PHYTOTHERAPY RESEARCH
Phytother. Res. (2016)
Published online in Wiley Online Library
(wileyonlinelibrary.com) **DOI**: 10.1002/ptr.5673

REVIEW

The Mechanisms of Pharmacological Activities of *Ophiocordyceps sinensis* Fungi

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The entomopathogenic fungus *Ophiocordyceps sinensis*, formerly known as *Cordyceps sinensis*, has long been used as a traditional Chinese medicine for the treatment of many illnesses. In recent years its usage has increased dramatically because of the improvement of people's living standard and the emphasis on health. Such demands have resulted in over-harvesting of this fungus in the wild. Fortunately, scientists have demonstrated that artificially cultured and fermented mycelial products of *O. sinensis* have similar pharmacological activities to wild *O. sinensis*. The availability of laboratory cultures will likely to further expand its usage for the treatment of various illnesses. In this review, we summarize recent results on the pharmacological activities of the components of *O. sinensis* and their putative mechanisms of actions. Copyright © 2016 John Wiley & Sons, Ltd.

Keywords: Ophiocordyceps sinensis; entomopathogenic fungus; traditional Chinese medicine; bioactive components; pharmacological functions; functional mechanisms.

INTRODUCTION

The ascomycete *Ophiocordyceps sinensis* (Berk.) Sung, Sung, Hywel-Jones & Spatafora (Sung *et al*., 2007), formerly known as *Cordyceps sinensis* (Berk.) Sacc., is a entomopathogenic fungus that parasitizes larvae of moths and produces a fruiting body valued as a herbal remedy (Chen *et al*., 2010a; He *et al*., 2013; Meng *et al*., 2014; Wang *et al*., 2011; Wu *et al*., 2014). *O. sinensis* is widely distributed on Tibetan Plateau and its surrounding regions (Liang *et al*., 2008; Quan *et al*., 2014; Zhang *et al*., 2009). In China, it is commonly called 'Dong Chong Xia Cao' (means 'winter worm summer grass') and has been used as a medication in China for over 300 years (Wang, 1694). Nowadays, this fungus is widely used in Traditional Chinese Medicine for the treatment of inflammation, cancer, chronic kidney disease (CKD), weakness after sickness, sexual dysfunction, etc. (Chang *et al*., 2015; Chen *et al*., 2010a; He *et al*., 2013; Meng *et al*., 2014; Wang *et al*., 2011; Wu *et al*., 2014).

O. sinensis has been officially classified as an endangered species in 2012 by the Convention on International Trade in Endangered Species (CITES) Management Authority of China because of limited wild population and over-harvesting (CITES Management Authority of China, 2012). A recent report (Shrestha *et al*., 2014) indicated that the per-capita harvest of *O. sinensis* has declined over the last 4 years because of the increasing number of harvesters and the decline in the stock. Fortunately, thanks to the biotechnology progress in recent years, artificially cultured and fermented mycelial products of *O. sinensis* have shown to have similar pharmacological activities to wild *O. sinensis* although the type and quantity of bioactive compounds synthesized by wild *O. sinensis* may differ from those by the cultured mycelium (Singh *et al*., 2014; Wang *et al*., 2015a; Zhou *et al*., 2014). These findings will not only reduce the pressure on natural resources of the species but also will increase the usage of this fungus in health treatment.

The pharmacological activities of *O. sinensis* have been noticed at least three hundred years ago in China (Nie *et al*., 2013) and have now attracted extensive attention in many parts of the world. However, robust scientific evidences on its health effects are still lacking and its functional mechanisms are still poorly understood. Nevertheless, great progress has been achieved in recent years on the study of pharmacological activities and mechanisms of action of *O. sinensis* (Chang *et al*., 2015; Chen *et al*., 2010a; He *et al*., 2013; Meng *et al*., 2014; Wang *et al*., 2011; Wu *et al*., 2014). Indeed, several recent reviews have been published on the biology, bioactive and pharmacological properties of *O. sinensis* (e.g. Liu *et al*., 2015; Nakamura *et al*., 2015; Shashidhar *et al*., 2015; Shashidhar *et al*., 2013; Zhou *et al*., 2014; Zhou *et al*., 2009). However, its functional mechanisms have been neglected to some extent. In this review, we summarize and discuss recent findings on functional activities and mechanisms in *O. sinensis* (summarized in Table 1 and reviewed in detail in the following).

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Received 20 January 2016
Revised 02 June 2016
Accepted 03 June 2016

