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Cordyceps Mushroom: A Potent Anticancer Nutraceutical

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Abstract: The *Cordyceps* mushrooms have a long history as medicinal fungi. In Traditional Chinese Medicine, *Cordyceps* have been used to treat several conditions including cancers for thousand of years. Extracts from both mycelium and fruiting bodies of *C. sinensis, C. militaris* and other *Cordyceps* species showed significant anticancer activities by various mechanisms such as, modulating immune system and inducing cell apoptosis. Some polysaccharide components and cordycepin (3'-deoxyadenosine) have been isolated from *C. sinensis* and *C. militaris,* which acted as potent anticancer components. This review article aims to further elucidate the importance of *Cordyceps* mushrooms by summarizing the findings of some of the important research works concerning possible mechanism of anticancer activity of this mushroom.

Keywords: *C. sinensis*, *C. militaris*, Cordycepin, Apoptosis, Immunomodulating, Anticancer activity.

INTRODUCTION

There is a common saying, ‘Medicines and foods have a common origin’. Mushrooms are a manifestation of this idea in constituting both a nutritionally functional food and a source of physiologically beneficial medicine. Many centuries ago, medicinal properties of mushrooms have been recognized in China, Korea and Japan. Although from ancient times, mushrooms have been treated as a special kind of nutraceutical, they have received a remarkable interest in recent decades. Major medicinal properties attributed to mushrooms include anticancer activity, antibiotic activity (directed against bacteria, fungi and protozoa), antiviral activity, immune response-stimulating effects, anti-hypertensive and blood lipid lowering effects [1, 2]. Some mushrooms have gained special consideration due to their various medicinal values in addition to nutritional importance. For example, *Lentinus edodes* were reported to possess anti-tumor, antihypertensive, hypocholesterolemic and antibacterial activities [3-6]. *Ganoderma lucidum* has been proved to have anti microbial and anti-HIV effects [7, 8]. The hepatic and renal protective effects of *G. lucidum* in mice were also evaluated [9]. The beta-glucan polysaccharide of this mushroom has potential application in immune surveillance and chemoprevention of cancer [2]. Mushrooms of *Pleurotus* species (*P. ostreatus*, *P. sajor-caju*, *P. florida*) were reported to have hypocholesterolemic activity in experimental rats [10, 11].

Cordyceps mushroom is a genus of ascomycete fungi that includes about 400 described species. All *Cordyceps* species are endoparasitoids, mainly on insects and other arthropods [12] These mushrooms have a long history as rare and exotic medicinal fungi. They have been a highly regarded cornerstone of traditional Chinese medicine for centuries; that apparently have a number of far reaching medicinal effects [13, 14]. *Cordyceps* mushrooms have been used to treat conditions including respiration and pulmonary diseases; renal, liver, and cardiovascular diseases; hyposexuality and hyperlipidemia. It is also used in the treatment of immune disorders and as an adjunct to modern cancer therapies (chemotherapy, radiation treatment and surgery) [12]. *C. sinensis* and *C. militaris* are the most common in use among the *Cordyceps* genus.

ANTICANCER ACTIVITY OF *CORDYCEPS SINENSIS*

C. sinensis is a fungus that has been used for over 2000 years in China as a treatment for a variety of conditions including cancer. Many available evidences suggest that the efficacy of *C. sinensis* as an anti-neoplastic therapeutic agent is due to its role as an activator of immune responses. Extracts from both mycelium and fruiting bodies from *C. sinensis* influence the immune system in various ways. In a study, water-extract from dried *C. sinensis* increased the median survival time of the allogeneic mice inoculated with Ehrlich ascites carcinoma cells (EAC) to 316% and syngeneic mice inoculated with Meth A fibrosarcoma (Meth A) to 312% of the control with no cytotoxic activity on either EAC or Meth A *in vitro* [15]. The water extract of this mushroom has been also proved beneficial in the prevention of tumor metastasis in mice as an adjuvant agent in cancer chemotherapy [16]. The natural killer cells (NK) activities of mouse were both *in vivo* and *in vitro* significantly augmented by intraperitoneal injection of the ethanol extract of *C. sinensis* [17]. The ethanol-extract also significantly decreased tumor weights and volumes in mice inoculated with Sarcoma-180 tumor cells. The extracts were demonstrated to exhibit phagocytosis enhancing activity as measured by carbon clearance in mice and caused a significant increase in an acid phosphatase

