Chapter 16
Cordycepin: A Biotherapeutic Molecule from Medicinal Mushroom

Mohammad Soltani, Roslinda Abd Malek, Nagib A. Elmarzugi, Mohamad Fawzi Mahomoodally, Davin Uy, Ong Mei Leng, and Hesham A. El-Enshasy

16.1 Introduction

For many years, mushrooms have been considered as important food based on its rich nutritional value. In addition, different mushrooms showed high potential application in medical applications. (El Enshasy et al. 2013) and pharmacological (Wasser 2002; Elenshasy 2010; El Enshasy et al. 2013). The "Chinese caterpillar

M. Soltani · R. A. Malek
Institute of Bioproduct Development (IBD), Universiti Teknologi Malaysia (UTM), Skudai, Malaysia

N. A. Elmarzugi
Department of Industrial Pharmacy, Faculty of Pharmacy & Biotechnology Research Center, Tripoli, Libya

M. F. Mahomoodally
Department of Health Sciences, Faculty of Science, University of Mauritius, Réduit, Mauritius

D. Uy
Research and Innovation Center, Tumnuptek, Chamkarmorn, Phnom Penh, Cambodia

O. M. Leng
Harita Go Green Sdn Bhd, Johor Bahru, Johor, Malaysia

H. A. El-Enshasy (✉)
Institute of Bioproduct Development, Universiti Teknologi Malaysia, Johor Bahru, Johor, Malaysia

School of Chemical Engineering, Faculty of Engineering, Universiti Teknologi Malaysia, Johor Bahru, Johor, Malaysia

City of Scientific Research and Technology Application, New Burg Al Arab, Alexandria, Egypt
e-mail: henshasy@ibd.utm.my

B. P. Singh et al. (eds.), *Biology of Macrofungi*, Fungal Biology,
https://doi.org/10.1007/978-3-030-02622-6_16

fungus", is one of the conventional medicinal mushrooms. This fungus attacks and expands inside a particular insect and kills the host in the later stage. During the time of winter passing inside the insect, the fruituning body grows on the surface of the corpse. However, there are numerous known types of Chinese caterpillar growths, which fit in with numerous distinctive such as *Cordyceps*, *Torrubiella* and *Paecilomyces*. One of the well-knownCordyceps species which is the, *Cordyceps sinensis*, become a parasite on the larvae of *Hepialus armoricanus*, been utilized to initiate the longevity, soothe fatigue and kill various ailments as Chinese traditional medicine (Pegler et al. 1994; Zhu et al. 1998; Buenz et al. 2005). Later contemplates showed that different species showed numerous pharmacological activities and showed potential application as **anticancer** (Leung et al. 2006; Wu et al. 2007; Lin and Chiang 2008; Lee et al. 2009; Lee et al. 2010; Choi et al. 2011; Jeong et al. 2011) **antioxidan**t (Wang et al. 2005; Ramesh et al. 2012; Ren et al. 2012;), **anti-diabetic** (hypoglycemic) (Yun et al. 2003; Ma et al. 2015), **anti-inflammatory** (Mizuno 1999; Won and Park 2005; Jeong et al. 2010; Choi et al. 2014), and **immunomodulator** (Ng and Wang 2005; El Enshasy & Hatti-Kaul, 2013; Jeong et al. 2013; Yao et al. 2014; Yang et al. 2015; Lee et al. 2015; Peng et al. 2015; Zhang et al. 2015;). Further investigations for isolation of bioactive molecules from this type of fungus showed that that low molecular weight compound, cordycepin is one of the potent active molecules with many **biotherapeutic** activities (Cunningham 1951; Suhadolnik and Cory 1964; Fujita et al. 1994; Kiho and Ukai 1995; Bok et al. 1999; Li et al. 2004; Yu et al. 2004a; Yu et al. 2004b; Yalin et al. 2006).

Cordycepin (3′-deoxyadenosine) well known as nucleoside analogue that have different type of bioactivities. The cordycepin will be converted into 5′-mono, di and triphosphates and thus hinder the movement of ribose-phosphate pyrophospho kinase and 5-phosphoribosyl-1-pyrophosphate amidotransferase in the de novo purines biosynthesis and/or the nucleic acids synthesis causing the antimetastatic, antitumor and antimicrobial results (Overgaard-Hansen 1964a; Rottman and Guarino 1964; Cory et al. 1965; Rich et al. 1965; Ahn et al. 2006; Yoshikawa et al. 2004; Nakamura et al. 2005). In addition, cordycepin with its antileukemic ability normally join with adenosine deaminase inhibitor and this will cause the inhibitoryn effect to take place which help to analogues of 2′, 5′- oligoadenylate towards the human immunodeficiency virus infection (Muller et al. 1991; Kodama et al. 2000;). Large scale culturing of mycelial through synthetic can be used a s a new source of cordycepin due to its limited amount in natural source. Tow stage control of dissolved oxygen or addition of NH4+ to the **submerged medium** can help to improve the production of cordycepin (Mao and Zhong 2004, 2006). In addition to production using fermentation technology, cordycepin could be also produced chemically. However, chemical synthesis has some limitation such as complication of the process and the utilization of large volume of organic solvents which decrease the attractiveness of this process (Hansske and Robins 1985; Aman et al. 2000).

The purpose of chapter is to give focus on the recent research for the production and therapeutic applications of cordycepin. However, weak and strong points of previous studies can be recognized through this review. Thus, it will be useful for further assessments to improve the production process and future medical applications of cordycepin in the treatment of different diseases.

16.2 Cordycepin Specifications

16.2.1 Chemical Structure, Molecular and Physical Properties

Cordycepin, or 3′-deoxyadenosine, is a subsidiary of the nucleoside adenosine, contrasting from the recent by the nonattendance of oxygen in the 3′ position of its ribose part. It was at first concentrated from fungi, family Cordyceps, yet is presently processed chemically. In light of the fact, as cordycepin is like adenosine, a few steps cannot separate between the two. Along these lines, it can take part in certain biochemical reactions (for instance, be joined into a RNA molecule, therefore initiating the untimely end of its amalgamation). Despite of other unknown bioactive ingredients (e.g. polysaccharides) extracted from various species of cordyceps with different molecular weights and biotherapeutic activities (Soltani et al. 2013). The molecular structure of adenosine, its analogues deoxyadenosine and cordycepin are presented in Fig. 16.1. Moreover, scientific data of cordycepin is indicated in Table 16.1.

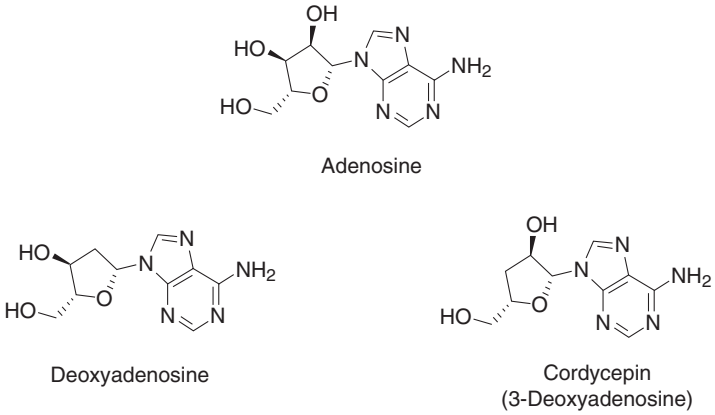

Fig. 16.1 Molecular structure of adenosine, deoxyadenosine and cordycepin (3′-deoxyadenosine)

Table 16.1 Cordycepin molecular information

Name	IUPAC name	9-(3-Deoxy-β-D-ribofuranosyl)adenine
	Other names	Cordycepine, 3′-Deoxyadenosine
Identifiers	CAS number	73–03-3
	PubChem	6303
	ChemSpider	6064
	ChEMBL	CHEMBL305686
	InChlKey	OFEZSBMBBKLLBJ-
Properties	Molecular formula	$C_{10}H_{13}N_5O_3$
	Molar mass	251.24 g mol^{-1}
	Melting point	225.5 °C, 499 K, 438 °F

Data are given for materials in their standard state (at 25 °C, 100 kPa)

16.2.2 Codycepin Biosynthesis

A **biosynthesis pathway** is frequently starts with a promptly accessible precursor particle that is like the product. The cell then joins together this antecedent with other small molecules, artificially adjusting the product along the way. At every step, the substrate will dynamically look like the final product. A multi-step biosynthesis pathway can have many steps along the way, experiencing consistent change by enzymes until the last compound is shaped. Concentrating on biosynthesis can yield numerous viable experiences into cures for human ailments. Comprehension the science of the human body plainly helps when a sickness results because of failing biosynthesis. Sometimes, on the other hand, concentrating on the biosynthetic pathways of different living beings can also turn up important hints for improving new drugs.

By picking up a comprehension of the biosynthesis pathway of a cordycepin, we can find that how it is synthesized and possibly mimic the synthesis in the laboratory. At last, it will be worthwhile to clone these genes to generate transgenic living organism, which might be designed to prepare natural product at more terrific concentration and purity at a division of the cost. Thus, in order to understand the synthesis of cordycepin deeply, the biosynthesis pathway of cordycepin from mushroom *Cordyceps militaris* is presented in detail on Fig. 16.2 (Zheng et al. 2012).

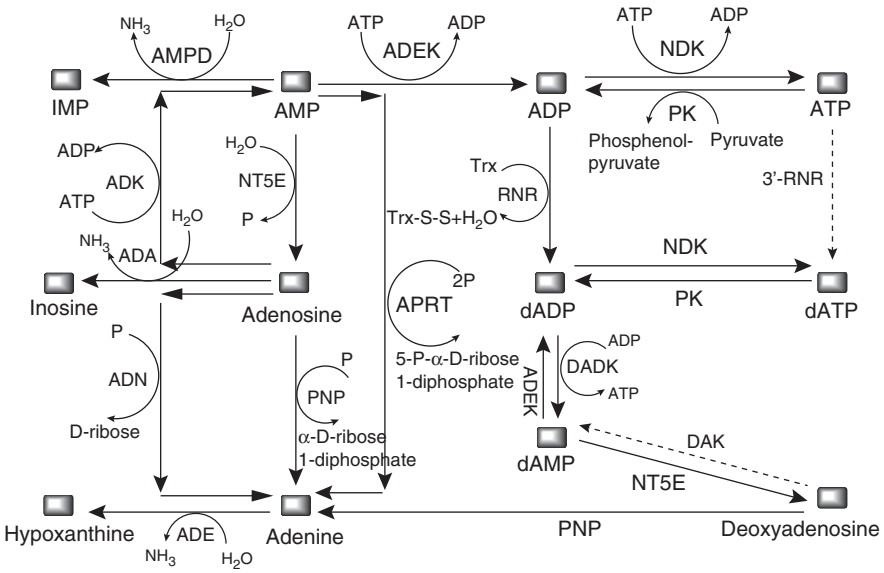

Fig. 16.2 *Cordyceps militaris* adenine metabolic pathway. Abbreviations for different enzymes: *ADA* adenosine deaminase, *ADE* adenine deaminase, *ADEK* adenylate kinase, *ADK* adenosine kinase, *ADN* adenosine nucleosidase, *AMPD*, *AMP* deaminase, *APRT* adenine phosphoribosyltransferase, *DADK* deoxyadenylate kinase, *DAK* deoxyadenosine kinase, *NDK* nucleoside-diphosphate kinase, *NT5E*, 5′-nucleotidase, *PK* pyruvate kinase, *PNP* purine nucleoside phosphorylase, 3'-RNR, ribonucleotide triphosphate reductase. The dashed lines exhibit metabolic pathways present in other organisms but absent in *Cordyceps militaris* (Zheng et al. 2012)

It can be clearly seen that several enzymes as significant agents are present during the metabolic pathway of cordycepin incorporating process until the final product is achieved.

16.3 Coducepin: Mechanism of Action

The structure of cordycepin is truly comparable with cellular nucleoside, adenosine (Fig. 16.1) and demonstrates like a nucleoside analogue.

16.3.1 Hinderance of Purine Biosynthesis Pathway

Inside the cells, Cordycepin get changed over into 5' mono-, di- and tri-phosphate that can decrease the catalyst action like ribose-phosphate pyrophosphokinase and 5-phosphoribosyl-1-pyrophosphate amidotransferase that being utilized within **de novo biosynthesis of purines** (Fig. 16.3) (Klenow 1963; Overgaard-Hansen 1964b; Rottman and Guarino 1964).

