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HEMEflow O2™
Blood Support Extract

Product Information Sheet

HEMEflow O2™ "Blood Support Extract" contains **Phytotherapeutic** Extracts of **Angelica sinensis, Cordyceps sinensis, *Curcuma longa*, Cnidum monerii, *Zingiber officinale*,** and ***Cinnamomum verum***. **HEMEflow O2** contains six of the most effective immune supporting herbs with proven effects in supporting blood flow and oxygenation. Hypoxia reduces production of ATP, leading to energy failure, anaerobic depolarization, functional damage of ion pumps, and receptor activation of the N-methyl-D-asparticacid receptor. This triggers Ca2þ influx leading to cell damage or even death.

***Angelica sinensis*,** known as Dong gui in TCM, has traditionally been used to treat a variety of blood-related ailments including menstrual cramps, blood deficiencies, uterine disorders, as well as ischemias of both the heart and brain. Angelica roots are used for tonifying, replenishing, and invigorating blood. More than 50 active components have been isolated from angelica root. These active compounds include polysaccharides, organic acids, and phthalates. Ferulic acid and *Z*-ligustilide are major bioactive components of angelica root.

***Cordyceps sinensis*,** is mainly beneficial for the kidneys and the lungs, and is indicated for chronic cough, hemoptysis, and impotence. An important traditional Chinese medicine that has been used for over one thousand years CS are mainly composed of cordycepin, cordymin, nucleoside, poly-saccharide, cordycepic acid, fatty acids, and amino acids. Studies have shown that CS has anti-cancer, immunoregulation, anti-oxidation, anti-diabetes, anti-aging effects. The mechanism of CSE may be related with reduction in oxygen free radicals, enhancement of scavenging ability of cells in response to oxygen free radicals, inhibition of the inflammatory reaction, or maintenance of endothelial function.

Curcuma longa. The curcumin in turmeric has antioxidant, antiseptic, antifungal and anti-inflammatory properties. Turmeric has often been used to treat and even prevent arthritis and other incidences of chronic inflammation.

***Cnidum monnieri*,** antioxidant, anticancer, anti-inflammatory, and immunomodulatory properties and has an anti-Inflammatory effect via blocking the activation of the NF-κB and MAPK/p38 pathways.

Zingiber officinale ginger extract was effective against several strains of drug-resistant bacteria. It may help inhibit the synthesis of certain markers of inflammation. It contains gingerol and other anti-inflammatory compounds like shogaol, paradol and zingerone.

Cinnamomum verum Contains antioxidants, including polyphenols, phenolic acid and flavonoids. These compounds work to fight oxidative stress in the body and aid in the prevention of chronic disease.

- **Highly bio-available due to heat and alcohol reflux extraction**
- Extracted in **Maui, Hawaii**.
- **Organic**, Non-GMO, Gluten free
- Extracted with **Maui-grown organic sugarcane alcohol** and deep ocean mineral water.

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Research Article

Cordyceps sinensis Increases Hypoxia Tolerance by Inducing Heme Oxygenase-1 and Metallothionein via Nrf2 Activation in Human Lung Epithelial Cells

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Cordyceps sinensis, an edible mushroom growing in Himalayan regions, is widely recognized in traditional system of medicine. In the present study, we report the efficacy of *Cordyceps sinensis* in facilitating tolerance to hypoxia using A549 cell line as a model system. Treatment with aqueous extract of *Cordyceps sinensis* appreciably attenuated hypoxia induced ROS generation, oxidation of lipids and proteins and maintained antioxidant status similar to that of controls via induction of antioxidant gene HO1 (heme oxygenase-1), MT (metallothionein) and Nrf2 (nuclear factor erythroid-derived 2-like 2). In contrast, lower level of NFκB (nuclear factor kappaB) and tumor necrosis factor-α observed which might be due to higher levels of HO1, MT and transforming growth factor-β. Further, increase in HIF1 (hypoxia inducible factor-1) and its regulated genes; erythropoietin, vascular endothelial growth factor, and glucose transporter-1 was observed. Interestingly, *Cordyceps sinensis* treatment under normoxia did not regulate the expression HIF1, NFκB and their regulated genes evidencing that *Cordyceps sinensis* per se did not have an effect on these transcription factors. Overall, *Cordyceps sinensis* treatment inhibited hypoxia induced oxidative stress by maintaining higher cellular Nrf2, HIF1 and lowering NFκB levels. These findings provide a basis for possible use of Cordyceps sinensis in tolerating hypoxia.