Table 1. Functional activities and mechanisms of *Ophiocordyceps sinensis*

Functional activities	Key functional mechanisms
Immunomodulating	Improve ovalbumin-specific IgG, IgG1 and IgG2b levels (Wu *et al.*, 2006).Promote proliferation and phagocytosis of macrophages, and stimulate macrophages to release IL-lβ, TNF-α and INF-γ (Hu *et al.*, 2016) and the reactive free radical NO and multiple chemokines and cytokines (Wu *et al.*, 2014), by stimulating the IkB–NF-kB pathway (He *et al.*, 2013).Activate myeloid dendritic cells by via a Toll-like receptor 9-dependent pathway (Xiao *et al.*, 2010) and induce differentiation of macrophages into dendritic-like cells and promoted phenotypic and functional maturation of dendritic cells in mouse (Meng *et al.*, 2014).Induce the cell proliferation of T-lymphocytes and the secretion of IL-2, IL-6 and IL-8 by these cells (Sheng *et al.*, 2011).
Antiinflammation	Reduce the production of NO, IL-12, TNF-α and pro-inflammatory cytokines (Liu *et al.*, 2011).Block nuclear NF-kB through decreasing extracellular signal-regulated kinase 1/2 signaling pathway (Chiou and Lin, 2012).
Antioxidant	Attenuate the changes of glutathione peroxidase and superoxide dismutase activities in cells (Li *et al.*, 2003).Promote the activities of glutathione peroxidase, catalase and superoxide dismutase but reduce the levels of lactate dehydrogenase and malondialdehyde (Shen *et al.*, 2011).Upregulation of the activity of copper-zinc-containing superoxide dismutase 1 and catalase and inhibition of lipofuscin accumulation (Zou *et al.*, 2015).
Inhibit apoptosis	Decrease reactive oxygen species production and regulating apoptotic signaling (Liu *et al.*, 2013).Inhibit apoptosis by down-regulating apoptotic genes, such as Fas, Fas ligand and TNF-α (Shahed *et al.*, 2001).
Induce apoptosis	Inhibit the tyrosine phosphorylation of Bcl-2 and Bcl-xL oncoproteins (Yang *et al.*, 2003).Induce apoptosis through caspase/MAPK pathways (Chen *et al.*, 2014b).By increasing p53 phosphorylation and expression followed by the downstream cleavage of caspase-7 and poly(ADP-ribose) polymerase (Chen *et al.*, 2014b).
Antitumor	By immunomodulating (Meng *et al.*, 2014), antioxidating (Lee *et al.*, 2015) and inducing tumor cell apoptosis (Chen *et al.*, 2014b).Stimulate phagocytic activity of macrophages and induce these cells to produce TNF-α, IL-2, IL-6 and other cytokines (Song *et al.*, 2013).By promoting of dendritic cells' maturation and activation through the inhibition of STAT3 phosphorylation (Song *et al.*, 2011).Regulation of signal pathway, such as inhibition of the MAPK signaling pathway that play important roles in the invasion and migration of cancer cells, activation of the PKC signaling pathways that has a direct antitumor effect (Pao *et al.*, 2012).By stimulating of adenosine A3 receptor, followed by the Wnt signaling pathway, including GSK-3b activation and cyclin D1 suppression (Nakamura *et al.*, 2015).Through inhibiting platelet aggregation induced by cancer cells and suppressing the invasiveness of cancer cells via inhibiting the activity of MMP-2 and MMP-9, and accelerating the secretion of TIMP-1 and TIMP-2 from cancer cells (Nakamura *et al.*, 2015).Inhibit the secretion of migration and invasion related factors by cancer cells, such as MMP-2, MMP-9 and uPA (Jiang and Sliva, 2010).
Cardiovascular protection	Prevent cholesterol deposition by inhibition of LDL oxidation mediated by free radicals (Yamaguchi *et al.*, 2000). Prohibit the activity of cholesterol esterase (Kim, 2010) and thus prohibit absorption of dietary cholesterol esters and lead to prevention of atherosclerosis (Jeon *et al.*, 2000).Cleave the Aα chain of fibrinogen and the α-chain of fibrin to prevent the thrombosis (Li *et al.*, 2007).Inhibit human platelet activation by initially activate adenylate cyclase/cyclic AMP and, subsequently, inhibit intracellular signals (such as MAPKs), ultimately inhibiting platelet activation (Lu *et al.*, 2014) to prevent the thrombosis.Antihypertension by stimulating the secretion of vasodilator NO, decreasing the level of endothelin-1, epinephrine, noradrenaline and angiotensin II, inhibiting the increase of TGF-β1 and lowering the level of inflammatory mediator of C-reactive protein (Xiang *et al.*, 2016).Therapeutic activity on ischemic stroke through the inhibiting damage induced by oxygen and glucose deprivation (Zou *et al.*, 2016).
Kidney protection	Inhibition of the pro-fibrotic cytokine TGF-β1/Smad signal pathway dependent epithelial to mesenchymal transdifferentiation and attenuate renal fibrosis (Yao *et al.*, 2014).Inhibition of the proliferation of glomerular mesangial cell (Zhao-Long *et al.*, 2000) through the PDGF/ERK and TGF-β1/Smad pathways (Wang *et al.*, 2014). Inhibiting PDGF homodimer BB induced inflammation and ROS production (Wang *et al.*, 2015b).
Reproduction protection	Stimulate leydig cells and granulosa-lutein cells to produce sex steroids (Chen *et al.*, 2005; Huang *et al.*, 2004b). Increased E_2 production (Huang *et al.*, 2004a). E_2 is the most important granulosa-derived hormone affecting the properties of oocytes.Activate both PKA and PKC signal transduction pathways to stimulate, StAR protein, to cell steroidogenesis (Chen *et al.*, 2005).Stimulate intracellular PLC/PKC and MAPK signal transduction pathways (Pao *et al.*, 2012) and associate with Ars to activate cAMP-PKA-StAR pathway (Leu *et al.*, 2011) to induce steroidogenesis.
Prevention of osteoporosis	Modulate ovarian steroidogenesis (Chen *et al.*, 2005) to prevent bone loss and osteoporosis caused by estrogen deficiency (Qi *et al.*, 2011).By decrease serum alkaline phosphatase activity, TRAP activity, CTX level and IFN-γ level (Zhang *et al.*, 2014a).

TAXONOMY, BIOLOGY, DISTRIBUTION AND ARTIFICIAL CULTURES OF *O. SINENSIS*

The *O. sinensis* was first named scientifically as *Sphaeria sinensis* in 1843 by Miles Berkeley and was then transferred to the genus *Cordyceps by* Pier Andrea Saccardo in 1878. The name *Cordyceps* is from the two Latin words 'cord' (means club) and 'ceps' (means head), and *sinensis* means from China. *Cordyceps* Fr. was historically classified in the family *Clavicipitaceae* and has been recognized as the most diverse genus in the family because of the great number of species (more than 400) (Stensrud *et al.*, 2005) and host range (ten orders of arthropods) (Kobayasi, 1982). Based on molecular phylogenetic techniques, Sung *et al.* (2007) emended the classification of the family Cordycipitaceae and the family Clavicipitaceae. Consequently, the original

megagenus Cordyceps was separated into four genera, *Ophiocordyceps*, *Cordyceps*, *Elaphocordyceps* and *Metacordyceps*. A new family *Ophiocordycipitaceae* also was proposed based on *Ophiocordyceps* Petch. The *C. sinensis* was transferred to the genus *Ophiocordyceps*, hence renamed as *O. sinensis*.

Ophiocordyceps are insect parasitizing fungi with a broad host range but most species are restricted to single or a set of closely related host species (reviewed in Yue *et al*., 2013). The *O. sinensis* parasitizes larvae of moths from the order Lepidoptera, particularly *Hepialus*/*Thitarodes* (Wang and Yao, 2011). The infection process has been studied in a limited number of species, but is generally thought to be similar across all known taxa (Clarkson and Charnley, 1996). An infection initiates with a spore or spores adhering to the exoskeleton of the host and then an infection peg develops and penetrates through the exoskeleton via mechanical pressure and the production of lipases and proteases. After invading, the fungus ramifies throughout the caterpillar and eventually converted it into a sclerotium to withstand the winter, which is called as 'winter worm'. In the next spring or summer, a sexual sporulating structure (a perithecial stroma) of the fungus grows from the larval head and grows upward to emerge from the soil, appearing as a herb, which is thus called as 'summer grass' (Xing and Guo, 2008).

