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Mushroom Nutrition as a Target for Novel Therapeutic Strategies: Relevance to Nutritional Approaches and Antioxidant Redox Modulation in Antiaging Medicine

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Comparative Enzyme Analysis of *Polyporus umbellatus, Agaricus blazei , Pleurotus osteratus* and *Hericium erinaceus*.

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***Coriolus versicolor* Supplementation as Immunonutrition in HPV Patients with Cervical Lesions (LSIL)**

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Rationale

The effects of edible mushrooms is an area of increasing interest associated with health benefits in a number of pathologies, mostly associated with oxidative stress and free-radical-induced cell damage[(1)]. Mushrooms have been valued throughout the world as both food and medicine for thousands of years. Especially in the Asian History the medical use of mushrooms is already described. There are a vast number of studies on their effect in cancer, AIDS, chronic fatigue, asthma and hepatitls.

Several researchers, both in laboratory and in clinical studies, have demonstrated that protein-bound polysaccharide complexes derived from *Coriolus versicolor*; or from *Letinula edodes* are the most important component responsible for immune-enhancing and anti-tumour activities associated with superoxide dismutase mimick activity, the latter due to a small peptide component responsible for oxidative stress mitigation in cancer patients. Moreover, mushrooms have become a new therapeutic target for cytoprotective strategies focusing on their nutritional potential capable of producing health benefits through modulation of immune function.

Mushrooms, in fact, contain a number of enzymes which may participate in several clinical conditions such as tumour and cancer invasion and cardiovascular disorders. However, other factors may be also involved in these biological processes which have not been fully characterized. It has been known that enzyme therapy plays an important role in several clinical conditions such as in cancer treatment, malignant lymphoma and cardiovascular disorders[(2)].

The majority of chemicals to which humans are exposed that increase the risk of cancer require metabolic activation by microsomal enzymes. This activation includes the formation of mutagenic and carcinogenic metabolites that may interact with DNA of target cells. Microsomal enzymes also metabolize steroids and drugs. These reactions and others may render reactive metabolites watersoluble and excretable by conjugating them with glutathione, sulphate and glucuronide[(3)].

Certain plant-derived compounds may afford protection against the formation and action of mutagenic and carcinogenic metabolites[(3)]. The human diet may include many of these plant products. Various studies indicate that the chemoprotective action of certain phytochemicals can be associated with one or more of the following mechanisms: **(a)** inhibition of metabolic activation; **(b)** preventing the interaction of reactive metabolites with cell DNA; **(c)** enhancement of the detoxification of reactive metabolites; **(d)** suppression of mechanisms of tumour progression[(4)].

In view of recent finding showing that mushroom enzymes are able to prevent oxidative stress as well as to inhibit cell growth in several diseases, enzyme and protein contents were investigated in various mushrooms by simulating the intestinal tract of the human body. These studies report that no laccase or peroxidase activities were found in all biomass from *Polyporus umbellatus* and *Agaricus blazei*. However, after cellular lysis significant levels of laccase and to a lesser extent peroxidase activity were measured in *Hericium erinaceus and Pleuortus osteratus*. Significant levels of tyrosinase activity were also detected in these different species of fungi, with the highest levels found in *Agaricus blazei*, compared to other species in which still significant levels of enzyme activity were observed.

Superoxide dismutase is essential to counteracting reactive oxygen species, or superoxide radicals. A number of pathological changes, including carcinogenesis and cellular degeneration related to aging, are due to reactive oxygen species. These reactive oxygen species are produced by sunlight, ultraviolet radiation, chemical reactions, as well as by metabolic processes, and are toxic to living cells since they oxidize and degrade important biological macromolecules such as lipids, sugars, proteins and nucleic acids.

Central to the ability of the body to resist the harmful effects of reactive oxygen species are a number of enzyme systems, prominent among which is Superoxide dismutase, which catalyses the destruction of superoxide radicals and hence protects oxygen-metabolizing cells from damage by these free radicals. Several researchers have shown that Superoxide dismutase is involved in diseases as diverse as Parkinson´s disease, cancer and anaemia.