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activity, representing lysosomal enzymes, suggesting that the anti-tumor activity of these fungi might be related to an immuno-stimulating function [18]. The ethyl acetate extract of *C. sinensis* mycelium was found to have strong anti-tumor activity on four cancer cell lines, MCF-7 breast cancer, B16 mouse melanoma, HL-60 human premyelocytic leukemia and HepG2 human hepatocellular carcinoma [19]. Some specific fractions of extracts of *C. sinensis*, especially polysaccharides have been found to modify immune response more precisely. The conditioned medium from the polysaccharide fraction of *C. sinensis* (PSCS)-stimulated blood mononuclear cells [PSCS-MNC-CM] had an activity that could significantly inhibit the proliferation of human leukemic U937 cells resulting in a growth inhibition rate of 78-83%. Furthermore, PSCS-MNC-CM treatment induced about 50% of the cells differentiating into mature monocytes/macrophages expressing nonspecific esterase (NSE) and the surface antigens of CD11b, CD14 and CD 68. The levels of interferon (IFN)-gamma, tumor necrosis factor (TNF)-alpha and interleukin (IL)-1 were very low in normal MNC-CM but they were greatly increased in MNC-CM prepared with PSCS stimulation. Antibody neutralization studies further revealed that the tumoricidal and differentiating effects of PSCS-MNC-CM were mainly derived from the elevated cytokines, especially IFN-gamma and TNF-alpha [20]. An exopolysaccharide fraction (EPSF), prepared from *C. sinensis* significantly enhanced the phagocytosis capacity of peritoneal macrophages and proliferation ability of spleen lymphocytes of H22 tumor (histocompatibility 22) and B16 melanoma bearing mice, as well as inhibited the tumor growth in separate study. EPSF significantly promoted macrophages' TNF-alpha expression and spleen lymphocytes' cytotoxicity. EPSF also significantly elevated TNF-alpha and IFN-gamma mRNA expression of splenic lymphocytes and thus elevated immunocytes' activity in H22 tumor bearing mice [21, 22]. The EPSF from *C. sinensis* also has inhibitory effect on oncogene expression. The c-Myc, c-Fos, and vascular endothelial growth factor (VEGF) levels in the lungs and livers of EPSF-treated mice were found to be significantly lower than those of untreated mice [23]. When lipopolysaccharide (LPS)-activated murine macrophage cell line R309 was exposed to the extracts *C. sinensis*, R309 induced significant levels of IL-1. IL-2 induction was recognized in T cell line LBRM-33 1A5 (1A5) cultures in the presence of IL-1 and phytohemagglutinin (PHA). However, no enhancement of IL-2 production by *C. sinensis* was discerned in 1A5 cultures with IL-1 and PHA, i.e., direct action of *C. sinensis* was not found on IL-2 production of 1A5. PHA-stimulated 1A5 exposed to *C. sinensis* induced IL-2 without IL-1 when co-cultured with LPS-activated R309 as a source of IL-1 [24]. *C. sinensis*, either alone or with IFN-gamma induction, increased the MHC class II antigen expression on hepatoma cell line HA22T/VGH, which makes the host immune surveillance more effective against tumor cells with down-regulated MHC class II antigen expression [25]. In leukemia patients, *C. sinensis* augmented the NK cell activity and also improved the CD16 marker expression on lymphocytes and the binding capacity to K562 cells [26]. In breast cancer, the oral *C. sinensis* did not reduce primary tumor growth but reduced lung metastasis occurrence in a surgical excision model of metastatic mammary carcinoma. The reduction in metastases growth is supposed to due to the effects of macrophage-derived factors on tumor cell cycle, NK cell activation and other immunostimulating activities [27, 28].

