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Anti-inflammatory Effects and Underlying Mechanisms of Epimedium Extracts*

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Abstract Herba Epimedii (yinyanghuo in Chinese) is a commonly used traditional Chinese medicine. Flavonoids are the major effective ingredient of Herba Epimedii, icariin is the most abundant component in Epimedium flavonoids, and icaritin is the metabolite of icariin. In the last decade, a number of studies on the pharmacological effects of Epimedium extracts have been conducted. Emerging evidence has shown that Epimedium flavonoids, especially icariin and its derivatives, have anti-inflammatory effects in the animal and cellular models on a variety of diseases related to chronic inflammation, including osteoporosis, osteoarthritis, neurological and psychiaric disorders, atherosclerosis, asthma and lung diseases, inflammatory bowel diseases, kidney diseases, skin diseases, autoimmune diseases, and cancer. The molecular mechanisms involved in anti-inflammation effects of icariin and its derivatives include reducing inflammatory cytokines, down-regulating NF-κB signaling, NLRP3/caspase-1/IL-1β axis, STAT signaling and MAPK pathway, as well as up-regulating Nrf2/ARE/HO-1 pathway and increasing glucocorticoid receptor and estrogen receptor signaling. In this review, we have updated the recent advances in this field, and these studies have suggested that Epimedii and its active compounds have potential to treat multiple diseases related to chronic inflammation.

Key words herba Epimedii, traditional Chinese medicine, inflammation, molecular mechanism
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Herba Epimedii is the dried leaves of medicinal plants Epimedii, named yinyanghuo in Chinese. According to the Chinese Pharmacopoeia, five Epimedii species are classified as *Epimedii*, including *Epimedium brevicornum* Maxim, *Epimedium sagittatum* (Sieb & Zucc.) Maxim, *Epimedium pubescens* Maxim, *Epimedium wushanense* T.S.Ying, and *Epimedium koreanum* Nakai. Herba Epimedii was first recorded in Shen Nong Ben Cao Jing, the oldest book of materia medica in China, about 2 000 years ago. Herba Epimedii has the effects of tonifying "the kidney and yang", strengthening muscles and bones, and relieving rheumatic conditions in the theory of traditional Chinese medicine (TCM), so it is used for the treatment of impotence, seminal emission, weakness of the limbs, and rheumatoid arthralgia according to the Chinese Pharmacopoeia. Flavonoids are the major effective ingredient of Herba Epimedii, and icariin is the most abundant component in Epimedium flavonoids. It has been reported that icariin can be transformed by human intestinal microflora, and its metabolites include icariside II, icaritin and desmethylicaritin[1]. Modern pharmacological studies have proved that Epimedium flavonoids and icariin have multiple beneficial biological effects, including anti-inflammatory,

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antioxidant and anti-apoptotic effects *in vivo* and *in vitro*[2-4].

Inflammation is associated with many diseases, including neurological and psychiaric disorders, metabolic syndrome and diabetes, atherosclerosis, osteoarthritis and osteoporosis, inflammatory bowel disease, kidney diseases, autoimmune diseases and cancer. After recognition of noxious stimuli such as pathogens or cellular and tissue damage, the innate immune receptors would initiate the inflammatory process, which is part of a complex biological immune response to protect the body. Inducers of inflammation trigger the production of numerous inflammatory mediators, such as inflammatory cytokines. These inflammatory mediators are over-expressed by stimulating factors such as lipopolysaccharide (LPS) or in chronic inflammatory response. Increased inflammatory mediators are in turn associated with increased oxidative stress and other maladaptive response, causing autoimmune diseases and other inflammatory disorders[5].

The present review summarizes the studies conducted in the last decade about the anti-inflammation effects and underlying mechanisms of Epimedii and its effective constituents in multiple diseases related to inflammation, so as to provide the reference for the further drug research and development.

1 Anti−inflammatory effects of Epimedium extracts in various diseases

1.1 Bone diseases

Inflammatory responses cause excessive bone resorption and disease progression by the interactions between the inflammatory mediators and the bone remodeling in chronic inflammatory diseases such as osteoporosis, osteoarthritis, septic and rheumatoid arthritis, and intervertebral disc degeneration. Thus, the inflammatory activity is one of top risk factors, and optimal control of inflammation is part of the prevention of these diseases[6].

1.1.1 Osteoporosis and bone loss

Osteoporosis is a progressive systemic skeletal disease characterized by osteopenia and microstructural degeneration of bone tissue. Estrogen plays a role in preventing inflammation induced osteoclast development and osteopenia, which are commonly medicated by TNF-α, IL-1β, macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor kappa beta ligand (RANKL) [7]. Thus, anti-inflammatory agents may have the beneficial effects on bone healing. In a recent study, sixteen different TCM plant extracts were tested for osteogenic and osteoclastic activities on an osteoporosis murine model. In addition, the results showed that extracts from Epimedium brevicornu inhibited the IL-1β-mediated activation of NF-κB and maintained bone mineral density in the ovariectomized rats[8]. In LPS-treated osteoblasts and osteoclasts co-culture cell model, icariin exhibited inhibitory effects on osteoclasts differentiation and inflammatory bone loss by suppressing activation of the p38 and c-Jun NH_2-terminal kinase (JNK) pathway[9]. Moreover, this result was verified in other researches using RANKL-induced osteoclast formation model in murine macrophages[10] and LPS-stimulated bone loss in mesenchymal stem cells[11]. These results highlight the potential use of icariin as a therapeutic agent in the treatment of bacteria-induced bone loss diseases and osteoporosis.

