

Potential applications of artemisinin in ocular diseases

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Abstract

• Artemisinin, also named *qinghaosu*, is a family of sesquiterpene trioxane lactone originally derived from the sweet wormwood plant (*Artemisia annua*), which is a traditional Chinese herb that has been universally used as anti-malarial agents for many years. Evidence has accumulated during the past few years which demonstrated the protective effects of artemisinin and its derivatives (artemisinins) in several other diseases beyond malaria, including cancers, autoimmune disorders, inflammatory diseases, viral and other parasite-related infections. Recently, this long-considered anti-malarial agent has been proved to possess anti-oxidant, anti-inflammatory, anti-apoptotic and anti-excitotoxic properties, which make it a potential treatment option for the ocular environment. In this review, we first described the overview of artemisinins, highlighting the activity of artemisinins to other diseases beyond malaria and the mechanisms of these actions. We then emphasized the main points of published results of using artemisinins in targeting ocular disorders, including uveitis, retinoblastoma, retinal neurodegenerative diseases and ocular neovascularization. To conclude, we believe that artemisinins could also be used as a promising therapeutic drug for ocular diseases, especially retinal vascular diseases in the near future.

• **KEYWORDS:** artemisinins; uveitis; retinoblastoma; retinal neurodegenerative diseases; ocular neovascularization

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INTRODUCTION

Artemisinin and its derivatives (artemisinins) are isolated from the one ancient Chinese plant *Artemisia annua* (more commonly known as sweet wormwood), which have

been used in traditional Chinese medicine (TCM) for fevers and chills^[1]. Following the isolation of the active agent by Dr. You-You Tu's group from the Chinese Academy of TCM in the 1970s, artemisinin-based combination therapies have joined the currently established standard treatments of malarial parasites around the world^[2-4]. Interestingly, abundant evidences have also demonstrated that artemisinins might also be of therapeutic value for many other diseases beyond malaria, including cancers, autoimmune disorders, inflammatory diseases as well as other infectious conditions^[5]. Recently, many ophthalmologists and researchers have also showed their great interest in artemisinins, especially artesunate and dihydroartemisinin (DHA) and their potential protective effects on ocular disorders. Herein, we present an overview of research advances of artemisinins as potential therapeutic methods for ocular diseases, including uveitis, retinoblastoma, retinal neurodegenerative diseases, especially ocular neovascularization (NV). In this review, we also emphasize some important points regarding the potential applications of artemisinins in ocular disorders to provide a platform for additional study.

OVERVIEW OF ARTEMISININS

History and Origins The medicinal herb *Artemisia annua* was first recognized by one Chinese physician, Hong Ge (born in the year 283) for its fever-reducing properties^[6]. Led by the Chinese project 523 in the 1970s, Dr. You-You Tu's group first successfully isolated artemisinin, a non-toxic extract of *Artemisia annua*, identified the active component of this extract in 1972 and further identified its stereostucture (sesquiterpene lactones) in 1975^[1]. In the 1980-90s, further studies conducted in humans confirmed the recognition of artemisinin-based combination therapies as the first-line option to treat malaria^[2-4]. This novel anti-malaria therapy has been used universally with great efficacy and safety for a long time and helped Dr. You-You Tu win the 2015 Nobel Prize in Physiology or Medicine for her outstanding achievements^[7].

Chemical and Pharmacological Characteristics It was Dr. You-You Tu who first clarified the molecule extracted from the herbaceous plant *Artemisia annua* to be a sesquiterpene lactone endoperoxide by using the combined method of mass spectroscopy, spectrophotometry, X-ray crystallography and polyarithmetic analysis^[8]. Those clinically important artemisinins include artesunate, artemether, arteether, and DHA (Figure 1), discovered and developed in 1986^[1]. Among which