1. Introduction

Acclimatization is a major problem for people travelling to high altitudes for the first time. Adverse environmental conditions such as extreme cold, hypoxia, low humidity, high wind velocity, and high intensity of solar radiation [1–3] result in the risk of altitude sickness worldwide. The common problems are acute mountain sickness (AMS), insomnia, lack of appetite, tiredness, lethargy, upset stomach, disinclination to work, bone and muscle degradation, high-altitude pulmonary edema (HAPE), high-altitude cerebral edema (HACE), and all resulted in decrease in physical and mental performance in unacclimatized individuals [4–8]. These problems may escalate rapidly and the results may also be lethal sometimes.

For an individual or cells to adapt to hypoxic conditions, they must be able to sense changes in oxygen tension and respond accordingly. The initiation of these responses can be rapid involving biochemical homeostasis, reprogramming of transcription factors and gene expression, and these changes lead to the production of proteins that exert a protective effect on the cell. Major oxygen and redox-sensitive transcriptional factors (TFs) are nuclear factor- (erythroid-derived 2-) like 2 (Nrf2), hypoxia-inducible factor 1 (HIF1), and nuclear factor kappa-B (NFκB). Expression of antioxidant-responsive-element- (ARE-) driven genes and enzymes is directed by Nrf2 cap'n'collar bZIP transcription factors. These include glutathione peroxidase (GPx), glutathione-S-transferase (GST), heme oxygenase (HO), superoxide dismutase (SOD), and ferritin. HIF1 is selectively stabilized in hypoxia and this in turn leads to activation of several genes such as erythropoietin (EPO) that promotes erythropoiesis, nitric oxide synthase (NOS) that modulates vascular tone, vascular endothelial growth factor (VEGF) that promotes angiogenesis, and glucose transporter-1 (GLUT1) that regulates energy metabolism. Conversely, NFκB activates genes

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Protective effect of *Cordyceps sinensis* extract on rat brain microvascular endothelial cells injured by oxygen–glucose deprivation

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KEYWORDS
Cordyceps sinensis extract;
Brain microvascular endothelial cells;
Oxygen–glucose deprivation;
Anti-oxidation;
Anti-inflammation

Abstract *Objective:* To investigate the protective effect of *Cordyceps sinensis* extract (CSE) on injury of primary cultured rat brain microvascular endothelial cells (rBMECs) induced by oxygen–glucose deprivation (OGD).
Methods: We isolated and cultured primary rBMECs in order to establish an *in vitro* OGD model. Cellular activity was detected using a cell counting kit to determine the appropriate dosage. The rBMECs were divided into control, model, low-, mid-, and high-dose (5, 10, 20 $\mu g \cdot mL^{-1}$) CSE groups under OGD for 6 hours. CSE was dissolved in cell culture medium to the appropriate concentration, passed through a 0.22 μm sterile filter, and administered for 12 hours before and during OGD. Cellular morphology was observed under a microscope. Lactate dehydrogenase level in cultural supernatant, superoxide dismutase activity, and the content of nitric oxide and malondialdehyde in cells were tested by colorimetric methods. Levels of tumor necrosis factor-α and interleukin-1 beta in cells were determined by enzyme-linked immunosorbent assay.
Results: After 12-hour administration of CSE at the concentration of 5, 10, 20 $\mu g \cdot mL^{-1}$ before and during OGD, compared with the model group, the CSE groups obviously alleviated the damage of rBMECs induced by OGD, inhibited the apoptosis and the necrosis of the cells, and improved cellular morphology of rBMECs. Additionally, compared with the model group, CSE also restrained lactate dehydrogenase leakage in hypoxic cells ($P < .01$), significantly increased superoxide dismutase activity ($P < .05$), and reduced the levels of nitric oxide, malondialdehyde, tumor necrosis factor-α, and interleukin-1 beta ($P < .05$).