16.3.2 Cordycepin Incites RNA Chain End

Cordycepin fails to offer 3′-hydroxyl group in its molecular form (Fig. 16.1), which is the main distinction from adenosine. Adenosine is a nitrogenous base and function as cell nucleoside, which is needed for the different molecular procedures in cells such as synthesis of RNA or DNA. Throughout the procedure of transcription (RNA combination), a few enzymes are not being recognize the adenosine and cordycepin, that prompts joining of 3′-deoxyadenosine, or cordycepin, in place of typical nucleoside avoiding further fuse of nitrogenous bases (A, U, G, and C), prompting untimely end of **transcription** (Chen et al. 2008; Holbein et al. 2009).

16.3.3 Cordycepin Meddles in mTOR Signal Transduction

Cordycepin has been used for its abbreviation of the **poly A tail** of m-RNA, which influences the strength within the cytoplasm. It was watched that restraint of polyadenylation by cordycepin of some m-RNAs made them touchier than alternate **mRNAs**. At higher dosages, Cordycepin represses cell connection and lessens focal attachment. Further rise in the usage of cordycepin may terminate **mTOR** (mammalian focus of rapamycin) signaling pathway (Fig. 16.4) (Wong et al. 2009). The name mTOR has been determined from the medication rapamycin, on the grounds that this medicine represses

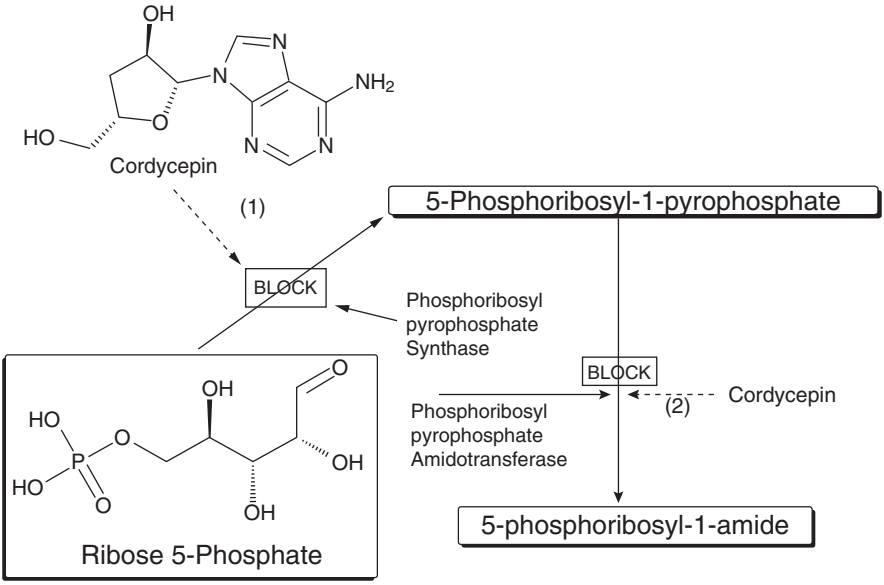

Fig. 16.3 The hindrance effect of cordycepin in mono- and tri- phosphate states on the catalyst enzymes, phosphoribosyl pyrophosphate synthase and phosphoribosyl pyrophosphate amidotransferase contain in biosynthesis pathway of purine (Tuli et al. 2014b)

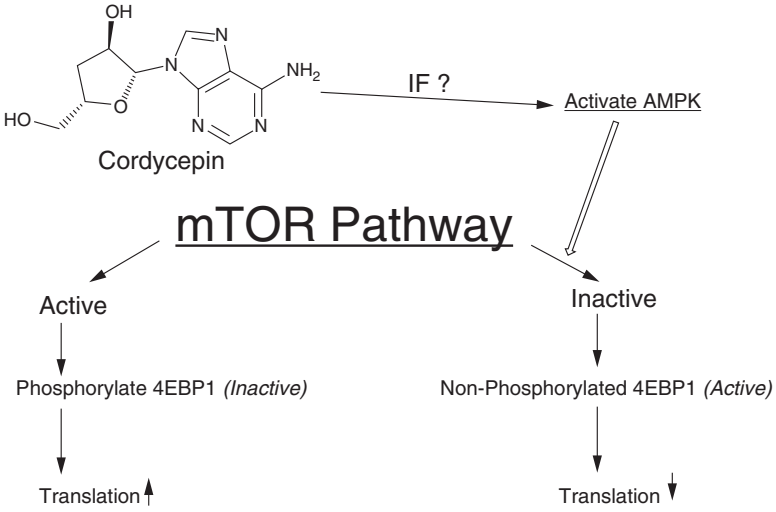

Fig. 16.4 Cordycepin presumably activates the AMPK by some unknown mechanism, which further negatively regulates the translation of mTOR signaling transduction pathway by the formation of a translational repressor, 4-E-binding protein-1 (4EBP1) (Tuli et al. 2014b)

mTOR action. The mTOR inhibitors, for example, rapamycin and CCI-779 have been tried as anticancer medicines, on the grounds that they repress or block mTOR pathway. mTORcan be defined as 298 kDa serine/threonine **protein kinase** from the family **PIKK** (Phosphatidylinositol 3-kinase-related kinase). The mTOR assumes an extremely imperative part to direct proteins production. Be that as it may, mTOR itself is controlled by different sorts of cell indicators such as factors of growth, nutritional environment, hormones, and energy level for cellular. As growth factor tie with cell receptor, Phosphatidyl inositol 3 kinase (PI3K) gets initiated, changes over phosphatidyl inositol bisphosphate (PIP2) to phosphatidyl inositol trisphosphate (PIP3). PIP3 further initiates PDK1 (phosphoinositide subordinate protein kinase 1). The actuated PDK1 then phosphorylates AKT 1 kinase and makes it somewhat initiated which is further made completely enacted by mTORC2 complex. The activated AKT 1 kinase now actuates mTORC1 complex that prompts the phosphorylation of 4EBP1 (translational repressor) and makes it inactive, exchanging on the protein production (Wong et al. 2009). The study confirmed that during the low level of nutritional stress affirmed, Cordycepin actuates AMPK, which hinders the action of mTORC1 and mTORC2 by some obscure component. The inactivated mTORC2 complex cannot enact AKT 1 kinase completely, which inhibits mTOR signal transduction hindering translation and more cell expansion and development (Fig. 16.4) (Tuli et al. 2014b).

16.4 Pharmacokinetic of Cordycepin

The cordycepin effects at molecular level represent comprehensive analysis on the cordycepin kinetics as well as ADME (Absorption, Distribution, Metabolism and Elimination at site of action of the biological target) studies. Therefore, promising investigations has been carried out in order to enhance the kinetic and the cordycepin quality of action at different active sites such as plasma.

In terms of cordycepin effects on the RNA synthesis, the termination of RNA transcription was recognized as a result of the absence of 3′ hydroxyl moiety (Holbein et al. 2009). Therefore, the impact of cordycepin investigated on the RNA metabolism of the yeast. The results presented that Cordycepin-triphosphate (CoTP) acted as a toxic and limiting factor on the growth of cells via RNA synthesis inhibition. However, the modulation of 3′ end heterogeneity of ASC1 and ACT1 mRNAs as well as rapid extended NEL025c loci and CYH2 transcript were another obtained findings (Holbein et al. 2009). In addition, the amelioration of poly (A) polymerase mutants growth defects together with pap1–1 mutation was reported to neutralize the effects of gene expression of cordycepin. It exhibited the epistatic relation of cordycepin function and poly (A) polymerase activity along with its potency to drop the efficiency of 3′ formations independently (Holbein et al. 2009). Advantageous impacts of cordycepin on the metabolic profiles of plasma and liver specifically in hyperlipidemic hamsters were studied thoroughly. Thus, ^{1}H NMR spectroscopy was applied on the intact liver tissues and plasma resulted high lipid level in the hyperlipidemic hamsters. At the end, the lipid-regulating activities and also the protective

effects of cordycepin on the plasma and liver especially in the fatty liver condition were recorded respectively (Sun et al. 2011).

Effects from cordycepin on the activation of Wnt/ß-catenin survival pathway to develop leukemia stem cells (LSCs), whether cordycepin regulates expression of ß-catenin in leukemia cells or not, was investigated (Ko et al. 2013). Cordycepin exhibited positive effects on the **malignant cancer** cells such as HepG2, A549, MCF7 and SK-Hep1 in a dose-dependent manner. Unexpectedly, reverse action with reduction on the levels of ß-catenin in the specific cancer cell line including THP1, K562 and U937 was resulted. GSK-3ß as pharmacological inhibitor was used in order to restore the negative effects of cordycepin on the above-mentioned group. The findings represented that cordycepin selectively reduced stability of **ß-catenin** in leukemia cells but not in other solid tumor cells (Ko et al. 2013). Another study by Wu et al. (2014), showed interaction of cordycepin with the γ1 subunit was found as activator for the AMP-activated protein kinase (AMPK) protein. The results clarified the mechanism and the role of cordycepin on the activation of AMPK in HepG2 cells. Therefore, circular and fluorescent dichroism as well as molecular docking measurements performed. The results showed the direct interaction of cordycepin with AMPK γ1 subunit. However, knock down of AMPKγ1 by siRNA and inhibition of AMPKγ1 expression has reported as significant factor leading abolishment of cordycepin actions in the lipid regulation especially in hyperlipidemia. Finally, cordycepin was introduced as promising agent to inhibit intracellular lipid accumulation by activating AMPK through the interaction with γ1 subunit (Wu et al. 2014).

16.5 Biological Activities of Cordycepin

16.5.1 Antioxidant Activity

In 2005, the **Supercritical carbon dioxide** (SC-CO2) was utilized as the elution dissolvable for fractioning ethanolic concentrate (E) of *Cordyceps sinensis* (CS), a customary Chinese natural cure, into R, F1, F2, and F3 parts. This extractive fractionation strategy was as an agreeable with extensive scale and as a nontoxic procedure. These four fractions were described regarding sum **polysaccharides** and cordycepin fixations; scavenge the free radicals and **antitumor** functions. Exploratory effects showed that fractionation changed the conveyances of aggregate polysaccharides and cordycepin in parts. Fraction R was the most dynamic portion to rummage free radicals and repress the expansion of carcinoma cells, emulated by the fraction F1 and the extract E. The impact of scavenging on 1, 1-diphenyl-2-picryl hydrazyl (**DPPH**) of *Cordyceps sinensis* concentrate and fractions at 2 mg/ml was R (93%), E (66%), F1 (75%), F2 (47%), and F3 (27%). The IC_{50} (half cell growth inhibitory concentration) of tumor cell expansion and colony structuring on human colorectal (HT-29 and HCT 116) and hepatocellular (**Hep 3b** and Hep G_2) carcinoma cells by fraction R were around 2 μg/ml. Then again, R did not influence the development of ordinary isolating human peripheral blood mononuclear cells (**PBMC**) by showing an extensive esteem of IC_{50} over 200 μg/ml. Tumor cells

accumulation at sub-G_1 stage and the fracture of DNA, regular characteristics of customized cell expiration, were watched in a time and manner of dose dependent. Totally, the discoveries recommended that fraction R containing cordycepin and polysaccharide, acquired by SC-CO_2 liquid extractive fractionation, indicated solid scavenging capability and specifically restrained the development of colorectal and hepatocellular growth cells by the procedure of apoptosis (Wang et al. 2005).

The most significant consequence led to aging process was introduced as **oxidative damage** induced by free radicals (Ramesh et al. 2012). The capacities and activities of antioxidant actions of cells decrease with increase in age. In addition, main reason for aging is steady loss of antioxidant/pro-oxidant equilibrium resulting considerable increase in oxidative stress. Current study was concentrated on cordycepin impacts on antioxidation and lipid peroxidation in elder rats. By comparison between old and young rats, decline in activities of catalase (**CAT**), superoxide dismutase (**SOD**), glutathione peroxidase (**GPx**), glutathione-S-transferase (**GST**), glutathione reductase (**GR**), vitamin C and vitamin E, reduced glutathione (**GSH**), and elevated levels of malondialdehyde (MDA) were entirely detected in the aged rats' lungs, heart, kidneys and liver. Moreover, the elevation of serum aspartate aminotransferase (**AST**), urea, alanine aminotransferase (**ALT**), and creatinine were significantly observed in old rats compared to younger ones. Cordycepin treatment on aged rats by increasing the activity of CAT, SOD, GST, GPx and GR and elevation of vitamins E, C and GSH level like the values of these parameters found in younger rats, demonstrated promising effects of cordycepin in antioxidant activities. In addition, decrease in the level of creatinine, ALT, AST and MDA was reported due to using cordycepin on the aged rats. Authors suggested cordycepin as effective agent for decreasing **lipid peroxidation** and restoring antioxidant status in the aged rats (Ramesh et al. 2012).

