *et al* (11) evaluated the protective effects of neferine against doxorubicin (DOX)-induced cardiomyopathy, and found that neferine pretreatment inhibited the NADPH oxidase system and reduced the production of ROS.

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a critical transcription factor that regulates the antioxidant response. In conjunction with its downstream targets, heme oxygenase-1 (HO-1) and NADPH: quinone oxidoreductase 1 (NQO1), Nrf2 plays a pivotal role in the defense against oxidative stress (37). Neferine has been shown to increase antioxidant activity in muscle cells under hypoxic conditions by promoting the nuclear translocation of Nrf2 (38). In addition, neferine significantly induced Nrf2 translocation and increased HO-1 and SOD1 expression in a H9c2 cardiomyoblast cell model of DOX-mediated cardiotoxicity (39). Furthermore, Liu *et al* (40) demonstrated that neferine alleviated high-glucose-induced oxidative stress injury in NRK-52E cells by inducing HO-1 expression and increasing its enzymatic activity. Consistent with these findings, neferine was found to increase the nuclear levels of Nrf2 in PC12 cells treated with tert-butyl hydroperoxide (t-BHP), accompanied by the upregulation of HO-1 and NQO1, suggesting that neferine is protective against t-BHP-induced neuronal injury (41). Moreover, the administration of neferine was demonstrated to inhibit angiotensin II-induced atrial fibrillation, atrial enlargement and atrial

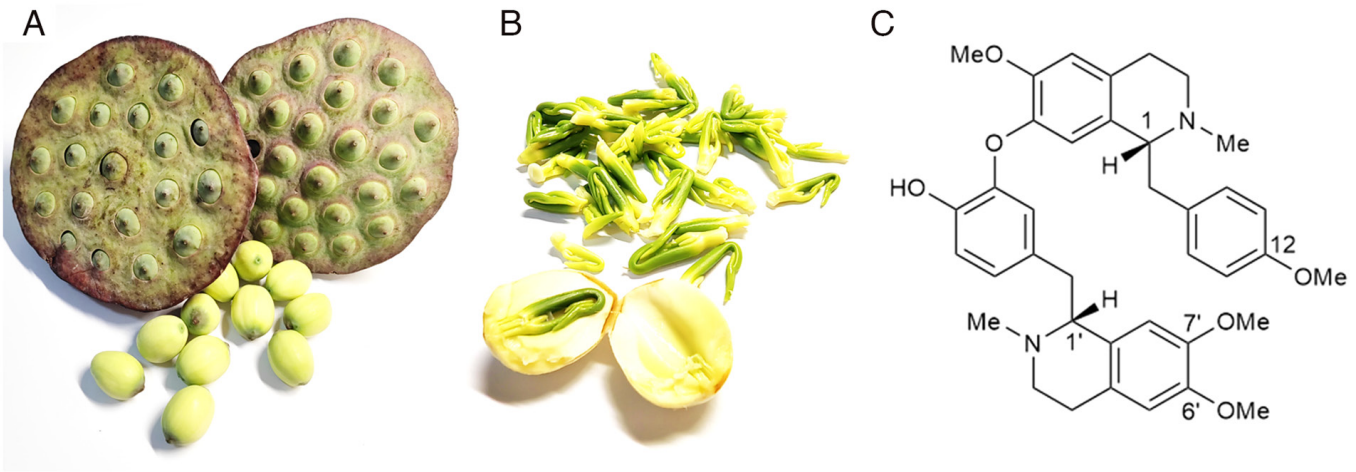

Figure 1. Lotus and its active component neferine. (A) Lotus seeds and (B) the radicle and young leaves of the mature lotus seed, known as the plumule. (C) Chemical structure of neferine. Me, methyl.

fibrosis in mice, with activation of the Nrf2/HO-1 pathway and inhibition of the TGF-β/phosphorylated (p-)Smad2/3 pathway identified as the underlying molecular mechanisms (42). In addition, Nrf2 serves as a link between oxidative stress and autophagy, which is further elaborated in the subsequent section.

