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Review

Mechanistic Insights into the Biochemical and Pharmacological Profile of Esculetin

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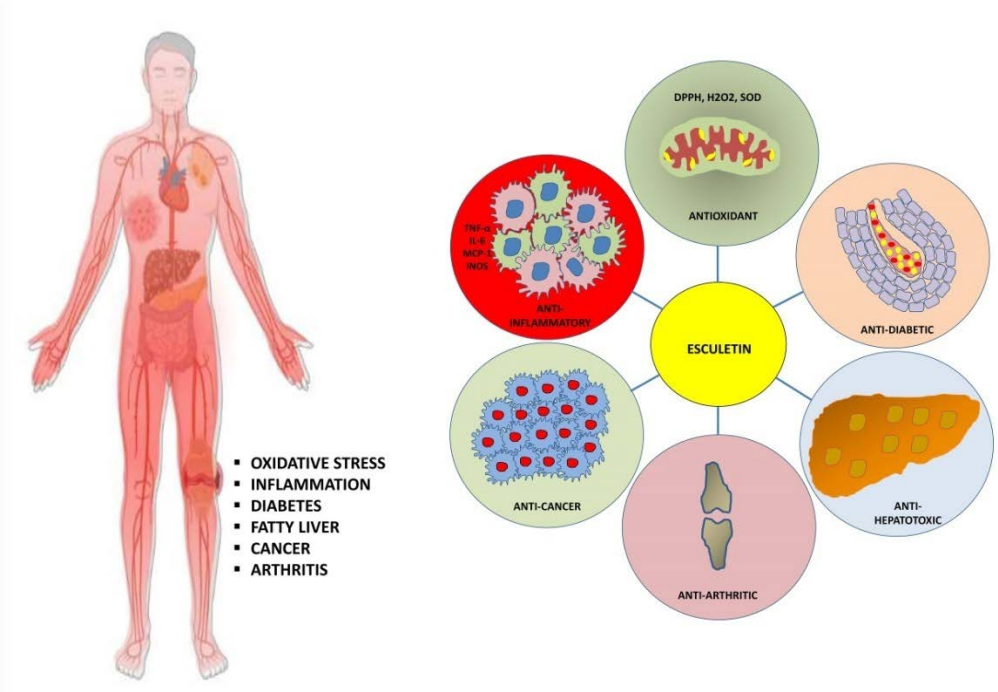
Abstract: Esculetin is a coumarin compound, which belongs to the class of benzopyrone enriched in various plants such as *Sonchus grandifolius*, *Aesculus turbinata*, and many others. Glycosides and caffeic acid conjugates are the common forms of esculetin present in medicinal plants. Esculetin acts as an antioxidant, anti-inflammatory, anti-diabetic, anti-hepatic, and anti-cancer agent by inhibiting the production of free radicals, inflammatory mediators, and genes that cause liver diseases and cancer. It also aids in the regulation of blood sugar. Scientists developing pharmaceutical formulations require some rationale and preliminary studies for drug design, but a small number of clinical studies on humans containing esculetin limit its potential for use as a safe alternative drug. Therefore, in this review article, the published studies have been reviewed to identify the pathogenesis of cancer, oxidative stress, inflammation, arthritis, diabetes and fatty liver along with the discussion on potential therapeutic strategies of esculetin. Advancements in our understanding of these diseases will aid in the development of new and innovative medications for treating many ailments. In conclusion, esculetin has immense potential to be used as a safe drug against many diseases but requires further testing and confirmation through clinical trials.



Graphical abstract: The role of esculetin in attenuating cancer, oxidative stress, inflammation, diabetes, fatty liver, and arthritis.

Keywords: esculetin; cancer; oxidative stress; inflammation; arthritis; diabetes; fatty liver

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1. Introduction

Esculetin (6, 7-dihydroxychromen-2-one), is one of the most-studied coumarin derivatives among other such compounds, namely umbelliferone, warfarin, scopoletin, etc [1]. Structurally, esculetin contains hydroxyl groups, at 6thand 7th carbon atoms. Cancer involves the uncontrolled proliferation of cells and results in tumor formation and metastasis [2]. Malignant cells display high levels of free radicals which further contribute to the occurrence of oxidative stress. Many genes and transcription factors are deregulated aiding in the cancer progression, esculetin has been shown to potentially limit the proliferation of abnormal or mutated cells inhibiting cancer growth [3].

