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The Clinical Journal of Mycology is dedicated to the dissemination of information on the scientific evidence of mushroom nutrition to health care practitioners.

Comparative Enzyme Analysis of *Inonotus obliquus* (Chaga), *Auricularia auricula* and *Poria cocos*

Professor Amin Karmali,
Chemical Engineering and Biotechnology Research Centre
and Department of Chemical Engineering of Instituto Superior de Engenharia de Lisboa (ISEL),
Lisbon Polytechnic Institute, Portugal
- akarmali@deq.isel.pl.pt

Introduction

Inflammation is a natural immune response that takes place in response to trauma, infection, tissue injury or noxious stimuli. During this process, activated inflammatory cells such as neutrophils, eosinophils, mononuclear phagocytes and macrophages increase the secretion of nitric oxide (NO), prostaglandin E2 (PGE2) and cytokines.

Macrophages cells have three main functions in inflammation: antigen presentation, phagocytosis, and immunomodulation through the production of several cytokines as well as growth factors, therefore, they play a crucial role in the initiation, maintenance, and resolution of inflammation[1].

Oxidative stress occurs when the equilibrium shifts in favor of reactive oxygen species (ROS) as a result of a depletion of anti-oxidant agents. Such overproduction of ROS can cause oxidative damage to biomolecules (eg. Lipids, proteins, DNA) which may be responsible to chronic diseases such as atherosclerosis, cancer, diabetes, rheumatoid arthritis, chronic inflammation, stroke, aging and other degenerative diseases in humans.

Oxidative damage is prevented by a defensive system that includes non-enzymatic and enzymatic anti-oxidants. Enzymatic anti-oxidants are widely used as markers of oxidative stress such as superoxide dismutase, catalase, and glutathione peroxidase[2]. Humans have extensively consumed mushrooms for several millennia. Mushrooms are a very rich source of enzymes, secondary metabolites, vitamins, minerals, proteins, polysaccharide, have high fiber content and low fat levels. Mushrooms contain several bioactive molecules such as terpenoids, steroids, phenols, nucleotides, glycoprotein derivatives, peptides, and free and protein-bound polysaccharides. Therefore, they have been considered as potential source of antioxidant and anti-inflammatory activity[3].

As discussed in Clinical Journal of Mycology Vol IV, it has been known for over a century that some enzymes can be used in the prevention and even treatment of several clinical conditions. Important immune-enhancing enzyme activity is found in the biomass form of mushroom nutrition and these enzymes are divided into the following activities:

a) Enzymes that prevent oxidative stress:

Superoxide dismutase

b) Enzymes that prevent cellular growth:

Protease, Glucoamylase

c) Enzymes that promote detoxification:

Peroxidase, Cytochrome P-450

Objective

The aim of the present work was to investigate the levels of enzymes and several secondary metabolites involved in coagulation as well as anti-oxidant agents present in the biomass form of *Inonotus obliquus* (Chaga), *Auricularia auricula* and *Poria cocos* in the presence and absence of proteolytic enzymes.

The following anti-oxidant parameters were investigated: peroxidase, glucoamylase, glucose2-oxidase, superoxide dismutase, cytochrome P-450, cytochrome P-450 reductase, catalase, protease, glutathione peroxidase, glutathione levels and secondary metabolites in all mushroom fractions[1].

Discussion

The data obtained reveal that all tested fungi products contain significant levels of several different enzymes and secondary metabolites including thrombin inhibitor.

The simulation of the gastro-intestinal tract was carried out in the presence and absence of pepsin and trypsin which revealed that the presence of these proteases did not significantly reduce the levels of several different enzymes and secondary metabolites.

Concerning to secondary metabolites, all tested mushroom products exhibited high levels of thrombin inhibitors. Results now obtained suggest that these mushrooms are a rich source of anti-oxidant agents which can protect the organism from the harmful effects of ROS.

Conclusions

The data presented in this study shows that the tested mushroom products are a rich source of enzymes and secondary metabolites including anti-oxidant agents which inactivates ROS. Moreover, immunonutrients in mycelia and primordia (young fruiting bodies) present in the biomass form of of *Inonotus obliquus* (Chaga), *Auricularia auricula* and *Poria cocos* are resistant to proteolytic enzymes (i.e simulation of digestive tract) since it is in a biomass form and not on cell extract.

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	Inonotus obliquus (Chaga)	*Auricularia auricula*	*Poria cocos*
Protein Content	49,5 mg	54,8 mg	59,3 mg
Peroxidase	35,8 mU	31,5 mU	39,5 mU
Glucoamylase	5,9 U	4,3 U	4,1 U
Glucose 2-oxidase	7,2 mU	4,9 mU	6,9 mU
Superoxide Dismutase	975 U	487,5U	446,9 U
Cytochrome P-450	2,9 nmole	2,1 nmole	2,5 nmole
Cytochrome P-450 reductase	10,2 mU	8,5 mU	9,8 mU
Catalase	18,1 mU	15,3 mU	21,5 mU
Protease	29,5 mU	27,1 mU	31,3 mU
Glutathione peroxidase	25,6 mU	18,5 mU	25,9 mU
Glutathione leves (ug/g)	30,3	32,5	19,1
Secondary metabolite (Thrombin inhibitors %)	7,80%	5,70%	6,30%

Table I -Comparative Differences in Enzyme Content between *Inonotus obliquus* (Chaga), *Auricularia auricula* and *Poria cocos*. (Absence of trypsin and pepsin.)

	Inonotus obliquus (Chaga)	*Auricularia auricula*	*Poria cocos*
Protein Content	41,4 mg	48,9 mg	52,6 mg
Peroxidase	30,9 mU	27,7 m U	35,1 m U
Glucoamylase	4,8 U	3,9 U	3,7 U
Glucose 2-oxidase	5,9 m U	4,2 m U	5,1 m U
Superoxide Dismutase	965,8 U	479,3 U	440,1 U
Cytochrome P-450	2,4 nmole	1,8 nmole	2,1 nmole
Cytochrome P-450 reductase	8,9 m U	7,1 m U	7,1 m U
Catalase	16,8 m U	12,8 m U	17,5 m U
Protease	23,9 m U	25,7 m U	28,1 m U
Glutathione peroxidase	21,8 m U	15,1 m U	21,1 m U
Glutathione leves (ug/g)	29,5	30,1	18,1
Secondary metabolite (Thrombin inhibitors %)	6,90%	4,70%	5,90%

Table II - Comparative Differences in Enzyme Content between *Inonotus obliquus* (Chaga), *Auricularia auricula* and *Poria cocos* with presence of pepsin

	Inonotus obliquus (Chaga)	*Auricularia auricula*	*Poria cocos*
Protein Content	43,7 mg	51,3 mg	53,1 mg
Peroxidase	32,7 mU	28,9 m U	32,9 m U
Glucoamylase	5,3 U	4,2 U	3,9 U
Glucose 2-oxidase	6,3 m U	4,4 m U	6,2 m U
Superoxide Dismutase	871,8 U	485,3 U	442,5 U
Cytochrome P-450	2,6 nmole	1,9 nmole	2,3 nmole
Cytochrome P-450 reductase	9,4 m U	7,5 m U	7,9 m U
Catalase	15,4 m U	13,8 m U	19,8 m U
Protease	25,8 U	24,8 m U	28,8 m U
Glutathione peroxidase	23,1 m U	16,7 m U	22,5 m U
Glutathione leves (ug/g)	31,1	30,9	19,5
Secondary metabolite (Thrombin inhibitors %)	7,10%	5,30%	6,10%