Superoxide dismutase measured in samples from edible mushrooms, revealed high activity, especially in *Agaricus blazei* and *Polyporus umbellatus*, The highest SOD activity, however, being measured in *Pleurotus ostreatus* and *Hericium erinaceus* (See following article)*.

Interestingly, there is strong evidence suggesting that important antioxidant and cytoprotective enzymes are present in various edible fungi, pointing out the importance of a therapeutic strategy based on nutritional interventions with mushroom supplementation to prevent and limit the deleterious consequences associated with free-radical induced damage of oxidant disorders such as cancer, coronary heart diseases and neurodegenerative disorders [(5)].

Neurodegenerative Processes

The brain has a large potential oxidative capacity but a limited ability to counteract oxidative stress [(6-8)]. Within the cell, reactive oxygen species (ROS) are physiologically present at minimal concentration as by-products of aerobic metabolism as well as second messengers in many signal transduction pathways and, in normal conditions, there is a steady-state balance between pro-oxidants and antioxidants which is necessary to ensure optimal efficiency of antioxidant defences [(9-12)].

However, when the rate of free radical generation exceeds the capacity of antioxidant defences, oxidative stress ensues with consequential severe damage to DNA, proteins and lipids[(13-15)]. Oxidative stress has been implicated in mechanisms leading to neuronal cell injury in various pathological states of the brain, including neurodegenerative disorders such as Alzheimer's disease (AD)[(16-20)]. Recently the term "nitrosative stress" has been used to indicate the cellular damage elicited by nitric oxide and occurs when intermediates are produced from nitrosate thiol, hydroxyl and amine groups as well as its congeners peroxynitrite,

Mushroom Nutrition *(cont)* ...

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N2O3, nitroxyl anion and nitrosonium (all can be indicated as reactive nitrogen species or RNS) [21-23].

From a molecular point of view, in the central nervous system (CNS) cells are able to fight against oxidant stress using many resources, including vitamins (A,C and E), bioactive molecules (glutathione, thioredoxin, flavonoids), lipoic acid, enzymes (e.g. heat shock protein-32, superoxide dismutase, catalase, glutathione peroxidases, thioredoxin reductase, etc) and redox sensitive protein transcriptional factors (e.g. AP-1, NFkB, Nrf2, HSF) [24-26]. The heat shock proteins (Hsps) are one of the more studied defence system active against cellular damage.

The idea of the pervasive nature of free radicals has been firmly entrenched in the minds of scientists ever since the group of Britton Chance[27] developed the basic biochemical techniques to show that in the resting state 2% of all oxygen consumed by cells is converted into reactive oxygen species (ROS) rather than water. McCord and Fridovich first described superoxide dismutase thus suggesting a physiological role of superoxide [28].

Although, there is now an appreciation that the physiological generation of ROS is likely to be an order of magnitude less, their impact on biomolecules has been amply documented. In response to this assault, the cell has developed a number of antioxidant defence systems such as superoxide dismutase, the peroxidases, the glutathione redox cycle with its associated constitutive enzymes as well as glutathione itself, whose concentration is higher in the cell than that of glucose [27]. Therefore, the cell has become well equipped to cope with the normal production of reactive species.

There is growing evidence that the continuous presence of a small stimulus such as low concentrations of ROS is in fact able to induce the expression of antioxidant enzymes and other defence mechanisms. The basis for this phenomenon may be encompassed by the concept of hormesis [29], a term for generally-favourable biological responses to low exposures to toxins and other stressors and which can be characterized as a particular dose–response relationship in which a low dose of a substance is stimulatory and a high dose is inhibitory. In this context, radicals may be considered to be beneficial since they act as signals to enhance defences rather than deleterious as they are when cells are exposed to high levels of ROS.

