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Review

Neuroprotective and Anti-Epileptic Potentials of Genus Artemisia L.

Bayan Sailike[1], Zhannur Omarova[1], Janar Jenis[2], Altynay Adilbayev[3], Burkitkan Akbay[3], Sholpan Askarova[4], Wei-Lin Jin[5] and Tursonjan Tokay[1*]

[1] Biology Department, School of Sciences and Humanities, Nazarbayev University, Kabanbay Batyr Ave. 53, 01000 Nur-Sultan, Kazakhstan; bayan.sailike@nu.edu.kz
[2] Research Center for Medicinal Plants of Al-Farabi Kazakh National University Al-Farabi Avenue 71, Almaty 050040, Kazakhstan; janarjenis@mail.ru
[3] Department of Biomedical Sciences, School of Medicine, Nazarbayev University, Kerei, Zhanibek khandar str. 5/1, 02000 Nur-Sultan, Kazakhstan; burkitkan.akbay@nu.edu.kz
[4] Laboratory of Bioengineering and Regenerative Medicine, National Laboratory of Astana, Nazarbayev University; shaskarova@nu.edu.kz
[5] Institute of Cancer Neuroscience, Medical Frontier Innovation Research Center, The First Hospital of Lanzhou University, The First Clinical Medical College of Lanzhou University, Lanzhou 730000, China; ldyy_jinwl@lzu.edu.cn
* Corresponding author: tursonjan.tokay@nu.edu.kz; Tel: +77172706610.

Abstract: Epilepsy is a chronic neuronal disorder characterized by periodic, unpredictable, and recurrent seizures due to either a genetically determined or an acquired brain disorder. Although many anti-epileptics drugs (AEDs) are developed to control epilepsy, 30% of patients still need additional drugs or experience recurrent seizures and psychiatric and behavioral side effects. Thus, the need for medical care for patients with uncontrolled epilepsy remains unmet. The Genus Artemisia L. is one of the largest genera in the Asteraceae family with more than 500 species widely distributed in Europe, Asia, and North America. Many Artemisia species have been used in various treatments since ancient times as folk remedies. They demonstrated strong antioxidant, anti-inflammatory, antimicrobial, antimalarial, and antitumor activity. Recent studies reveal that some species of Artemisia demonstrated a therapeutic benefit for epilepsy by its anti-oxidant, anti-inflammatory, neuroprotective, and anticonvulsant properties. In this review, we investigate the current state of the literature regarding the neuroprotective and antiepileptic potentials of the genus Artemisia and its possible underlying mechanisms.

Keywords: Genus Artemisia; anticonvulsant; antioxidant; antiapoptosis; anti-neuroinflammatory; neuroprotective; cognitive function

1. Introduction

Epilepsy is a type of neurological disorder characterized by symptoms of periodic, unpredictable, and recurrent seizures [1]. According to the International League Against Epilepsy (ILAE) classification in 2017, epilepsies are classified as focal, generalized, combined generalized and focal epilepsy or unknown types [2]. Although the cause of epilepsy is still unknown in most cases, seizures can be caused by any damage associated with metabolic disorders, infectious diseases, genetic mutation, or immune disorders that disrupt brain function [3].

Currently available antiepileptic drugs (AEDs) are only partially efficient for epilepsy. Although AEDs control nearly 70% of patients with epilepsy, 5-10% of patients still require additional medications and more than 20% of patients continue to have seizures after treatment. They can only treat the symptoms of epilepsy without making any significant progress in neither reversing its underlying mechanisms nor offering neuroprotection caused by the pathogenesis of epilepsy, resulting in about 15-40% of all patients with epilepsy, unfortunately, continue to have seizures that impair their daily

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living [4]. Moreover, most AEDs have shown cognitive, psychiatric, and behavioral side effects on patients [5]. For example, cognitive abnormality, especially impairment in learning and memory, is one of the serious comorbidities caused by epileptic seizures and/or AEDs that has a significant negative impact on patients' quality of life [6]. The neuronal damage and death caused by seizure-associated excitotoxicity are most likely responsible for both epilepsy and epilepsy-induced cognitive impairment. Therefore, dampening excitotoxicity, which is resulting from an imbalance between the excitatory and inhibitory neurotransmitters, has become the main target of currently available AEDs, the most widely used treatment methods for epilepsy now [4]. Hence, the development of alternative, more effective but with fewer side effects and clinically relevant novel therapeutic approaches for epilepsy are in urgent demand.