Moreover, icariin represented a potential treatment for wear particle-induced osteolysis with inhibitory effects on inflammatory osteoclastogenesis in titanium particle-induced osteolysis models *in vivo* and *in vitro*[12-13]. Besides, Baohuoside I, another active component of Epimedium flavonoids, inhibited the expression of inflammatory cytokines in the tissues and showed anti-osteoporotic activity in osteoporosis rat model induced by ovariectomy[14].

1.1.2 Osteoarthritis

Osteoarthritis (OA) is the most prevalent arthritic disease and a leading cause of disability, characterized by progressive cartilage degradation, osteophyte formation, and joint stiffness. Low-grade inflammatory is considered highly associated with OA, involves the interplay of the innate immune system and inflammatory mediators[15]. In TNF-α-induced chondrocytes and OA patient-derived human fibroblast-like synoviocytes, icariin protected against OA by suppressing inflammatory cytokines and apoptosis, through activation of autophagy *via* NF-κB inhibition[16-17]. In another study, LPS-treated chondrocytes and monosodium iodoacetate-treated Wistar rats were used as models of OA *in vitro* and *in vivo*, respectively. The results indicated that icariin suppressed LPS-induced inflammation and inhibited OA *via* repressing NLRP3/caspase-1 signaling-

mediated pyroptosis in models of OA[18]. In a bioinformatics analysis investigating the KEGG pathways of icariin-targeted genes involved in OA, the results showed that icariin played a role in OA by regulating inflammatory cytokine production, cell survival and insulin resistance through modulation of the NF-κB, mitogen-activated protein kinase (MAPK), and Akt signaling pathways[19].

1.1.3 Septic arthritis and intervertebral disc degeneration

Septic arthritis is an inflammatory arthropathy characterized by degeneration of articular cartilage and causing permanent joint damage. In LPS-simulated chondrocytes mimicking inflammatory response during septic arthritis, icariin protected chondrocytes from LPS-induced inflammation and extracellular matrix degradation[20]. Intervertebral disc degeneration is a main cause of low back pain, characterized by highly expression of pro-inflammatory cytokines and loss of the extracellular matrix. Icariin showed anti-inflammatory effects and protection on IL-1β-stimulated nucleus pulposus cells, which indicated the potential therapeutic action of icariin in intervertebral disc degeneration[21].

These results demonstrated that Epimedium extracts had beneficial effects on various bone diseases, which is consistent with "strengthening muscles and bones" in the theory of TCM, proving that the clinical experience of TCM for thousands of years is correct and valuable. Modern pharmacology studies further elucidate their mechanisms of action.

1.2 Neurological and psychiaric disorders

Epimedii and its main component icariin have shown anti-inflammation effect in nervous system both *in vitro* and *in vivo*, which may contribute to the preventive and/or therapeutic benefits in various neurological and psychiaric disorders, such as Alzheimer's disease, Parkinson's disease, depression, multiple sclerosis, cerebral ischemia, and spinal cord injury[22]. In LPS-activated microglia, icariin could inhibit the release of nitric oxide (NO), prostaglandin E (PGE) -2 and reactive oxygen species (ROS), decrease the protein expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2, and reduce the mRNA expression of proinflammatory cytokines such as tumor necrosis factor (TNF) - α, interleukin (IL) -1β and IL-6[23-24]. *In vivo*, icariin improved spatial learning and memory abilities in rats with brain dysfunction induced by LPS, and decreased the levels of TNF-α, IL-1β and COX-2[25]. Icariside II, an active component of Epimedium flavonoids and also a metabolite of icariin in intestinal microflora, was found to attenuate LPS-induced neuroinflammation through inhibiting Toll-like receptor 4 (TLR4) / myeloid differentiation factor 88 (MyD88) / nuclear factor-kappa B (NF-κB) pathway in rats[26].

1.2.1 Alzheimer's disease

Alzheimer's disease (AD) is a common neurodegenerative disease, characterized by progressive deterioration in cognitive functions. Extracellular senile plaques consisted of amyloid-β (Aβ) and intracellular neurofibrillary tangles (NFT) accumulated by hyper-phosphorylated tau (p-tau) are considered as two hallmarks of AD. Both Aβ and p-tau protein can lead to neuroinflammation in AD brain, and eventually promote neuron death and cognitive function decline[27]. Our study showed that Epimedium flavonoids significantly improved the learning and memory ability, inhibited the activation of microglia and astrocytes, decreased IL-1β and TNF-α content, inhibited COX-2 expression, and increased glutathione content and superoxide dismutase (SOD) activity in the hippocampus and cerebral cortex of $A\beta_{1-40}$ intracerebroventricular injection mouse model[28-29]. Other investigators found that administration of icariin ameliorated cognitive deficits in APP/PS1 transgenic mice, *via* modulating the differentiation of $CD4^+$ T cells and the release of inflammatory cytokines in plasma and brain tissue[30] and/or attenuating microglial activation and transforming growth factor (TGF) - β1 immunoreactivity at amyloid plaques in brain[31]. Moreover, icariin attenuated M1 activation of microglia and Aβ plaque accumulation by up-regulating peroxisome proliferators-activated receptor γ (PPARγ), and ameliorated the depressive-like behaviors and spatial memory damage in restraint/isolation-stressed APP/PS1 mice[32]. Treatment with icariside II also exhibited anti-neuroinflammation effects by inhibiting microglial and astrocytic activation, and/or reducing expression of inflammatory cytokines on AD models induced by injection of Aβ[33], LPS[34] or streptozotocin[35].

