Antioxidant and anti-inflammatory effects of esculin and esculetin (Review)

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Abstract. *Fraxinus chinensis Roxb* is a deciduous tree, which is distributed worldwide and has important medicinal value. In Asia, the bark of *Fraxinus chinensis Roxb* is a commonly used traditional Chinese medicine called Qinpi. Esculetin is a coumarin compound derived from the bark of *Fraxinus chinensis Roxb* and its glycoside form is called esculin. The aim of the present study was to systematically review relevant literature on the antioxidant and anti-inflammatory effects of esculetin and esculin. Esculetin and esculin can promote the expression of various endogenous antioxidant proteins, such as superoxide dismutase, glutathione peroxidase and glutathione reductase. This is associated with the activation of the nuclear factor erythroid-derived factor 2-related factor 2 signaling pathway. The anti-inflammatory effects of esculetin and esculin are associated with the inhibition of the nuclear factor κ-B and mitogen-activated protein kinase inflammatory signaling pathways. In various inflammatory models, esculetin and esculin can reduce the expression levels of various proinflammatory factors such as tumor necrosis factor-α, interleukin (IL)-1β and IL-6, thereby inhibiting the development of inflammation. In summary, esculetin and esculin may be promising candidates for the treatment of numerous diseases associated with inflammation and oxidative stress, such as ulcerative colitis, acute lung and kidney injury, lung cancer, acute kidney injury.

Contents

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1. Introduction

Oxidative stress is caused by the disruption of the balance between oxidative and antioxidant responses in the body. When exposed to noxious stimuli, the body is in a state of oxidative stress, and the level of free radicals is elevated, leading to apoptosis and tissues damage (1). Inflammation is the fundamental response of the body to injury and infection. However, if inflammation is not controlled, various proinflammatory factors (Interleukins, Tumor Necrosis Factor, etc.) released by persistent inflammation can induce further pathological responses (2). Previous research shows that oxidative stress and inflammation are associated with the occurrence and development of a number of diseases such as diabetes, cardiovascular disease and cancer (3-5). Therefore, studies have been attempting to reveal additional effective drugs with antioxidant and anti-inflammatory effects. As an important source of drugs, natural products serve a key role in the identification and development of new drugs (6). Therefore, finding new antioxidant and anti-inflammatory drugs from natural products may become an important research direction in the future. An overview of the antioxidant and anti-inflammatory effects of *Fraxinus chinensis Roxb* in Fig. 1.

Fraxinus chinensis Roxb is a deciduous tree, which is distributed worldwide and has important medicinal value. In Asia, the bark of *Fraxinus* is a commonly used traditional Chinese medicine called Qinpi (7). The bark of *Fraxinus* contains 1.0~1.4%, with esculetin and its glycoside form, esculin, being the most studied active ingredients (Fig. 2) (8). The present study shows that esculetin and esculin have a wide range of pharmacological activities, including antioxidant, anti-inflammatory, antibacterial, antitumor, antidiabetic, anti-atherosclerotic, and immunomodulatory effects. And esculetin and esculin are expected to be used in the treatment of various diseases, such as cancer, diabetes and neurodegenerative diseases. Further studies have shown that esculetin and esculin have prominent antioxidant and anti-inflammatory effects, which may underlie their broad pharmacological activities (9,10). Antioxidant effects of esculetin and esculin are associated with the activation of the nuclear factor erythroid-derived factor 2-related factor 2 (Nrf2) signaling pathway (11). As a key signaling pathway for antioxidation, the Nrf2 signaling pathway can regulate the expression levels of various endogenous antioxidant proteins, such as superoxide

dismutase (SOD), glutathione peroxidase (GPx) and glutathione (GSH) (11,12). The anti-inflammatory effects of esculetin and esculin are associated with the inhibition of nuclear factor κ-B (NF-κB) and mitogen-activated protein kinase (MAPK), two important inflammatory signaling pathways. In various inflammatory models, esculetin and esculin can reduce the expression levels of a number of proinflammatory factors such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β and IL-6, thereby inhibiting the development of inflammation (13,14). In summary, as natural products, esculin and esculetin may be promising candidates for the potential use as complementary and alternative medicines. The present study aimed to systematically review studies that investigated the anti-inflammatory and antioxidant properties of esculetin and esculin, as well as to provide current and useful information.

2. Antioxidant activities of esculin and esculetin

It is considered that the oxidative and antioxidant reactions of the normal body are in a state of dynamic equilibrium. Oxidative stress is caused by the breakdown of this balance and the accumulation of oxidative substances such as reactive oxygen species (ROS) or reactive nitrogen species such as $O_2^{\bullet-}$, $NO^{\bullet}$, $ONOO^-$ and $OH^{\bullet}$ (15). A study confirmed that esculin and esculetin effectively scavenge free radicals (16). In addition, experimental results show that esculin and esculetin can improve various pathological processes that are associated with oxidative stress. This is associated with the activation of the Nrf2 signaling pathway and the promotion of the expression of various antioxidant proteins such as GSH and SOD (15,16).

Antioxidant activities of esculetin
Free radical scavenging effects of esculetin. At present, a number of studies have determined the scavenging effect of esculetin on 2,2-Diphenyl-1-picrylhydrazyl (DPPH), but there are differences between the results. Wang *et al* (16) find that the clearance rates of DPPH with different concentrations of esculetin (0.02, 0.05, 0.08 and 0.1 mg/ml) are 28.13, 59.94, 79.68 and 100%, respectively. The SC_{50} values of esculetin for scavenging DPPH as determined by Jeong *et al* (17) and Kim *et al* (18) are both 14.68 μM. The SC_{50} value of esculetin for scavenging DPPH as determined by Vianna *et al* (19) is 25.18 μM, but is determined by Lee *et al* (20) to be 40 μM. In addition to scavenging DPPH, esculetin also has a scavenging effect on hydroxyl radicals with an SC50 value of 0.091 mg/ml (16). Hsia *et al* (21) also find that esculetin (50-80 μM) inhibits hydroxyl radical formation in washed human platelets. In addition, Lee *et al* (22) demonstrated that esculetin has a scavenging effect on superoxide anion radicals with an SC50 value of 0.6 μg/ml. In addition, esculetin inhibits peroxidase-induced oxidation in a non-competitive manner with a Ki value of 9.5 μM in the methemalbumin-H2O2-tetramethylbenzidine pseudoperoxidase system (23). By scavenging the free radicals, esculetin (10-100 mg/ml) protects human dermal fibroblasts from UVB-induced oxidative stress damage (22). Furthermore, by scavenging the O2-accumulation caused by H_2O_2, esculetin (100 μM) protects human leukemia NB4 cells from oxidative stress damage (24,25).

Regulated antioxidant protein action of esculetin. Studies using cells further confirm the antioxidant effect of esculetin, which regulates the expression of various antioxidant proteins (26-28). For example, in L-buthionine-sulfoximine-induced mouse cortical cells, esculetin (10-100 μM) increases GPx, glutathione reductase (GR) and GSH activities and inhibits oxidized glutathione (glutathione disulfide; GSSG) accumulation (26). In addition, esculetin (10 μM) can inhibit the accumulation of ROS in *Helicobacter pylori* urease-induced human vascular endothelial cells by promoting the expression of heme oxygenase-1 (HO-1) (27). Additionally, esculetin (10-20 μM) can increase the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), SOD and GSH and inhibit the accumulation of ROS and malondialdehyde (MDA) in tert-butyl hydroperoxide (t-BHP)-induced HepG2 cells (28,29).