O. sinensis is distributed mainly in the Tibetan Plateau and its surrounding regions, including Tibet, Gansu, Qinghai, Sichuan and Yunnan provinces in China and in certain areas of the southern flank of the Himalayas, in the countries of Bhutan, India and Nepal. Its habitat is very restricted, usually found in the soil of a prairie at an altitude from 3500 to 5000 m with 3000 m as the lowest altitude for the distribution (Quan *et al*., 2014). A substantial intraspecific genetic diversity has been observed in *O. sinensis*, which may be because of the diversity of terrains and climates on the Tibetan Plateau and the broad insect host range (more than 50 species in the family *Hepialidae*) (Quan *et al*., 2014; Zhang *et al*., 2009). The genetic divergence of *O. sinensis* was significantly greater in southern isolates than that of northern isolates (Zhang *et al*., 2009). However, in the four tested host insects, quite lower genetic diversity were found in comparison with that of *O. sinensis* populations (Quan *et al*., 2014). In a recent study by Zhang *et al*. (2014b), both significant congruence and significant incongruences were found on phylogenies between *O. sinensis* and its host insects, suggesting that the occurrence of cospeciation events, and the host shifts were also prevalent. These findings provide valuable information to protect and sustainable use of *O. sinensis* and as well as its host insects.

O. sinensis has a sexual stage (teleomorph) and an asexual stage (anamorph). Because of overharvest and limited yield, the market price of natural *O. sinensis* (teleomorph) has increased by up to 2300% over the last 10 years, which is more expensive than gold. Commercially attempts to develop an efficient technology for cultivation of fruiting bodies have yet to succeed. Anamorphic mycelia produced by fermentations thus was widely used as the alternative of natural *O. sinensis*. Industrial cultured mycelial products of *O. sinensis* have shown to have similar pharmacological activities to wild *O. sinensis* although the type and quantity of bioactive compounds synthesized by wild *O. sinensis* may differ from those by the cultured mycelium (Singh *et al*., 2014; Wang *et al*., 2015a; Zhou *et al*., 2014). For example, the levels of polysaccharide, adenine and adenosine in cultured sample amounted to 10.43%, 0.50 mg/g and 2.61 mg/g, respectively, considerably higher than the natural ones (8.90%, 0.11 mg/g and 0.96 mg/g) (Wang *et al*., 2015a). On the contrary, the content of mannitol in natural samples was significantly higher, ranging from 9.85% to 11.51%, while in the cultured sample the value was less than half.

BIOACTIVE COMPOUNDS OF *O. SINENSIS*

Scientists have extracted and purified a variety of bioactive compounds from *O. sinensis*, such as cordycepin, cordycepic acid, polysaccharides, ergosterol, nucleosides and other compounds. Many studies have shown that these compounds have obvious pharmacological property either in single or mixed conditions. A recent study by Wang *et al*. (2015a) has demonstrated that the artificially cultured *O. sinensis* produced higher levels of polysaccharide, adenine and adenosine than those of the natural ones. In addition, their study also showed that water extracts of cultured sample had a stronger antioxidant capacity.

Cordycepin and cordycepic acid

Cordycepin, which formula is 3′-deoxyadenosine, was first extracted from *Cordyceps militaris in* 1951 and from *O. sinensis* in 1996. In *O. sinensis*, the content of cordycepin in the fermented mycelia is 40.8 mg/g (Wang *et al*., 2005), which is higher than that in the fruiting bodies (<5.4 mg/g) (Hsu *et al*., 2002). Cordycepin has been reported to have numerous biological activities and been used to alleviate a large variety of ailments, such as the inhibition of inflammation (Kim *et al*., 2006), cell proliferation (Nakamura *et al*., 2006) and platelet activation (Chang *et al*., 2015). Wong *et al*. (2010) have suggested that many of the reported biological effects of cordycepin are likely to be because of its effects on mammalian target of rapamycin (mTOR) and the AMP-activated kinase (AMPK) signaling.

Cordycepic acid is an isomer of quinic acid, which was first isolated from *O. sinensis* in 1957 (Chatterjee *et al*., 1957) and was identified as d-mannitol soon after (Sprecher and Sprinson, 1963). Mannitol is a major bio-product of plants, particularly in edible fungus, lichens and carrot. In *O. sinensis*, the content of cordycepic acid is 7–29%, differing in the various growing stages (Jiang, 1987); the cordycepic acid content in the mycelia is higher than in fruiting bodies (Xu, 1997). Cordycepic acid has been shown as one of the main active medicinal compounds, and it is now used in injections as raw material and as a supplement in other medicines.

Polysaccharides

Polysaccharides are a class of structurally diverse macromolecules with demonstrable bioactivities such as anti-tumor, antioxidation and immunological properties.

Polysaccharides are multi-branched galactomannan, which have a multi-branched structure and multifarious linkages between adjacent monosaccharides to form small rings and helical structures. Polysaccharides usually are colorless and odorless and have good solubility and stability in water. The structures of the polysaccharides from natural *O. sinensis* were first studied by Miyazaki *et al.* (1977). Since then, much of scientific investigation in China and Japan has been performed to discover possible functional polysaccharides.

Polysaccharides are well known also in all other fungi because they are mainly the components of normal fungal cell membrane and cell wall. The most common class of immunomodulating and antitumor polysaccharides from medicinal fungi is comprised by the glucans with various glycosidic linkages, such as β-(1→3)-, β-(1→6)-, α-(1→3)- and α-(1→4)-glucans (Borchers *et al.*, 1999; Vannucci *et al.*, 2013).

Polysaccharides usually can found in the fruiting bodies, the fermented mycelia and the fermentation cultures and broth, with a concentration of 3–8% in *O. sinensis* (Li *et al.*, 2002).

More and more studies have demonstrated that polysaccharides from *O. sinensis* have multiple bioactivities, such as immunomodulating, antioxidant, inhibit and induce apoptosis, antitumor and cardiovascular protection. Therefore, further studies upon the isolation and purifying of polysaccharides and the functional processes and mechanisms of polysaccharides will lead to greater findings and the usage of *O. sinensis*.

Other bioactive compounds

Besides the above-mentioned key bioactive compounds, many other bioactive components have been extracted from *O. sinensis*, including nucleotides, ergosterol, fatty acids, crude protein, amino acids and metal elements, and they manifest a wide range of pharmacological functions.

Nucleotides, including adenosine, uridine and guanosine, are effective components in *O. sinensis* (Xiao *et al.*, 2010).

Ergosterol is a sterol unique to fungi and is an important precursor of vitamin D2, which has great medicinal value. Ergosterol is present in two forms, as free ergosterol or esterified ergosterol, which have different physiological functions (Yuan *et al.*, 2007).

