Neuroprotective Potential of *Cordyceps* Extracts

Subjects: Tropical Medicine

Contributor: Himadri Sharma , Niti Sharma , Seong Soo A. An

Cordyceps, also known as “zombie fungus”, is a non-poisonous mushroom that parasitizes insects for growth and development by manipulating the host system in a way that makes the victim behave like a “zombie”. These species produce promising bioactive metabolites, like adenosine, β-glucans, cordycepin, and ergosterol. *Cordyceps* has been used in traditional medicine due to its immense health benefits, as it boosts stamina, appetite, immunity, longevity, libido, memory, and sleep. Neuronal loss is the typical feature of neurodegenerative diseases (NDs) (Alzheimer’s disease (AD), Parkinson’s disease (PD), multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS)) and neurotrauma. Both these conditions share common pathophysiological features, like oxidative stress, neuroinflammation, and glutamatergic excitotoxicity.

Cordyceps zombie fungus neuroprotection neuroinflammation

1. Introduction

Of all *Cordyceps* species, only 35 have been characterized [1], of which *C. militaris* and *C. sinensis* are the two most widely studied. *C. sinensis* is a rare and expensive species that is difficult to cultivate, whereas *C. militaris* is a successful commercially grown species and is considered an alternative to *C. sinensis* [2]. By changing the culture conditions, the concentrations of bioactive compounds can be manipulated.

Several extraction methods and solvents have been employed for the isolation of selective bioactive compounds [3][4], with each extract exhibiting specific activity. As polar molecules, the aqueous extract contains functional concentrations of nucleosides and polysaccharides. In contrast, alcoholic extracts are rich in nucleosides, polysaccharides, and proteins with a high antioxidant potential.

2. Cordyceps militaris

C. militaris is a valuable TCM that grows on moth larvae (*Lepidoptera*). This fungus has been reported to treat respiratory, renal, hepatic, and cardiovascular diseases and has antiaging, antiviral, anti-inflammatory, and antitumor potentials [5]. Recently, *C. militaris* has become an economical alternative to *C. sinensis* in TCM because it can be easily cultivated under artificial conditions using diverse media [2]. Analyses of the compositions revealed that the concentrations of cordycepin and polysaccharides in the media of cultured *C. militaris* were higher than those in *C. sinensis* from the natural site [6]. The major bioactive components of *C. militaris* are nucleosides (adenosine, uridine, and cordycepin), myriocin, ergosterol, polysaccharides, L-arginine, and L-proline [2][7][8].

Previous studies have shown the presence of GABA (γ-aminobutyric acid), ergothioneine, D-mannitol (cordysepic acid), glycolipids, glycoproteins, xanthophylls (like carotenoids), sterols, statins, phenolic compounds, vitamins, and biominerals in *C. militaris* [6][9]. A previous study reported differences in the concentrations of cordycepin, cordycepic acid, and ergothioneine between fruiting bodies and mycelial biomass. The concentrations of cordycepin, cordycepic acid, and carbohydrates are higher in mycelial biomass, whereas those of ergothioneine and total amino acids are higher in fruiting bodies [9]. The reported optimal drying temperature for *C. militaris* is 60 °C, over which, cordycepin and phenolic compounds are lost [10]. Pentostatin, used as an antileukemia drug, is also produced by *C. militaris* through the same biosynthetic gene cluster for cordycepin production [11]. Similar to other chemotherapeutic drugs, it also has side effects such as diarrhea, nausea, and neurological toxicities [12]. Cordymin is an antifungal peptide that inhibits the mycelial growth of various fungi, including *Candida albicans*, *Bipolaris maydis*, and *Rhizoctonia solani* [13]. Ergosta-7,9(11),22-trien-3β-ol isolated from *C. militaris* shows anti-inflammatory and antioxidative activity [14].

Selective deterioration of cholinergic neurons in AD diminishes acetylcholine (ACh) levels, contributing to cognitive decline [15]. In addition to acting as a neurotransmitter, ACh also induces neurite outgrowth [16][17]. The methanolic extract of *C. militaris* promoted neurite outgrowth and ACh expressions in Neuro 2A mouse neuroblastoma cells in a dose-dependent manner (5–20 μg/mL). It also reversed scopolamine-induced memory deficits in rats and increased central cholinergic function at a dose of 300 mg/kg [18]. The ethanolic extract has been known to promote neurite outgrowth in Neuro 2A cells [19], provide protection from amyloid beta (Aβ)-induced toxicity [20], reduce the expression of inflammatory markers (cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS)), and downregulate mitogen-activated protein kinases/c-Jun *N*-terminal kinase/extracellular signal-regulated kinase (MAPK/JNK/ERK) pathway in C6 glial cells [21], which helped to reduce stress, inflammation, and apoptosis [22]. In addition, the extract restores recognition and memory functions by inhibiting oxidative stress (nitric oxide (NO) and lipid peroxidation) caused by toxic peptides [23]. Moreover, it upregulated the dopaminergic system in vivo and in vitro by upregulating tyrosine hydroxylase, an enzyme that catalyzes the rate-limiting steps in the biosynthesis of dopamine and other catecholamines [24].