Further studies show that the antioxidant activity of esculetin is associated with the regulation of multiple signaling pathways, such as inhibiting the NF-κB and MAPK signaling pathways (13,14,30). The Nrf2 signaling pathway is the primary defense mechanism for inhibiting oxidative stress, and it regulates the expression of a number of important antioxidant proteins such as HO-1, SOD and GPx. A large number of studies show that the antioxidant effect of esculetin is associated with the activation of the Nrf2 signaling pathway (15,30,31). Sen *et al* (30) find that esculetin (10-20 μM) reduces the production of ROS in the high glucose-induced human renal tubular epithelial HK-2 cells and that the Nrf2 signaling pathway is activated. Furthermore, esculetin (20-500 μM) can induce SOD expression in the leukemia NB4 cells by activating the Nrf2 signaling pathway (31).

Scavenging ROS effects of esculetin. Accumulation of ROS can activate the mitochondrial apoptotic pathway and induce apoptosis, and esculetin can block this process by scavenging ROS (32). In zinc oxide nanoparticles-induced human neuroblastoma SH-SY5Y cells, esculetin (10-30 μM) can decrease GPx and GSH depletion by inhibiting lipoxygenase expression, which ameliorates the ROS accumulation-induced apoptosis (33). Esculetin (0.1-10 μM) can also protect Aβ25-35-induced human neuroblastoma SH-SY5Y cells from apoptosis caused by ROS accumulation by increasing SOD, GSH and catalase (CAT) expression levels, which reduces caspase-3, cytochrome c and Bax expression levels and increases Bcl-2 expression levels (34,35). Additionally, esculetin (5-20 μM) increases SOD levels and decreases ROS levels and thus decreases lactate dehydrogenase (LDH), Bax and cleaved caspase-3 levels in hypoxia/reoxygenation-induced myocardial H9c2 cells (36).

In H2O2-induced human corneal epithelial cells, esculetin (20-100 μM) activates the Nrf2 signaling pathway, inducing the expression of multiple antioxidant genes, including HO-1, quinone 1 (NQO1), glutamate cysteine ligase modifier subunit (GCLM), SOD1 and SOD2, thereby inhibiting ROS generation and accumulation (37). By activating the Nrf2 pathway and promoting the expression of HO-1, SOD, GPx and CAT, esculetin (12.5-100 μM) inhibits the production and accumulation of ROS in 3T3-L1 preadipocytes (38). Furthermore, in H2O2-induced Caco2 cells, esculetin (100-300 μg/ml) activates the Nrf2 pathway and increases the expression of SOD,
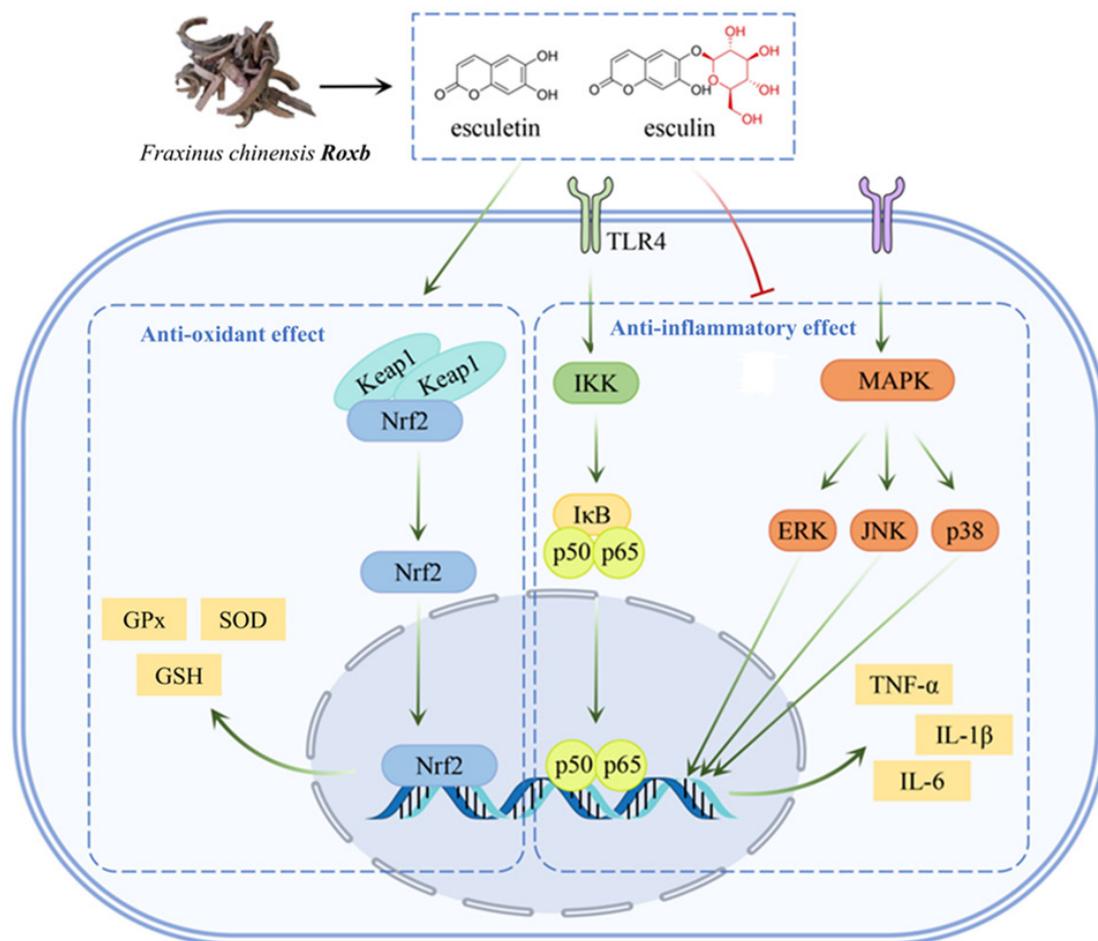
Figure 1. Antioxidant and anti-inflammatory effects of *Fraxinus chinensis Roxb*. TLR4, toll-like receptor 4; GPx, glutathione peroxidase; SOD, superoxide dismutase; GSH, glutathione; IKK, IκB kinase.
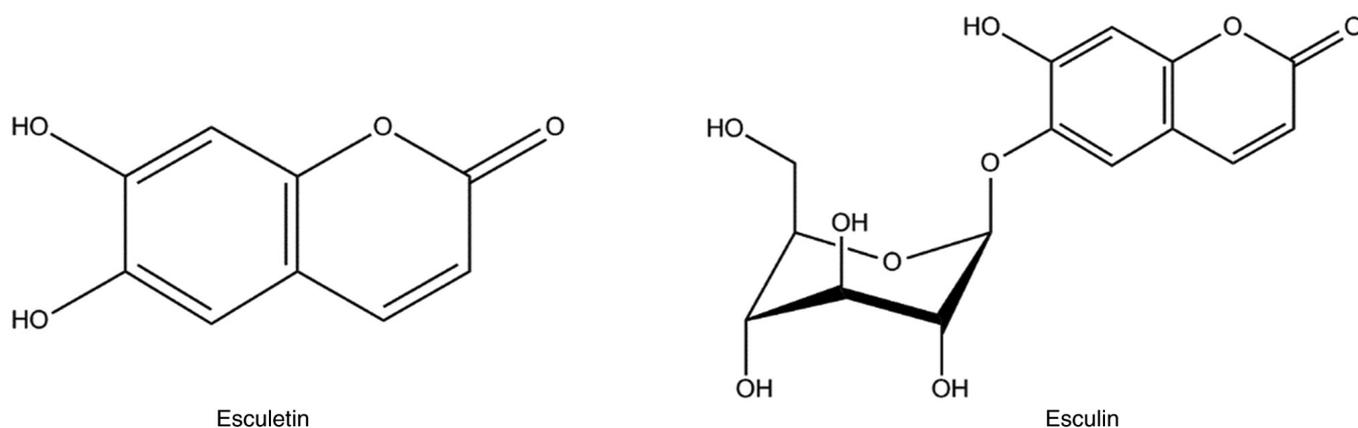
Figure 2. Structural formula of esculetin and esculin.