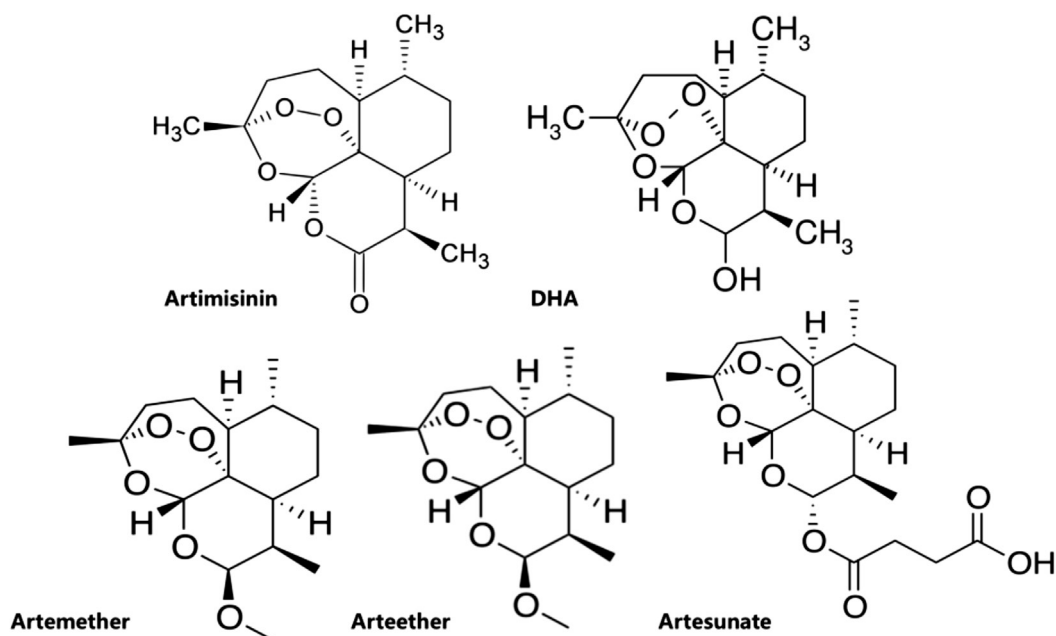


Figure 1 Chemical structures of artemisinins.

artemisinin is the most important analog, which shows a more favorable pharmacological profile because of its greater water-solubility and high oral bioavailability due to the additional hemisuccinate group^[9].

Beyond Malaria: Activity of Artemisinins to Other Diseases While the efficacy and low toxicity of artemisinins to treat malaria is well-recognized around the world, they have currently been reported to have a great therapeutic value beyond malaria^[10]. These capacities include protective functions in non-malaria parasitic infections^[11-14], anti-viral^[15-17] and anti-fungal properties^[18-19], anti-cancer functions^[20-24], as well as anti-inflammatory^[25-27] and anti-allergic effects^[28-29] (Figure 2). Recent results further indicated that artemisinins might also reduce glucose, thus exerting a protective effect on diabetes mellitus^[30].

Mechanisms of Actions of Artemisinins Although artemisinins are long known and effectively used as anti-malaria drugs, their specific biological action is poorly identified and understood. Current *in vivo* and *in vitro* studies have proposed numerous possible mechanisms of the actions, which include 1) oxidative stress, 2) induction of apoptosis^[31], 3) inhibition of angiogenesis^[32-33], 4) arrest of cell cycle at G₀/G₁^[34] (Figure 3). As a matter of fact, these functional pathways may overlap in a number of ways.

Oxidative Stress Reactive oxygen species (ROS) are the natural byproduct of aerobic metabolism, whose levels can dramatically elevate during times of environmental stress. Studies in various tumor cell lines have proved ROS to have an important role in artemisinins-induced apoptosis^[31]. These studies covered neuroblastoma^[32], breast cancer^[33], T-cell lymphoma^[34], embryonal rhabdomyosarcoma cells^[35], and

glioblastoma^[36]. In a recent study on human hepatocellular carcinoma cells, artesunate was shown to be able to induce ROS-dependent apoptosis *via* Bax-mediated intrinsic pathway^[37]. Similarly, DHA was shown to alleviate oxidative stress in bleomycin-induced pulmonary fibrosis^[38].