Oxidative stress (OS) might play a role in epilepsy and associated disorders. The mechanisms by which a normal brain develops spontaneous seizures as well as the cognitive abnormality seen in chronic epilepsy have not been completely elucidated [7]. However, a wide range of studies has shown that oxidative stress is involved in the development of recurrent seizures and SE as well as learning abnormalities in animals by causing neural damage and death [8]. Consistent neuronal excitation associated with seizure causes increased production of reactive oxygen species (ROS), which contribute to seizure-induced brain damage by either apoptotic or necrotic pathways [9]. Thus, oxidative stress is likely an important pathogenic factor of epilepsy.

The genus *Artemisia*, one of the most distributed genera in the Asteraceae family, is mostly distributed in Europe, Asia, and North America [10]. Many of them have been used since ancient times as folklore remedies for various diseases, such as malaria, fever, cough, and gut parasitic diseases [11, 12]. Pharmacological studies demonstrated that Artemisia species possess a wide range of antioxidant, anti-inflammatory, antimalarial, antimicrobial, antiviral, antitumor, antipyretic, antihemorrhagic, anticoagulant, antianginal, antihepatitic, antiulcerogenic, and antispasmodic activities [10].

Despite their wide diversity, distribution, and application, most Artemisia species investigated to date have similar chemical compositions. The principal bioactive constituents found in this genus include isoprenoids, flavonoids, phenolic acids, coumarins, glycosides, sterols, polyacetylenes, and caffeoylquinic acids [13, 14], and the presence of these bioactive compounds in varying proportions in different plants might be attributed to *Artemisia*'s diverse pharmacological activities. For example, flavonoids, which are rich in *Artemisia annua* [15], possess numerous antioxidant, anti-inflammatory, and anti-apoptotic activities. There is increasing evidence to suggest that some *Artemisia* species might have therapeutic potential for epilepsy due to their antioxidant, anti-inflammatory, and anticonvulsant properties [16]. Thus, the main objectives of this mini-review are to explore the therapeutic potential of *Artemisia* species for the prevention and treatment of epilepsy and its associated comorbidities.

2. Anticonvulsant activity of Artemisia

In recent years, more and more evidence has shown that oxidative stress and neuroinflammation play important roles in the pathophysiology of acquired epilepsy. A line of experimental studies has demonstrated that oxidative stress and neuroinflammation are implicated in the development of recurrent seizures and status epilepticus (SE) [17, 18, 19, 20] as well as cognitive abnormalities caused by recurrent seizures by inducing oxidative damage to neural tissue [8, 21, 23]. Therefore, the focus of the epilepsy research and treatment strategy has now shifted to seeking alternative approaches, such as herbal plants, with better efficacy and minimal side effects [24, 25, 26]. It is postulated that the use of plant-based antioxidants (e.g., flavonoids) to reduce or eliminate neuroinflammation and oxidative damage might be a desirable strategy in the treatment of epilepsy and epilepsy-/AEDs-induced cognitive impairment.

Several Artemisia species have been used in folklore medicine to treat epileptic seizures, and the anticonvulsant efficacy of these plants was confirmed by in vivo animal

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experiments using acute and chronic epilepsy [27, 28, 29, 30, 31]. Studies indicated that essential oil, crude extracts, and different fractionations of Artemisia species can prevent seizures. De Lima et al [27] reported earlier that a high dose of the hydroalcoholic extract (HE) of *Artemisia verlotorum* prevented non-invasive electroshock (75 mA, 60 Hz) and the seizure-induced by pentylenetetrazole. In addition, the *Artemisia* extracts also increased the latencies of seizures in both pilocarpine and 3-mercaptopropionic acidpilocarpine induced mice (De Lima et al., 1993). These results suggest that extracts of *A. verlotorum* are able to protect against experimental convulsions elicited by various agents. *Artemisia dracunculus L.* is used as an antiepileptic remedy in Iranian folklore medicine. Essential oil of *Artemisia dracunculus L.* demonstrated the anti-seizure activity in mouse models of acute epilepsy [28]. The extracted *Artemisia dracunculus L.* essential oil showed a dose and time-dependent anticonvulsant activity in both maximal electroshock (MES; ED_{50} 0.84 ml/kg) and pentylenetetrazole (PTZ; ED_{50} 0.26 ml/kg) mice models of seizure, respectively. After gas chromatography (GC)/mass spectrometry (MS) analysis of the essential oil, authors postulate that monoterpenoids in the essential oil may be responsible for the anticonvulsant effects [28]. The potential anti-epileptic effect of *Artemisia copa Phil.* was explored by Mio et al [29] as a part of their psychopharmacological studies. They evaluated the anticonvulsant activity of plant water extracts on a pentylenetetrazole-induced mouse model and found that *A. copa Phil.* extract significantly increased the latency time and decreased the duration of seizures and mortality in mice. Khan et al [31] studied the anticonvulsive effect of A*rtemisia indica* fractions and the involvement of GABA-A receptors using electrophysiological methods. The results showed that the isolated carnosol, ursolic acid, and oleanolic acid had significant anticonvulsant activity in a pentylenetetrazole-induced epilepsy mouse model by positively regulating the $\alpha 1\beta 2\gamma 2L$ GABA-A receptor. Kediso et al [30] evaluated the effect of an aqueous ethanolic extract of *Artemisia afra* on pentylenetetrazole-induced seizures in mice. They noted that extracts of *A. afra* showed a delay in seizure onset and a reduction in the duration of convulsions in a dose-dependent manner.