CAT and GPx (39). Esculetin (10-25 μM) also protects human hepatoma HepG2 cells from hydrogen peroxide-induced oxidative damage by activating the Nrf2 signaling pathway and promoting the expression of NQO1 and glutathione (40). Additionally, esculetin (10-100 μM) can restore depleted GSH levels and inhibit the production of ROS and MDA in ethanol-induced HepG2 cells by activating the Nrf2 signaling pathway (41).

Amelioration of oxidative stress damage by esculetin. The results of mouse studies further show that esculetin can improve oxidative stress and associated injuries, such as nervous system injury, liver injury and kidney injury: For example, esculetin can improve nervous system injury caused by oxidative stress (42-45). Esculetin (0.5% w/w) also ameliorates 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity in the substantia nigra pars compacta in mice

by preventing nitrosative stress, increasing GSH levels, inhibiting the activation of caspase 3 and preventing neuronal apoptosis (42). Additionally, esculetin (20-80 mg/kg) inhibits tBCCAO-induced activation of the mitochondrial apoptosis pathway and autophagy in mice by reducing the expression levels of autophagy-related factors (such as Bnip3, Beclin1, Pink1 and parkin) and apoptosis-related factors (such as p53, Bax and caspase 3) by activating the Nrf2 signaling pathway (43). In addition, esculetin (25 mg/kg) decreases immobility time and climbing time in the forced swim task elicited in acute restraint stress-induced rats by restoring the activities of antioxidant enzymes including CAT, SOD, GPx and GSH, and reducing the levels of GSSG in rat cortical areas (44). Another study shows that esculetin can inhibit oxidative stress in liver injury (45). In CCl4-induced liver injury rats, esculetin (35 mg/kg) increases SOD and CAT levels and decreases MDA levels (45). Furthermore, in N-nitrosodiethylamine-induced liver injury rats, esculetin (0.5% w/w) ameliorates hepatic lipid peroxidation by restoring the total glutathione levels and increasing the levels of NQO1, HO-1 and glutathione S-transferase Pi (GSTP1) (46). Esculetin can also inhibit oxidative stress in kidney injury. In high-fat diet-induced kidney injury mice, esculetin (40 mg/kg) increases the expression levels of SOD, CAT, GPx, glutathione-S-transferase (GST), non-enzymic antioxidants vitamin C and GSH, and reduces the levels of thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides and co-conjugated diene (47).

Other antioxidant oxidative effects of esculetin. In addition, He *et al* (36) find that esculetin (5-20 μM) protects hypoxia/reoxygenation-induced myocardial H9c2 cells from oxidative stress-induced apoptosis. This protective effect is produced by activating the Janus kinase/signal transducer and activator of transcription (JAK2/STAT3) pathway (36). Notably, studies show that activation of the Nrf2 pathway is associated with the extracellular regulated protein kinases (ERK) signaling pathway. Esculetin (5 μM) can activate the Nrf2 signaling pathway and promote the expression of NQO1 by activating the ERK signaling pathway in H2O2-induced C2C12 myoblasts (48,49).

The results of animal studies further show that esculetin can improve the oxidative stress caused by ischemia-reperfusion. For example, esculetin (20-40 mg/kg) restores SOD activity and reduces the levels of MDA in myocardial ischemia-reperfusion rats (50). In addition, esculetin can improve the oxidative stress in testicular tissue damage. Esculetin (50 mg/kg) ameliorates AlCl3-induced rat testicular tissue damage by activating the Nrf-2 signaling pathway, increasing the activities of GSH, GPx and CAT and preventing the activation of the apoptotic pathway (51).

In conclusion, the antioxidant effect of esculetin stems from increasing the expression levels of endogenous antioxidants such as NQO1, HO-1, GSH and SOD by activating the Nrf2 signaling pathway, which enables the free radicals generated by the body to be scavenged in time to avoid lipid oxidation and apoptosis.

Antioxidant activities of esculin
Free radical scavenging effects of esculin. Free radical scavenging experiments show that esculin has scavenging effects

on a variety of free radicals including O2•-, NO• and DPPH, and the SC50 values are 69.27 μg/ml, 8.56 μg/ml and 0.141 μM, respectively (52,53). Similar to the antioxidant mechanism of esculetin, the activation of the Nrf2 signaling pathway is also associated with the antioxidant effect of esculin. Studies demonstrate that esculin (50-200 μmol/l) can disrupt the interaction of the Kelch-like ECH-associated protein 1 (Keap1), a key negative regulator of the Nrf2 signaling pathway, with Nrf2 to activate the Nrf2 signaling pathway (54,55).

Regulated antioxidant protein action of esculin. Esculin has significant antioxidant modulating effects and ameliorates oxidative stress in kidney injury. Esculin (150 mg/kg) promotes the expression of SOD, GR and CAT in prooxidant aflatoxin B-1-induced nephrotoxicity mice (56). Similarly, esculin can inhibit the oxidative stress in pancreatic injury (57). Esculin (10-50 mg/kg) increases the expression levels of GPx, SOD and CAT in streptozotocin (STZ)-induced type 2 diabetic mice (57).

Scavenging ROS effects of esculin. The scavenging ROS effects of esculin have been widely reported. For example, esculin (1-100 μM) protects dopamine-induced human neuroblastoma SH-SY5Y cells from ROS accumulation-induced apoptosis by increasing SOD and GSH expression levels and inhibiting the expression of cytochrome c, apoptosis-inducing factor, p53, Bax and caspase 3 (58). Myeloperoxidase is able to mediate oxidative stress by promoting ROS production and promoting inflammation-related signaling pathways (59). In human neuroblastoma SH-SY5Y cells, esculin reduces the dopamine-induced ROS overproduction, scavenges ROS and enhances the activities of SOD and GSH (58).

Amelioration of oxidative stress damage by esculin. Experiments using cells further confirm the antioxidant effect of esculin. For example, esculin (20-100 μM) can protect linoleic acid hydroperoxide-induced human umbilical vein endothelial cells from free radical-induced oxidative stress damage (60).

The results of experiments using mice further show that esculin can decrease oxidative stress and the associated damage. Esculin can inhibit oxidative stress in liver injury, as esculin (10-40 mg/kg) can activate the Nrf2 signaling pathway to increase HO-1 expression levels and decrease the MDA content in lipopolysaccharide (LPS)/D-galactosamine-induced acute liver injury mice (61). Another study demonstrates that the esculin can improve oxidative stress in gastric injury, as in ethanol-induced gastric mucosal injury rats, esculin (12.5-50 mg/kg) increases the SOD levels and decreases the MDA levels (52).