Induction of Apoptosis Apoptosis, or programmed cell death, is a regulated cellular suicide mechanism involving the degradation of cellular components, which can be initiated *via* the intrinsic pathway and the extrinsic pathway^[39]. Artemisinins could trigger apoptotic cell death through both pathways^[40-41]. In human colon cancer cell line (HT29), B-cell lymphoma 2 associated X protein (BAX) was proved to be activated by artemisinins, inducing the release of cytochrome C, which led to apoptosis in cancer cells^[42]. In human prostate cancer cell line (DU145), cleavage of procaspases 3 and 9 was found to be induced by artesunate, inducing the release of cytochrome C and the subsequent caspase-dependent apoptosis^[43]. In human breast cancer cell line (MCF-7), apoptosis was also induced *via* a caspase-related mechanism under the effect of a semi-synthetic derivative of artemisinin^[44].

Inhibition of Angiogenesis Various models have accumulated mounting evidences, demonstrating the involvement of inhibiting aberrant angiogenesis in the actions of artemisinins^[45-46]. In mouse embryonic stem cells, artemisinin was shown to be able to reduce the levels of hypoxia inducible factor (HIF)-1 α and vascular endothelial growth factor (VEGF), suggesting the mechanism of artemisinin might involve the inhibition of angiogenesis^[47]. Artemisinin was also found to be able to significantly reduce lymph-angiogenesis *via* downregulating the expression of VEGF-C in C57BL/6 mouse Lewis lung carcinoma model^[48]. Similarly, in a rat glioma model,