3. Potential anti-inflammatory pathways of neferine

NF-κB pathway. The NF-κB family comprises pleiotropic transcription factors that regulate various biological processes, including inflammation, immunity and apoptosis (43). This pathway involves canonical and non-canonical signaling mechanisms. The canonical NF-κB pathway is activated by diverse external stimuli, including inflammation, immune responses and cell proliferation. In its inactive state, the NF-κB transcription factor, which is typically a RelA/p50 heterodimer, is retained in the cytoplasm by inhibitor of κB(IκB) protein. Upon activation, inhibitor of κB kinase (IKK)β induces the phosphorylation and subsequent degradation of IκB, enabling the RelA/p50 heterodimer to translocate to the nucleus where it regulates the expression of proinflammatory genes (44). Therefore, targeting the NF-κB pathway represents a potential strategy for the treatment of inflammatory diseases.

In lung injury, the NF-κB pathway is persistently activated, driving the transcription of harmful cytokines and promoting fibroblast proliferation. However, neferine has been shown to reduce elevated levels of TNF-α and IL-6 in mice with bleomycin-induced lung injury by inhibiting NF-κB activity in the nucleus (28). In addition, neferine has been found to ameliorate scopolamine-induced cognitive impairment in animal models via the inhibition of lipid peroxidation and NF-κB activation (45). It has also been shown to suppress receptor activator of NF-κB ligand-induced osteoclast formation by inhibiting the NF-κB signaling pathway (46,47), and to attenuate the IL-1β-induced inflammatory response in endothelial cells by inhibiting NF-κB nuclear translocation (23).

In a study of human endothelial cells exposed to LPS, neferine significantly prevented the formation of NO, TNF-α, COX-2, iNOS and IL-1β and inhibited NF-κB pathway signaling in a concentration-dependent manner, with reduction of the phosphorylation of IKKα, IKKβ and IκB-α and the expression of NF-κB p65 (20). Additionally, neferine exhibited anti-inflammatory effects against acute kidney injury (AKI) in *in vivo* and *in vitro* models by inhibiting cytokine production, which was achieved through the inhibition of IκB-α phosphorylation and NF-κB p65 nuclear translocation (24) (Fig. 2). This mechanism is similar to its action in CCl_4-induced liver fibrosis (30). However, these studies did not investigate the mechanism of NF-κB p65 activation.

In a study performed by Ni *et al* (48), neferine alleviated IL-1β-induced inflammation in rat chondrocytes by preventing NF-κB p65 phosphorylation and nuclear translocation. Furthermore, in another study, neferine inhibited LPS-mediated microglial activation by preventing the phosphorylation and nuclear translocation of the NF-κB p65 subunit (25). Nevertheless, the precise mechanisms by which neferine modulates NF-κB remain to be clarified. Toll-like receptor 4 (TLR4) has been identified as an upstream regulator of the NF-κB pathway (49). Building on this, a study on LPS-treated HepG2 cells and mice with nonalcoholic steatohepatitis induced by a high-fat diet and CCl_4, indicated that neferine reduces hepatic inflammation, potentially by suppressing the TLR4/NF-κB signaling pathway (50). Recently, findings from the present research group suggested that the renoprotective effects of neferine against AKI are partially mediated through the reversal of renal peroxisome proliferator-activated receptor α (PPAR-α) deficiency, leading to inhibition of the NF-κB pathway. This suggests that PPAR-α may function upstream of NF-κB in the regulatory mechanisms of neferine in the kidney (51).

MAPK pathways. MAPKs are a group of serine/threonine protein kinases that play a crucial role in the regulation of inflammation and various cellular processes (52). Each MAPK pathway involves a cascade of at least three kinases: A MAPK kinase kinase (MAPKKK), a MAPK kinase (MAPKK) and a MAPK. In mammals, the primary MAPKs include extracellular signal-regulated kinases 1/2 (ERK1/2), p38 MAP kinase, and c-Jun N-terminal kinases (JNK) (53). Typically,