Furthermore, NF-κB signaling pathway suppression by cordycepin was investigated (Ren et al. 2012). Dose-dependent decrease in activity of NF-κB induced by TNF-α in HEK-293 T cells by cordycepin was detected. Although cordycepin in its high concentration decreased the NF-κB transcriptional activities and DNA-binding, cordycepin did not limit nuclear translocation of p65 induced by TNF-α. Moreover, in order to suppress IκBα degradation, cordycepin inhibited **phosphorylation** of IκBα. Additional examination showed that activation of NF-κB mediated by IKKs suppressed by cordycepin. Also, inhibition of IKKγ ubiquitination was reported as the effect of cordycepin. Researchers proposed cordycepin as an agent, which strongly inhibits NF-κB signaling through IKK, IκB and NF-κB activity suppression. In addition, They suggested potential medical applications of cordycepin in treating disorders associated with inflammation and cancer therapy (Ren et al. 2012).

16.5.2 Anticancer Activity

A research study with the aim at examination and exhibition of high chemical constituents and antitumor effects of Cs-HK1 mycelium were conducted. When compared with natural *Cordyceps sinensis*, the mycelium of fungal had much higher

contents of cordycepin (65/7 μg vs 20/8 μg) (per gram dry weight). Low cytotoxic effect was shown by the fungal extract from the hot water extraction towards the B16 melanoma cells in culture (about 25% inhibition) but significant antitumor effect in animal tests, that cause 50% inhibition on the B16 cell-induced tumor growth in mice. Major bioactive compounds like cordycepin and polysaccharides of *Cordyceps sinensis*, can be found in mycelium biomass and the mycelium extract had significant antitumor activity. The Cs-HK1 fungus was introduced as a new and promising medicinal fungus and as an effective and good substitute of economical of the wild *Cordyceps sinensis* for health care (Leung et al. 2006). One year later, the cultured mycelium of a *Cordyceps sinensis* (CS) organism was successively removed by petroleum ether (PE), ethyl acetic acid derivation (EtOAc), ethanol (EtOH) and heated water. All dissolvable concentrates with the exception of high temperature water extract demonstrated a huge and dose-dependent inhibitory impact on the cell growth of four cancer cell lines, MCF-7 breast growth, Hl-60 human premyelocytic leukemia, B16 rodent melanoma and Hepg2 human hepatocellular carcinoma, with IC_{50} values underneath 132 mg/ml. The EtOAc extract, specifically, had the most intense impact against every one of the four cancer cell lines, with IC_{50} between 12 mg/ml (on B16) and 45 mg/ml (on MCF-7). Conversely, it had lowest cytotoxicity on the ordinary rodent bone marrow cells. The EtOAc extract included significant amount of cordycepin cooperating the *in vitro* cytotoxicity. In an animal experiment, the EtOAc extract demonstrated noteworthy hindering impact on B16-induced melanoma in C57BL/6 mice, bringing on around the range of 60% abatement of tumor size over 27 days. The outcomes proposed that the EtOAc concentrate of *Cordyceps sinensis* mycelium has solid antitumor activity and can be positively applied as a potential anticancer and/or antitumor product (Wu et al. 2007).

In 2008, the study utilized Radix Astragali (RA) as the media for cultivation of *Cordyceps militaris* to explore the antitumor function of the fermentation stock. The cordycepin production through the culturing of *Cordyceps militaris* in Radix Astragali medium was found higher in antitumor activity than that cultured in artificial or synthesized medium. The broth used for fermentation purpose hindered the development of four different tumor cells incorporating human breast cancer MCF-7 cells, human gastric cancer AGS cells, murine colorectal adenocarcinoma CT26 cells and human hepatocellular carcinoma Hep G2 cells with IC50 37 mg/ml, 465 mg/ml, 20 mg/ ml, and 25 mg/ml, respectively. Despite the fact that cordycepin as the main bioactive segment with the strong antitumor functions in the fermentation medium of *Cordyceps militaris* in RA culture, there were different constituents, which improved the antitumor function of fermentation synergistically. To assess and accept the antitumor function, the BALC/c mice were embedded with CT26 cells and after that encouraged with different measurements of the fermentation broth. Clearly it was reported that 20-mg/kg-body weight (Bw)/day group had no noteworthy antitumor movement as contrasted with the control group. The measurements of 100 mg/kg Bw/day and 200 mg/kg Bw/day hindered the tumor size by 43.81% and 48.89%. Tumor weight was additionally decreased by 31.21% and 39.48% contrasted with the control bunch. Moreover, the fermentation broth

demonstrated low cytotoxicity against essential rodent hepatocytes, and did not cause any genuine symptom or any side effects on the key organs of the mice as contrasted with the chemotherapeutic medicine 5-FU (Lin and Chiang 2008).

Other research on antitumor activity of cordycepin mechanism have been studied in two various bladder cancer cell lines, T-24 and 5637 cells (Lee et al. 2009). Important and dose-dependent tumor growth inhibition by cordycepin action at a dose of 200 μM (IC50) through cell-cycle progression, was reported as a main result of G2/M-phase arrest and led to up-regulation of p21WAF1 expression, free of the p53 pathway as well as cordycepin-induced phosphorylation treatment of JNK (c-Jun N- terminal kinases). **Small interfering RNA** (si-JNK1) and SP6001259 (JNK-specific inhibitor) blocked the JNK function and saved cell growth inhibition, cordycepin-dependent p21WAF1 expression and decreased cell cycle proteins. It demonstrated cordycepin as a positive and effective healing agent for the treatment of bladder cancer (Lee et al. 2009).

Regarding the molecular mechanism and molecular targets of cordycepin, a research study was explored promising molecular systems for the antitumor impacts of cordycepin on the human colon malignancy HCT116 cells. After using cordycepin to treat the cells, the dose-dependent cell was watched at an IC50 esteem of 200 μm. Cordycepin medicine demonstrated G2/m-stage cell cycle arrest, which was connected with expanded p21WAF1 levels and decreased measures of cyclin Cdc2, B1, and Cdc25c in a p53-free pathway. Additionally, treatment by cordycepin instigated enactment of JNK (c-Jun N-terminal kinases). SP600125 pretreatment as a JNK-particular inhibitor, annulled cordycepin-intervened p21WAF1 expression, cell development restraint, and diminished proteinscell cycle. Besides, JNK1 restraint by small meddling RNA (siRNA) handled comparative results: concealment of cordycepin-actuated p21WAF1 expression, diminished cell growth, and decreased cell cycle proteins. Together, these effects prescribed a basic role for G2/m-stage arrest in human colon growth cells and JNK1 activation in cordycepin-instigated restraint of cell development (Lee et al. 2010).

Other investigation was also carried out to determine the mechanism of cell death induced by cordycepin in the breast cancer cells. Reduction in cell viability and dose-responsive cell growth inhibition were resulted due to contact of both MCF-7 and MDA-MB-231 breast cancer cells with cordycepin. Moreover, Some particular properties of mitochondria-mediated apoptotic pathway confirmed by biochemical assays, TUNEL and **DNA fragmentation** was associated with cell death induced by cordycepin in MDA-MB-231 cells (Choi et al. 2011).

In addition, **Caspases-3** and **Caspases -9** activation and cytosolic release of cytochrome c was triggered by cordycepin-associated dose-dependent growth of mitochondrial translocation of Bax. Interestingly, in cytoplasm, large membranous vacuole ultrastructure morphology and autophagosome-specific protein were detected which confirmed the autophagy-associated cell death of MCF-7 cells. Regardless of ER response, MCF-7 cells with apoptotic defects can be treated by employing cordycepin-induced autophagic cell death. While survival roles of autophagy in tumorigenesis of other cancer cells have been confirmed, the significant function of autophagy on the cordycepin-induced MCF-7 cell death is also

observed. Finally, results showed the strong killing of MCF-7 and MDA-MB-231 human breast cancer cells by the cordycepin. Further study was suggested to evaluate more about clinical usage, ER dependency and therapeutic actions of cordycepin on human breast cancer (Choi et al. 2011).

However, the recent research of (Pan et al. 2015) reported on the strong antitumor activity of cordycepin when treating MA-10 cells in different combined immunodeciency (SCID) mice *in vivo*. The outcome of studyshows that cordycein is significantly selective treatment highly effective in inductionof MA-10 cells apoptosis through p38 MAPKs signaling.

16.5.3 Anti-Inflammatory Activity

In 2005, a study was carried out to illustrate **anti-inflammatory** functions of *Cordyceps militaris*. The 70% ethanolic extract concentrates of fruiting bodies (FBE) and cultivated mycelia (CME) of *Cordyceps militaris* were ready. Both FBE and CME demonstrated topical anti-inflammatory in the croton oil-prompted ear edema in mice. CME was reported to hold intense anti-inflammatory movement, which was assessed by the carrageenan-instigated edema, and additionally solid antinociceptive action in writhing experiment. FBE and CME hold powerful inhibitory action on the chick embryo chorioallantoic membrane (CAM) angiogenesis in a dose-dependent manner. Besides, cordycepin as famous product from *Cordyceps militaris* was reported to be responsible for only part of anti-angiogenic and anti-inflammatory activities (Won and Park 2005).

Kim et al. (2006) investigated the anti-inflammatory effect of cordycepin on lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage cells. The key component of endotoxin, Lipopolysaccharide (LPS), which is formed by lipid A (phosphoglycolipid) is connected to hydrophilic heteropolysaccharide covalently. Cytokines production such as TNF-α, IL-1γ, GM-CSF, and nitric oxide was increased by considerable LPS-induced macrophage activation and modulated by up-regulation of inducible NOS2 (**nitric oxide synthase**). In addition, butanol fraction of *Cordyceps militaris* was inhibited by the production of nitric oxide (NO) in LPS-activated macrophage. Moreover, Cordycepin was reported as main component of *Cordyceps militaris* identified by high performance liquid chromatography. The activation of MAP and Akt kinases in LPS-activated macrophage was evaluated to elucidate the mechanism of inducible nitric oxide synthase (iNOS) expression and NO production inhibition by cordycepin. Furthermore, the phosphorylation of p38 and Akt in dose-dependent manners LPS-activated macrophage strong inhibition by cordycepin was reported obviously. Tumor necrosis factor (TNF-α) expression, translocation of nuclear factor- B (NF-κB) and IκB alpha phosphorylation were reported by considerable suppressing property of cordycepin. In contrast, Cordycepin demonstrated decreasing effect on the expressions of inducible nitric oxide synthase (iNOS) and cycloxygenase-2 (COX-2) in RAW 264.7 cell significantly.

Results presented that inhibition of NO production was occurred by cordycepin by down-regulation of COX-2 and iNOS gene expression via p38 and Akt phosphorylation and suppression of NF-κB activation as antioxidant inhibitor. Hence, the conclusion drawn by authors is that the cordycepin may afford a potential therapeutic action for inflammation-associated illnesses (Kim et al. 2006).

Recently, cordycepin anti-inflammatory action on the inflammatory mediator's production in murine BV2 microglia stimulated by **lipopolysaccharide** (LPS) was assessed by Jeong et al. (2010). In addition, the cordycepin secretion results on phosphorylation of **mitogen-activated protein kinases** (MAPKs) and nuclear factor-kappaB (NF-κB) activation induced by LPS was studied. After LPS stimulation, Pro-inflammatory cytokine production, nitric oxide (NO) and prostaglandin E2 (PGE2) was sensed in BV2 microglia. However, significant inhibitory effect of cordycepin on the extreme production of PGE2, pro-inflammatory cytokines and NO in a concentration-dependent manner without producing cytotoxicity was reported. In addition, cordycepin illustrated the suppressing effect on NF-κB translocation by blocking the degradation of IkappaB-α (IκB-α) and inhibition of Akt, ERK-1/2, JNK, and p38 kinase phosphorylation. Results presented that cordycepin inhibition on LPS-stimulated inflammatory mediator production in BV2 microglia is related to Akt, NF-κB, and MAPK signaling pathway suppression. Therefore, authors proposed cordycepin as useful agent for the treatment of neurodegenerative diseases by inflammatory mediator production inhibition in activated microglia (Jeong et al. 2010).