The free radicals affect living cells by oxidative damage to DNA, lipids, and proteins [4]. Oxidative damage induced by free radical generation triggers a cascade of oxidative stress in the body that serves as the source of many other diseases pertaining to cancer, chronic inflammation, diabetes, and liver damage [5]. Superoxide dismutase, catalase, and glutathione peroxidase are the naturally occurring physiological antioxidant enzymes with the ability to disrupt the cellular oxidative damage caused by reactive oxygen species (ROS) [6]. Oxidative stress develops with an increase in the ROS level or a decrease in the antioxidant enzyme activity in the body [7]. Esculetin, an antioxidant compound has the ability to scavenge free radicals generated during oxidative stress and thus enhance the activation of such enzymes.

Macrophages responsible for pathogen elimination trigger the generation of free radicals during causing inflammation [8]. Esculetin which is a naturally occurring coumarin compound exhibits anti-inflammatory properties through the inhibition of inflammatory cytokines [9]. Arthritis is a condition in which one or more joints enlarge and become tender [10]. Injury, abnormal metabolism, genetic makeup, infection, and immune system malfunction are the factors that result in arthritis [11]. Inflammation is a major factor in arthritis as it makes it difficult for the joints and bones to function properly. Symptoms of arthritis include pain, redness, stiffness, swelling, tenderness, and warmth.

The activity of several antioxidant enzymes is found to be dysregulated in diabetes triggering a cascade of hyperglycemia-induced oxidative stress. Esculetin is reported to control the fluctuations in the glycemic index thus managing the blood sugar levels in diabetes and also enhancing the activity of antioxidant enzymes to prevent oxidative stress [12]. In non-alcoholic fatty liver disease, fat accumulates in the liver. People with obesity, high blood sugar, high blood pressure, and high cholesterol have a higher risk of developing fat in the liver [13-20]. Esculetin treatment has shown a significant therapeutic effect against hepatic failure [21]. In this review, we have attempted to discuss the biochemical and pharmacological profile of esculetin in detail (Table 1).

Table 1: The pharmacological activity of esculetin and its major mechanism of action

S. No.	Pharmacological activity	Mechanism of action

Anti caner activity	MAPK signaling pathways, caspase-3 and 9 leading to apoptosis
Antioxidant activity	Inhibits H_2O_2, superoxide and DPPH radical activity
Antioxidant activity	Increases the glutathione levels
Anti-inflammatory activity	Downregulates pro-inflammatory cytokines and obstructs NF-kB pathway
Anti-arthritic activity	Protects cartilage degradation by inhibiting matrix metalloproteases (MMPs)
Anti-diabetic activity	Attenuates the cascade of hyperglycemia-induced oxidative stress in diabetes
Protection from fatty liver disease	Ameliorate TGF-β mediated hepatic fibrosis

2. Biochemical mechanism of esculetin

Oxidative stress plays an important in the pathogenesis of chronic ailments like cancer, diabetes, liver disease, and inflammatory disorders. Esculetin participates in a wide range of biochemical activities including scavenging of free radicals, suppression of

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dysregulated transcription factors in cancer, inhibition of inflammatory pathways involved in arthritis, management of glycemic index, fatty liver disease, etc.

2.1. Esculetin in cancer

Cancer is a fatal disease that develops in the body in response to abnormal cell growth, and metastasis leading to organ failures and death. A vast number of advancements have been made either to control or cure this lethal disease. Coumarin compounds have always been considered the first choice for researchers because of their excellent biological activity and low toxicity [22]. A study showed that esculetin arrests the growth of cancer cells in the G1 phase of the cell cycle. It impedes the binding interaction between Nrf2 and KEAP1 by activating the ARE pathway and attenuating the NF-κB activity leading to apoptosis which is one of the primary goals of cancer treatment [23]. Esculetin inhibits leukemia cell proliferation and induces autophagy through the formation of autophagic vesicles. In addition, it downregulates the expression of cyclin D1, D3, DK4, and DK2 causing a cell cycle arrest at the G0/G1 phase. This coumarin compound was shown to block the phosphorylation of MEK and ERK, thereby inhibiting the activation of Raf/MEK/ERK signaling [24], and also reported downregulating the JNK and ERK pathways in leukemia U937 cells suggesting its potent anticancer property [25].

Benzo[a]pyrene is known to play an important role in lung cancer [26] and esculetin treatment showed an arrest in the cancerous cell proliferation by downregulating Bcl-2 and NF-κB causing apoptosis [27]. Esculetin not only inhibits pancreatic and lung cancer but also prevents colon cancer by activating MAPK signaling pathways, caspase-3 and 9 leading to apoptosis [28]. In the oral squamous cancer cell, esculetin has been shown to downregulate the expression of specificity protein 1 (Sp1), p27, cyclin D1, Mcl-1, and survivin thus inducing apoptosis [29]. In larynx cancer, esculetin was found to suppress the phosphorylation of STAT3 and subsequent translocation into the nucleus resulting in the inhibition of the JAK/STAT pathway. Furthermore, esculetin causes the cell cycle arrest at the G1/S phase and thus supports apoptosis [30].

