Therapeutic targets of thunder god vine (*Tripterygium wilfordii hook*) in rheumatoid arthritis (Review)

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Abstract. Celastrol and triptolide, chemical compounds isolated from *Tripterygium wilfordii hook* (also known as thunder god vine), are effective against rheumatoid arthritis (RA). Celastrol targets numerous signaling pathways involving NF-κB, endoplasmic reticulum Ca^{2+}-ATPase, myeloid differentiation factor 2, toll-like receptor 4, pro-inflammatory chemokines, DNA damage, cell cycle arrest and apoptosis. Triptolide, inhibits NF-κB, the receptor activator of NF-κB (RANK)/RANK ligand/osteoprotegerin signaling pathway, cyclooxygenase-2, matrix metalloproteases and cytokines. The present review examined the chemistry and bioavailability of celastrol and triptolide, and their molecular targets in treating RA. Clinical studies have demonstrated that *T. wilfordii* has several promising bioactivities, but its multi-target toxicity has restricted its application. Thus, dosage control and structural modification of *T. wilfordii* are required to reduce the toxicity. In this review, future directions for research into these promising natural products are discussed.

Contents

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Key words: rheumatoid arthritis, thunder god vine, *Tripterygium wilfordii hook*, molecular targets, metabolism, bioavailability, celastrol, triptolide, signaling pathway

1. Introduction

Rheumatoid arthritis (RA) is a complicated disease caused by environmental and genetic factors that involves a hyperactive immune system and synovial inflammation in multiple joints, which if left untreated irreversibly destroys joints and leads to severe disability (1). The common drugs used to treat RA, including methotrexate, cannot prevent the disease and cause severe complications in many patients (2); therefore, the development of safer, cost-effective therapeutics is required.

RA involves the action of pro-inflammatory cytokines and chemokines produced by synoviocytes and infiltrating immune cells (3), including interleukin (IL)-1, IL-2, IL-3, IL-4, IL-6, IL-8, IL-17, interferon (IFN)-α and IFN-β, tumor necrosis factor (TNF)-α, transforming growth factor β, granulocyte-macrophage colony-stimulating factor and macrophage inflammatory protein (MIP)-3α (2,4-7). These cytokines and chemokines are able to activate NF-κB, upregulate the expressions of cyclooxygenase-2 (COX-2) and nitric oxide synthase, and promote the production of prostaglandin E2 (PGE2) and nitric oxide. Moreover, these changes contribute to synovial inflammation accompanied by arthrosis, swelling, hyperplasia, angiogenesis, bone destruction and arthritic decay (8,9). Natural products that target these molecules are less likely to induce adverse effects may be used as therapeutic agents owing to their high therapeutic potential (2,10-13). For example, bioactive natural compounds extracted from plants, such as polyphenolic compounds, commonly exert multiple therapeutic effects (14-17).

Tripterygium wilfordii hook (also known as thunder god vine) is a common plant species, which has been used for a variety of purposes in traditional Chinese medicine (TCM) (18). Previous studies in the TCM literature suggest that *T. wilfordii* can treat several autoimmune and inflammatory conditions including RA, and improvements to its efficacy and safety have been made (14,19).

Currently, >380 secondary metabolites have been isolated from *T. wilfordii*, and ≥350 of these are structurally diverse terpenoids with a wide range of pharmacological activities, including anti-inflammatory, immunosuppressive and anti-neoplastic effects (20). It has been shown that two diterpenoid

tri-epoxides, celastrol and triptolide (Fig. 1), are primarily responsible for the anti-inflammatory and immunosuppressive effects of *T. wilfordii* preparations (21,22). Furthermore, these two compounds are the most abundant and most pharmacologically active of the metabolites found in *T. wilfordii* extracts (23).

The present study critically reviews the chemistry, bioavailability, bioactivities, multi-target toxicities and molecular targets of celastrol and triptolide for the treatment of RA (Fig. 2; Table I). The present review also discussing future directions for research into the aforementioned promising natural products.

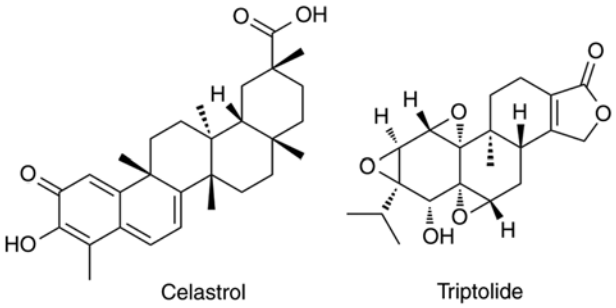

Figure 1. Molecular structures of celastrol and triptolide.