One of the most conspicuous age-related diseases is ischemia, which is a common form of neurodegeneration that leads to cognitive impairment in the elderly [25]. The post-ischemic brain induces hippocampal neuronal death, neuroinflammation, and neuropathy, similar to AD [26]. Post-ischemic treatment with the butanolic extract of the fungus (WIB-801C: 50 mg/kg) decreased the inflammatory cell infiltration into ischemic lesions by inhibiting chemotaxis through adenosine receptor A3 (A3AR), thus providing neuroprotection in the middle cerebral artery occlusion (MCAO) rat model [27]. Moreover, after spinal cord injury (SCI), it mitigated blood–spinal cord barrier (BSCB) disruption by inhibiting matrix metalloprotease-9 (MMP-9), downregulating the expression of chemokine and promoting that of pro-nerve growth factor (NGF) in microglia (MG) [28]. The fungus also improved memory impairment caused by global cerebral ischemia and memory deterioration by delaying neuronal death, decreasing MG expression in the CA1 region of the hippocampus in rats [29], and increasing the expression of brain-derived neurotrophic factor (BDNF) and tyrosine kinase B (TrkB) in gerbils [30].

C. militaris aqueous extract showed beneficial effects in a D-galactose (Gal)-induced aging mouse model by improving memory [31]. Extract supplementation improved the levels of antioxidants (superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione (GSH)) and reduced malondialdehyde (MDA) and monoamine oxidase (MAO), which play important roles in the progression of aging. These results suggest a role for the antioxidant action of the fungus in recovering memory impairments in mice with D-Gal-induced aging [31].

In a recent study, nanoencapsulated *C. militaris* extract relieved neuronal pathology in SH-SY5Y cells (human neuroblastoma cells) by significantly improving dopamine secretion and the expression of dopaminergic-specific genes such as leucine-rich repeat kinase 2 gene (LRRK2), LIM homeobox transcription factor 1 beta (LMX1B), Forkhead Box (FOXA2), engrailed homeobox 1(EN1), and nuclear receptor-related 1 protein (NURR1) [32]. In line with this, *C. militaris* treatment enhanced the expression of neuronal protein paired box 6 (PAX6), a crucial player in brain development and function [33], and neuron-specific class III beta-tubulin (nestin), a marker of neuronal progenitor cells in the adult brain [34], indicating the role of *C. militaris* in enhancing neuronal maturation. Furthermore, it reduced amyloid precursor protein (APP) secretion by promoting autophagy [32]. As autophagy helps clear Aβ and tau aggregates in brain cells [35], *C. militaris* is considered important in AD treatment. The downregulated expression of AD-related genes presenilin 1 (PSEN1), presenilin 2 (PSEN2), and APP and the increased expression of the non-amyloidogenic pathway, ADAM metallopeptidase domain 10 (ADAM10), and sirtuin1 (SIRT1) by nanoencapsulated *C. militaris* extract suggest its potential in improving AD pathology at both the gene and protein levels [32].

These results suggest that the fungus is highly effective in protecting against memory-related neuronal degeneration in the brain and in retarding the progression of memory deficits associated with various NDs by its antioxidant, anti-inflammatory, and anti-apoptotic properties.

3. Cordyceps ophioglossoides

C. ophioglossoides, commonly known as the “golden thread *Cordyceps*”, is colonized on fruiting bodies of truffle-like *Elaphomyces* [36]. The fungus contains a variety of polysaccharides (antioxidant nature), ophiocordin (antibiotic), peptibiotics (antibiotic and antifungal properties), sesquiterpenes (antitumor activity), balanol (a protein kinase inhibitor with antitumor activity), and arsenocholine-O-sulfate (a nontoxic form of arsenic) [36][37][38][39][40].

$A\beta_{(25–35)}$ represents the biologically active region of Aβ, since it is the shortest fragment that displays large β-sheet aggregated structures, keeping the toxicity of the full-length peptide [41]; hence, it is often used as a model for inducing toxicity and memory deficits. The neuroprotective effect of *C. ophioglossoides* (methanolic extract) has been observed in vitro (extract: 100 µg/mL) and in vivo (extract:100 mg/kg) in $A\beta_{(25–35)}$ AD models, where the fungal extract protected SK-N-SH human neuroblastoma cells from cell death and helped in the restoration of spatial memory loss in induced memory deficit by $A\beta_{(25–35)}$ in rats probably by suppressing Aβ-induced oxidative stress [42].

4. Cordyceps sinensis

C. sinensis is the most popular *Cordyceps*, which parasitizes the larva of *Hepialus armoricanus*. This fungus has long been used in TCM to promote longevity and has anti-inflammatory and antitumor activities [43]. The major biochemical markers of nucleosides are adenosine and cordycepin [3][44], with immunomodulatory and antioxidant activities. In 2008, Yuan et al. reported the presence of other nucleosides (thymine, adenine, cytosine, uracil, uridine, hypoxanthine, ionosine, guanosine, and thymidine) in aqueous extracts of *C. sinensis* [45]. Polysaccharides are major contributors to the biological activities of *C. sinensis*. Guan et al. identified several monosaccharides (fructose, mannitol, galactose, arabinose, ribose, rhamnose, mannose, xylose, glucose, and sorbose) using GC-MS [46]. Ergosterol is the main identified sterol [47] and is present either as free or esterified ergosterol [3][48][49] with antitumor activity [50]. Other compounds, such as polyamines and free fatty acids, have also been identified in *C. sinensis* extracts [51]. Two peptides (cordymin and cordycedipeptide) and an ergosterol (H1-A) with biological activities were also isolated from the fungus [52].