Fatty acids can be classified as saturated or unsaturated fatty acids. The unsaturated fatty acid content reached 57.84% in *O. sinensis*, including C16:1, C17:1, C18:1 and C18:2, while the saturated fatty acid content was 42.16%, including C14, C15, C16, C17, C18, C20 and C22 (Zhou *et al.*, 2009). Unsaturated fatty acid is an effective physiologically active component, which has the unique function of decreasing blood lipids and protecting against cardiovascular disease.

Crude protein was composed of 18 amino acids, including aspartic acid, threonine, serine, glutamate, proline, glycine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, cystine, cysteine and tryptophan, with the content of crude protein in the range of 29.1–33% (Hsu *et al.*, 2002).

Amino acids are mostly reported as 20–25% after hydrolysis, the lowest being 5.53%, the highest being 39.22%. The highest contents are glutamate, arginine and aspartic acid, and the major pharmacological components are arginine, glutamate, tryptophan and tyrosine (Ji and Yi, 1999).

Metal elements, such as Zn, Mg, Mn and so on, are of great significance to the development and maintenance of function of the gonads (Zhu and Wang, 1993).

FUNCTIONAL ACTIVITIES AND MECHANISMS OF *O. SINENSIS*

Thus far, more than 300 species of *Ophiocordyceps* have been found. In addition to *O. sinensis*, some other entomopathogenic fungus, such as *C. militaris*, *Cordyceps pruinosa* and *Elaphocordyceps ophioglossoides*, also have been shown to possess pharmacological property. However, the research into pharmacological properties of *Ophiocordyceps* have mainly focused on *O. sinensis* because of its obvious medical activity and the huge market demand particularly in China, Tibet, Nepal and Himalayan region.

Immunomodulating

O. sinensis and its extracts and isolated components have long been used in preventing and treating many age related diseases. However, the mechanisms of its action are still poorly understood. The most possible answer for this is that there are immunomodulators in *O. sinensis*, which may treat or prevent diseases and illnesses caused by certain immunodeficiencies and other age or illness related decline of immunity (Koh *et al.*, 2002).

Quite a number of studies, mostly in the past five years, have tested the possibility and mechanism of *O. sinensis* on immunomodulating using various models (Table 1). The components that may function as immunomodulators from *O. sinensis* are mostly found as polysaccharides (Chen *et al.*, 2010a; Cheung *et al.*, 2009; He *et al.*, 2013; Meng *et al.*, 2014; Wang *et al.*, 2011; Wu *et al.*, 2014), and sometimes deoxynucleic acids (He *et al.*, 2014; Xiao *et al.*, 2010) and other unknown bioactive compounds (Shashidhar *et al.*, 2013).

Wu *et al.* (2006) investigated the pharmacological potentials of polysaccharides from the mycelia of *O. sinensis* on the humoral and cellular immune responses of ICR mice against ovalbumin and found ovalbumin-specific IgG, IgG1 and IgG2b levels in serum were significantly improved by polysaccharide treatment. Later studies generally found that polysaccharides from the mycelia of *O. sinensis* can promote proliferation and phagocytosis of macrophages, and stimulate macrophages to release the reactive free radical nitric oxide (NO) and multiple chemokines and cytokines (Chen *et al.*, 2010a; He *et al.*, 2013; Hu *et al.*, 2016; Meng *et al.*, 2014; Wang *et al.*, 2011; Wu *et al.*, 2014). Further cytokine assays of interleukin (IL)-1b, tumor necrosis factor (TNF)-α, nuclear factor (NF)-kB and NO suggested that polysaccharides probably stimulated macrophage activities by stimulating the IkB–NF-kB pathway (Chen *et al.*, 2010a; He *et al.*, 2013). NF-kB is a protein complex that controls the transcription of DNA and plays a key role in regulating the immune response to

infection (Gilmore, 2006). IkB is a cytoplasmic retention protein. Activation of NF-kB involves its dissociation from IkB, allowing free NF-kB to be transported into the nucleus where it can regulate genes involved primarily in immune and inflammation responses (Beg and Baldwin, 1993).

A recent study also found that polysaccharides induced differentiation of macrophages into dendritic-like cells and promoted phenotypic and functional maturation of dendritic cells (DCs) in mouse (Meng *et al*., 2014). DCs are the professional antigen-presenting cells, which encounter the invading pathogens at the earlier stage of the infection process and then trigger the immune response (Wu and Liu, 2007). Some other studies also found that application of polysaccharides induced the cell proliferation of T-lymphocytes and the secretion of IL-2, IL-6 and IL-8 by these cells (Sheng *et al*., 2011). Further information on immunomodulating properties and action mechanisms of fungal polysaccharides can be found in other detailed reviews on this field (Borchers *et al*., 1999; Vannucci *et al*., 2013).

Nucleotides (including adenosine, uridine and guanosine) in *O. sinensis* also may function as immunomodulators. Yu *et al*. (2007) demonstrated that adenosine and guanosine decreased NO but increased IL-lβ release of macrophage, and guanosine augmented TNF-α release of macrophage. Xiao *et al*. (2010) found that deoxynucleic acids from *O. sinensis* activated mouse bone marrow-derived DCs via a Toll-like receptor 9-dependent pathway. The Toll-like receptors (TLRs) play a key role in the innate immune system in vertebrate, which function in recognizing pathogen-associated molecular patterns (PAMPs), such as unmethylated CpG DNA (Krieg *et al*., 1995), and then activates antipathogen responses characterized by T helper cell type 1 (Th1) or Th2 cytokines (Qi *et al*., 2003).

Antiinflammation

Inflammation is part of the nonspecific immune response of tissues to harmful stimuli, such as injury, pathogens and irritants (Ferrero-Miliani *et al*., 2007). Inflammation often manifests as pain, swelling, vasodilatation and release of soluble mediators, such as NO, IL-12, TNF-α and pro-inflammatory cytokines (Linton and Fazio, 2003). These mediators are important for the organism to remove the injurious stimuli and to initiate the healing process but overproduction of these inflammatory mediators in some disorders may result in inflammatory diseases (Clancy *et al*., 1998).

Several studies have shown that *O. sinensis* extracts have significant antiinflammation activities by reducing the production of NO, IL-12, TNF-α, and pro-inflammatory cytokines (Liu *et al*., 2011). Yang *et al*. (2011) analyzed 50 compounds from the mycelia *O. sinensis* and found one compound, 1-(5-Hydroxymethyl-2-furyl)-β-carboline, displayed the most significant antiinflammatory activity. A recent study (Wang *et al*., 2012b) indicated that the cordymin (a peptide purified from the medicinal mushroom *O. sinensis*) may also have antiinflammatory and antioxidants activities.