In the case of hepatocellular carcinoma, esculetin promotes apoptosis by arresting the cells at the S-phase of the cell cycle. In addition, esculetin was found to elevate the mechanism of caspase-3 and 9 and reduced the mitochondrial membrane potential. Esculetin significantly increased the Bax expression thereby reducing the Bcl-2 expression, thus exhibiting its anti-cancerous potential [31]. Overactivation of IGF-1/PI3K/Akt and IGF-1/MAPK signaling pathways contributed to the development of tumors however esculetin was found to diminish the mitochondrial membrane potential with simultaneously activating the mitochondrial apoptotic pathway in the MGC-803 gastric cancer cell line. It increased the cytochrome c release from mitochondria, Bax/Bcl-2 index, and activated the activity of caspase-3 and 9. The mechanism includes the suppression of the IGF-1/PI3K/Akt pathway [32]. Esculetin was reported to prevent the proliferation, migration, and invasion of renal carcinoma cells by cell cycle arrest at G0/G1 and G2 phase, downregulating the expression of Cyclin D1, CDK4, CDK6, and c-Myc resulting in apoptosis. Levels of E-cadherin were increased whereas the expressions of N-Cadherin and vimentin were downregulated with esculetin treatment (Fig 1) [33].

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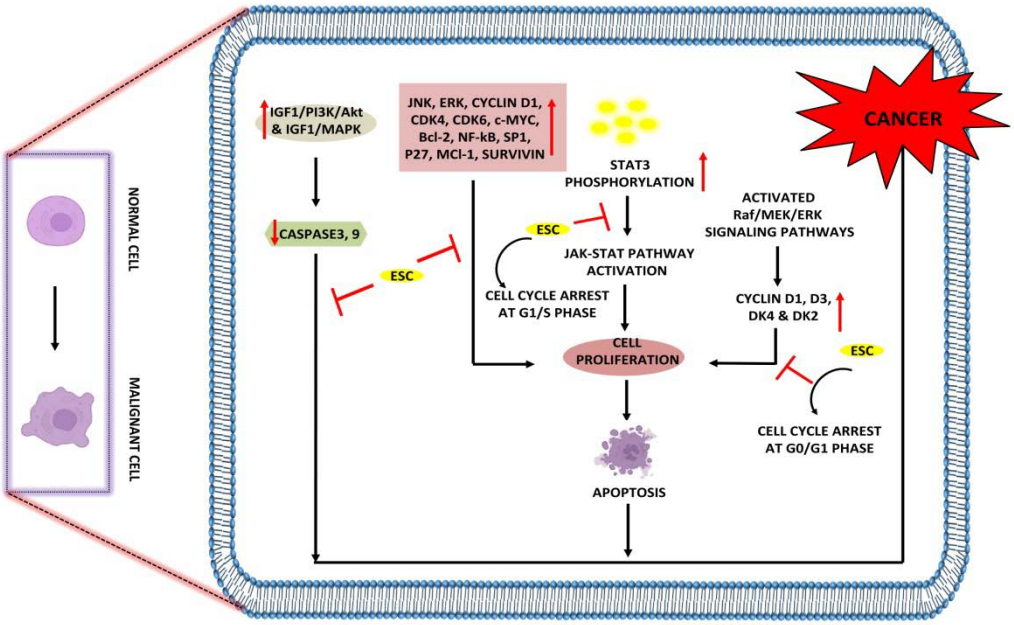

Figure 1. Anti-cancer effects of esculetin: Esculetin downregulates the IGF1/P13/AKT, IGF1/MAPK, JNK, ERK, NF-κB, P27, Survivin, and Bcl-2. Esculetin also inhibits the cell-proliferation by inhibiting the STAT3 phosphorylation and cyclin D1, D3, DK4, and DK2, leading to apoptosis and suppression of cancer.

2.2. Esculetin in oxidative stress

Free radicals are the single unpaired electron species that exist independently in the body and are capable of causing oxidative damage to DNA, proteins, and carbohydrates. Therefore, antioxidants act as an eminent tool against oxidative damage. Many coumarin compounds including esculetin have been evaluated using in vitro and in vivo models of oxidative stress. Hydrogen peroxide (H2O2) is known to play a crucial role in generating oxidative stress. Esculetin has been reported to inhibit the H2O2 in the generated radicals attenuating the cascade of oxidative stress in the myoblasts cell line [34]. A similar study highlighted that cells treated with H2O2 showed an increase in lipid peroxidation whereas esculetin inhibits the oxidative DNA damage caused by it [35]. Increased serum levels of alkaline phosphatase, aspartate transaminase, and alanine transaminase in carbon tetrachloride-induced injury in rat liver were reduced with esculetin dosage due to its lipid peroxidation inhibition activity [36].