To sum up, certain Artemisia species have been found to be effective anticonvulsants. However, the exact pharmacologically active compounds of Artemisia and the underlying cellular and molecular mechanisms through which Artemisia exerts antiepileptic effects are not fully understood.

3. Antioxidant activity of Artemisia

Oxidative stress generated by ROS may play a role in the occurrence and development of epilepsy, and changes in mitochondria-related oxidative stress status can lead to neuronal apoptosis. Various plants from the *Artemisia* species demonstrate protective activities against oxidative damage to neural tissue *in vitro* and *in vivo.* Treatment with *Artemisia* extracts is able to protect neurons or recover the oxidative stress-induced damage by preventing the formation of free radicals, enhancing the endogenous antioxidant system, and regulating apoptosis signaling pathways.

One initial study with *Artemisia* extracts indicated that the methanol extract of *Artemisia absinthium* has free radical scavenging activity *in vitro* and antioxidant capacity in the animal brain (in vivo) by decreasing thiobarbituric acid reactive substances (TBARS) and restoring levels of superoxide dismutase (SOD) and glutathione (GSH) [14]. Another recent study using rats showed that the levels of the pro-oxidants MDA (an indicator of lipid peroxidation) and nitric oxide (NO) were significantly reduced, while the levels of GSH and gene expression of the antioxidant enzymes were significantly increased in cortical tissue in STZ-induced diabetic rats exposed to ethanol extract of *A. judaica* [32]. More recently, Rashidi et al [33] demonstrated that ethanolic extract of *Artemisia absinthium* has antioxidant and neuroprotective effects on 6-hydroxydopamine (6-OHDA)-induced oxidative stress in SH-SY5Y cells. The extract of the plant at concentrations ranging from 6.25 to 25 μg/mL has been found to significantly slow

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increases in intracellular ROS formation induced by 6-OHDA. The plant extract also increases the GSH level and SOD activity. Similarly, various fractions of two *Artemisia* species—*A. turanica* and *A. turcomanica*—were found to effectively suppress H_2O_2-induced oxidative stress and apoptosis of PC12 cells as well as restored the H_2O_2-induced GSH depletion [34]. Besides the crude *Artemisia* extracts, the essential oil from *Artemisia campestris* has been shown to be a potent antioxidant and neuroprotectant [35]. Pretreatment of animals with the essential oil significantly reduces deltamethrin-induced oxidative damage in rat brain tissue by alleviating lipid peroxidation, oxidative stress, and degeneration of brain tissue [35].

The antioxidant and cytoprotective activities of *Artemisia* were also investigated using secondary metabolites of the genus. Caffeoylquinic acids and their derivatives isolated from *A. princeps Pampanini* showed potent antioxidant and neuroprotective effects on β -amyloid-induced oxidative stress in PC12 cells in a dose-dependent manner. Under oxidative stress conditions, PC-12 cells treated with whole extract and 3,5-diCQA increased their cell viability by approximately 1.6 and 2.4 times, respectively, compared to the control without treatments [36]. DSF-52, a sesquiterpene dimer isolated from Artemisia argyi, suppressed NADPH oxidase blocking ROS production in lipopolysaccharide (LPS)-induced BV-2 cells [37]. In addition, 3,5-dicaffeoylquinic acid (3,5-diCQA) isolated from *A. argyi H.* possesses potent antioxidant, neuroprotective, and precognitive properties. It demonstrated that 3,5-diCQA can restore trimethyltin (TMT)-induced cognitive dysfunction by increasing Acetylcholine (ACh) and decreasing acetylcholinesterase (AChE) activity and antioxidant capacity by reducing the amount of pro-oxidant malondialdehyde (MDA) and augmenting the levels of oxidized GSH in the brain tissue of ICR mice [38].

Artemisia amygdalina showed neuroprotective action on differentiated N2a and SH-SY5Ycells [39]. The oxidative stress and cell death induced by H_2O_2 in these cell lines were attenuated by different extracts of *Artemisia amygdalina* by upregulation of the Nrf2 signaling pathway, which is known to be an emerging modulatory pathway of cellular resistant to oxidants [40].