However, the possible mechanisms by which the compounds from the *O. sinensis* suppress the expression of inflammatory mediators are still poorly understood. Chiou and Lin (2012) found that *O. sinensis* extracts reduced airway inflammation in ovalbumin-induced allergic mice, probably by blocking NF-kB through decreasing extracellular signal-regulated kinase 1/2 signaling pathway. As mentioned above, NF-kB is one of the most ubiquitous transcription factors and regulates the expression of genes involved in inflammatory responses (Lawrence and Fong, 2010). Further studies may lead to new findings on the antiinflammation mechanisms of *O. sinensis*.

Antioxidant

Oxidative stress is imposed by the reactive oxygen species (ROS), including H_2O_2, hydroxyl radical and superoxide radical, which can attack lipid membranes, proteins and nucleic acids, resulting in cell injury and apoptosis (Simon *et al*., 2000). This stress has been generally recognized as the direct or indirect cause of tissue damage and aging and many human illnesses, such as atherosclerosis, cancer, inflammation and neurodegenerative diseases (Victor *et al*., 2009).

Studies to date have shown that the chemical components from *O. sinensis* with antioxidant activities mostly are polysaccharides (Deng *et al*., 2015; Shen *et al*., 2011; Yan *et al*., 2009; Zheng *et al*., 2014). Two studies also showed that certain peptides (Wang *et al*., 2012b) and adenosine (Yan *et al*., 2013) also have such effects.

In an early study, Li *et al*. (2001) showed that the antioxidant activity was increased 10–30-folds in the partially purified polysaccharide fractions compared to water extracts of *O. sinensis* using an assay of superoxide anion, indicating the importance of polysaccharides on antioxidant property. A later study further found that the hydrolysed polysaccharide fractions (hydrolysed in dilute sulphuric acid solution at pH 1 and 90 °C) had much higher (30–80%) antioxidant and radical-scavenging activities than that of non-hydrolysed polysaccharide fractions (Yan *et al*., 2009). Polysaccharides pretreated rat pheochromocytoma cells revealed strong protective effect against H_2O_2-induced insult and significantly increased the survival of cells, probably by attenuating the changes of glutathione peroxidase and superoxide dismutase activities in cells (Li *et al*., 2003) or promoting the activities of glutathione peroxidase, catalase and superoxide dismutase but reducing the levels of lactate dehydrogenase and malondialdehyde (Zheng *et al*., 2014).

Some other studies also suggested that one peptide (Wang *et al*., 2012b) and adenosine (Yan *et al*., 2013) from *O. sinensis* mycelia possess cardiovascular- and neuro-protective effects, possibly because of the increase of antioxidant activities and inhibition of inflammation.

Zou *et al*. (2015) recently showed that oral administration of *O. sinensis* significantly prolonged the lifespan of fruit flies through an anti-oxidative stress pathway from upregulation of the activity of copper-zinc-containing superoxide dismutase 1 and catalase and inhibition of lipofuscin accumulation. *O. sinensis* provides novel means for slowing aging process in human.

Effect on apoptosis of cells

Apoptosis, or programmed cell death, is essential for organisms' development and homeostasis (Vaux and Korsmeyer, 1999). Many studies have suggested that *O.*

sinensis can be used to inhibit apoptosis as an important potential therapy for some diseases (Cheng *et al*., 2014) but induce apoptosis to treat cancer (Chen *et al*., 2014b).

Although the evidence is still scarce, two possible mechanisms have been suggested for the inhibition of apoptosis by *O. sinensis* (Fig. 1). Studies in liver diseases have suggested that heightened production of ROS may cause hepatocyte apoptosis, which plays a central role in liver fibrosis (Koek *et al*., 2011). Liu *et al*. (2013) found that polysaccharides from *C. militaris* markedly inhibited H_2O_2-induced hepatocyte apoptosis, probably by decreasing ROS production and regulating apoptotic signaling. Therefore, antioxidant compounds from *O. sinensis*, including polysaccharides (Shen *et al*., 2011; Zheng *et al*., 2014), peptide (Wang *et al*., 2012b) and adenosine (Yan *et al*., 2013) may also function in inhibiting H_2O_2-induced apoptosis. Alternatively, *O. sinensis* may inhibit apoptosis by down-regulating apoptotic genes, such as Fas, Fas ligand and TNF-α (Shahed *et al*., 2001).

While there is robust evidence for the inhibition of apoptosis by *O. sinensis*, a greater number of studies also identified evidence that *O. sinensis* can induce apoptosis (Fig. 1). Yang *et al*. (2003) found that a 410-kDa polysaccharide from *O. sinensis* induced apoptosis, possibly by inhibiting the tyrosine phosphorylation of Bcl-2 and Bcl-xL oncoproteins. Bcl-2 and Bcl-XL have been identified as transcripts that function in blocking apoptosis and mediating transforming activity (Fernandez-Sarabia and Bischoff, 1993). A later study by Lee *et al*. (2006) showed that *C. militaris* water extract inhibited cancer cell proliferation by inducing cell apoptosis through the activation of caspase-3 in the cytosol. Recent studies found cordycepin from *O. sinensis* also induced apoptosis in mouse and human cells, likely through the caspase/MAPK pathways (Chen *et al*., 2013; Chen *et al*., 2014b). Because the chemical structure of cordycepin is similar to adenosine, the apoptotic effect of cordycepin may be mediated by adenosine receptors (ARs). There are four types of ARs, all of which are G protein-coupled receptors, including A1R, A2AR, A2BR and A3R (Englert *et al*., 2002). Chen *et al*. (2010b, 2014b) found that cordycepin-induced apoptosis in rat glioma cells is initiated by increased p53 phosphorylation and expression followed by the downstream caspase-7 activation and poly(ADP-ribose) polymerase (PARP) cleavage, which was A2AR dependent. PARP also is one of the oncogenic anti-apoptotic proteins (Strosznajder *et al*., 2005). P53 is considered a master regulator of apoptosis in response to various stimuli (Haupt *et al*., 2003). It triggers expression of the Bcl-2 family proteins Bax and p53-upregulated modulator of apoptosis (PUMA), targeting mitochondria to induce cytochrome c release and subsequent activation of key regulators of apoptosis, including caspase-3, -6, -7 and -9, and then to activate the intrinsic apoptotic pathway (Mrass *et al*., 2004).