Table III Comparative Differences in Enzyme Content between *Inonotus obliquus* (Chaga), *Auricularia auricula* and *Poria cocos* with presence of trypsin

Note: One enzyme unit (U) is defined as the amount of enzyme required to convert one micromole of substrate to product per minute under certain experimental conditions. One milli-enzyme unit (mU) is defined as the amount of enzyme required to convert one nanomole of substrate to product per minute under certain experimental conditions

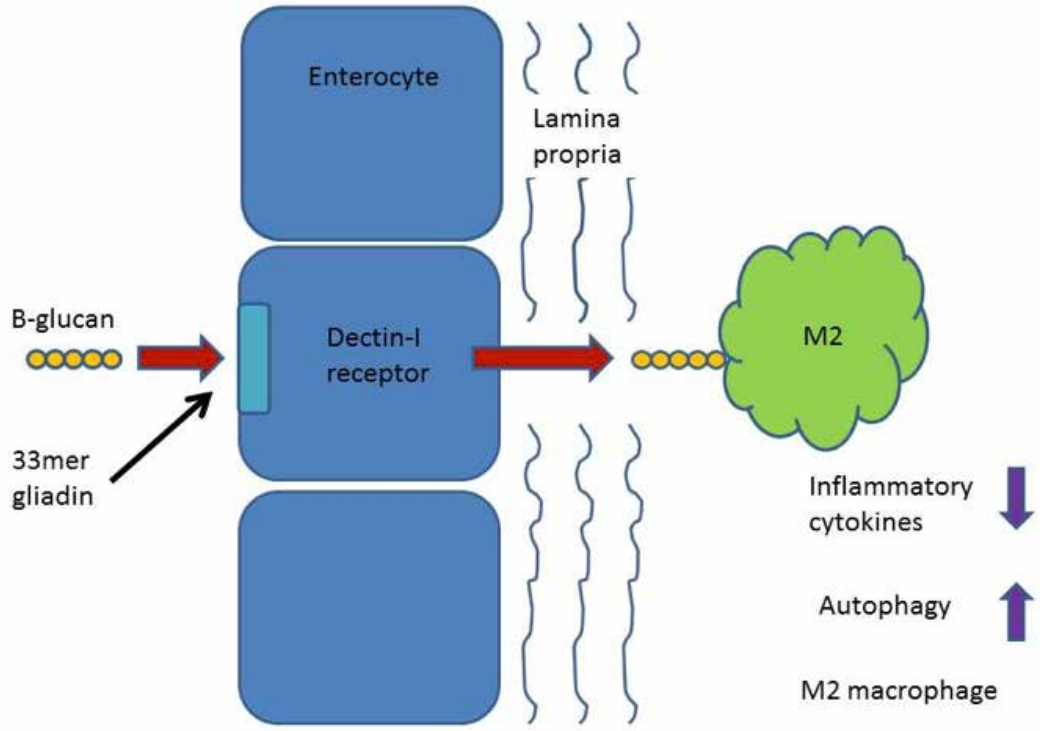
The the biomass form of *Inonotus obliquus* (Chaga), *Auricularia auricula* and *Poria cocos* was supplied by Mycology Research Laboratories Ltd.-United Kingdom. (www.mycologyresearch.com)

Mushroom Nutrition, Dectin-1 and Autophagy: Implications for Celiac Disease?

Chris J. Newton Ph.D.
Centre for Immuno-metabolism,
Microbiome and Bio-Energetic Research (CIMMBER)
and CytoGenex Ltd, UK
- chris.newton@icloud.com

The latest studies on the aetiology of Celiac Disease (CD) provide fresh opportunities to explore the role of mushroom nutrition in the protection of the gut from the pro-inflammatory stimuli. The rationale behind this assertion is two-fold; **i)**, the Dectin-1 receptor contributes (along with changes in gut barrier function and the transferrin receptor-TfR) to the uptake of gliadin peptides by the lamina propria and **ii)**, mushroom-derived β-glucans might directly modulate macrophage activity (phenotype) by up-regulating autophagy. The assertion that mushroom polysaccharides play a role in reducing the outcome (and indeed the risk) of stress-induced inflammation might be viewed as counter-intuitive, as the majority of studies have focussed on the pro-inflammatory role of fungal-derived β-glucans. However, our suggestion is that mushroom nutrition will target enterocyte Dectin-1 receptors, decreasing the uptake of antigens; in other words mushroom-derived β-glucans will compete for binding with the 33-mer form of gliadin peptide to the Dectin-1 receptor. Further to this, recent studies have demonstrated that β-glucans, acting via the Dectin-1 receptor, up-regulate autophagy (Ohman et al., 2014). The process of autophagy is fundamental to cellular homeostasis and in particular, to maintaining the immune system in a state of tolerance. So not only is the activity of the autophagosomal-lysosomal pathway essential to maintaining the regulatory phenotype of T lymphocytes (Treg cells) (Wei et al., 2016) it is also important in maintaining macrophages in what is termed an alternatively activated form or M2 (as opposed to a pro-inflammatory M1 form).

Figure 1 – B-glucans out-compete gliadin for binding to the Dectin – I receptor



Editor´s Note: The Importance of Autophagy

Professor Yoshinori Ohsumi of Japan won the 2016 Nobel Prize in medicine for discovering the mechanisms of autophagy - how cells break down and recycle their biochemical components. Disrupted autophagy has been linked to Parkinson´s, Alzheimer´s and type-2 diabetes and cancer; disorders that appear late in life.

Autophagy controls many physiological functions where cellular components need to be degraded and recycled. It can rapidly provide energy and chemical building blocks when these are needed in response to starvation and other types of stress (ie viral infection). After infection, autophagy helps to eliminate invading germs. It contributes to embryo development and, at the other end of life, cells use autophagy to eliminate damaged components-a quality control mechanism that counteracts the negative consequences of ageing. Mutations in autophagy genes can cause genetic disease an disturbances in the autophagic manchinery have also been linked to cancer.

Reference: "Japanese scientist wins Nobel Prize in medicine for cell studies" Clive Cookson, *Financial Times*,

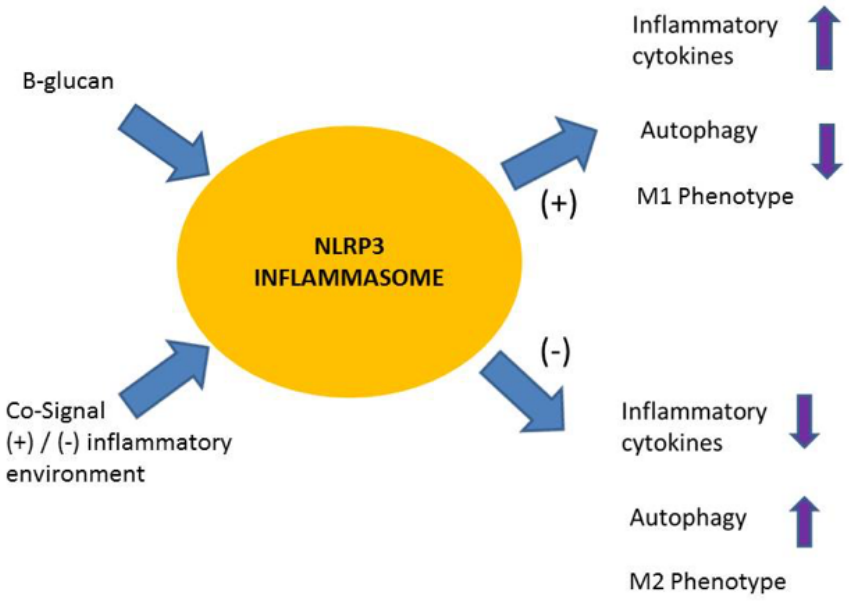


Figure 2 – The NLRP3 inflammasome drives inflammation or autophagy depending on the environment.

