Pharmacological Actions of Cordyceps militaris

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The medicinal mushroom *C. militaris* has been widely consumed in China for medication purpose since ancient times (3000 years). It is used for therapeutic treatment of lung and kidney malfunction, hyperglycemia and hyperlipidemia, respiratory disorders, fatigue, treatment of night sweating, fertility issues, cardiac arrhythmias, and other heart diseases. On a broader scale, *C. militaris* has an array of pharmacological properties, including as inflammation inhibition, and antioxidant, antitumor, antimetastatic, immunomodulatory, hypoglycemic, and steroidogenic activities.

Keywords: Cordyceps militaris ; inflammation ; polysaccharides ; pharmacokinetics

1. Immune Boosting Activity

Several reports have suggested the immune regulation activities of extracts of this medicinal mushroom[1]. The oral administration of aqueous extracts from the *C. militaris* fruiting body at a concentration of 20 mg/kg resulted in induced interferon (IFN) secretion by macrophages via IL-18 [2]. Both fresh and dried *C. militaris* extracts have been observed to have equivalent immunomodulatory effects in clophosphamide (cy)-activated immunosuppressed experimental animals. Quantitative investigation of phytochemicals revealed that levels of cordycepin and adenosine in fresh and dried *C. militaris* were similar, whereas fresh extracts contained more polysaccharides, total polyphenol, and total flavonoids compared to dried ones. Both types of extracts reversed the inhibition of the thymus and spleen index in a dose-dependent manner in a diseased mice model. Additionally, these extracts were able to increase the levels of IL-2 and IFN-γ secretion levels in test animals [3]. An ethyl alcohol extract of *C. militaris* administered to healthy Korean male volunteers enhanced cell-mediated immunity at a dose of 1.5 g/day via an oral route. Researchers observed no side effects after a treatment duration of 4 weeks along, with significant enhancement in the levels of IL-2 and the IFN-γ compared to the placebo group. Further, due to enhanced T cell proliferation and improved natural killer cell activity, this fungus was promoted as a safe immunomodulator to boost cell-mediated immunity [4]. In another study, *C. militaris* fruiting body extracts displayed immune modulation potential and antioxidative activity in healthy Kunming mice. Oral administration of different extracts concentrations i.e., 50, 100, or 200 mg/kg on a daily basis caused a significant increase in thymic and splenic indices. Total white blood cell count, as well as monocytes and lymphocytes, were enhanced, neutrophils were decreased, but eosinophils and basophils did not undergo any changes. Augmented IL and TNF-α levels were reported in the spleen and increased total antioxidant capacity, glutathione peroxidase, and SOD were observed in different organs such as the heart, kidney, and liver. These results suggest an immune boosting response in heathy organisms [5] and indicate the positive impact of *C. militaris* as an immunomodulatory agent.

2. Antiviral Potential

C. militaris extract has been used to determine its protective effects in influenza A/NWS/33 (H1N1) virus-infected mouse. The virus preventive effects of *C. militaris* extracts were investigated by administering different doses at 30, 100, or 300 mg/kg per day for seven days to H1N1-infected animals. The results inferred that the protective effect of the extracts could be attributed to suppressed TNF-α level along with increased IL-12, natural killer (NK) cells in experimental mice models [6]. Viral hepatitis occurs commonly worldwide, and when untreated causes hepatocellular carcinomas and cirrhosis. *C. militaris* showed moderate anti-HCV potential in conjunction with standard antivirals (IFN-α or ribavirin) in a cell-based HCV RNA replication assay system used to investigate antiviral activity [7].

3. Anticoagulant Activity

Fibrin is formed by fibrinogen by the activity of thrombin. The accumulation of fibrin in blood vessels can lead to clot formation resulting in thrombosis and cardiovascular diseases. A number of reports have confirmed the presence of fibrinolytic enzymes in *C. militaris* with anti-coagulant or thrombolytic activities. Cui et al., successfully purified a novel fibrinolytic enzyme from culture broth of the species and named it *C. militaris* fibrinolytic enzyme (CMase) [8]. Similarly, Liu

et al. purified a fibrinoyltic enzyme, which had the ability to hydrolyze fibrin or fibrinogen by cleaving the α-chains more efficiently than β- and γ-chains, revealing its plasmin like nature. It was able to degrade thrombin, indicating its benefits as an anticoagulant and antithrombotic protein [9]. The same authors reported the biochemical characterization of another fibrinolytic protease from *C. militaris* [10]. Hence, *C. militaris* can be suggested as good source of novel thrombolytic agents, although work related to the anticoagulant activities of crude extracts is scarce.