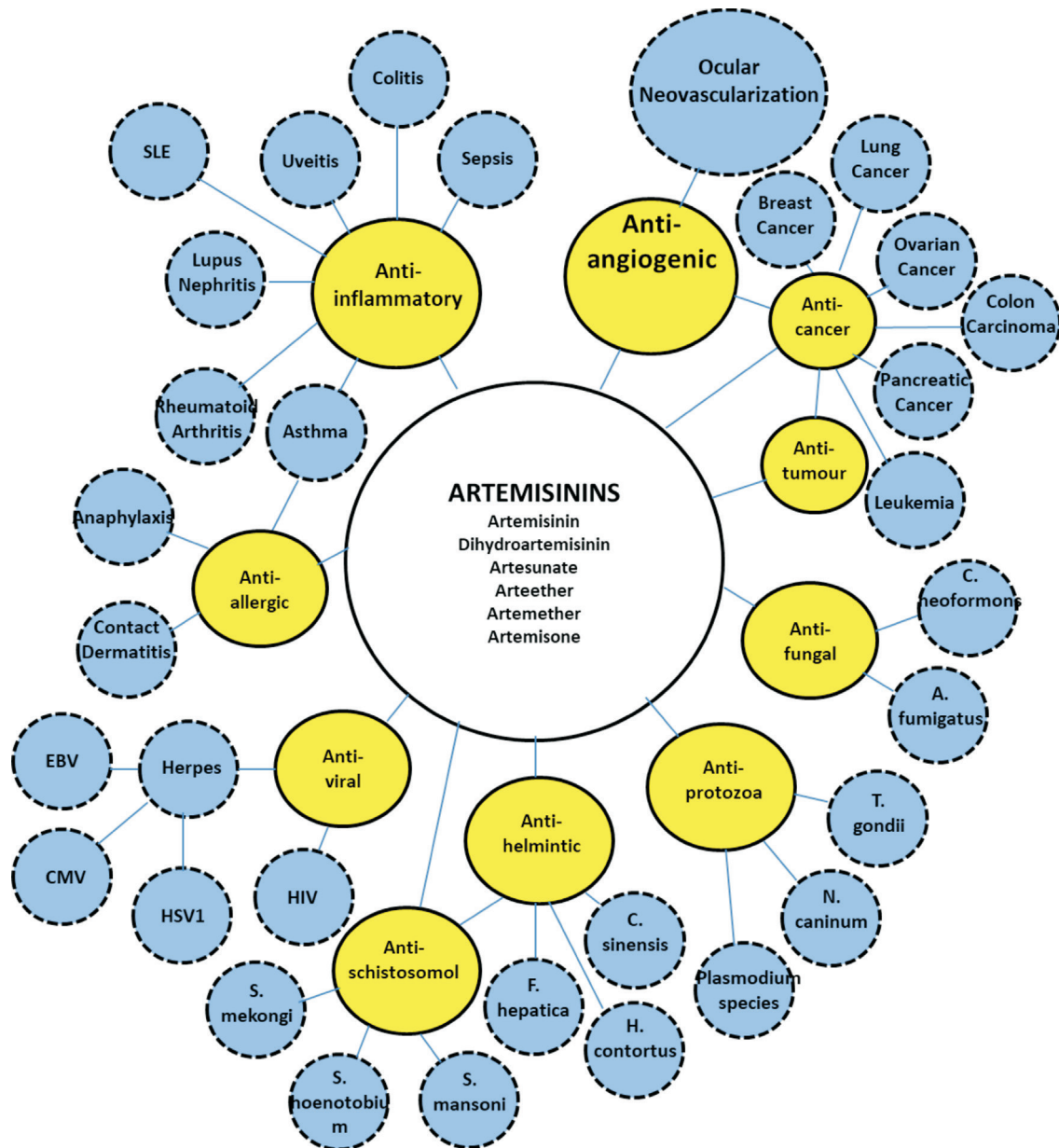


Figure 2 Various biological activities of artemisinins and potential applications in different diseases (Bubble map).

artemisinins were shown to have the effect of reducing VEGF and angiogenesis^[49]. Moreover, artesunate was proved to be able to suppress osteoclastogenesis and aberrant angiogenesis, thus attenuating anterior cruciate ligament transection (ACLT)-induced osteoarthritis^[50].

Arrest of Cell Cycle at G₀/G₁ Artemisinins have been shown by accumulating current studies to have the potential application in cancer drug development for its action on inducing growth arrest at various stages of cell division cycle^[51-53]. In prostate cancer cells (LNCaP), phosphorylated retinoblastoma protein (pRB), a mediator cooperating with E2F transcription factors and cyclin-dependent kinases (CDKs) to push forward the cell cycle progression through G₁ into S phase was shown to be ablated by artemisinin, inducing G₁ cell cycle arrest, thus inhibiting cell division^[54]. Willoughby *et al*^[55] has also demonstrated that artemisinin could disrupt

specificity protein 1 (Sp1) transcription factor from binding to CDK4 promoter and inhibiting CDK4 gene expression, thus blocking prostate cancer growth and cell cycle progression. Wu *et al*^[56] have further proved the growth inhibition effect of artemisinin in nasopharyngeal carcinoma cell lines by suppressing the level of cyclin D1, cyclin E, CDK2, CDK4, CDK6 and upregulating the inhibitors of cell cycle division (p16, p27).

POTENTIAL APPLICATION IN OCULAR DISEASES

Recently, many ophthalmologists and researchers have noticed the potential protective effects of artemisinins on ocular disorders. Recent findings have shed light on the potential applications of artemisinins as promising therapeutic agents in ocular diseases. In this review, we are going to highlight the main points of published results of using artemisinins in targeting ocular disorders.