2. Metabolism and bioavailability

Oral administration of triptolide is recommended for to treat inflammation, autoimmune diseases and tumors (24). In rats, triptolide at a dose of 1 mg/kg exhibits a bioavailability of 81.6% after intravenous injection and 63.9% after oral administration (25). Moreover, it achieves a maximum concentration of 293.19±24.43 ng/ml within ~10 min. Triptolide distributes into the liver, followed by plasma, kidney, lung, spleen, heart and testicular tissue, and is quickly excreted through the biliary, urinary and fecal routes with a half-life of 0.42 h (26). Moreover, the metabolism and bioavailability of triptolide is not fully understood as clinical trials investigating the use of triptolide to treat RA have not been performed (27,28).

Celastrol has low aqueous solubility (13.25±0.83 μg/ml at 37˚C) and poor intestinal absorption, which results in low oral bioavailability and limits its clinical application (29). To overcome these constraints, the compound can be delivered using solid lipid nanoparticles, liposomes, micelles or nanostructured lipid carriers (30,31).

3. Molecular targets of celastrol

Celastrol inhibits activation of NF-κB by targeting inhibitor of NF-κB (IκB) kinase. NF-κB regulates the transcription of numerous genes that are involved in immune, inflammatory and anti-apoptotic responses (32). Furthermore, the function of NF-κB is regulated by rapid degradation of its endogenous inhibitory molecule IκB. Inflammatory stimuli, such as cytokines, initiate a signaling cascade that leads to the activation of two IκB kinases (IKK), IKK-1 and IKK-2, which then phosphorylate IκB at two N-terminal serine residues (33). The IKK complex is expressed in fibroblast-like synoviocytes (FLS) and is activated by IL-1 and TNF-α (34-36). These studies demonstrated that celastrol suppresses NF-κB activation by inhibiting IKK activity, possibly by targeting cysteine 179 in the activation loop of IKK (34,35,37). Moreover, celastrol inhibits the activation of NF-κB by targeting IκB kinase, which is not specific in the RA (38). It has been revealed that the NF-κB signaling pathway is involved in a number of diseases, including RA, systemic lupus erythematosus and ankylosing spondylitis (32,34-37). Moreover, myeloid differentiation factor 2 (MD2) and Toll-like receptor 4 (TLR4) are associated with RA, thus it is likely that celastrol targets MD2 (39).

Celastrol inhibits endoplasmic reticulum (ER) Ca^{2+}-ATPase. In RA, Ca^{2+} signaling mediates the expression of pro-inflammatory cytokines through autoreactive T- and B-lymphocytes after autoantigen stimulation (40). Various pumps maintain Ca^{2+} homeostasis, among which is the ER Ca^{2+}-ATPase, which can be inhibited by celastrol (41). Therefore, celastrol may alter Ca^{2+} signaling pathways to downregulate inflammatory response genes (42) and promote Ca^{2+}-mediated autophagic cell death (43,44), as shown by studies in RA-FLS. Thus, it is speculated that resistance of FLS to apoptosis may be a characteristic of RA.

Celastrol induces DNA damage, cell cycle arrest and apoptosis. Celastrol blocks RA-FLS at G2/M phase (45), which may be a potential mechanism for its ability to inhibit proliferation and induce apoptosis. It has also been demonstrated that celastrol inhibits cell cycle progression by blocking the association of cyclins with cyclin-dependent kinases (33). Furthermore, celastrol leads to an increase in cell division cycle protein 2 homolog (Cdc2) phosphorylation and downregulation of Cdc2 and Cyclin-b1, which may reduce the number of Cdk1-Cyclin-b1 complexes and arrest cells at the G2/M phase (45). Celastrol also increases phosphorylation of Cdc25, which may contribute to G2/M phase arrest (46).

It has been shown that celastrol activates cleaved caspases 3 and 9, as well as cleaved poly (ADP-ribose) polymerase, downregulates FasR and increases the Bax/Bcl-2 ratio (46). Therefore, celastrol may induce apoptosis in RA-FLS, which express a variety of death-inducing surface receptors of the TNF receptor family such as Fas/CD95, TNF-related apoptosis-inducing ligand-receptor (TRAIL-R1), TRAIL-R2 and TNF receptor 1 (47).

