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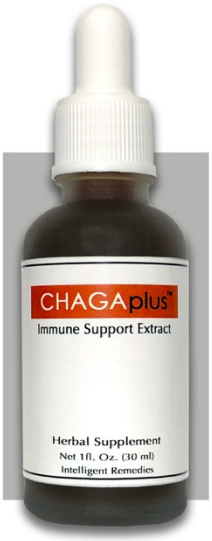
CHAGAplus™

Immune Support Extract

Product Information Sheet



CHAGAplus™ "Immune Support Extract" contains **Phytotherapeutic** Extracts of ***Inonotus obliquus, Ganoderma lingzhi, Curcuma longa, Zingiber officinale, Withania somnifera*** and ***Cinnamomum verum***. **CHAGAplus** contains six of the most effective immune supporting herbs with proven effects against colds and influenza.

Inonotus obliquus Chaga mushroom is a type of fungus that grows mainly on the bark of birch trees in cold climates. By promoting the formation of beneficial cytokines (specialized proteins that regulate the immune system) chaga stimulates white blood cells, which are essential for fighting off harmful bacteria or viruses, As a result, this mushroom could help fight infections. Studies demonstrate that chaga can prevent the production of harmful cytokines, which trigger inflammation and are associated with disease.

Ganoderma lingzhi, the Reishi mushroom, includes triterpenoids, polysaccharides and peptidoglycans, that may be responsible for its health effects. Studies have shown that reishi can affect the genes in white blood cells, in the immune system. Reishi may also alter inflammation pathways in the white blood cells.

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.

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.

Withania somnifera, also known as Ashwaganda, is an adaptogen that can boost brain function, lower blood sugar and cortisol levels, and help fight symptoms of anxiety and depression. Studies in humans have found that it increases the activity of natural killer cells, which are immune cells that fight infection. It has also been shown to decrease markers of inflammation, such as C-reactive protein (CRP).

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.

PLOS ONE

RESEARCH ARTICLE

Inonotus obliquus attenuates histamine-induced microvascular inflammation

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OPEN ACCESS

Citation: Javed S, Mitchell K, Sidsworth D, Sellers SL, Reutens-Hernandez J, Massicotte HB, et al. (2019) Inonotus obliquus attenuates histamine-induced microvascular inflammation. PLoS ONE 14 (8): e0220776. https://doi.org/10.1371/journal.pone.0220776

Editor: Ferenc Gallyas, Jr., University of PECS Medical School, HUNGARY

Received: June 16, 2018

Accepted: July 23, 2019

Published: August 22, 2019

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This work was funded by UNBC internal grants to GWP, CHL and SJ. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Abstract

Cell-to-cell communication is a key element of microvascular blood flow control, including rapidly carrying signals through the vascular endothelium in response to local stimuli. This cell-to-cell communication is negatively impacted during inflammation through the disruption of junctional integrity. Such disruption is associated with promoting the onset of cardiovascular diseases as a result of altered microvascular blood flow regulation. Therefore, understanding the mechanisms how inflammation drives microvascular dysfunction and compounds that mitigate such inflammation and dysfunction are of great interest for development. As such we aimed to investigate extracts of mushrooms as potential novel compounds. Using intravital microscopy, the medicinal mushroom, *Inonotus obliquus* was observed, to attenuate histamine-induced inflammation conducted vasodilation in second-order arterioles in the gluteus maximus muscle of C57BL/6 mice. Mast cell activation by C48/80 similarly disrupted endothelial junctions and conducted vasodilation but only histamine was blocked by the histamine antagonist, pyrilamine not C48/80 suggesting the importance of mast cell activation. Data presented here supports that histamine induced inflammation is a major disruptor of junctional integrity, and highlights the important anti-inflammatory properties of *Inonotus obliquus* focusing future assessment of mast cells as putative target for *Inonotus obliquus*.

