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The Clinical Journal of Mycology is dedicated to the dissemination of information on the clinical use of mushroom nutrition to health care practitioners

Central Nervous System Profiling of *Hericium erinaceus* Biomass Powder by an Electropharmacogram Using Spectral Field Power in Conscious Freely Moving Rats

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SUMMARY

Mushroom extracts seem to exert an action on brain function. In order to objectify such an effect changes of the electric activity of the brain have been successfully used in earlier experiments in the presence of diverse food extracts.

Changes of field potentials recorded from implanted electrodes into the depth of the brain of rats served to analyse the action of plant-derived extracts in comparison to food supplements and reference drugs. Frequency analysis of the data and feeding of the results into discriminant analysis allowed indication dependent classification of the effects. The present investigation aimed at the neuro-physiological characterization of the effect of a preparation of *Hericium erinaceus* in this model.

The presently tested mycological preparation "MRL's *Hericium erinaceus* hyphal powder" induced a pattern of frequency changes consisting in a statistically significant attenuation of delta, theta, alpha2 and beta1 spectral power, but not alpha1 power in all brain regions during the first 2 hours after administration. The lack of alpha1 spectral power attenuation in combination with attenuation of delta, theta and alpha2 power is shared by some other preparations tested earlier under identical conditions like Zembrin®, Acetylsalicylic acid, Methylphenidate, Taxifolin and Ginkgo extract. From this, calming, analgesic, antidepressive and cognition enhancing properties might be deduced for the tested mushroom biomass. However, due to the fact, that only one dosage was tested, interpretation of the results is limited. As active ingredients recently discovered erinacines from *Hericium ericaneus* mycelia might be considered, which show up in the brain as early as 30 min after oral administration.

INTRODUCTION

Drugs, food supplements and functional food exert their action within the organism by interaction with targets defined biochemically (e.g. receptors, enzymes, channels transporters, large protein molecules sometimes also sitting at the outer surface of cells). With respect to the central nervous system neurotransmitter receptors represent main targets. Interaction of drugs with these molecules induces a signalling cascade, which finally ends up with the control of ion channel conductance. Since the electric activity of single neurons depends on the set of momentarily active ion channels, communication between neurons is governed by channel activity. From here, it is obvious that field potentials contain the information of larger local networks of electrically active neurons, by it reflecting the interaction of externally administered molecules with their targets within the concert of neurotransmission.

Frequency analysis of the field potentials in the presence of drugs leads to the so-called electropharmacogram, which has been widely used in the past to characterize drug actions on rat (Dimpfel, 2007) and human brains (Dimpfel, 2011)(Dimpfel, 2015). Interpretation of the results was performed with respect to neurotransmitter activity as well as aiming at possible clinical indications in humans. A relation between EEG delta waves and cholinergic neurotransmission has been suggested for the first time by Dimpfel (Dimpfel, 2005). Theta waves have been recognized as being influenced by drugs acting at the biochemically defined norepinephrine alpha2 receptor (Dimpfel & Schober, 2001). Presynaptic interaction with this receptor leads to drowsiness and sleep and increases of theta waves have been used as part of a formula describing depth of sleep in humans (Dimpfel et al., 1989). Dopaminergic activity is reflected by changes in alpha2 frequencies (Dimpfel, 2008). Drug induced changes in the beta1 frequency domain relate to glutamatergic transmission, whereas drugs acting at GABA receptors induce increases in beta2 frequencies.

Hericium erinaceus (Bull.: Fr.) Pers. is an edible and medicinal basidiomycete fungus belonging to the class Agaricomycetes, order Russulales and family Agaricomycetes (Kirk et al. 2008). It is commonly known as *Shishigashira* or *Houtou* (meaning "monkey head") in China and *Yamabushitake* (meaning "mountain priest") in Japan. English names for the fungus include Lion's Mane, Monkey's Mushroom, Bear's Head, Hog's Head Fungus, White Beard, Old Man's Beard and Pom Pom (Thongbai et al., 2015). The fruiting body has historically been prescribed as part of traditional Chinese medicine (TCM) and Kampo medicine in Japan, including for treating neurasthenia and general debility (Ying et al., 1987).

Hericium is found across the northern hemisphere in Asia, Europe and North America (Thongbai et al., 2015). In recent years the fruiting bodies and cultured mycelia of Hericium have become increasingly popular in North America and Europe in the form of nutraceuticals and food supplements for improving health and well-being, including for enhancing cognitive function. Hericium fruiting bodies and mycelium can be grown on industrial scale on diverse substrates, including inexpensive agricultural wastes. Both the fruiting body and the cultured mycelia have been reported to produce several classes of bioactive molecules, including polysaccharides, proteins, lectins, sterols, phenols, and terpenoids (Thongbai et al., 2015).

The following *in vitro, in vivo* and human clinical studies have demonstrated that powders, extracts and fractions of Hericium have activities on the central and peripheral nervous systems:

In vitro studies

Secretion of nerve growth factor (NGF) from astrocytes has been noted to be increased with 150µg/mL of the ethanolic extract. Lion's Mane has been noted to increase mRNA expression of NGF in isolated astrocytes to around 5-fold that of control at 100-150µg/mL

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of ethanolic extract in a concentration dependent manner (Mori et al., 2008). Isolated erinacines (A-C), present in the mycelium, are known to stimulate NGF secretion at 1mM concentrations (Kawagishi et al., 1994). Glutamate neuronal excitability appears to be attenuated in the presence of Hericium extracts *in vitro* (Moldavan et al., 2007).

In vivo studies

An increase in NGF mRNA has been detected in the hippocampus, but not cortex, of mice given 5% of the diet as lion's mane for a period of seven days to around 1.3-fold of control (Mori et al., 2008). Hericium appears to protect rats against cognitive decline caused by β-amyloid pigmentation at 5% of the diet (Mori et al., 2011).

In an *in vivo* study in rats, Hericium aqueous extract of fruiting body was able to promote neuronal regrowth after crushing injury to the gluteal nerve. Rats that had induced gluteal nerve damage were able to walk better after ingestion of the extract (Wong et al., 2011).

Compared with saline-treated mice, dietary administration of Hericium ethanolic fruit body extracts at 60 mg/kg once a day for 4 weeks reduced anxiety and depressive-like behaviour in healthy mice assessed through elevated plus-maze, tail-suspension and forced swimming tests. This was associated with increased proliferation of hippocampal progenitors and enhanced neurogenesis (Ryu et al., 2018).