On the other hand, oxidants, when in excess can, over long term, disrupt redox homeostasis, impose oxidative stress and subsequently lead to a dramatic loss of molecular fidelity which is the major cause for accumulation of unfolded or misfolded proteins in brain cells. Alzheimer's (AD), Parkinson's (PD), and Huntington's diseases, but also amyotrophic lateral sclerosis and Friedreich ataxia belong to the so called "protein conformational diseases" and affect several millions of aged people in all the world [26].

Cells have evolved mechanisms such as the unfolded protein response, where chaperons can rescue misfolded proteins by breaking up aggregates and assisting the refolding process, while proteins that cannot be rescued by refolding are delivered to the proteasome by other chaperones to be recycled[30]. In general, an unfolded protein response conformational diseases are conditions that arise from the dysfunctional aggregation of proteins in non-native conformations. This often is associated with multiple metabolic derangements that result in the excessive production of ROS and oxidative stress[30].

The ability of a cell to deal with ROS and RNS requires the activation of pro-survival pathways as well as the production of molecules endowed with anti-oxidant and anti-apoptotic activities.

It is plausible to exploit the possibility that mushroom nutrition can activate signaling processes within brain cells leading to augmented cellular stress resistance, thereby opening novel therapeutic windows to withstand deleterious effects of oxidative damage in vulnerable neurons and consequently cell death-mediated degenerative diseases [31-53].

*Mycology Research Laboratories Ltd supplied the biomass samples of *Agaricus blazei*, *Pleurotus ostreatus*, *Polyporus umbellatus* and the *Hericium erinaceus* for the study. (www.mycologyresearch.com)

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Comparative Enzyme Analysis of *Polyporus umbellatus, Agaricus blazei , Pleurotus osteratus* and *Hericium erinaceus.*

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INTRODUCTION

Some of the adverse effects of increasing oxidative stress are offset by antioxidants, present either naturally or added as supplements in the diet. Consistently, mushrooms contain a number of enzymes which may participate in several clinical conditions such as tumour and cancer invasion and cardiovascular disorders.

It has been known that enzyme therapy plays an important role in several clinical conditions such as in cancer treatment, malignant lymphoma and cardiovascular disorders[1,2]. All the above evidence supports the notion that nutritional approaches with mushroom biomass can be a novel target for preventive medicine actions based on the modulation of endogenous redox state to withstand conditions of oxidative stress which is the main pathogenic factor operating in aging and neurodegenerative disorders, as well as in the promotion and progression of malignant cells.

Accordingly, a variety of proteins have been isolated and characterized from mushrooms and fungi including lectins, ribonucleases, ribosome-inactivating proteins, anti-fungal proteins, laccases and ubiquitin-like peptides Some of these proteins exhibit anti-proliferative/anti-tumour, anti-microbial and human immunodeficiency virus (HIV)-1 reverse transcriptase (RT) inhibitory activities[3]. These mushroom enzymes mentioned below are thought to prevent oxidative stress as well as to inhibit cell growth in several diseases.

In view of recent findings showing that mushrooms are effective in the treatment of oxidative stress, we have determined the levels of various enzymes associated with the removal of ROS (superoxide dismutase, catalase, peroxidase, GSH-reductase, NADPH-cytochrome C reductase, laccase) as well as tyrosinase and in the following mushrooms: *Polyporus umbellatus, Agaricus blazei, Pleurotus ostreatus* and *Hericium erinaceus.*

METHOD

Antioxidant enzyme activities in these select mushrooms were investigated by simulating the intestinal tract of the human body with the following proteolytic enzymes:

1.Pepsin (500 IU/tablet) at pH 2 for 30 min. at 37°C in an incubator with orbital shaking

2.Trypsin (500 IU/tablet) at pH 7.6 for 30 min. at 37°C in an incubator with orbital shaking.

