Chapter 16
Cordycepin: A Biotherapeutic Molecule from Medicinal Mushroom

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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
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 fruiting 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-known *Cordyceps* 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) *antioxidant* (Wang et al. 2005; Ramesh et al. 2012; Ren et al. 2012;), *antidiabetic* (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 adenine deaminase inhibitor and this will cause the inhibitory 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 as 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.

![Molecular structure of adenosine, deoxyadenosine and cordycepin (3′-deoxyadenosine)](image)

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: Cordycepin, 3′-Deoxyadenosine          |
| Identifiers                                | CAS number: 73–03-3                                  |
|                                             | PubChem: 6303                                       |
|                                             | ChemSpider: 6064                                    |
|                                             | ChEMBL: CHEMBL305686                                |
|                                             | InChIKey: OFEZSMBBKLBJ-                             |
| Properties                                 | Molecular formula: C_{10}H_{13}N_{5}O_{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).

![Diagram of Codyceps militaris adenine metabolic pathway](image-url)

**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 Coduceptin: 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 polyA 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. mTOR can 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 nutrutional 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, 1H 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/β-catenin survival pathway to develop leukemia stem cells (LSCs), whether cordycepin regulates expression of β-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 β-catenin in the specific cancer cell line including THP1, K562 and U937 was resulted. GSK-3β 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 β-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 IC50 (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 G2) 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 IC50 over 200 μg/ml. Tumor cells
accumulation at sub-G1 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-CO2 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 IkBα degradation, cordycepin inhibited phosphorylation of IkBα. 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, IkB 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, annull ed cordycepin-intervened p21WAF1 expression, cell development restraint, and diminished proteins cell 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 immunodeficiency (SCID) mice in vivo. The outcome of study shows that cordycepin is significantly selective treatment highly effective in induction of 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 cyclooxygenase-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 IκB-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 Cordycepin

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 exsistanceof different wide range of concentrations of CaCl2 was reported thoroughly. Of thromboxane A2 (TXA2) and cytosolic freeCa2+ ([Ca2+]i), as two aggregation-inducing molecules, up to 74% of up-regulation of [Ca2+]i was blocked with cordycepin (500 μM) while for TXA2 production, it was suppressed by 46%. Afterward, phosphorylation of Ca2+- 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+}]. (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 and extracellular 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 cyclooxygenase-2 (COX-2) correlated to hyperplasia 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 enhanced in reperfusion/ischemia set, 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 adenosinedeaminase 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 nonspecific 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 focus on the in vitro cordycepin effects on osteoclastogenesis as well as 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 as an osteoclast inhibitor and have big ability therapeutic effect especially effective in avoiding bone 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 cultivation of a *Cordyceps militaris* 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, \( \text{NH}_4^+ \) has significant role in cordycepin production. To improve production of cordycepin, in this, \( \text{NH}_4^+ \) 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 was 4.1 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 production in 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 to 596.59 ± 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), IMP dehydrogenase (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 (\( \text{K}_2\text{HPO}_4 \)) 0.3%, Potassium phosphate monobasic (\( \text{KH}_2\text{PO}_4 \)) 0.1%, sodium chloride (NaCl) 0.05%, Magnesium Sulfate (\( \text{MgSO}_4 \)) 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, \([\text{Mg–Al–NO}_3]\). 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 \((T_{\text{max}})\) might be stretched with the alkyl chain length expansion, however concentration time curve \((\text{AUC})\) and maximum concentration \((C_{\text{max}})\) enhanced at first, then diminished when the number of alkyl carbon surpassed eight. The \(C_{\text{max}}\), \(T_{\text{max}}\) 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 acylation with an immobilized species of \(\text{Candida antartica}\) 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 \(\text{Cordyceps militaris}\) 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 \(\text{Cordyceps militaris}\) (Zheng et al. 2015).

In recent years, several methods have been applied in command to enhance the 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 \(\text{Cordyceps militaris}\) 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 established 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 determined 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/mL varieties of cordycepin concentration, linearity was found clearly. It should be noted that enhanced technique has already been practical for 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-C18 column 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 CO2as 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, ¹H NMR, ¹³C NMR and MS (Xie Huichun et al. 2011). Recent study showed simple optimized solvent system composed of: water (23%), ethanol (30%), methanol (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
| 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 3986** | 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.

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