Antidepressant-like effects of ethanolic extract of Hericium mycelium enriched in erinacine A were studied in depressive mice challenged by repeated restraint stress (RS) . The extract at 100, 200 or 400 mg/kg body weight/day was orally given to mice for 4 weeks. After 2 weeks of Hericium administration, all mice except the control group went through with 14 days of RS protocol. Stressed mice exhibited behavioural alterations, including extended immobility time in the tail suspension test (TST) and forced swimming test (FST), and increasing the number of entries in open arm (POAE) and the time spent in the open arm (PTOA). The levels of norepinephrine (NE), dopamine (DA) and serotonin (5-HT) were decreased in the stressed mice, while the level-s of interleukin (IL)-6 and tumour necrosis factor (TNF)-α were increased. These changes were significantly reversed by the administration of Hericium extract, especially at the dose of 200 or 400 mg/kg body weight/day. Additionally, the extract was shown to activate the BDNF/TrkB/PI3K/Akt/GSK-3β pathways and block the NF-κB signals in mice. Taken together, this erinacine A-enriched Hericium mycelium extract could reverse the depressive-like behaviour caused by induced RS and was accompanied by the modulation of monoamine neurotransmitters as well as pro-inflammatory cytokines, and regulation of BDNF pathways. Thus the erinacine A-enriched Hericium mycelium extract has potential for the treatment of depressive disorders (Chiu et al., 2018).

Hericium extracts with known amounts of erinacine A and hericenones C and D were tested in a frail mouse model of physiological aging. Two-months oral supplementation with Hericium reversed the age-related decline in recognition memory. Proliferating cell nuclear antigen (PCNA) and doublecortin (DCX) immunohistochemistry in the hippocampus and cerebellum in treated mice supported a positive effect of the extract on neurogenesis in frail mice (Ratto et al., 2019).

Clinical studies

A double-blind, parallel-group, placebo-controlled trial performed on 50 to 80 year old Japanese men and women diagnosed with mild cognitive impairment, 30 subjects were randomized into two 15 person groups. The subjects of the active group took four 250 mg tablets containing 96% of Hericium hyphal and dry powder 3 times a day for 16 weeks. After termination of the intake, the subjects were observed for the next 4 weeks. At weeks 8, 12 and 16 of the trial, the Hericium group showed significantly increased scores on the cognitive function scale compared with the placebo group; at week 4 after the termination of the 16 weeks intake, the cognitive function scores decreased significantly. The results indicated that Hericium is well tolerated, and improves mild cognitive impairment (Mori et al., 2009).

A recent study carried out on 77 overweight or obese volunteers reported that a daily, 8-week oral supplementation with Hericium (80% mycelium extract and 20% fruiting body extract), coupled with a low calorie diet regimen improved depression, anxiety, sleep, and binge eating compared with subjects undergoing low calorie diet only. This improvement was correlated with increased circulating pro-BDNF levels and pro-BDNF/BDNF ratio, despite the lack of any significant changes in BDNF circulating levels (Vigna et al., 2019).

The present preparation consisting of mycelium powder from *Hericium erinaceus* biomass (Hericium-MRL) was tested as the first study in the animal model of field potential analysis in order to see whether any ingredients can pass the blood barrier and exert an activity on the electric activity of the central nervous system. The electropharmacogram of this preparation should also provide more insight into the effectiveness with respect to time dependence. Since many publications also deal with so-called EEG gamma activity -representing frequencies above 35 Hz-, this parameter was also measured.

Materials and Methods

The *Hericium erinaceus* used in this study was derived from a specific superior isolate and cultivated and homogenised in the form of a biomass on sterile (autoclaved) substrate in Europe, under ISO 22000:2018 standards. The proprietary technology used in the cultivation process ensures that the resulting standardised biomass is free from contamination by other fungi. The obtained biomass contains mycelium and primordia (young fresh fruiting body) of

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this respective mushroom and was supplied by Mycology Research Laboratories Ltd (Luton, United Kingdom).

EEG signals were recorded from frontal cortex, hippocampus, striatum and reticular formation of freely moving rats from inside a totally copper shielded room. Signals were wirelessly transmitted by a radio-telemetric system (Rhema Labortechnik, Hofheim, Germany, using 40 Megahertz as carrier frequency) and were amplified and processed as described earlier to give power spectra with a resolution of 0.25 Hz (Dimpfel et al.,1986) (Dimpfel et al.,1988) (Dimpfel et al., 1989), (Dimpfel, 2003). In short, after automatic artefact rejection electric signals were collected in sweeps of 4 s duration and Fast Fourier transformed using a Hanning window. Sampling frequency was 512 Hz. Four values were averaged to give a final sampling frequency of 128 Hz, well above the Nyquist frequency. The resulting electrical power spectra were divided into 8 specially defined frequency ranges (delta: 1.50 - 4.00 Hz; theta: 4.25 - 6.75 Hz; alpha1: 7.00 - 9.50 Hz; alpha2: 9.75 - 12.25 Hz; beta1: 12.50 - 17.75 Hz; beta2: 18.00 - 34.25 Hz; gamma: 34.50 - 81.00 Hz). Spectra were averaged in steps of 3 min each and displayed on-line. In off-line procedure spectra were averaged to give longer periods for further analysis and data presentation.

The "Tele-Stereo-EEG" animal model consisting of continuous recording of intracerebral field potentials was used in combination with a video tracking system for detection of changes in motility (GJB Datentechnik GmbH, D-98704 Langewiesen, Germany). This system recognized locomotion as well as stereotyped behaviour by following a contrast difference of the black transmitter on the head of the animal in comparison to its environment. The system has been validated in previous studies, for example with different dosages of caffeine. Solutions were prepared fresh for each experimental day and administered orally by gavage after 45 minutes of pre-drug Vehicle recording. Vehicle was water.

The study was performed at the preclinical laboratories of NeuroCode AG, Sportparkstr. 9, D-35578 Wetzlar/ Germany. Allowance according German guidelinesfor animal protection was received (Genehmigung gem. §8 Abs.1 des Tierschutzgesetzes, Ref. # 17 0736 540 13 00007), dated 24th of May 2017).

Table 1
Test compounds

Hericium erinaceus Biomass (Hericium-MRL)	LOT 16K118	150 mg/kg	Mycology Research Laboratories The Spires, Suite 8, Adelaide Str., Luton.UK LU1 5BB
VEHICLE 0.9% NaCl		1.0 ml/kg	Braun, Melsungen, Germany

Nine adult Fisher 344 rats (5 months of age and day - night converted, weight about 350 - 400 g, provided by Charles River Laboratories, D-97633, Sulzfeld, Germany) were used in this experimental series. Animals were implanted with electrodes into the brain and were given 2 weeks for recovery from surgery. After this, the transmitter was plugged in for adaptation and control experiments. During the recording rats were not restricted and could move freely, but did not have food available (chewing would have produced too many artefacts). The principles of laboratory animal care were followed in all trials.

The animals were allowed to acclimatise for at least 4 weeks before the study started. There was automatic control of light cycle, temperature and humidity. Animals were day-night reversed (12h/12h). Daily monitoring indicated that temperature and humidity remained within the target ranges of 22 degree Celsius and 44, 5% humidity, respectively. Cages, bedding, and water bottles were changed at regular intervals, i.e. every 2-3 days. Standard Diet (Nohrlin H10, Altromin, D-32791 Lage, Germany) was available ad libidum. The animals had access to domestic quality mains water ad libidum.