Celastrol targets MD2 and inhibits TLR4 activation. TLR4 exists as a complex with a co-receptor, MD2, in the plasma membrane of various immune cells (48). Celastrol blocks the most upstream step in TLR4 activation (49), and thus it likely targets MD2. Moreover, celastrol may function similar to the anti-inflammatory phytochemicals sulforaphane and caffeic acid phenethyl ester, which interfere with the interaction between lipopolysaccharide (LPS) and the TLR4/MD2 complex (50,51). Furthermore, it is speculated that celastrol may have intracellular targets, including MD2 and TLR4 (48,49).

Celastrol modulates pro-inflammatory chemokines. Chemokines are a superfamily of cytokines that are associated with cell migration and recruitment to sites of inflammation (5). Chemokines are categorized into four groups, CXC,

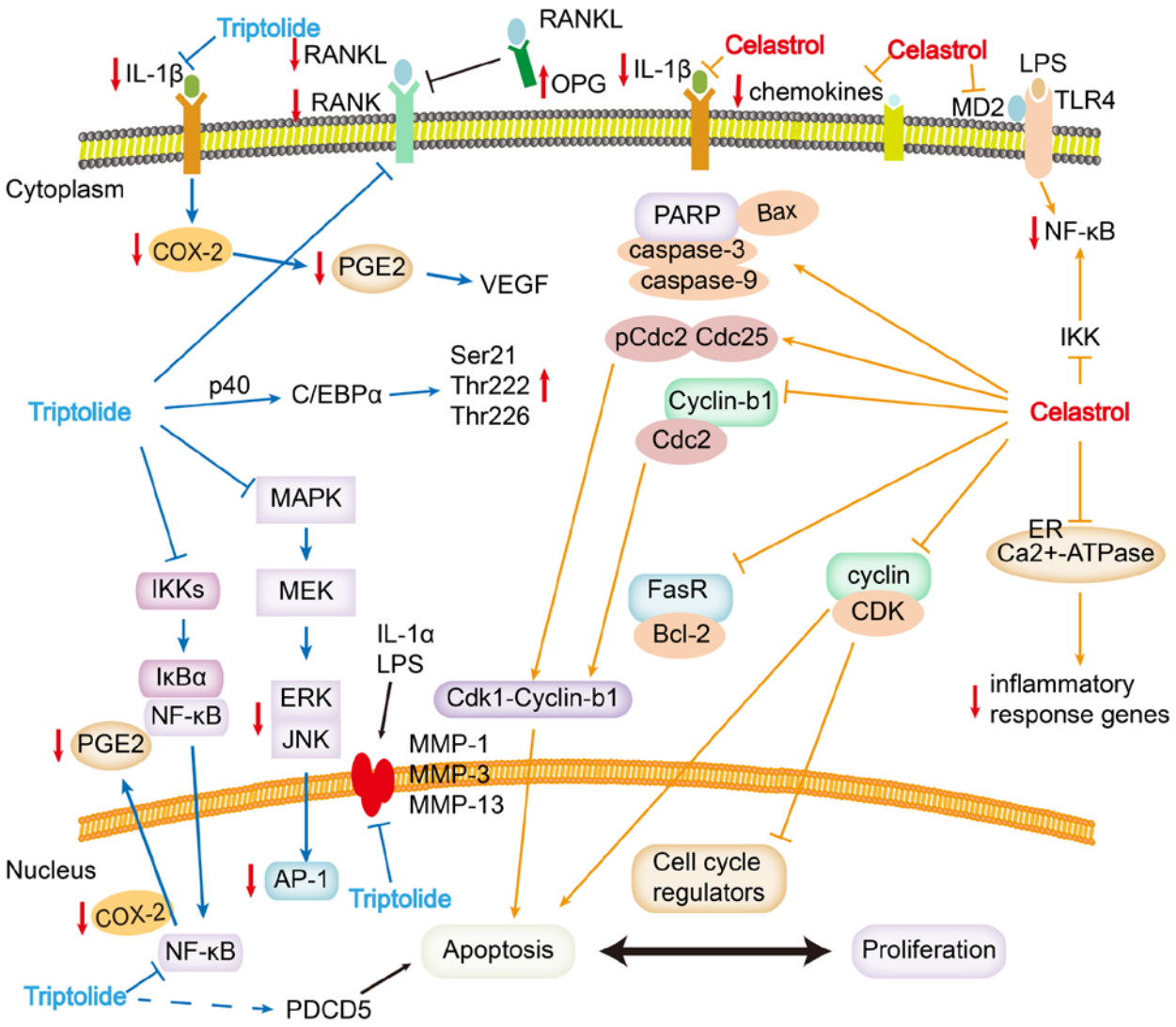

Figure 2. Schematic of the molecular targets of celastrol and triptolide in the treatment of RA. Arrows indicate activating effects; horizontal lines indicate inhibitory effects. Celastrol targets numerous signaling pathways associated with RA, including those involving NF-κB, endoplasmic reticulum Ca^{2+}-ATPase, MD2, TLR4, pro-inflammatory chemokines, DNA damage, cell cycle arrest and apoptosis. Triptolide inhibits NF-κB, RANKL/RANK/OPG signaling, COX-2, matrix metalloproteases and cytokines. AP-1, activating protein-1; C/EBPα, CCAAT/enhancer binding protein-α; cdc, cell division cycle protein; COX-2, cyclooxygenase-2; ER, endoplasmic reticulum; IκB, inhibitor of NF-κB; IKK, IκB kinase; IL, interleukin; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MD2, myeloid differentiation factor 2; MEK, MAPK/ERK kinase; MMP, matrix metalloproteinase; OPG, osteoprotegerin; p, phosphorylated; PARP, poly (ADP-ribose) polymerase; PDCD5, programmed cell death protein 5; PGE2, prostaglandin E2; RA, rheumatoid arthritis; RANK, receptor activator of NF-κB; RANKL, RANK ligand; TLR4, Toll-like receptor 4; VEGF, vascular endothelial growth factor.