Other antioxidant oxidative effects of esculin. In addition, esculin can decrease oxidative stress in colitis. For example, esculin (5-25 mg/kg) promotes the expression of GPx and partially ameliorates the intestinal injury in rats with 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis (53). Furthermore, studies associated with acute lung injury (62) and diabetic nephropathy (63) demonstrate the antioxidant activity of esculin. Esculin can improve oxidative stress in arthritis and, in adjuvant-induced arthritis rats, esculin (10-40 mg/kg) promotes the expression of GPx (64).

Table I. Antioxidant and anti-inflammatory effects of esculetin.

A, Antioxidant

Detail	Cell lines/model	Dosage	(Refs.)
Increase GPx, GR and GSH activities and inhibit GSSG accumulation	L-buthionine-sulfoximine-induced mouse cortical cells	10-100 μM	(26)
Promote the expression of HO-1	*Helicobacter pylori* urease-induced human vascular endothelial cells	10 μM	(27)
Increase the levels of AST, ALT, SOD and glutathione and inhibit the accumulation of ROS and MDA	t-BHP-induced HepG2 cells	10-20 μM	(28, 29)
Decrease GPx and GSH depletion by inhibiting lipoxygenase expression	Zinc oxide nanoparticles-induced human neuroblastoma SH-SY5Y cells	10-30 μM	(33)
Increase SOD, GSH and CAT expression levels, which inhibit activation of mitochondrial apoptosis pathway	$A\beta_{25-35}$-induced human neuroblastoma SH-SY5Y cells	0.1-10 μM	(34, 35)
Increase SOD levels and decrease ROS levels and thus, inhibit activation of mitochondrial apoptosis pathway	Hypoxia/reoxygenation-induced myocardial H9c2 cells	5-20 μM	(36)
Induce SOD expression levels by activating the Nrf2 signaling pathway	Leukemia NB4 cells	20-500 μM	(31)
Activate the Nrf2 signaling pathway and induce the expression levels of multiple antioxidant genes, including HO-1, NQO1, GCLM, SOD1 and SOD2	H_2O_2-induced human corneal epithelial cells	20-100 μM	(37)
Activate the Nrf2 pathway and promote the expression of HO-1, SOD, GPx and CAT	3T3-L1 preadipocytes	12.5-100 μM	(38).
Activate the Nrf2 pathway and increase the expression levels of SOD, CAT and GPx	H_2O_2-induced Caco2 cells	100-300 μg/ml	(39)
Activate Nrf2 signaling pathway and promote the expression of NQO1 and glutathione	Hydrogen peroxide-induced human hepatoma HepG2 cells	10-25 μM	(40)
Restore the depleted GSH level and inhibit the production of ROS and MDA by activating the Nrf2 signaling pathway	Ethanol-induced HepG2 cells	10-100 μM	(41)
Activate the Nrf2 signaling pathway and promote expression of NQO1 by activating the ERK signaling pathway	H_2O_2-induced C2C12 myoblasts	5 μM	(48, 49)
Activate JAK2/STAT3 pathway	Hypoxia/reoxygenation-induced myocardial H9c2 cells	5-20 μM	(36)
Prevent nitrosative stress, increase GSH levels and inhibit the activation of caspase 3	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity in mice	0.5% w/w	(42)
Reduce the expression levels of autophagy-related factors (such as Bnip3, Beclin1, Pink1 and parkin) and apoptosis-related factors (such as p53, Bax and caspase 3) by activating the Nrf2 signaling pathway	tBCCAO-induced mice	20-80 mg/kg	(43)
Decrease immobility time and climbing time by restoring the antioxidant enzyme activities of CAT, SOD, GPx and GSH, and reducing GSSG levels	Forced swim task elicited in acute restraint stress-induced rats	25 mg/kg	(44)
Increase SOD and CAT levels, and decrease MDA levels	CCl_4-induced liver injury rats	35 mg/kg	(45)
Restore total glutathione levels and increase the levels of NQO1, HO-1 and GSTP1	N-nitrosodiethylamine-induced liver injury rats	0.5% w/w	(46)
Increase expression levels of SOD, catalase, GPx, GST, non-enzymic antioxidants vitamin C and GSH, and reduce levels of TBARS, lipid hydroperoxides and co-conjugated diene	High-fat diet-induced kidney injury mice	40 mg/kg	(47)
Restore SOD activity and reduce the amount of MDA	Myocardial ischemia-reperfusion rats	50 mg/kg	(50)
Activate the Nrf-2 signaling pathway, and increase the activities of GSH, GPx and CAT	$AlCl_3$-induced rat testicular tissue damage	50 mg/kg	(51)

Table I. Continued.

B, Anti-inflammatory

Detail	Cell lines/model	Dosage	(Refs.)
Inhibit the production of NO	IL-1β stimulated rat hepatocytes	IC$_{50}$, 34 μM	(66)
Inhibit IL-6 production	TNF-α stimulated human osteosarcoma MG-63 cells	10-100 μg/ml	(67)
Inhibit the release of NO, TNF-α, IL-1β, IL-6 and MCP-1	LPS-induced RAW 264.7 cells	1-10 μg/ml	(68-71)
Inhibit the production of IL-6, IL-8 and mucin-5AC by inhibition of the NF-κB signaling pathway	Histamine-induced human nasal epithelial cells	10-40 μM	(72)
Inhibit expression of iNOS, COX-2, TNF-α and IL-1β by inhibiting the NF-κB signaling pathway	LPS-induced RAW 264.7 cells	12 μg/ml	(73)
Inhibit activation of the NF-κB signaling pathway and reduce expression of MMP-9 and the binding activity of AP-1	TNF-α induced vascular smooth muscle cells	12.5-25 μg/ml	(74, 75)
Reduce the IL-1β, COX-2 and TNF-α levels by inhibiting the NF-κB signaling pathway	Trimethyltin-induced human neuroblastoma SH-SY5Y cells	30-200 μM	(76)
Decrease the expression levels of IL-1β, IL-12, TNF receptor and TRAIL by inhibiting the ERK1/2 and NF-κB signaling pathways	LPS-induced human retinal pigment epithelium cells	0.78-50 μM	(77)
Inhibit the production of TNF-α, IL-1β, IL-6, IL-23, myeloperoxidase and MPO by inhibiting the AKT/ERK/ NF-κB and RORγt/IL-17 signaling pathways	LPS-induced acute lung injury mice	20-40 mg/kg	(80, 81)
Reduce the levels of MMP-9, macrophage inflammatory protein 2, IL-8 and IL-1β	Polyhexamethylene guanidine-induced pulmonary fibrosis mice	10 mg/kg	(82)
Reduce the levels of IL-4, IL-5, IL-13, IL-17A, IgE, GATA3 and RORγt	Ovalbumin-induced asthma mice	20 mg/kg	(83)
Inhibit the TLR4 signaling pathway and the expression levels of COX-2 and NOS2	uPM10-exposed mice	10 mg/kg	(84)
Decrease the levels of COX-2, iNOS and CINC-3	TNBS-induced colitis rats	100 mg/kg	(85)
Inhibit the production of NO, TNF-α, IL-6, iNOS and NLRP3 by inhibiting the NF-κB and MAPK signaling pathways	DSS-induced colitis rats	5 mg/kg	(86, 87)
Inhibit the expression of iNOS, TNF-α and IL-6 by inhibiting the NF-κB signaling pathway	Ethanol-induced gastric injury rats	20-40 mg/kg	(88)
Downregulate the expression of *Tlr4*, *Myd88*, *Nfkb*, *Tnfα*, *Il6*, blood HbA1c, TNF-α, IL-6 and MCP-1	HFD-induced fatty liver mice	0.01% w/w	(89)
Inhibit the expression of IL-1β and TNF-α	Zinc oxide nanoparticles-induced rats	5-25 mg/kg	(90)
Reduce the levels of IL-6, IL-1β, TNF-α, iNOS and COX-2 by inhibiting the NF-κB signaling pathway	LPS-induced mice	20-40 mg/kg	(91, 92)
Reduce serum IgE, IgG2a and histamine levels, and inhibit the production of TNF-α, IFN-γ, IL-4, IL-13, IL-17 and IL-31 by inhibiting the STAT1 and NF-κB signaling pathways	House dust mite (dermatophagoides farinae extract) and 2,4-dinitrochlorobenzene-induced dermatitis mice	2-50 mg/kg	(93)
Reduce the levels of IL-6, IL-17A, IL-22, IL-23, TNF-α and IFN-γ by inhibiting the NF-κB signaling pathway	Imiquimod-induced psoriasis-like mice	50-100 mg/kg	(94)
Inhibit the expression of IL-1β, IL-6, TNF-α, CCL2 and iNOS by inhibiting the NF-κB and STAT1/STAT3 signaling pathways	*Escherichia coli*-induced sepsis mice	20-60 mg/kg	(95)
Inhibit the expression of IL-1α, IL-1β and TNF-α by inhibiting the ERK1/2 signaling pathway	Dry eye rabbits	0.05% of the diet	(96)
Inhibit the expression of TNF-α and IL-6 by inhibiting the NF-κB signaling pathway	Isoproterenol induced myocardial toxicity rats	10-20 mg/kg	(97)