The antioxidant properties and underlying mechanisms of Artemisia might be attributed to the bioactive secondary metabolites of the plants. Most studies associated with the antioxidant and neuroprotective activities of *Artemisia* extracts focused on Artemisinin, principal bioactive isoprenoids isolated from *Artemisia annua.* Zheng et al [41] demonstrated that Artemisinin has a neuroprotective effect on sodium nitroprusside-induced oxidative damage to primary cortical neurons and PC12 cells in vitro. They found that pretreatment of PC12 cells with Artemisinin could protect cell viability by reducing oxidation and preventing the decline of mitochondrial membrane potential. In addition, they also demonstrated by Western blotting analysis that the neuroprotective effect of Artemisinin is associated with the activation of the extracellular regulated protein kinases (ERK) pathway, which is responsible for intracellular signaling. The involvement of the ERK/CREB signaling pathway was also supported by other studies [42], which demonstrated that H_2O_2-induced oxidative damage can be suppressed by Artemisinin in retinal pigment epithelial cells D407 through restoring cell morphology, mitochondrial membrane potential, and slowing intracellular ROS generation. In line with those findings, Yan et al [43] demonstrated that Artemisinin has a protective function against H_2O_2-induced oxidative stress in retinal neuronal cells RGC-5. In this study, a decreased ROS accumulation and cell apoptosis, as well as an increased mitochondrial membrane potential has been observed following Artemisinin treatment of cells. The western blotting analysis indicated that the P38 and ERK1/2 kinase pathway phosphorylation were upregulated by H_2O_2-induced oxidative stress in RGC-5 cells, and the inhibitory effect of H_2O_2 on two kinases was reversed by the Artemisinin. Moreover, the inhibitors PD169316 or PD98059 were blocked by the protection of Artemisinin. Interestingly, flash

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electroretinogram showed that intravitreous injection of Artemisinin in a concentration-dependent manner could protect retinal function from light-exposed damage [43]. The neuroprotective function of Artemisinin against glutamate-induced oxidative stress was also reported by Lin et al [44]. They investigated the effects of artemisinin on a mouse hippocampal cell line (HT-22) damaged by glutamate-induced oxidative stress. The results demonstrated that overproduction of ROS and the collapse of mitochondrial membrane potential caused by glutamate-induced oxidative stress could be rescued by activating protein kinase B (Akt)/Bcl-2 signaling in the cell line treated with artemisinin. In addition to artemisinin, administration of 3,5-dicaffeoylquinic acid (3,5-diCQA), a phenolic compound extracted from *Artemisia argyi H.,* prevented neuronal apoptosis from the mitochondrial pathway in the brain tissue of ICR mice exposed to trimethyltin (TMT) [38]. Similar to artemisinin, 3,5-diCQA also reduced TMT-induced mitochondrial ROS production and increased TMT-lowered mitochondrial membrane potential and ATP levels thus providing significant protection against TMT-induced mitochondrial dysfunction and cellular apoptosis [38]. Phosphorylation of microtubule-associated protein tau (p-tau) by protein kinase B/Akt (Akt), plays an essential role in Akt-mediated anti-apoptotic signaling [45]. Kang et al. [38] found that treatment with 3,5-diCQA increased the ratio of phosphorylated-Akt (p-Akt)/Akt that was reduced in the TMT mice as well as the decreased levels of phosphorylated tau (p-tau) and Bax, which were increased in the TMT group.

Taken together, Artemisia extracts exert their antioxidant effects by modulating signaling pathways such as ERK, CREB, and Akt/Bcl-2 or altering enzyme levels to reduce oxidation. Therefore, these signaling pathways may be potential targets for the treatment of epilepsy.

3. Anti-neuroinflammatory effects of Artemisia

Microglia-associated neuroinflammation is presumed to contribute to neuronal injury in various neurodegenerative disorders including epilepsy. Neuroinflammation in epilepsy is primarily characterized by robust astrogliosis, microglial activation, and the production of cytokines and chemokines [18, 20]