4. Anticancer Activity

C. militaris is one of the important medicinal species and well documented for its anticancer effects. *C. militaris* ethanolic extract was orally administered to a xenograft in mice bearing murine T cell lymphoma (RMA) cell-derived cancers, which resulted in significant anticancer activity by the suppressing the size and mass of cancer. Furthermore, reduced proliferation of RMA cells and C6 glioma cells, downregulation of phosphorylation of AKT, p85 and augmented cleaved caspase-3, phosphoglycogen synthase kinase 3β (p-GSK3β) were reported. The extract significantly increased the proapoptotic cell population and reduced viability compared to control cells. The finding indicates the anticancerous activity of *C. militaris* occurred by regulating of p85/AKT- or GSK3β-related caspase 3-dependent apoptosis [11]. Similarly, methanolic extracts showed good cytotoxic activity via the MTT assay against Hep-2 cancer cell lines with an IC_{50} value of 20 µg/mL [12]. In another study, the effect of fluoride was monitored in the culture medium of *C. militaris*, and positive effects were observed on the synthesis of secondary bioactive metabolites and growth of fruiting bodies, which eventually caused reduced proliferation and apoptosis in a human osteosarcoma (U2OS) cell line [13]. Another study discussed the decreased apoptotic activity of aqueous extract of *C. militaris* (AECM) on MDA-MB-231 cells. It showed significant induction of mitochondrial dysfunction and loss of mitochondrial membrane permeability by modulating Bcl2/Bax proteins, and also caspase activation [14]. Another report showed the tumor inhibitory effects of an ethanolic extract of *C. militaris* in xenograft Balb/c nude mice transfected with human colorectal carcinoma RKO cells. The oral administration of test extracts led to delayed growth of RKO cell-derived tumors. It also stimulated cell cycle arrest in G2/M phase (66.33% at 300 µg/mL) and enhanced early apoptosis (18.07% at 300 µg/mL). Western blot analysis indicated an increase in the expression levels of p53, cleaved caspase 9, cleaved caspase-3, cleaved PARP, and Bim, Bak, and Bad proteins [15]. A mechanistic based study conducted by Chou et al., revealed that anticancerous effects of *C. militaris* on leukemia cell lines might be attributed to activation of AKT and p38 mitogen activated protein kinase (MAPK), during the course of apoptosis induction, suggesting the possible use of its extracts against leukemia by activating the p38 MAPK pathway [16]. Another mechanism of the apoptosis of lung carcinoma by *C. militaris* extracts is related to downregulation of TCTN3 expression, which affected the hedgehog signaling cascade and contributed to the serial activation of caspases. Additionally, the extract negatively modulated GLI1 transcriptional activity by inhibiting SMO/PTCH1 molecules, subsequently regulating the intrinsic apoptotic signaling cascade [17]. All these findings support the possible use of *C. militaris* extracts as anticancer agents in future studies.

5. Anti-Obesity Activity

C. militaris extracts possess lipid lowering activities. A novel extract of mulberry leaves fermented with *C. militaris* was exploited to detect its effect on lipid metabolism. Administration of an extract in a high fat diet fed to (HFD)-activated obese C57BL/6 mice for 12 weeks showed significantly decreased concentrations of triglyceride, glucose, total cholesterol and low-density lipoprotein, and induced production of levels of high-density lipoproteins were observed. The amount of abdominal fat and the size of adipocytes were reduced compared to control groups. Moreover, the sample reduced the Fas cell surface death receptor for lipogenesis and inhibited adipocyte protein 2 and peroxisome proliferator-activated receptor-γ mRNA expression [18]. Recently, strawberry extracts fermented with *C. militaris* showed enhanced levels of secondary metabolites as well as different extents of inhibition of adipogenesis in a 3T3-L1 cell line [19][20]. The extract also showed dose-dependent suppressed differentiation of 3T3-L1 preadipocytes into mature adipocytes and did not show any toxic effects on cells. An associated reduction in lipid accumulation, increased levels of adipocyte markers including peroxisome proliferator-activated receptor-γ, adiponectin, and CCAAT/enhancer binding protein-α, as well as continuous expression of monocyte chemoattractant protein (pre adipocytes marker) were observed [21].