In Alzheimer's disease, esculetin activates the Nrf2, increases the glutathione level, and thus protects cells from oxidative stress-induced damage by amyloid proteins [37]. Similarly, esculetin also showed scavenging activity for superoxide, and DPPH radicals [38]. It has been found that H2O2 upregulates the expression of MMP-1 promoting skin aging, also oxidative stress by activating the MAPK and AP1 signaling pathways. The mechanism involves esculetin inhibiting the expression of phospho-MEK1, phospho-ERK1/2, phospho-SEK1, and phospho- JNK1/2 along with the intracellular Ca2+ levels induced by H2O2. [39]. In addition, esculetin was reported to protect human fibroblast from oxidative stress-induced DNA damage induced by linoleic acid hydroxide and iron (III) ion [40]. Figure 2 summarizes the above-mentioned events.

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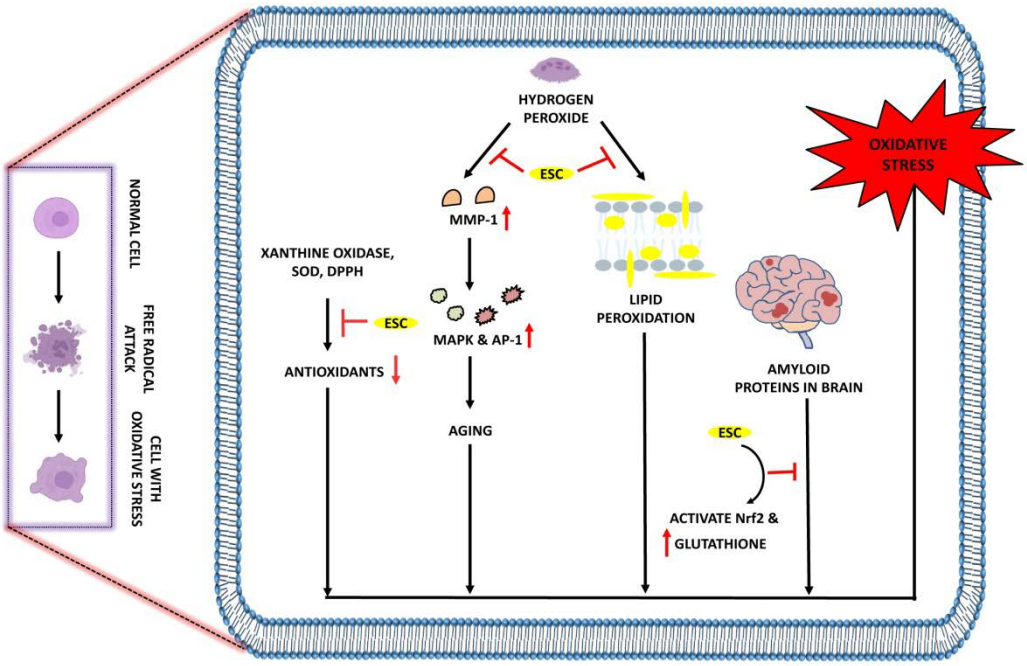

Figure 2. Antioxidant effect of esculetin: Esculetin blocks the activity of hydrogen peroxide which further inhibits the MMP-1 and lipid peroxidation in cell membranes. Esculetin prevents the brain from amyloid-induced oxidative stress via activating the Nrf2 and increasing the levels of glutathione, thus inhibiting oxidative stress.

2.3. Esculetin in inflammation

Inflammation is defined as the defense mechanism that occurs in response to infection or injury and helps the body to maintain homeostasis. During infection or injury, the damaged cell triggers the release of various physiological messengers including histamine, prostaglandins, nitric oxide, and leukotrienes, which promote the cascade of inflammation [41], resulting in the development of debilitating diseases like rheumatoid arthritis (RA) and osteoarthritis (OA) [42]. Much research has been done to reveal the molecular mechanism of esculetin as a potent inhibitor of inflammation.

The transcription factor nuclear factor kappa B (NFκB) is involved in the pathogenesis through the regulation of inflammatory genes [43]. Briefly, the cytosol is a primary site of occurrence of NFκB and under inflammatory stimuli, IκB is phosphorylated as well as degraded by the 26S proteasome resulting in the release of NFκB. Consequently, the released NFκB translocates to the nucleus, resulting in its binding to the promoter region and upregulation of inflammatory genes. In response to LPS stimulation, esculetin downregulates the phosphorylation of IKKα and IκBα. It also inhibits the JMJD3 phosphorylation, thus exhibiting anti-inflammatory properties [44]. Previous findings revealed that esculetin inhibits the nuclear translocation of NFκB by downregulating the LPS-mediated IκBα degradation, thus inhibiting inflammation [45].