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Effect of Cs-4® (*Cordyceps sinensis*) on Exercise Performance in Healthy Older Subjects: A Double-Blind, Placebo-Controlled Trial

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Abstract

Objective: The objective of this study was to examine the effect of Cs-4® (*Cordyceps sinensis*) on exercise performance in healthy elderly subjects.
Design: Twenty (20) healthy elderly (age 50–75 years) subjects were enrolled in this double-blind, placebo-controlled, prospective trial. The subjects were taking either Cs-4 333 mg or placebo capsules 3 times a day for 12 weeks.
Measurement: Subjects received baseline screening including physical examination and laboratory tests. Maximal incremental exercise testing was performed on a stationary cycle ergometer using breath-by-breath analysis at baseline and at the completion of the study.
Results: After receiving Cs-4 for 12 weeks, the metabolic threshold (above which lactate accumulates) increased by 10.5% from 0.83 ± 0.06 to 0.93 ± 0.08 L/min ($p < 0.02$) and the ventilatory threshold (above which unbuffered H^+ stimulates ventilation) increased by 8.5% from 1.25 ± 0.11 to 1.36 ± 0.15 L/min. Significant changes in metabolic or ventilatory threshold were not seen for the subjects in the placebo group after 12 weeks, and there were no changes in $\dot{V}O_2$ max in either group.
Conclusion: This pilot study suggests that supplementation with Cs-4 (*Cordyceps sinensis*) improves exercise performance and might contribute to wellness in healthy older subjects.

Introduction

Cordyceps sinensis (Berk) Sacc is a natural herbal medicine that has been popular in China for centuries for invigoration, health preservation, and reduction of fatigue.[1] Naturally occurring *Cordyceps sinensis* is a wild fungus found on the Qinghai-Tibetan Plateau of China at an altitude of about 10,000 feet. The fungus is parasitic and colonizes the larvae of moths until their inner body is filled with mycelium.[2] Wild *Cordyceps* is a composite consisting of the stroma of the parasite together within the larva of the Hepialidae moths.[3] Wild cordyceps is increasingly rare in its natural habitat, and the price is now completely out of reach for clinical practice.[4] For this reason and because of the scarcity of natural sources, a refined standardized fermentation product, Cs-4,® was produced from the mycelial strain *Paecilomyces hepiali* Chen at Dai that was isolated from wild *C. sinensis*. A close similarity between this fermentation product and natural *Cordyceps* has been demonstrated with respect to their chemical constituents (Cs-4 contains not less than 0.14% adenosine and 5% mannitol) and pharmacologic properties.[2,5]

The mechanisms of action of *Cordyceps* and its fermentation product Cs-4 in improving general well-being and physical ability have yet to be fully investigated.[6] Improvements in quality of life have been suggested in patients with chronic heart failure,[7] renal failure,[8] and chronic pulmonary disease.[9] *Cordyceps* gained world attention in 1993 when Chinese female runners achieved records in 1500 m, 3000 m, and 10,000 m events.[10] Their coach attributed their success to a diet containing *Cordyceps*. It was suggested that *Cordyceps* helped improve exercise capacity in these athletes via antioxidant effects. Despite these reports, the ability of *Cordyceps* or Cs-4 to enhance aerobic capacity has not been tested objectively. Because of the popularity of *Cordyceps* among the older population in China,[2] but recognizing the advantages

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RESEARCH ARTICLE Open Access

Polysaccharides from the root of *Angelica sinensis* promotes hematopoiesis and thrombopoiesis through the PI3K/AKT pathway

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Abstract

Background: Dozens of Traditional Chinese Medicine (TCM) formulas have been used for promotion of "blood production" for centuries, and we are interested in developing novel thrombopoietic medicines from these TCMs. Our previous studies have demonstrated the hematopoietic effects of DangGui BuXue Tong (DBT), a formula composed of *Radix Angelicae Sinensis* and *Radix Astragali* in animal and cellular models. As a step further to identify and characterize the active chemical components of DBT, we tested the hematopoietic and particularly, thrombopoietic effects of polysaccharide-enriched fractions from the root of *Radix Angelicae Sinensis* (APS) in this study.