Aqueous and different alcoholic extracts (CSEs) from the fungus revealed the presence of the antioxidants hesperidin, rutin, and ascorbic acid by high-performance thin-layer chromatography (HPTLC). Hesperidin, rutin, and ascorbic acid were present at high concentrations in the aqueous extract. However, the highest hesperidin content was observed in the 25% alcoholic extract in comparison to others [53]. Additionally, adenosine, adenine, and uracil are present at higher concentrations in the aqueous extract than in the other extracts [54]. The protective effects of the extracts against hypoxia-induced oxidative stress and inflammation were studied in mouse hippocampal (HT22) cells. CSEs (250 µg/mL) show neuroprotection by increasing the expression of endogenous antioxidants (GSH, GPx, and SOD), limiting lipid oxidation by decreasing MDA levels and reducing the level of inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) as well as transcription factor nuclear factor-κB (NF-κB) to various extents. The aqueous extract is more effective as an antioxidant in hypoxia, whereas the alcoholic extract prevented oxidative stress and inflammation [53], owing to the presence of more phenolics and flavonoids [54].

The aqueous [55] and ethanolic [56] extracts of the fungus were also evaluated for anti-inflammatory effects in an experimental middle cerebral artery occlusion/reperfusion (MCAO/R) model, as ischemic brain injury is associated with inflammatory reactions. In addition, neuronal apoptosis is triggered by increased expression of Bcl2-associated X (Bax), an apoptosis regulator, which in turn activates caspase-3 and inhibits B-cell lymphoma-2 (Bcl-2) expression [57]. The extract provided neuroprotection by downregulating the expression of inflammatory cytokines and other inflammatory mediators (IL-1β, TNF-α, Myeloperoxidase (MPO), and adhesion molecules ICAM-1, COX-2, and iNOS), blocking polymorphonuclear cell (PMNC) infiltration, thereby subsiding neurological deficits and infarct volume. Moreover, the aqueous extract also revealed the decreased Bax, cytochrome c (Cyt c), and caspase-3 protein expressions, which in turn improved mitochondrial membrane potential (ψ_m), thus modulating the electron transport chain in the mitochondria in vivo and in vitro [58].

Multiple sclerosis (MS) is an autoimmune demyelinating disease of the CNS that involves a variety of immune cells [59]. Inflammation resulting from MS is mediated by the infiltration of autoreactive T cells into the CNS through the

blood–brain barrier (BBB) [60]. Amongst the T cells, primarily interferon-gamma (IFN-γ)-producing T-helper 1 (Th1) cells and IL-17-producing Th17 cells had an important role in the pathogenesis of the disease [61]. Effector molecules secreted by Th1 cells directly affect the phenotype, function, and recruitment of MG, whereas Th17 cells upregulate chemokines during the inflammatory process [62]. *C. sinensis* extract was reported to reduce the number of Th1 cells in a mouse model of MS/experimental autoimmune encephalomyelitis (EAE), thus relieving EAE severity and the associated pathology [63].

The neuroprotective effect of fermented fungus powder (Cs-C-Q80 or 'corbrin capsule') was evaluated in subcortical ischemic vascular dementia induced in a mouse model of right unilateral common carotid artery occlusion (rUCCAO) [64], which damaged the white matter region in the brain, resulting in myelin loss, glial activation, neuroinflammation, and dementia [65]. However, both the prophylactic and therapeutic administration of corbrin (1 g/kg) significantly reduced white matter lesions and improved learning and memory loss through anti-inflammatory actions [64]. A lower dose of corbrin (1 mg/kg) was effective in reducing the pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), improving the levels of oxidative stress parameters (SOD, MDA), increasing ATP concentration, and alleviating neurological deficits in an MCAO mice model [66].

5. Cordyceps cicadae

C. cicadae is the oldest known therapeutic fungus that feeds on *Lepidoptera* species larvae [67]. It has been used in TCM for the treatment of asthma, cancer, convulsions, dizziness, palpitations, and chronic renal disease. Natural *C. cicadae* is a slow-growing fungus in high demand, whereas its anamorph, *Paecilomyces cicadae,* can be cultured easily and used as a substitute for *C. cicadae* to accommodate market requirements [68]. Various bioactive compounds, such as cyclopeptides, myriocins, polysaccharides, nucleosides, and mannitol have been identified in *C. cicadae* [68][69]. LC-MS analyses have detected adenosine and adenosine analogs, N6-(2-hydroxyethyl)-adenosine (HEA), a Ca^{2+} antagonist, and an anti-inflammatory agent [70][71][72]. HEA is a major bioactive compound in *C. cicadae* that exhibits antidiabetic, sedative, analgesic, antitumor [73], and renoprotective activities [74][75]. Another isolated bioactive compound, ergosterol peroxide, exhibits immunomodulatory and anti-inflammatory effects [76][77].

Trauma to the CNS and NDs initiate a torrent of cellular and molecular reactions that result in neuronal loss and regenerative failure. To understand the associated mechanisms, the rodent optic nerve crush (ONC) model can be used and later extrapolated to NDs [78]. *C. cicadae* mycelium extract provided neuroprotection in the ONC rat model through anti-apoptotic and anti-inflammatory effects by improving retinal ganglion cell (RGC) density and P1-N2 amplitude [79], which intensified with visual–spatial attention in the visual cortex. The butanol fraction protected rat adrenal pheochromocytoma (PC12) cells against glutamate-induced oxidative damage. Additionally, the extract restored the mitochondrial function, suppressed ROS accumulation, upregulated the antioxidant enzymes (GPX and SOD), increased cell viability, decreased lactase dehydrogenase (LDH) release, and reduced apoptosis [80][81]. Subsequently, adenosine was identified as the main nucleoside responsible for this neuroprotective action [81]. The anti-inflammatory activities of three bioactive nucleosides (adenosine, cordycepin,