6. Anti-Allergic Activity

Allergic responses are associated with disorders related to the immune system, where intense immune reactions occur in response to various triggers such as foods, chemicals, pollens and particulate matter[22]. Primarily, production of CD4+ specific allergen cells i.e., type 2 helper, Th2 are accompanied by the generation of interleukins (IL-4, IL-5, IL-9, and IL-13) by the effector Th2 cells, which subsequently results in the generation of IgE i.e., allergen related immunoglobins from B cells. IgE reactions with allergen cytokines generate the allergic reaction. Therefore, suppression of both allergen

cytokines and IgE are effects of therapeutic agents for allergies. Aqueous extracts of *C. militaris* have demonstrated asthma-preventing potential in ovalbumin (OVA)-activated experimental animals at a dose of 4 g/kg/day. Results revealed a decreased concentration of serum immunoglobulin E (IgE) as well as fewer infiltrating cells in the airways of mice treated with test extracts, although efficiency was less compared to montelukast and steroids, which are standard drugs for the treatment of asthma [23]. In another study, *C. militaris* (ethyl acetate extract) inhibited allergic reactions in a concentration-dependent manner in basophilic leukemia (RBL-2H3) cells. Extracts inhibited antigen-activated degranulation in RBL-2H3 cells with an IC_{50} value of 28.5 µg/mL. The extract prevented antigen-induced passive cutaneous anaphylaxis in experimental animals in a concentration-dependent manner [24]. In another study, an extract of *C. militaris* cultured on germinated soybean extract showed inhibitory potential in 2,4-dinitro-1-fluorobenzene (DNFB)-activated contact dermatitis mice models at a concentration of 300 mg/kg. It not only led to reduced ear swelling but also reduced infiltration of T, CD4 and CD8 cells in the ear tissues of the mice [25]. A study was performed to determine the molecular mechanisms of allergy prevention. An ethyl alcohol extract prepared from silkworm pupa-cultivated *C. militaris* fruiting bodies in immunogen triggered RBL-2H3 mast cells inhibited the release of β-hexosaminidase (a degranulation marker) and mRNA levels of TNF-α, as well as IL-4. Western blotting results revealed the inhibition of the Syk/phosphatidylinositol 3-kinases (PI3K)/MEKK4/JNK/c-Jun signaling cascade associated with the expression of various allergic cytokines in stimulated RBL-2H3 cells. Additionally, inhibited PLCγ evocation, and Erk activation were involved in stimulating the synthesis of lipid mediators and Ca^{2+} mobilization, which favor degranulation in activated RBL-2H3 cells [26].

7. Other

Several studies have documented the antihyperglycemic potential of *C. militaris*. Oral administration of aqueous and ethanolic extracts of *C. militaris* to diabetic Sprague-Dawley rats caused significant reduction in blood glucose levels. These results were due to increased glucose metabolism and suppression of total cholesterol and triglyceride concentrations [27]. The diabetic preventive potential of different fractions of *C. militaris* in streptozotocin-induced diabetic animals was determined in another study, resulting reduced blood glucose levels in which *C. militaris* extract acted as an insulin sensitizer (enhanced insulin secretion and insulin resistance in type II diabetic rats) [28]. This medicinal fungus has also proved its importance as a fertility enhancer, antimicrobial and antiaging species [29].