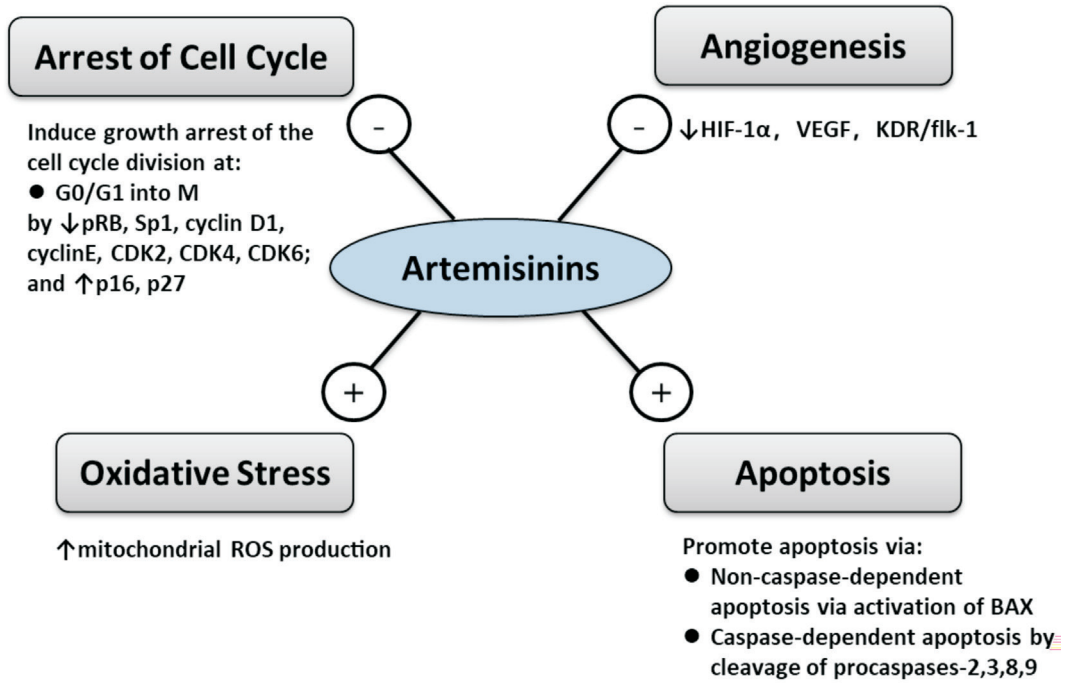


Figure 3 Overview of mechanisms of actions of artemisinins - indicates inhibition and + indicates activation.

Uveitis Uveitis is the inflammation of the uvea whereas the anti-inflammatory effects of artemisinins have already been recognized in the past few decades^[57]. Artesunate has been reported by Li *et al*^[58] to have a protective effect on sepsis mouse model by decreasing serum endotoxin release and toll-like receptors (TLR)4, TLR9 expressions, also suppressing nuclear factor-kappa B (NF-κB) activation. Xu *et al*^[59] also reported that in human rheumatoid arthritis fibroblast-like synoviocytes, artesunate was able to inhibit TNF-α expression and decrease the secretion of pro-inflammatory cytokines. Based on those experimental results, the question of if artesunate could reduce the release of inflammatory cytokines in some type of inflammatory ocular diseases was raised and further investigated. Wang *et al*^[60] studied the protective effect of artesunate by using endotoxin-induced uveitis (EIU) rat model, which has been generally considered as an experimental model for human uveitis^[61]. In their study, artesunate of three concentrations (1, 10, 100 mg/kg) were intravenously injected in male Long-Evans rats whereas prednisolone (10 mg/kg) was used as positive control and their results showed that artesunate (10 mg/kg and 100 mg/kg) could suppress infiltrating cells and protein concentration in the aqueous humor, suggesting that artesunate treatment could suppress the inflammation of EIU by inhibiting the production of inflammatory mediators^[60]. More future studies will be needed to clearly define the specific cellular mechanisms of the therapeutic effects. The role of artemisinins in modulating ocular inflammatory responses might be of great interest in the future.

Retinoblastoma In recent years, artemisinins have been shown to exert protective effects in various types of cancer^[62-66].

Retinoblastoma (RB) is an eye cancer, which is most common among children^[67]. Zhao *et al*^[68] tested the anti-neoplastic activity of artesunate against RB to see whether artesunate might be a good candidate to treat RB. Using epithelial retina cell line as normal counterpart, the cytotoxic activity and specificity of artesunate were analyzed in an RB cell line, which showed a dose-dependent manner concerning the cytotoxic activity specific to RB cells, with low toxicity in normal retina cells and high cytotoxicity in RB cells^[68]. Their results also demonstrated that artesunate, even at low doses, could block the cell cycle progression at the G₁ phase^[68]. Artesunate is practically suitable for long-term treatments with few side-effects. Therefore, artesunate could be considered as a promising option for RB treatment. Further randomized studies *in vivo* need to be done to provide better insights regarding the efficacy as well as efficiency of the novel treatment.

Retinal Neurodegenerative Diseases Retinal neurodegeneration is a retinopathy which consists in the deterioration of the retina caused by the progressive death of its neuronal cells^[69]. There are several reasons for retinal neurodegeneration, including age-related macular degeneration (AMD), diabetic retinopathy (DR), and retinal artery or vein occlusion^[69]. Zeng *et al*^[70] studied the neurogenic effects of artemisinin and their findings indicated that artemisinin at low concentration could induce neurite outgrowth as well as promote neuronal differentiation in PC12 cells.

Accordingly, Chong and Zheng^[71] demonstrated that artemisinin was able to suppress hydrogen peroxide (H₂O₂)-induced oxidative stress in D407 retinal pigment epithelium (RPE) cells, which are first damaged in retinal diseases owing

to their critical support functions for photoreceptors. The findings of Yan *et al*^[72] also demonstrated that artemisinin could prevent RPE cells from oxidative stress *via* the MAPK/CREB pathway.