and HEA) isolated from wild-type and artificially cultured *C. cicadae* were evaluated. Cordycepin was found to be more potent than other nucleosides in limiting the release of pro-inflammatory cytokines by lipopolysaccharide (LPS)-stimulated RAW 264.7; however, no synergistic effect of the three compounds was observed. LPS-induced pro-inflammatory responses were attenuated by HEA through the suppression of the toll-like receptor (TLR)-4-mediated NF-κB signaling pathway [82]. The effects of the hydroalcoholic fungal extract on cisplatin toxicity have also been evaluated. Cisplatin is an anticancer agent involved in multi-organ toxicity, including neurotoxicity. It accumulates in the dorsal root ganglion (DRG) and causes oxidative stress, neuronal apoptosis, and inflammation [83]. The nucleoside-rich extract of *C. cicadae* ameliorated memory impairment and neuropathy by reducing oxidative stress, acetylcholinesterase enzyme (AChE) levels, and inflammation in cisplatin-treated rats [84].

In a recent study, increased levels of bioactive compounds were obtained from cultured *C. cicadae* in deep ocean water (DOW) and minerals, thus increasing their therapeutic value [85]. The effect of DOW-cultured fungus (DCC) was investigated on D-Gal-induced brain damage and memory impairment in rats. DCC (100–500 mg/kg), in turn, improved cognition by alleviating the expressions of antioxidants and inflammatory genes (iNOS, TNF-α, IL-6, IL-1β, COX-2), along with reduced expressions of the aging-related proteins (glial fibrillary acidic protein (GFAP) and PS1) [85].

References

1. Olatunji, O.J.; Tang, J.; Tola, A.; Auberon, F.; Oluwaniyi, O.; Ouyang, Z. The genus Cordyceps: An extensive review of its traditional uses, phytochemistry and pharmacology. Fitoterapia 2018, 129, 293–316.
2. Jędrejko, K.J.; Lazur, J.; Muszyńska, B. Cordyceps militaris: An Overview of Its Chemical Constituents in Relation to Biological Activity. Foods 2021, 10, 2634.
3. Shashidhar, M.G.; Giridhar, P.; Udaya Sankar, K.; Manohar, B. Bioactive principles from Cordyceps sinensis: A potent food supplement—A review. J. Funct. Foods 2013, 5, 1013–1030.
4. Chen, P.X.; Wang, S.; Nie, S.; Marcone, M. Properties of Cordyceps sinensis: A review. J. Funct. Foods 2013, 5, 550–569.
5. Zhang, J.; Wen, C.; Duan, Y.; Zhang, H.; Ma, H. Advance in Cordyceps militaris (Linn) Link polysaccharides: Isolation, structure, and bioactivities: A review. Int. J. Biol. Macromol. 2019, 132, 906–914.
6. Cohen, N.; Cohen, J.; Asatiani, M.D.; Varshney, V.K.; Yu, H.T.; Yang, Y.C.; Li, Y.H.; Mau, J.L.; Wasser, S.P. Chemical composition and nutritional and medicinal value of fruit bodies and submerged cultured mycelia of culinary-medicinal higher Basidiomycetes mushrooms. Int. J. Med. Mushrooms 2014, 16, 273–291.

7. Sun, T.; Dong, W.; Jiang, G.; Yang, J.; Liu, J.; Zhao, L.; Ma, P. Cordyceps militaris Improves Chronic Kidney Disease by Affecting TLR4/NF-κB Redox Signaling Pathway. Oxid. Med. Cell Longev. 2019, 2019, 7850863.
8. Deacon, J.W. Fungal Biology; John Wiley & Sons: Hoboken, NJ, USA, 2005.
9. Chan, J.S.; Barseghyan, G.S.; Asatiani, M.D.; Wasser, S.P. Chemical Composition and Medicinal Value of Fruiting Bodies and Submerged Cultured Mycelia of Caterpillar Medicinal Fungus Cordyceps militaris CBS-132098 (Ascomycetes). Int. J. Med. Mushrooms 2015, 17, 649–659.
10. Wu, X.-F.; Zhang, M.; Li, Z. Influence of infrared drying on the drying kinetics, bioactive compounds and flavor of Cordyceps militaris. LWT 2019, 111, 790–798.
11. Xia, Y.; Luo, F.; Shang, Y.; Chen, P.; Lu, Y.; Wang, C. Fungal Cordycepin Biosynthesis Is Coupled with the Production of the Safeguard Molecule Pentostatin. Cell Chem. Biol. 2017, 24, 1479–1489.e4.
12. Margolis, J.; Grever, M.R. Pentostatin (Nipent): A review of potential toxicity and its management. Semin Oncol. 2000, 27, 9–14.
13. Wong, J.H.; Ng, T.B.; Wang, H.; Sze, S.C.; Zhang, K.Y.; Li, Q.; Lu, X. Cordymin, an antifungal peptide from the medicinal fungus Cordyceps militaris. Phytomedicine 2011, 18, 387–392.
14. Huang, Y.-P.; Chen, D.-R.; Lin, W.-J.; Lin, Y.-H.; Chen, J.-Y.; Kuo, Y.-H.; Chung, J.-G.; Hsia, T.-C.; Hsieh, W.-T. Ergosta-7, 9 (11), 22-trien-3β-ol attenuates inflammatory responses via inhibiting MAPK/AP-1 induced IL-6/JAK/STAT pathways and activating Nrf2/HO-1 signaling in LPS-stimulated macrophage-like cells. Antioxidants 2021, 10, 1430.
15. Terry, A.V.; Buccafusco, J.J. The cholinergic hypothesis of age and Alzheimer's disease-related cognitive deficits: Recent challenges and their implications for novel drug development. J. Pharmacol. Exp. Ther. 2003, 306, 821–827.
16. De Jaco, A.; Augusti-Tocco, G.; Biagioni, S. Muscarinic acetylcholine receptors induce neurite outgrowth and activate the synapsin I gene promoter in neuroblastoma clones. Neuroscience 2002, 113, 331–338.
17. Anelli, T.; Mannello, F.; Salani, M.; Tonti, G.A.; Poiana, G.; Biagioni, S. Acetylcholine induces neurite outgrowth and modulates matrix metalloproteinase 2 and 9. Biochem. Biophys. Res. Commun. 2007, 362, 269–274.
18. Lee, B.; Park, J.; Park, J.; Shin, H.-J.; Kwon, S.; Yeom, M.; Sur, B.; Kim, S.; Kim, M.; Lee, H. Cordyceps militaris improves neurite outgrowth in Neuro2a cells and reverses memory impairment in rats. Food Sci. Biotechnol. 2011, 20, 1599–1608.
19. Phan, C.-W.; David, P.; Naidu, M.; Wong, K.-H.; Sabaratnam, V. Neurite outgrowth stimulatory effects of culinary-medicinal mushrooms and their toxicity assessment using differentiating Neuro-