16.5.4 Hypoglycemic (Anti-Diabetic) Activity

Anti-diabetic impact of different fractions of *Cordyceps militaris* was compared in pure compounds and crude extracts. In addition, the effect of the various fractions, CMESS (ethanol soluble supernatant), CCCA (crude cordycepin containing adenosine) and cordycepin were studied in diabetic mice (Yun et al. 2003). Regarding to starch-loaded mice, Potential inhibitory activity of 34.7% and decreased blood glucose level by 35.5% was reported for the CMESS. Nevertheless, cordycepin and CCCA presented no difference. It is reported that administration of cordycepin and CMESS for a period of 7 days, considerably decreased the level of blood glucose. But, the reduction of blood glucose did not occurred by utilizing of CCCA with high concentration of cordycepin. T-lymphocyte proliferation was expressively reduced; while NO production was augmented above than two-fold in the cordycepin-administered group. Additionally, NO production and macrophage proliferation were meaningfully reduced in the group administrated by CMESS. Authors suggested that cordycepin and CMESS might be useful kits use to control the blood glucose level in diabetes and encouraging new medicine as an anti-hyperglycemic instrument without the deficiencies of decreased immune responses and other side effects (Yun et al. 2003).

16.5.5 Immunomodulatory and Protective Effects of Codycepin

A research conducted on medicine by Zhou et al. (2002), which resulted in the discriminating up-regulation of interleukin-10. Authors focused on the immunoregulatory effects of cordycepin derived from Cordyceps spp. measuring the Interleukin-2 and interleukin-10 secretion of human peripheral blood mononuclear cells, which were incubated through cordycepin was performed. Furthermore, the expression of surface markers on T lymphocytes, the proliferative response and the effect of cordycepin on the expression of interleukin-10 mRNA were evaluated completely. In addition, cytotoxicity of cordycepin, interleukin-10-secreting cells and the impact of anti-interleukin-10 neutralizing antibody were assessed. Results showed that cordycepin has an expressively up-regulative impact on the interleukin-10 production and interleukin-10 **mRNA** expression. Moreover, Cells of CD8+, CD4+, CD56+, CD14+ and CD19+ were confirmed as Interleukin-10-producing cells. In parallel, Proliferation of peripheral blood mononuclear cells cordycepin and **phytohaemagglutinin**-induced interleukin-2 production was inhibited considerably. It is reported that decrease in expression of the surface markers CD45PRO, CD25, CD71, CD54 and HLA DR also reflected restricted T lymphocyte activation. Furthermore, Anti-interleukin-10 neutralizing antibody was reported, as component not completely hindered the cordycepin suppressive effect on production of interleukin-2. Authors stated an effective concentration (above 24 μg/ml) of cordycepin, which presented minor cytotoxicity but did not raise apoptosis. In contrast, high cordycepin concentration was stated as it may have exerted widely inhibitory effect on DNA synthesis or caused in cytotoxicity remarkably. However, in range of cordycepin concentration between 0 and 24 μg/ml, the strong up-regulation of interleukin-10 expression and immediate down-regulation of interleukin-2 expressions signified that an inhibitory result on **DNA synthesis** or a **cytotoxicity** mechanism might not be essential. Results indicated that cordycepin uses immunoregulativeoutcomes. Additional study on the method for the improvement of novel immunomodulatory medicines, which precisely adjust the excretion of cytokines, was suggested (Zhou et al. 2002).

Cho et al. (2007) carried out a research on the new effect of collagen stimulated human platelet accumulation resulted by cordycepin (3′-deoxyadenosine). Inhibition of measure-dependently collagen-induced platelet aggregation by the cordycepin in existanceof different wide range of concentrations of CaCl2 was reported thoroughly. Of thromboxane A2 (TXA2) and cytosolic freeCa^{2+} ($[Ca^{2+}]i$), as two aggregation-inducing molecules, up to 74% of up-regulation of $[Ca^{2+}]i$ was blocked with cordycepin (500 μM) while for TXA_2 production, it was suppressed by 46%. Afterward, phosphorylation of Ca^{2+}- for 20-kDa and 47-kD aproteins in collagen treated platelets was effectively reduced by by cordycepin. But, upstream routes such as formation of inositol1,4,5-triphosphate (IP3) and the activation of pphospholipase C- γ2 (PLC-γ2) (assessed by phosphotyrosine level) that normally used for producing these two inducers were not changed by cordycepin. Furthermore,

the level of second messengers guanosine 3′, 5′-cyclic monophosphate (cGMP) and adenosine 3′,5′-cyclic monophosphate (cAMP) in collagen-stimulated platelets was increased in presence of cordycepin. However, cordycepin-induced up-regulation of cGMP did not changed by the NO-sensitive guanylyl cyclase inhibitor ODQ and the cAMP enhancement mediated by cordycepin was totally blocked by adenylyl cyclase inhibitor SQ22536, demonstrating the various cordycepin modes of action. Hence, Authors suggested that their results show the inhibitory effect of cordycepin on platelet aggregation could be related to the boost of cAMP/cGMP production and the down-regulation of $[Ca^{2+}]_i$ (Cho et al. 2007).

One of the common techniques for curing the **atherosclerosis** is Percutaneous Transluminal Coronary Angioplasty (PTCA). However, it has limited efficacy due to manifestation of restonesis during 3–6 months after angioplasty(Chang et al. 2008). Restenosis is stimulated in cells andextracellular matrix (ECM) inside the intimal layer and/or alteration of the vessel wall. Thus, one of the potential therapeutic agents for the atherosclerosis or restenosis treatment can be **matrix metalloproteinase** (MMP) system. In this study, the effect of cordycepin on the MMP system within vascularmuscle cells was investigated particularly. Cordycepin (20 μM/day, i.p) led to inhibition of rat aortic smooth muscle cells (RaoSMCs) proliferation. Thus, neointimal development in the carotid artery of a balloon injured-Spraque–Dawley (SD) rat was decreased considerably. In addition, the triggering of MMPapproachincollagentypeI activated RaoSMCs was evaluated in order to conduct on cordycepin inhibition mechanism on the buildupof both, cells and ECM and/or remodeling of thou thee vessel wall. The positive effect of cordycepin was reported as a strong agent to inhibit the activation of MMP-2 and extracellular matrix **metalloproteinaseinducer** (EMMPRIN) expression in a dose-dependent within RAoSMCs activated by collagen type I. Furthermore, the expression of cycloxygenase-2 (COX-2) correlated tohyperplasia of RAoSMCs was strongly suppressed by cordycepin. Taken together, anti-proliferation property of cordycepin in RAoSMCs through the varietypf vessel wall remodeling concluded by the authors. They also stated that cordycepin can be a probable use medicinal for restenosis treatment (Chang et al. 2008).

Previous investigation was also obtained by (Yao et al. 2011) to investigate the effects of cordycepin on CA1 pyramidal neurons exist in the rat hippocampal brain slices by whole-cell patch clamp method. The result exhibited the drop in the both evoked and spontaneous action potential (AP) firing frequencies. The hyperpolarization of neuronal membrane potential by cordycepin and the stability of either membrane input resistance or the amplitude of fast after hyperpolarization (fAHP) in presence of cordycepin was reported as opposed with AP, which spiked width. Collectively, Membrane hyperpolarization induced by cordycepin led to diminish neuronal activity presenting cordycepin as potential therapeutic approach to treat excitotoxic disorders such as ischemic.

Oxygen–glucose deprivation (OGD) injury in mice brain slices was investigated in the presence of cordycepin. Study outcomes illustrated that brain slice injury and especially postischemic neuronal degeneration can be prevented by cordycepin.

Also, two types of excitatory amino acids in brain homogenized supernatant, aspartate and glutamate, were enhancedin reperfusion/ischemiaset, were sensed by **HPLC**. The results also showed that the significant reduction in the extracellular level of aspartate and glutamate was the positive effect of cordycepin. Furthermore, Ameliorating the extent of oxidation, dropping malondialdehyde (MDA) level and increasing the superoxide dismutase (SOD) activity were reported as cordycepin activity on the system. The remarkable increase in the expression of matrix metalloproteinase-3 (MMP-3 as main enzyme involved in inflammation) after ischemia reperfusion was also inhibited by the protective activity of cordycepin. It was concluded that the potential neuroprotective action of cordycepin, in both *in vivo* and *in vitro* studies, was exerted subsequently after cerebral reperfusion/ ischemia (Cheng et al. 2011).

Drugs, mostly viable throughout the initial phase of trypanosomosis, however crudely enter the blood brain restraint, are ineffectual when parasites spread to the brain and reason cencephalitis. Therefore, a study with intended to assess the weakness of species*T. evansi* to cordycepin invitro and in rats tentatively affected was carried out. In vitro, a critical reduction ($P < 0.01$) in living trypanosomes in the doping between 5.0 and 10 mg/ ml was recorded one hour afterward the start of the investigation, and also at three, six, nine and twelve hours in all concentrations contrasted with control. Despite the fact that no remedial impacts were found in the in vivo assessments in the most of groups, the treatment was fit to keep the parasitemia at low levels, along these lines expanding the life span of rats when contrasted with positive control. Rats that gained cordycepin only or in combination by adenosimedeaminase inhibitor (ADA: EHNAhydrochloride), did not indicate trypomastigote manifestations of the parasite in the bloodstream 24 h after the consumption. Averages of 8 days were assigned to remain animals negative in blood smears, yet from that point had a repeat of parasitemia. Around all the tainted creatures, just 3 rats cured with the mixture of EHNA hydrochloride (2 mg/kg) and cordycepin (2 mg/kg) persisted negative throughout the trial period. The adequacy of 42.5% was affirmed by PCR utilizing *Trichoderma evansi* particular primers. Subsequently, authors concluded that cordycepin requires natural impact to against *T. evansi*. The cordycepin treatment, when secured by an inhibitor of ADA, be able to delay the *T. evansi*-contaminated rats survival then furnish therapeutic viability (Da Silva et al. 2011).

Jeong et al. (2011) carried out a research on the specific polyadenylation inhibitory effect of cordycepin as an operative compound in *Cordyceps militaris.* Authors believed that cordycepin has possessed many immunological activities, which is efficient for **cancer therapy**. However, anticancer mechanism still needs more investigation. In this study, cordycepin apoptotic effects were conducted on human leukemia cells. Apoptosis induction (not necrosis) by cordycepin treatment led to celldevelopment inhibition in a suitable concentration manner. Poly (ADP-ribose) polymerase protein cleavage, caspases activation, dysfunction of mitochondrial and initiation of **reactive oxygen species** (ROS) were reported as activities related to induction process. However, significant inhibitory effect of caspases, in the responses of cordycepin, attenuated cordycepin-induced apoptosis. N-acetyl-L-cysteine, a ROS scavenger, also inhibited caspases activation and apoptosis induced

by cordycepin. Results supported a mechanism whereby human leukemia cells apoptosis was induced by cordycepin via a signaling cascade containing a caspase pathway mediated by ROS (Jeong et al. 2011).

As knowledge goes far, *Cordyceps sinensis* is a parasite utilized as conventional Chinese drug as a tonic to alleviate the lung for the medication of exhaustion and respiratory ailments. Idiopathic pulmonary fibrosis is a persistent, irreversible and incapacitating lung infection demonstrating fibroblast/myofibroblast development and extreme deposition of extracellular framework in the interstitium causing breathing issues. Recently, some results uncovered an incomplete help of lung fibrosis in patients experiencing intense respiratory syndrome (SARS). Subsequently, the theory that cordyceps has some benefits for lung fibrosis was expressed and the idea of more current study was pointed at investigating the target(s) of cordyceps in the alleviation of lung fibrosis in rats and cell unit models and understanding of its mechanisms. A rodent model of bleomycin (BLM)-incited lung fibrosis and a fibrotic cell pattern with converting growth factor beta −1 actuation were used in the studies. Lessening of invasion of inflammatory cells, accumulation of fibroblastic loci and collagen, creation of reactive oxygen species (ROS), and cytokines production, together with recuperation from unbalance of MMP-9/TIMP-1, were seen in fibrotic rats after medication with cordyceps in prophylactic (from the day of BLM consumption) and remedial (from 14 days after BLM) diets. In a fibrotic cell pattern with changing growth factor beta-1 infusion, the human lung epithelial A549 gained a mesenchymal phenotype with an expansion of vimentin manifestation with an associative lessening of E-cadherin. This epithelial–mesenchymal move could be returned in part by cordycepin as a significant component of Cordyceps. The results give an understanding of the prophylactic and remedial possibilities of Cordyceps for the cure of lung fibrosis (Chen et al. 2012).

