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Comparative Differences in ß-1,3-1,6 Glucan content between *Ganoderma lucidum* (Reishi) mushrooms (Biomass vs Extracted) in the Presence of Proteolytic Enzymes

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INTRODUCTION

Some mushrooms have been known to exhibit several medicinal properties for thousands of years in Japanese and other Asian cultures. The Reishi mushroom, also known as *Ganoderma lucidum*, is well characterized (in Traditional Chinese Medicine) for the prevention and treatment of several disease states such as cancer, allergies and asthma.

In the West, *Reishi* is sold in an extracted form (extracted specifically for β-glucan content) or in a biomass form (mycelium and primordial (young fruit body)). The biomass form contains several substances of clinical interest such as enzymes, secondary metabolites and β-glucans. The specific quantification of β-glucans in mushrooms (extracted or biomass forms) with anti-tumour activity is of great clinical importance[1]

There are several types of β-glucans in mushroom species such as β-1,3 glucans and β-1,3-1,6 glucans[2]. As far as anti-tumour immune enhancing and modulating activities, these three activities are attributed to β-1,3-1,6 glucans which exhibit a triple helix as their tertiary structure [3,4].

METHODS

Two samples (1g) of Reishi MRL and Reishi Myco were compared to detect and to quantify enzymes, β-glucans and secondary metabolites. These two samples are different since the former is a biomass that contains mycelia and primordia whereas the latter is a concentrated extract (20x) of fruiting bodies.

The β-1,3-1,6 glucan content was determined by a colorimetric method recently established in Germany [2]. The enzyme and secondary metabolite contents were determined by methods commonly used.

In order to assess the impact of digestive enzymes on the constituents of each sample, both samples were compared *in vitro*; **a)** in the absence of proteolyic enzymes, **b)** in the presence of pepsin and **c)** in the presence of trypsin.

DISCUSSION

The data obtained reveal that, in the absence of proteolytic enzymes, both forms of Reishi contain significant levels of β 1,3-1,6 glucans with anti-tumour activity with Reishi Myco exhibiting higher values (hot water fraction and NaOH fraction).However, in the presence of pepsin and trypsin, Reishi-MRL (biomass form) exhibited higher β1,3-1,6 glucan values than Reishi Myco (extracted form) (See Table I and Fig. 7-11).

When comparing the enzyme values, in the absence of, and presence of, proteolytic enzymes, between the two forms of Reishi, (See Table II and Figures 1-6), there was an absence of important immune-enhancing enzyme activity, such as peroxidase, glucoamylase and protease activities in Reishi Myco (extracted form) when compared to Reishi-MRL (biomass form). The Reishi biomass form demonstrated a greater overall enzyme activity level over the extracted form of Reishi.

It has been known for over a century that some enzymes can be used in the prevention and even treatment of several clinical conditions. These enzymes are divided into the following activities::

Enzymes that prevent oxidative stress:

Superoxide dismutase

Enzymes that prevent cellular growth:

Protease

Glucoamylase

Enzymes that promote detoxification:

Cytochrome P-450

Peroxidase

Glucose 2 oxidase

Reishi Myco exhibits low levels of secondary metabolites compared with the Reishi-MRL product.

CONCLUSIONS

The differences in β-1,3-1,6 glucan content between both samples may be due to differences in biological material in these samples since one contains mycelia and primordia whereas the other consists of concentrated (20x) extract of fruiting bodies. In fact, immunonutrients in mycelia and primordia (young fruiting bodies) in MRL product are more resistant to proteolytic enzymes (i.e simulation of digestive tract) since it is in a biomass form and not on cell extract. Therefore, the concentrated extract of the fruiting bodies is more exposed and available to the action of proteolytic enzymes (i.e simulation of digestive tract) since there are no physio-chemical barriers to prevent such exposure.

In addition to the importance of β-1,3-1,6 glucan content, one should assess the advantages of enzyme supplementation afforded by the biomass form of mushroom nutrition.