1.2.2 Parkinson's disease

Parkinson's disease (PD) is a multifactorial

neurodegenerative disease characterized by depleted dopamine (DA) level, α-synuclein aggregation and the death of dopaminergic neurons in the substantia nigra. Increasing evidence suggests an essential role of inflammation in driving the initiation and progression of PD[36]. In 6-hydroxydopamine (6-OHDA) -induced PD mouse model, icariin treatment attenuated glial cells-mediated neuroinflammation and evoked dopaminergic neuroprotection *via* a nuclear factor erythroid-2-related factor 2 (Nrf2) -dependent manner[37]. Icariin could protect DA neurons against 6-OHDA and LPS-induced neurotoxicity both *in vivo* and *in vitro*, which might be closely associated with the inhibition of microglia-mediated neuroinflammation[38].

1.2.3 Depression

Depression is a chronic, multi-causal and life-threatening psychiatric disorder. Accumulating evidence has revealed that depression is obviously correlated with inflammation processes, and anti-inflammatory treatment has beneficial effects on depression[39]. In LPS-induced and social defeat (SD) stress inflammation mouse model, icariin and its derivative icaritin ameliorated neuroinflammation in hippocampus *via* suppressing high mobility groupprotein box 1 (HMGB1) - receptor for advanced glycation end products (RAGE) signaling, resulting in improving depressive behaviors in mice[40]. In an unpredictable chronic mild stress (CMS) model of depression in rats, icariin showed anti-inflammatory effects on the brain tissue *via* the inhibition of NF-κB signaling activation and the nod-like receptor protein 3 (NLRP3) inflammasome/caspase-1/IL-1β axis, resulting in improvement of physical state of CMS depression model[41].

1.2.4 Ischemic stroke and spinal cord injury

Stroke is one of the largest causes of death and permanent disability worldwide. Inflammation post stroke or cerebral ischemia-reperfusion injury is well known to contribute to the expansion of the ischemic lesion, and influence the susceptibility of stroke patients to overcome the disease[42]. Icariin and Icariside II treatment showed neuroprotective effect on ischemic stroke through inhibition of inflammatory responses mediated by NF-κB, PPARα and PPARγ in middle cerebral artery occlusion (MCAO) model rats and mice[43-44].

Spinal cord injury is a severe, often life-threatening, and debilitating clinical condition. Apart from the immediate injury, the pathophysiological events consist of a cascade of vascular, inflammatory, biochemical, and glial processes, causing neuron death and progressive degeneration. In a mouse model of spinal cord injury, icariin enhanced motor recovery through inhibiting oxidative stress, neuronal apoptosis and pro-inflammatory factors such as IL-1β, TNF-α and NO[45].

1.3 Cardiovascular diseases

Inflammatory response is involved in the pathogenesis of several common forms of cardiovascular diseases, such as myocarditis, cardiomyopathy, myocardial infarction, atherosclerosis, and diabetic vascular disorders. Several articles have indicated that icariin can inhibit the inflammatory response and subsequent injury in cardiovascular disease[46].

1.3.1 Heart diseases

The inflammatory response is involved in pathogenesis of several heart diseases, and prolonged inflammation commonly leads to additional cardiomyocyte loss and cardiac injury. In LPS-stimulated H9c2 rat cardiomyocytes, icariin reduced the mRNA expression of TNF-α, IL-1β and IL-6 by inhibition of the reactive oxygen species-dependent JNK/NF-κB pathway[47]. Moreover, icariin showed protective effects on a takotsubo syndrome like model (an acute cardiac syndrome associated with sudden physical or psychiatric episodes) induced by selective β-adrenergic agonist isoprenaline. Icariin prevented isoprenaline-induced takotsubo-like cardiac dysfunction, through reducing pro-inflammatory factor secretion and suppressing Toll-like receptor 4/NF-κB signaling[48]. Icaritin inhibited the pro-inflammatory cytokine TNF-α production, increased the anti-inflammatory cytokine IL-10 level, improved cardiac function, and limited infarct size following myocardial ischemia and reperfusion injury[49]. Another flavonoid compound Icariside II improved left ventricular function and decreased the left ventricular myocardial collagen area in spontaneously hypertensive rats through suppression of NF-κB signaling and the TGF-β1/Smad2 signaling pathway[50].

1.3.2 Atherosclerosis

Atherosclerosis is a chronic vascular inflammatory disease, characterized by rigidity and narrowing of the lumen because of endothelial

dysfunction, cholesterol and lipid accumulation. Inflammatory cells including monocytes and macrophages in atherosclerotic plaque are playing a key role in the pathogenesis of atherosclerosis, *via* secreting pro-inflammatory cytokines and fibrous cap-degrading matrix metalloproteinase[46]. In aged murine aortic endothelial cell and heart tissue, icariin had an inhibitory effect on the mRNA levels of inflammatory factors TNF-α, intercellular cell adhesion molecule-1 (ICAM-1), IL-2, and IL-6 through NF-κB signaling pathway[51]. In a high-cholesterol diet (HCD)-induced rat model, icariin inhibited the HCD-induced dyslipidemia, partly through the anti-inflammation effects such as decreasing the serum and tissue mRNA levels of IL-6 and TNF-α, and down-regulating p38 MAPK signaling pathway[52].