Introduction

Inflammation is a protective mechanism that is activated to combat invading pathogens or to reverse tissue injury. The activation of inflammatory pathways stimulates the release of pro-inflammatory mediators including nitric oxide, reactive oxygen species, interleukins (eg. IL-1, I-L6), tumor necrosis factor-alpha (TNF-α), cyclo-oxygenase (COX-2), prostaglandins (PGE2), nuclear factor (NF-κβ)[1]. Although the initial inflammatory response is protective, a chronic and unresolved response can result in cellular damage and facilitates the

Mol. Cells *31*, 423-429, May 31, 2011
DOI/10.1007/s10059-011-0256-7

Molecules and Cells

Inonotus obliquus Protects against Oxidative Stress-Induced Apoptosis and Premature Senescence

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In this study, we investigated the cytoprotective effects of *Inonotus obliquus* against oxidative stress-induced apoptosis and premature senescence. Pretreatment with *I. obliquus* scavenged intracellular ROS and prevented lipid peroxidation in hydrogen peroxide-treated human fibroblasts. As a result, *I. obliquus* exerted protective effects against hydrogen peroxide-induced apoptosis and premature senescence in human fibroblasts. In addition, *I. obliquus* suppressed UV-induced morphologic skin changes, such as skin thickening and wrinkle formation, in hairless mice *in vivo* and increased collagen synthesis through inhibition of MMP-1 and MMP-9 activities in hydrogen peroxide-treated human fibroblasts. Taken together, these results demonstrate that *I. obliquus* can prevent the aging process by attenuating oxidative stress in a model of stress-induced premature senescence.

INTRODUCTION

Reactive oxygen species (ROS), which are constantly produced in biological tissues, play significant roles in various cellular signaling pathways. Homeostasis between oxidants and antioxidants is necessary to minimize molecular, cellular and tissue damage. However, upsetting the balance in favor of oxidants results in oxidative stress and eventually oxidative damage (Rhee, 2006). The free radical theory of aging provides much support for ROS, such as superoxide, hydrogen peroxide and hydroxyl radicals, playing a role in the initiation and progression of the aging process (Harman, 1956). As evidence, aged animals have been shown to produce higher levels of ROS compared to younger animals. In addition, increased oxidative damage of DNA, proteins and lipids has been reported in aged animals (Chen, 2000).

Direct exposure of various cell types to oxidants such as hydrogen peroxide or ultraviolet (UV) radiation can directly induce apoptosis (Nobel et al., 1995). Enhanced apoptosis and elevated levels of ROS play a major role in aging (Schindowski et al., 2001). Apoptosis, or programmed cell death, is an important physiological process and occurs during tissue remodeling. Cells undergoing apoptosis show a sequence of cardinal morphological features, including membrane blebbing, cellular shrinkage and condensation of chromatin (Saraste and Pulkki, 2000). The mitochondria-mediated pathway, when stimulated, leads to the release of cytochrome c from the mitochondria and to the activation of the death signal (Scorrano, 2009; Yu et al., 2010). Apoptotic signaling and execution through this pathway depends on caspases, or aspartate-specific cysteine proteases, which are the key effector molecules in the apoptotic process (Cohen, 1997).

Oxidative stress has been shown to induce stress-induced premature senescence in fibroblasts (Dasari et al., 2006; Frippiat et al., 2001). Stress-induced premature senescence was recently invoked as an explanation of irreversible growth arrest characterized by senescence-specific cell morphology and gene expression, similar to the phenomenon of replicative senescence (Touissant et al., 2000). The number of senescence-associated β-galactosidase (SA-β-gal)-positive cells increases in older animals, and oxidative stress can be increased SA-β-gal activity *in vivo* (Serrano and Blasco, 2001). These data support stress-induced premature senescence models as representative tools for the investigation of aging.

Data on extracts isolated from *Inonotus obliquus* is highly impressive, and these extracts show promise for use as pharmacological therapeutics (Kim et al., 2006; 2007; Nakajima et al., 2009). However, the preventive effects of *I. obliquus* extracts on oxidative stress-induced apoptosis and premature senescence have never been investigated.

In the present study, we investigated the protective effects of *I. obliquus* on fibroblast senescence and apoptosis induced by oxidative stress, as well as the underlying mechanism of cytoprotection.