Methods: A myelosuppression mouse model was treated with APS (10 mg/kg/day). Peripheral blood cells from APS, thrombopoietin and vehicle-treated samples were then counted at different time-points. Using the colony-forming unit (CFU) assays, we determined the effects of APS on the proliferation and differentiation of hematopoietic stem/progenitor cells and megakaryocytic lineages. Using a megakaryocytic cell line M-07e as model, we analyzed the cellular apoptosis progression with and without APS treatment by Annexin V, Mitochondrial Membrane Potential and Caspase 3 assays. Last, the anti-apoptotic effect of APS on cells treated with Ly294002, a Phosphatidylinositol 3-Kinse inhibitor (PI3K) was also tested.

Results: In animal models, APS significantly enhanced not only the recovery of platelets, other blood cells and their progenitor cells, but also the formation of Colony Forming Unit (CFU). In M-07e cells, we observed the anti-apoptotic effect of APS. Treatment by Ly294002 alone increased the percentage of cells undergoing apoptosis. However, addition of APS to Ly294002-treated cells significantly reduced the percentage of cells undergoing apoptosis.

Conclusions: APS promotes hematopoiesis and thrombopoiesis in the mouse model. This effect likely resulted from the anti-apoptosis activity of APS and is likely to involve the PI3K/AKT pathway.

Background

Thrombocytopenia (an abnormal decrease in the number of platelets in circulatory blood) is frequently developed in hematological and cancer patients who undergo bone marrow suppression or infiltration resulting from chemotherapy or radiotherapy. This condition may lead to haemorrhage and fatality [1]. In severe cases, platelet transfusion may be required to prevent or stop bleeding. However, platelet transfusion may induce the formation of anti-platelet antibodies, and the transmission of both viral and bacterial infection. Until today, no effective treatments for thrombocytopenia are clinically available. Our long term goal is to identify novel thrombopoietic agents from Traditional Chinese Medicine (TCM) formulations or products for further development.

Dozens of TCM formulations have been used for promotion of "blood production" for centuries and have

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RESEARCH ARTICLE

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Integrated metabonomic–proteomic studies on blood enrichment effects of *Angelica sinensis* on a blood deficiency mice model

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ABSTRACT

Context: *Angelica sinensis* (Oliv.) Diels (Umbelliferae) (AS) is a well-known Traditional Chinese Medicine (TCM) that enriches and regulates the blood.

Objective: An integrated metabonomic and proteomic method was developed and applied to study the blood enrichment effects and mechanisms of AS on blood deficiency (BD) mouse model.

Materials and methods: Forty mice were randomly divided into the control, BD, High-dose of AS (ASH), Middle-dose of AS (ASM), and Low-dose of AS (ASL) groups. BD model mice were established by injecting *N*-acetylphenylhydrazine (APH) and cyclophosphamide (CTX) (ip). The aqueous extract of AS was administered at three dose of 20, 10, or 5 g/kg b. wt. orally for 7 consecutive days before/after APH and CTX administration. Gas chromatography–mass spectrometry (GC-MS) combined with pattern recognition method and 2D gel electrophoresis (2-DE) proteomics were performed in this study to discover the underlying hematopoietic regulation mechanisms of AS on BD mouse model.

Results: Unlike in the control group, the HSP90 and arginase levels increased significantly ($p < 0.05$) in the BD group, but the levels of carbonic anhydrase, GAPDH, catalase, fibrinogen, GSTP, carboxylesterase and hem binding protein in the BD group decreased significantly ($p < 0.05$). Unlike the levels in the BD group, the levels of these biomarkers were regulated to a normal state near the control group in the ASM group. Unlike in the control group, L-alanine, arachidonic acid, L-valine, octadecanoic acid, glycine, hexadecanoic acid, L-threonine, butanoic acid, malic acid, L-proline and propanoic acid levels increased significantly ($p < 0.05$) in the BD group, the levels of D-fructose in the BD group decreased significantly ($p < 0.05$). The relative concentrations of 12 endogenous metabolites were also significantly affected by the ASL, ASM, and ASH treatments. Notably, most of the altered BD-related metabolites were restored to normal state after ASM administration.