Several bioactive molecules isolated from Artemisia extract were able to significantly eliminate neuroinflammation in brain tissue and neural cell lines. Artemisinin B isolated from *Artemisia annua* and DSF-52 isolated from *Artemisia argyi* were found to exhibit significant anti-neuroinflammatory effects on LPS-activated BV2 cells (microglial cell model) [37], [46]. Both artemisinin B and DSF-52 significantly downregulated LPS-induced increase in NO production, and gene expression levels of inflammatory cytokines IL-1β, IL-6, TNF-α and upregulated gene expression levels of anti-inflammatory cytokine IL-10 [37, 46]. DSF-52 also downregulated pro-inflammatory Prostaglandin E2 (PGE2), iNOS, COX-2 and granulocyte-macrophage colony-stimulating factor (GM-CSF) cytokines [37]. Another important finding of their studies is that both artemisinin B and DSF-52 inhibited major inflammatory transcription factor NF-κB in a dose-dependent manner [37,46]. DSF-52 also inhibited the phosphorylation of NF-kB, IkB, and Akt, the activation and translocation of NF-kB from the cytoplasm into the nucleus, and NF-kB-DNA binding activity [37]. In addition to the Akt/IkB/NF-kB signaling pathway, DSF-52 also blocked JNK/p38 MAPKs and Jak2/Stat3 inflammatory signaling pathways by inhibiting phosphorylation of respective molecules. Artemisinin B also reduced glial cell surface receptor TLR4 and its downstream adaptor protein MyD88 levels that were abnormally high in the model group [46]. Both TLR4 and MyD88 are important because their association eventually leads to the activation of the NF-κB and MAPK signaling pathways and thus to the expression of inflammatory cytokines and chemokines [47]. Based on these findings, the study suggests that artemisinin B inhibits neuroinflammation via the TLR4-MyD88-NF-kB signaling pathway [46]. The ethanol extract of *Artemisia judaica* significantly reduced the streptozotocin (STZ)-induced increase in the

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proinflammatory TNF-α and iNOS levels and expression in the cortical tissue of diabetic rats, comparable to the positive control metformin [32]. The study also suggests that Artemisia judaica inhibits the development of cortical inflammation in diabetic rats [32]. In line with those findings, Artemisinin isolated from *Artemisia annua* significantly reduced the immunoreactivity of glial cells, as detected by a decrease in their GFAP and Iba1 markers, cleaved caspase 1, reduced IL-1β levels, and restored microglia morphology in the cerebral cortex region of 3xTg AD mouse model [48].

Anti-apoptotic effects

Although whether apoptosis is a cause or a consequence of epilepsy remains controversial, activation of apoptotic signaling pathways has been widely demonstrated to exacerbate seizure-induced brain damage and lead to seizure prolongation. The Bcl-2 family is a well-characterized protein family involved in the modulation of apoptotic cell death, consisting of anti-apoptotic Bcl-2 protein and pro-apoptotic Bax and Bak, which are key modulators of the intrinsic (mitochondrial) pathway of apoptosis. In response to cellular stress, Bax creates pores in the mitochondrial outer membrane, leading to depolarization of the mitochondrial membrane potential and release of cytochrome c into the cytoplasm [49], [50]. Cytosolic cytochrome c further activates caspases and leads to cell apoptosis [49]. In contrast, Bcl-2 binds/inactivates Bax and prevents Bax/Bak oligomerization, which would otherwise cause the mitochondrial release of cytochrome c and several other apoptotic molecules[51]

Recently, various studies have documented the neuroprotective and antiapoptotic effects of Artemisia. The ethanol extract of *Artemisia judaica* increased the level of the anti-apoptotic marker Bcl2 and decreased the level of the pro-apoptotic marker Bax by blocking the neuronal apoptosis induced by injection of STZ into the rat brain [32]. 3,5-diCQA-has also been reported to prevent trimethyltin (TMT)-induced neuronal apoptosis in mouse brain via the intercellular mitochondrial pathway [38]. In this study, 3,5-diCQA restored TMT-induced increase in the mitochondrial ROS production and the decrease in mitochondrial membrane potential and ATP levels thus providing significant protection against mitochondrial dysfunction and cellular apoptosis [38]. Besides the regulatory activity of *Artemisia judaica* on the intrinsic apoptotic pathway, Kang et al. [38] also found that 3,5-diCQA treatment can increase the phosphorylated-Akt (p-Akt)/Akt ratio which was reduced in the TMT model, and decrease tau (p-tau) phosphorylation which was increased in the TMT model. [38].