Table I. Continued.

B, Anti-inflammatory

Detail	Cell lines/model	Dosage	(Refs.)
Inhibit monoamine oxidase-A activity, and downregulate the IL-1β, TNF-α and TBARS levels	Reserpine-induced fibromyalgia mice	2.5-10 mg/kg	(98)

AP-1, Activating protein-1; COX-2, Cyclooxygenase 2; DPPH, 1,1-Diphenyl-2-Picrylhydrazyl; DSS, dextran sulfate sodium; CINC-3, Cytokine-induced neutrophil chemoattractant-3; GATA3, GATA binding protein 3; GCLM, glutamate-cysteine ligase modifier subunit; GR, Glutathione reductase; GSSG, Oxidized glutathione; HFD, High-fat diet; ICAM-1, Intracellular adhesion molecule-1; LDH, lactic dehydrogenase; LPS, Lipopolysaccharide; MCP-1, Monocyte chemoattractant protein-1; MMP-9, matrix metalloproteinase-9; MPO, Myeloperoxidase; Myd88, Myeloid differentiation primary response gene 88; NLRP3, NOD-like receptor (NLR) family member pyrin domain-containing protein 3; NOS2, nitric oxide synthase 2; RORγt, Retinoid-related orphan nuclear receptor γt; ROS, Reactive oxygen species; STAT1, Signal transducer and activator of transcription 1; STZ, streptozotocin; tBCCAO, Transient bilateral common carotid artery occlusion; TBARS, Thiobarbituric acid reactive substances; t-BHP, tert-butyl hydroperoxide; TNBS, 2,4,6-trinitrobenzenesulfonic acid; TRAIL, TNF-related apoptosis-inducing ligand; UVB, Ultraviolet radiation b.

In conclusion, esculin, as the glycoside form of esculetin, has similar antioxidant activities to esculetin. By activating the Nrf2 signaling pathway and promoting the expression of various endogenous antioxidants such as HO-1, GSH and SOD, esculin serves a protective role in the process of various tissue injuries.

3. Anti-inflammatory activities of esculetin and esculin

Inflammation is the basic response of the body to injury and infection. However, if inflammation is not controlled, persistent inflammatory responses can induce new pathological responses (such as tissue damage, fibrosis, metabolic disorders, cardiovascular disease, etc.) and lead to the development of various diseases, such as cancer, metabolic diseases, neurodegenerative diseases and vascular diseases (65). Previous research shows that esculetin and esculin can inhibit the expression of proinflammatory factors, such as TNF-α, IL-1β and IL-6 in various inflammatory models (13,14,61). This is associated with the inhibition of inflammatory signaling pathways, such as NF-κB and MAPK.

Anti-inflammatory activities of esculetin. Studies using cells show that esculetin can inhibit the expression of various inflammatory markers (66). For example, esculetin can inhibit the production of nitric oxide (NO) in IL-1β stimulated rat hepatocytes with an IC50 value of 34 µM (66). Additionally, esculetin (10-100 µg/ml) inhibits IL-6 production in TNF-α stimulated human osteosarcoma MG-63 cells (67). Esculetin (1-10 µg/ml) can also inhibit the release of inflammatory mediators, such as NO, TNF-α, IL-1β, IL-6 and monocyte chemoattractant protein-1 (MCP-1) in LPS-induced RAW 264.7 cells (68-71). Further studies demonstrate that the anti-inflammatory effect of esculetin is associated with inhibiting the NF-κB signaling pathway (72-77). Sun *et al* (72) demonstrate that esculetin (10-40 µM) inhibits the histamine-induced production of IL-6, IL-8 and mucin-5AC in human nasal epithelial cells, which is partly mediated by inhibition of the NF-κB signaling pathway (72). Esculetin (12 µg/ml) also inhibits the expression of inducible isoform of NO synthase (iNOS), cyclooxygenase

2 (COX-2), TNF-α and IL-1β in LPS-induced RAW 264.7 cells by inhibiting the NF-κB signaling pathway (73). Additionally, esculetin (12.5-25 µg/ml) inhibits the activation of the NF-κB signaling pathway in TNF-α induced vascular smooth muscle cells, which reduces the expression levels of mitochondrial membrane potential-9 (MMP-9) and the binding activity of activating protein-1 (AP-1) (74,75). Furthermore, esculetin (30-200 µM) reduces the IL-1β, COX-2 and TNF-α levels in trimethyltin-induced human neuroblastoma SH-SY5Y cells by inhibiting the NF-κB signaling pathway (76). In addition to inhibiting the NF-κB signaling pathway, esculetin can also decrease the inflammatory response by inhibiting the MAPK signaling pathway. In LPS-induced human retinal pigment epithelium cells, esculetin (0.78-50 µM) decreases the expression levels of IL-1β, IL-12, TNF receptor and TNF-related apoptosis-inducing ligand by inhibiting the ERK1/2 and NF-κB signaling pathways (77). In addition, lipoxygenases are a family of non-heme iron-containing enzymes and are involved in the synthesis of inflammatory cytokines such as leukotrienes. A previous study demonstrated that the inhibition rate of esculetin on soybean 15-lipoxygenase at a concentration of 28.1 µM is 40.50% (78). Esculetin can also inhibit the activity of 5-lipoxygenase with an IC50 value of 6.6 µM (79).