Professor Amin Karmali

Fig. 1- Peroxidase

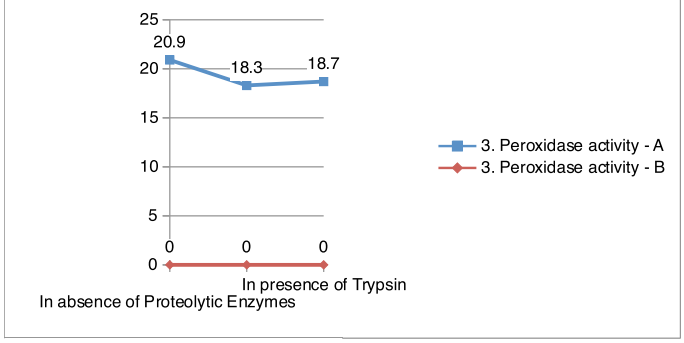


Fig. 2- Glucoamylase

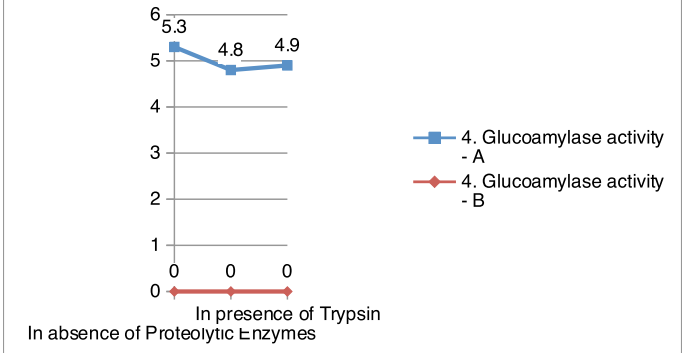


Fig. 3 - Protease

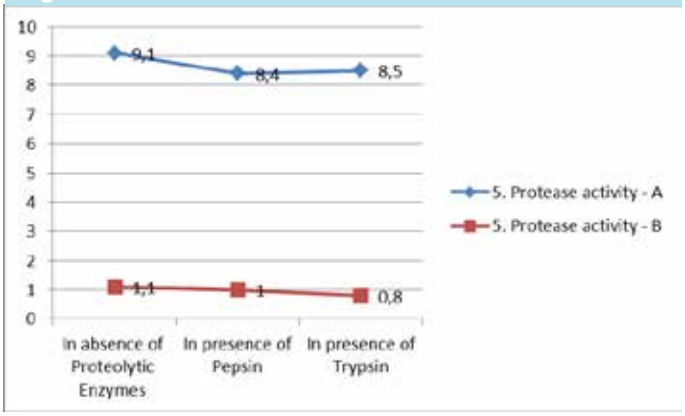


Fig. 4 - Glucose 2-Oxidase

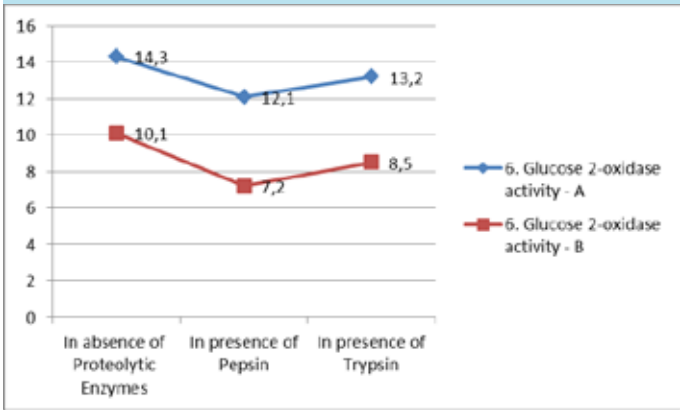


Fig. 5 - SOD

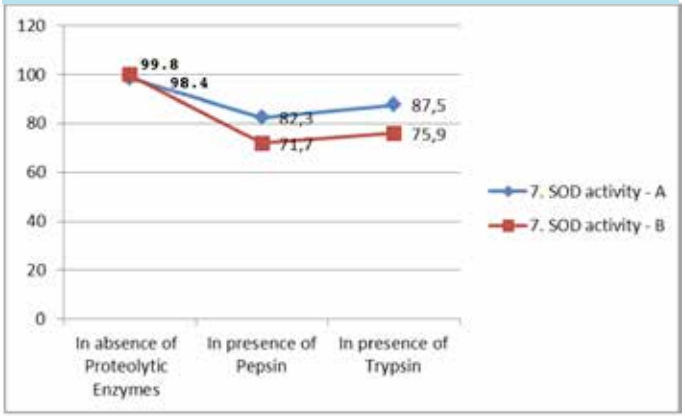
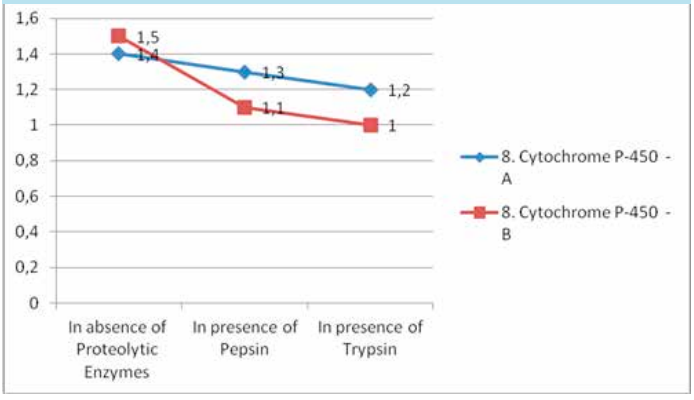


Fig. 6 - Cytochrome 450



Comparative Differences in ß-1,3-1,6 Glucan content between *Ganoderma lucidum* (Reishi) mushrooms (Biomass vs Extracted) in the Presence of Proteolytic Enzymes