1.3.3 Diabetic vascular disorders

Endothelial dysfunction induced by high glucose is closely associated with the development and progression of diabetic complications, such as diabetic cardiovascular disorders. Inflammation is a key factor in the onset and progression of endothelial dysfunction, interplayed with oxidative stress and cell apoptosis[53]. In skeletal muscle C2C12 cells, icariin improved palmitate-induced skeletal muscle insulin resistance through a proteasome-dependent mechanism, and inhibited inflammation and STAT3 phosphorylation[54]. In human umbilical venous endothelial cells, icariin exerted a beneficial effect on high glucose-induced endothelial dysfunction by decreasing the expression levels of inflammatory factors and cell adhesion molecules[55]. In autoimmune-mediated destruction of insulin-producing pancreatic β cells, icariin abrogated the pro-apoptotic effect of inflammatory cytokines and significantly suppressed the activation of NF-κB[56]. *In vivo* study found that icariside II improved diabetic cardiomyopathy in streptozotocin-induced diabetic rats by activating the Akt/NOS/NF-κB pathway and reducing inflammation[57].

1.4 Asthma and Lung diseases

Asthma is a common, chronic respiratory inflammatory disease of the lung and airway, involving many cellular elements and cells[58]. In ovalbumin-induced murine asthma models, icariin alleviated the infiltration of inflammatory cells and cytokines, reduced airway hyper-responsiveness through regulating Th1/Th2 or Th17/Treg imbalance[59-60]. Moreover, in an asthma mouse model established by ovalbumin (OVA) sensitization and respiratory syncytial virus (RSV) infection, icariin decreased the degree of airway inflammation, reduced leukocytes and inflammatory cells infiltration[61]. In another ovalbumin-induced murine model of asthma combined with psychosocial stress, icariin reduced the anxiety behavior, reversed airway hyper-responsiveness, and reduced inflammatory cytokine infiltration to the lung[62].

Apart the protective effects on asthma, icariin decreased mRNA expressions of IL-6, COX-2 and iNOS, inhibited secretion of TNF-α, PGE2 and NO, and suppressed NF-κB p65 activation of LPS-induced acute inflammatory responses in lung tissue and macrophages, indicating the protective effects of icariin on acute lung injury[63]. Besides, icariin also showed effects on chronic obstructive pulmonary disease by decreasing inflammatory cells and production of TNF-α, IL-8 and matrix metalloproteinase-9 (MMP-9) in cigarette smoke-induced inflammatory model in mice[64].

1.5 Kidney diseases

1.5.1 Chronic kidney disease

Chronic kidney disease (CKD) is a chronic disease and global health burden with irreversible pathological processes that causes the loss of renal function, tubulointerstitial fibrosis and end-stage renal disease. During the progress of CKD, injured cells attract the infiltration of inflammatory cells, and accelerate the progression of renal fibrosis. Chronic inflammation is considered to be a common comorbid condition in CKD[65]. In a unilateral ureteral obstruction mouse model, icariin protected against CKD-associated renal fibrosis *via* its antifibrotic and anti-inflammatory properties[66]. In adenine-induced chronic renal failure rat model, icariin combined with human umbilical cord mesenchymal stem cells (huMSCs) decreased inflammatory responses, promoted the expression of growth factors, and protected injured renal tissues[67].

1.5.2 Acute renal damage

Acute renal damage, characterized by acute tubular cell injury and kidney dysfunction, mainly develops following toxic insults such as cisplatin. Cisplatin, an effective and commonly used chemotherapeutic agent, could selectively accumulate and damage in proximal tubular cells, contributing to

nephrotoxicity that seriously limits its clinical application[68]. In acute renal injury mouse model induced by cisplatin, icariin ameliorated the nephrotoxicity *via* improving renal oxidant status, consequent NF-κB activation and inflammation cascade and apoptosis[69]. *In vitro*, icariin prevented cisplatin-induced human embryonic kidney (HEK)-293 cell injury by inhibiting oxidative stress, inflammatory response, and cellular apoptosis partly *via* regulating NF-κB and PI3K/Akt signaling pathways[70].

Acute kidney injury is a frequent complication of sepsis, a complex dysregulated inflammatory response to infection. In a mouse model of cecal ligation and perforation (CLP)-induced sepsis, icariin improved sepsis-induced mortality and acute kidney injury by inhibiting renal oxidant damage, inflammatory responses, apoptosis, and vascular permeability[71].

1.6 Skin diseases

Atopic dermatitis is a common, chronic inflammatory skin disease, due to an immune reaction and inflammatory responses in keratinocytes. In human keratinocytes (HaCaT cells), icariin reduced TNF-α/IFN-γ-induced IL-6, IL-8, IL-1β, and monocyte chemo-attractant protein-1 (MCP-1) production *via* inhibition of the substance P and p38-MAPK signaling pathway[72]. In another study, icariin treatment blocked the secretion of the inflammatory cytokines and prevented initiation of steroidogenesis in TNF-α/IFN-γ-stimulated HaCaT cells[73]. Besides, icariin inhibited inflammation and necrosis in skin flap, which is used widely in burn injury repair and reconstruction, plastic surgery, and tissue defect repair[74]. Ultraviolet (UV) radiation is a key risk factor facilitating the initiation and development of various skin diseases. In ultraviolet B-irradiated human keratinocytes, icariin and icaritin inihibited ultraviolet B-induced oxidative stress, inflammation and photoaging, indicating the possible use of icariin and icaritin on this kind of skin disease[75].