The results of study with mice further indicate that esculetin can exhibit an anti-inflammatory effect in various inflammatory models (61,63,74). This suggests that esculetin may be promising for the treatment of a variety of inflammation-related diseases, such as ulcerative colitis, chronic kidney disease, cardiovascular disease.

Lung inflammation. A study confirms that esculetin can improve the inflammatory response in lung injury (80). Esculetin (20-40 mg/kg) reduce the histopathological changes (such as alveolar wall thickening, interstitial edema, and pulmonary congestion) and the infiltration of inflammatory cells, and inhibits the production of TNF-α, IL-1β, IL-6, IL-23 and myeloperoxidase (MPO) in LPS-induced acute lung injury mice. This is associated with the inhibition of the activation of the AKT/ERK/NF-κB and retinoid-related orphan nuclear receptor γt (RORγt)/IL-17 signaling pathways (80,81).

Table II. Antioxidant and anti-inflammatory effects of esculin.

A, Antioxidant

Detail	Cell lines/model	Dosage	(Refs.)
Increase SOD and GSH expression levels and inhibit the expression of cytochrome c, apoptosis-inducing factor, p53, Bax and caspase 3	Dopamine-induced human neuroblastoma SH-SY5Y cells	1-100 μM	(58)
Activate the Nrf2 signaling pathway to increase HO-1 expression levels and decrease the MDA content	LPS/D-galactosamine-induced acute liver injury mice	10-40 mg/kg	(61)
Increase SOD levels and decrease MDA levels	Ethanol-induced gastric mucosal injury rats	12.5-50 mg/kg	(104)
Promote the expression of GPx	Adjuvant-induced arthritis rats	10-40 mg/kg	(64)
Promote the expression of GPx	TNBS-induced colitis rats	5-25 mg/kg	(53)
Promote the expression of SOD, GR and catalase	Prooxidant aflatoxin B-1-induced nephrotoxicity mice	150 mg/kg	(56)
Increase the expression levels of GPx, SOD and catalase	STZ-induced type 2 diabetic mice	10-50 mg/kg	(57)

B, Anti-inflammatory

Detail	Cell lines/model	Dosage	(Refs.)
Reduce the levels of TNF-α, IL-1β and IL-6	Free fatty acid-induced HepG2 cells	25-200 μM	(99)
Reduce the expression levels of IL-1β, TNF-α and iNOS by inhibiting the NF-κB signaling pathway	LPS-stimulated RAW264.7 cells	300-500 μM	(100)
Inhibit p38/MAPK signaling pathway and downregulate MMP-9 levels and AP-1 binding activity	LPS-stimulated RAW264.7 cells	10-50 μM	(101)
Inhibit p38/MAPK, JNK and ERK1/2 signaling pathways and reduce the TNF-α and IL-6 expression levels	Mouse peritoneal macrophages	1 μg-1 mg	(102)
Inhibit the expression levels of IL-12, IL-6 and TNF-α	Reserpine-induced depression mice	50 mg/kg	(105)
Inhibit the activation of the NF-κB signaling pathway and the expression of IL-6 and TNF-α	LPS-induced depressed mice	20-40 mg/kg	(106)
Inhibit the MAPK signaling pathway and reduce the levels of TNF-α, IL-6, ICAM-1 and MCP-1	STZ-induced diabetic rats	30-90 mg/kg	(63)
Decrease the levels of NO and TNF-α	Hyperoxia-induced lung injury rats	10-50 mg/kg	(61)
Inhibit the TLR4/MyD88/NF-κB signaling pathway and downregulate the expression levels of TNF-α, IL-1β and IL-6	LPS-induced acute lung injury mice	20-40 mg/kg	(103)
Inhibit the NF-κB signaling pathway and downregulate the levels of IL-1β and TNF-α	DSS-induced colitis mice	5-50 mg/kg	(100)
Inhibit the NF-κB signaling pathway and downregulate the production of NO, TNF-α and IL-6	Ethanol-induced gastric injury mice	5-20 mg/kg	(104)
Inhibit the TLR4/MyD88 signaling pathway and downregulate the expression of IL-1β, IL-6, TNF-α, MCP-1 and ICAM-1	LPS-induced acute kidney injury mice	25-100 mg/kg	(107)
Decrease the expression levels of IL-1β, IL-6, ICAM-1 and NO	STZ-induced diabetic rats	30-90 mg/kg	(108)
Inhibit the NF-κB signaling pathway and reduce the levels of TNF-α and IL-1β	LPS/D-galactosamine-induced acute liver injury mice	10-40 mg/kg	(62)
Inhibit the NF-κB signaling pathway, downregulate the expression of TNF-α and IL-6, and increase the expression of the silent information regulator 1	Non-alcoholic steatohepatitis mice	20-40 mg/kg	(99)
Decrease the levels of IL-1β, TNF-α and IL-6	Adjuvant-induced arthritis rats	10-40 mg/kg	(64)
Inhibit p38/MAPK, JNK and ERK1/2 signaling pathways and decrease the TNF-α and IL-6 levels	Xylene-induced rat paw swelling	5-20 mg/kg	(102)

Table II. Continued.

B, Anti-inflammatory

Detail	Cell lines/model	Dosage	(Refs.)
Inhibit the NF-κB signaling pathway and reduce the levels of TNF-α and IL-6	LPS-induced sepsis mice	30 mg/kg	(109)

SOD, superoxide dismutase; GSH, glutathione; HO-1, heme oxygenase-1; MDA, malondialdehyde; GPx, glutathione peroxidase; TNBS, 2,4,6-trinitrobenzenesulfonic acid; TLR4, toll-like receptor 4; Myd88, Myeloid differentiation primary response gene 88; ICAM-1, intracellular adhesion molecule-1; MCP-1, monocyte chemoattractant protein-1; LPS, Lipopolysaccharide.

In polyhexamethylene guanidine-induced pulmonary fibrosis mice, esculetin (10 mg/kg) antagonizes macrophage infiltration, ameliorates alveolar epithelial barrier disruption and reduces the levels of MMP-9, macrophage inflammatory protein 2, IL-8 and IL-1β (82).

Esculetin decreases the inflammatory response in asthma. For example, esculetin (20 mg/kg) reduces the levels of IL-4, IL-5, IL-13, IL-17A, immunoglobulin E (IgE), GATA binding protein 3 and RORγt in the lung tissue of ovalbumin-induced asthma mice (83). In addition, esculetin (10 mg/kg) improves airway inflammation, and inhibits airway eosinophilia, collagen fiber deposition and goblet cell hyperplasia in mice exposed to particles <10 μm. This is associated with the inhibition of the activation of the toll-like receptor 4 (TLR4) signaling pathway and the expression of COX-2 and nitric oxide synthase 2 (84).

Inflammation of the gastrointestinal tract. Esculetin can reduce the inflammatory response in gastrointestinal injury. For example, esculetin (100 mg/kg) decreases the levels of COX-2, iNOS and cytokine-induced neutrophil chemoattractant-3 in TNBS-induced colitis rats (85). Furthermore, esculetin (5 mg/kg) can inhibit the production of NO, TNF-α, IL-6, iNOS and nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain-containing in DSS-induced colitis rats by inhibiting the activation of the NF-κB and MAPK signaling pathways (86,87). Additionally, esculetin (20-40 mg/kg) inhibits the expression of iNOS, TNF-α and IL-6 in ethanol-induced gastric injury rats by inhibiting the activation of the NF-κB signaling pathway (88).