Rats were implanted with 4 bipolar concentric steel electrodes within a stereotactic surgical procedure during anaesthesia with Ketamine. All 4 electrodes were placed 3 mm lateral within the left hemisphere. Dorso-ventral coordinates were 4, 6, 4.2 and 8 mm and anterior coordinates were 3.7, 9.7, 5.7 and 12.2 mm for frontal cortex, striatum, hippocampus, and reticular formation, respectively (according to the atlas of (Paxinos & Watson, 1982)). A pre-constructed base plate carrying 4 bipolar stainless steel semi-micro electrodes (neurological electrodes "SNF 100" from Rhodes Medical Instruments, Inc., Summerland, CA 93067, USA) and a 5-pin-plug was fixed to the skull by dental cement interacting with 3 steel screws placed on distance into the bone. The distant recording spot of the electrode was the active electrode, whereas the proximal spots of the 4 electrodes were connected to each other to give a common reference. The base plate was carrying a plug to receive later on the transmitter during the experimental session (weight: 5.2 g including battery, 26x12x6 mm of size).

A crossover design with at least 1 week of drug holidays in between the administrations was used. Oral administration of 1.0 ml/kg of vehicle (0.9% NaCl) or *Hericium erinaceus* biomass preparation was performed. After a pre-drug period of 45 min for pre-drug recording, drug effects were observed continuously on the screen (artefact control) for 300 minutes subdivided into 15 min periods after a lag time of 5 minutes for calming of animals after oral administration. Changes of electric power µV2 are expressed as % of the 45 min lasting absolute pre-drug spectral power values within each frequency band. Data were averaged from 8-9 animals. Data are expressed as mean values ± S.E.M. Statistics were calculated by means of the Wilcoxon, Mann, Whitney U-test.

Dosage was chosen by taking in account the human dose

recommendation and a relationship factor of 5–10:1 based on kilogram body weight as recommended in the literature (Reagan-Shaw et al, 2008). Dose level of *Hericium erinaceus* biomass 150 mg/kg was discussed with the Sponsor on the base of his experience. The animals were dosed orally using a solution of a constant volume of 1.0 ml/kg body weight for vehicle and active preparation. The dosage administered to each animal was determined every day by the weight of that animal at the time of administration.

Statistically, the Wilcoxon-Mann-Whitney U-test was used for comparison to results obtained by vehicle administration at the particular timing. Comparison of data to reference compounds tested earlier under identical conditions was performed using linear discriminant analysis according to Fischer. A total of the classic 24 variables (6 frequency ranges times 4 brain areas) was used for analysis. Please note, that this analysis does not contain gamma activity for historical reasons (gamma activity was not recorded earlier!) Firstly, projection of the results from reference compounds was performed using the 3 spatial coordinates for the results of the first 3 discriminant functions. Secondly, coding of the result of the fourth to sixth discriminant analysis into red, green and blue was followed by an additive colour mixture in analogy to the so-called RGB mode (as used in TV). A reference matrix of earlier tested drug actions is kept constant (frozen) for classification of unknown preparations. Only data from 20 to 65 min after administration were classified. Data are archived as raw data on hard disk and magneto-optic devices for backup.

RESULTS: Oral administration of the vehicle (0.9% NaCl) did only result in very minor changes of spectral gamma power within the striatum and reticular formation from the second hour on. A complete time course is given in Fig. 1. Oral administration of the *Hericium erinaceus* biomass preparation (150 mg/kg) resulted in a statistically significant attenuation of spectral delta and theta power in the hippocampus and reticular formation. Theta and alpha2 spectral power were statistically significantly attenuated in all brain areas. Also, beta1 spectral power was significantly attenuated in all brain regions. Changes were still visible during the second hour after administration but did not reach statistical significance (Fig. 2).

Fig.1: Effect of Vehicle:

Time dependence of changes of spectral power (Ordinate) in % of the 45 min lasting pre-drug baseline values in four brain regions of the freely moving rat in the presence of Vehicle (0.9% NaCl 1.0 ml/kg). Frequency ranges are depicted as coloured bar graphs on the abscissa representing delta (red), theta (orange), alpha1 (yellow), alpha2 (green), beta1a (light blue) and beta1b (dark blue) and gamma spectral power (violet) from left to right within the four brain areas as mentioned on top of the graph.

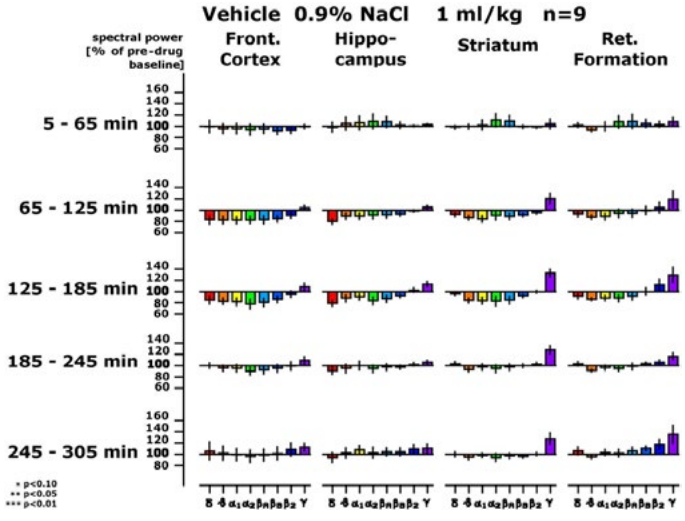

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Fig.2 Effects of *Hericium erinaceus* biomass (150 mg/kg) bodyweight)

For other details see legend to Fig. 1. Data from 1 animal were not evaluated due to technical problems. Statistical significance in comparison to control (vehicle) is documented by stars: *=p<0.10; **=p<0.05; ***=p<0.01.

With respect to motion no statistically relevant differences were observed in comparison to vehicle administration during the first 3 hours. Only during the 4th hour a significant reduction was seen (Tab.3).