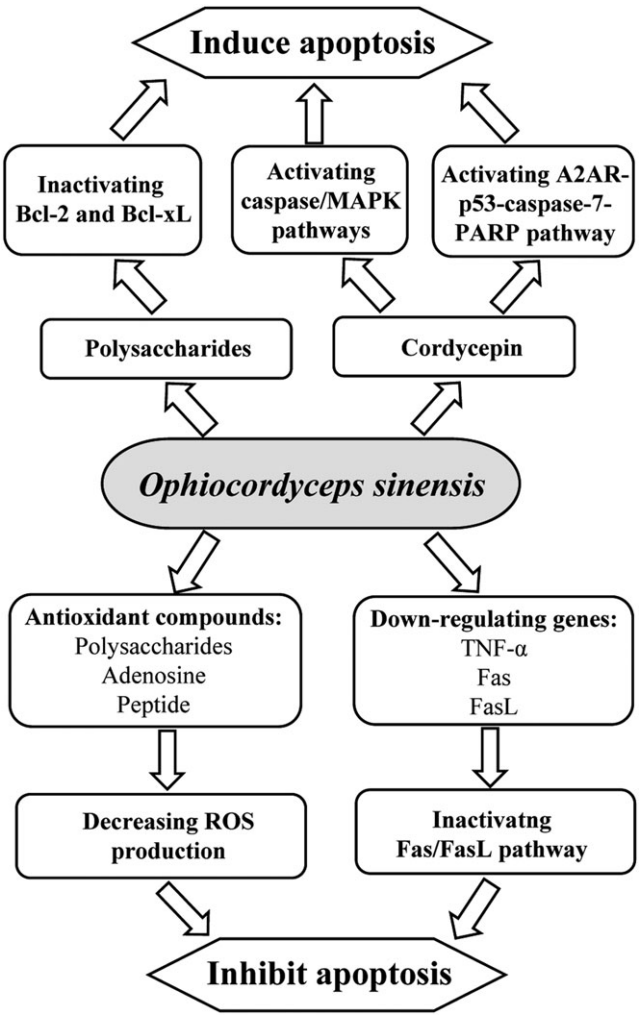

Figure 1. Scheme showing the mechanisms of the effect of *Ophiocordyceps sinensis* on cell apoptosis. A2AR: adenosine 2A receptor; Bcl-2: B-cell lymphoma-2; Bcl-xL: B-cell lymphoma-extra large; caspase: cysteine aspartic-specific protease; FasL: a tumor necrosis factor; Fas: the receptor of FasL; MAPK: mitogen-activated protein kinases; p53: tumor protein p53; PARP: poly (ADP-ribose) polymerase; ROS: reactive oxygen species; TNF-α: tumor necrosis factor α.

Antitumor

Cancer, after cardiovascular diseases, is the second most predominant cause of death in the modern world. Cancer treatments, including surgical operation, radiotherapy and chemotherapy, usually cause serious damage and suffering to patients. Many studies have demonstrated that *O. sinensis*, its extracts and isolated compounds have obvious antitumor activities in inhibiting proliferation of tumor cells (Lee *et al*., 2015) and in preventing the invasion and migration of cancer cells (Pao *et al*., 2012). A number of recent studies (Ko *et al*., 2013) also showed that combinations of cordycepin with other treatments can have synergistic effects and may be used as a novel strategy to eradicate leukemia via the elimination of leukemia stem cells. These findings provide a very promising start for new approaches to treat cancer.

As mentioned above, immunomodulation (He *et al*., 2013; Meng *et al*., 2014; Wang *et al*., 2011), antioxidant activity (Ji *et al*., 2014; Lee *et al*., 2015) and induction of tumor cell apoptosis (Chen *et al*., 2013; Chen *et al*., 2014b) should be the main channels by which *O. sinensis* inhibits the grows of cancer cells.

Immunotherapy has been recognized as an alternative treatment and is now gaining more attention than ever. Jordan *et al*. (2010) found that oral *O. sinensis* reduced the occurrence of lung metastases associated with breast cancer, other previous immunotherapies have not shown to be effective against this cancer. Macrophages

play an important role in immune-surveillance against malignant cells and pathogens (Gordon, 2007). Yamaguchi *et al*. (1990) showed that administration of *O. sinensis* extract significantly enhanced phagocytic activity of macrophages and the survival time of tumor-bearing mice. Polysaccharides in *O. sinensis* mycelia were found to be the main immune stimulating compounds (He *et al*., 2013; Meng *et al*., 2014; Wang *et al*., 2011; Wu *et al*., 2014). These polysaccharides not only stimulate the phagocytic activity of macrophages but also induce these cells to produce TNF-α, IL-2, IL-6 and other cytokines that have antitumor activities (Sheng *et al*., 2011; Song *et al*., 2013; Yan *et al*., 2011).

Tumor progression usually induces maturation defects in DCs, from which tumor-bearing hosts exhibit immunosuppression and tumor escape (Idoyaga *et al*., 2007). Zhang *et al*. (2005) found that exopolysaccharides from *O. sinensis* may inhibit tumor growth by modulating the hosts' immunity. Their later study (Song *et al*., 2011) further demonstrated that exopolysaccharides promoted the expressions of cytokines (IL-12p40 and TNF-α) and inducible NO synthase but decreased the expression level of phosphorylated signal transducers and activators of transcription 3 (p-STAT3) of DCs. These results suggest that exopolysaccharides may inhibit tumor growth by promoting DC maturation and activation through the inhibition of STAT3 phosphorylation.

Cordycepin from *O. sinensis* has been shown possess an anticancer action, probably through the stimulation of adenosine A3 receptor, followed by the Wnt signaling pathway, including glycogen synthase kinase (GSK)-3b activation and cyclin D1 suppression (Nakamura *et al*., 2015). Cordycepin also has an antimetastatic effect through inhibiting platelet aggregation induced by cancer cells and suppressing the invasiveness of cancer cells via inhibiting the activity of matrix metalloproteinase (MMP)-2 and MMP-9, and accelerating the secretion of tissue inhibitor of metalloproteinase (TIMP)-1 and TIMP-2 from cancer cells (Nakamura *et al*., 2015).

Although sufficient evidence is still to come, some other possible mechanisms also have been hypothesized on how *O. sinensis* inhibits the grows of cancer cells: (i) regulation of signal pathway, such as inhibition of the mitogen-activated protein kinase (MAPKs) signaling pathway that play important roles in the invasion and migration of cancer cells, activation of the protein kinase C (PKC) signaling pathways that has a direct antitumor effect (Pao *et al*., 2012); (ii) inhibition of the migration and invasion related factors by cancer cells, such as MMP-2, MMP-9 and urokinase plasminogen activator (uPA) (Chia *et al*., 2010; Jiang and Sliva, 2010; Nakamura *et al*., 2015); and (iii) inhibition of angiogenesis (Chia *et al*., 2010). Angiogenesis plays a prominent role in tumor growth and metastasis inhibition of angiogenesis is considered to be an important strategy for cancer therapy.