Nitric oxide (NO) is one of the key mediators of inflammation [46]. Nitric oxide synthase (NOS) is an enzyme that exists in three different forms neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS). This class of enzyme is responsible for the conversion of L-arginine into nitric oxide [47]. But when there is an imbalance in this reaction, nitric oxide levels rise resulting in exacerbated inflammation. The effect of esculetin in a hippocampus animal model had a significant impediment on the NFκB signaling pathway thereby attenuating iNOS expression [48]. Moreover, a study showed that esculetin decreased the level of LPS-induced iNOS in a murine macrophage cell line (RAW 264.7) [49, 50]. It also inhibits the inflammatory cascade by attenuating the NO formation in the cartilage osteoarthritis animal model [51]. In the

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adipose tissues of obese mice, macrophages are known to release potent inflammatory agents, especially nitric oxide, prostaglandins, and tumor necrosis factor-α causing systemic inflammation. Esculetin upregulates the level of heme oxygenase 1 (HO-1) in cocultured macrophages and adipocytes restricting the liberation of TNFα, and MCP-1, suggesting its protective role against the obesity-induced inflammation [52].

Proinflammatory cytokines such as TNF-α, IL-1β, IL-2, IFN-γ, IL-8, and IL-6 are produced by macrophage activation causing inflammatory diseases. [53]. Esculetin has been shown to obstruct the inflammatory cascade by downregulating the expression of IL-1β, IL-2, IFN-γ, and TNF-α in inflammatory bowel disease [54] and fibromyalgia [55]. Furthermore, esculetin exhibits anti-allergic and anti-inflammatory activity by downregulating the expression of IL-6 and IL-8 in the nasal epithelial cells under in vitro conditions [56]. Esculetin isolated from Fraxinus rhynchophylla was found to attenuate the expressions of TNF-α, IL-6, and IL-1β in acute skin inflammation [57]. This chemical compound constrains phosphorylation of ERK1/2 and thereby restricting inflammation [58]. Esculetin has been reported to inhibit the RhoA/Rho kinase pathway, followed by the downregulation of TNF-α, IL-1β, and IL-6 thereby downregulating the inflammation-causing pathways in the acute lung injury model [59]. The TNBS-induced ulcerative colitis has also been treated successfully with esculetin revealing the attenuation of COX2 in the inflamed colon [60]. Esculetin reduces the release of soluble intracellular adhesion molecule-1 inhibiting leukocyte and endothelial cell adhesion events therefore inflammation [61]. Esculetin decreases the expression of TNF-α, IL-6, IL-22, IL-23, IL-17A, and IFN-γ cytokines in psoriatic mouse skin, reducing inflammation [62] as shown in figure 3.

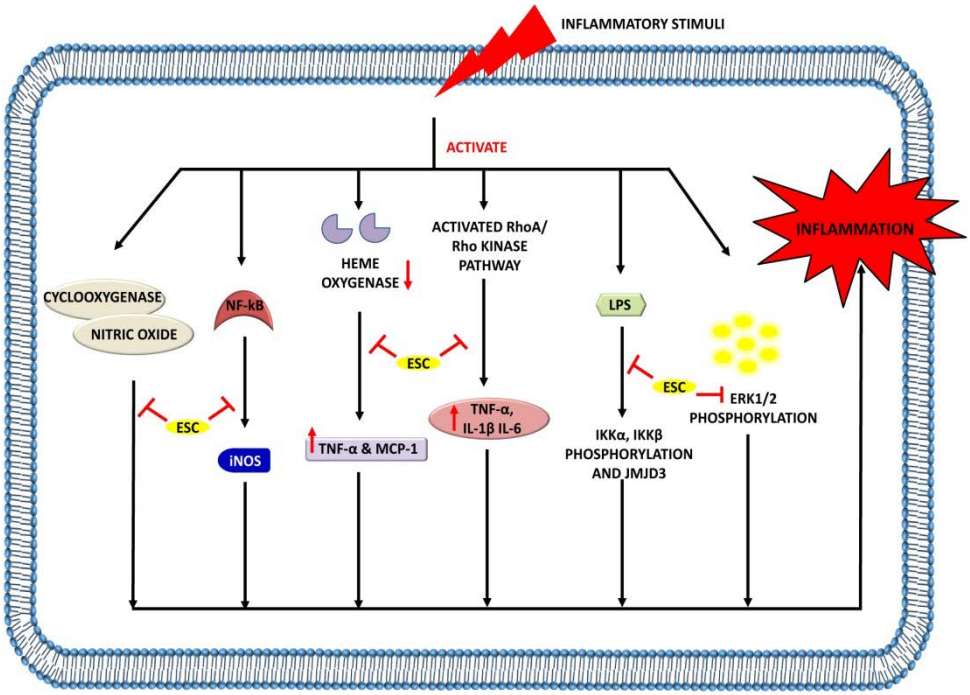

Figure 3. Anti-inflammatory effects of esculetin: Esculetin inhibits the action of cyclooxygenase, NF-κB, Nitric oxide, LPS, heme oxygenase, RhoA/Rho kinase pathway, and ERK1/2 phosphorylation, inhibiting the inflammatory reactions.