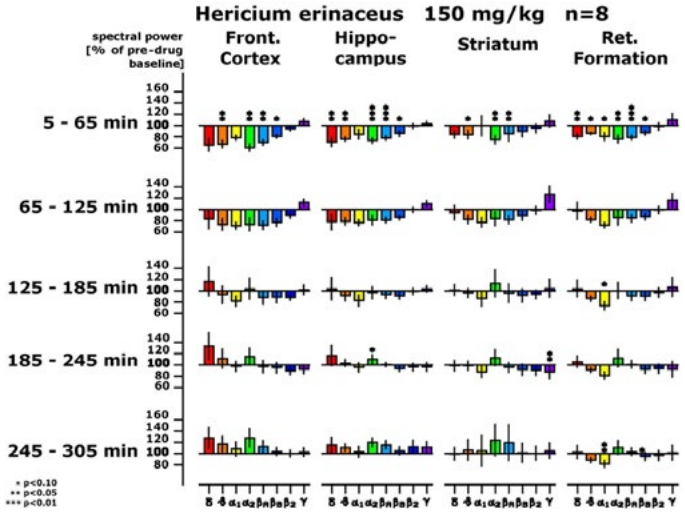


Table 3. Effects of Motion

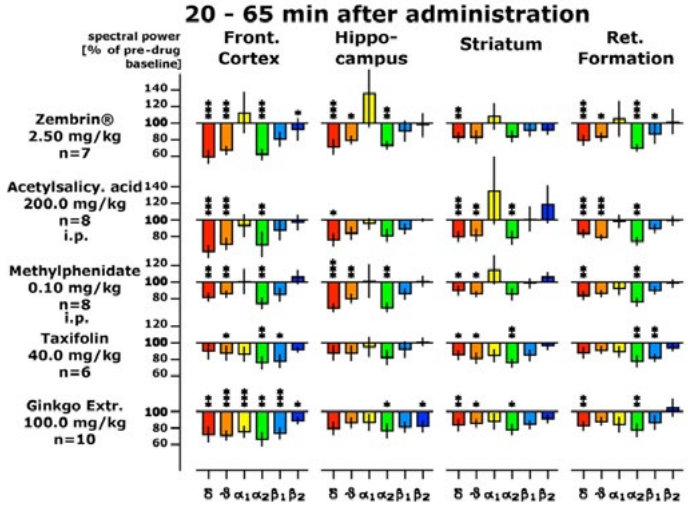
Changes in motion (cm/h) given for the whole time line of 5 hours after drug administration in hourly intervals. Mean average values are given ± S.E.M. Statistical comparison to the results with control (Vehicle) were determined using the Wilcoxon, Mann, Whitney U-test (p values are given on the right side).

Motion [cmlh]							
Time (min)	VEHICLE 0.9% NaCl 1.0ml/kg n ±9			Hericium 150mg/kg n=8			
-45-0	626.67	±	120	884.22	±	48	
5-65	676.49	±	117	899.44	±	50	
65-125	794.39	±	97	984.71	±	68	
125-185	1051.10	±	81	975.87	±	67	
185-245	913.30	±	89	540.38	±	46	0.027
245-305	695.38	±	80	811.92	±	70	

Fig.3 Effects of reference preparations

For other details see legend to Fig. 1. Statistical significance in comparison to control (vehicle) is documented by stars: *=p<0.10; **=p<0.05; ***=p<0.01. Please note, that analysis of the data is referring to the time period of 20 to 65 minutes after administration.

Feeding the data into linear discriminant analysis revealed, that classic drugs with well-known clinical indications grouped together according to their prescription in patients (Dimpfel, 2003). Analysis of the presently tested *Hericium erinaceus* biomass (MRL) confirmed the observed similarity to some reference preparations since the *Hericium erinaceus* biomass was projected into the vicinity of Zembrin®, Acetylsalicylic acid, Taxifolin and Ginkgo (Fig. 4). For comparison to reference drugs the first time period of 20 to 65 min. was chosen.



DISCUSSION

The animal model "Tele-Stereo-EEG" (Dimpfel et al., 1986) has been used to characterize more than 200 preparations with respect to changes of the frequency content of field potentials recorded from different regions of the depth of the brain, namely frontal cortex, hippocampus, striatum and reticular formation. In general, drugs produced different individual patterns of spectral changes. However, drugs with similar clinical indications induced similar changes among each other. Therefore, unknown preparations can be compared to drugs with well-established use.

The presently tested herbal preparation *Hericium erinaceus* biomass induced a pattern of frequency changes consisting in a significant attenuation of delta, theta, alpha2 and beta1 spectral power, but not alpha1 power (except for the reticular formation). The lack of alpha1 spectral power attenuation in combination with attenuation of delta, theta and alpha2 power is shared by some other preparations like Zembrin®, Acetylsalicylic acid, Methylphenidate, Taxifolin except for Ginkgo extract (Fig. 3). Due to the similarity to these drugs with well-known clinical efficacy calming, analgesic, antidepressive and cognition enhancing properties might be deduced for the *Hericium erinaceus* biomass. However, due to the fact, that only one dosage was tested, interpretation of the results is limited.