Inflammation in liver injury. Esculetin decreases the inflammatory response in liver injury. For example, esculetin (0.01%, w/w) can downregulate the expression of inflammatory genes including TLR4, myeloid differentiation primary response gene 88 (MyD88), NF-κB, TNF-α and IL-6, and decrease the levels of blood glycated hemoglobin, TNF-α, IL-6 and MCP-1 in high-fat diet-induced fatty liver mice (89).

Neuroinflammation. Esculetin can reduce neuroinflammation. For example, esculetin (5-25 mg/kg) can inhibit the zinc oxide nanoparticles-induced expression of IL-1β and TNF-α in rat brain tissue (90). Furthermore, esculetin (20-40 mg/kg) ameliorates LPS-induced neuroinflammation and depression-like behavior in mice by reducing the levels of IL-6, IL-1β, TNF-α, iNOS and COX-2 in serum and the hippocampus by inhibiting the activation of the NF-κB signaling pathway (91,92).

Skin-related inflammation. Esculetin decreases skin-related inflammation. In house dust mite (*Dermatophagoides farinae* extract) and 2,4-dinitrochlorobenzene-induced dermatitis mice, esculetin (2-50 mg/kg) reduces serum IgE, IgG2a and histamine levels, reduce the infiltration of inflammatory cells, and inhibits the production of TNF-α, interferon-γ (IFN-γ), IL-4, IL-13, IL-17 and IL-31 by inhibiting the STAT1 and NF-κB signaling pathways (93). Furthermore, in imiquimod-induced psoriasis-like mouse, esculetin (50-100 mg/kg) was able to significantly reduce the mRNA levels of pro-inflammatory cytokines (including IL-6, IL-17A, IL-22, IL-23, TNF-α and IFN-γ) in the skin of mice, and inhibited the phosphorylation of IKK alpha and P65 in the skin of psoriasis, consequently, inhibited the NF-KB signaling pathway (94).

Other inflammation. Esculetin can improve the inflammatory response in other pathological processes. For example, esculetin (20-60 mg/kg) can inhibit the production of inflammatory factors such as IL-1β, IL-6, TNF-α, C-C motif chemokine ligand 2 and iNOS in *Escherichia coli*-induced sepsis mice by inhibiting the NF-κB and STAT1/STAT3 signaling pathways (95). In a rabbit model in which the primary lacrimal gland, sclerotial gland, and cornea were removed to induce dry eye, esculetin (0.05% of the diet) was able to inhibit the expression of IL-1α, IL-1β, and TNF-α by inhibiting the ERK1/2 signaling pathway and thereby suppressing IL-1α, IL-1β, and TNF-α (96). Furthermore, esculetin (10-20 mg/kg) inhibits the expression of TNF-α and IL-6 in isoproterenol induced myocardial toxicity in rats by inhibiting the activation of the NF-κB signaling pathway (97). Additionally, esculetin (2.5-10 mg/kg) can reduce reserpine-induced fibromyalgia in mice, which is associated with the inhibition of monoamine oxidase-A activity, and downregulated the levels of IL-1β, TNF-α and TBARS (98). In summary, the aforementioned studies show that esculetin can reduce the expression levels of various proinflammatory factors and inhibit the infiltration of inflammatory cells in a number of inflammatory models. In addition to inhibiting the two most common inflammatory signaling pathways, NF-κB and MAPK, the anti-inflammatory effect of esculetin is also associated with inhibiting the STAT signaling pathway.
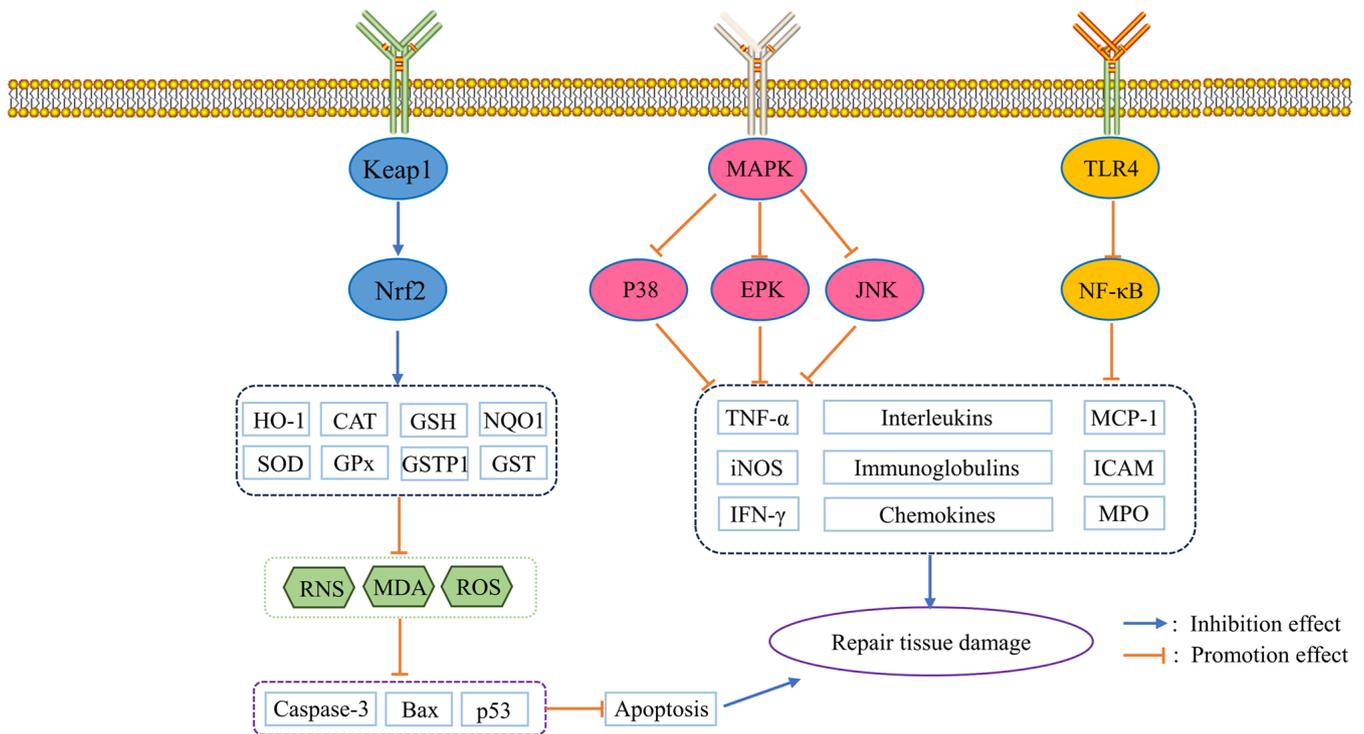
Figure 3. Schematic diagram of the antioxidant and anti-inflammatory mechanisms of esculetin and esculin. TLR4, toll-like receptor 4; Keap1, Kelch-like ECH-associated protein 1; Nrf2, nuclear factor erythroid 2-related factor 2; HO-1, heme oxygenase-1; CAT, chloramphenicol acetyltransferase; GSH, glutathione; NQO1, NADPH quinone oxidoreductase; SOD, superoxide dismutase; GPx, glutathione peroxidase; GSTP1, glutathione S-transferase Pi; GST, glutathione-S-transferase; RNS, reactive nitrogen species; MDA, malondialdehyde; ROS, reactive oxygen species; ERK, extracellular regulated protein kinase; JNK, c-Jun N-terminal kinase; MCP-1, monocyte chemoattractant protein-1; iNOS, inducible isoform of nitric oxide synthase; ICAM, intracellular adhesion molecule; MPO, myeloperoxidase.