Kwon et al [52] reported the neuroprotective effect of *Artemisia capillaris* and the possible involvement of apoptotic mechanisms. They showed that ethanol extract of *Artemisia capillaris* rescued BCCAO-induced neuronal degeneration and death and increased the caspase-3-positive cells in the mouse hippocampus [52]. Furthermore, Artemisinin isolated from *Artemisia annua* was also found to reduce p-tau levels and protect brain tissue as assessed by using 3xTg mice, a well-known transgenic animal model of Alzheimer's disease, and SH-SY5Y cells [48]. In the brain tissue of 3xTg mice, Artemisinin treatment resulted in a significant reduction of Aβ plaques in the cerebral cortex and hippocampus, damage to neurons and Nissl bodies, and the number of apoptotic cortical neurons in 3xTg mice. Moreover, p-ERK1/2, P-CREB levels, and Bcl-2/Bax ratio were significantly increased in the artemisinin-treated 3xTg animal group compared to the non-treated 3xTg group. In the SH-SY5Y cell line, pre-treatment with artemisinin prevented the Aβ (1-42)-induced SH-SY5Y cell death in a dose-dependent manner by protecting the mitochondrial membrane potential and decreasing cellular ROS production [48]. Moreover, artemisinin treatment resulted in a time- and dose-dependent increase in phosphorylated ERK1/2 and CREB levels in SH-SY5Y cells. However, artemisinin did not affect Aβ-induced neuronal apoptosis and the expression of apoptosis regulators in SH-SY5Y cells pretreated with the ERK inhibitor PD98059 [48]. Therefore, this study suggests that artemisinin exerts a potential anti-apoptotic effect on neural cells mediated by regulating the ERK/p-CREB/Bcl2 pathway.

Artemisia effect on cognitive function

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Cognitive abnormality, especially impairment in learning and memory, is one of the serious comorbidities of epilepsy that has a significant negative impact on patients' quality of life [6]. Despite some reports, the mechanisms underlying epilepsy-associated cognitive impairment have not been completely elucidated. According to the literature, it is likely that many factors are contributed, including the dysregulation of neurotransmitter systems (cholinergic, glutamatergic, GABAergic), microglial activation and neuroinflammation, and oxidative stress, which ultimately result in neural tissue damage and cognitive impairment. Several *Artemisia* species (some of them mentioned in previous sections) have been identified as having neuroprotective potential and the capacity of restoring cognitive impairment by suppressing oxidative stress and neuroinflammation. For instance, methanol extract of *Artemisia absinthium* significantly reduced cerebral infarct volume and short-term memory impairment in a mouse model of cerebral ischemia induced by middle cerebral artery occlusion (MCAO) via regulation of oxidative stress/antioxidant parameters [53]. In this study, *Artemisia* treatment resumed levels of antioxidant enzymes (GSH, SOD, and CAT) that were decreased in the brain of the MCAO model, and the concentration of the thiobarbituric acid reactive substance (TBARS)—a lipid peroxidation marker—that was increased in the brain of MCAO model [53]. Interestingly, administration of the plant extract before focal cerebral ischemia also prevented short-term memory impairment [53] suggesting that *Artemisia absinthium* not only restores already damaged neural tissue but also protects them from damage. In another study using a mouse model of Alzheimer's disease established by intra-cerebroventricular injection of the toxic fragment of Aβ (Aβ25-35), artemisinin B—a type of artemisinins derived from *Artemisia annua*—significantly ameliorated learning and memory of this mouse model in water-maze and step-through tasks. In the same model group, artemisinin B also inhibited the activation of microglia as well as the loss of the Nissl bodies and synaptophysin in the CA1 neurons of the hippocampus [46]. These studies indicated that oxidative stress, microglial activation, and neuroinflammation are involved in neural damage and *Artemisia* extracts could protect and restore neural damage by suppressing those processes.

Artemisia species can also restore cognitive impairment by modulating neurotransmitter and neuromodulator activity. Pre-treatment with 3,5-diCQA from *Artemisia argyi* H. significantly inhibited trimethyltin (TMT)-induced impairment of the cholinergic system [52]. It lowered AChE activity and increased Ach levels in the brain tissue of ICR mice compared to negative controls [52]. In line with those findings, treatment with the ethanol extract of *Artemisia capillaries* significantly inhibited AChE activity in mouse hippocampus in a dose-dependent manner [52]. They showed that nonselective neuronal nicotinic ACh receptor antagonist mecamylamine markedly blocked the neuroprotective effect of *Artemisia capillaries* against BCCAO-mediated neurodegeneration [52]. Hence, the study suggests that the neuroprotective and procognitive action of *Artemisia capillaries* is due to the activation of the acetylcholinergic neurotransmitter system in the brain. The authors also hypothesize that Artemisia capillaries activate the PI3K-Akt signalling cascade, which leads to the activation of neuronal nicotinic acetylcholine receptors [52]. Moreover, *Artemisia judaica* extract significantly recovered the STZ-induced decline in dopamine, norepinephrine, and BDNF levels in diabetic rats [32].

4. Discussion

Epilepsy is defined as one of the World Health Organization (WHO) public health priorities for its effective care, prevention, and treatment. It is one of the fundamental causes of disability and death in most countries [54]. Although AEDs are the primary treatment method for controlling epilepsy, most of these drugs are considered to be associated with temporary seizure control, drug resistance, and cognitive abnormalities [55]. Growing evidence reveals that oxidative stress and neuroinflammation contribute to the pathophysiology of epilepsy. Neuronal damage and death are most likely to be

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directly caused by neuronal hyperexcitability and oxidative stress. Therefore, antioxidant and neuroprotective actions could prevent epileptogenesis.