2.4. Esculetin in arthritis

Arthritis is characterized by acute or chronic inflammation of the joints accompanied by severe pain and structural damage. The two most frequent types of arthritis that afflict the global population are osteoarthritis (OA) and rheumatoid arthritis (RA) [63]. OA is a degenerative disease characterized by progressive cartilage loss and

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bone deterioration [64] whereas rheumatoid arthritis is a systemic, persistent inflammatory condition driven by an autoimmune response to stimuli in the environment mainly affecting the synovial joints [65]. Rapid loss of articular cartilage, degradation of collagen and proteoglycans, upregulation of matrix metalloproteinases, leukotrienes (particularly leukotriene B4), and thickening of the subchondral plate are the main contributors to the OA and RA [66-72]. Various studies have reported that esculetin acts on cartilage and results in the inhibition of matrix degradation by suppressing the MMP production, secretion, and its activity in rabbit joint cartilage, which serves as a lead therapeutic compound in the treatment of OA and RA [73, 74]. Esculetin effectively reduces the level of leukotriene B4 in plasma of rats with adjuvant-induced arthritis [75].

An esculetin derivative, 4-Methylesculetin was reported to modulate the extracellular matrix and hyaluronidase enzymes thus inhibiting articular cartilage and subchondral bone degradation, and restoring joint homeostasis [76]. The interleukin-1α with oncostatin M upregulates the expression of MMP-1, MMP-3, MMP-13, and TIMP-1 mRNA whereas esculetin treatment potentially downregulates the expression of these proteases. Moreover, esculetin also suppresses the collagen and proteoglycan degradation from cartilages and stops collagen depletion [77] as depicted in figure 4.

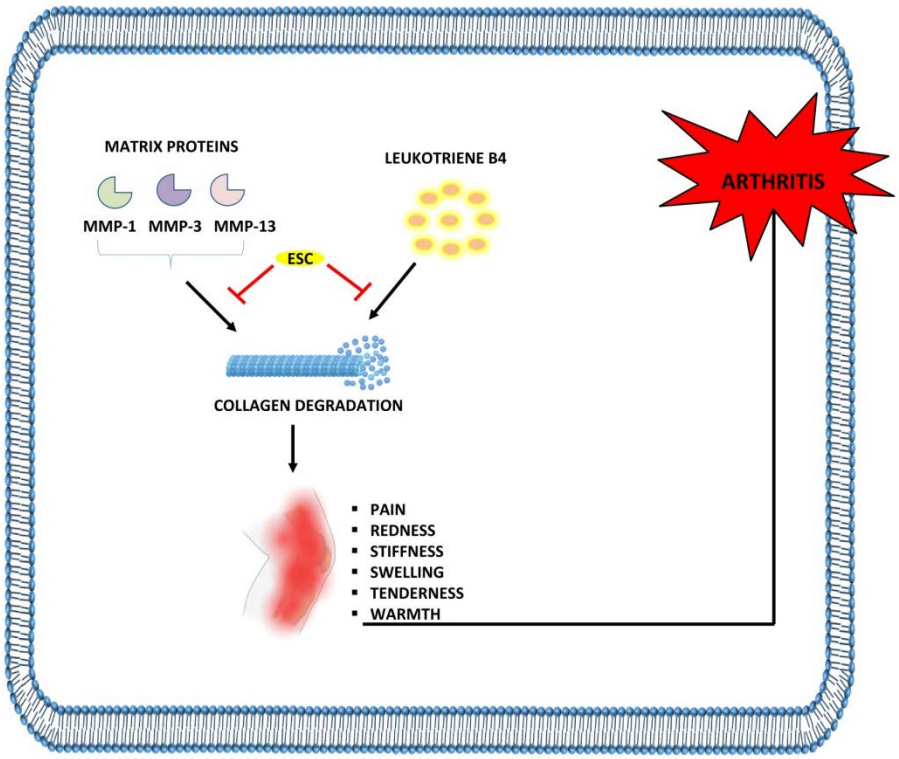

Figure 4. Anti-arthritic effects of esculetin: Esculetin inhibits the degradation of cartilage through the suppression of MMP-1, MMP-3, MMP-13, and leukotriene B4, leading to the inhibition of arthritis.