1.7 Autoimmune disease

Autoimmune disease refers to the disease caused by the immune reactions of the body to the autoantigen and the damage. Inflammation plays an important role in the pathogenesis of some autoimmune diseases, such as systemic lupus erythematosus (SLE), rheumatoid arthritis, multiple sclerosis, and inflammatory bowel disease.

1.7.1 Multiple sclerosis

Multiple sclerosis (MS) is an autoimmune disease characterized by inflammatory demyelination of the central nervous system. In MS or experimental autoimmune encephalomyelitis (EAE, *in vivo* model for MS), immune effector mechanisms such as clonal expansion of B cells, $CD8^+$ cytotoxic T lymphocytes, microglia and astrocyte activation can in turn stimulate microglia/astrocytes to increase their activities and release more proinflammatory cytokines and chemokines[76]. Our study found that Epimedium flavonoids ameliorated neurological deficits, alleviated demyelination and inflammatory infiltration in the brain and spinal cord of EAE model rats *via* inhibiting astrocytes activation and decreased the levels of proinflammatory molecules such as IL-1β, TNF-α, NO and NF-κB, meanwhile enhanced the expression of nerve growth factor (NGF) and increased the number of oligodendrocytes[77-78]. Other mechanism studies showed that icariin ameliorated EAE *via* suppressing Th1 and Th17 cell differentiation[79], or mediating estrogen receptor β (ERβ), modulating hypothalamic–pituitary–adrenal (HPA) function and up-regulating the expression of glucocorticoid receptor (GR) in cerebral white matter[80].

1.7.2 Inflammatory bowel diseases

Inflammatory bowel disease (IBD), a term for two conditions (Crohn's disease and ulcerative colitis), is an autoimmune disease characterized by a chronic inflammation of the gastrointestinal tract with unknown etiology, and triggered by numerous inflammatory cytokines[81]. In LPS-induced intestinal epithelial cell injury model, icariin and phosphorylated icariin increased cell viability reduced the levels of IL-1β, IL-6, IL-8 and TNF-α, and attenuated oxidative stress, apoptosis and intestinal permeability[82]. In intestinal inflammation mouse model induced by dextran sulfate sodium, icariin attenuated the disease progression and alleviated the pathological changes of colitis, as well as inhibited the production of pro-inflammatory cytokines and Th1/Th17 responses *via* suppression of STAT1 and STAT3 activation[83]. Icariin also reduced the colonic injury and the inflammatory response in ulcerative colitis rat model induced by trinitrobenzene sulfonic acid/ethanol[84]. Moreover, icaritin, an intestinal metabolite of icariin, exhibited significant anti-

inflammatory effects that might be mediated through the regulation of inflammatory cytokines and phosphorylation of p38 and JNK in LPS-stimulated mouse peritoneal macrophages *in vitro* and zymosan-induced peritonitis model *in vivo*[85]. These results indicated that icariin might be developed as a potential drug for treatment of inflammatory bowel disease.

1.7.3 Rheumatoid arthritis and lupus nephritis

Rheumatoid arthritis is a chronic autoimmune inflammatory disease that causes deformity of the joints and physical disability. In type II collagen-induced arthritis mouse model, icariin decreased Th17 cells and suppressed the production of IL-17 through the inhibition of signal transducer and activator of transcription 3 (STAT3) activation, contributing to the alleviated rheumatoid arthritis[86].

Lupus nephritis is a kidney inflammatory disease caused by systemic lupus erythematosus. Icariin alleviated murine lupus nephritis *via* inhibiting NF-κB activation pathway and NLRP3 inflammasome in MRL/lpr mouse model developing systemic lupus erythematosus-like phenotype[87]. Immunoglobulin A nephropathy (IgAN) is a common form of glomerulonephritis, characterized by glomerular proliferation and renal inflammation. In IgAN rats, icariin ameliorated renal damage *via* inhibition of NF-κB-mediated NLRP3 inflammasome activation[88].

1.8 Cancer

The imbalance between inflammation and immune suppression involves in many diseases, including autoimmunity and cancer. Inflammatory microenvironment induced by immune cells is favorable for tumor metastasis by facilitating epithelial-mesenchymal transition progression[89]. The effective compounds in Epimedii has showed therapeutic effects on cancer by anti-inflammation trait. Icariin and icaritin exerted anti-inflammatory and anti-tumor effects, and modulated the functions of myeloid-derived suppressive cells (MDSCs), a major component of the immune suppressive network responsible for T cell defects in cancer. In these cells, icariin and icaritin treatment down regulated the production of IL-6 and TNF-α, decreased expression of S100A8/9, and inhibited activation of STAT3 and Akt[90]. In transgenic adenocarcinoma mouse prostate (TRAMP) mice, icaritin increased the mice survival, reduced inflammation scores and cytokine levels, indicating that icaritin could inhibit the progression of prostate cancer in TRAMP mice *via* inhibiting proinflammatory cytokines[91]. A similar study on human PC-3 prostate cancer cells, icariside II initiated the inhibition of COX-2/PGE2 pathway and induced apoptosis of PC-3 prostate cancer cells[92]. Further studies on the invasion of cancer cells, icariside II inhibited invasion and epithelial-mesenchymal transition of human non-small cell lung cancer A549 cell line and H1299 cells induced by LPS or by pro-inflammatory factor TNF-α, through inactivating Akt/NF-κB pathway[93]. These studies provided evidence of the anti-tumor effects of icariin and its derivatives *via* inhibiting inflammation response and the progression/invasion of cancer cells.