Cordycepin exhibited an anti-atherogenic impact on exploratory animals. Notwithstanding, the impacts of cordycepin on the signaling pathway and cell-cycle regulation in **vascular smooth muscle units** (VSMC) maintain largely obscure; hence, in an alternate study, surprising function of cordycepin-actuated hindrance in VSMC development were examined. Cordycepin treated VSMC mechanisms were explored through a MTT test, an uptake trial of thymidine, FACS investigation, immunoblot examination, kinase assay, immunoprecipitation test, and transient transfection evaluation. Cordycepin repressed cell growth, actuated G1-phase cell-cycle arrest, down the cyclins and **cyclin-subordinate kinase** (CDK) secretion, and up-regulated p27KIP1 expression in VSMC. Cordycepin actuated JNK activity, ERK1/2 and p38MAPK. Obstructing of the ERK activity by either ERK1/2-particular inhibitor U0126 or a little meddling RNA (si-ERK1) restraint of cell development, switched p27KIP1 expression, and diminished cell-cycle proteins in VSMC treated by cordycepin. Ras initiation was expanded by cordycepin. Cells transfection with overwhelming negative Ras (RasN17) mutant genes expanded p27kip1 declaration, saved cordycepin-instigated ERK1/2 movement, decreased cell cycle proteins and restrained cell multiplication. In conclusion, Authors stated that findings of research demonstrated that Ras/ERK1 pathways take part in p27KIP1-intervened G1-phase cell cycle arrest incited by cordycep in through cyclin/CDK complexes reduction in VSMC (Jung et al. 2012).

Recently, novel research was accepted to examine the cordycepin impact on the normal sleep in rats, and intervened by **adenosine receptors** (ARs). Thus, **electroencephalogram** (EEG) was applied in order to record the sleep about 4 h later than cordycepin oral administration by rats. Analyses on the sleep structure and EEG effect spectra were performed. As a result, Cordycepin diminished wake cycles of sleep and expanded non rapideye movement (NREM) rest. Fascinatingly, cordycepin expanded (theta) waves force density throughout NREM rest. Furthermore, AR subtypes (A2A, A1 and A2B) protein levels were risen later the cordycepin consumption, particularly in the rodent hypothalamus which assumes a vital part in regulation of sleep. Subsequently, authors prescribed that cordycepin grows theta waves force density throughout NREM rest through nonspesifiv AR in rats. Authors declared that results were presented to give fundamental proof that cordycepin may be accommodating for sleep-bothered issues (Hu et al. 2013).

Lately, a research study with focuson the *in vitro* cordycepin effects on osteoclastogenesis as well asthe*in vivo* effects on ovariectomized (OVX) mice were conducted (Dou et al. 2016). An in vivo result showed the significant role of cordycepin on prevention of several diseases such as bone loss and bonemicroarchitecture as well as fixes bone mineral in OVX mice. The authors proposed the cordycepin asanosteoclastinhibitor and have big ability therapeutic effect especially effective inavoidingbone loss between postmenopausal osteoporosis victims.

16.6 Production of Cordycepin

16.6.1 Cultivation Conditions for Cordycepin Production

Optimal cultural environments for the creation of cordycepin through submerged cultures of *Cordyceps sinensis* and *Cordyceps militaris*was investigated by Kim and Yun (2005) in shake flask level cultivation and in 5 literstirred tankbioreactors. The mycelial biomass concentration of cordycepin attained in the submerged culture conditions of *Cordyceps militaris* was greater than *Cordyceps sinensis.*

In same year, production of cordycepin (3′-deoxyadenosine) in submerged cultivationof a *Cordyceps militariss* was performed and the effect of different types of carbon source and carbon: nitrogen (C:N ratio) on cordycepin was studied by Mao et al. (2005). Glucose (carbon source) was discovered to be the suitable for cordycepin production. The maximum cordycepin production of 245.7 was found in medium of 40 gL^{-1} glucose. In addition, response surface analysis as well as central composite design was used to study the impact of carbon: nitrogen ratio on cordycepin production. The highest cordycepin production of 345.4 mg/l was reached in culture media of 42.0 g/l glucose and 15.8 g/l peptone. (Mao et al. 2005).

One year later, *Cordyceps militaris* NBRC 9787 surface culture was used to study the production of cordycepin (Masuda et al. 2006). In this study, 98% of extracted cordycepin from *Cordyceps militaris* was excrete into medium (extracellular). Mixture of yeast extract and peptone was the best for the production of

cordycepin. Glucose was utilized as the carbon source with the optimum carbon: nitrogen ratio of 2:1 (w/w). The maximum concentration and productivity of cordycepin in the medium under the optimal condition were reported as 640 mg/l and 32 mg/day, respectively (Masuda et al. 2006).

Mao et al. (2005) described that Peptone was the best nitrogen source involved in complex medium for the production of cordycepin. In addition, NH_4^+has significant role in cordycepin production. To improve production of cordycepin, in this, NH_4^+ was added during fed-batch culture along with complex medium in the presence of peptone. Furthermore, Maximum concentration of cordycepin (420.5 ± 15.1 mg/l) was obtained by optimizatation of feeding and ammonium feeding rate.

Different compounds such as glycine, l-glutamine, l-aspartic acid, adenosine and adenine were also supplemented to the base medium to enhance the cordycepin production, which illustrated positive effects on the production rate. The mixture of 16 g/l glycine and 1 g/l of adenine was the most suitable concentration and supported cordycepin production up to 2.5 g/l, which was4.1 times significant than the one in the basal medium. The results showed that guanine and cordycepin production may be connected to each other and also about 97% of *Cordyceps militaris* synthesized cordycepin was excreted into the medium (Masuda et al. 2007).

Fan et al. (2012) investigated the cordycepin productionin submerged cultivation of *Cordyceps militaris*, in shake flasks level. Adding of ferrous sulfate in concentration of 1 g/l at zero time supports volumetric production of cordycepin up to596.59 ± 85.5 mg/L (70% greater than control without addition of ferrous sulfate). Meanwhile consumption of a potential cordycepin precursor, inosine 5′-monophosphate (IMP), was dropped significantly. In addition, study on transcription levels of key genes encrypting IMP cyclohydrolase (purH), IMPdehydrogenase (guaB) and adenylosuccinate synthetase (purA) in the purine nucleotide biosynthetic pathway were done. In comparison with controlled sample, the transcription level of guaB and purH were slowly down-regulated, while in ferrous sulfate supplemented cultures, purA was expressively up-regulated (Fan et al. 2012). Recent research showed that the optimum culture condition for production of cordycepin were pH at 5.5, temperature 25 °C, inoculum size 8% v/v, inoculum age 72 h, incubation time 24 d. The optimum culture medium in the same study was composed of: 1.5% dextrose, 0.8% yeast extract, potassium phosphate dibasic (K_2HPO_4) 0.3%, Potassium phosphate monobasic (KH_2PO_4) 0.1%, sodium chloride (NaCl) 0.05%, Magnesium Sulfate ($MgSO_4$) 0.05% and NaCl 0.05%. The maximal cordycepin production in this medium was 846 mg/L (Tuli et al. 2014c).

16.7 Synthesis and Delivery of Cordycepin

A novel study on layered double hydroxides (LDHs) as nanocarriers for delivering of cordycepin was investigated. Using **XRD, CZE, TEM, FT-IR** and electrophoretic mobility confirmed negatively charged biomolecule-cordycepin intercalated in

the gallery spaces of charge-compensating species, [Mg–Al–NO3]. Decomposition of cordycepin by adenosine deaminase was prevented by new bio-LDH nanohybrid particles strongly. This new preparation suggested as a new form cordycepin arterial injection (Yang et al. 2006).

In 2008, a research study on the preparation of 4′-benzoyloxy precursor (4′-substituted cordycepin from adenosine) was carried out. In order to make the precursor, an electrophilic supplementation (iodo-benzoyloxylation) to the 4′, 5′-unsaturated byproducts was performed. Furthermore, radical mediated removal of the 3′-iodine atom of the consequential adducts was done. The 4′-substituted cordycepin effectiveness was concisely proved through the synthesis of 4′-cyano and 4′-allyl cordycepin analogues (Kubota et al. 2008).

Moreover, for easing off the rapid metabolic velocity and also expanding the cordycepin bioavailability, four N-acyl-(octanoyl-, propionyl-, stearoyl- and lauroyl-) cordycepin formatives were produced artificially and their pharmacokinetic profiles were examined. The outcomes demonstrated that half-life time ($t_{1/2}$) and maximum concentration time (T_{max}) might be stretched with the alkyl chain length expansion, however concentration time curve (AUC) and maximum concentration (C_{max}) enhanced at first, then diminished when the number of alkyl carbon surpassed eight. The C_{max}, T_{max} and AUC of N-octanoyl-cordycepin were about 30, 4 and 68 times, respectively, greater than that of cordycepin. It showed that N-octanoyl modification could expand the cordycepin bioavailability and diminish the metabolic activity (Wei et al. 2009).

Recently, an investigation on region selective and extremely efficient cordycepin acyltion with an immobilized species of *Candida antartica* lipase B (Novozym 435)-catalyzed vinyl acetate in the solvent consumed, which is 2-methyltetrahydrofuran (MeTHF) was carried out. High operational stability and excellent region selectivity during the transformation displayed by Novozym 435 as biocatalyst, and 96.2% isolated yield of cordycepin acetate 25-g scale syntheses by biocatalyst introduced Novozym 435 as useful and effective agent on production of cordycepin. In addition, 5′-substituted cordycepin derivative was described as the mainly product from acylation effects. Finally, Recycling of Novozym 435, as biocatalyst, for the cordycepin derivative synthesis on a 25 g scale and maintaining of 63% of its unique activity after reusing for 7 batches production (Chen et al. 2013). Moreover, the identification of genes involved in formation of cordycepin within the fungus *Cordyceps militaris* was investigated through bioinformatic analysis method. The results showed important role of glucose methanol choline oxido reductase and telomerase reverse transcriptase on the development of cordycepin in fruting body of fungus *Cordyceps militaris* (Zheng et al. 2015).

In recent years, several methods have been applied in command to enhancethe rate of cordycepin creation within the cell as first level biofactory unit (Lia et al. 2015) or in total manufacturing rate including the coupling of *Cordyceps militaris* cells with various additives in medium culture (Das et al. 2009; Leung and Wu 2007), repeated batch culture method (Das et al. 2016), optimization of bioprocess parameters in order to achieve higher rate of production (Tang et al. 2007; Jiapeng et al. 2014), using mixed static-liquid culture technique (Kang et al. 2014), and

improved feeding strategy (Velut et al. 2007) as well as cordycepin extraction process enhancement (Wang et al. 2014; Park 2015).

Another research was conducted on the optimization of solvent-solvent extraction method in order to efficiently extract the cordycepin from fermented broth. The hexane, chloroform and n-butanol were used in different parameters such as solvent-solvent ratio, extraction time and extraction temperature. Results showed the maximum yield of extraction (95% cordycepin) was establish at solvent-solvent ratio (1:2 v/v), 90 min of extraction at 40 °C (Tuli et al. 2014a).

16.8 Extraction, Separation, Purification and Determination of Cordycepin

Hsu et al. (2002) investigated on characterization of the bioactive components containing cordycepin and adenosine from *Cordyceps sinensis*. The mycelium of *Cordyceps sinensis* was cultured in potato dextrose broth (PDB) for 5 day incubation period at 22 °C. The fermentation process was performed in a 5-L fermenter with a working volume of 3-L and 10 percent of inoculum at 22 °C for 5 days. The overall levels of amino acids were found considerably diverse, varying from 4–17% obtained by an automatic amino acid analyzer device. Aspartic and glutamic acid were found as two major amino acids significantly in all samples verified. The amount of overall amino acids in the mycelium was determine only half that found in the natural type. HPLC analysis proved that no bioactive components adenosine and cordycepin found in the mimic and counterfeit types. Regarding to Adenosine, it was exist rich in the fruiting body, more than in the mass of the ordinary type and also in the *Cordyceps sinensis* mycelium. In terms of cordycepin, it was found in both fermented and natural *Cordyceps* extracts. Results suggested that cordycepin and adenosine can be applied as indexing elements for distinguishing cordyceps from the mimic and counterfeit (Hsu et al. 2002).