2.5. Esculetin in diabetes and its associated complications

Diabetes is a metabolic disorder in which changes in plasma levels of glucose occur as a result of defects in insulin secretion or insulin action [78]. In such conditions, the activity of several antioxidant enzymes is found to be downregulate, resulting in the development of diabetes-induced-oxidative stress [22, 79-80]. Many natural and synthetic compounds have been used to ameliorate oxidative stress, and esculetin is one of them.

Esculetin has been reported to reduce blood glucose levels and increase plasma insulin levels in diabetes. Moreover, esculetin ameliorates the level of antioxidant enzymes, particularly catalase, superoxide dismutase, glutathione-S-transferase, and

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glutathione peroxidase, and thereby attenuates the cascade of hyperglycemia-induced oxidative stress in diabetic rats [81]. As streptozotocin (STZ) and a high-fat diet results in an increase in triglyceride and plasma glucose concentrations, esculetin improves the insulin level under in vivo hyperinsulinemic conditions [82]. So, esculetin not only reduces blood glucose levels and diabetes-mediated oxidative stress but also lessens the associated complications such as diabetic nephropathy suggesting esculetin as an anti-diabetic compound. In addition, PPARγ is upregulated in the diabetic kidney which is inhibited by esculetin treatment and the latter also inhibits the TGF-β1-mediated fibronectin expression in the diabetic kidney [83].

At the molecular level, various modulated epigenetic markers such as H3S10phospho, H3S28phospho, H3K9Ac, H3K4me2, and H3K9me2are responsible for the pathogenesis of insulin resistance and type 2 diabetes. Some reports suggest that esculetin reversed these modified epigenetic markers mitigating the risk of type 2 diabetic cardiomyopathy [84]. Similarly, another in vivo study highlighted that hyperacetylation of histone H3 lysine (K) 14 and 18 could be reversed with the esculetin treatment. Furthermore, a high-fat diet and STZ are reported to increase H2AK119Ub levels which were potently reversed by esculetin preventing type 2 diabetes and associated nephropathy [85] as shown in figure 5.

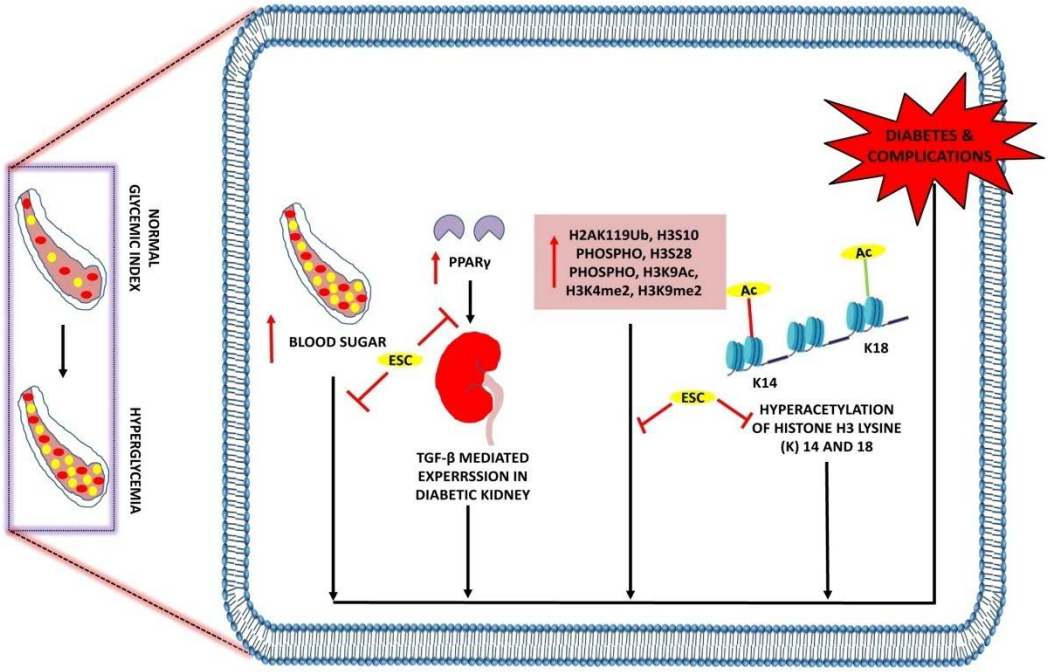

Figure 5. Anti-diabetic effects of esculetin: In the figure, red circles represent RBCs in vessels and yellow circles represent sugar. Esculetin downregulates the expression of PPARγ, H3S10phospho, H3S28phospho, H3K9Ac, H3K4me2, and H3K9me2, hyperacetylation of histone H3 lysine (K) 14 and 18 and attenuates diabetes and its associated complications.