References

1. Abdul-Rehman Phull; Madiha Ahmed; Hye-Jin Park; *Cordyceps militaris* as a Bio Functional Food Source: Pharmacological Potential, Anti-Inflammatory Actions and Related Molecular Mechanisms. *Microorganisms* **2022**, *10*, 405, 10.3390/microorganisms10020405.
2. Kim, C.S.; Lee, S.Y.; Cho, S.H.; Ko, Y.M.; Kim, B.H.; Kim, H.J.; Park, J.C.; Kim, D.K.; Ahn, H.; Kim, B.O.; et al. Cordyceps militaris induces the IL-18 expression via its promoter activation for IFN-gamma production. J. Ethnopharmacol. 2008, 120, 366–371.
3. Zhu, S.J.; Pan, J.; Zhao, B.; Liang, J.; Ze-Yu, W.; Yang, J.J. Comparisons on enhancing the immunity of fresh and dry Cordyceps militaris in vivo and in vitro. J. Ethnopharmacol. 2013, 149, 713–719.
4. Kang, H.J.; Baik, H.W.; Kim, S.J.; Lee, S.G.; Ahn, H.Y.; Park, J.S.; Park, S.J.; Jang, E.J.; Park, S.W.; Choi, J.Y.; et al. Cordyceps militaris Enhances Cell-Mediated Immunity in Healthy Korean Men. J. Med. Food 2015, 18, 1164–1172.
5. Liu, Y.; E, Q.; Zuo, J.; Tao, Y.; Liu, W. Protective effect of Cordyceps polysaccharide on hydrogen peroxide-induced mitochondrial dysfunction in HL-7702 cells. Mol. Med. Rep. 2013, 7, 747–754.
6. Lee, H.H.; Park, H.; Sung, G.H.; Lee, K.; Lee, T.; Lee, I.; Park, M.S.; Jung, Y.W.; Shin, Y.S.; Kang, H.; et al. Anti-influenza effect of Cordyceps militaris through immunomodulation in a DBA/2 mouse model. J. Microbiol. 2014, 52, 696–701.
7. Ueda, Y.; Mori, K.; Satoh, S.; Dansako, H.; Ikeda, M.; Kato, N. Anti-HCV activity of the Chinese medicinal fungus Cordyceps militaris. Biochem. Biophys. Res. Commun. 2014, 447, 341–345.
8. Cui, L.; Dong, M.S.; Chen, X.H.; Jiang, M.; Lv, X.; Yan, G. A novel fibrinolytic enzyme from Cordyceps militaris, a Chinese traditional medicinal mushroom. World J. Microbiol. Biotechnol. 2008, 24, 483–489.
9. Liu, X.; Kopparapu, N.K.; Shi, X.; Deng, Y.; Zheng, X.; Wu, J. Purification and biochemical characterization of a novel fibrinolytic enzyme from culture supernatant of Cordyceps militaris. J. Agric. Food Chem. 2015, 63, 2215–2224.
10. Liu, X.; Kopparapu, N.K.; Li, Y.; Deng, Y.; Zheng, X. Biochemical characterization of a novel fibrinolytic enzyme from Cordyceps militaris. Int. J. Biol. Macromol. 2017, 94 Pt B, 793–801.