In the same year, **Capillary zone electrophoresis** (CZE) method was applied to separate adenosine and cordycepin by Ling et al. (2002). In addition, determination of concentrations of both adenosine and cordycepin in the stroma of Cordyceps spp. was made by CZE. Outcomes presented that the CZE technique is a rapid, simple and sensitive system to quantify the cordycepin and adenosine concentration with upright repeatability (Ling et al. 2002).

Regarding to cordycepin determination, an innovative capillary electrophoresis (CE) technique with UV detection at 254 nm was advanced and enhanced (Rao et al. 2006). Ideal settings initiate were 20 mM sodium borate buffer plus 28.6% methanol, separation voltage20 kV, pH 9.5, temperature 25 °C and hydrodynamic injection time 10s. Above the 20–100 μg/mLvarieties of cordycepin concentration, linearity was found clearly. It should be noted that enhanced technique has already been practicalfor cordycepin determination in different pharmaceutical products. By comparing the results obtained by either CE or high performance liquid chromatography (HPLC) methods, it can be found that both techniques were offered

comparable outcomes regarding cordycepin concentration in either natural types purchased from China and Taiwan or cultured fruiting body within the solid-state fermentation. At the end, Authors claimed that low running cost and short analysis time are two main advantages of CE technique were proposed (Rao et al. 2006).

In addition, Quantitative and qualitative evaluation of bases, nucleosides and their analogues in cultured and natural *Cordyceps* was done utilizing high performance liquid chromatography electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) and rapid pressurized liquid extraction (PLE) (Fan et al. 2006). Extractions were done using PLE technique while ZORBAX Eclipse XDB-C18column with 5 mM aqueous ammonium acetate as mobile phase and gradient elution of methanol was used for separation purposes. Precursor ions, product ions, and retention times were characterized to identify target compounds. Compounds were analyzed quantitatively operating time programmed selective reaction monitoring (SRM) or selective ionmonitoring (SIM) with10segmentsinpositive (or negative foruridine) ion mode. The total numbers of 43 nucleosides, bases and their analogues were discovered in cordyceps, which among them 16compoundswere known separately. PLE and HPLC–ESI–MS/MS techniques explained above, were demonstrated good selectivity, linearity, recovery, precision, short analysis time plus LOQ and LOD in the ng/ml range (Fan et al. 2006).

In order to investigate pharmacological and physiological effects of bioactive ingredients from mushrooms, nucleoside determination and its metabolic compounds are important concepts should be considered initially. In other study, a fast-response **ultra-performance liquid chromatography** (UOLC) technique was created for the cordycepin determination. The detachment was done on Water AcquityUPLC framework with Acquity UPLC BEH-C18 column with 0.5 mm acetic acid gradient elution and acetonitrile (5 min). In addition, high value of analyte correlation coefficient was recorded at $R2 > 0.9995$. The LOQ and LOD were lower to 47.0 and 11.9 ng/ml along with 1 µl of injection volume, respectively (Yang et al. 2007).

For extracting the cordycepin from the solid state fermentation, a novel column chromatographic extraction (CCE) technique was developed and presented. Imbibition of dried wastes (4times its volume of water) for6 h, transferring to the columns and washing with water were the initial steps for preparations. Separation step was done using macro-porous resinDM130columnsfollowedby precipitation phenomenon, crystallization process, and polyamide column chromatography as purification steps. The results were 97% extraction rates of cordycepin gained by 4 volumes of water for washed materials distributed through 3 different columns and 12 volumes of water for a single column aimed to concentrate cordycepin (Ni et al. 2009).

In other study, cordycepin and adenosine were extracted from *Cordyceps kyushuensis* using **supercritical fluid extraction** (SFE) technique. In order to get the optimal extraction conditions anorthogonal array strategy (OAD) test, L9(3), including different parameter synch as temperature, pressure and flow rate of CO_2as critical factors (Ling et al. 2009). In addition, scaling up the procedure up to 30 times using a SFE system was successfully achieved under the following conditions

40 °C, pressure of 40 MPa and a flow rate of CO2 (2.0lmin^{-1}). The same study showed also that **High-speed counter-current chromatography** (HSCCC) with a two-phase solvent system constituted of collected fractions analyzed by HPLC and ethyl acetate–n-butyl alcohol–water at ratio of 1:4:5 (v/v/v) was used successfully for cordycepin separation (Ling et al. 2009).

Moreover, **capillary electrophoresis mass spectrometry** (**CE–MS**) technique was improved in order to concurrent resolution of cordycepin in wild and artificially cultured *Cordyceps* by 5 chlorocytosine arabinoside as standard. The CE division environments and MS related parameters were enhanced methodically for obtaining exceptional CE and MS results of the explored compound. The 100 mM formic acid including 10% (v/v) methanol was reported as the optimal CE electrolyte. In addition, the optimal MS parameters were explained as 75%(v/v)methanol including 0.3% formic acid with a rate of 3 μl/min was chosen (Yang et al. 2009).

Xie et al. (2010) established a consistent, selective and sensitive **liquid chromatography mass spectrometry** (LCMS) method using electrospray ionization interface technique to separate and determine bioactive compounds adenine, thymine, cordycepin and adenosine in mushroom *Cordyceps sinensis.* In this method, gradient elution technique using a 2.0X150mm VP-OD Scolumn was used for separation (Xie et al. 2010).

Recently, One-step separation and purification of cordycepin from *Cordyceps militaris*(L.) Link, in a preparative measure, has been applied using **high speed counter current chromatography** (HSCCC) method (Xie Huichun et al. 2011). A two-phase solvent method of combinations of n-hexane-n-butanol-methanol-water (23,80,30:155, v/v/v/v) was used to perform HSCCC separation with high efficiency. The identification of product was done using IR, UV, ^{1}H NMR, ^{13}C NMR and MS (Xie Huichun et al. 2011). Recent study showed simple optimized solvent system composed of: water (23%), ethanol (30%), methanoll (25%), ethylacetate (22%) was successfully used for cordycepin extraction (Soltani et al. 2017).

In order to get better overall look on the recent investigations on the cordycepin determination techniques as provided above, data were summarized and presented in Table 16.2 as follows.

16.9 Conclusion

As we have recently faced by various hard-treating diseases such as cancer among the people in different societies, the researchers were encouraged to find the alternate treatment with potential therapeutic activity. Chinese medicinal mushrooms especially *Cordyceps* spp. are one of those with long history background about 2000 years which are used to treat many type of these diseases. Cordycepin is low molecular weight bioactive compound isolated from the mushroom *Cordyceps militaris* and/or *Cordyceps sinensis* and possess different biotherapeutic activities. Therefore, the method of production and its therapeutic actions on the different types of cancer cell lines were investigated extensively. However, more studies are

Table 16.2 Cordycepin determination using different methods

Cordyceps species	Objectives	Cultivation mode	Methods	Results	References
Cordyceps sinensis	Characterization	Submerged (cultured in Potato Dextrose Broth, PDB)	Automatic amino acid analyzer, HPLC	Cordycepin found in both cultured and natural *Cordyceps sinensis*, Cordycepin was introduced as an indicator for *Cordyceps* from its mimic and counterfeit	Hsu et al. (2002)
Cordyceps sinensis	Separation and concentration determination	Submerged (shake flask)	Capillary Zone Electrophoresis (CZE)	Rapid, Simple and Sensitive system to quantify the cordycepin	Ling et al. (2002)
Cordyceps militaris	Cordycepin determination	Solid-state fermentation	Capillary Electrophoresis (CE) + UV detection, HPLC	Low running cost and short analysis time are obtained as advantages of this method	Rao et al. (2006)
Cordyceps sinensis	Quantitative and qualitative evaluation of cordycepin	Submerged	HPLC-ESI-MS/MS, PLE, SRM and SIM	Good selectivity, linearity, recovery, precision, short analysis time + LOQ and LOD in the ng/ml range	Fan et al. (2006)
Cordyceps sinensis	Determination of cordycepin	Submerged	Ultra-performance liquid chromatography (UPLC)	Fast determination of analytes in pharmaceutical products and biological fluids	Yang et al. (2007)
Cordyceps militaris	Cordycepin extraction from wastes, propose technique for purification and separation	Solid fermentation	Column chromatographic extraction method (CCE)	High effective extraction method, minimal volume of solvent consumption, energy-saving, simple, environmentally friendly and low operating cost	Ni et al. (2009)
Cordyceps kyushuensis	Optimal extraction of cordycepin	Submerged	Surface fluid extraction (SFE), HSCCC + HPLC	Considerable amount of cordycepin with purity of 98.5% was obtained	Ling et al. (2009)
Cordyceps militaris and *Cordyceps sinensis*	Cordycepin analysis in natural and cultured Cordyceps	Submerged	Capillary electrophoresis-Mass spectroscopy (CE-MS)	Cordycepin was found in both natural and cultured *Cordyceps Militaris* but solely detected in natural *Cordyceps sinensis* with very low content	Yang et al. (2009)

Cordyceps sinensis	Determination of cordycepin and its transformation product	Submerged	IP-RP-LC-MS	No transformation was found in both natural and commercial cultured *Cordyceps sinensis*	Yang et al. (2010)
Cordyceps sinensis	Determination of cordycepin	Submerged	Liquid chromatography-Mass spectroscopy (LCMS)	Cordycepin was recovered from 98.47–99.32%	Xie et al. (2010)
Cordyceps militaris	Separation and purification of cordycepin	Submerged	High-speed counter-current chromatography (HSCCC)	Cordycepin with purity of 98.6% and 91.7% was produced	Zhang et al. (2016)
Cordyceps militaris Mutant G81–3	Correlation determination between adenosine and cordycepin	Surface-liquid culture of Mutant	High-energy proton beam irradiation	Significant overproduction of cordycepin at 0.48 g/l/day	Masuda et al. (2011)
Cordyceps militaris Mutant G81–3	Cordycepin production using crystallization method	Surface-liquid culture of Mutant	High-energy proton beam irradiation	High level production of cordycepin at 14.3 g/l	Masuda et al. (2014)
Cordyceps militaris 3936	Optimization of cordycepin extraction	Submerged cultivation broth	Solvent-Solvent extraction	High yield extraction (95%)	Tuli et al. (2014a)

still required to improve the production process in both upstream and downstream. In addition, more investigations are still needed for scaling up and industrialization of this process. Finally, finding the correlation between cordycepin and other bioactive ingredients in the extract need further investigations to improve the production process.

Acknowledgement The financial support from the Institute of Bioproduct Development (IBD), is gratefully acknowledged. We would like also to acknowledge the support of MOHE and UTM-RMC through HICOE grant no.R.J130000.7846.4 J262.