In a study to investigate the effect of a high fat diet (HFD) on autophagy in macrophages, Liu et al, (2015) demonstrated impaired macrophage autophagy in obese mice. They also demonstrated that knockout of essential autophagy genes enhanced systemic and liver inflammation when mice were fed a HFD in combination with lipopolysaccharide. By measuring gene and protein expression within Kupffer cells (liver macrophage-like cells) and other macrophages, they were able to demonstrate that this effect was due to a change in phenotype from an M2-like, alternative activated form, to a pro-inflammatory M1 type. The apparent role of the Dectin-1 receptor in activating inflammatory pathways as well as activating autophagy (a process that is often considered as anti-inflammatory) may be explained by the observations that inflammasomes, that are activated early in the inflammatory response, also seem to be involved in autophagy. The knock-out of the NLRP6 inflammasome blocks autophagy in intestinal goblet cells (Wlodarska et al., 2014) and blockade of NLRP3 function decreases lipopolysaccharide-induced inflammatory cytokine expression and increases autophagy in macrophages (Abderrazak et al., 2015).

These findings open up the exciting possibility that mushroom-derived β-glucans might up-regulate autophagy in gut-associated macrophages and in so doing, increase tolerance to food derived antigens such as gliadin. So not only might mushroom nutrition reduce the likelihood of developing Celiac disease in genetically susceptible individuals, it may be helpful as nutritional support during the early stages of a gluten free diet.

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A Safety Assessment of *Coriolus versicolor* Biomass as Food Supplement

Ana B Barros,
Inst. of Hygiene and Tropical Medicine,
Nova University of Lisbon,
Rua da Junqueira 100, Lisboa
- anabdebarros@gmail.com

Professor Jorge Ferrão,
Ministry of Education,
Av. 24 de Julho, Maputo,
Mozambique

Professor Tito Fernandes,
ACIVET Faculty of Veterinary Medicine, Lisbon University, Portugal.
Alto da Ajuda, Rua Prof. Cid. dos Santos,
Lisboa, Portugal
- procattitofernandes@gmail.com

Abstract

Background: *Coriolus versicolor* (CV) is a common mushroom with antitumor, anti-inflammatory, antioxidant, antiviral, antibacterial and immunomodulatory properties. This has been extensively proven mainly using CV extract form while research on the biomass form is scarce. Objective: The aim of this study was to investigate the safety of the CV biomass form as it is commonly used as a food supplement. Design: The CV biomass powder was dissolved in distilled water and administered daily (2.5, 5.0 and 7.5 g/kg live weight) in single doses by gavage to both female and male rats. Results: No adverse or lethal effects were observed as a consequence of the daily administration of CV biomass. Also, compared with the control group, no abnormal findings were observed at necropsy and histopathological examination. Conclusions: The safe profile of *Coriolus versicolor* biomass for human consumption can be inferred from the absence of any remarkable adverse effect in rats.

Introduction

Mushrooms such as *Coriolus versicolor* (CV) increases the activity of the lymphocytes – B cells, T cells and especially NK (natural killer) cells. CV biomass contains mycelia and primordia and is more resistant to proteolytic enzymes (i.e. simulation of digestive tract) than the extracted form [12]. The biomass form also incorporates important immune-enhancing enzyme activity, such as superoxide dismutase, peroxidase, glucoamylase and protease activities that are not detected in extracted forms of mushrooms. The biomass form of mushrooms provide not only β-glucans (e.g. lentinan, schizophyllan and grifolan), also supplied in the extracted form, but also important immune-enhancing enzyme activity (e.g. cytochrome P-450, cytochrome reductase, peroxidase, glucoamylase, β-glucanase, gluco 2-oxidase, laccase, superoxide dismutase and protease) and secondary metabolites (e.g. terpenes, steroids, anthraquinones, benzoic acid derivatives and quinolones) which are not detected in significant quantities in extracted forms of mushrooms[14]. Therefore, biomass forms of mushrooms are considered more beneficial in promoting detoxification and preventing oxidative stress and cellular growth[15]. The purpose of this study was to produce solid scientific evidence on the safety assessment of CV biomass with regard to extrapolate data to humans according to international guidelines. In order to achieve it, the present study was conducted in laboratory animals (rats) using different and increasing levels of inclusion of CV biomass, doses related to animal weight and calculated from previous knowledge of administration in humans. The study complies with European guidelines.[28-31]

Materials and Methods

Coriolus versicolor (CV) biomass used on the present trial was purchased from Mycology Research Laboratories Ltd.

Male and female Wistar Han (RccHan: WIST) rats were used for the present study. The rats were maintained at 25 ± 5°C under a light/dark cycle of 12h and relative humidity 70% ± 10%. All procedures in this study were performed in accordance with Directive 2010/63/UE and the Portuguese National Regulation (Decree Law 113/2013, of 7th August, 2013) and with approval (n° 08/2014) from the Animals Ethics Committee of the Faculty of Veterinary Medicine of Lisbon University.

Safety Assessment

The CV biomass powder was dissolved in distilled water and administered daily (2.5g, 5.0g and 7.5 g/kg live weight/day) in single doses by gavage to both female and male rats (n = 60; 10 males and 10 females per treatment), whereas the control group (n=10; 5 males and 5 females) received only the same feed with no addition of CV. The dose of the rat exposure was calculated to ensure a safety factor of 100 corresponding to some 2g/kg for a 60kg human individual. The general behaviour of the rat was continuously monitored during the 90 days (13 weeks) of the experiment and their body weight was weekly measured and intake adjusted.

Results and Discussion

Main findings and discussion

During the 90 days (13 weeks) of the study no lethal effects were observed as a consequence of the daily administration of CV biomass (2.5g/kg, 5.0g/kg and 7.5g/kg live weight). Animal's appearance and behaviour were similar in all groups during the 90 days (one male rat of the control group died during the study experiment). Normal body weight gains were observed in males and females of all groups and there were no significant differences between relative organ weights of the test groups compared to controls. No abnormal findings were observed at the necropsy.

Although *Coriolus versicolor* being one of the commonly used medicinal products in Japan and China [36], little toxicological information is available regarding its safety either for the extract or the biomass forms. Accumulating evidence suggests that the polysaccharopeptides in the extracted forms are nontoxic even when administered at several times the therapeutically effective dosage and over extended periods. Extended use of PSP at 100-fold the normal clinical dose has not induced acute and chronic toxicity in animals and is not teratogenic [17]. Polysaccharopeptides appear to be safe during pregnancy and no adverse effects of PSP have been observed in female reproductive and embryonic development in mice [37]. According to Loomis and Hayes classification [38], substances with LD50 between 5 and 15g/kg are regarded as being practically nontoxic. As such, in the present study with rats, the NOAEL (no observed adverse effect level) of CV-OH1 biomass was 7.5 g/kg live weight and therefore the Acceptable Daily Intake of CV-OH1 biomass, using a safety factor of 100, can be established at 4.5 g for a human being of average 60kg.