2a and embryonic fibroblast BALB/3T3. BMC Complement. Altern. Med. 2013, 13, 261.

20. He, M.T.; Park, C.H.; Cho, E.J. Caterpillar Medicinal: Mushroom, Cordyceps militaris (Ascomycota), Attenuates Aβ 1− 42− Induced Amyloidogenesis and Inflammatory Response by Suppressing Amyloid Precursor Protein Progression and p38 MAPK/JNK Activation. Int. J. Med. Mushrooms 2021, 23, 2634.
21. He, M.T.; Lee, A.Y.; Park, C.H.; Cho, E.J. Protective effect of Cordyceps militaris against hydrogen peroxide-induced oxidative stress in vitro. Nutr. Res. Pr. 2019, 13, 279–285.
22. Bogoyevitch, M.A.; Kobe, B. Uses for JNK: The many and varied substrates of the c-Jun N-terminal kinases. Microbiol. Mol. Biol. Rev. 2006, 70, 1061–1095.
23. He, M.T.; Lee, A.Y.; Kim, J.H.; Park, C.H.; Shin, Y.S.; Cho, E.J. Protective role of Cordyceps militaris in Aβ(1-42)-induced Alzheimer's disease in vivo. Food Sci. Biotechnol. 2019, 28, 865–872.
24. Sapkota, K.; Kim, S.; Park, Y.L.; Choi, B.-S.; Park, S.-E.; Kim, S.-J. Enhancement of tyrosine hydroxylase expression by Cordyceps militaris. Cent. Eur. J. Biol. 2010, 5, 214–223.
25. Lucke-Wold, B.P.; Logsdon, A.F.; Turner, R.C.; Rosen, C.L.; Huber, J.D. Chapter Fourteen—Aging, the Metabolic Syndrome, and Ischemic Stroke: Redefining the Approach for Studying the Blood–Brain Barrier in a Complex Neurological Disease. In Advances in Pharmacology; Davis, T.P., Ed.; Academic Press: Cambridge, MA, USA, 2014; Volume 71, pp. 411–449.
26. Pluta, R.; Furmaga-Jabłońska, W.; Januszewski, S.; Czuczwar, S.J. Post-ischemic brain neurodegeneration in the form of Alzheimer's disease proteinopathy: Possible therapeutic role of curcumin. Nutrients 2022, 14, 248.
27. Hwang, S.; Cho, G.-S.; Ryu, S.; Kim, H.J.; Song, H.Y.; Yune, T.Y.; Ju, C.; Kim, W.-K. Post-ischemic treatment of WIB801C, standardized Cordyceps extract, reduces cerebral ischemic injury via inhibition of inflammatory cell migration. J. Ethnopharmacol. 2016, 186, 169–180.
28. Lee, J.Y.; Choi, H.Y.; Baik, H.H.; Ju, B.G.; Kim, W.K.; Yune, T.Y. Cordycepin-enriched WIB-801C from Cordyceps militaris improves functional recovery by attenuating blood-spinal cord barrier disruption after spinal cord injury. J. Ethnopharmacol. 2017, 203, 90–100.
29. Kim, Y.O.; Kim, H.J.; Abu-Taweel, G.M.; Oh, J.; Sung, G.H. Neuroprotective and therapeutic effect of Cordyceps militaris on ischemia-induced neuronal death and cognitive impairments. Saudi J. Biol. Sci. 2019, 26, 1352–1357.
30. Lee, S.-H.; Ko, I.-G.; Kim, S.-E.; Hwang, L.; Jin, J.-J.; Choi, H.-H.; Kim, C.-J. Aqueous extract of Cordyceps alleviates cerebral ischemia-induced short-term memory impairment in gerbils. J. Exerc. Rehabil. 2016, 12, 69.