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Fig. 7 - Water Soluble Fraction

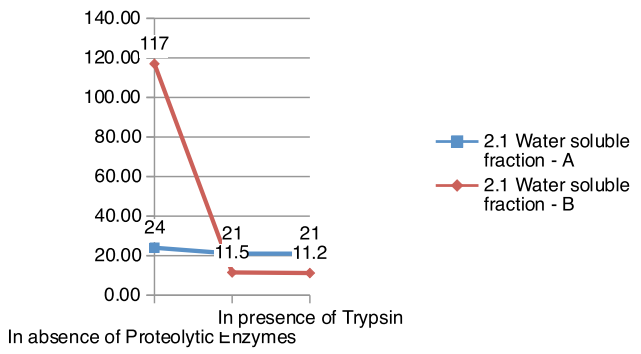

Fig. 8 - Hot Water Fraction

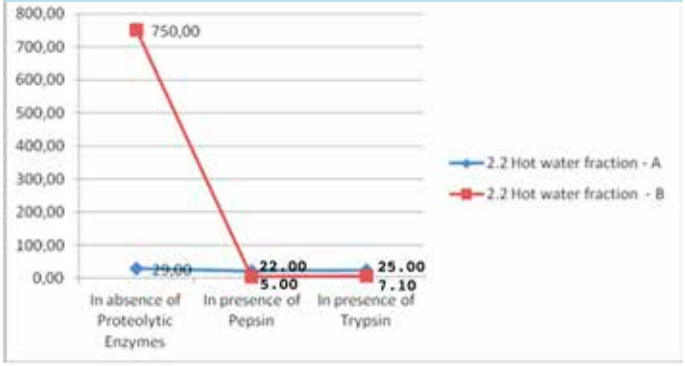

Fig. 9 - NaoH Fraction

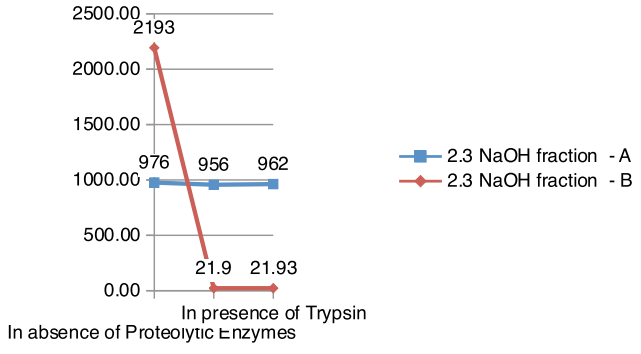

Fig. 10- KOH Fraction

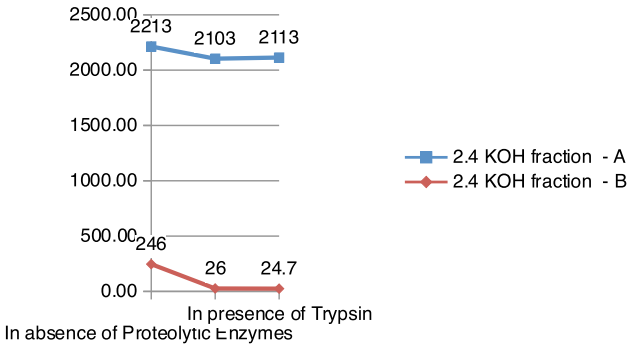

Fig. 11 - HCL Fraction

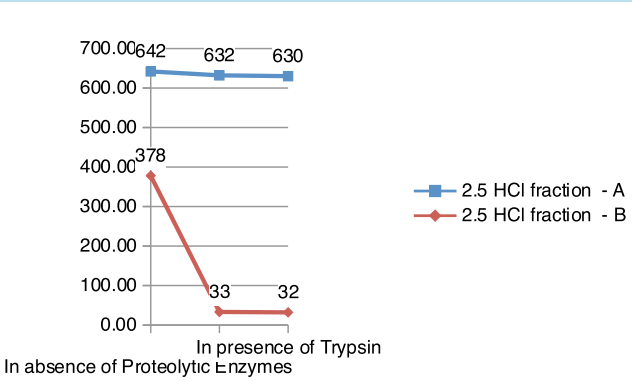

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Table I: Comparision of Impact of Proteolytic Enzymes on Beta 1,3-1,6 Glucan Activity between Reishi-MRL (biomass) and Reishi Myco (extract) (one gram of product in powder form)

	I. IN ABSENCE OF PROTEOLTIC ENZYMES (per gram of product)		
	In absence of Proteolytic Enzymes	Reish A MRL	Reshi B Myco
2.1	Water soluble fraction	24.0µg	117.0µg
2.2	Hot water fraction	29.0µg	750.0µg
2.3	NaOH fraction	976.0µg	2193.0µg
2.4	KOH fraction	2213.0µg	246.0µg
2.5	HCl fraction	642.0µg	378.0µg