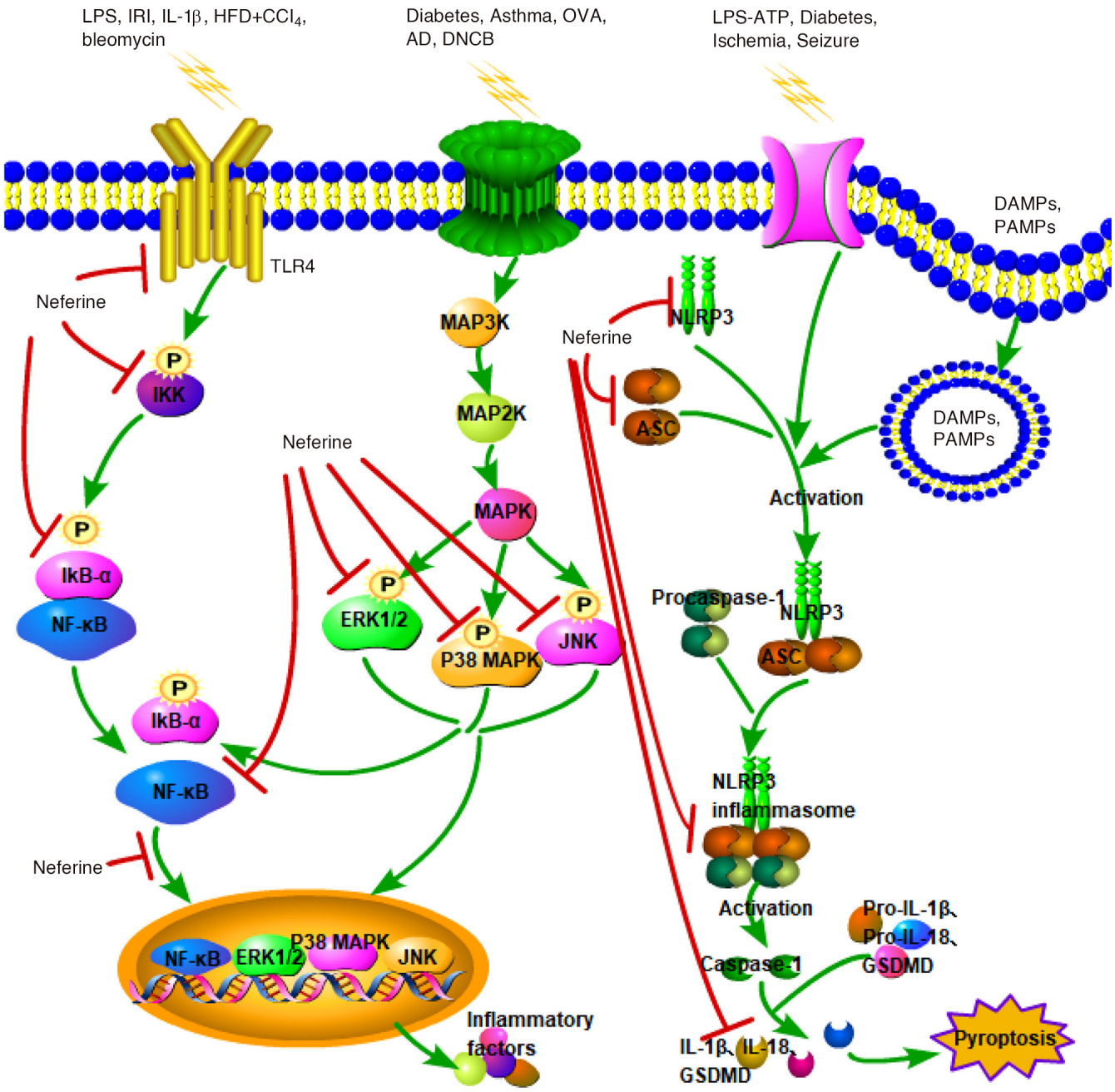

Figure 2. Schematic presentation of the major signaling cascades underlying the anti-inflammatory effect of neferine. Green arrows signify the activation of signal pathways, while red T-bars denote that neferine inhibits the activation of these pathways. LPS, lipopolysaccharide; IRI, ischemia/reperfusion injury; IL-1β, interleukin-1β; HFD, high-fat diet; CCl_4, carbon tetrachloride; OVA, ovalbumin; AD, atopic dermatitis; DNCB, 2,4-dinitrochlorobenzene; TLR4, Toll-like receptor 4; P, phosphorylation; IKK, inhibitor of κB kinase; IκB-α, inhibitor of κB a; NF-κB, nuclear factor-κB; MAPK, mitogen-activated protein kinase; ERK1/2, extracellular-signal-regulated kinases 1/2; JNK, c-Jun N-terminal kinases; MAP3K, MAPK kinase kinase; MAP2K, MAPK kinase; NLRP3, NOD-like receptor protein 3; ASC, apoptosis-associated speck-like protein containing a CARD; GSDMD, gasdermin D; DAMPs, danger-associated molecular patterns; PAMPs, pathogen-associated molecular patterns; IL, interleukin.

MAPKKKs activate MAPKKs through phosphorylation, which in turn activate MAPKs, ultimately leading to the regulation of inflammatory cytokine expression and the initiation of inflammatory responses (54).

In vivo research has revealed that neferine can prevent diabetes-induced cardiac fibrosis. This effect was evidenced by a reduction of the expression levels of collagen I, collagen III and TGF-β1 in diabetic mice treated with neferine, which was suggested to be mediated by the inhibition of p38, ERK and Smad 2/3 phosphorylation (55) (Fig. 2). In addition, neferine has been demonstrated to inhibit cell proliferation and migration in retinal pigment epithelial cells exposed to epidermal growth factor, potentially by reducing the phosphorylation of p38 MAPK (56). Furthermore, in an ovalbumin (OVA)-induced asthma model, neferine decreased various inflammatory factors in the serum and bronchoalveolar lavage fluid of OVA-treated animals and reduce the phosphorylation of p38, JNK, and ERK. These findings suggest that neferine mitigates asthma-induced inflammation via the inhibition of MAPK pathways (57).