RESULTS

It was found the following:

1) Highest levels of superoxide dismutase (SOD) (Tables 1,2) were recorded in *Hericium erinaceus* and *Pleurotus ostreatus* (19,430x103 U/500g biomass and 13,043x103 U/500g biomass, respectively), followed by *Agaricus blazei* (143.5x103 U/500g biomass) and Polyporus umbellatus (11.8x103 U/500g biomass). Incubation with pepsin induced a 10 to 20% decrease in enzyme activity, while trypsin decreased by 6-10%, in all species examined but *Hericium erinaceus* where the decrease was 20%.

2) NADPH-Cyt P450 reductase activity (Tables 1,2) was detected in all four mushroom species with Polyporus umbellatus exhibiting the highest activity (10,2 mU/500g biomass), followed by *Pleurotus ostreatus* (8,33 mU/500g biomass), *Agaricus blazei* (7,5 mU/500g biomass) and *Hericium erinaceus* (4,62 mU/500g biomass. In the presence of proteolytic enzymes, enzyme activity was decreased by 40% after pepsin treatment in *Agaricus blazei, Pleurotus ostreatus* and *Hericium erinaceus.* No change in the activity was found in *Polyporus umbellatus.* Interestingly under trypsin exposure only *Pleurotus ostreatus* showed a 50% reduction in the enzyme activity while in the other mushrooms no changes were measured.

TABLE 1	*Polyporus umbellatus*	*Agaricus blazei*	*Pleurotus ostreatus*	*Hericium Erinaceus*
Superoxide dismutase (SOD)	11.8 10^3 U	143.5 10^3 U	13.043 10^3 U	19.430 10^3 U
NADPH Cyt. "P-450" reductase	10,200 uM	7,500 uM	8,330 uM	4,620 uM
GSH Reductase	15,4 U	510 U	69.6 U	21.74 U
Catalase	279.1 U	996.5 U	22.61 U	96.1 U
Laccase			8.15 U	75.6 U
Tyrosinase	3274 U	6849 U	3717 U	2369 U
Peroxidase			0.68 U	4.77 U

Table 1. Enzyme Activity (U/ 500 mg Biomass)

Comparative Enzyme Analysis... *(cont)*

3) Reduced glutathione, most commonly called glutathione or GSH, is a relatively small molecule ubiquitous in living systems. Significant levels of GSH reductase activity were measured *Agaricus blazei* (510 U/500g biomass), *Pleurotus ostreatus* (69.6 U/500g biomass) and *Hericium erinaceus* (21.74 U/500g biomass), with the lowest activity found in *Polyporus umbellatus* (15.4 U/500g biomass) (Tables 1,2). In the condition of the intestinal tract no change in the activity in *Hericium erinaceus* was detected under pepsin. However it was found a significant reduction of 70-80% under pepsin in *Agaricus blazei*, *Pleurotus ostreatus* and *Polyporus umbellatus.* With regards to trypsin effects no reduction of the enzyme activity was measured in *Hericium erinaceus* as well as in *Agaricus blazei. Polyporus umbellatus* showed a 12% reduction and *Pleirozus ostreatus* 34% decrease (Tables 1,2).4)

4) Significant high catalase activity was found in *Agaricus blazei* (996.5 U/500g biomass), followed by *Polyporus umbelatus* (279.1 U/500g biomass). Lower enzymes activity levels were measured in *Hericium. erinaceus* (96.1 U/500g biomass) and *Pleurotus ostreatus* (22.61 U/500g biomass) (Table 1).

5) Searching for laccase activity and peroxidase activities (Tables 1,3), laccase activity was detected in *Hericium erinaceus* (75.6 U/ 500 g of biomass) and *Pleurotus ostreatus* (8.15 U/500g biomass).

6) In the same mushrooms the peroxidase activity was 4.77U/500g biomass and 0.68 U/500g biomass respectively. Conversely, no measurable levels of enzyme activity was found either in *Agaricus blazei* or *Polyporus umbellatus*.