	II IN THE PRESENCE OF PEPSIN (per gram of product)		
		Reish A	Reshi B
2.1	Water soluble fraction	21.0µg	11.5µg
2.2	Hot water fraction	22.0µg	5.0µg
2.3	NaOH fraction	956.0µg	21.9µg
2.4	KOH fraction	2103.0µg	26.0µg
2.5	HCl fraction	632.0mg	33.0mg

	III IN THE PRESENCE OF TRYPSIN (per gram of product)		
		Reish A	**Reshi B**
2.1	Water soluble fraction	21.0µg	11.2µg
2.2	Hot water fraction	25.0µg	7.1µg
2.3	NaOH fraction	962.0µg	21.93µg
2.4	KOH fraction	2113.0µg	24.7µg
2.5	HCl fraction	630.0µg	32.0µg

Comparative Differences in ß-1,3-1,6 Glucan content between *Ganoderma lucidum* (Reishi) mushrooms (Biomass vs Extracted) in the Presence of Proteolytic Enzymes

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Table II: Comparison of Impact of Proteolytic Enzymes on Beta 1,3-1,6 Glucan , Enzyme and Secondary Metabolite Activity between Reishi-MRL (biomass) and Reishi Myco (extract) in one gram of powder.

	Enzymes, polysacharides and secondary metabolites per gram of product	In absence of Proteolytic Enzymes		In presence of Pepsin		In presence of Trypsin	
		Reish A MRL	Reshi B Myco	Reish A MRL	Reshi B Myco	Reish A MRL	Reshi B Myco
1	Protein content	44.8mg	40.5 mg	35.9 mg	30.5mg	37.1mg	33.2mg
2	b-1,3-1,6- glucans with anti-tumour activity						
2.1	Water soluble fraction	24.0mg	117.0mg	21.0mg	11.5mg	21.0mg	11.2mg
2.2	Hot water fraction	29.0mg	750.0mg	22.0mg	5.0mg	25.0mg	7.1mg
2,3	NaOH fraction	976.0mg	2193.0mg	956.0mg	21.9mg	962.0mg	21.93mg
2.4	KOH fraction	2213.0mg	246.0mg	2103.0 mg	26.0mg	2113.0mg	24.7mg
2.5	HCl fraction	642.0mg	378.0mg	632.0mg	33.0mg	630.0mg	32.0mg
3	Peroxidase activity	20.9mU	0.0mU	18.3mU	0.0mU	18.7mU	0.0mU
4	Glucoamylase/Beta-glucanasase activity	5.3 U	0.0 U	4.8 U	0.0mU	4.9 U	0.0mU
5	Protease activity	9.1mU	1.1mU	8.4mU	1.0mU	8.5mU	0.8mU
6	Glucose 2-oxdase activity	14.3mU	10.1mU	12.1mU	7.2mU	13.2mU	8.5mU
7	Superoxide dismutase (SOD) activity	98.4mU	99.8mU	82.3mU	71.7mU	87.5mU	75.9mU
8	Cytochrome "P-450"	1.4 nmoles	1.5nmoles	1.3nmoles	1.1nmoles	1.2nmoles	1.0nmoles
9	Cytochrome P 450 reductase	15.5mU	12.5mU	12.6mU	8.7mU	13.3mU	6.2mU
10	Secondary metabolites (Thrombin inhibitors)	4.9%	1.1%	4.6%	1.0%	4.7%	1.0%

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

*Centro de investigação de Engenharia Química e Biotecnologiaia - ISEL

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Coriolus-MRL supplementation in patients infected with low-risk and high-risk HPV subtypes - Bulgarian experience

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INTRODUCTION:

Coriolus-MRL is a food supplement which contains biomass of the fungus *Coriolus versicolor*. Recent studies showed a positive effect of this non-specific immunomodulator in HPV patients[4]. The specialists in Bulgaria have a five-year experience with the use of Coriolus-MRL and the results show high effectiveness in HPV carrying patients[1,2].

Study 1 (2009-2010)

Materials and methods:
100 women aged 16-50, infected with low-risk and high-risk HPV subtypes; Period of treatment: 6 months,

Conservative treatment: Coriolus-MRL 2 x 3 tablets;

Combined treatment: Surgical intervention + Coriolus-MRL 2x3 tablets;

Table 1. Results of a 6-month study: number of the patients and the PAP smears in the respective groups.

1ST MONTH		6TH MONTH		
		PAP I	PAP II	PAP III a
47	PAP I И PAP II	47		–
29	PAP III a	9	20	–
9	PAP III b	3	6	–
15	PAP III d	6	6	3
100				

Table 2. Results of a 6-month study: patients who were found to carry high-risk or low-risk HPV subtypes.