CX3C, CC and C, based on the location of conserved cysteine residues (21). In inflammatory disorders such as RA, chemokines bind to their receptors leading to leukocyte trafficking into the joints, where leukocytes exacerbate inflammation and lead to pannus formation and tissue damage (4). Moreover, several chemokines have been implicated in RA and experimental arthritis, including T cell specific protein RANTES [RANTES; also known as C-C motif chemokine 5 (CCL5)], monocyte chemotactic protein-1 (MCP-1; also known as CCL2), MIP-1α (also known as CCL3) and growth-related oncogene/keratinocyte chemoattractant (GRO/KC) (51-53). It has been revealed that treating arthritic rats with celastrol significantly reduces expression levels of RANTES, MCP-1, MIP-1α and GRO/KC, as well as the pro-inflammatory cytokines TNF-α and IL-1β (54), and this may inhibit leukocyte migration into joints (55).

4. Molecular targets of triptolide

Triptolide inhibits the NF-κB pathway. The NF-κB family comprises Rel domain-containing proteins that regulate inflammatory and immune responses (56). In resting cells, these proteins are retained in the cytosol by a group of inhibitory proteins, such as IκBα (57). Upon activation, IKKs, including IKK-1 and IKK-2, phosphorylate IκBα, which is subsequently ubiquitinated and destroyed by the proteasome. This liberates NF-κB to translocate into the nucleus, where it activates several genes associated with RA (56). It has been shown that triptolide regulates IKK-1 and IKK-2 activities induced by various stimuli (57). The purified *T. wilfordii* component PG490 inhibits both IKK-1 and IKK2 activities with similar potency (58). As NF-κB transcription factors upregulate the expression of several genes involved in

Table I. Molecular targets, signaling pathways and potential biological effects of celastrol and triptolide in the treatment of rheumatoid arthritis.