7) Readily detectable levels of tyrosinase were observed in all four mushrooms, particularly, *Agaricus blazei* (6849 U/500g biomass). This enzyme activity was 3717 U/500g biomass in Pleurotus ostreatus, 3274 U/500g biomass in *Polyporus umbellatus* and 2369 U/500g biomass in *Hericium erinaceus* (Table 1).

Table 2 Enzyme Activity in presence of Proteolytic Enzymes (U /500 mg Biomass)

TABLE 2	*Polyporus umbellatus*	*Agaricus blazei*	*Pleurotus ostreatus*	*Hericium erinaceus*
Superoxide dismutase (SOD)	11.8 10^3 U	143.5 10^3 U	13.043 10^3 U	19.430 10^3 U
Superoxide dismutase (SOD) + Pepsin	10.4 10^3 U	115.9 10^3 U	10,671 10^3 U	17,544 10^3 U
Superoxide dismutase (SOD) + Trypsin	11.1 10^3 U	128.7 10^3 U	11,940 10^3 U	14.961 10^3 U
NADPH Cyt. "P-450" reductase	10,200 uM	7,500 Um	8,330 uM	4,620 uM
NADPH Cyt. "P-450" reductase + Pepsin	10,800 uM	4,725 uM	4,957 uM	2,772 uM
NADPH Cyt. "P-450" reductase + Trypsin	11,000 uM	7,510 uM	3,716 uM	5,108 uM
GSH Reductase	15,4 U	510 U	69.6 U	21.74 U
GSH Reductase + Pepsin	4,3 U	84 U	6.9 U	20.9 U
GSH Reductase + Trypsin	13.55 U	517 U	18.55 U	21.80 U

Table 3. Enzyme Activity in presence of Proteolytic Enzymes (U /500 mg Biomass)

TABLE 3	*Polyporus Umbellatus*	*Agaricus blazei*	*Pleurotus ostreatus*	*Hericium Erinaceus*
Laccase			8.15 U	75.6 U
Laccase + Pepsin			6,99 U	62.48 U
Laccase + Trypsin			8.86 U	78.23 U
Peroxidase			0.68 U	4.77 U
Peroxidase + Pepsin			0.57 U	4.32 U
Peroxidase + Trypsin			0.61 U	4.08 U

Comparative Enzyme Analysis... *(cont)*

CONCLUSIONS

In conclusion, these studies suggest that important antioxidant and cytoprotective enzymes are present in all the different fungi examined, suggesting considerable potential for therapeutic strategies based on nutritional interventions with mushrooms to limit and/or prevent the adverse consequences associated with free-radical induced damage in neurodegenerative disorders.

* Mycology Research Laboratories Ltd supplied the biomass samples of *Agaricus blazei, Pleurotus ostreatus , Polyporus umbellatus* and the *Hericium erinaceus* for the study. (www.mycologyresearch.com)

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Coriolus versicolor Supplementation as Immunonutrition in HPV Patients with Cervical Lesions (LSIL)


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The results of a year long clinical trial examining the effects of mushroom supplementation in patients with Human Papillomavirus (HPV) have recently been presented at congress. Dr. Jose Silva Couto and Dr. Daniel Pereira da Silva of the Cervical Pathology Unit of the Portuguese Institute of Oncology in Coimbra, Portugal presented their findings at the 20th European Congress of Obstetrics and Gynaecology, in Lisbon Portugal. This study provides a promising set of results and demonstrates proof-of concept for the question as to whether immunonutrition supplements can be successfully used to improve HPV status in patients.

The poster presentation detailed the results of the evaluation of the efficacy of *Coriolus versicolor* supplementation in patients infected with HPV with low-grade squamous intraepithelial lesions (LSIL). The *Coriolus versicolor* mushroom biomass was in a tablet form (500 mg/tablet).*

Dr. Silva Couto et al. found that *Coriolus versicolor* supplementation (3g /day) over a period of one year substantially increased regression of the dysplasia (LSIL) and induced clearance of the high risk sub-types of the HPV virus responsible for cervical cancer.