Although the immunomodulating activity of *C. sinensis* is mainly responsible for its anticancer activity, it is not so simply described and only exact mechanism. Directly or indirectly many other metabolic and genetic pathways are responsible. Extensive research works have been done to evaluate the mechanism of anticancer activity of *C. sinensis* and the most significant mechanism has been found to influence apoptosis. In a study, the ethyl acetate extract of mycelium of *C. sinensis* induced the characteristic apoptotic symptoms in human premyelocytic leukemia cells (HL-60), DNA fragmentation and chromatin condensation. The activation of caspase-3 and the specific proteolytic cleavage of poly ADP-ribose polymerase were detected during the course of apoptosis induction. These results suggest that this extract inhibited cancer cell proliferation by inducing cell apoptosis [29]. The involvement of caspase-8 with caspase-3 was also found in *C. sinensis* induced apoptosis in MA-10 mouse Leydig tumor cells [30]. Components from *Cordyceps* induce tumor cell apoptosis through both extrinsic and intrinsic pathways. Two new epipolythiodioxopiperazines, named gliocladicillins A and B, from *Cordyceps*-colonizing fungi inhibited growth of HeLa, HepG2 and MCF-7 tumor cells by arresting the cell cycle at G(2)/M phase and induced apoptosis through up-regulation of expression of p53, p21, and cyclin B and activation of caspases-8, -9 and -3 [31]. Furthermore, EPSF of *C. sinensis* decreased the levels of Bcl-2 in the lungs and livers [22]. The antitumor activity of *C. sinensis* by inducing apoptosis was also found in human colorectal (HT-29 and HCT 116), hepatocellular (Hep 3B and Hep G2) carcinoma cells [32] and human oral squamous cancer cell line (OEC-M1) [33]. Interestingly, Tang *et al.* [34] reported that *C. sinensis* reduced Angiotensin II induced NRK-52E cell apoptosis, which may be part of its mechanism of the protective effects on hypertensive renal damage.

The antioxidant activity of *C. sinensis* components is also responsible for its anticancer property. The ethanolic extract of *C. sinensis* was found to have inhibitory effect on lipid peroxidation and protective effect on 4-nitroquinoline oxide-triggered DNA lesion in V79 hamster cells [35]. The polysaccharide component of *C. sinensis* has been found to inhibit the tumor growth of H22-bearing mice by modulating the antioxidant enzymes activity such as enhancing superoxide dismutase (SOD) activity of liver, brain and serum as well as glutathione peroxidase (GPx) activity of liver and brain in tumor-bearing mice [36]. Wang *et al.* [32] reported the free radical scavenging activity of *C. sinensis*. As free radicals are responsible for oxidative damage of apoptotic genes, by scavenging free radicals, *C. sinensis* protects apoptotic genes and induces the apoptosis of cancer cells.

ANTICANCER ACTIVITY OF *CORDYCEPS MILITARIS*

The mushroom *C. militaris* has been used for a long time in eastern Asia as a nutraceutical and in traditional Chinese medicine as a therapeutic agent for cancer patients. *C. militaris* has been found good for inhibiting the growth of tumor, prolonging the survival period of mice implanted with S180 sarcoma cells and inhibiting the growth and metastasis of Lewis pneumonic cancer in the implanted mice [37]. Water extract of *C. militaris* inhibited growth of human umbilical vein endothelial cells (HUVEC) and human sarcoma cell line HT1080. This mushroom extract also reduced metalloproteinase 2 (mmp2) gene expression in HT1080 and basic fibroblast growth factor (bFGF) gene expression in HUVECs [38]. Recently, a cytotoxic protease was purified from the dried fruiting bodies of *C. militaris*, which exhibited cytotoxicity against human breast and bladder cancer cells [39].