Anti-inflammatory activity of esculin. Cellular research shows that esculin (25-200 μM) reduces the levels of TNF-α, IL-1β and IL-6 in free fatty acid-induced HepG2 cells (99). Further studies show that the anti-inflammatory effect of esculin is associated with the inhibition of the MAPK and NF-κB signaling pathways. For example, esculin (300-500 μM) can reduce the expression levels of IL-1β, TNF-α and iNOS in LPS-stimulated RAW264.7 cells by inhibiting the NF-κB signaling pathway (100). In LPS-stimulated RAW264.7 cells, esculin (10-50 μM) also inhibits the p38/MAPK signaling pathway and downregulates the MMP-9 level and AP-1 binding activity (101). Additionally, esculin (1 μg-1 mg) can inhibit the p38 MAPK, c-Jun N-terminal kinase (JNK) and ERK1/2 signaling pathways and reduce the TNF-α and IL-6 expression levels in mouse peritoneal macrophages (102).

Studies using animals further indicates that esculin can exhibit an anti-inflammatory effect in various inflammatory models (95,96,99,100). This suggests that esculin may be promising for the treatment of a variety of inflammation-related diseases (including ulcerative colitis, chronic kidney cardiovascular disease).

Lung inflammation. Esculin can reduce lung injury-related inflammation. For example, esculin (10-50 mg/kg) decreases the levels of NO and TNF-α in hyperoxia-induced lung injury rats (61). Additionally, esculin (20-40 mg/kg) can inhibit the TLR4/MyD88/NF-κB signaling pathway and downregulate the expression of TNF-α, IL-1β and IL-6 in LPS-induced acute lung injury mice (103).

Inflammation of the gastrointestinal tract. Esculin can reduce the inflammatory response in gastrointestinal injury. For example, esculin (5-50 mg/kg) inhibits the activation of the NF-κB signaling pathway and downregulates the levels of IL-1β and TNF-α in DSS-induced colitis mice (100). In ethanol-induced gastric injury mice, esculin (5-20 mg/kg) also inhibits the activation of the NF-κB signaling pathway and downregulates the production of NO, TNF-α and IL-6 (104).

Inflammation in liver injury. Esculin can reduce the inflammatory response in liver injury. For example, esculin (10-40 mg/kg) inhibits the NF-κB signaling pathway and reduces the levels of TNF-α and IL-1β in LPS/D-galactosamine-induced acute liver injury mice (62). In methionine choline-deficient diet-induced non-alcoholic steatohepatitis mice, esculin (20-40 mg/kg) also inhibits the NF-κB signaling pathway, downregulates the expression of TNF-α and IL-6, and increases the expression level of the silent information regulator 1 (99).

Neuroinflammation. Esculin can improve nervous system inflammation. For example, esculin (50 mg/kg) inhibits the expression of IL-12, IL-6 and TNF-α in the hippocampus of reserpine-induced depression mice (105). Furthermore, esculin (20-40 mg/kg) inhibits the activation of the NF-κB signaling pathway and the expression of IL-6 and TNF-α in LPS-induced depressed mice hippocampus, which increases the number of selectively activated microglia and decreases the number of classically activated microglia (106). In addition, esculin (30-90 mg/kg) can inhibit the MAPK signaling

pathway and reduce the levels of TNF-α, IL-6, intracellular adhesion molecule-1 (ICAM-1) and MCP-1 to improve memory impairment in STZ-induced diabetic rats (63).

Kidney inflammation. Esculin can improve the inflammatory response in renal injury. For example, esculin (25-100 mg/kg) inhibits the TLR4/MyD88 signaling pathway and downregulates the expression of IL-1β, IL-6, TNF-α, MCP-1 and ICAM-1 in LPS-induced acute kidney injury mice (107). Similarly, esculin (30-90 mg/kg) decreases the expression levels of IL-1β, IL-6, ICAM-1 and NO in the kidney tissue of STZ-induced diabetic rats (108).

Other inflammation. Esculin can also improve the inflammatory response in other pathological processes. For example, esculin (10-40 mg/kg) significantly improved body weight, decreased paw volume, and the levels of IL-1β, TNF-α, and IL-6 levels in adjuvant-induced arthritis rats compared to arthritic controls (64). Additionally, esculin (5-20 mg/kg) can ameliorate xylene-induced rat paw swelling by inhibiting the activation of the p38, MAPK, JNK and ERK1/2 signaling pathways and decreasing TNF-α and IL-6 levels (102). Furthermore, in LPS-induced sepsis mice, esculin (30 mg/kg) inhibits the NF-κB signaling pathway and reduces the levels of TNF-α and IL-6 (109). In conclusion, esculin (the glycoside form of esculetin) has anti-inflammatory effects and the mechanisms of action are similar to esculetin, inhibiting the activation of the NF-κB and MAPK signaling pathways and inhibiting the expression of various proinflammatory factors.

4. Conclusion and future perspectives

In conclusion, esculetin and esculin have been extensively studied, and studies utilizing cells and/or animals show that both possess a variety of pharmacological activities, including anti-inflammatory, antioxidant, anti-tumor, antidiabetic, antibacterial, antiviral, anti-atherosclerotic and immuno-modulatory pharmacological effects (110). The antioxidant effects of esculetin and esculin have been shown in a variety of diseases, their underlying mechanisms of action and biological activities include scavenging of free radicals, promotion of the expression of endogenous antioxidant proteins (such as SOD, GPx, and GSH), modulating the nuclear factor erythroid 2-related factor 2 pathway, regulating the cell cycle, inhibiting tumor cell proliferation and migration, etc.. Further studies reveal that this is associated with the activation of the Nrf2 signaling pathway (Fig. 3). The anti-inflammatory effects of esculetin and esculin are associated with the inhibition of two important inflammatory signaling pathways, NF-κB and MAPK. In various models of inflammation, including diabetes, liver injury, tumors, bacterial, fungal, neurological, lung and respiratory inflammation, both esculetin and esculin are able to reduce the expression of various proinflammatory factors, such as TNF-α, IL-1β and IL-6, thereby inhibiting the tissues damage caused by inflammation (Fig. 3, Table I and II). In conclusion, esculetin and esculin both have antioxidant and anti-inflammatory effects, and may be used for the treatment or improvement of various diseases in the future.

Currently, the most studied components in the Chinese medicine Qinpi are esculetin and esculin, both of which belong to the coumarin group of components. It's worth looking into esculetin and esculin have relatively abundant experimental data from animals (including mice, rats, rabbits, etc.) in the treatment of a variety of diseases related to inflammation and oxidative stress, and therefore, these two components have a better prospect of clinical application. In the future research, the clinical study can be carried out to determine the esculetin and esculin in the clinical environment. antioxidant and anti-inflammatory activities. The development of esculetin and esculin as novel drugs for the treatment of inflammation and other associated diseases has the potential to advance the development of natural drug formulations for the treatment of inflammation.

Availability of data and materials

Not applicable.

Authors' contributions

SJ conceived and revised the review. YT and QW performed the literature. LZ and KW drafted the review article and revised the manuscript. CW and MW reviewed and helped to modify the manuscript. Data authentication is not applicable. All authors have 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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