31. Li, Z.; Zhang, Z.; Zhang, J.; Jia, J.; Ding, J.; Luo, R.; Liu, Z. Cordyceps militaris extract attenuates D-galactose-induced memory impairment in mice. J. Med. Food 2012, 15, 1057–1063.
32. Kaokaen, P.; Sorraksa, N.; Phonchai, R.; Chaicharoenaudomrung, N.; Kunhorm, P.; Noisa, P. Enhancing Neurological Competence of Nanoencapsulated Cordyceps/Turmeric Extracts in Human Neuroblastoma SH-SY5Y Cells. Cell Mol. Bioeng. 2023, 16, 81–93.
33. Kikkawa, T.; Casingal, C.R.; Chun, S.H.; Shinohara, H.; Hiraoka, K.; Osumi, N. The role of Pax6 in brain development and its impact on pathogenesis of autism spectrum disorder. Brain Res. 2019, 1705, 95–103.
34. Krishnasamy, S.; Weng, Y.-C.; Thammisetty, S.S.; Phaneuf, D.; Lalancette-Hebert, M.; Kriz, J. Molecular imaging of nestin in neuroinflammatory conditions reveals marked signal induction in activated microglia. J. Neuroinflamm. 2017, 14, 45.
35. Rahman, M.A.; Rahman, M.H.; Mamun-Or-Rashid, A.; Hwang, H.; Chung, S.; Kim, B.; Rhim, H. Autophagy modulation in aggresome formation: Emerging implications and treatments of Alzheimer's disease. Biomedicines 2022, 10, 1027.
36. Kneifel, H.; Konig, W.A.; Loeffler, W.; Müller, R. Ophiocordin, an antifungal antibiotic of Cordyceps ophioglossoides. Arch. Microbiol. 1977, 113, 121–130.
37. Braeuer, S.; Borovička, J.; Glabonjat, R.A.; Steiner, L.; Goessler, W. Arsenocholine-O-sulfate: A novel compound as major arsenic species in the parasitic mushroom Tolypocladium ophioglossoides. Chemosphere 2021, 265, 128886.
38. Quandt, C.A.; Bushley, K.E.; Spatafora, J.W. The genome of the truffle-parasite Tolypocladium ophioglossoides and the evolution of antifungal peptaibiotics. BMC Genom. 2015, 16, 553.
39. He, X.; Zhang, M.; Guo, Y.-Y.; Mao, X.-M.; Chen, X.-A.; Li, Y.-Q. Revelation of the balanol biosynthetic pathway in Tolypocladium ophioglossoides. Org. Lett. 2018, 20, 6323–6326.
40. Xu, Q.; Liu, Z.; Sun, Y.; Ding, Z.; LÜ, L.; Li, Y. Optimization for Production of Intracellular Polysaccharide from Cordyceps ophioglossoides L2 in Submerged Culture and Its Antioxidant Activities in vitro. Chin. J. Chem. Eng. 2012, 20, 294–301.
41. Sato, K.; Wakamiya, A.; Maeda, T.; Noguchi, K.; Takashima, A.; Imahori, K. Correlation among secondary structure, amyloid precursor protein accumulation, and neurotoxicity of amyloid β (25–35) peptide as analyzed by single alanine substitution. J. Biochem. 1995, 118, 1108–1111.
42. Jin, D.-Q.; Park, B.-C.; Lee, J.-S.; Choi, H.-D.; Lee, Y.-S.; Yang, J.-H.; Kim, J.-A. Mycelial extract of Cordyceps ophioglossoides prevents neuronal cell death and ameliorates β-amyloid peptide-induced memory deficits in rats. Biol. Pharm. Bull. 2004, 27, 1126–1129.
43. Paterson, R.R. Cordyceps: A traditional Chinese medicine and another fungal therapeutic biofactory? Phytochemistry 2008, 69, 1469–1495.

44. Li, S.; Yang, F.; Tsim, K.W. Quality control of Cordyceps sinensis, a valued traditional Chinese medicine. J. Pharm. Biomed. Anal. 2006, 41, 1571–1584.
45. Yuan, J.P.; Zhao, S.Y.; Wang, J.H.; Kuang, H.C.; Liu, X. Distribution of nucleosides and nucleobases in edible fungi. J. Agric. Food Chem. 2008, 56, 809–815.
46. Guan, J.; Yang, F.Q.; Li, S.P. Evaluation of carbohydrates in natural and cultured Cordyceps by pressurized liquid extraction and gas chromatography coupled with mass spectrometry. Molecules 2010, 15, 4227–4241.
47. Li, Y.H.; Li, X.L. Determination of ergosterol in Cordyceps sinensis and Cordyceps black-bone chicken capsules by HPLC. Yao Xue Xue Bao 1991, 26, 768–771.
48. Li, S.P.; Li, P.; Lai, C.M.; Gong, Y.X.; Kan, K.K.; Dong, T.T.; Tsim, K.W.; Wang, Y.T. Simultaneous determination of ergosterol, nucleosides and their bases from natural and cultured Cordyceps by pressurised liquid extraction and high-performance liquid chromatography. J. Chromatogr. A 2004, 1036, 239–243.
49. Yuan, J.-P.; Wang, J.-H.; Liu, X.; Kuang, H.-C.; Zhao, S.-Y. Simultaneous determination of free ergosterol and ergosteryl esters in Cordyceps sinensis by HPLC. Food Chem. 2007, 105, 1755–1759.
50. Bok, J.W.; Lermer, L.; Chilton, J.; Klingeman, H.G.; Towers, G.H. Antitumor sterols from the mycelia of Cordyceps sinensis. Phytochemistry 1999, 51, 891–898.
51. Mishra, R.; Upadhyay, Y. Cordicepssinensis: The Chinese Rasayan-current research scenario. Int. J. Res. Pharm. Biomed. Sci. 2011, 2, 1503–1519.
52. Liu, Y.; Wang, J.; Wang, W.; Zhang, H.; Zhang, X.; Han, C. The Chemical Constituents and Pharmacological Actions of Cordyceps sinensis. Evid.-Based Complement. Altern. Med. 2015, 2015, 575063.
53. Pal, M.; Bhardwaj, A.; Manickam, M.; Tulsawani, R.; Srivastava, M.; Sugadev, R.; Misra, K. Protective efficacy of the caterpillar mushroom, Ophiocordyceps sinensis (Ascomycetes), from India in neuronal hippocampal cells against hypoxia. Int. J. Med. Mushrooms 2015, 17, 829–840.
54. Mehrotra, S.; Kirar, V.; Vats, P.; Nandi, S.P.; Negi, P.; Misra, K. Phytochemical and Antimicrobial Activities of Himalayan Cordyceps Sinensis (Berk.) Sacc; NISCAIR-CSIR: New Delhi, India, 2015.
55. Liu, Z.; Li, P.; Zhao, D.; Tang, H.; Guo, J. Anti-inflammation Effects of Cordyceps sinensis Mycelium in Focal Cerebral Ischemic Injury Rats. Inflammation 2011, 34, 639–644.
56. Kong, R.; Zhang, Y.; Zhang, S.; Liu, M.; Sun, W.; Xing, Y.; Guan, Y.; Han, C.; Liu, Z. Protective Effect of Ethanol Extracts of the Chinese Caterpillar Mushroom, Ophiocordyceps sinensis (Ascomycetes), on the Experimental Middle Cerebral Artery Occlusion/Reperfusion (MCAO/R) Model. Int. J. Med. Mushrooms 2015, 17, 997–1003.