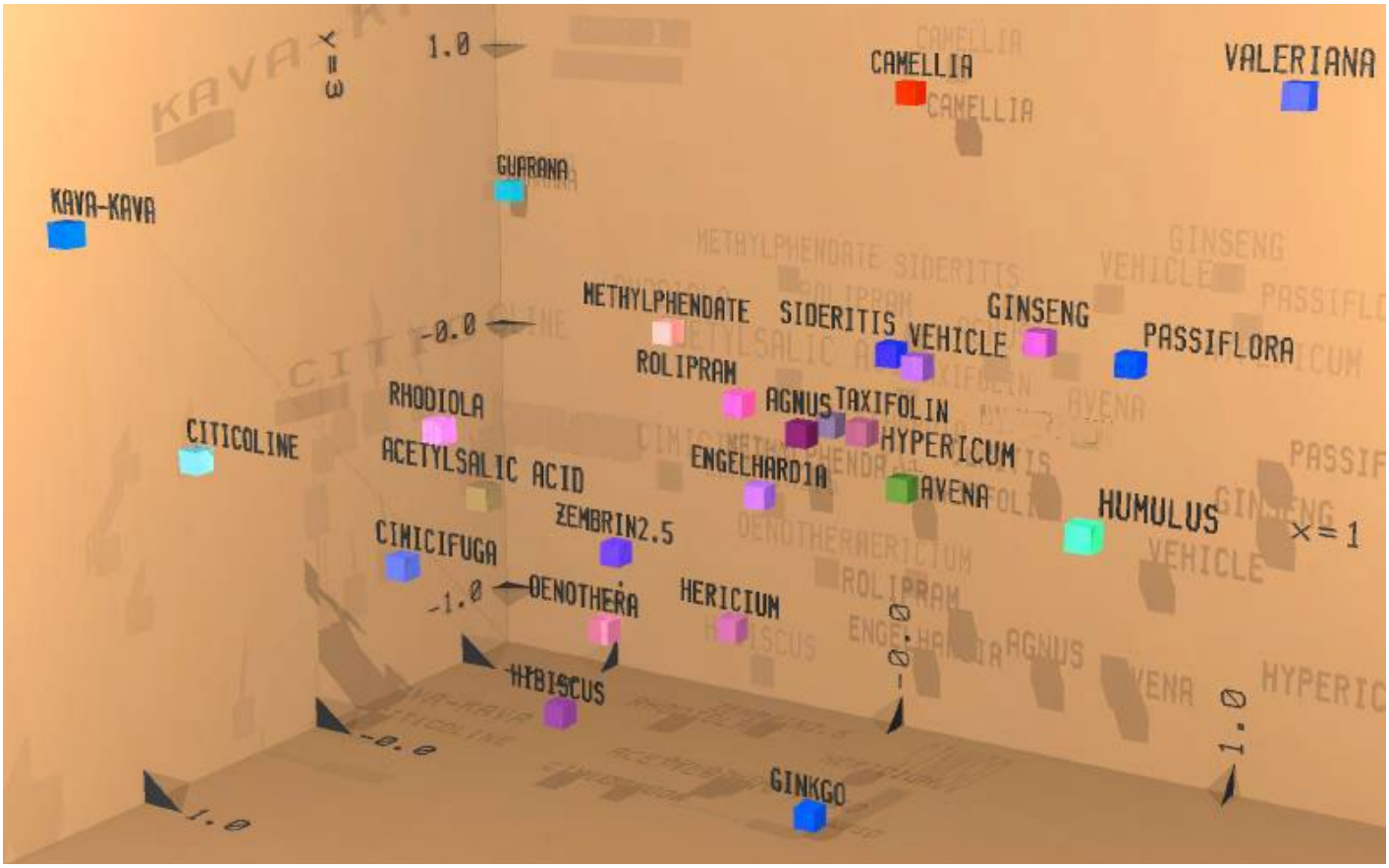


Fig. 4 Discriminant analysis of electropharmacograms

Comparison of the electro-pharmacogram of orally given ***Hericium erinaceus*** biomass MRL (150 mg/kg) with patterns of reference drugs. It provides similar spectral frequency changes according to the results of the first 3 discriminant functions.

A great similarity with respect to space and colour to some reference drugs signalizes similar net effects with respect to clinical indications (Dimpfel, 2003, 2013). Data from the first recording period 20 to 65 min. after administration (s. Tab. 2).

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Table. 2 Listing of reference compounds used for discriminant analysis. Doses and time of recording are given.

Substance Definition	Dose [mg/kg	Application	Time
Kava-Kava	200	orally	20 - 65 min
Guarana	25	orally	20 - 65 min
Humulus	50	orally	125 - 185 min
Valeriana	100	orally	125 - 185 min
Ginkgo	100	orally	20 - 65 min
Agnus-Castus	50	orally	20 - 65 min
Rhodiola	100	orally	20 - 65 min
Hypericum	250	orally	20 - 65 min
Substance Analysis	**Dose [mg/kg]**	**Application**	**Time**
Avena	100	orally	20 - 65 min
Ginseng	100	orally	20 - 65 min
Passiflora	100	orally	20 - 65 min
Oenothera	50	orally	20 - 65 min
Cimicifuga	75	orally	20 - 65 min
Camellia sin.	25	orally	20 - 65 min
Citicoline	48	orally	20 - 65 min
Rolipram	0.1	orally	20 - 65 min
Sideritis	100	orally	20 - 65 min
Taxifolin	40	orally	20 - 65 min
Zembrin	2.5	orally	20 - 65 min
Acetylsalicylic acid	200	i.p	20 - 65 min
Metylphenidate	0.1i.p.	i.p	20 - 65 min
Vehicle	1 ml	orally	20 - 65 min
Engelhardia	75	orally	20 - 65 min
Hericium-MRL150	orally	orally	20 - 65 min
Hibiscus (SUP_EEG_HSA50)75	orally	orally	20 - 65 min

Due to similarity to some reference preparations tested earlier under identical conditions, calming, analgesic, antidepressive and cognition enhancing properties might be deduced. Potential antidepressive effects of *Hericium erinaceus* have been described recently besides those of *Scutellaria baicalensis* and *Rhodiola rosea* within a review (Limanaqi et al., 2020). At some matter of fact a reduction of depression and anxiety in 30 females has been reported by (Nagano et al., 2010) after intake of Hericium cookies for 4 weeks. A review on the therapeutic potential of *Hericium erinaceus* for depressive disorder has been published recently (Chong et al., 2019), stating that Hericium ameliorates depressive like behaviour through the modulation of monoamine transmitters. According to the catecholamine hypothesis of affective disorders (Schildkraut, 1965) (Bunney & Davis, 1965) norepinephrine, serotonin and dopamine activity are disturbed in depressed patients. Main effects of Hericium in the present investigation are seen on theta and alpha2 frequencies, which correspond to norepinephrine (theta) and dopamine (alpha2) neurotransmission. This is in line with our interpretation of a potential positive effect of Hericium on depression.

Available information on Hericium, including its taxonomy, phylogeny, health-promoting benefits, and medicinal properties is reviewed by (Thongbai et al., 2015). Studies on secondary metabolites have resulted in the isolation of an exceptionally large amount of structurally different and potentially bioactive components including erinacines, hericerins, steroids, alkaloids, and lactones (Friedman, 2015). Biologically active ingredients of Hericium have been mainly recognized to be polysaccharides. Over the past decade, it has been demonstrated that Hericium polysaccharides possess various promising bioactivities, including antitumor and immunomodulation, anti-gastric ulcer, neuroprotection and neuroregeneration, anti-oxidation and hepatoprotection, anti-hyperlipidemia, anti-hyperglycemia, anti-fatigue and anti-aging (He et al., 2017).

Lipoxin A4 (LXA4) is an endogenously produced eicosanoid that acts as an endogenous "breaking signal" in the inflammatory process. *Hericium erinaceus* biomass (MRL) supplementation has been shown to significantly up-regulate Lipoxin A4 in the brain of rats within 90 days when compared to a separate control group (Trovato et al., 2016). In the brain of rats receiving Hericium maximum induction of Lipoxin A4 was observed in cortex and hippocampus followed by substantia Nigra, striatum and cerebellum. These brain regions correspond very much to those where effects of Hericium were observed in our acute study (Cortex and hippocampus).

With respect to active compounds of Hericium only erinacine A has confirmed pharmacological actions in the central nervous system in rats and to date only erinacines have been documented to cross the blood brain barrier (BBB). Therefore, current effects of Hericium on brain activity as observed in this study might very well derive from erinacines. However, no direct evidence has yet shown that other compounds of the whole extract could pass through the blood-brain barrier. Erinacines are groups of cyathin diterpenoids that show biological activities as stimulators of NGF synthesis and could be useful as a treatment for neurodegenerative disorders and peripheral neuropathy. To date, 15 erinacines (erinacines A–K and P–S) have been identified and further investigations have demonstrated that 8 of them have various neuroprotective properties, such as enhancing NGF release (erinacines A–I), reducing amyloid-β deposition, increasing insulin-degrading enzyme (IDE) expression (erinacines A and S), or managing neuropathic pain (erinacine E), while others are either being currently discovered or have other pharmacological activities (Li et al., 2018). Erinacine S, so far known to have been produced only in *Hericium erinaceus* mycelia, has just recently

been discovered and is able to reduce amyloid plaque growth and improve neurogenesis in aged brain of rats (Hu et al., 2019). Erinacine S was detected in the brain, as early as half hour after administration (2.069 ± 0.503 µg/g), peaked at 2h after administration (11.294 ± 9.662 µg/g) (Hu et al., 2019). These values correspond quite well with the early effects of Hericium as observed on brain electricity in the present study. Preclinical studies have also shown that there can be improvements in ischemic stroke, Parkinson's disease, Alzheimer's disease, and depression if *Hericium erinaceus* mycelia enriched with erinacines are included in daily meals (Li et al., 2018).