2 Molecular mechanisms of anti-inflammatory effects of Epimedium extracts

2.1 Inflammatory cytokines

In inflammation response, the initial recognition of stimuli is mediated by macrophages and mast cells, leading to the production of a variety of inflammatory cytokines (including IL-6, IL-1β, TNF-α and many others) and other mediators (such as PEG2, COX-2 and NO)[94]. Then these cytokines and mediators alter the functionality or homeostasis of many tissues and organs, including activation of the leukocytes and endothelium and induction of the acute-phase inflammatory response[5]. As summarized in the present review, Epimedii showed anti-inflammatory effects on several disease models *in vitro* and *in vivo*. The water extract from Epimedii had anti-inflammatory effects by inhibiting production of inflammatory cytokines and mediators including IL-1β, IL-6, TNF-α, NO, and PGE2 induced by LPS both *in vitro* and *in vivo*[2-3]. The ethanol extract and ethyl acetate extracts of Epimedii also exhibited anti-inflammatory effects by inhibition of LPS-induced inflammatory response through TLR4/MD-2-mediated NF-κB pathway[95-96]. Epimedium flavonoids are main effective compounds in regulating inflammatory cytokines, in which the most studied is icariin, followed by icaritin. They showed significant anti-inflammatory effects *via* suppressing inflammatory cytokines and mediators in most of the studies described in this review article. In addition, several other derivatives of icariin extracted from Epimedii also exhibited anti-inflammatory effects on

LPS-stimulated macrophages, such as icariside II[92], desmethylanhydroicaritin[97], Ikarisoside A[98], 2'-hydroxy-3'-en-anhydroicaritin[99-100], 3, 5, 7-trihydroxy-4'-methoxy-8- (3-hydroxy-3-methylbutyl) -flavone[4,101], 3, 5-dihydroxy-4-methoxy-6, 6-dimethy1-4, 5-dihydropyrano[2, 3: 7, 8] -flavone[102], and 8-O-4' neolignan[103]. As these compounds share similar chemical structure and showed common anti-inflammatory effects, they may have homogeneous mechanisms or signaling pathways against inflammatory responses.

2.2 TLR4/MyD88/NF−κB signaling pathway

NF-κβ is a family of transcription factors, which regulate the expression of genes influencing a broad range of biological processes including inflammation, innate and adaptive immunity, B-cell development, lymphoid organogenesis and stress responses[104]. Icariin and its derivatives showed anti-inflammatory effects on several disease models by blocking NF-κB-dependent inflammatory processes and decreasing the transcription and expression of inflammatory factors[3-4,17,56,87,93,95,97-98]. In NF-κB signaling pathway, icariin and its derivatives exhibited inhibition of IκB kinase (IKK) activation, IκB phosphorylation and degradation, and NF-κB nuclear translocation[43,97].

Toll-like receptor 4 (TLR4) predominately functions in the initiation of pro-inflammatory responses after forming a complex with CD14, leads to the activation of the adapter MyD88 and the ubiquitination of its downstream protein TNF receptor associated factor 6, further degrades IκB-α levels and triggers NF- κB signaling pathways[105]. The ethyl acetate extract of Epimedium[95], icariin[48], Icariside II[26] and 3, 5, 7-Trihydroxy-4'-methoxy-8- (3-hydroxy-3-methylbutyl) -flavone[4] were found to attenuate inflammation or neuroinflammation through inhibiting TLR4/MyD88/NF- κB pathway in LPS-induced models resembling varied diseases.

Besides, Icariside II regulated tumor invasion and inflammatory microenvironment induced by LPS *via* down-regulation of Akt/NF- κB signaling pathway[93]. Another study in diabetic rats, icariside II improved diabetic cardiomyopathy by activating the Akt/NOS/NF-κB pathway[57].

2.3 NLRP3/caspase−1/IL−1β axis

The cytosolic innate immune signaling receptor NLRP3 (NOD- , LRR- and pyrin domain-containing 3) plays a notable role in inflammatory responses, leading to caspase 1-mediated proteolytic activation of IL-1β, and induces an inflammatory response and cell death[106-107]. In the LPS-induced chondrocyte model and monosodium iodoacetate-induced rat model of osteoarthritis, icariin alleviated inflammation and osteoarthritis by inhibiting NLRP3 inflammasome/caspase-1 signaling pathway-mediated pyroptosis[18]. In an unpredictable chronic mild stress (CMS) model of depression in rats, icariin exhibited anti-inflammatory effects on the brain tissue *via* the inhibition of the NLRP3 inflammasome/caspase-1/IL-1β axis[41]. Icariin also suppressed NLRP3 inflammasome activation and IL-1β production in MRL/lpr mice developing the Lupus nephritis-like phenotype[87]. Besides, NF- κB can act as an initial signaling that induces transcription and translation of pro-IL-18 and pro-IL-1β and activation of NLRP3 inflammasome[108]. In immunoglobulin A nephropathy rats, icariin ameliorates IgA nephropathy by inhibition of NF-κB/NLRP3 pathway[88].