References

Ahn YJ, Park SJ, Lee SG, Shin SC, Choi DH (2006) Cordycepin: selective growth inhibitor derived from liquid culture of *cordyceps militaris* against *clostridium* spp. J Agric Food Chem 48(7):2744–2748

Aman S, Anderson DJ, Connolly TJ, Crittall AJ, Ji G (2000) From adenosine to 3 '-deoxyadenosine: development and scale up. Org Process Res Dev 4(6):601–605

Bok JW, Lermer L, Chilton J, Klingeman HG, Towers GN (1999) Antitumor sterols from the mycelia of *Cordyceps sinensis*. Phytochemistry 51(7):891–898

Buenz EJ, Bauer BA, Osmundson TW, Motley TJ (2005) The traditional Chinese medicine *Cordyceps sinensis* and its effects on apoptotic homeostasis. J Ethnopharmacol 96(1–2):19–29

Chang W, Lim S, Song H, Song BW, Kim HJ, Cha MJ, Sung JM, Kim TW, Hwang KC (2008) Cordycepin inhibits vascular smooth muscle cell proliferation. Eur J Pharmacol 597(1–3):64–69

Chen LS, Stellrecht CM, Gandhi V (2008) RNA-directed agent, cordycepin, induces cell death in multiple myeloma cells. Br J Haematol 140(6):682–391

Chen M, Cheung FW, Chan MH, Hui PK, Ip SP, Ling YH, Che CT, Liu WK (2012) Protective roles of *Cordyceps* on lung fibrosis in cellular and rat models. J Ethnopharmacol 143(2):448–454

Chen ZG, Zhang DN, Cao L, Han YB (2013) Highly efficient and region selective acylation of pharmacologically interesting cordycepin catalyzed by lipase in the eco-friendly solvent 2-methyltetrahydrofuran. Bioresour Technol 133:82–86

Cheng Z, He W, Zhou X, Lv Q, Xu X, Yang S, Zhao C, Guo L (2011) Cordycepin protects against cerebral ischemia/reperfusion injury in vivo and in vitro. Eur J Pharmacol 664(1–3):20–28

Cho HJ, Cho JY, Rhee MH, Park HJ (2007) Cordycepin (3′-deoxyadenosine) inhibits human platelet aggregation in a cyclic AMP-and cyclic GMP-dependent manner. Eur J Pharmacol 558(1–3):43–51

Choi S, Lim MH, Kim KM, Jeon BH, Song WO, Kim TW (2011) Cordycepin-induced apoptosis and autophagy in breast cancer cells are independent of the estrogen receptor. Toxicol Appl Pharmacol 257(2):165–173

Choi YH, Kim GY, Lee HH (2014) Anti-inflammatory effects of cordycepin in lipopolysaccharide-stimulated RAW 264.7 macrophages through Toll-like receptor 4-mediated suppression of mitogen-activated protein kinases and NF-κB signaling pathways. Drug Des Devel Ther 8:1941

Cory JG, Suhadolnik RJ, Resnick B, Rich MA (1965) Incorporation of cordycepin (3′-deoxyadenosine) into ribonucleic acid and deoxyribonucleic acid of human tumor cells. Biochim Biophys Acta 103(4):646–653

Cunningham KG (1951) Cordycepin, a metabolic product from cultures of Cordyceps militaris (Linn) Link. Part I. Isolation and characterization. J Chem Soc 2:2299–2302

Da Silva AS, Wolkmer P, Nunes JT, Duck MR, Oliveira CB, Gressler LT, Costa MM, Zanette RA, Mazzanti CM, Lopes ST, Monteiro SG (2011) Susceptibility of Trypanosoma evansi to cordycepin. Biomed Pharmacother 65(3):220–223

Das S, Masuda M, Sakurai A, Sakakibara M (2009) Effects of additives on cordycepin production using a *Cordyceps militaris* mutant induced by ion beam irradiation. Afr J Biotechnol 8(13):3041–3047

Das SK, Fujihara S, Masuda M, Sakurai A (2016) Efficient production of anticancer agent cordycepin by repeated batch culture of *Cordyceps militaris* mutant. WCECS 2010, San Francisco, USA. World Congress on Engineering and Computer Science 2010

Dou C, Cao Z, Ding N, Hou T, Luo F, Kang F, Yang X, Jiang H, Xie Z, Hu M, Xu J (2016) Cordycepin prevents bone loss through inhibiting osteoclastogenesis by scavenging ROS generation. Nutrients 8(4):231

El Enshasy H, Elsayed EA, Aziz R, Wadaan MA (2013) Mushrooms and truffles: historical biofactories for complementary medicine in Africa and in the middle East. Evid Based Complement Alternat Med 2013:1–10

El Enshasy HE (2010) Immunomodulators. In: Hofrichter M (ed) Mycota, 2nd edn. Springer Verlag, Heidelberg, pp 165–194

El Enshasy HA, Hatti-Kaul R (2013) Mushroom immunomodulators: unique molecules with unlimited applications. Trends Biotechnol 31(12):668–677

Fan DD, Wang W, Zhong JJ (2012) Enhancement of cordycepin production in submerged cultures of *Cordyceps militaris* by addition of ferrous sulfate. Biochem Eng J 60:30–35

Fan H, Li SP, Xiang JJ, Lai CM, Yang FQ, Gao JL, Wang YT (2006) Qualitative and quantitative determination of nucleosides, bases and their analogues in natural and cultured *Cordyceps* by pressurized liquid extraction and high performance liquid chromatography–electrospray ionization tandem mass spectrometry (HPLC–ESI–MS/MS). Anal Chim Acta 567(2):218–228

Fujita T, Inoue K, Yamamot OS, Ikumoto T, Sasaki S, Toyama R, Chiba K, Hoshino Y, Okumoto T (1994) Fungal metabolites. Part 11. A potent immunosuppressive activity found in *Isaria sinclairii* metabolite. J Antibiot (Tokyo) 47(2):208–215

Hansske F, Robins MJ (1985) Regiospecific and stereoselective conversion of ribonucleosides to 3-deoxynucleosides. A high yield three-stage synthesis of cordycepin from adenosine. Tetrahedron Lett 26(36):4295–4298

Holbein S, Wengi A, Decourty L, Freimoser FM, Jacquier A, Dichtl B (2009) Cordycepin interferes with 3′ end formation in yeast independently of its potential to terminate RNA chain elongation. RNA 15(5):837–849

Hsu TH, Shiao LH, Hsieh C, Chang DM (2002) A comparison of the chemical composition and bioactive ingredients of the Chinese medicinal mushroom Dong Chong XiaCao, its counterfeit and mimic, and fermented mycelium of *Cordyceps sinensis*. Food Chem 78(4):463–469

Hu Z, Lee CI, Shah VK, Oh EH, Han JY, Bae JR, Lee K, Chong MS, Hong JT, Oh KW (2013) Cordycepin increases nonrapid eye movement sleep via adenosine receptors in rats. Evid Based Complement Alternat Med:2013

Jeong JW, Jin CY, Kim GY, Lee JD, Park C, Kim GD, Kim WJ, Jung WK, Seo SK, Choi IW, Choi YH (2010) Anti-inflammatory effects of cordycepin via suppression of inflammatory mediators in BV2 microglial cells. Int Immunopharmacol 10(12):1580–1586

Jeong JW, Jin CY, Park C, Hong SH, Kim GY, Jeong YK, Lee JD, Yoo YH, Choi YH (2011) Induction of apoptosis by cordycepin via reactive oxygen species generation in human leukemia cells. Toxicol In Vitro 25(4):817–824

Jeong MH, Lee CM, Lee SW, Seo SY, Seo MJ, Kang BW, Jeong YK, Choi YJ, Yang KM, Jo WS (2013) Cordycepin-enriched *Cordyceps militaris* induces immunomodulation and tumor growth delay in mouse-derived breast cancer. Oncol Rep 30(4):1996–2002

Jiapeng T, Yiting L, Li Z (2014) Optimization of fermentation conditions and purification of cordycepin from *Cordyceps militaris*. Prep Biochem Biotechnol 44(1):90–106

Jung SM, Park SS, Kim WJ, Moon SK (2012) Ras/ERK1 pathway regulation of p27KIP1-mediated G1-phase cell-cycle arrest in cordycepin-induced inhibition of the proliferation of vascular smooth muscle cells. Eur J Pharmacol 681(1–3):15–22

Kang C, Wen TC, Kang JC, Meng ZB, Li GR, Hyde KD (2014) Optimization of large-scale culture conditions for the production of cordycepin with *Cordyceps militaris* by liquid static culture. Sci World J 2014:2014

Kiho T, Ukai ST (1995) (Semitake and others), Cordyceps species. Food Rev Int 11(1):231–234
Kim HG, Shrestha B, Lim SY, Yoon DH, Chang WC, Shin DJ, Han SK, Park SM, Park JH, Park HI, Sung JM (2006) Cordycepin inhibits lipopolysaccharide-induced inflammation by the suppression of NF-κB through Akt and p38 inhibition in RAW 264.7 macrophage cells. Eur J Pharmacol 545(2–3):192–199
Kim HO, Yun JW (2005) A comparative study on the production of exopolysaccharides between two entomopathogenic fungi *Cordyceps militaris* and *Cordyceps sinensis* in submerged mycelial cultures. J Appl Microbiol 99(4):728–738
Klenow H (1963) Formation of the mono-, di-and triphosphate of cordycepin in Ehrlich ascites-tumor cells in vitro. Biochim Biophys Acta (BBA) 76:347–353
Ko BS, Lu YJ, Yao WL, Liu TA, Tzean SS, Shen TL, Liou JY (2013) Cordycepin regulates GSK--3β/β-catenin signaling in human leukemia cells. PLoS One 8(9):e76320
Kodama EN, McCaffrey RP, Yusa K, Mitsuya H (2000) Antileukemic activity and mechanism of action of cordycepin against terminal deoxynucleotidyl transferase-positive (TdT+) leukemic cells. Biochem Pharmacol 59(3):273–281
Kubota Y, Kunikata M, Ishizaki N, Haraguchi K, Odanaka Y, Tanaka H (2008) Synthesis of 4-benzoyloxycordycepin from adenosine. Tetrahedron 64(10):2391–2396
Lee DH, Kim HH, Lim DH, Kim JL, Park HJ (2015) Effect of cordycepin-enriched WIB801C from *Cordyceps militaris* suppressing fibrinogen binding to glycoprotein IIb/IIIa. Biomol Ther (Seoul) 23(1):60–70
Lee SJ, Kim SK, Choi WS, Kim WJ, Moon SK (2009) Cordycepin causes p21WAF1-mediated G2/M cell-cycle arrest by regulating c-Jun N-terminal kinase activation in human bladder cancer cells. Arch Biochem Biophys 490(2):103–109
Lee SJ, Moon GS, Jung KH, Kim WJ, Moon SK (2010) c-Jun N-terminal kinase 1 is required for cordycepin-mediated induction of G2/M cell-cycle arrest via p21WAF1 expression in human colon cancer cells. Food Chem Toxicol 48(1):277–283
Leung PH, Wu JY (2007) Effects of ammonium feeding on the production of bioactive metabolites (cordycepin and exopolysaccharides) in mycelial culture of a *Cordyceps sinensis* fungus. J Appl Microbiol 103(5):1942–1949
Leung PH, Zhang QX, Wu JY (2006) Mycelium cultivation, chemical composition and antitumour activity of a *Tolypocladium* sp. fungus isolated from wild *Cordyceps sinensis*. J Appl Microbiol 101(2):275–283
Li SP, Li P, Lai CM, Gong YX, Kan KK, Dong TT, Tsim KW, Wang YT (2004) 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 1036(2):239–243
Lia J, Guanb M, Lic Y (2015) Effects of cooking on the contents of adenosine and cordycepin in *Cordyceps militaris*. In: The 7th World Congress on Particle Technology (WCPT7). Elsevier Ltd, China, pp 485–491
Lin YW, Chiang BH (2008) Anti-tumor activity of the fermentation broth of *Cordyceps militaris* cultured in the medium of Radix astragali. Process Biochem 43(3):244–250
Ling JY, Sun YJ, Zhang H, Lv P, Zhang CK (2002) Measurement of cordycepin and adenosine in stroma of *Cordyceps* sp. by capillary zone electrophoresis (CZE). J Biosci Bioeng 94(4):371–374
Ling JY, Zhang GY, Lin JQ, Cui ZJ, Zhang CK (2009) Supercritical fluid extraction of cordycepin and adenosine from *Cordyceps kyushuensis* and purification by high-speed counter-current chromatography. Sep Purif Technol 66(3):625–629
Ma L, Zhang S, Du M (2015) Cordycepin from *Cordyceps militaris* prevents hyperglycemia in alloxan-induced diabetic mice. Nutr Res 35(5):431–439
Mao XB, Eksriwong T, Chauvatcharin S, Zhong JJ (2005) Optimization of carbon source and carbon/nitrogen ratio for cordycepin production by submerged cultivation of medicinal mushroom *Cordyceps militaris* (2005). Process Biochem 40(5):1667–1672
Mao XB, Zhong JJ (2004) Hyperproduction of cordycepin by two-stage dissolved oxygen control in submerged cultivation of medicinal mushroom *Cordyceps militaris* in bioreactors. Biotechnol Prog 20(5):1408–1413

Mao XB, Zhong JJ (2006) Significant effect of NH4+ on cordycepin production by submerged cultivation of medicinal mushroom *Cordyceps militaris*. Enzym Microb Technol 38(3–4):343–350

Masuda M, Das SK, Fujihara S, Hatashita M, Sakurai A (2011) Production of cordycepin by a repeated batch culture of a *Cordyceps militaris* mutant obtained by proton beam irradiation. J Biosci Bioeng 111(1):55–60

Masuda M, Das SK, Hatashita M, Fujihara S, Sakurai A (2014) Efficient production of cordycepin by the *Cordyceps militaris* mutant G81-3 for practical use. Process Biochem 49(2):181–187

Masuda M, Urabe E, Honda H, Sakurai A, Sakakibara M (2007) Enhanced production of cordycepin by surface culture using the medicinal mushroom *Cordyceps militaris*. Enzym Microb Technol 40(5):1199–1205