57. Liu, G.; Wang, T.; Wang, T.; Song, J.; Zhou, Z. Effects of apoptosis-related proteins caspase-3, Bax and Bcl-2 on cerebral ischemia rats. Biomed. Rep. 2013, 1, 861–867.
58. Bai, X.; Tan, T.-Y.; Li, Y.-X.; Li, Y.; Chen, Y.-F.; Ma, R.; Wang, S.-Y.; Li, Q.; Liu, Z.-Q. The protective effect of cordyceps sinensis extract on cerebral ischemic injury via modulating the mitochondrial respiratory chain and inhibiting the mitochondrial apoptotic pathway. Biomed. Pharmacother. 2020, 124, 109834.
59. Dendrou, C.A.; Fugger, L.; Friese, M.A. Immunopathology of multiple sclerosis. Nat. Rev. Immunol. 2015, 15, 545–558.
60. Arima, Y.; Kamimura, D.; Sabharwal, L.; Yamada, M.; Bando, H.; Ogura, H.; Atsumi, T.; Murakami, M. Regulation of Immune Cell Infiltration into the CNS by Regional Neural Inputs Explained by the Gate Theory. Mediat. Inflamm. 2013, 2013, 898165.
61. Goverman, J. Autoimmune T cell responses in the central nervous system. Nat. Rev. Immunol. 2009, 9, 393–407.
62. Prajeeth, C.K.; Kronisch, J.; Khorooshi, R.; Knier, B.; Toft-Hansen, H.; Gudi, V.; Floess, S.; Huehn, J.; Owens, T.; Korn, T.; et al. Effectors of Th1 and Th17 cells act on astrocytes and augment their neuroinflammatory properties. J. Neuroinflamm. 2017, 14, 204.
63. Zhong, S.S.; Xiang, Y.J.; Liu, P.J.; He, Y.; Yang, T.T.; Wang, Y.Y.; Rong, A.; Zhang, J.; Liu, G.Z. Effect of Cordyceps sinensis on the Treatment of Experimental Autoimmune Encephalomyelitis: A Pilot Study on Mice Model. Chin. Med. J. 2017, 130, 2296–2301.
64. Chen, Y.; Fu, L.; Han, M.; Jin, M.; Wu, J.; Tan, L.; Chen, Z.; Zhang, X. The Prophylactic and Therapeutic Effects of Fermented Cordyceps sinensis Powder, Cs-C-Q80, on Subcortical Ischemic Vascular Dementia in Mice. Evid.-Based Complement. Altern. Med. 2018, 2018, 4362715.
65. Yoshizaki, K.; Adachi, K.; Kataoka, S.; Watanabe, A.; Tabira, T.; Takahashi, K.; Wakita, H. Chronic cerebral hypoperfusion induced by right unilateral common carotid artery occlusion causes delayed white matter lesions and cognitive impairment in adult mice. Exp. Neurol. 2008, 210, 585–591.
66. Wu, J.; Yan, W.; Wu, X.; Hong, D.; Lu, X.; Rao, Y. Protective effects of Corbrin Capsule against permanent cerebral ischemia in mice. Biomed. Pharmacother. 2020, 121, 109646.
67. Li, I.C.; Lin, S.; Tsai, Y.T.; Hsu, J.H.; Chen, Y.L.; Lin, W.H.; Chen, C.C. Cordyceps cicadae mycelia and its active compound HEA exert beneficial effects on blood glucose in type 2 diabetic db/db mice. J. Sci. Food Agric. 2019, 99, 606–612.
68. Li, L.; Zhang, T.; Li, C.; Xie, L.; Li, N.; Hou, T.; Wang, Y.; Wang, B. Potential therapeutic effects of Cordyceps cicadae and Paecilomyces cicadae on adenine-induced chronic renal failure in rats and their phytochemical analysis. Drug Des. Devel Ther. 2019, 13, 103–117.