	1ST MONTH	6TH MONTH	
		Negative	Positive
Low-risk HPV subtypes	4	4	–
High-risk HPV subtypes	96	96	11

Results: The results showed that 64 (88%) out of 73 patients on conservative treatment and 25 (93%) out of 27 patients on combined treatment were HPV-negative.
All PAP group IIIa and IIIb patients reverted to PAP group I and/or II (Table 1).

At the end of the study only 11 patients were still positive for 1 or more HPV subtypes (Table 2).

HPV subtype 16 was found to be most treatment resistant [2].

Study 2 (2010-2012)

Materials and methods: 200 patients;

Period of treatment:
6 months; Treatment: Coriolus-MRL 2 x 3 tablets.

Results: 95% of the patients reverted to HPV-negative status. HPV subtype 16 was again most resistant. HPV-positive patients without histological changes reverted to HPV-negative status in 3 months. In patients with mild dysplasia (CIN I) Coriolus-MRL supplementation can help the body clear the infection. In cases with severe dysplasia (CIN II and CIN III) Coriolus-MRL supplementation can strengthen the immune system and reduce the risk of relapse.

Coriolus-MRL have been taken also by the women's partners in about 70% of the couples. 90% of these couples reverted to HPV-negative status after 6 months[1]. 1st month 6th month Negative Positive Low-risk

HPV subtypes 4 4 – High-risk HPV subtypes 96 85 11

Conclusions:
Coriolus-MRL has no adverse reactions and drug interactions with the main drugs used for the treatment and it could be taken during pregnancy. It is an addition to the methods for prevention and treatment (both combined and conservative) of HPV infection

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Coriolus versicolor – Innovation in Prevention of Oncogynecological Diseases, especially HPV.

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ABSTRACT

Coriolus-MRL is a nutrient adjuvant, which contains biomass of the fungus *Coriolus versicolor* and is studied to reverse early stages of cervical cancer and to reduce risk factors of reoccurring HPV virus.

(PMID:19449722 / Akush Ginekol (Sofiia). 2008; 47 Suppl 3:51-3. [PubMed – indexed for MEDLINE [Original Article in Bulgarian; the following an English Translation approved by author.]

SUMMARY

Coriolus-MRL is a nutrient adjuvant, which contains biomass of the fungus *Coriolus versicolor* with immune-modulating components – β-glucans and proteoglucans. Research has been carried out in recent years, establishing the effects of the mushroom biomass in the prevention of HPV patients from developing cervical cancer as well as the prevention against other cancer-causing viruses.

Immuno-modulating nutrition is a new concept, representing the potential for modulating the activities of the immune system through intake of specific nutrients and strengthening the capability of the organism for self-defense and prevention.

Fungotherapy (treatment with medicinal mushrooms) is an important part of immune-modulating nutrition. The biological activity of the fungi is connected with improvement in cardiac activity, strengthening of the immune system, alleviation of allergic symptoms, normalization of sugar blood levels and detoxification of the organism. One of the most important effects is the prevention from cancer-inducing viruses. Such viruses are the Human Herpes virus type 8 (HHV-8), Human papillomavirus (HPV), Epstein-Barr virus (EBV) and hepatitis B virus (HBV)[3].

The relation between HPV and the development of cervical cancer has been proved. It has been established that 80% of all women get infected with HPV within 4 years after they start their sexual life, and that 90 % of all cases of cervical cancer are caused by HPV. Thirteen out of 100 subtypes of HPV are considered high-risk for the development of cervical cancer. Studies by many authors point out a relation between HPV and prostate cancer [7]; some esophagus cancer cases may be also resulting from same virus both in man and women, possibly through oral sex.

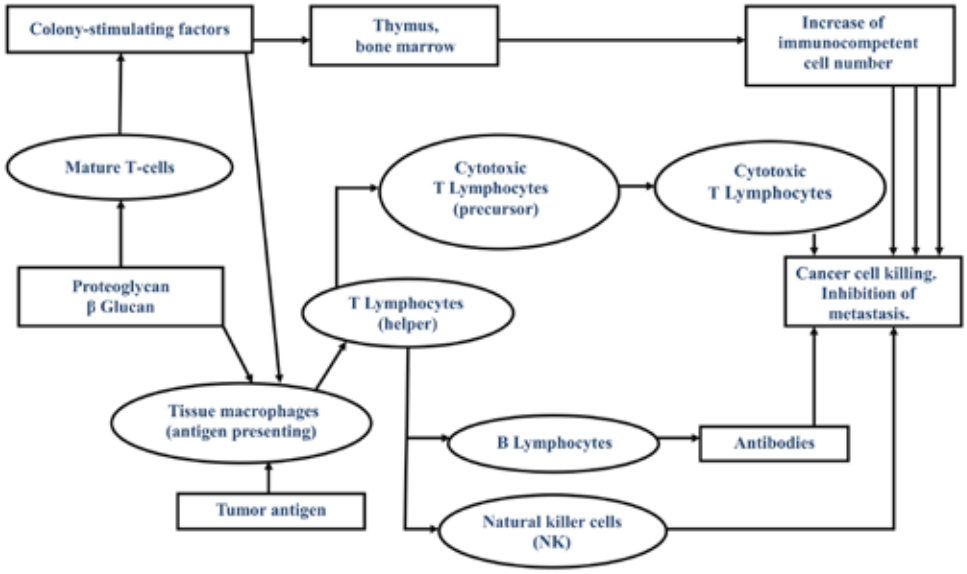
The anti-HPV vaccines introduced in the 1990s are effective against 2 to 4 of the high-risk subtypes HPV and are effective only in HPV-uninfected women. Surgical treatment of HPV lesions is not possible in the early stages and in many cases there is a risk of recurrence of the process.