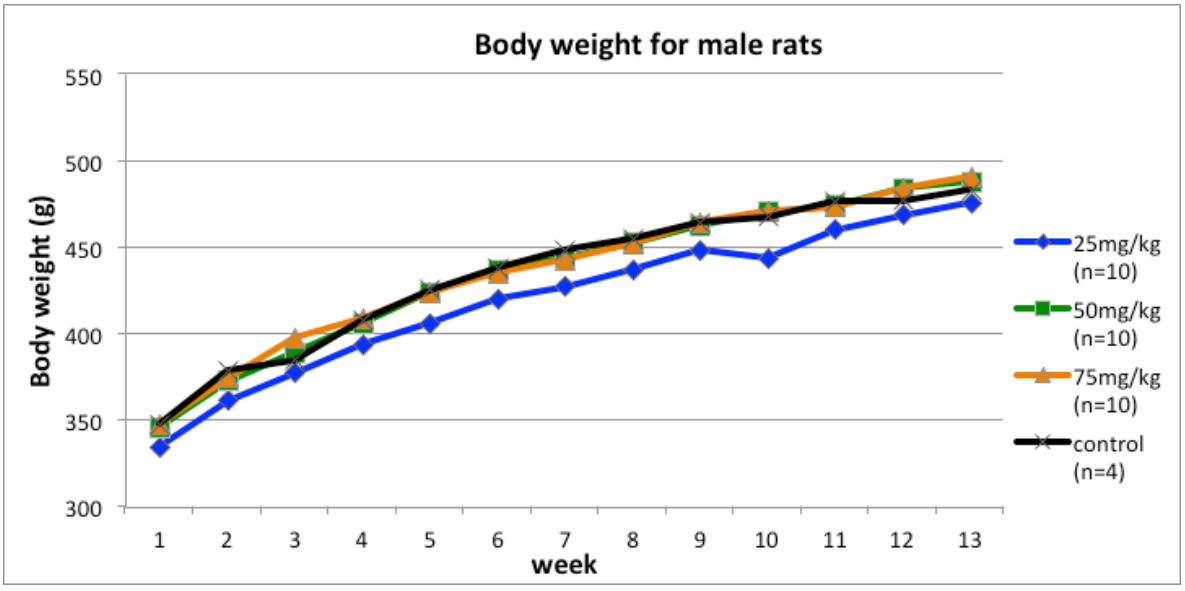
After some exposure to potentially toxic substances, normally there will be a slight reduction in body weight gain and internal organ weights. However, in the repeated dose 90-day oral study, no deaths and no treatment related signs were observed in animals of all groups. All rats at each dosage group continued to gain weight throughout the 90-day study (see graphics below), from juvenile to adult stage [39]. Indeed, in the present study, the absolute organ weights in all treated groups of both sexes were not significantly different from those of the control groups (see table 1 below). This suggests no grossly toxic effect from CV-OH1 biomass.

Although present trial did not aim at carcinogenicity studies, as described by OECD TG 451[31], data here obtained showed no development of neoplastic lesions therefore indicating a non-potential carcinogenicity effect. Several other experiments involving CV extract showed that the antitumor action is due to the enhancement and potentiation of cell mediated immune system through the regulation of immunomodulatory cytokines and activation of the complement system and Natural Killer Cells.[24;40-42]

Conclusion

A safety assessment of the test substance as a food supplement was conducted in male and female rats in three different levels of orally inclusion of CV-OH1 biomass. The level of exposure of 7.5g/kg live weight for 90 days for both male and female rats showed no biologically or statistically significant adverse effects of the biomass consumption. Results demonstrated the safe profile of *Coriolus versicolor* biomass for human consumption inferred from the absence of any remarkable adverse effect in rats.

Editor´s Note: The paper was originally published with the following reference: Barros AB Ferrão, J and Fernandes T. (2016). A safety assessment of *Coriolus versicolor* biomass as a food supplement. Journal of Food and Nutrition Research 2016, Vol 60:29953: http://dxdoi.org/10.3402/fnr.v60.29953



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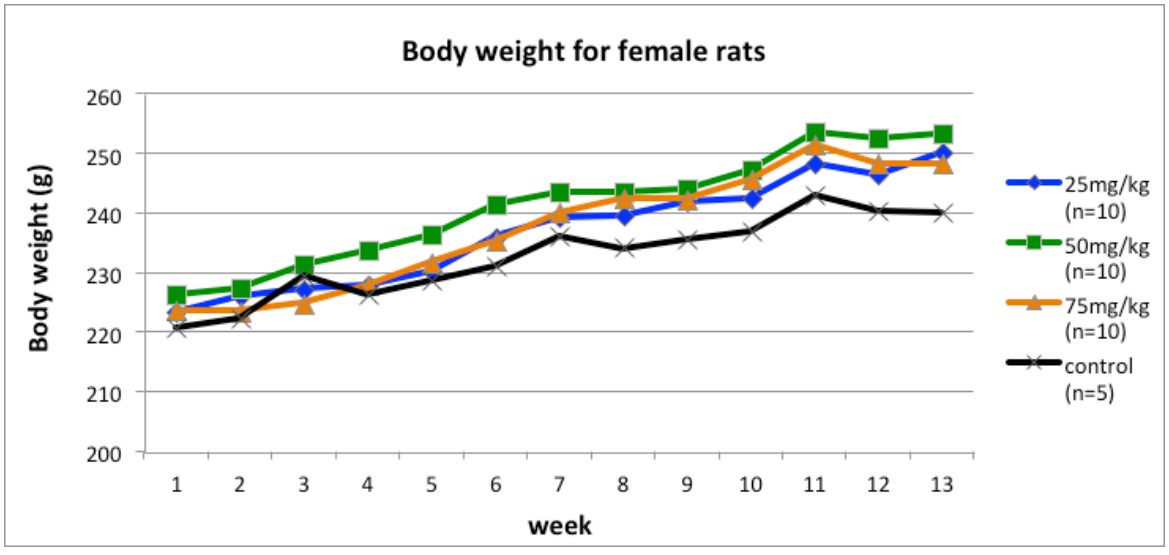

	Administered dose							
	Control		*Coriolus versicolor* biomass					
% Organ weight/body weight			2.5g/kg		5.0g/kg		7.5g/kg	
	mean	± SEM	mean	± SEM	mean	± SEM	mean	± SEM
Male								
Bladder	0.066	0.0144	0.046	0.003	0.054	0.004	0.050	0.006
Heart	0.257	0.0053	0.265	0.011	0.265	0.010	0.272	0.009
Kidneys	0.533	0.0300	0.531	0.009	0.539	0.015	0.553	0.012
Liver	2.481	0.0232	2.751	0.081	2.488	0.134	2.805	0.093
Lungs	0.314	0.0102	0.350	0.016	0.429	0.071	0.386	0.035
Spleen	0.163	0.0122	0.160	0.007	0.184	0.011	0.170	0.006
Stomach	0.423	0.0119	0.467	0.014	0.437	0.010	0.431	0.014
Female								
Bladder	0.069	0.003	0.068	0.003	0.071	0.006	0.081	0.006
Heart	0.357	0.013	0.368	0.015	0.362	0.010	0.367	0.007
Kidneys	0.671	0.019	0.680	0.013	0.658	0.016	0.667	0.017
Liver	2.990	0.043	2.965	0.088	3.111	0.079	3.025	0.078
Lungs	0.559	0.048	0.499	0.012	0.505	0.027	0.485	0.015
Spleen	0.283	0.012	0.275	0.010	0.257	0.011	0.251	0.009
Stomach	0.666	0.029	0.666	0.026	0.637	0.017	0.673	0.027