Nowadays, the growing need for more natural sources of medicine and certain prominent pharmacological activities of the Artemisinin has driven scientific interest in Artemisia species. In recent years, a series of studies have demonstrated that Artemisia has anticonvulsant effects in animal models of epilepsy *in vivo* **(Table 1)** and antioxidant, anti-neuroinflammatory, and neuroprotective effects in neural cell lines *in vitro* **(Table 2)** in which oxidative stress is induced and seizures are developed.

Table 1. Anticonvulsant activities of Artemisia species in vivo in animal models.

Species	Material	Model	Dose	Authors
Artemisia verlotorum	Crude extracts	Mice	200 mg/kg	[27]
Artemisia dracunculus L	Essential oil	Mice	MES; 0.84ml/kg PTZ; 0.26ml/kg	[28]
Artemisia copa Phil.	Crude extracts	Mice	150 mg/kg	[29]
Artemisia absinthium	Methanolic extract	Mice	100 or 200 mg/ml	[14]
Artemisia Indica Lin	Carnosol, ursolic acid, and oleanolic acid compounds	Mice	10–100 mg/kg	[31]
Artemisia campestris	Essential oil	Rats	200 mg/ml	[35]
Artemisia. afra	Hydroethanolic extract	Mice	250 –1000 mg/kg	[30]

Table 2. Antioxidant, anti-inflammatory, and neuroprotective effects of Artemisia species in vitro/ in vivo and its mechanisms of action.

Species	Compounds	Models	Mechanisms	References
Artemisia absinthium	Methanolic extract	Cerebral I/R-induced mice	Restore SOD and GSH levels and decrease TBARS	[14]
	Ethanolic extract	SH-SY5Y cells	Needs further investigation	[33]
Artemisia princeps Pampanini	Phenolics	PC12 cells	Decreasing intracellular oxidative stress	[36]
Artemisia argyi	DSF-52	LPS-mediated BV-2 microglial	Suppression of NF-κB, JNK/p38 MAPKs, and Jak2/Stat3 signaling pathways Reduction of the expression levels of the inflammatory cytokines IL-1β, IL-6, and TNF-α	[37]
	3,5-diCQA	TMT-induced cognitive dysfunction mice	Increasing the GSH ratio Protection of mitochondrial activities and the repression of apoptotic signaling molecules such as p-Akt, BAX, and p-tau (Ser 404)	[38]
	Artemisinin	PC12 cells	Activation of ERK pathway	[41]

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Artemisia annua		D407 retinal pigment epithelial cells	Activation of ERK/CREB signaling pathway	[42]
		Neuronal cells RGC-5	Activation of P38 and ERK1/2 kinase pathway	[43]
		LPS-mediated BV-2 microglial	Reduction of the expression levels of the inflammatory cytokines IL-1β, IL-6, and TNF-α Inhibition of TLR4-MyD88-NF-kB signaling pathway	[46]
		HT-22 cells	Activation of kinase B (Akt)/Bcl-2 pathway	[44]
		SH-SY5Y	activation of the ERK/CREB pathway and inhibition of apoptosis pathway	[48]
Artemisia capillaries	Ethanol extracts	Ischemic brain injury-induced mice	Activation of nicotinic acetylcholine receptors	[52]
Artemisia turanica	Crude extracts	PC12 cells	Reduction of caspase-3 activity	[34]
Artemisia campestris	Essential oil	Brain tissue of deltamethrin treated rats	Change antioxidant enzymes level	[35]
Artemisia amygdalin	Methanol, aqueous, and ethyl acetate extracts	Differentiated N2a and SH-SY5Ycells	Upregulate the Nrf2 signaling pathway	[39]
Artemisia judaica	Ethanol extracts	STZ-induced diabetic rats	Improvement of gene expression of the antioxidant enzymes Decreasing the level and expression of TNF-α and iNOS in diabetic rats	[32]

However, the exact pharmacologically active compounds of Artemisia (except artemisinin) and the underlying mechanisms through which those bioactive compounds exert antiepileptic effects have not been extensively investigated.

Based on all the data included and analyzed in the review, we can conclude that Artemisia has potential antioxidant, anti-inflammatory, antiapoptotic, antiepileptic, and neuroprotective activities **(Figure 1).** The extracts of the Artemisia plant are likely to act through modulating multiple signaling pathways, such as extracellular regulated protein kinases (ERK/CREB) pathway and intracellular (mitochondrial) pathway, as well as an emerging nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway.