	With *Coriolus versicolor*		Without supplementation		Total
TABLE 1	Negative after 1 year	Positive after 1 year	Negative after 1 year	Positive after 1 year	
Citology	13 (72.5%)	5 (27.5%)	10 (47,5%)	11 (52.5%)	39
HPV	9 (91.5%)	1 (10%)	1 (8,5%)	11 (91.5%)	22

Table 1. Results of the treatment of LSIL lesions. *Coriolus versicolor* supplementation demonstrated a 72.5% regression rate in LSIL lesions compared to 47.5% without supplementation.

What do these results mean for HPV patients?

The results from this study are encouraging and provide insight into the effectiveness of *Coriolus versicolor* as a useful immunonutrition agent. Using Coriolus supplementation for one year resulted in 72.5% of recipients reverting to normal cytology state compared with only 47.5% of the control group (non supplemenented) . Encouragingly, 91.5% of the Coriolus recipients reverted to a HPV- status compared with only 8.5% in the control group.

Simply stated the skin texture of the cervical area returned to normal in 72.5% of the patients taking Coriolus supplementation, while the viral load of HPV was not detected (0) in 91.5% of the patients taking Coriolus supplementation. Given that the HPV virus is responsible for the cervical lesions; the impact of the Coriolus supplementation on HPV viral load reduction is considered significant.

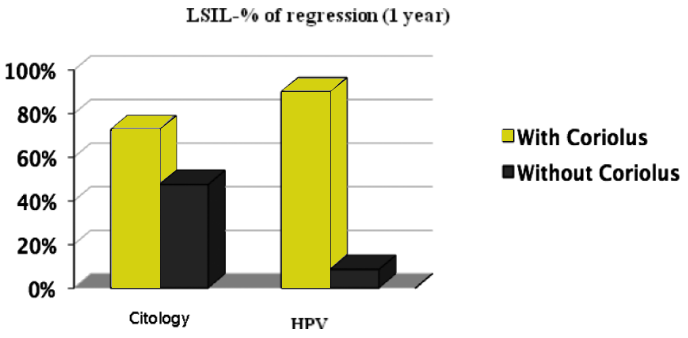

Fig.1 – Percentage of regression of cytologies LSIL and HPV + In LSIL lesions *Coriolus versicolor* supplementation demonstrated a 91.5% regression rate in the high risk HPV virus sub-types compared to 8.5% without supplementation.

While the study sample size is limited in number, the results strongly suggest that using *Coriolus versicolor* as a supplementation agent offers doctors a useful nutritional tool when treating HPV (LSIL) patients over the age of 35 or those HPV (LSIL) patients with compromised immune systems.

It is also likely that *Coriolus versicolor* could be beneficial in HSIL patients who have undergone surgery but who experience recurrent lesions caused by persistent HPV viral infection; the eradication or "control" of the viral infection is key to both LSIL and HSIL patient care.

The estimated cost per day for *Coriolus versicolor* supplementation under this protocol would be €52.00 per month (€1.75 per day) or £ 41.60 per month (£1.40 per day), making Coriolus treatment a viable treatment without undue increases in the cost of therapy.

The use of *Coriolus Versicolor* for 1 year revealed a great efficacy, whether in the regression of the displasia (LSIL), or in the disappearance of the High Risk HPV. It seems therefore, to be a very useful food supplementation with positive therapeutic impact, either in the reversion of LSIL (with High Risk HPV+), or in those HSIL patients, who have undergone surgery but experience continued High Risk HPV viral count.

For more information on mechanism of action, please review *Clinical Journal of Mycology* Vol 1. at www.mycologyresearch.com

* The *Coriolus versicolor* biomass for the study was supplied by Mycology Research Laboratories Ltd in tableform (500 mg/tablet).

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