MATERIALS AND METHODS

Materials

Fetal bovine serum, penicillin G, streptomycin, and Dulbecco's modified Eagle's medium (DMEM) were obtained from GIBCO

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Received October 13, 2010; accepted January 31, 2011; published online February 22, 2011

Keywords: antioxidant, apoptosis, *Inonotus obliquus*, reactive oxygen species (ROS), stress-induced premature senescence

Mycobiology 33(3): 158-162 (2005)

Immunomodulatory Activity of the Water Extract from Medicinal Mushroom *Inonotus obliquus*

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(*Received August 29, 2005*)

The immunomodulatory effect of aqueous extract of *Inonotus obliquus*, called as Chaga, was tested on bone marrow cells from chemically immunosuppressed mice. The Chaga water extract was daily administered for 24 days to mice that had been treated with cyclophosphamide (400 mg/kg body weight), immunosuppressive alkylating agent. The number of colony-forming unit (CFU)-granulocytes/macrophages (GM) and erythroid burst-forming unit (BFU-E), increased almost to the levels seen in non-treated control as early as 8 days after treatment. Oral administration of the extract highly increased serum levels of IL-6. Also, the level of TNF-α was elevated by the chemical treatment in control mice, whereas was maintained at the background level in the extract-treated mice, indicating that the extract might effectively suppress TNF-α related pathologic conditions. These results strongly suggest the great potential of the aqueous extract from *Inonotus obliquus* as immune enhancer during chemotherapy.

KEYWORDS: Bone marrow, Cytokine, Hematopoiesis, Immune modulator, *Inonotus obliquus*

Inonotus obliquus, a white rot fungus in the family *Hymenochaetaceae* has only generative hyphae with no clamp connections and pore instead of gills. It has been known as the clinker polypore because the fruitless form of a fruiting body resembles clinkers, the irregular lumps of black material that remains after coal has been burned, and also known as Chaga throughout the Russia (Wasser, 2002). It is a parasitic fungus growing on birch, alder, beech and other hardwood trees throughout Russia, North America, Eastern Europe, and Japan. Since ancient times, Chaga has been a medicinal fungus in Siberian folk medicine to treat a variety of pharmacological activities such as stomach disease, liver-heart problems, blood purification, and pain relief. It has been also used as Russian and Siberia folk remedy for cancers, including inoperable breast cancer, lip cancer, gastric, parotid gland, pulmonary, stomach, skin, and rectal cancers, and Hodgkin's disease. Today, Chaga is well known for its antimicrobial, antiviral, antitumor activity (Borchers *et al*., 1999). Therefore, scientific research regarding the effects of Chaga has been centered around its common folk uses.

Many bioactive compounds have been reported from the Chaga mushroom. The active substances among these are thought to be oxygenated triterpenes (Park *et al*., 2004). The mushroom contains large amounts of betulin or betulinic acid, a chemical that is being studied for use as a chemotherapeutic agent because of its anti-cancer properties and also the full spectrum of immune-stimulating phytochemicals found in other medicinal mushrooms such as maitake and shitake mushrooms. Although Chaga mushroom may turn out to be one of the most useful medicinal mushrooms, its biological action as immune-modulator has not yet been validated (Kim *et al*., 2005).

Chemotherapy and/or radiotherapy often results in hematopoietic and immune dysplasia as hematopoietic stem cells are damaged during the procedure, and subsequently, committed hematopoietic and immune cells are depleted. Consequently, patients often experience anemia, lymphocytopenia, thrombocytopenia, and/or granulocytopenia, leading to serious and lethal infections and increasing the mortality and morbidity of these patients. Chemotherapeutic agent affects almost all subpopulations undergoing cell division, including early blasts, present in the bone marrow. Therefore, how rapidly patients recover from chemotherapy and/or radiotherapy greatly depends on the percentage of resting stem cells remaining after such treatment. As a means to protect stem cells or help damaged stem cells to recover, the use of biological response modifiers (BRMs) has received attention. Various compounds, especially carbohydrates isolated from mushrooms, yeasts, and plants were reported to affect bone marrow and peripheral blood cells, and to induce hematopoiesis (Hoefer *et al*., 1993). For example, a single peritoneal injection of Scleroglucan, derived from *Sclerotium glucanicum*, enhanced the bone marrow cellularity (Pretus *et al*., 1991), and OL-2 from *Omphalia lapidescens* increased the number of lymphocytes and various immune cells in both the peritoneum and the spleen (Ohno *et al*., 1993). Intravenous injections of glucan-F, a soluble glucan, increased the overall number of granulocyte/macrophage (GM)-col-