Conclusion:

From this preliminary study in a small number of animals (n=8) it can be concluded that MRL's *Hericium erinaceus* hyphal powder contains compounds that are bioavailable and cross the blood brain barrier resulting in an EEG signature that can be interpreted by discriminant analysis to have potential calming, analgesic, antidepressant and cognitive-enhancing activities. Based upon many years in the evaluation of electropharmacogram studies in both pharmaceutical and natural products, the dose of 150 mg/kg body weight used in the present study may translate to a human dose of 15 mg/kg body weight, or 1 050 mg in a 70 kg adult, within the daily dose range of 1-3g recommended by the Sponsor (Nektium)).

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Conflict of interest

The study was financially supported by Nektium Pharma Julia Wiebe is co-worker at Nektium Pharma. There was no conflict of interest.

Contributions:

Dr Julia Wiebe designed the experimental set up of the study. Prof Wilfried Dimpfel performed the experiments and wrote part of the manuscript. Dr Nigel Gericke wrote part of the manuscript and provided many references.

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Targeting Neurogenesis with Mushroom Nutrition: A Mini Review

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Neurogenesis is the process by which new neurons are formed in the brain. Neurogenesis is crucial when an embryo is developing, but also continues in certain brain regions after birth and throughout lifespan. The mature brain has many specialised areas of function, and neurons that differ in structure and connections. The hippocampus, for example, which is a brain region that plays an important role in memory and spatial navigation, alone has at least 122 different types of neurons [1]

Adult neurogenesis in the dentate gyrus of the hippocampus is highly regulated by several external conditions and cell-intrinsic factors to adapt to environmental changes.

Accumulating evidence suggests that adult-born neurons may play distinct physiological roles in hippocampus-dependent functions, such as memory encoding and mood regulation [2].

Adult hippocampal neurogenesis is a process that describes the generation of new functional dentate granule cells (DGCs) from adult neural stem cells through the amplification of intermediate progenitors and neuroblasts, as well as the differentiation and integration of these new neurons into the existing neural circuits [3].

Diagram I
Outline of the scheme of lineage progression and fate decisions during adult hippocampal neurogenesis.

A astrocyte;
GC granule cell;
NSC neural stem cell [4].

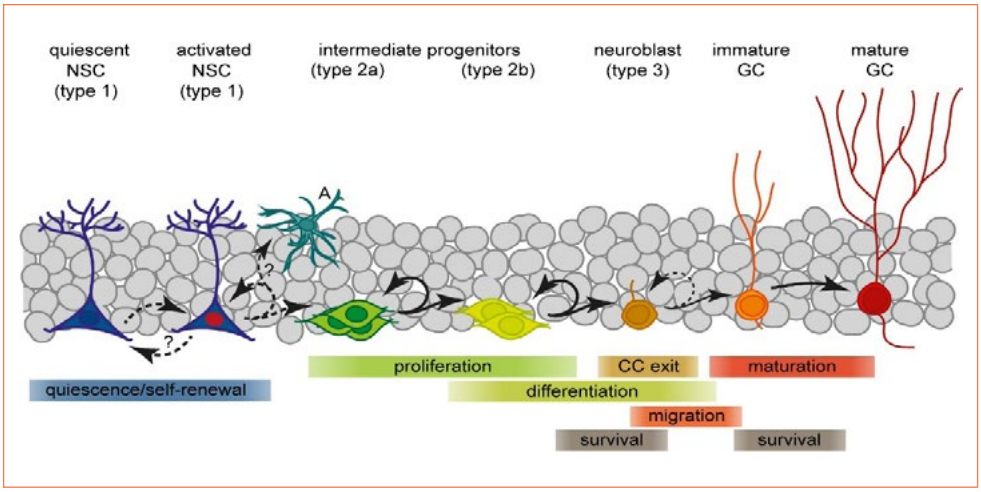
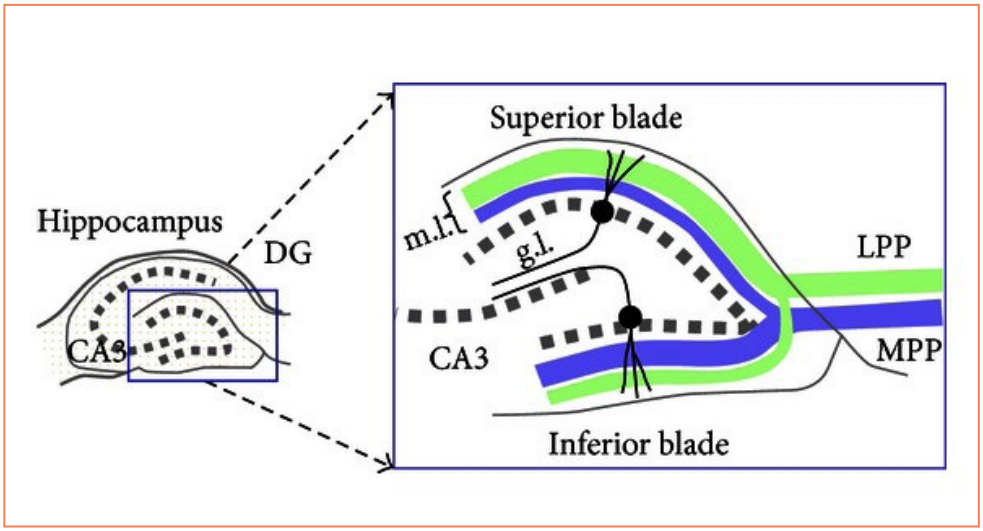


Diagram II
As part of this process the newly generated granule cells then extend axons or dendritic branches to integrate themselves in the pre-existing network of cells that compose the hippocampal. Again, the integration relies on newly formed dendritic branches of pre-matured neurons that reach the dentate gyrus (DG) molecular layer (ML) from granule cell layer (GCL) and upon reaching the molecular layer (ML) layer these dendritic branches reach out to entorhinal cortex (EC) and lateral (LPP) and medial (MPP) path, forming novel synaptic connections [5].



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Targeting Neurogenesis with Mushroom Nutrition: A Mini Review

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These cells can then project their axons to the *Cornu Ammonis* 3 (CA3), further establishing the hippocampal circuitry; hence maintaining or improving dendritic complexity is key to hippocampal function, a brain region important for spatial information, learning and memory functions.

When there is a malfunction in any of the processes, then a subsequent breakdown can be expected in either the quiescent pool (stem cell generator), loss of dendritic complexity **(Diagram III)**, angiogenesis or neuroplasticity leading to a loss of memory.

Diagram III displays impaired morphology and activity in dentate gyrus granule cells in depression. In the healthy state, the granule cells have long thick apical dendrites [6].