Coriolus versicolor is a fungus whose biomass acts as a non-specific immunomodulator and is a suitable adjuvant in cancer patients for the strengthening of the immune system, especially after chemotherapy and radiotherapy [1].

This medicinal mushroom is widely distributed in the woodlands of North America, Asia and Europe. It is parasitic on deciduous and coniferous tree species or saprophytic on tree stumps. It consists of mycelium (made up of hyphae) and fruit bodies, where spores for reproduction are formed.

The immunostimulating effect of *Coriolus versicolor* is due to the β-glucans and proteoglucans it contains. β-glucans are polysaccharides, consisting only of glucose residues, and proteoglucans are proteins connected with polysaccharide chains. They have the selective ability to cause apoptosis (programmed cell death) in cancer cells without damaging the healthy ones [4].

Fig.1 Selective ability of the β-glucans to cause apoptosis in cancer cells



Yuliyana Bogdanova PhD

Back in the 1970s two proteoglucans, typical of this fungus species were isolated – polysaccharide K (PSK) and polysaccharopeptide (PSP). A number of studies prove the activity of these compounds in stimulating the effect of natural killer cells and the increase of the number of T-lymphocytes.

The activity of enzymes preventing from oxidizing stress, inhibiting cellular reproduction and contributing for the detoxification of the organism was established. Immunostimulating effect is strengthened by the content of some secondary metabolites (lectines, terpenoids, chelates).

A specially created sort (CV-OH1), a result of 15 years of work of scientists from Mycology Research Laboratories and Gourmet Mushrooms Inc., California, USA is used for the industrial cultivation of *Coriolus versicolor*. The mycelium of this sort has very high bioactivity and vitality and is genetically identical with the initial parent forms. The cultivation of the fungus and the whole process of tablet production ensure preservation of β glucans, proteoglucans and enzymes as major immune-modulating factors. The required microbiological control avoids the presence of impurities and accumulation of pesticides and heavy metals in the biomass. The nutrient adjuvant Coriolus-MRL is not an extract; it contains mycelium and young fruit bodies of the fungus. In this way the synergistic effect of all biologically active compounds in the biomass is ensured.

Over 350 scientific and clinical studies have been published since 1971, when polysaccharide K was discovered. The activity of *Coriolus versicolor* in various diseases, especially oncological diseases was shown. Recent studies have established its positive effect in HPV patients.

Dr. Silva Couto from the Institute of Oncology- Coimbra, Portugal carried out clinical trial on the effect of *Coriolus versicolor* in HPV patients with cervical lesions[2]. 39 patients with low-grade squamous intraepithelial lesions (LSIL), 22 of them with high-risk subtype HPV, were included. Two groups were formed: control group, without any treatment, and a group with an intake of 6 tablets (3g) Coriolus-MRL per day in the course of 1 year. All patients were submitted to colposcopy, biopsy and HPV tipification in the beginning and at the end of the study. Results showed normal cervical cytology in 13 (72.5%) of the 18 women taking Coriolus-MRL. In the control group the percentage was 47.5%. Of the 10 high-risk patients taking Coriolus-MRL, 9 moved from HPV(+) to HPV(-) status. Only one of the 12 women in the control group was HPV(-) at the end of the study. This researcher has reported regression of dysplasia and reversion from HPV(+) to HPV(-) status in high-risk patients with LSIL. In patients with high-grade squamous intraepithelial lesions (HSIL), who were still high-risk HPV(+) after conization, Dr Couto also reports a positive effect.

Dr. Jean Monro – Medical Director in Breakspear Hospital, UK applied Coriolus-MRL in 36 patients with Chronic Fatigue Syndrome and immune deficiency manifested by irregular differentiation of T-lymphocytes, low level of natural killer cells, active viruses and high titres of antibodies, IgG or IgM to a range of viruses[6]. Patients took Coriolus-MRL – 6 tablets of 500mg for 15 days, and 3 tablets of 500mg for 45 days. At the end of the study a doubling of the levels of natural killer cells and decreases in viral loads was established. On the basis of these results and previous studies on the influence of folic acid in HPV control, Dr. Monro recommends a scheme of intake of Coriolus-MRL and folic acid for 8 weeks with the aim of preventing HPV patients from developing cervical cancer and reversing the process of dysplasia. During the treatment period patients are given 3g Coriolus-MRL daily as well as 300mg folic acid daily for 1 week and 10mg folic acid daily for 7 weeks. Control of microbiological and immune markers is recommended in order to evaluate the synergistic effect of Coriolus-MRL and folic acid [5].