Like *C. sinensis*, the most significant anticancer mechanism of *C. militaris* was found by inducing cell apoptosis. In a study, Park *et al.* [40] observed that the aqueous extract of *C. militaris* (AECM) inhibited cell growth of human leukemia U937 cells by morphological change and apoptotic cell death such as formation of apoptotic bodies and DNA fragmentation. They also observed the down-regulation of anti-apoptotic gene bcl-2 expression and proteolytic activation of caspase-3 in AECM-treated U937 cells. But AECM did not affect the pro-apoptotic gene bax expression and activity of caspase-9. The hot water extract of *C. militaris* was also found to induce apoptosis in the human premyelocytic leukemia HL-60 cells and the activation of caspase-3 and the specific proteolytic cleavage of Poly ADP-ribose polymerase *(*PARP*)* were detected during the course of apoptosis [41]. In addition to the activation of caspase-3, the AECM-induced apoptosis may relate to the inactivation of Akt (an oncogene) in human breast cancer MDA-MB-231 cells [42]. In another study, the growth inhibition and apoptosis induction by the water extract of *C. militaris* (WECM) treatment in human lung carcinoma A549 cells were found associated with the induction of Fas expression, catalytic activation of caspase-8, and Bid cleavage. Activation of caspases, downregulation of anti-apoptotic gene bcl-2 expression, and upregulation of pro-apoptotic Bax protein were also observed in WECM-treated cancer cells. In addition, WECM exerted a dose-dependent inhibition of telomerase activity via down-regulation of human telomerase reverse transcriptase (hTERT), c-Myc and Sp1 expression. The data indicated that WECM induced the apoptosis of A549 cells through a signaling cascade of death receptor-mediated extrinsic and mitochondria-mediated intrinsic caspase pathways and diminishing the telomerase activity through the inhibition of hTERT transcriptional activity [43].

ANTICANCER ACTIVITY OF OTHER *CORDYCEPS* SPECIES

Three different polysaccharide-peptide complexes (PPCs) were produced by submerged mycelial culture of a rare entomopathogenic fungus *Cordyceps sphecocephala* and their anticancer activities were investigated in human hepatocarcinoma (HepG2) and neuroblastoma (SK-N-SH) cells. In this study, PPC-induced apoptosis of both cancer cells was found which was associated with intracellular events including DNA fragmentation, activation of caspase-3, and modulation of Bcl-2 and Bax and no cytotoxicity against normal cells was reported [44].

A water-insoluble extracellular glucan (CO-1) was isolated from the precipitate formed on incubation of the culture filtrate of *Cordyceps ophioglossoides* and this CO-1 strongly inhibited the growth of Sarcoma 180 solid-type tumor [45]. The effects of protein-bound polysaccharide (SN-C) extracted from *C. ophioglossoides* on the growth of transplanted allogeneic and syngeneic murine tumors were studied and it was found that SN-C given by intraperitoneal administration suppressed the growth of sarcoma-180 transplanted subcutaneously in mice. SN-C also showed a significant cytocidal effect on cultured tumor cells but did not affect delayed-type hypersensitivity (DTH) in normal mice [46]. A protein-bound galactosaminoglycan (CO-N) was isolated from SN-C of *C. ophioglossoides.* When given intraperitoneally to mice, CO-N inhibited the proliferation of sarcoma 180 cells inoculated into the peritoneal cavity and exhibited a marked life-prolonging effect against ascitic tumors such as Ehrlich carcinoma. CO-N also showed an inhibitory effect against solid Ehrlich carcinoma when given intratumorally and significantly inhibited the growth of a syngeneic solid tumor (MM46 mammary carcinoma) upon intravenous administration at a low dose [47].

CORDYCEPIN: AN ANTICANCER AGENT FROM *CORDYCEPS* SPECIES

Cordycepin, or 3'-deoxyadenosine, is a derivative of the nucleoside adenosine, differing from adenosine by the absence of oxygen in the 3' position of its ribose part. Cordycepin was isolated from the water extract of *C. sinensis* [33, 48]. Later the major component of the butanol fraction of *C. militaris* was also identified as cordycepin by high performance liquid chromatography [49]. Because cordycepin is similar to adenosine, RNA polymerase cannot discriminate between the two and when incorporated into a growing RNA molecule, cordycepin prevents further elongation, thus producing a prematurely terminated RNA molecule [50].