Masuda M, Urabe E, Sakurai A, Sakakibara M (2006) Production of cordycepin by surface culture using the medicinal mushroom *Cordyceps militaris*. Enzym Microb Technol 39(4):641–646

Mizuno T (1999) Medicinal effects and utilization of Cordyceps (Fr.) Link (Ascomycetes) and Isaria Fr. (Mitosporic Fungi) Chinese Caterpillar Fungi, "Tochukaso". Int J Med Mushrooms 1(3):251–261

Muller WE, Weiler BE, Charubala R, Pfleiderer W, Leserman L, Sobol RW (1991) Cordycepin analogues of 2′, 5′ -oligoadenylate inhibit human immunodeficiency virus infection via inhibition of reverse transcriptase. Biochemistry 30:2027–2033

Nakamura K, Konoha K, Yoshikawa N, Yamaguchi Y, Kagota S, Shinozuka K, Kunitomo MN (2005) Effect of cordycepin (3′-deoxyadenosine) on hematogenic lung metastatic model mice. In Vivo 19(1):137–141

Ng TB, Wang HX (2005) Pharmacological actions of *Cordyceps*, a prized folk medicine. J Pharm Pharmacol 57(12):1509–1519

Ni H, Zhou XH, Li HH, Huang WF (2009) Column chromatographic extraction and preparation of cordycepin from Cordyceps militaris waster medium. J Chromatogr B 877(22):2135–2141

Overgaard-Hansen K (1964a) The inhibition of 5-phosphoribosyl-1-pyrophosphate formation by cordycepin triphosphate in extracts of Ehrlich ascites tumor cells. Biochim Biophys Acta 80(3):504–507

Overgaard-Hansen K (1964b) The inhibition of 5-phosphoribosyl-1-pyrophosphate formation by cordycepin triphosphate in extracts of Ehrlich ascites tumor cells. Biochim Biophys Acta 80:504–507

Pan BS, Wang YK, Lai MS, Mu YF, Huang BM (2015) Cordycepin induced MA-10 mouse Leydig tumor cell apoptosis by regulating p38 MAPKs and PI3K/AKT signaling pathways. Sci Rep 25(5):13372

Park HJ (2015) Ethanol extract of *Cordyceps militaris* grown on germinated soybeans inhibits 2, 4-dinitrophenolfluorobenzene-induced allergic contact dermatitis. J Funct Foods 1(17):938–947

Pegler DN, Yao YJ, Li Y (1994) The Chinese 'caterpillar fungus'. Mycologist 8(1):3–5

Peng J, Wang P, Ge H, Qu X, Jin X (2015) Effects of cordycepin on the microglia-overactivation-induced impairments of growth and development of hippocampal cultured neurons. PLoS One 10(5):e0125902

Ramesh T, Yoo SK, Kim SW, Hwang SY, Sohn SH, Kim IW, Kim SK (2012) Cordycepin (3′-deoxyadenosine) attenuates age-related oxidative stress and ameliorates antioxidant capacity in rats. Exp Gerontol 47(12):979–987

Rao YK, Chou CH, Tzeng YM (2006) A simple and rapid method for identification and determination of cordycepin in *Cordyceps militaris* by capillary electrophoresis. Anal Chim Acta 566(2):253–258

Ren Z, Cui J, Huo Z, Xue J, Cui H, Luo B, Jiang L, Yang R (2012) Cordycepin suppresses TNF-α-induced NF-κB activation by reducing p65 transcriptional activity, inhibiting IκBα phosphorylation, and blocking IKKγ ubiquitination. Int Immunopharmacol 14(4):698–703

Rich MA, Meyers P, Weinbaum G, Cory JG, Suhadolnik RJ (1965) Inhibition of human tumor cells by cordycepin. Biochim Biophys Acta 95(2):194–204

Rottman F, Guarino AJ (1964) The inhibition of phosphoribosyl-pyrophosphate amidotransferase activity by cordycepin monophosphate. Biochem Biophys Acta 89:465–472

Soltani M, Kamyab H, El-Enshasy HA (2013) Molecular weight (Mw) and Monosaccharide composition (MC): Two major factors affecting the therapeutic action of polysaccharides extracted from *Cordyceps sinensis*. J Pure Appl Microbio 7(3):160111613

Soltani M, Abd Malek R, Ware I, Ramli S, Elsayed EA, Aziz R (2017) Optimization of cordycepin extraction from *Cordyceps militaris* fermentation broth. J Sci Ind Res 76:355–361

Suhadolnik RJ, Cory JG (1964) Further evidence for the biosynthesis of cordycepin and proof of the structure of 3-deoxyribose. Biochim Biophys Acta 91:661–662

Sun Y, Wang YH, Qu K, Zhu HB (2011) Beneficial effects of cordycepin on metabolic profiles of liver and plasma from hyperlipidemic hamsters. J Asian Nat Prod Res 13(06):534–546

Tang YJ, Zhu LW, Li HM, Li DS (2007) Submerged culture of mushrooms in bioreactors-challenges, current state-of-the-art, and future prospects. Food Technol Biotechnol 45(3): 221–229

Tuli HS, Sandhu SS, Kashyap D, Sharma AK (2014a) Optimization of extraction conditions and antimicrobial potential of a bioactive metabolite, cordycepin from *Cordyceps militaris* 3936. WJPPS 3:1525–1535

Tuli HS, Sandhu SS, Sharma AK (2014b) Pharmacological and therapeutic potential of *Cordyceps* with special reference to Cordycepin. 3 Biotech 4(1):1–2

Tuli HS, Sharma AK, Sandhu SS (2014c) Optimization of fermentation conditions for cordycepin production using *Cordyceps militaris* 3936. J Biol Chem Sci 1:35–47

Velut S, de Maré L, Hagander P (2007) Bioreactor control using a probing feeding strategy and mid-ranging control. Control Eng Pract 15(2):135–147

Wang B-J, Won S-J, Yu Z-R, Su C-L (2005) Free radical scavenging and apoptotic effects of *Cordyceps sinensis* fractionated by supercritical carbon dioxide. Food Chem Toxicol 43:543–552

Wang H-J, Pan M-C, Chang C-K, Chang S-W, Hsieh C-W (2014) Optimization of ultrasonic-assisted extraction of cordycepin from *Cordyceps militaris* using orthogonal experimental design. Molecules 19:20808–20820

Wasser SP (2002) Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Appl Microbiol Biotechnol 60:258–274

Wei HP, Ye XL, Chen Z, Zhong YJ, Li PM, Pu SC, Li XG (2009) Synthesis and pharmacokinetic evaluation of novel N-acyl-cordycepin derivatives with a normal alkyl chain. Eur J Med Chem 44:665–669

Won S-Y, Park E-H (2005) Anti-inflammatory and related pharmacological activities of cultured mycelia and fruiting bodies of *Cordyceps militaris*. J Ethnopharmacol 96:555–561

Wong YY, Moon A, Duffin R, Barthet-Barateig A, Meijer HA, Clemens MJ, de Moor CH (2009) Cordycepin inhibits protein synthesis and cell adhesion through effects on signal transduction. J Biol Chem 23:jbc-M109

Wu C, Guo Y, Su Y, Zhang X, Luan H, Zhang X, Zhu H, He H, Wang X, Sun G, Sun X, Guo P, Zhu P (2014) Cordycepin activates AMP-activated protein kinase (AMPK) via interaction with the γ1 subunit. J Cell Mol Med 18:293–304

Wu JY, Zhang QX, Leung PH (2007) Inhibitory effects of ethyl acetate extract of *Cordyceps sinensis* mycelium on various cancer cells in culture and B16 melanoma in C57BL/6 mice. Phytomedicine 14:43–49

Xie Huichun YL, Lao D, Zhang T, Ito Y (2011) Preparative separation of high-purity cordycepin from *Cordyceps militaris*(L.) link by high-speed countercurrent chromatography. J Liq Chromatogr Relat Technol 34:491–499

Xie J-W, Huang L-F, Hu W, He Y-B, Wong KP (2010) Analysis of the Main Nucleosides in *Cordyceps Sinensis* by LC/ESI-MS. Molecules 15:305–314

Yalin W, Cuirong S, Yuanjiang P (2006) Studies on isolation and structural features of a polysaccharide from the mycelium of a Chinese edible fungus (*Cordyceps sinensis*). Carbohydr Polym 63:251–256

Yang F-Q, Ge L, Yong JWH, Tan SN, Li S-P (2009) Determination of nucleosides and nucleobases in different species of *Cordyceps* by capillary electrophoresis–mass spectrometry. J Pharm Biomed Anal 50:307–314

Yang FQ, Guan J, Li SP (2007) Fast simultaneous determination of 14 nucleosides and nucleobases in cultured *Cordyceps* using ultra-performance liquid chromatography. Talanta 73:269–273

Yang QZ, Yang J, Zhang CK (2006) Synthesis and properties of cordycepin intercalates of Mg–Al–nitrate layered double hydroxides. Int J Pharm 326:148–152

Yang FQ, Li DQ, Feng K, Hu DJ, Li SP (2010) Determination of nucleotides, nucleosides and their transformationproducts in Cordyceps by ion-pairing reversed-phase liquid chromatography-mass spectrometry. J Chromatogr A.34:5501–5510. https://doi.org/10.1016/j.chroma.2010.06.062.

Yang X, Li Y, He Y, Li T, Wang W, Zhang J, Wei J, Deng Y, Lin R (2015) Cordycepin alleviates airway hyperreactivity in a murine model of asthma by attenuating the inflammatory process. Int Immunopharmacol 26:401–408

Yao L-H, Li C-H, Yan W-W, Huang J-N, Liu W-X, Xiao P (2011) Cordycepin decreases activity of hippocampal CA1 pyramidal neuron through membrane hyperpolarization. Neurosci Lett 503:256–260

Yao L-H, Meng W, Song R-F, Xiong Q-P, Sun W, Luo Z-Q, Yan W-W, Li Y-P, Liu X-P, Li H-H, Xiao P (2014) Modulation effects of cordycepin on the skeletal muscle contraction of toad gastrocnemius muscle. Eur J Pharmacol 726:9–15

Yoshikawa N, Nakamura K, Yamaguchi Y, Kagota S, Shinozuka K, Kunitomo M (2004) Antitumor activity of cordycepin in mice. Clin Exp Pharmacol Physiol 31:S51–S53

Yu R, Song L, Zhao Y, Bin W, Wang L, Zhang H (2004a) Isolation and biological properties of polysaccharide CPS-1 from cultured *Cordyceps militaris*. Fitoterapia 75:465–472

Yu R, Wang L, Zhang H, Zhou C, Zhao Y (2004b) Isolation, purification and identi- fication of polysaccharides from cultured *Cordyceps militaris*. Fitoterapia 75:662–666

Yun YH, Han SH, Lee SJ, Ko SK, Lee CK, Ha NJ, Kim KJ (2003) Anti-diabetic effects of CCCA, CMESS, and cordycepin from *Cordyceps militaris* and the immune responses in Streptozotocin-induced diabetic mice. Nat Prod Res 9:291–298

Zhang DW, Deng H, Qi W, Zhao GY, Cao R (2015) Osteoprotective effect of cordycepin on estrogen deficiency-induced osteoporosis in vitro and in vivo. Biomed Res Int 2015:423869

Zhang Z, Tudi T, Liu Y, Zhou S, Feng N, Yang Y, Tang C, Tang Q, Zhang J (2016) Preparative isolation of cordycepin, N 6 -(2-hydroxyethyl)-adenosine and adenosine from Cordyceps militaris by macroporous resin and purification by recycling high-speed counter-current chromatography. Journal of Chromatography B 1033-1034:218–225

Zheng P, Xia Y, Xiao G, Xiong C, Hu X, Zhang S, Zheng H, Huang Y, Zhou Y, Wang S, Zhao GP (2012) Genome sequence of the insect pathogenic fungus *Cordyceps militaris*, a valued traditional Chinese medicine. Genome Biol 12(11):R116

Zheng ZL, Qiu XH, Han RC (2015) Identification of the genes involved in the fruiting body production and cordycepin formation of *Cordyceps militaris* fungus. Mycobiology 43:37–42

Zhou X, Meyer CU, Schmidtke P, Zepp F (2002) Effect of cordycepin on interleukin-10 production of human peripheral blood mononuclear cells. Eur J Pharmacol 453:309–317

Zhu JS, Halpern GM, Jones K (1998) The scientific rediscovery of an ancient Chinese herbal medicine: *Cordyceps sinensis*: part I. J Altern Complement Med 4:289–303