Dr. L. J. Standish from the School of Naturopathic Medicine at Bastyr University has received funding from the Cancer Treatment Research Foundation (CTRF) to perform a placebo-controlled clinical trial looking at the effects of *Coriolus versicolor* supplementation on the immune system, quality of life and fatigue of women with breast cancer after completing radiation therapy.

Coriolus-MRL is appropriate as a dietary supplement for patients with immune deficit after illness and after surgical intervention, for prevention of cancer-causing viruses and as adjuvant therapy in patients with cervical cancer, breast cancer and prostate cancer.

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Link between Herpes Simplex Virus and Alzheimer´s Disease
Potential Role of Mushroom Nutrition Supplementation in Prevention

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Herpes exists in two common forms. The majority of the population acquires Herpes Simplex Virus (HSV-1), which causes cold sores, during childhood from non-sexual contact. Herpes Simplex Virus 2 (HSV-2), also known as genital herpes, is transmitted by sexual contact.

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, a toxic agent that accumulates in the brains of Alzheimer patients.[1]

Genetic sequencing revealed that two-thirds of the viral protein is identical to the β-amyloid protein. The researchers showed that, like β-amyloid, it could destroy brain neurons, a key feature in the development of Alzheimer´s. Moreover, in laboratory experiments, the viral protein formed abnormal twisted fibres (neurofibrillary tangles, called 'tau') like those found in the brains of Alzheimer´s patients-the definitive hallmark of the disease.[2]

According to Dr. LaFerla "Most people are exposed to HSV-1, but do not develop Alzheimer´s. However, recent studies show that people genetically predisposed to Alzheimer´s are more likely to develop the disease if they are exposed to herpes".[3]

In support of Dr. LaFerla´s hypothesis, in December 2008, Professor Ruth Itzhaki and her team in the United Kingdom, at the Manchester University´s Faculty of Life Sciences published in the Journal of Pathology that the HSV-1 DNA is located very specifically in amyloid plaques: 90% of plaques in Alzheimer´s disease sufferers´ brains contain HSV-1 DNA, and most of the viral DNA is located within amyloid plaques[4].

The team had previously shown that HSV-1 infection of nerve-type cells induces deposition of the main component, β-amyloid, of amyloid plaques. Together these findings strongly implicate HSV-1 as a major factor in the formation of amyloid deposits and plaques, abnormalities thought by many in the field to be major contributors of Alzheimer´s disease.

The University of Manchester´s data strongly suggest that HSV-1 has a major role in Alzheimer´s disease and point to the usage of antiviral agents for treating the disease, in fact in preliminary experiments they have shown that acyclovir reduces the amyloid disposition and also reduces certain other features of the disease which they have found are caused by HSV-1 infection. Further research has been conducted on the potential use of acyclovir, penciclovir and foscarnet as therapeutic agents for the treatment of Alzheimer`s disease [4][5][6].

In addition to the use of anti-HSV1 antiviral agents to disrupt the HSV1 virus, researchers at the Medical University of South Carolina have suggested the use of immunoglobulin (IG) GM genes based on their putative role as the modulators of host immune response. In some studies, the intravenous use of immunoglobulin acts synergistically with the antiviral, acyclovir.[7][8]

More recently, in May of 2013, Dr. LaFerla and his associates at the Institute of Memory Impairments and Neurological Disorders at the University of California, Irvine, Irvine California published in the American Journal of Pathology, that there could be another method to reduce Alzheimer´s disease (AD).

The researchers demonstrated that aspirin–triggered Lipoxin A4 (LXA4) (15 µg/kg) s c, twice a day, reduced 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). 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.[9] In effect the researchers contend that activating LXA4 signaling may represent a novel therapeutic approach for AD[10].

Given the potential gastrointestinal discomfort associated with aspirin intake, is there another manner to achieve to trigger LXA4 as well as provide both anti-viral protection and anti-oxidant protection in the form of SOD (Super-oxide dismutase) supplementation?

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.

Given this information, there are four reasons to consider the use of mushroom nutrition in the preventative use for patients with Alzheimer´s disease if HSV-1 induced:

Coriolus versicolor has a clinically verified use in the reduction of viruses (EBV, CMV and HHV-6) related to the Chronic Fatigue Syndrome condition[11,12] .

Coriolus versiclor has been used to increase the regression rate of LSIL lesions in HPV patients and to reduce the viral load in HPV patients. [13]

Hericium erinaceus has an extremely high SOD content, which in the presence of proteolytic enzymes per 500 mg tablet has a SOD content of $19.430x10^3$ U[14]. 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 of the central nervous system, and 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)[15].