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Supplementation with *Hericium erinaceus* and *Coriolus versicolor* to Inhibit Progression of Alzheimer´s Disease

Professor Vittorio Calabrese,
Faculty of Medicine, University of Catania, Catania, Italy -
Tel:+39 3288310716 - calabres@unict.it

Maria Laura Ontario PhD.
University of Catania, Catania, Italy

Introduction

The World Health Organization reports that 47.5 million people are affected by dementia worldwide. With ageing populations and 7.7 million new cases each year, the burden of illness due to dementia approaches crisis proportions. Due to increased life expectancy, the prevalence of cognitive decline related to neurodegenerative diseases and to non-neurological conditions is increasing in western countries. Within this context, dementia is a syndrome associated with progressive declines in cognitive capacities and impairments that interfere with daily functioning. These conditions are the primary cause of dependency, disability and institutionalization among older populations.[1]

In 2014, one of the objectives of *Global Action Against Dementia* was to identify a cure or disease-modifying therapy for dementia by 2015. The objective of this paper is to propose a disease-modifying nutritional therapy for early stage dementia based on the use of mushroom nutrition .[2]..

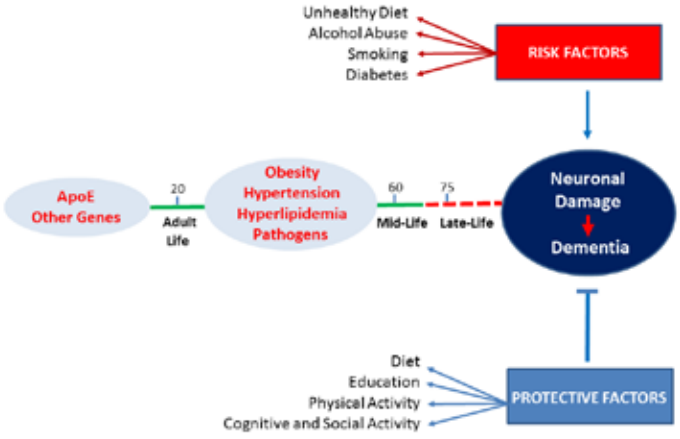

Figure 1: Risk Factors and Protective Factors

***Editors note:** This paper is drawn from the following two papers recently published in 2016 and published with the permission of the authors:

Redox modulation of cellular stress response and lipoxin A4 expression by *Coriolus versicolor* in rat brain: Relevance to Alzheimer´s disease pathogenesis.
Trovato A, Siracusa R, Di Paola R, Scuto M,Fronte V, Koverech C, Luca M, Serra A, Toscano M.A., Petralia A, Cuzzocrea S, Calabrese V. Neurotoxicology. 53:350-8. doi: 10.1016/j.neuro.2015.09.012. 2016.

Redox modulation of cellular stress response and lipoxin A4 expression by *Hericium Erinaceus* in rat brain: relevance to Alzheimer's disease pathogenesis
Trovato A, Siracusa R, Di Paola R, Scuto M, Ontario ML, Bua O, Di Mauro P, Toscano MA, Petralia CC, Maiolino L, Serra A, Cuzzocrea S, Calabrese V.. Immun Ageing. 13:23. doi: 10.1186/s12979-016-0078-8. Jul 9 2016.

What causes Dementia?

Dementia can be caused by:

1. Cerebrovascular diseases (Silent stroke, micro-infarcts, arteriosclerosis)
2. Traumatic Brain Injury (TBI)
3. Hypertension
4. Alzheimer´s disease (AD)

In all these conditions an increased burden of beta amyloid occurs and neuro-inflammations ensues. The most common cause of dementia is Alzheimer´s disease (AD).[3]

Dementia and AD are multifactorial disorders (Figure 1). Hypotheses regarding the cause of dementia have also changed over time. As recently as the 1960s, a vascular aetiology was the prevailing view, while now it is increasingly reported that mixed pathology dementias account for half or more of all dementia cases, with beta-amyloid and vascular disease constituting the most frequent combination of pathologies.

Atherosclerosis, arteriosclerosis, micro-infarcts, silent stroke, and diffuse white matter disease are all associated with increased risk of dementia. Recent evidence suggests an association between mid-life hypertension, a major risk factor for stroke and diffuse white matter disease, and mid-life obesity with future risk of dementia.

Diverse environmental factors, cerebrovascular dysfunction, and epigenetic phenomena, together with structural and functional genomic dysfunctions lead to amyloid deposition, neurofibrillary tangle formation and premature neuronal death, the major neuro-pathological hallmarks of AD.[4,5]

Two major hypotheses have been implicated in the pathogenesis of AD. namely the cholinergic hypothesis which ascribed the clinical features of dementia to the deficit cholinergic neurotransmission and the amyloid cascade hypothesis which emphasized on the deposition of insoluble peptides formed due to the faulty cleavage of the amyloid precursor protein. Current pharmacotherapy includes mainly the acetylcholinesterase inhibitors and N-methyl-D-aspartate receptor agonist which offer symptomatic therapy and does not address the underlying cause of the disease.

The disease-modifying therapy has garnered a lot of research interest for the development of effective pharmacotherapy for AD. β and γ-Secretase constitute attractive targets that are focused in the disease-modifying approach. Potentiation of α-secretase also seems to be a promising approach towards the development of an effective anti-Alzheimer therapy. Additionally, the ameliorative agents that prevent aggregation of amyloid peptide and also the ones that modulate inflammation and oxidative damage associated with the disease are focused upon. On the other hand, development in the area of the vaccines is in progress to combat the characteristic hallmarks of the disease.[5]

The genetic, cellular, and molecular changes associated with Alzheimer disease provide evidence of immune and inflammatory processes involvement in its pathogenesis. These are supported by epidemiological studies, which show some benefit of long-term use of NSAID. The hypothesis that AD is in fact an immunologically mediated and even inflammatory pathological process may be in fact scientifically intriguing.