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Nutrition Research and Practice 2015;9(2):111-116

http://e-nrp.org

Ethanol extract of *Innotus obliquus* (Chaga mushroom) induces G_1 cell cycle arrest in HT-29 human colon cancer cells

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BACKGROUND/OBJECTIVES: *Inonotus obliquus* (*I. obliquus*, Chaga mushroom) has long been used as a folk medicine to treat cancer. In the present study, we examined whether or not ethanol extract of *I. obliquus* (EEIO) inhibits cell cycle progression in HT-29 human colon cancer cells, in addition to its mechanism of action.
MATERIALS/METHODS: To examine the effects of *Inonotus obliquus* on the cell cycle progression and the molecular mechanism in colon cancer cells, HT-29 human colon cancer cells were cultured in the presence of 2.5 - 10 μg/mL of EEIO, and analyzed the cell cycle arrest by flow cytometry and the cell cycle controlling protein expression by Western blotting.
RESULTS: Treatment cells with 2.5 - 10 μg/mL of EEIO reduced viable HT-29 cell numbers and DNA synthesis, increased the percentage of cells in G_1 phase, decreased protein expression of CDK2, CDK4, and cyclin D1, increased expression of p21, p27, and p53, and inhibited phosphorylation of Rb and E2F1 expression. Among *I. obliquus* fractions, fraction 2 (fractionated by dichloromethane from EEIO) showed the same effect as EEIO treatment on cell proliferation and cell cycle-related protein levels.
CONCLUSIONS: These results demonstrate that fraction 2 is the major fraction that induces G_1 arrest and inhibits cell proliferation, suggesting *I. obliquus* could be used as a natural anti-cancer ingredient in the food and/or pharmaceutical industry.

Nutrition Research and Practice 2015;9(2):111-116; doi:10.4162/nrp.2015.9.2.111; pISSN 1976-1457 eISSN 2005-6168

Keywords: *Inonotus obliquus,* cell cycle, Rb, colon cancer, anti-cancer

INTRODUCTION

Inonotus obliquus (*I. obliquus*), known as chaga mushroom, is a white rot fungus [1]. *I. obliquus* can be made into tea decoctions, extracts, syrup, injections, hip bath agent, and aerosol and has been used as a folk medicine for treating cancer in many areas such as Russia, Asia, and North America [2,3].

Prior studies have reported that *I. obliquus* contains bioactive compounds such as polysaccharides, and polyphenols, which include triterpenoids, steroids, ergosterol peroxides, inotodial, and 3β-hydroxy-lanosta-8,24-dien-21-al, a lignin-like substance. *I. obliquus* has also been shown to possess biological activities, including antioxidant, anti-viral, anti-inflammatory, hepatoprotective, platelet aggregation inhibitory, and anti-tumor effects [4-18]. However, the molecular mechanisms responsible for the anti-cancer effects of *I. obliquus* are not well understood, despite its increasing usage.

Cell proliferation and death are involved in maintenance of homeostasis in normal cells, however, in cancer cells, homeostasis is often disrupted due to deregulation of cell cycle mechanisms [19]. Anti-tumor effects can be attributed to changes in biochemical mechanisms, such as inhibition of proliferation, induction of cell cycle arrest at various cell cycle checkpoints, induction of apoptosis, and regulation of signal transduction pathways, all of which are related to altered expression of key enzymes [20]. The mammalian cell cycle is divided into 4 separate phases: G_1, S, G_2, and M phases. During G_1 phase, cells respond to extracellular signals by either advancing toward another division or withdrawing from the cell cycle into a resting state (G_0) [21]. Cyclin-dependent kinases (CDKs), CDK inhibitors (CDKIs), and cyclins are all important regulators of mammalian cell cycle progression [22]. Each phase of the cell cycle is controlled by different CDKs, each of which is associated with their individual regulatory cyclin. The G_0/G_1 phases of the cell cycle is regulated by CDK4 and CDK 6 associated with cyclin D, late G_1 into early S phase by CDK2 with cyclin E, S phase by CDK2 with cyclin A, and G_2/M phase by CDK1 (CDC2 kinase) with cyclin A or B [23]. Increased

This work was supported by the research grant of the Kongju National University in 2011 and the National Research Foundation of Korea grant funded the Korea government (N0. 2011-0028637).
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Received: August 5, 2014, Revised: October 20, 2014, Accepted: October 21, 2014
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