2.4 ERK/p38/JNK MAPK signaling pathway

A group of enzymes called mitogen-activated protein kinases (MAPKs), including extracellular signal-regulated kinase1/2(ERK1/2), c-Jun N-terminal kinase (JNK) and p38 MAPK, can selectively phosphorylates serine and threonine residues in response to extracellular stimuli and plays important role in LPS-induced production of pro-inflammatory cytokines[109]. In LPS-stimulated human innate immune cells or mouse macrophages, 3, 5-dihydroxy-4-methoxy-6,6-dimethy1-4,5-dihydropyrano [2,3:7,8]-flavone and 2″-hydroxy-3″-en-anhydroicaritin inhibited the expression and activation of ERK, p38, and JNK phosphorylation protein [99,102]. In a high-cholesterol diet (HCD) -induced rat model of atherosclerosis, icariin demonstrated significant anti-inflammatory effect *via* suppressing p38-MAPK signaling pathway[52]. The phosphorylation of JNK has been reported to activate NF-κB pathway, through degradation of IκB and the nuclear translocation of NF- κB[9]. Icariin treatment blocked JNK/NF- κB pathway and prevented cardiomyocytes from apoptosis and inflammatory response in H9c2 cells[47].

2.5 STAT and immune response

The Janus kinase-signal transduction and activator of transcription (JAK-STAT) signaling pathway is involved in the pathogenesis of inflammatory and autoimmune diseases, *via*

regulating essential transcription factors of T helper (Th) cells and the expression of IL-6, IL-17, IL-21, and IL-23 receptor (IL-23R)[110-112]. In a heterogeneous group of myeloid cells, icariin down-regulated production of IL-6 and TNF-α *via* inhibition of activation of STAT3[90]. Besides, icariin inhibited intestinal inflammation in mice induced by dextran sulfate sodium *via* inhibiting the phosphorylations of STAT1 and STAT3 in Th cells[83].

2.6 Nrf2/ARE/HO−1 signaling pathway

Nuclear factor erythroid-2 related factor 2 (Nrf2) is an important factor in endogenous antioxidant defense systems, controlling redox homoeostasis and inflammation[113]. Activated Nrf2 binds to antioxidant response elements, regulating the expression of antioxidant genes such as heme oxygenase-1 (HO-1). Activation of Nrf2 signaling has been revealed to attenuate induced inflammatory response *via* regulating key innate immunity-regulating adaptor MyD88 and NF-κB, and suppressed neuroinflammation through regulating microglial polarization[114-115] .

In microglia BV-2 cell lines, icariin suppressed LPS-induced microglial pro-inflammatory factors production through activating Nrf2/HO-1 signaling pathway[24]. In 6-hydroxydopamin-induced PD model mice, icariin attenuated glial cells-mediated neuroinflammation and protected dopaminergic neurons *via* an Nrf2-dependent manner[37]. In ultraviolet B (UVB) -irradiated human keratinocytes, icariin and icaritin activated Nrf2/ARE signaling to improve the anti-oxidative stress capacity and suppress NF-κB activation, exhibiting potentials to treat UVB-induced oxidative stress and inflammation[75]. These results suggest that activation of Nrf2 signaling by icariin and icaritin are linked to anti-inflammatory activities.

2.7 Glucocorticoid receptor and estrogen receptor signaling

Glucocorticoid receptor (GR) shows anti-inflammatory effect through interacting with signaling pathways such as PI3K, JNK and pro-inflammatory gene expression[116]. In LPS-induced inflammatory cell models, icariin up-regulated the amount of GRα and promoted its nucleus translocation, thus silencing pro-inflammatory transcription factors NF-κB, c-Jun, and STAT3[117]. In a murine model combined psychosocial stress with allergic exposure, icariin prevented the development of depression behaviors and inflammatory cytokine infiltrations *via* increasing expression of GR and recovering glucocorticoid responsiveness[62]. Besides, icariin also showed anti-inflammatory effects in cigarette smoke-induced inflammatory model by modulating GR protein expression[64].

Estrogen receptors (ER) are expressed on monocyte-derived cells, and estrogens have been reported to prevent and control the inflammatory response[118]. Activated estrogen receptor by estrogen can be translocate into the nucleus and bind to DNA to regulate the activity of different genes, such as glucocorticoid receptor[119]. In experimental autoimmune encephalomyelitis (EAE) mice, icariin induced estrogen-like activity *via* mediating ERβ, enhancing HPA function and increasing GR expression in cerebral white matter[80].

3 Conclusion and prospect

Emerging evidence from the last decade has indicated that Epimedium flavonoids, especially icariin and its derivatives, have anti-inflammatory effects in the animal and cellular models of various diseases related to chronic inflammation, including osteoporosis, osteoarthritis, Alzheimer's disease, Parkinson's disease, depression, cardio-cerebrovascular diseases, atherosclerosis, asthma and lung diseases, chronic kidney disease, skin diseases, cancer, and inflammatory autoimmune diseases (including multiple sclerosis, inflammatory bowel diseases, rheumatoid arthritis, and lupus nephritis) [3,37,73,120].