Importance to Maintain Healthy Hippocampal Neurogenesis

Brain cognitive reserve reflects the brains capacity to preserve normal functions, and has been used to explain the gap between tissue damage and clinical symptoms that has been observed in dementia and Alzheimer´s disease (AD)[8]. In early/moderate stages of Alzheimer´s disease, cognitive reserve compensates neurodegeneration and allows the maintenance of patients' cognitive performance [7]. As outlined in **Table I**, there are several factors that modulate cognitive reserve:

Diagram III

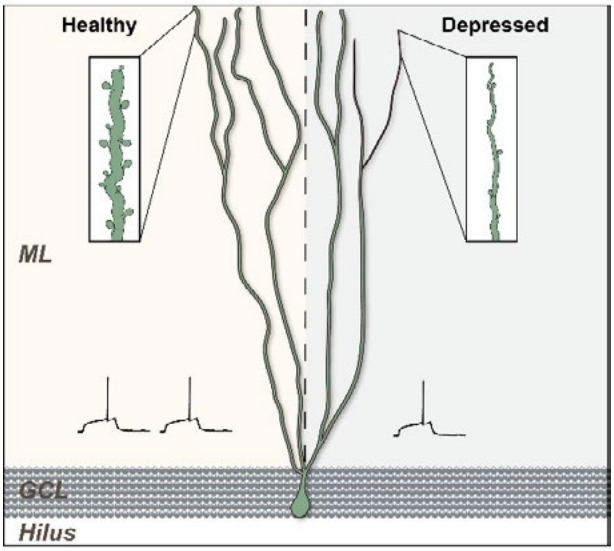

Table I

The impact of the aforementioned factors can translate into physical changes in brain morphology as outlined in Table II [9].

Beneficial Factors		Deleterious Factors
Cognitive Exercises i.e. crossword, puzzles		Sedative Mental Activity i.e. excessive television viewing
Education levels		Illiteracy
Creative activities		Inadequate diet
Diet		Solitude
Regular Physical Exercise		Little Physical Exercise
Social stimulus i.e. Bridge club		

Table II

Physical changes in brain morphology

Improvement of cognitive reserve		Decline of cognitive reserve
Increased brain size		Reduction in brain size
Increase in the number of neurons		Reduction in the number of neurons
Increased synaptic density		Reduction in synaptic density

Neurogenesis decreases with aging and declines may be linked to compromised cognitive-emotional resilience. Older individuals have less angiogenesis and neuroplasticity and a smaller quiescent progenitor pool.

In these AD patients there is a malfunction of the growth stages of hippocampal neurons or an unexplained severe deletion of these neurons or inability of these neurons to establish an integration within the existing circuitry of hippocampal function.

Due to the aforementioned malfunction and unexplained deletion of these neurons the continued pharmaceutical focus on the reduction of Tau and β-Amyloid proteins may be in need of review. Attempts to treat Alzheimer´s disease with a "reduction" approach to Tau and β-Amyloid proteins has been disappointing [9].

Until a pharmaceutical solution can be proven, could mushroom nutrition act as a disease modifying therapy by nutritionally targeting neurogenesis? How?

By focusing on:

1. Reduction of neuroinflammation by increasing Lipoxin A4.

Lipoxin A4 (LXA4) is an endogenously produced eicosanoid that inhibits neutrophil recruitment and activation and blocks the generation of pro-inflammatory proteins. LXA4 acts as an endogenous "breaking signal" in the inflammatory process. Biomass

forms of *Coriolus versicolor* and *Hericium erinaceus* increased Lipoxin A4 and Aβ uptake by phagocytic cells in rats which is recognized as a potential therapeutic target for AD treatment [10][11].

2. Combating viral infections that could be responsible for triggering dementia

Mushroom nutrition can be a source of natural bioactive compounds responsible for prevention and treatment of viral diseases through improvement of human immunomodulation. They boost antiviral cytokines or prevent the entry of the virus into the human cell that are responsible for triggering neurodegeneration [12][13][14][15].

3. Rebalancing the microbiota in dementia diagnosed patients

Food needs to cover the requirements of the human body and of the microbiota and ensure homeostasis. Mushrooms supply numerous enzymes, nucleosides, nucleotides and secondary metabolites essential for microbiota contributing to cell cycle regulation, apoptosis, autophagy, angiogenesis, metastasis, and signal transduction cascades [16].

4. Reducing oxidative and cellular stress response

Mushroom nutrition reduces oxidative stress and free radical-induced cell damage in neurodegenerative conditions [17].

5. Maintaining hippocampal neurogenesis by supporting Wnt/β-catenin signalling?

The Wnt/β-catenin signalling pathway involves secreted glycoproteins acting through transduction pathways signalling cascade and ligands, controlling carcinogenesis, embryonic development, and tissue regeneration [18].

They are dual function proteins, involved in regulation and coordination of cell–cell adhesion and gene transcription [19], playing an important role in neurogenesis by enhancing the maturation of the newly generated dendritic network [20]. In fact, β-catenin is a protein with numerous functions in different cellular locations and its regulation is dependent of the action of several interplayers.

An important regulator of β-catenin is the glycogen synthase kinase-3β (GSK3β) that promotes β-catenin degradation through its phosphorylation [21]. In pathological conditions in which GSK3β is highly activated, β-catenin is degraded, impairing the cellular processes dependent on its normal function. This degration is evident in AD where both Wnt/β-catenin signalling pathway and β-catenin levels were shown to be decreased in the brain of AD patients [22].

Thus, β-catenin is a powerful therapeutic target, as its overexpression may function as a promoter of maturation and functional integration of immature neurons into the hippocampus. This possible therapeutic intervention may thus counterbalance scenarios where β-catenin is abnormally degraded and dysfunctional.

Study Design

In 2018, researchers at the Institute for Pharmacology and Experimental Therapeutics (IBILI), Faculty of Medicine at the University of Coimbra Portugal and in the Centre for Neuroscience and Cell Biology at the University of Coimbra led by Professors Frederico C. Pereira and A. Cristina Rego performed the following study on mice to evaluate the impact of mushroom nutrition on hippocampal neurogenic reserve [23].

Using *Coriolus versicolor* (CV) biomass supplementation, the researchers took two groups of 2.5-month-old mice divided into one group taking 200 mg/kg every day for 2.5 months while the control group took saline. After 2.5 months the mice were sacrificed, and the following parameters were measured and compared between the two groups [23].

While there was no change in the dentate gyrus volume or proliferation in newly generated neurons, it was found that mice treated with *Coriolus versicolor* biomass supplementation had a significant increase in the complexity of the long and short immature neurons (increase in dendritic complexity) [23].