Conclusion: AS can promote hematopoietic activities, inhibit production of reactive oxygen species, regulate energy metabolism, increase antiapoptosis, and potentially contribute to the blood enrichment effects of AS against APH- and CTX-induced BD mice.

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KEYWORDS
GC-MS; 2D gel electrophoresis; hematopoietic activities

Introduction

Anaemia is a common disease characterized by a decrease in haemoglobin (HGB). Anaemia frequently occurs because of fatigue, pressure, and radiation. Different types of this disease include blood loss anaemia, aplastic anaemia, sickle-cell anaemia, and iron-deficiency anaemia (Gupta 2014). The theory of Traditional Chinese Medicine (TCM) states that blood loss anaemia is similar to blood deficiency (BD) in TCM (Shi et al. 2014), such as the condition of postoperative and postpartum women with chronic bleeding, excessive menstruation, prolonged menstrual periods or uterine bleeding.

Angelica sinensis (Oliv.) Diels (Umbelliferae) (AS) is a well-known TCM that enriches and regulates the blood. AS is a popular herb commonly used in clinics for the treatment of BD syndrome in China. AS mainly consists of polysaccharides and different bioactivities, such as haematopoietic (Yang et al. 2009; Zhao et al. 2012), immunomodulatory (Ko & Cho 2003), antitumor (Cao et al. 2006), antioxidant (Ai et al. 2013) and antiulcer (Ye et al. 2003) effects. Several researchers have investigated the distribution of AS components, including phthalides, these components exhibit antifungal, antibacterial, anti-inflammatory, smooth-muscle relaxant, and vasodilatory properties (Chao et al. 2010; Su et al. 2011). AS is also a basic component of many Chinese drugs used for BD, such as DangguiBuxuetang (Shi et al. 2014) and Siwutang (Su et al. 2008). Although, AS has been applied clinically in animal models, the mechanism of AS blood enrichment effects remains unclear.

Proteomics and metabonomics are two well-established '-omic' techniques in the post-genomic era. Proteomics is the study of large-scale proteins in an organism encoded by its genome. It is a powerful tool and has been widely used to elucidate protein profile changes in response to drug treatment, as well as to identify disease-relevant biomarkers (Hsiao et al. 2012). Metabonomics focuses on a global profile of metabolites with low molecular weight (1000 Da), which are the end products of metabolisms in biofluids, tissues and even whole organisms (Sheridan et al. 2012). This approach is consistent with the integrity and systemic feature of TCM, which is increasingly utilized

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Polysaccharide from *Angelica sinensis* protects H9c2 cells against oxidative injury and endoplasmic reticulum stress by activating the ATF6 pathway

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Abstract

Objectives: *Angelica sinensis* exerts various pharmacological effects, such as antioxidant and anti-apoptotic activity. This study aimed to investigate the active ingredients in *A. sinensis* with antioxidant properties and whether *A. sinensis* polysaccharide (ASP) protects H9c2 cells against oxidative and endoplasmic reticulum (ER) stress.

Methods: The ingredients of *A. sinensis* and their targets and related pathways were determined using web-based databases. Markers of oxidative stress, cell viability, apoptosis, and ER stress-related signalling pathways were measured in H9c2 cells treated with hydrogen peroxide (H_2O_2) and ASP.