MAPKs are crucial signaling molecules in the inflammatory response. Components of the MAPK pathway, including JNK1/2, p38 MAPK and ERK1/2, can influence upstream signaling events that modulate NF-κB activation, thereby affecting the expression and activity of inflammatory factors (58). Studies have also highlighted the therapeutic effects of neferine on atopic dermatitis (AD) in various models, including HaCaT keratinocyte cells (59), mast cells (60) and mouse models. Notably, in a 2,4-dinitrochlorobenzene-induced AD model, neferine significantly reduced cytokine expression and inhibited the phosphorylation of p38, ERK and IκB, supporting the hypothesis that neferine exerts anti-inflammatory effects through inhibition of the MAPK/NF-κB pathway (59).

NLRP3 inflammasome pathway. Overactivation of the NLRP3 inflammasome pathway contributes to various inflammatory diseases. Formation of the NLRP3 inflammasome involves the oligomerization of NLRP3, and the recruitment of apoptosis-associated speck-like protein containing a CARD (ASC) and caspase-1. This complex activates caspase-1, which subsequently cleaves pro-IL-1β, pro-IL-18 and gasdermin D (GSDMD). Activated GSDMD then disrupts the cell membrane, leading to pyroptosis, a form of cell death that releases proinflammatory cellular contents (61). Consequently, NLRP3 inflammasomes are crucial targets for anti-inflammatory treatments (62).

Tang *et al* (22) were the first to investigate the role of neferine in the prevention of endothelial cell pyroptosis. In their study, neferine was revealed to inhibit oxidative stress and the activation of the NLRP3 inflammasome pathway triggered by LPS-ATP. Using NLRP3 small interfering RNA and overexpression techniques, they also demonstrated that neferine prevented the LPS-ATP-induced pyroptosis of endothelial cells by blocking the ROS/NLRP3/caspase-1 signaling pathway. Furthermore, in a diabetic db/db mouse model, neferine treatment reduced oxidative stress and inflammation in the hippocampus (63). This was evidenced by decreased levels of thioredoxin-interacting protein, NLRP3 inflammasomes, ASC and IL-1β, suggesting that neferine alleviated memory and cognitive dysfunction in diabetic mice by modulating the NLRP3 inflammasome pathway.