69. Zhang, X.; Hu, Q.; Weng, Q. Secondary metabolites (SMs) of Isaria cicadae and Isaria tenuipes. RSC Adv. 2019, 9, 172–184.
70. Lu, Y.; Luo, F.; Cen, K.; Xiao, G.; Yin, Y.; Li, C.; Li, Z.; Zhan, S.; Zhang, H.; Wang, C. Omics data reveal the unusual asexual-fruiting nature and secondary metabolic potentials of the medicinal fungus Cordyceps cicadae. BMC Genom. 2017, 18, 668.
71. Chyau, C.-C.; Wu, H.-L.; Peng, C.-C.; Huang, S.-H.; Chen, C.-C.; Chen, C.-H.; Peng, R.Y. Potential protection effect of ER homeostasis of N6-(2-hydroxyethyl) adenosine isolated from Cordyceps cicadae in nonsteroidal anti-inflammatory drug-stimulated human proximal tubular cells. Int. J. Mol. Sci. 2021, 22, 1577.
72. Latini, S.; Pedata, F. Adenosine in the central nervous system: Release mechanisms and extracellular concentrations. J. Neurochem. 2001, 79, 463–484.
73. Furuya, T.; Hirotani, M.; Matsuzawa, M. N6-(2-hydroxyethyl) adenosine, a biologically active compound from cultured mycelia of Cordyceps and Isaria species. Phytochemistry 1983, 22, 2509–2512.
74. Wang, X.; Qin, A.; Xiao, F.; Olatunji, O.J.; Zhang, S.; Pan, D.; Han, W.; Wang, D.; Ni, Y. N(6) -(2-hydroxyethyl)-adenosine from Cordyceps cicadae protects against diabetic kidney disease via alleviation of oxidative stress and inflammation. J. Food Biochem. 2019, 43, e12727.
75. Meng, Z.; Kang, J.; Wen, T.; Lei, B.; Hyde, K.D. Cordycepin and N6-(2-hydroxyethyl)-adenosine from Cordyceps pruinosa and their interaction with human serum albumin. PLoS ONE 2015, 10, e0121669.
76. Zhu, R.; Zheng, R.; Deng, Y.; Chen, Y.; Zhang, S. Ergosterol peroxide from Cordyceps cicadae ameliorates TGF-beta1-induced activation of kidney fibroblasts. Phytomedicine 2014, 21, 372–378.
77. Kobori, M.; Yoshida, M.; Ohnishi-Kameyama, M.; Shinmoto, H. Ergosterol peroxide from an edible mushroom suppresses inflammatory responses in RAW264.7 macrophages and growth of HT29 colon adenocarcinoma cells. Br. J. Pharmacol. 2007, 150, 209–219.
78. Sharma, T.P.; McDowell, C.M.; Liu, Y.; Wagner, A.H.; Thole, D.; Faga, B.P.; Wordinger, R.J.; Braun, T.A.; Clark, A.F. Optic nerve crush induces spatial and temporal gene expression patterns in retina and optic nerve of BALB/cJ mice. Mol. Neurodegener. 2014, 9, 14.
79. Wen, Y.T.; Jhou, B.Y.; Hsu, J.H.; Fu, H.I.; Chen, Y.L.; Shih, Y.C.; Chen, C.C.; Tsai, R.K. Neuroprotective Effects of Cordyceps cicadae (Ascomycetes) Mycelium Extract in the Rat Model of Optic Nerve Crush. Int. J. Med. Mushrooms 2022, 24, 41–48.
80. Wang, D.; Wang, J.; Wang, D.; Yu, X.; Olatunji, O.J.; Ouyang, Z.; Wei, Y. Neuroprotective effects of butanol fraction of Cordyceps cicadae on glutamate-induced damage in pc12 cells involving oxidative toxicity. Chem. Biodivers. 2018, 15, e1700385.

81. Olatunji, O.J.; Feng, Y.; Olatunji, O.O.; Tang, J.; Ouyang, Z.; Su, Z.; Wang, D.; Yu, X. Neuroprotective effects of adenosine isolated from Cordyceps cicadae against oxidative and ER stress damages induced by glutamate in PC12 cells. Environ. Toxicol. Pharmacol. 2016, 44, 53–61.
82. Lu, M.Y.; Chen, C.C.; Lee, L.Y.; Lin, T.W.; Kuo, C.F. N(6)-(2-Hydroxyethyl)adenosine in the Medicinal Mushroom Cordyceps cicadae Attenuates Lipopolysaccharide-Stimulated Pro-inflammatory Responses by Suppressing TLR4-Mediated NF-κB Signaling Pathways. J. Nat. Prod. 2015, 78, 2452–2460.
83. Santos, N.A.G.d.; Ferreira, R.S.; Santos, A.C.d. Overview of cisplatin-induced neurotoxicity and ototoxicity, and the protective agents. Food Chem. Toxicol. 2020, 136, 111079.
84. Liu, N.; Zhou, S.; Olatunji, O.J.; Wu, Y. Nucleosides rich extract from Cordyceps cicadae alleviated cisplatin-induced neurotoxicity in rats: A behavioral, biochemical and histopathological study. Arab. J. Chem. 2022, 15, 103476.
85. Chang, C.Y.; Yang, P.X.; Yu, T.L.; Lee, C.L. Cordyceps cicadae NTTU 868 Mycelia Fermented with Deep Ocean Water Minerals Prevents D-Galactose-Induced Memory Deficits by Inhibiting Oxidative Inflammatory Factors and Aging-Related Risk Factors. Nutrients 2023, 15, 1968.