Results: The ingredient–pathway–disease network showed that *A. sinensis* exerted protective effects against oxidative injury through its various active ingredients on regulation of multiple pathways. Subsequent experiments showed that ASP pretreatment significantly decreased H_2O_2-induced cytotoxicity and apoptosis in H9c2 cells. ASP pretreatment inhibited H_2O_2-induced reactive oxygen species generation, lactic dehydrogenase release, and malondialdehyde

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Angelica sinensis and its Alkylphthalides Induce the Detoxification enzyme NAD(P)H: Quinone OxidoReductase 1 by Alkylating KEAP1

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Abstract

The roots of *Angelica sinensis* (Oliv.), Diels (Dang Gui; Apiaceae) have a long history in traditional Chinese medicine as a remedy for women's disorders, and are often called “lady's ginseng”. Currently, extracts of *A. sinensis* are commonly included in numerous dietary supplements used for women's health and as anti-aging products. In the present study, we examined the potential chemopreventive activity of *A. sinensis* extracts by measuring the relative ability to induce the detoxification enzyme, NAD(P)H:quinone oxidoreductase 1 (NQO1). The lipophilic partitions showed strong NQO1 induction with concentrations to double the enzyme activity (CD) of 5.5 ± 0.7 μg/mL (petroleum ether) and 3.9 ± 0.5 μg/mL (chloroform). Fractionation led to the isolation of phenolic esters and alkylphthalides, especially *Z*-ligustilide, the main lipophilic compound, which showed strong NQO1 inducing properties (CD = 6.9 ± 1.9 μM). Transcription of many detoxifying enzymes is regulated through the antioxidant response element (ARE) and its transcription factor Nrf2, which is repressed under basal conditions by Keap1. However, exposure to electrophilic inducers that alkylate Keap1 results in a higher concentrations of free Nrf2 and ARE activation. The ARE reporter activity was therefore analyzed in HepG2-ARE-C8 cells after incubation with lipophilic extracts of *A. sinensis* or ligustilide for 24 h. Under these conditions, both the extract and ligustilide increased ARE-luciferase reporter activity in a dose-dependent manner. Incubation of ligustilide with GSH and subsequent LC-MS-MS analysis revealed that ligustilide as well as oxidized ligustilide species covalently modified GSH. In addition, using MALDI-TOF mass spectrometry and LC-MS-MS, it was demonstrated that the lipophilic extracts, ligustilide, and monooxygenated ligustilide alkylated important cysteine residues in human Keap1 protein, thus activating Nrf2 and transcription of ARE regulated genes. These observations suggest that *A. sinensis* dietary supplements standardized to ligustilide have potential as chemopreventive agents through induction of detoxification enzymes.

Keywords

Angelica sinensis; alkylphthalides; cancer chemoprevention; Dang Gui; detoxification enzymes; Keap1; ligustilide; NAD(P)H: quinone reductase 1; Nrf2; oxidative stress

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Effect of Cs-4® (*Cordyceps sinensis*) on Exercise Performance in Healthy Older Subjects: A Double-Blind, Placebo-Controlled Trial

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Abstract

Objective: The objective of this study was to examine the effect of Cs-4® (*Cordyceps sinensis*) on exercise performance in healthy elderly subjects.
Design: Twenty (20) healthy elderly (age 50–75 years) subjects were enrolled in this double-blind, placebo-controlled, prospective trial. The subjects were taking either Cs-4 333 mg or placebo capsules 3 times a day for 12 weeks.
Measurement: Subjects received baseline screening including physical examination and laboratory tests. Maximal incremental exercise testing was performed on a stationary cycle ergometer using breath-by-breath analysis at baseline and at the completion of the study.
Results: After receiving Cs-4 for 12 weeks, the metabolic threshold (above which lactate accumulates) increased by 10.5% from 0.83 ± 0.06 to 0.93 ± 0.08 L/min ($p < 0.02$) and the ventilatory threshold (above which unbuffered H^+ stimulates ventilation) increased by 8.5% from 1.25 ± 0.11 to 1.36 ± 0.15 L/min. Significant changes in metabolic or ventilatory threshold were not seen for the subjects in the placebo group after 12 weeks, and there were no changes in $\dot{V}O_2$ max in either group.
Conclusion: This pilot study suggests that supplementation with Cs-4 (*Cordyceps sinensis*) improves exercise performance and might contribute to wellness in healthy older subjects.