There are several obstacles that suggest the need for more complex view, in the process of targeting inflammation and immunity in AD. In 2000, researchers led by Dr Frank M LaFerla at the Department of Neurobiology and Behaviour at the University of California Irvine, Irvine, USA, demonstrated that a synthetic

protein that resembles the Herpes Simplex Virus (HSV-1) mimics the structure and function of a protein called β-amyloid, the toxic agent that accumulates in the brains of Alzheimer patients.[6] Moreover, genetic sequencing revealed that two-thirds of the viral protein is identical to the β-amyloid protein, and also, the viral protein generates abnormally twisted fibers similar to those found in AD brain brains (neurofibrillary tangles, formed of hyper-phosphorylated 'tau' protein) representing one of the hallmark of the disease.[7]
Several data indicate that neuronal infection with herpes simplex virus type 1 (HSV-1) causes biochemical alterations reminiscent of Alzheimer's disease (AD) phenotype. They include accumulation of amyloid-β (Aβ), which originates from the cleavage of amyloid precursor protein (APP), and hyper-phosphorylation of tau protein, which leads to neurofibrillary tangle deposition. HSV-1 infection triggers APP processing and drives the production of several fragments including APP intracellular domain (AICD) that exerts trans-activating pro-inflammatory properties. Although a recent study indicated unequivocally lack of evidence for a role of HHV-6 in the pathogenesis of Alzheimer's disease,[8] still there are evidence indicating that, for instance, HSV-1 infection might induce early upstream events in the cell that may eventually lead to Aβ deposition and tau hyper-phosphorylation and further suggest HSV-1 as a possible risk factor for AD.[9-14]

Increasing evidence indicates that aspirin-triggered Lipoxin A4 (LXA4) (15 μg/kg) s c, twice a day, reduced both NF-kB activation and levels of pro-inflammatory cytokines and chemokines, as well as increased levels of anti-inflammatory IL-10 and transforming growth factor B (beta). Basically, LXA4 seems to reduce brain inflammation.[15] Such changes in the cerebral milieu resulted in recruitments of microglia in an alternative phenotype as characterized by the up-regulation of Ym1 and arginase-1 and the down-regulation of inducible nitric oxide synthase expression.[16]

In effect, the researchers contend that activating LXA4 signalling may represent a novel therapeutic approach for AD. Given the potential gastrointestinal discomfort associated with aspirin intake, is there another manner to increase LXA4 in the brain as well as provide both anti-viral protection and anti-oxidant protection?

Why Mushroom Nutrition?

In the past ten years, the clinical development of mushroom nutrition has determined that *Coriolus versicolor* (biomass) has viral protective properties, while *Hericium erinaceus* (biomass) is extremely high in SOD content. Consistent with this notion *Coriolus versicolor* biomass has a clinically verified use in the reduction of viral load of EBV, CMV and HHV-6. These viruses are related to the onset of Chronic Fatigue Syndrome condition.[17,18] In addition, *Coriolus versicolor* has been used to increase the regression rate of LSIL lesions in HPV patients and to significantly reduce the viral load in HPV patients.[19]

Hericium erinaceus biomass has an extremely high super-oxide dismutase (SOD) content which in the presence of in vitro proteolytic enzymes (per 500 mg tablet) has a SOD content of 19.430 10^3 U.[20] This high SOD content is important given that with Herpes Simplex virus infection, apoE4 intensifies virus latency and is associated with the increased oxidative damage to the central nervous system. In addition there is some evidence that herpes simplex virus infection in combination with the apoE4 genotype may be associated with increased risk of Alzheimer´s disease (AD).[21]

Assessing the Capacity of *Coriolus versicolor* and *Hercium erinaceus* to Increase LXA4. LXA4, a metabolic product of arachidonic acid, is considered an endogenous 'stop signal' for inflammation and demonstrates strong anti-inflammatory properties in many inflammatory disorders, such as nephritis, periodontitis or arthritis.[22] Chronic brain inflammation sustains the progression of Alzheimer´s disease, so the objective is to find molecules that can reduce brain inflammation; thereby providing a disease-modifying therapy for dementia.

Research was conducted at Catania and Messina Universities to evaluate if the biomass form of *Coriolus versicolor* and *Hericium erinaceus* stimulates Lipoxin A4 (LXA4) activation in peripheral blood and in the CNS of male rats treated with an equivalent human dose of 3g per day given, orally. One group of rats were supplemented with ***Coriolus versicolor*** biomass and another group (Control) that was not supplemented over 30 days (N=10) [23]. This same protocol was conducted in a separate study with *Hericium erinaceus* biomass over 90 days.[24].

At the end of experimental period animals were sacrificed and the activity of LXA4 was determined in serum, lymphocytes and in different brain regions (cortex, striatum, substantia nigra, hippocampus and cerebellum) and compared with LXA4 of untreated animals, as control.[23,24]

The researchers focused on the impact of *Coriolus versicolor* and *Hericium erinaceus* supplementation on redox-dependent genes, called vitagenes, including heat shock proteins (Hsps), sirtuins, thioredoxin and lipoxin A4 (LXA4).

The differences in the up-regulation of the following vitagenes were measured:
a. Lipoxin A4 (LXA4)

b. Heme Oxygenase-1 (HO-1);

c. Heat Shock Protein 70 (Hsp 70).

d. Thioredoxin

Results: LXA4-Coriolus versicolor vs Control

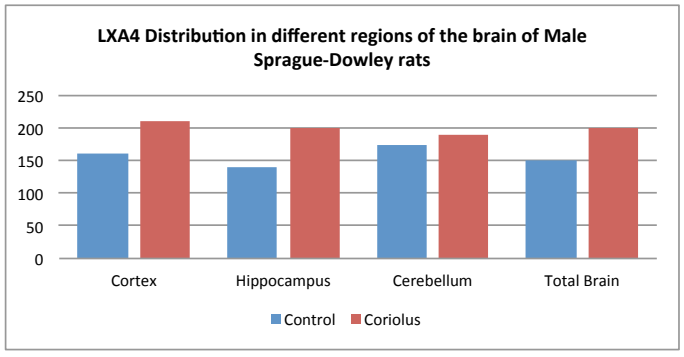

Figure 2

Densitometric Units	Control	Coriolus
Cortex	160	210
Hippocampus	140	200
Cerebellum	175	190
Total Brain	150	200

Table 1

As outlined in Figure 2 and Table I, regional distribution of Lipoxin A4 protein levels in different brain regions and in total brain of control or Coriolus-fed rats. Values are expressed as mean SEM of three independent analyses on 10 animals per group. CX: cortex; Hp: hippocampus; Cb: cerebellum; TB: total brain.

Administration of *Coriolus versicolor* for 30 days at the oral daily dose of 200 mg/kg induced an increase in the protein levels of LXA4 in all brain regions examined. This effect was significant (P<0.05) in the cortex, hippocampus and in the total brain compared to control group, but not in the cerebellum[23].

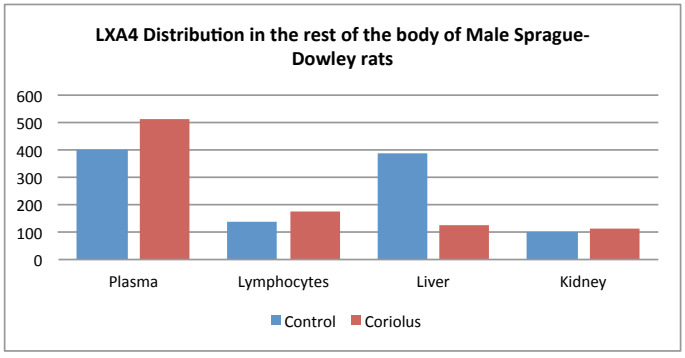

Figure 3

Densitometric Units	Control	Coriolus
Plasma	400	510
Lymphocytes	140	175
Liver	387	125
Kidney	100	110

Table II

In Figure 3 and Table II, demonstrates the distribution of LXA4 levels in plasma from rats fed Coriolus biomass preparation as compared to the control group. Data are expressed as mean SEM of 10 animals per group. *P < 0.05 vs controls; LXA4 levels in liver, kidney and in lymphocytes from rats fed Coriolus biomass preparation as compared to control group. Data are expressed as mean SEM of 10 animals per group. *P < 0.05 vs controls.