Given the neuroprotective properties of neferine, Zhu *et al* (64) explored its effects on hypoxic-ischemic brain injury in neonatal rats. The study demonstrated that neferine reduced neuroinflammation and oxidative stress damage by inhibiting the NLRP3 inflammasome pathway and pyroptosis (Fig. 2). In addition, a follow-up study of ischemia/reperfusion injury in mice revealed that neferine inhibited pyroptosis, thereby improving the integrity of the blood-brain barrier via regulation of the peroxisome proliferator-activated receptor γ coactivator 1α/NLRP3/GSDMD signaling pathway (65). In addition, in a kainic acid-induced seizure rat model, neferine alleviated seizure severity and reduced neuroinflammation in the hippocampus, likely by inhibiting NLRP3 inflammasome activation and reducing inflammatory cytokine levels (66).

Autophagy. Autophagy is a crucial cellular process involving the formation of autolysosomes that degrade intracellular pathogens and damaged organelles. Key regulators of autophagy include AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) (67). Neferine has been shown to have regulatory effects on autophagy. For example, one study demonstrated that it not only promoted the accumulation of autophagy-related proteins, such as microtubule-associated protein 1A/1B-light chain (LC3-II) and p62/sequestome 1, but also hindered lysosome maturation, suggesting that neferine inhibits macroautophagic flux (68). In hypoxic muscle cells, neferine inhibited autophagy by downregulating beclin 1, class III PI3K and LC3B-II while activating the Akt/mTOR pathway (38). Similarly, in H9c2 cells exposed to DOX, neferine reduced the expression of Unc-51 like autophagy activating kinase 1, beclin 1, autophagy-related gene 7 and LC3B, possibly through the insulin-like growth factor 1 receptor/PI3K/Akt/mTOR pathway (39). Furthermore, neferine significantly alleviated cerebral artery occlusion-induced cerebral ischemia in rats by reducing the upregulation of LC3-II, beclin 1 and p62, as well as the formation of autophagosomes, through regulation of the Ca^{2+}-dependent AMPK/mTOR pathway (69). By contrast, when preventing cisplatin-induced nephrotoxicity, neferine appears to promote autophagy via the AMPK/mTOR pathway (70). Therefore, it appears that neferine may regulate autophagy through different mechanisms depending on the disease context.

Autophagy plays a role in clearing inflammasomes, cytokines and bacteria, and is influenced by factors including cytokines, ROS and danger/pathogen-associated molecular patterns, as well as pharmacological inhibitors (71), indicating a close relationship between autophagy and inflammation. Defects in autophagy are associated with susceptibility to several autoimmune and inflammatory disorders (72). Consequently, neferine may exert anti-inflammatory effects via the regulation of autophagy, consistent with findings in Graves' orbitopathy (GO) (73). The study of GO showed that neferine suppressed both IL-13-induced autophagosome formation and inflammation in GO orbital fibroblasts, as evidenced by attenuation of the both the increased LC3-II/LC3-I ratio and downregulated p62, and suppression of the upregulated TNF-α, IL-1β, IL-6 and monocyte chemoattractant protein-1 levels. In addition, the suppression of these inflammatory factors was partially reversed by 3-methyladenine, an autophagy inhibitor, suggesting that the anti-inflammatory effects of neferine were mediated, at least in part, through the regulation of autophagy.

Nrf2 serves as a link between oxidative stress and autophagy. In the non-canonical activation of Nrf2, p62 competes with Nrf2 to interact with Keap1, leading to the sequestration of p62-Keap1 in autophagosomes and promoting the nuclear translocation of Nrf2 to induce antioxidant gene expression. Experiments involving p62 knockdown have demonstrated that p62 is crucial for Nrf2 activation (74). Neferine has been shown to upregulate Nrf2 expression and suppress autophagy in diabetic wound models (17) and *in vitro* models of GO (73). Future research is required to elucidate the mechanisms underlying the effects of neferine on Nrf2 and autophagy.