A, Celastrol

Molecular targets	Signaling pathways	Potential biological effects	(Refs.)
IκB kinase	NF-κB	NF-κB function is regulated via rapid degradation of its endogenous inhibitory molecule IκB	(32-39)
Ca^{2+}-ATPase	Ca^{2+} signaling	Alter Ca^{2+} signaling pathways to downregulate inflammatory response genes	(40-44)
Cyclins with CDKs	DNA damage, cell cycle arrest and apoptosis	Inhibit cell cycle progression by blocking the association of cyclins with cyclin-dependent kinases	(33,45-47)
MD2 or TLR4	Interaction between LPS and the TLR4/MD2 complex	Block the most upstream step in TLR4 activation, maybe targets MD2.	(48-51)
Pro-inflammatory chemokines	Reduces expression levels of RANTES, MCP-1, MIP-1α and GRO/KC, as well as the pro-inflammatory cytokines tumor necrosis factor-α and IL-1β	Inhibit leukocyte migration into joints	(4,5,51-55)

B, Triptolide

Molecular targets	Signaling pathways	Potential biological effects	(Refs.)
IκB kinase	NF-κB	Rapid degradation of its endogenous inhibitory molecule IκB	(56-64)
RANKL	RANKL/RANK/OPG signaling	Reduce the number of osteoclasts in areas of bone destruction by downregulating RANKL and RANK, and upregulating OPG	(65-68)
COX-2 and matrix metalloproteinases	NF-κB	Downregulate COX-2 and PGE2, and alleviate LPS-induced inflammation	(69-79)
Cytokines	Cytokines pathway	Inhibit cytokines	80-83
VEGF	Infiltration of the synovial membrane	Inhibit several downstream effects of IL1-β, including cell adhesion of human FLS, upregulate several angiogenic activators and activate MAPK signaling	(5,84-86)

MD2, myeloid differentiation factor 2; TLR4, Toll-like receptor 4; RANK, receptor activator of NF-κB; RANKL, RANK ligand; OPG, osteoprotegerin; COX-2, cyclooxygenase-2; IL, interleukin; IκB, inhibitor of NF-κB; RANTES, regulated upon activation, normally T cell expressed and secreted chemokine protein; VEGF, vascular endothelial growth factor; MAPK, mitogen-activated protein kinase; PGE2, prostaglandin E2; GRO/KC, growth-related oncogene/keratinocyte chemoattractant; MCP-1, monocyte chemotactic protein-1; MIP-1α, macrophage inflammatory protein-3α. LPS, lipopolysaccharide.

inflammatory responses, the targeting of components of NF-κB signaling is a major therapeutic strategy for treating autoimmune diseases (59).

Similar to NF-κB, activating protein-1 (AP-1) transcription factors, comprising Jun and Fos family proteins, regulate cell proliferation, transformation and death, and may be potential therapeutic targets for the control of inflammation (60-62). Triptolide also inhibits mitogen-activated protein kinase (MAPK)/AP-1 signaling pathways, effectively suppressing MAP kinases, including JNK, p38 and ERK activities (59). Therefore, triptolide is a promising candidate immunomodulatory drug for autoimmune disorder therapy (63,64).

Triptolide alters RANKL/RANK/OPG signaling. Osteoclasts are the primary bone resorptive cells, and are located mainly in the synovial inflammatory tissue; RANKL stimulates osteoclast-mediated bone destruction in RA by binding to its receptor RANK (65). Under physiological conditions, osteoblasts and activated T cells express RANKL, which binds to RANK on osteoclasts to trigger osteoclast maturation and bone resorption. Osteoblasts counteract the action of osteoclasts in the balance between bone formation and destruction; osteoblasts express osteoprotegerin (OPG), which 'mops up' RANKL and prevents it from binding to RANK, thus inhibiting bone resorption (66). However,

under pathological conditions such as RA, this balance is shifted toward bone destruction (67). In mice with collagen-induced arthritis, triptolide significantly reduces the number of osteoclasts in areas of bone destruction by downregulating RANKL and RANK, and upregulating OPG (68).

Triptolide inhibits COX-2 and matrix metalloproteinases (MMPs). It has been revealed that injury, tumorigenesis and invasion from the joint into multiple organs upregulate COX-2 via NF-κB to produce prostaglandins, which induce inflammation and increase capillary permeability (69). Moreover, triptolide downregulates COX-2 and PGE2, thus alleviating LPS-induced inflammation (70).

MMPs participate in tumorigenesis, tumor metastasis and inflammatory diseases such as RA (57,71-75). In human synovial fibroblasts and mouse macrophages, triptolide inhibits IL-1α-induced phosphorylation of MMP-1 and LPS-induced phosphorylation of MMP-3. By inhibiting MMP-3 and MMP-13, triptolide slows the degradation of extracellular matrix and cartilage degeneration in mice with collagen-induced arthritis, as well as in primary human osteoarthritis and bovine chondrocytes (26,76-79).