These studies have demonstrated that *O. sinensis* can inhibit both the growth and migration of various cancer cells, which may be related to the complicated processions and mechanisms that worth further investigations.

Cardiovascular protection

Cardiovascular diseases represent the major burden of morbidity and mortality and are rapidly becoming the major health issue in the modern world. A worldwide estimation of 17 million deaths because of cardiovascular diseases in 2008 showed that the majority deaths were because of atherosclerotic cardiovascular disease (Ginsberg, 2013).

Orally administered water extracts of the fruiting bodies of *O. sinensis* have shown to prevent cholesterol deposition in the aorta of mice. The effect was likely achieved by inhibition of low-density lipoprotein (LDL) oxidation mediated by free radicals rather than by reduction in serum lipid level (Yamaguchi *et al*., 2000). A heteropolysaccharide, namely PS-A, was isolated from water extracts of *O. sinensis* and has shown to possess strong inhibitory activity against cholesterol esterase (Kim, 2010). Inhibition of cholesterol esterase enzyme activities could prohibit the absorption of dietary cholesterol esters and lead to prevention of atherosclerosis (Jeon *et al*., 2000). Similarly, *O. sinensis* extracts attenuated aortic transplant arteriosclerosis in rats (Zhang *et al*., 2012b).

Platelets play a complex role in hemostasis and thrombosis. Initiation of an intraluminal thrombosis is believed to involve platelet adherence, aggregation and activation, and therefore antiplatelet drugs are beneficial in controlling cardiovascular diseases (e.g. myocardial infarction, ischemic stroke and vascular death) (von Hundelshausen and Weber, 2007). *Ex vivo* and *in vivo* studies have demonstrated that polysaccharides from *O. sinensis* may inhibit human platelet activation by initially inhibiting the PLCgamma2-PKC-p47 cascade, and subsequently preventing PI3-kinase/Akt and MAPK phosphorylation through activating adenylate cyclase/cyclic AMP, then inhibiting intracellular Ca^{2+} mobilization and, ultimately, inhibit platelet activation (Chang *et al*., 2015).

A novel serine protease with fibrinolytic activity named CSP was purified from the culture supernatant of *O. sinensis* (Li *et al*., 2007). CSP was found to be a plasmin-like protease, but not a plasminogen activator, and it preferentially cleaved the Aα chain of fibrinogen and the α-chain of fibrin. Therefore, the extracellular protein CSP may represent a potential new therapeutic agent for the treatment of thrombosis.

O. sinensis also is a potential therapeutic agent for hypertension (Xiang *et al*., 2016), occlusion-induced focal cerebral ischemia of the middle cerebral artery (Zou *et al*., 2016) and other cardiovascular diseases (Yan *et al*., 2013). Xiang *et al*. (2016) found that *O. sinensis* had antihypertensive effect by stimulating the secretion of vasodilator NO, decreasing the level of endothelin-1, epinephrine, noradrenaline and angiotensin II, inhibiting the increase of transforming growth factor β1 (TGF-β1) and lowering the level of inflammatory mediator of C-reactive protein. Zou *et al*. (2016) demonstrated that the therapeutic activity of *O. sinensis* on ischemic stroke is likely through the inhibiting damage induced by oxygen and glucose deprivation.

Kidney protection

It has been suggested that the Traditional Chinese herbal preparation *O. sinensis* may have a beneficial effect in patients with renal diseases (Wang, 1694). CKD is a global public health problem with a high mortality. Renal fibrosis is the final pathological manifestation of CKD, characterized by tubulointerstitial fibrosis and

glomerulosclerosis, and it leads to the deterioration and eventual loss of renal function, independent of the primary cause (Liu, 2006). Some studies have demonstrated that *O. sinensis* have the ability to suppress the epithelial to mesenchymal trans-differentiation by regulating the pro-fibrotic cytokine TGF-β1/Smad signal pathway and eventually attenuates renal fibrosis (Pan *et al.*, 2013; Yao *et al.*, 2014; Zhang *et al.*, 2012a).

O. sinensis inhibited, to a certain degree, the proliferation of cultured human glomerular mesangial cells induced by LDLs (Zhao-Long *et al.*, 2000). Recent studies indicated that *O. sinensis* polysaccharide may protect human mesangial cells from reducing platelet-derived growth factor (PDGF)-induced cell proliferation through the PDGF/ERK and TGF-β1/Smad pathways (Wang *et al.*, 2014) and inhibiting PDGF homodimer BB induced inflammation and ROS production (Wang *et al.*, 2015b).

The changes in blood urea nitrogen and serum creatinine revealed that a water-soluble polysaccharide (named CPS-2) significantly relieved renal failure caused by fulgerizing kidney (Wang *et al.*, 2010).

Reproduction protection

O. sinensis has also been used as a tonic supplement for sexual dysfunction (Zhu *et al.*, 1998). Studies have illustrated that *O. sinensis* can stimulate leydig cells and granulosa-lutein cells (GLCs) to produce sex steroids (Chen *et al.*, 2005; Hsu *et al.*, 2003; Huang *et al.*, 2004b) and several possible mechanisms have been suggested for this effect (Fig. 2).

A few studies have shown that *O. sinensis* activates both PKA and PKC signal transduction pathways to stimulate the production of steroidogenic acute regulatory (StAR) protein (Chen *et al.*, 2005; Hsu *et al.*, 2003; Huang *et al.*, 2001).

In a study to test whether and how *O. sinensis* enhanced female reproduction, treatment of human GLCs with *O. sinensis* resulted in increased 17b-estradiol (E_2) production (Huang *et al.*, 2004a). E_2 is the most important granulosa-derived hormone affecting the properties of oocytes and there is increasing evidence that it directly influences the quality of maturing oocytes. These data may help in the development of treatment regimens to improve the success rate of in vitro fertilization.

Recent studies showed cordycepins from *O. sinensis* induced steroidogenesis through stimulating intracellular PLC/PKC and MAPK signal transduction pathways (Pao *et al.*, 2012) and associating with adenosine receptors to activate cAMP–PKA–StAR pathway (Leu *et al.*, 2011).