As outlined in Table II, animals receiving chronic administration of Coriolus compared to untreated controls, brain changes in LXA4 protein were associated with a significant (P<0.05) increase in plasma (Figure 4 A), lymphocytes and peripheral organs, such as liver and kidney [23].

Heme Oxygenase-1/ Hsp-70 /TrX -*Coriolus versicolor* vs Control

In both Figure 4 and Table III, the Heme oxygenase-1 (HO-1) protein levels in the brain of rats fed Coriolus biomass preparation are compared to the control group. Total brain homogenates from control and mushroom-supplemented rats were assayed for HO-1 expression by Western blot.

As demonstrated in Table III, Coriolus supplementation resulted in up-regulation of brain cellular stress response protein heme oxygenase-1 (HO-1).

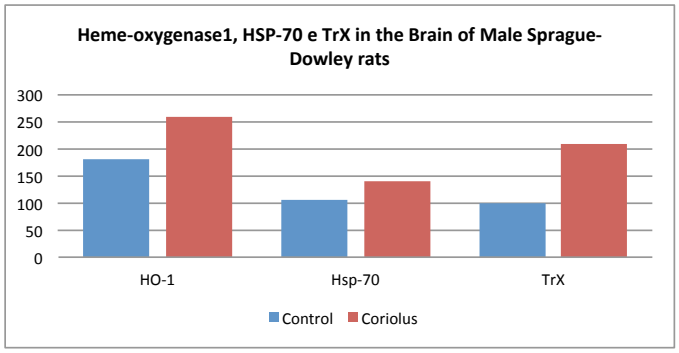

Figure 4

Densitometric Units	Control	Coriolus
HO-1	180	260
Hsp-70	105	140
TrX	100	210

Table III

Inducible Heat shock protein (Hsp-70) protein levels in the brain of rats fed Coriolus biomass preparation are compared to control group. Total brain homogenates from control and mushroom supplemented rats were assayed for Hsp70 expression by Western blot.

As demonstrated in Table III, levels of Hsp 70 were significantly increased.

Thioredoxin (TrX) protein levels in the brain of rats fed Coriolus biomass preparation are compared to the control group. Total brain homogenates from control and mushroom-supplemented rats were assayed for thioredoxin (Trx) by Western blot.

As outlined in Table III, there was a significant increased expression of redox-sensitive thioredoxin in total brain homogenate of Coriolus fed rats when compared to the Control group of rats [23] .

Results II: LXA4 - *Hericium erinaceus* vs Control

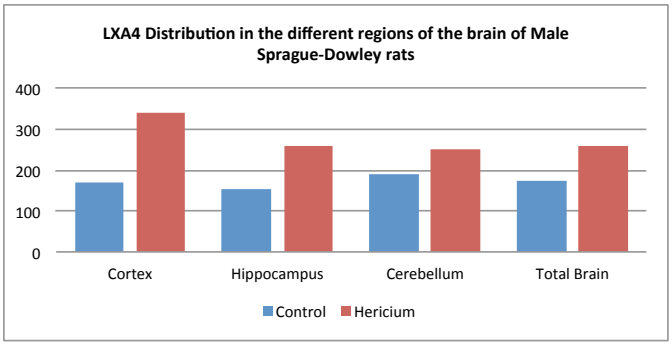

Figure 5

Densitometric Units	Control	Hericium
Cortex	170	340
Hippocampus	155	260
Cerebellum	190	250
Total Brain	175	260

Table IV

As outlined in Figure 5 and Table IV, the regional distribution of Lipoxin A4 protein levels in different brain regions and in total brain of control vs Hericium-fed rats. Values are expressed as mean SEM of three independent analyses on 10 animals per group. CX: cortex; Hp: hippocampus; Cb: cerebellum; TB: total brain. Hericium, was given orally at the dose of 200 mg/kg for 90 days.

Administration of *Hericium erinaceus* for 90 days at the oral daily dose of 200 mg/kg induced an increase in the protein levels of LXA4 in all brain regions examined. This effect was significant (P<0.05) in the cortex, hippocampus, cerebellum and in the total brain compared to control group [24].

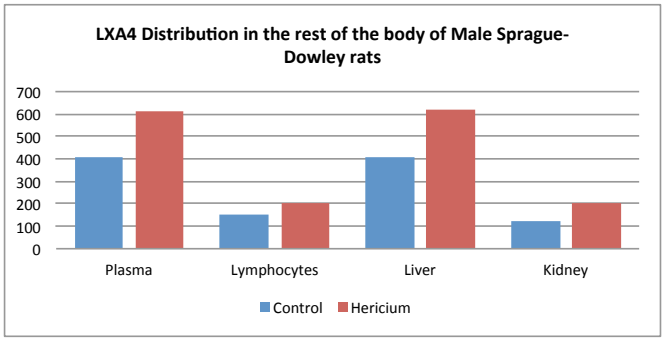

Figure 6

Densitometric Units	Control	Hericium
Plasma	410	610
Lymphocytes	150	200
Liver	410	620
Kidney	125	200

Table V

Outlined in Figure 6 and Table V, provides a comparision of the LXA4 levels in plasma from rats fed Hericium biomass preparation to the control group after 90 days. Data are expressed as mean SEM of 10 animals per group. *P < 0.05 vs controls; LXA4 levels in liver, kidney and in lymphocytes from rats fed Hericium biomass preparation as compared to control group. Data are expressed as mean SEM of 10 animals per group. *P < 0.05 vs controls.

As outlined in Table V, animals receiving chronic administration of Hericium compared to untreated controls, brain changes in LXA4 protein were associated with a significant (P<0.05) increase in plasma , lymphocytes and peripheral organs, such as liver and kidney [24].

Heme Oxygenase-1/ Hsp-70 /TrX –*Hericium erinaceus* vs Control

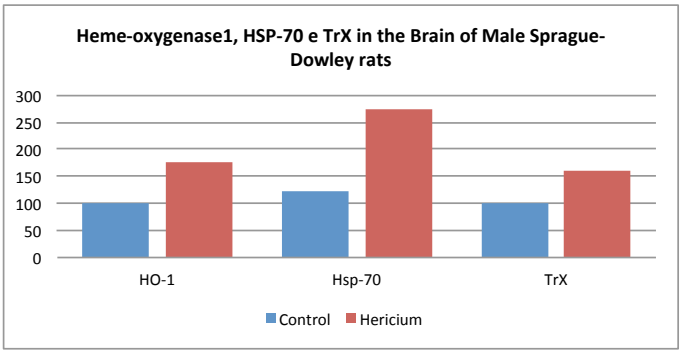

Figure 7

Densitometric Units	Control	Hericium
HO-1	100	175
Hsp-70	124	275
TrX	100	160

Table VI

Outlined in Figure 7 and Table VI are the Heme oxygenase-1 (HO-1), the Inducible Heat Shock (Hsp-70) and Thiorexdoxin (TrX) protein levels in the brain of rats fed Hericium biomass preparation as compared to the control group after 90 days. Total brain homogenates from control and mushroom-supplemented rats were assayed for HO-1 expression by Western blot.
As demonstrated in Table VI, Hericium supplementation resulted in up-regulation of brain cellular stress response protein heme oxygenase-1 (HO-1).