Triptolide inhibits cytokines. Antigen-presenting cells produce IL-12 and IL-23, which are heterodimeric cytokines sharing a p40 subunit; these cytokines stimulate the generation and functions of T helper (Th)1 and Th17 cells, respectively. These cytokines are involved in the pathogenesis of several autoimmune disorders, including RA and systemic lupus erythematosus (80). Triptolide downregulates p40, in part, by activating the expression and phosphorylation of CCAAT/enhancer binding protein-α (C/EBPα) via the kinases ERK1/2 and AKT-glycogen synthase kinase 3β (81). This phosphorylation allows C/EBPα to bind antagonistically to the p40 promoter (81). Furthermore, programmed cell death 5 enhances the ability of triptolide to induce FLS apoptosis in RA, and therefore may be a potential therapeutic target in RA (29,82,83).

Triptolide targets vascular endothelial growth factor (VEGF). VEGF-driven angiogenesis promotes RA progress by allowing inflammatory cell infiltration of the synovial membrane (5). Triptolide prevents the formation of new blood vessels *in vitro* and *in vivo*, and it inhibits several downstream effects of IL-1β, including cell adhesion of human FLS in RA and human umbilical vein endothelial cells (HUVECs) (84). Furthermore, triptolide upregulates several angiogenic activators, including TNF-α, IL-17, VEGF, VEGFR, Angiopoietin (Ang)-1, Ang-2 and Tie2, and activates the MAPK signaling pathway involving phosphorylated (p)-ERK, p-p38 and p-JNK (85). Moreover, triptolide disrupts tube formation in HUVECs on Matrigel, and suppresses VEGF-induced chemotactic migration of HUVECs and human FLS in RA (86).

5. Adverse effects of *T. wilfordii*

T. wilfordii is a Chinese herb that has been traditionally used in clinics for RA treatment (3). Numerous preclinical studies have demonstrated that extracts from *T. wilfordii* roots inhibit the expression levels of RA-related inflammatory factors secreted by macrophages, lymphocytes, synovial fibroblasts and chondrocytes (87-91). Moreover, *T. wilfordii* suppresses lymphocytes and synovial fibroblast proliferation by inducing apoptosis (46). The anti-angiogenesis property of synovial fibroblasts has been shown in a previous study (83). Although *T. wilfordii* has several promising bioactivities *in vivo* and *in vitro,* its multi-target toxicity has restricted its clinical application (88). Data from the China Food and Drug Administration catalogue at least 633 instances of adverse reactions (53 severe) involving reproductive organ, liver and renal toxicity. Furthermore, clinical studies have concluded that *T. wilfordii* can damage the digestive system, including liver injury and stomachache, as well as the endocrine and reproductive systems (30,32,92). Moreover, 271 patients with RA have reported digestive tract symptoms and irregular menstruation. As a compound of *T. wilfordii,* triptolide-induced toxicity was shown to be dependent on dosage and administration times (30,93). To avoid toxicity, previous studies have attempted to alter the dosage and structure, and to assess its compatibility with other drugs (34). For example, Freag *et al* (94) developed self-assembled celastrol phytosomal nanocarriers (celastrol-PHY) to improve celastrol solubility and oral bioavailability; these were confirmed through an *in vitro* release study and a pharmacokinetic study in rabbits (94). Structural modification and alternation of triptolide can produce derivatives of triptolide with lower toxicity and relative higher activity. Apart from structural alterations, the development of novel triptolide delivery systems is a valuable strategy to improve water solubility, and the efficiency of absorption and metabolism, and to reduce toxicity (95).

6. Conclusions

Celastrol and triptolide from *T. wilfordii* are effective against RA; they target numerous signaling pathways, proteases and cytokines. The present review examined the chemistry and bioavailability of celastrol and triptolide, and their molecular targets in treating RA, which may be potential effective drugs. However, owing to the strong toxicity of *T. wilfordii*, novel approaches are required for the safe application of this TCM. These may include investigating new triptolide formulations or its combination with other drugs. Furthermore, defining early toxicity markers, investigating dosage ranges for different target organs and establishing a toxicity warning system are required.

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Availability of data and materials

Not applicable.

Authors' contributions

XS conceived and designed the study, and wrote the manuscript. YZ and ED analyzed the data. All authors read and approved the final manuscript.

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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