TGF-β/Smad signaling pathway. TGF-β is a cytokine that regulates numerous cellular processes, including growth,

proliferation, differentiation, senescence, apoptosis, adhesion, migration and the synthesis and remodeling of the extracellular matrix. Smad proteins function as downstream transcription factors in the TGF-β signaling pathway (75,76). Disruptions in TGF-β signaling are implicated in various conditions, such as developmental abnormalities (77), cancers, tissue fibrosis, cardiovascular diseases (78) and immune disorders (79).

Neferine has been investigated for its effects on fibrotic diseases. For example, in a fibrotic endometriosis model in mice (80), neferine significantly reduced the expression of extracellular matrix components, including fibronectin, collagen type I, connective tissue growth factor and smooth muscle actin, and decreased the levels of TGF-β and p-ERK. These results suggest that neferine inhibited extracellular matrix deposition and fibrosis in this model by blocking the TGF-β/ERK signaling pathway. Similarly, neferine exhibited anti-fibrotic effects on testosterone-induced benign prostatic hyperplasia in mice (81); the study suggested that neferine prevented the epithelial-mesenchymal transition and prostate enlargement caused by testosterone by modulating the TGF-β/Smad pathway. Furthermore, neferine was shown to reduce vascular remodeling in spontaneously hypertensive rats via the inhibition of TGF-β1/Smad2/3 signaling (82). Collectively, these studies highlight neferine as a promising therapeutic agent for the management of fibrosis in various diseases.

4. Side effects of neferine

Research on neferine has predominantly focused on cell and animal experiments, with a conspicuous absence of clinical trials. This has severely restricted comprehensive investigations into its potential side effects. In Cell Counting Kit-8 assays, when the concentration of neferine reached 100 μM, the proliferation activity of NRK-52E cells remained >50% (24), indicating that neferine exhibits a low level of toxicity. However, in a study conducted by Yu *et al* (83), neferine inhibited myocardial contractility and disrupted the calcium homeostasis in cardiomyocytes, but had no significant effect on cell viability. These results demonstrate the potential cardiac side effects of neferine. Notably, *in vivo* data on the neurological or reproductive toxicity of neferine are lacking. Consequently, further research is necessary to comprehensively evaluate the safety profile of neferine across diverse physiological systems.

5. Summary and perspectives

Numerous studies have underscored that neferine exerts anti-inflammatory activity through various signaling pathways, establishing a foundational basis for its potential as a novel therapeutic agent for inflammatory diseases. The wide availability of neferine and minimal side effects further indicate its promise for disease treatment and prevention. However, exploration of the therapeutic efficacy of neferine has primarily been confined to cell and animal disease models. There are several reasons why it has not been widely used clinically. i) Lack of sufficient clinical trial data. The translation from models to human clinical applications requires extensive and well-designed clinical trials to evaluate its efficacy, safety, optimal dosage and potential side effects in humans. Without comprehensive clinical trial results, it is challenging to determine its true value and applicability in clinical practice. ii) Pharmacokinetic and pharmacodynamic uncertainties. Limited research has been performed to fully elucidate these aspects, which leads to uncertainties in dosing regimens and potential drug-drug interactions, hindering its immediate clinical adoption. iii) Competition from existing treatments.

Neferine has been demonstrated to modulate multiple cellular and molecular mediators involved in inflammation. However, the molecular mechanisms underlying the actions of neferine are complex, and its precise drug targets remain inadequately understood. Future research should focus on elucidating the detailed mechanisms of action of neferine under diverse physiological and pathological conditions, and identifying specific molecular targets to advance neferine as a viable therapeutic strategy for the prevention and treatment of inflammatory diseases. In addition, enhancing the solubility and bioavailability of neferine through structural modifications or the development of nanomedicine formulations may also be beneficial. Additionally, the active components of *Nelumbo nucifera* should be further explored to promote and preserve the legacy of traditional Chinese medicine.

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Authors' contributions

HL and QZho designed and conceived the review. QZha and HL wrote the original draft of the manuscript. HL, QZha and QZho contributed to editorial changes in the manuscript. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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