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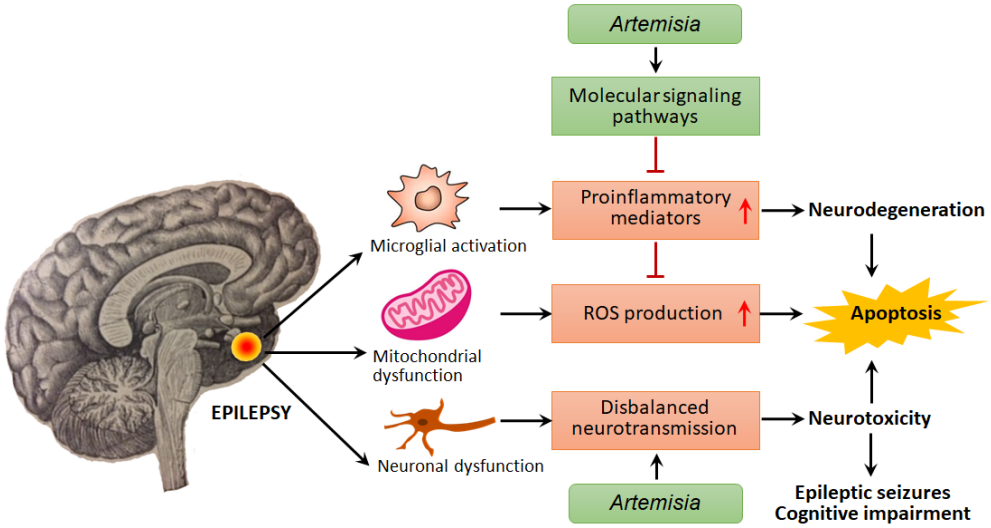

Figure 1. Neuroprotective effects of *Artemisia* through modulating neuroinflammation, reactive oxygen species (ROS) levels, and neuronal dysfunction to prevent epilepsy and cognitive impairment. *Artemisia* exerts its indirect neuroprotective effect through the regulation of molecular signalling pathways involving neuroinflammation and ROS production. It also regulates disbalanced neurotransmission.

Therefore, the use of natural bioactive compounds isolated from *Artemisia* as potential pharmacological modulators targeting the aforementioned signaling pathways might be an alternative option for the treatment of epilepsy and other neurodegenerative disorders.

However, although many studies have demonstrated anticonvulsant and neuroprotective activities of *Artemisia* species, most of them have emphasized crude plant extracts or fractions, and only a few pure bioactive compounds have been used in the studies (see **Table 1 and Table 2**). In addition, there are other pitfalls in using Artemisia: long-term and low-dose Artemisia exposure may induce free radical scavengers that disrupt the endoperoxide bridge structure of Artemisia. In addition, unexpected metabolic dysfunction or other abnormalities may occur after excessive use of Artemisia, including neurotoxicity and/or sperm DNA damage induced genotoxicity [56, 57]. Therefore, to ensure safety at appropriate doses, long-term testing should be evaluated. Finally, there is currently no clinical trial evidence showing that Artemisia has a therapeutic effect on epilepsy.

5. Conclusions

This review suggests neuroprotective and antiepileptic potentials of the genus Artemisia, which stem from their strong antioxidant, anti-inflammatory, anti-apoptotic, and neuromodulatory activity. Although some fundamental research results have been achieved, there is still a lot of work to be done for further research on Artemisia and its neuroprotective effects. Many Artemisia species have not been tested biologically and pharmacologically for their neuroprotective and antiepileptic activity. As the investigation continues, this genus might prove to be a rich source of bioactive compounds needed for the development of new chemotherapeutical agents that alleviate neural tissue damage in epilepsy and other neurodegenerative disorders. Thus, more investigations are still needed.

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Abbreviations:

ILAE	International League Against Epilepsy
AED	Anti-epileptics drugs
SE	Status epilepticus
OS	Oxidative stress
ROS	Reactive oxygen species
HE	Hydroalcoholic extract
GC/MS	Gas chromatography/mass spectrometry
MES	Maximal electroshock
PTZ	Pentylenetetrazole
TBARS	Thiobarbituric acid reactive substance
GSH	Glutathione
SOD	Superoxide dismutase
6-OHDA	6-hydroxydopamine
NO	Nitric oxide
TMT	Trimethyltin
ACh	Acetylcholine
AChE	Acetylcholinesterase
DiCQA	Dicaffeoylquinic acid
Nrf2	Nuclear factor erythroid 2-related factor 2
PGE2	Prostaglandin E2
GMSCF	Granulocyte-macrophage colony-stimulating factor

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