11. Park, J.G.; Son, Y.J.; Lee, T.H.; Baek, N.J.; Yoon, D.H.; Kim, T.W.; Aravinthan, A.; Hong, S.; Kim, J.H.; Sung, G.H.; et al. Anticancer Efficacy of Cordyceps militaris Ethanol Extract in a Xenografted Leukemia Model. Evid. Based Complement. Altern. Med. 2017, 2017, 8474703.
12. Joshi, M.; Sagar, A.; Kanwar, S.S.; Singh, S. Anticancer, antibacterial and antioxidant activities of Cordyceps militaris. Indian J. Exp. Biol. 2019, 57, 15–20.
13. Li, X.; Wang, J.; Zhang, H.; Xiao, L.; Lei, Z.; Kaul, S.C.; Wadhwa, R.; Zhang, Z. Low Dose of Fluoride in the Culture Medium of Cordyceps militaris Promotes Its Growth and Enhances Bioactives with Antioxidant and Anticancer Properties. J. Fungi 2021, 7, 342.
14. Jin, C.Y.; Kim, G.Y.; Choi, Y.H. Induction of apoptosis by aqueous extract of Cordyceps militaris through activation of caspases and inactivation of Akt in human breast cancer MDA-MB-231 Cells. J. Microbiol. Biotechnol. 2008, 18, 1997–2003.
15. Lee, H.H.; Lee, S.; Lee, K.; Shin, Y.S.; Kang, H.; Cho, H. Anti-cancer effect of Cordyceps militaris in human colorectal carcinoma RKO cells via cell cycle arrest and mitochondrial apoptosis. Daru 2015, 23, 35.
16. Chou, S.M.; Lai, W.J.; Hong, T.; Tsai, S.H.; Chen, Y.H.; Kao, C.H.; Chu, R.; Shen, T.L.; Li, T.K. Involvement of p38 MAPK in the Anticancer Activity of Cultivated Cordyceps militaris. Am. J. Chin. Med. 2015, 43, 1043–1057.
17. Jo, E.; Jang, H.J.; Shen, L.; Yang, K.E.; Jang, M.S.; Huh, Y.H.; Yoo, H.S.; Park, J.; Jang, I.S.; Park, S.J. Cordyceps militaris Exerts Anticancer Effect on Non-Small Cell Lung Cancer by Inhibiting Hedgehog Signaling via Suppression of TCTN3. Integr. Cancer 2020, 19, 1534735420923756.
18. Lee, M.R.; Kim, J.E.; Choi, J.Y.; Park, J.J.; Kim, H.R.; Song, B.R.; Choi, Y.W.; Kim, K.M.; Song, H.; Hwang, D.Y. Anti-obesity effect in high-fat-diet-induced obese C57BL/6 mice: Study of a novel extract from mulberry (Morus alba) leaves fermented with Cordyceps militaris. Exp. Ther. Med. 2019, 17, 2185–2193.
19. Liu, Q.; Hong, I.P.; Ahn, M.J.; Yoo, H.S.; Han, S.B.; Hwang, B.Y.; Lee, M.K. Anti-adipogenic activity of Cordyceps militaris in 3T3-L1 cells. Nat. Prod. Commun. 2011, 6, 1839–1841.
20. Guo, L.; Li, K.; Kang, J.S.; Kang, N.J.; Son, B.G.; Choi, Y.W. Strawberry fermentation with Cordyceps militaris has anti-adipogenesis activity. Food Biosci. 2020, 35, 100576.
21. Shimada, T.; Hiramatsu, N.; Kasai, A.; Mukai, M.; Okamura, M.; Yao, J.; Huang, T.; Tamai, M.; Takahashi, S.; Nakamura, T.; et al. Suppression of adipocyte differentiation by Cordyceps militaris through activation of the aryl hydrocarbon receptor. Am. J. Physiol. Endocrinol. Metab. 2008, 295, E859–E867.
22. Abdul-Rehman Phull; Kyu-Ree Dhong; Hye-Jin Park; Lactic Acid Bacteria Fermented *Cordyceps militaris* (GRC-SC11) Suppresses IgE Mediated Mast Cell Activation and Type I Hypersensitive Allergic Murine Model. *Nutrients* **2021**, *13*, 3849, 10.3390/nu13113849.
23. Hsu, C.H.; Sun, H.L.; Sheu, J.N.; Ku, M.S.; Hu, C.M.; Chan, Y.; Lue, K.H. Effects of the immunomodulatory agent Cordyceps militaris on airway inflammation in a mouse asthma model. Pediatr. Neonatol. 2008, 49, 171–178.
24. Oh, J.Y.; Choi, W.S.; Lee, C.H.; Park, H.J. The ethyl acetate extract of Cordyceps militaris inhibits IgE-mediated allergic responses in mast cells and passive cutaneous anaphylaxis reaction in mice. J. Ethnopharmacol. 2011, 135, 422–429.
25. Park, D.K.; Choi, W.S.; Park, H.J. Antiallergic activity of novel isoflavone methyl-glycosides from Cordyceps militaris grown on germinated soybeans in antigen-stimulated mast cells. J. Agric. Food Chem. 2012, 60, 2309–2315.
26. Wu, T.F.; Chan, Y.Y.; Shi, W.Y.; Jhong, M.T. Uncovering the Molecular Mechanism of Anti-Allergic Activity of Silkworm Pupa-Grown Cordyceps militaris Fruit Body. Am. J. Chin. Med. 2017, 45, 497–513.
27. Dong, Y.; Jing, T.; Meng, Q.; Liu, C.; Hu, S.; Ma, Y.; Liu, Y.; Lu, J.; Cheng, Y.; Wang, D.; et al. Studies on the antidiabetic activities of Cordyceps militaris extract in diet-streptozotocin-induced diabetic Sprague-Dawley rats. BioMed Res. Int. 2014, 2014, 160980.
28. De Silva, D.D.; Rapior, S.; Hyde, K.D.; Bahkali, A.H. Medicinal mushrooms in prevention and control of diabetes mellitus. Fungal Divers. 2012, 56, 1–29.
29. Mehra, A.; Zaidi, K.U.; Mani, A.; Thawani, V.J.K. The health benefits of Cordyceps militaris—A review. Kavaka 2017, 48, 27–32.