For those patients not able to both tolerate the side effects of anti-HSV1 virals and/or afford intravenous immunoglubin IG therapy, having an alternative.

The following is a proposed two part study to determine if biomass form of both *Coriolus versicolor* and *Hericium erinaceus* are able to trigger LXA4 stimulation of microglia and indirectly reduces Alzheimer disease symptoms in early stage patients. Working with the University of Catania, the protocol structure is the following:

Protocol to determine if *Coriolus versicolor* and *Hericium erinaceus* stimulates Lipoxin A4 (LXA4) activation in peripheral blood and in the CNS of rats treated with an equivalent human dose of 3 g per day MRLs given, orally, separately or in combination, morning and evening, for 1 and, respectively, 3 months. At the end of experimental period animals will be sacrificed and the activity of LXA4 will be 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.


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20 control subjects and 20 patients with early stage AD diagnosis will be treated with MRLs (*Coriolus versicolor* and *Hericium erinaceus*) for a period of 180 days (See section III). For each mushroom (*Coriolus versicolor* and *Hericium erinaceus*) the supplementation schedule should be six tablets (500 mg) per day or 3 g per day (3 tablets in mornings before breakfast and 3 tablets in evening before dinner). Supplementation period: 180 days. After the treatment period blood samples will be taken and LXA4 activity will be determined in serum and limphocytes together with heme oxygenase-1 expression as marker of anti-inflammatory and antioxidant potential.

Upon determination of the potential for mushroom nutrition to trigger LXA4 activity, the next step will be to clinically test the hypothesis in early stage Alzheimer´s patients.

Protocol Outline for use of Mushroom Nutrition in Early Stage Alzheimer Patients.

A. Patient Selection:

1. Test for Lyme disease and or associated conditions with Lyme disease.*
2. Patients with genetic precondition (based on family history) to Alzheimer's.
3. Patients with high HSV-1 viral load. (Need to define).
4. Cognitive testing to determine a baseline (see point 5).
5. Determine number of "senior moments" per week.
6. Supplement for six months and retest against HSV-1 viral load, cognitive baseline test and number of "senior moments" per week.

B. Supplementation Schedule:

For each mushroom (*Coriolus versicolor* and *Hericium erinaceus*) the supplementation schedule should be six tablets (500 mg) per day or 3 g per day (3 tablets in mornings before breakfast and 3 tablets in evening before dinner). Supplementation period: 180 days.

C. Success Criteria after 180 days:

Testing for:

Table I: Proposed Human Trial Protocol and Corresponding Cost per Day for Use of Mushroom Nutrition in AD.

	Coriolus versicolor 500mg tablets 6 tablets/day = 3g	*Hericium erinaaecus* 500mg tablet 6 tablets/day = 3g	
MONTH 1	180 TABLETS	180 TABLETS	
MONTH 2	180 TABLETS	180 TABLETS	
MONTH 3	180 TABLETS	180 TABLETS	
MONTH 4	180 TABLETS	180 TABLETS	
MONTH 5	180 TABLETS	180 TABLETS	
MONTH 6	180 TABLETS	180 TABLETS	
TOTAL	1080 TABLETS	1080 TABLETS	
Bottle of 90 Tablets	12	12	
Assumed cost per bottle 26.00	318	318.00	
cost per day (€)	**1.77**	**1.77**	**3.53**

*If due to untreated Lyme disease, then one has to understand that the *Borrelia burgdorferi* bacterium is not the only potential cause of the neurological disorder as the disorder is multi-factorial due to potential of co-infections caused by the initially weakened immune state of the individual. In Lyme disease, one has to diagnose the bacterium and then address the immune state of the patients with specific supplementation and diet change [16].

1. Significant changes in cognitive state from day 0 to dayw 180.

2. Significant changes in HSV-1 viral load.

3. Significant changes in "well-being" i.e. rate of change in "senior moments experienced in a week".

D. Potential Daily Cost per day of Mushroom Supplementation to Patient

Supplementation at the price of a full treatment for six months would be equal to two "Big Macs" and soft drinks per day.

Concluding Remarks:

In sum, given the fact that there may be a viral trigger for the onset of Alzheimer´s disease and given the recently confirmed profile of mushroom nutrition supplying both immune modulation and SOD supplementation, it seems with worth the effort to

a) determine if mushroom nutrition does trigger lipoxin A4 activitiy in the microglia; and if so:

b) conduct a clinical trial in early stage patients with an established (and recognized) parameters for success. Such parameters could be a) decrease in onset of AD and b) decrease in rate of increase "tau" production.

Conclusion

Given the expanding number of patients with Alzheimer´s disease and the increasing cost of care for Alzheimer patients, government healthcare services require safe, clinically reliable and cost effective protocols that treat the symptoms of Alzheimer´s disease; the aforementioned two part protocol may offer a cost effective tool for the management of this condition in developing and developed countries.

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