These recent results all shed light on the promising therapeutic value of artemisinin as a candidate drug for the treatment of many retinal neurodegenerative disorders. Though, its specific effects on the retinal neuronal cells need to be further explored.

Ocular Neovascularization Ocular NV is one of the major causes of blindness among ocular disorders. Substantial evidences have demonstrated that VEGF played an essential part in its pathogenesis^[73]. Currently for the treatment of ocular NV, anti-VEGF agents such as ranibizumab and bevacizumab are widely used^[74-75]. However, these drugs both have a large molecular weight and resistance to these drugs is usually seen in approximately 20%-30% ocular NV patients^[76]. Moreover, because of the short aqueous half-life, the recurrence rate is high after anti-VEGF treatments which may also increase the risk of endophthalmitis owing to frequent intravitreal injections^[77]. Abundant studies have already demonstrated the anti-angiogenic effects of artemisinins in tumors^[78]. The known mechanisms of artemisinins in inhibiting angiogenesis include downregulating several growth factors, inducing apoptosis of vascular endothelial cells, upregulating angiogenesis inhibitors, depleting the levels of the flt-1 and KDR/flk-1-receptors^[79-80]. In human umbilical vein endothelial cell (HUVEC) lines, artesunate was shown to inhibit angiogenesis through downregulating the levels of the VEGF receptors^[81]. Similar protective effects were also investigated in lymphatic endothelial cells and Lewis lung carcinoma cells with the treatment of DHA^[82]. In the science of ophthalmology, Cheng *et al*^[83] demonstrated that artesunate could inhibit corneal NV by inducing ROS-dependent apoptosis in animal models. Their results suggested that artesunate could markedly inhibit angiogenesis by specifically inducing apoptosis *via* an iron/ROS-dependent p38 MAPK-mitochondrial pathway in vascular endothelial cells^[83]. Zong *et al*^[84] further investigated the use of artesunate in retinal NV and found that retinal NV could be remarkably inhibited under the effect of artesunate *via* downregulating the expression of VEGFR2, and PDGFR. Compared to bevacizumab, artesunate could remarkably inhibit retinal NV in rabbits with more durable efficacy. These two published animal evidences indicated the potential role of artesunate as a promising drug candidate to manage ocular NVs. As a newly-discovered anti-angiogenesis drug, artemisinins are worthwhile to be further explored due to a host of advantages.

Compared to the currently used anti-VEGF drugs, the advantages of artesunate are as follows: 1) Small molecule size: artesunate is a 384 Da molecule less than one-hundredth

the size of bevacizumab (149 kDa); 2) Safety and low toxicity: artesunate has been widely used for many years as anti-malarial agents, with few adverse side effects and proven safety records; 3) Multi-targets: artesunate was proved to possess not only anti-angiogenic effects targeting multi-growth factors (VEGF, FGF, HIF-1 α , and Ang-1), but also anti-inflammatory and anti-apoptotic effects.

Thus, we postulate that artesunate might be a potential novel treatment option for retinal vascular diseases such as AMD, DR, retinal artery or vein occlusion, especially when given intravitreally or being formulated into eye drops.

LIMITATIONS OF ARTEMISININS

The present studies of artemisinins have several limitations. While applying artemisinins for treatments beyond malaria, different research groups have reported inconsistent effective doses even for similar cell lines or animal models. Progress for further clinical trials could be hampered for the lack of a concerted effort to confirm the efficacies of artemisinins in different models. Another limitation is the lack of acute and chronic toxicological studies for acute as well as chronic exposure to artemisinin in ocular diseases, which is necessary for future application in ocular diseases.

CONCLUSION

To date, researches on artemisinins and its applications in ocular diseases are still limited, and much more will need to be studied. Further understanding of the protective activities of artemisinins beyond malaria might lead to improved treatments for ocular disorders.

In this review, we summarized recent studies on artemisinins in treating ocular diseases and we believe that this anti-malaria agent could also be used as a promising therapeutic drug for ocular diseases, especially retinal vascular diseases.

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