Orally administered cordycepin inhibited B16-BL6 melanoma cell growth in mice with no adverse effects [48]. Further study demonstrated that cordycepin inhibited the proliferation of B16-BL6 cells by stimulating adenosine A3 receptors followed by the Wnt signaling pathway, including GSK-3beta activation and cyclin D1 inhibition [51]. Cordycepin markedly inhibited the phosphorylation of Akt and p38 in dose-dependent manners in LPS-activated macrophage. Moreover, cordycepin suppressed TNF-alpha expression, Ikappa B alpha phosphorylation and translocation of nuclear factor-kappa B (NF-kappa B) [49]. Cordycepin-induced apoptosis is also reported. In MA-10 cells (a mouse Leydig tumor cell line), cordycepin induced DNA fragmentation, declined the percentage of G1 and G2/M phase cells, increased the percentages of subG1 phase cells suggesting cordycepin induced MA-10 cell apoptosis. Moreover, western blotting analysis showed that cordycepin induced caspase-9, caspase--3 and caspase--7 protein expressions, but not caspase-8 [52]. In another study, cordycepin significantly induced cell apoptosis in OEC-M1 human oral squamous cancer cells [23]. It was also suggested that the effect of cordycepin on the growth of tumor cells was significantly related to the metabolism-associated protein expression induced by cordycepin [53]. Platelet aggregation induced by cancer cells is an indispensable step for hematogenic metastasis and it was showed that cordycepin had an inhibitory effect on hematogenic metastasis of B16-F1 melanoma cells via blocking of ADP-induced platelet aggregation *in vivo* [54]. A novel molecular mechanism for the anti-tumor effects of cordycepin in two different bladder cancer cell lines, 5637 and T-24 cells has been revealed by Lee *et al.* [55]. They reported that cordycepin treatment during cell-cycle progression resulted in significant growth inhibition, which was largely due to G2/M-phase arrest and resulted in an up-regulation of p21WAF1 expression, independent of the p53 signaling pathway. Moreover, treatment with cordycepin induced phosphorylation of JNK (c-Jun N-terminal kinases). Blockade of JNK function using SP6001259 (JNK-specific inhibitor) and small interfering RNA (si-JNK1) rescued cordycepin-dependent p21WAF1 expression, inhibited cell growth and decreased cell cycling proteins.

Derivatives of cordycepin have also showed anticancer activity. In an experiment, 1-O-Acetyl-2,5-di-O-p-chlorobenzoyl-3-deoxy-D-ribofuranose, derived from cordycepin was coupled with trimethylsilylated derivatives of N4-propionylcytosine, N4-p-toluoyl-5-fluorocytosine and 5-fluorouracil in the presence of trimethylsilyl trifluoromethanesulfonate (TMS-triflate) to give fully acylated nucleosides. Selective removal of the N4-propionyl group of these nucleosides by treatment with hydrazine hydrate gave 2',5'-di-O-p-chlorobenzoyl-3'-deoxycytidine, deamination of which with sodium nitrite in trifluoroacetic acid afforded 2',5'-di-O-p-chlorobenzoyluridine in good yield. The acylated nucleosides and 2',5'-di-O-p-chlorobenzoyluridine were saponified to give free 3'-deoxycytidine, 5-fluoro-3'-deoxycytidine, 3'-deoxyuridine, and 5-fluoro-3'-deoxyuridine, respectively. These 3'-deoxyribonucleosides were then examined for growth-inhibitory effects on mouse leukemic L5178Y cells, and their IC50 (inhibitory concentration 50) values were found 1.8, 33, 6.5, and 18 (microgram/ml) respectively [56]. Other nucleotide derivatives also exist which showed anticancer activities, such as, forodesine (also known as Immucillin H; IUPAC name: 7-[(2*S*,3*S*,4*R*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)-2-pyrrolidinyl]-1,5-dihydropyrrolo[2,3-*e*]pyrimidin-4-one) is a inhibitor of purine nucleoside phosphorylase, which is recognized as a potential target for the treatment of patients with T-cell malignancies and also B-cell chronic lymphocytic leukemia [57, 58].

CONCLUSIONS

Currently, in some parts of the world, there is a renaissance of interest in traditional remedies. Most of the investigators believe that traditional medicine is a promising source of new therapeutics. Mushrooms have a notable place in folklore or traditional medicine throughout the world. Medicinal mushrooms are now the subject of study for many ethnobotanists and medical researchers. The ability of some mushrooms, especially *Cordyceps* to inhibit tumor growth and enhance aspects of the immune system has been a subject of research for approximately 50 years. As cancer is one of the most complicated diseases and its pathogenic mechanism is diverse, therapeutic attempts are being taken against cancer from different angles. The cancer protecting activities of *Cordyceps* are widely reported and established and now it is the time to look for the cancer preventing activity of this mushroom more precisely. Extensive research with *Cordyceps* mushrooms may contribute to the discovery of new anticancer strategies.

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