As demonstrated in Table VI, levels of Hsp 70 were significantly increased.
As outlined in Table VI, there was a significant increased expression of redox-sensitive thioredoxin in total brain homogenate of Hericium fed rats when compared to the Control group of rats [24].

Conclusion

Coriolus versicolor biomass and *Hericium erinaceus* biomass supplementation has been shown to significantly up-regulate LXA4 in the brain in rats (in 30 days and 90 days respectively) when compared to separate control groups. In addition, there was a significant increase in heme oxygenase-1, Hsp 70 and thioredoxin in the total brain of both Coriolus-fed rats and Hericium-fed rates when compared to their respective control groups.[23,24]

These results could have implications for the development of a mushroom nutrition based, disease-modifying therapy, for the treatment of patients with Mild Cognitive Impairment (MCI) or pre-Alzheimer´s disease. In such a patient group, the objective is to reduce the first signs of brain inflammation while testing for both viral infections (HSV1, HSV2 or CMV) and genetic susceptibility to AD [24].
This finding has been further refined and consolidated in a subsequent study indicating the powerful therapeutic potential of a supplementation with mushroom nutrition in the control of neuroinflammatory alterations sustaining the pathogenesis of MCI or pre-Alzheimer's disease with potential impact on the course and the progression of the disease.[23,24]
This nutritional approach is not a cure, but a stop-gap approach until a pharmaceutical alternative can be discovered and confirmed.
The next step is to construct a clinical trial that provides a 'proof of concept' in patients.

Supplementation with *Hericium erinaceus* and *Coriolus versicolor* to Inhibit Progression of Alzheimer´s Disease *continued…*

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Note: The *Coriolus versicolor* and *Hericium erinaceus* biomass was supplied by Mycology Research Laboratories Ltd.-United Kingdom. (www.mycologyresearch.com)

Editors Note:
For more information, we recommend that readers review the article entitled "*Link Between Herpes simplex Virus and Alzheimer´s Disease: Potential Role of Mushroom Nutrition Supplementation in Prevention.*" Fernandes, T, Calabrese, V. Clinical Journal of Mycology Vol IV, (Nov 2013).
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Bioactive Properties of Mushroom *Coriolus versicolor*

Aritson Cruz MSc[a], Lígia Pimentel PhD[b], Prof. Tito Fernandes[c] and Prof. Manuela Pintado[d]

The growing consumer concern for health issues has led to an increased interest in functional foods. Besides the nutritional properties, mushrooms have attracted market attention because they are a potential source of bioactive compounds able to perform several positive functions on the health of the consumer. *Coriolus versicolor (CV)*, also known in the literature by *Tramates versicolor* or *Polyporus versicolor,* belongs to the genus *Coriolus,* family *Polyporaceae*, order *Polyporales* and division *Basidiomycotina* (Chen, J., Jin, X., Zhang, L. & Yang, L.).

This mushroom rises up from lignocellulosic wastes and has a fan-shaped wavy margin and may exist in nature in several different colours. Polysaccharides of *C. versicolor* are physiologically active: polysaccharopeptide (PSP) and polysaccharopeptide Krestin (PSK) were isolated and used during the last years as a supplement to support cancer treatments due to its immunostimulatory properties (Sakamoto et al., 2006; Jiménez-Medina et al., 2008).

Furthermore, it seems that these polysaccharides may also act as prebiotics by stimulating the growth and/or activity of probiotic bacteria in the colon (Yu, Liu, Muckherjee & Newburg, 2013). Since most studies have focused on the PSP and the PSK from *C. versicolor* the objective of the present work was to evaluate bioactive properties of mushroom biomass, namely the prebiotic activity.

To evaluate this activity, a sample of MRL-CV (a nutrient adjuvant which contains biomass of the fungus *C. versicolor*) was submitted to the conditions of the gastrointestinal tract (GIT) from the mouth to the intestine.

Species	Growth with 1 % of sample*	Growth with 1 % of sample after GI tract*	Growth with 1% of FOS*
Lactobacillus acidophilus L10	-	-	-
Lactobacillus casei L26	++	+	+
Bifidobacterium longum BG6	_____	+	-
Bifidobacterium animalis B0	++	++	_____

*Bacterial growth after the 48th hour of incubation at 37°C. Growth was measured by enumeration of viable microorganisms (CFUmL). ++, same level of growth compared to glucose; +, weaker growth compared to glucose; - no growth.

Table 1. Summary of the bacterial growth of the species tested

The experimental data showed a potential strain-dependent prebiotic effect with higher activity on the *B. animalis Bo*. Fermentation of *C. versicolor* biomass by *L. paracasei* L26 increased the concentrations of organic acids particularly acetic acid.

Prebiotic agents can have an indirect inhibitory effect on pathogenic bacteria through its selective fermentation by the probiotic bacteria in the colon. However antiadhesive components are another potential strategy to inhibit undesirable bacteria (Rhoades, Gibson, Formentin, Beer, & Rastall, 2006). The adhesion of pathogens can be inhibited through two processes: receptor analogs, which are usually carbohydrates that can mimic the epithelial receptor sites and bind to the bacterial adhesin receptors preventing the bacteria from adhering to the host cells, and adhesin analogs that bind to the host cells surface receptors blocking the pathogens (Gibson & Roberfroid, 2008). Mushrooms may constitute a new source of bioactive molecules with the ability inhibit pathogen infection.

The adhesion of undesirable bacteria to host tissue is the first step in pathogenesis. The effect of the *C. versicolor* biomass upon *Salmonella enterica* (ATCC 13076), *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC CRM 8739) adhesion to mucin was evaluated *in vitro* using mucin (Type II Sigma-Aldrich) as a model of the intestinal mucus. The results showed a potential inhibitory effect of the substrate, especially in the case of *Salmonella enterica*. However, additional studies are needed in mixed cultures and faecal samples in order to assess the bioactivity in an environment involving complex intestinal microbiota.

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Note: The *Coriolus versicolor* was supplied by Mycology Research Laboratories Ltd.-United Kingdom. (www.mycologyresearch.com)

Editors Note:

Prebiotics are substances that induce the growth or activity of microorganisms (e.g., bacteria and fungi) that contribute to the well-being of their host. In diet, **prebiotics** are typically non-digestible fibre and mineral compounds that pass undigested through the upper part of the gastrointestinal tract and stimulate the growth or activity of advantageous bacteria that colonize the large bowel by acting as substrate for them.

Probiotics are preparations of or a product containing viable mono- or mixed microbial culture in sufficient numbers, which applied to animal or man, beneficially affects a compartment of the host nutrition and health by improving the properties of the indigenous microflora. The term **Synbiotic** is used when a product contains both probiotics and prebiotics. This term should be reserved for products in which the prebiotic compound selectively favours the probiotic compound.

[a] **Aritson Cruz**,
Universidade Católica Portuguesa,
CBQF - Centro de Biotecnologia e Química Fina - Laboratório Associado, Escola Superior de Biotecnologia,
Porto, Portugal
- arycrz19@hotmail.com

[b] **Lígia Pimentel**,
Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina - Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal
- lpimentel@porto.ucp.pt

[c] **Prof. Tito Fernandes**,
ACIVET Faculty of Veterinary Medicine,
Lisbon University,
Portugal.
- procattitofernandes@gmail.com

[d] **Prof. Manuela Pintado**,
Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal
- mpintado@porto.ucp.pt

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