2.6. Esculetin in fatty liver

Non-alcoholic fatty liver disease is becoming the primary cause of chronic liver disease due to the increasing evidence of obesity and type 2 diabetes mellitus. Esculetin has been reported to improve the phospho-FOXO1 expression thus acting on the Akt/P13K/FOXO1 pathway; these results in the amelioration of TGF-β mediated hepatic fibrosis followed by the high-fat diet induced non-alcoholic fatty liver [86]. The upregulation of PPARγ, Fasn, Pap, and Dgat2 are actively responsible for the development of non-alcoholic fatty liver disease in diabetes.Esculetin exhibits hepatoprotective activity by downregulating the level of genes responsible for hepatic

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fatty acid and triglyceride synthesis [87]. The probable mechanism of the prevention of non-alcoholic fatty liver disease by esculetin is the induction of decreased expression of sterol regulatory element-binding protein-1c (SREBP1c) and fatty acid synthase through the activation of adenosine monophosphate-activated protein kinase (AMPK) signaling pathway. So, esculetin attenuates the cascade of development of non-alcoholic fatty liver disease [88] as summarized in Figure 6.

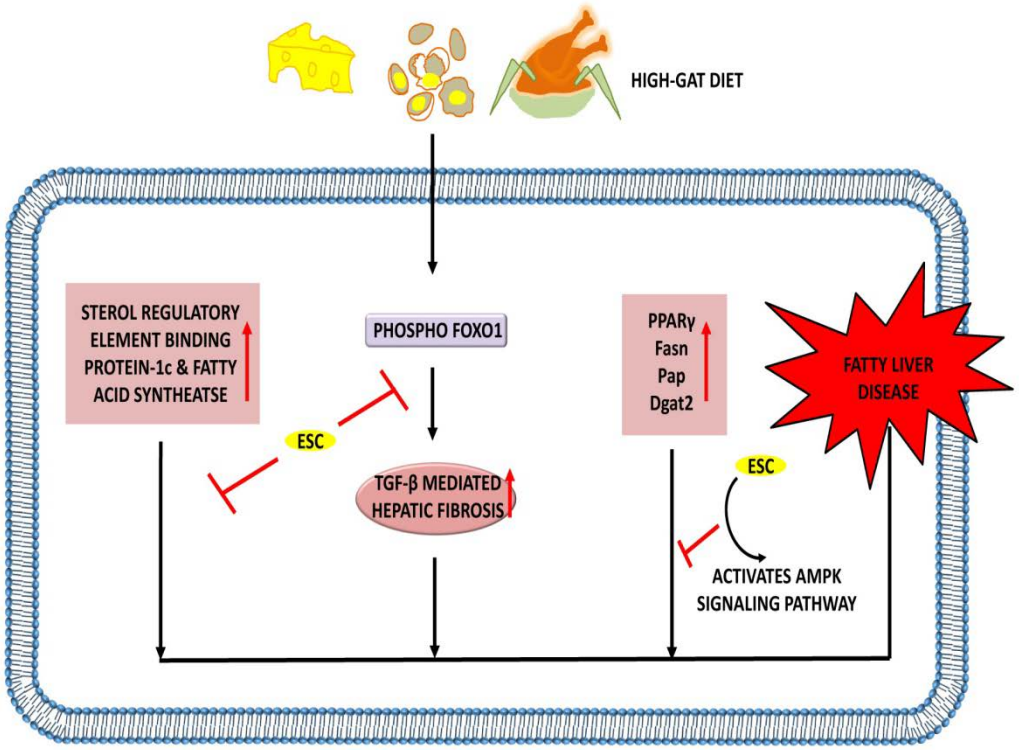

Figure 6. Hepatoprotective effects of esculetin: Esculetin downregulates the expression of sterol regulatory element-binding protein-1c, fatty acid synthetase, and TGF-β mediated hepatic fibrosis. Esculetin also activates AMPK signaling pathway and inhibits the action of PPARγ, Fasn, Pap, and Dgat2, thus inhibits the pathogenesis of the fatty liver disease.

3. Conclusion

A large number of studies have been conducted to reveal the pharmacological and biochemical mechanism of action of coumarin compounds, especially esculetin. As oxidative stress is a known trigger for the onset of many diseases, this coumarin compound scavenges free radicals produced during oxidative stress and thus protects against other diseases. In this review article, we discussed a variety of biochemical modes of action of this molecule, with an emphasis on cancer, oxidative stress, inflammation, arthritis, diabetes, and fatty liver. The given table shows the major pathways affected by esculetin to exhibit various pharmacological properties. This suggests its potential to become a safer drug for the treatment of other ailments as well. A much deeper understanding is needed with the development of a more clinically effective formulation of esculetin targeting cancer, oxidative stress, inflammation, arthritis, diabetes, and fatty liver to reducing mortality worldwide.

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