Among these diseases, we think that inflammatory autoimmune diseases, such as systemic lupus erythematosus (SLE), multiple sclerosis and inflammatory bowel diseases, should be paid great attention to in the future. These diseases are characterized by chronic inflammation and often involve multiple organs, while long term use of glucocorticoid and immunosuppressant has serious side effects. Epimedium flavonoids and icariin have anti-inflammatory and immunoregulatory effects in multiple organs as presented above, and are natural compounds with less side effects, thus may have advantages in the treatment of inflammatory autoimmune diseases.

The recent studies have shown that the

mechanisms involved in anti-inflammation effects of icariin and its derivatives include reducing inflammatory cytokines, down-regulating NF- κB signaling, NLRP3/caspase-1/IL-1β axis, STAT signaling and ERK/p38/JNK MAPK pathway, as well as up-regulating Nrf2/ARE/HO-1 pathway and increasing glucocorticoid receptor and estrogen receptor signaling (Figure 1).

These results have demonstrated that the mechanisms of icariin and its derivatives are complex and there are crosstalks among them. NF- κB has a central role in inflammatory diseases as mentioned above, by means of the large number of important pro- and anti-inflammatory factors under its transcriptional control[121]. NF- κB also collaborates with other signaling pathways, such as NLRP3, MAPK and Nrf2, inducing inflammation responses[106,122]. Thus, we infer that NF- κB may be the main target of icariin and its derivatives in inflammation-related diseases.

There are also several aspects that need to be investigated in future studies: to investigate novel effects of Epimedii extracts on new disease models based on their anti-inflammatory effects; to identify the therapeutic targets of icariin and its derivatives; and to comprehensively elucidate their molecular pharmacological mechanisms by using new techniques such as genomics, proteomics, metabolome or herbal decoctosome[123]. Further understanding of mechanisms of Epimedii and its effective compounds will help us gain a more comprehensive understanding of the anti-inflammatory effects, and discover the potential use of Epimedii and its effective compounds. In addition, because Herba Epimedii is a clinically available traditional Chinese medicine (TCM), it can also be combined with other TCM to enhance the therapeutic effects.

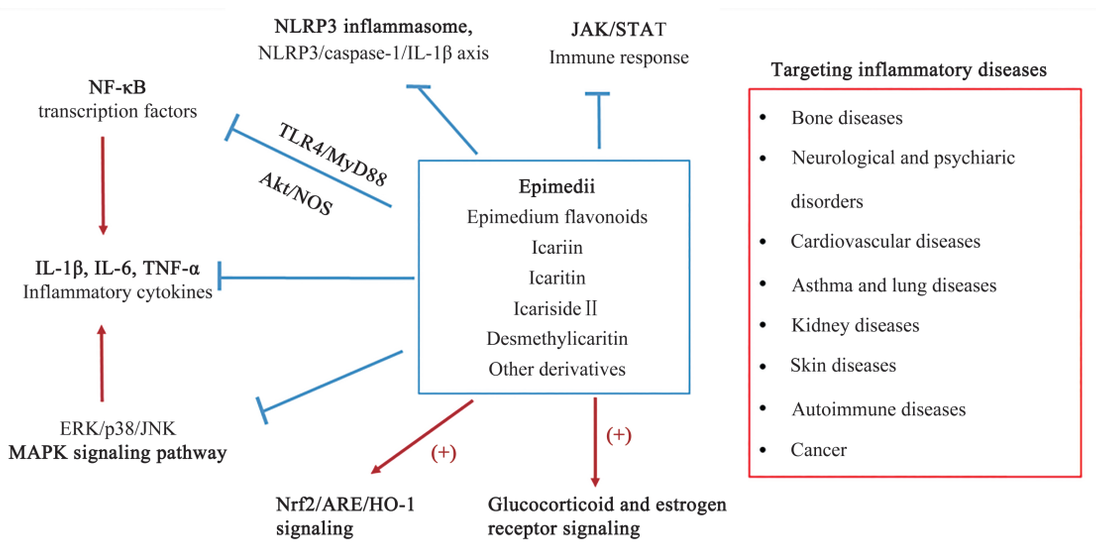

Fig. 1 Mechanisms and signaling pathways involved in anti−inflammation effects of Epimedii

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淫羊藿提取物的抗炎作用及其机制*

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摘要　淫羊藿是一种常用的传统中药. 黄酮类成分是淫羊藿的主要有效成分，其中淫羊藿苷是含量最高的单体成分，淫羊藿素是淫羊藿苷的代谢产物. 最近10余年来，学者对淫羊藿提取物的药理作用进行了许多研究，表明淫羊藿黄酮，尤其是淫羊藿苷及其衍生物，在骨质疏松症、骨关节炎、神经和精神疾病、动脉粥样硬化、哮喘和肺部疾病、炎症性肠病、肾脏疾病、皮肤病、自身免疫性疾病和癌症等多个与慢性炎症有关的疾病模型中显示了良好的抗炎作用. 淫羊藿苷及其衍生物发挥抗炎作用的分子机制主要包括降低炎症细胞因子释放和NF-κB信号通路激活，抑制NLRP3/caspase-1/IL-1β、STAT和MAPK介导的信号传导通路，上调Nrf2/ARE/HO-1信号通路以及糖皮质激素受体和雌激素受体下游信号通路等. 本文综述了该领域的近期研究进展，提示淫羊藿及其所含的活性化合物具有治疗多种慢性炎症相关疾病的潜力.

关键词　淫羊藿，中药，炎症，分子机制
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