Introduction

Cordyceps sinensis (Berk) Sacc is a natural herbal medicine that has been popular in China for centuries for invigoration, health preservation, and reduction of fatigue.[1] Naturally occurring *Cordyceps sinensis* is a wild fungus found on the Qinghai-Tibetan Plateau of China at an altitude of about 10,000 feet. The fungus is parasitic and colonizes the larvae of moths until their inner body is filled with mycelium.[2] Wild *Cordyceps* is a composite consisting of the stroma of the parasite together within the larva of the Hepialidae moths.[3] Wild cordyceps is increasingly rare in its natural habitat, and the price is now completely out of reach for clinical practice.[4] For this reason and because of the scarcity of natural sources, a refined standardized fermentation product, Cs-4,® was produced from the mycelial strain *Paecilomyces hepiali* Chen at Dai that was isolated from wild *C. sinensis*. A close similarity between this fermentation product and natural *Cordyceps* has been demonstrated with respect to their chemical constituents (Cs-4 contains not less than 0.14% adenosine and 5% mannitol) and pharmacologic properties.[2,5]

The mechanisms of action of *Cordyceps* and its fermentation product Cs-4 in improving general well-being and physical ability have yet to be fully investigated.[6] Improvements in quality of life have been suggested in patients with chronic heart failure,[7] renal failure,[8] and chronic pulmonary disease.[9] *Cordyceps* gained world attention in 1993 when Chinese female runners achieved records in 1500 m, 3000 m, and 10,000 m events.[10] Their coach attributed their success to a diet containing *Cordyceps*. It was suggested that *Cordyceps* helped improve exercise capacity in these athletes via antioxidant effects. Despite these reports, the ability of *Cordyceps* or Cs-4 to enhance aerobic capacity has not been tested objectively. Because of the popularity of *Cordyceps* among the older population in China,[2] but recognizing the advantages

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The in Vitro and in Vivo Anti-Inflammatory Effect of Osthole, the Major Natural Coumarin From Cnidium Monnieri (L.) Cuss, via the Blocking of the Activation of the NF-κB and MAPK/p38 Pathways

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Abstract

Background: Ulcerative colitis (UC) is a chronic inflammatory condition of the intestines and is difficult to cure once diagnosed. The efficacy of the current clinical treatment for UC is limited. Common anti-inflammatory drugs are prone to adverse effects, while novel biological agents are expensive, although tolerated by patients. Therefore, an urgency exists to find more safe and effective drugs to treat UC. Osthole is an active constituent isolated from the fruit of Cnidium monnieri (L.) Cuss. Osthole has anti-inflammatory activities and offers certain intestinal protection. These characteristics indicate that osthole has the potential to inhibit UC.

Purpose: The study was conducted to investigate the anti-inflammatory potential of osthole in LPS-induced RAW 264.7 cells and dextran sulphate sodium (DSS)-induced ulcerative colitis in mice.

Methods: In in vitro experiments, mouse monocyte-macrophage RAW 264.7 cells were stimulated by 1 μg/ml LPS to produce inflammatory mediators. Griess reagent was used to determine Nitric Oxide (NO) production, and ELISA kits were used to determine the levels of PGE_2, TNF-α, and IL-6. The anti-inflammatory mechanisms of osthole were detected using western blot. In in vivo experiments, UC was induced via the intragastric administration of 3.5% DSS to BALB/C mice for 7 days. During the experiment, clinical signs and body weight were monitored and recorded daily to calculate the DAI score. At the end of the experiment, the colon lengths were measured. The colonic histopathological lesions were evaluated. MPO activity and TNF-α levels were determined using the corresponding kits. The protein expression of TNF-α and NF-κB pathways were analysed using western blot.

Results: In an in vitro study, osthole inhibited the production of NO, PGE2, TNF-α, and IL-6 in LPS-induced RAW 264.7 cells. The results of western blot showed that osthole inhibited the expression of iNOS, COX-2, p38 MAPK and IκB α in RAW 264.7 cells. On this basis, in DSS-induced UC mice, it was found that osthole relieved the symptoms of UC by inhibiting weight loss, colon shortening and the DAI score, and simultaneously alleviating colon tissue lesions. It was also found that osthole reduced the levels of TNF-α in serum and colon tissues and effectively inhibited the activity of MPO. The western blot results showed that osthole reduced the expression of NF-κB p65 and p-IκB α and increased the content of IκB α in colon tissues.