Prevention of osteoporosis

According to ancient records, *O. sinensis* also was used in the modulation of cytoskeleton restructuring and

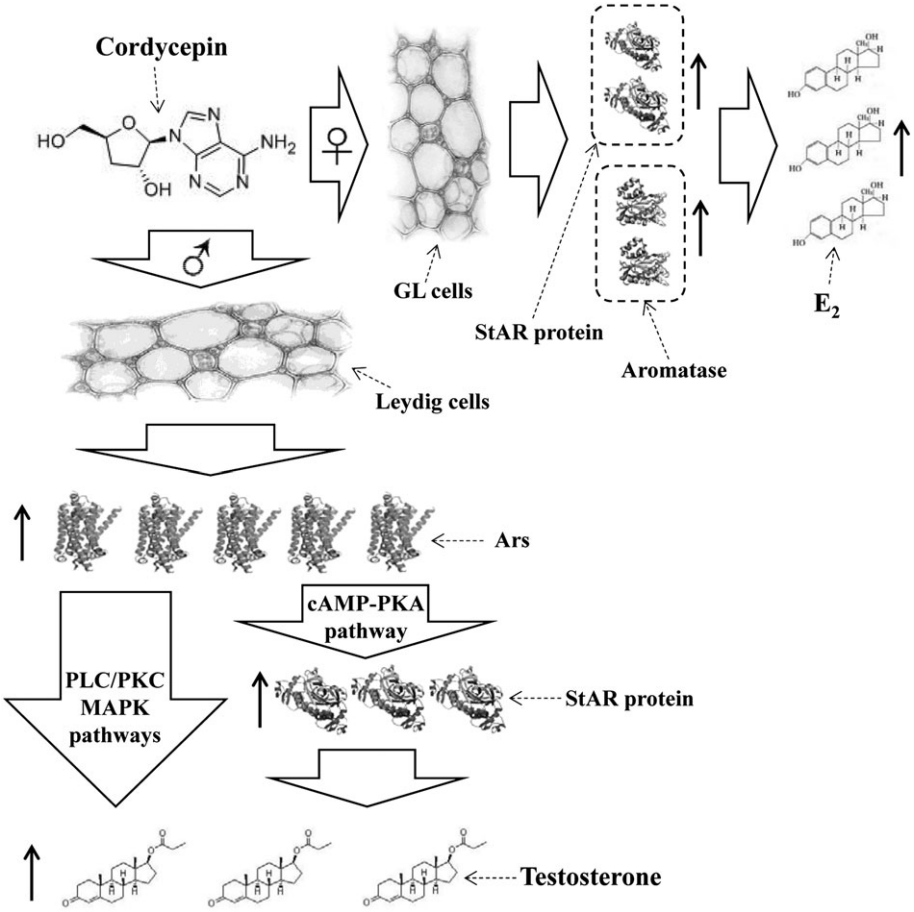

Figure 2. Scheme showing the mechanisms of the effect of Cordycepin on reproduction. Ars: adenosine receptors; E_2: 17b-estradiol; GL cells: granulosa-lutein cells; MAPK: mitogen-activated protein kinases; PKA: protein kinase A; PKC: protein kinase C; PLC: phospholipase C; StAR: steroidogenic acute regulatory; ↑: indicates the synthesis of these substances is improved.

prevention of osteoporosis (reviewed in Qi *et al*., 2012). Osteoporosis is characterized by a reduction in bone mass and micro-architectural deterioration of bone tissue, which increases dramatically with aging and may result in skeletal fragility and fractures (Garber *et al*., 2000).

It was reported that strontium administration at low dose increases bone formation and bone mass in normal animals (Reginster *et al*., 2005). There is experimental evidence that estrogen deficiency leads to excessive bone loss and eventually osteoporosis (Canalis, 2010) and *O. sinensis* modulated ovarian steroidogenesis (Chen *et al*., 2005; Hsu *et al*., 2003). Qi *et al.* (2011) thus tested the effect of *O. sinensis* and strontium on the prevention of osteoporosis in ovariectomized (OVX) rats and found *O. sinensis* plus strontium did prevent bone loss and osteoporosis caused by estrogen deficiency. Their further studies (Zhang *et al*., 2014a) also found that the beneficial effects of *O. sinensis* on improvement of osteoporosis in rats were attributable mainly to decrease the serum alkaline phosphatase (ALP) and tartarate-resistant acid phosphatase (TRAP) activities as well as the C-terminal crosslinked telopeptides of collagen type I (CTX) and interferon-gamma (IFN-γ) levels. Further studies based on these results should lead to valuable findings.

Other therapeutic and health care potentialities

O. sinensis also has obvious therapeutic and health care effects on other organ systems and diseases, such as antimicrobial activities (Mamta *et al*., 2015), protection of lung (Chen *et al*., 2012) and liver (Wang *et al*., 2012a) functions, therapeutic effects on diabetes (El Ashry *et al*., 2012; Liu *et al*., 2016) and asthma (Kuo *et al*., 2001). Studies also found that *O. sinensis* can increase hypoxia tolerance (Singh *et al*., 2013) and ATP levels on muscle strength, power output and endurance (Chen *et al*., 2014a; Kumar *et al*., 2011). Recent studies also demonstrated fascinating potentialities of *O. sinensis* on the improved of memory and learning abilities (Dong *et al*., 2014) and lifespan (Zou *et al*., 2015).

CONCLUSIONS AND PERSPECTIVES

O. sinensis has long been used as a medication in China for the treatment of many illnesses and in the recent years its usage has increased dramatically because of the improvement of people's living standard and the emphasis on health. Such huge requirement has resulted in the overharvest of this fungus in the wild. Accordingly, *O. sinensis* has now attracted scientists' extensive attention, worldwide. Recent studies have demonstrated that artificially cultured and fermented mycelial products of *O. sinensis* have similar pharmacologically activities to wild ones, which will likely reduce the price of this medication and further expand its usage for the treatment of various illnesses. As reviewed above, great progress has been achieved in recent years on the pharmacological properties and the functional mechanisms of action of *O. sinensis*. Further studies will not only provide deep insights into functional activities and mechanisms of *O. sinensis* but also will facilitate the development of better or novel means for disease therapy and health regulation using this fungus.

Acknowledgements

We are very grateful to Dr. Jian-Ping Xu from McMaster University for his kind help in writing assistant and good advice to improve our manuscript. This work was funded jointly by projects from the National Basic Research Program of China (2013CB127506), NSFC (31560606, 31460028, 31260020), Department of Science and Technology of Yunnan Province (2012BA015), CNTC (110201201009 BR-03) and Yunnan of CNTC (HYHH2012HX06).

Conflict of Interest

The authors have declared that there is no conflict of interest.

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