Table III: Summary of changes in the parameters

1	Changes in volume of granular cell layer (GCL) and sub granular zone (SGZ) of the hippocampal dentate gyrus (DG)	None
2	Changes in proliferation of DCX-positive cells	None
3	Changes on number and nucleus area of immature neurons in the DG of mouse hippocampus	None
4	Changes in differentiation features of DG short immature neurons of mouse hippocampus	Yes
5	Changes in differentiation features of DG long immature neurons of mouse hippocampus	Yes
6	Changes in Wnt/ β-catenin signalling	Yes

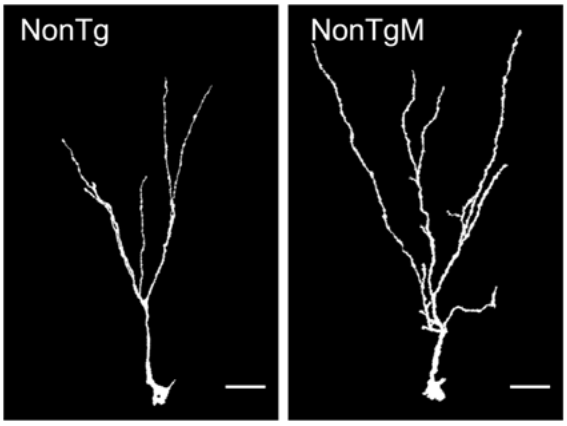

Image I

Of additional interest there was the fact that mice treated with *Coriolus versicolor* biomass had increased cytoplasmic and nuclear levels of β-catenin in immature neurons from hippocampal DG, suggesting that this protein may be a key molecule responsible for the increase in dendritic complicity in these cells.

Conclusion

The researchers demonstrated that supplementation with *Coriolus versicolor* biomass in mice promotes:

a) Increased dendritic arborization and complexity of newly generated neurons (short and long); and

b) Increase in the levels of β-catenin in the nucleus and cytoplasm of hippocampal newly generated neurons.

This indicates that *Coriolus versicolor* biomass promotes hippocampal neurogenic reserve in mice by increasing levels of β-catenin that may translate into enhanced cognitive reserve essential for learning and memory [24].

Further clinical research is required to reconfirm these results.

The *Coriolus versicolor* biomass used in this study was supplied by Mycology Research Laboratories Ltd. (Luton, UK). (www.mycologyresearch.com).

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Mushroom Nutrition in Neurodegenerative Diseases

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Neuroinflammation is a specialized immune response that occurs in the central nervous system, mainly in older adulthood, and has been connected to chronic neurodegenerative disorders and characterized by a gradual loss of neurons from specific regions in the brain.

Brain inflammation has been linked to:

- Amyotrophic Lateral Sclerosis (ALS)
- Parkinson´s disease (PD)
- Dementia with Lewy bodies (DLB)
- Psychosis
- Ageing
- Multiple Sclerosis (MS)
- Alzheimer´s disease (AD)
- Depression and Stress
- Cognitive Functions

The effects of mushroom-preparations 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]. Of particular note is the potential use of mushroom-preparation as a disease modifying therapy in neurodegenerative conditions.

The brain has a large potential oxidative capacity but a limited ability to counteract oxidative stress[2-4]. 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 defenses[5-8]. However, when the rate of free radical generation exceeds the capacity of antioxidant defenses, oxidative stress ensues with consequential severe damage to biomolecules such as proteins, lipids, nucleic acids, and carbohydrates[9-11].

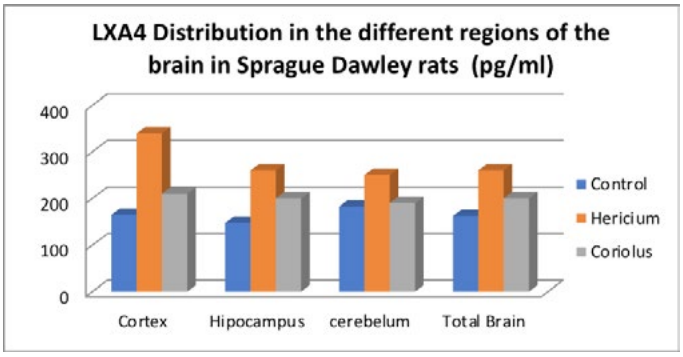
Increase Lipoxin A4

One approach to reduce neuroinflammation is to increase Lipoxin A4 (LXA4). This is an endogenously produced eicosanoid, that inhibits neutrophil recruitment and activation, reduces many cell responses evoked by pathogens and pro-inflammatory cytokines (IL-1, IL-6, and TNF-α), blocks the generations of these pro-inflammatory proteins from Th1 cells, CD4+ cells, macrophages, and dendritic cells, and toxic compounds including ROS, thereby promoting resolution of inflammation, and acts as an endogenous "breaking signal" in the inflammatory process[12].

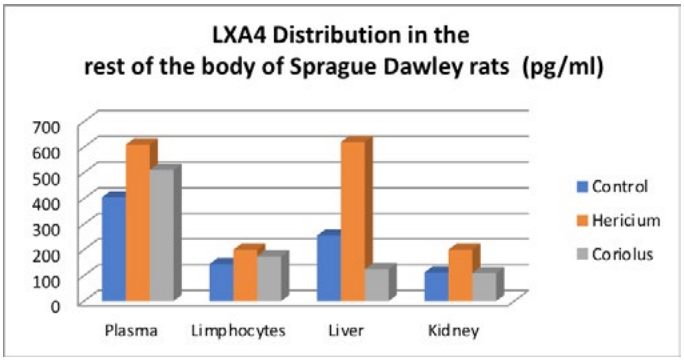
In 2015 and 2016 a team of researchers at the University of Catania, Italy, led by Professor Vittorio Calabrese demonstrated that mushroom-preparations, such as *Hericium erinaceus* and *Coriolus versicolor* can increase Lipoxin A4 in Sprague Dawley rats when compared to a control group in 90 and 30 days respectively[12-13]. The supplementation was an equivalent human dose of 3 g per day.

The following graphs A and B outline the LXA4 distribution in Sprague Dawley rats over 90 days with *Hericium erinaceus* biomass and over 30 days with *Coriolus versicolor* biomass in the brain and in the rest of the body.

GRAPH A



GRAPH B



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Increases in stress biomarkers

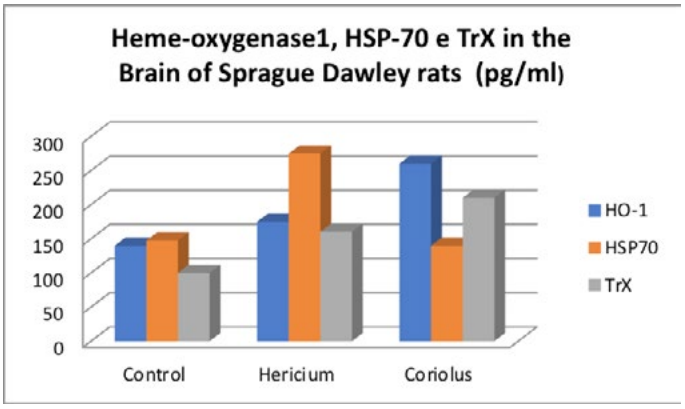
In addition to measuring LXA4, additional oxidative stress biomarkers were measured:

1) Heme-Oxygenase –1(HO-1) – an Nrf2-regulated gene that plays a critical role in the prevention of vascular inflammation, especially in atherogenesis.

2) Heat Shock Protein 70 (Hsp-70 – a cell protector from thermal or oxidative stress; such stresses cause proteins to "unfold" and possible aggregation; Hsp-70 binds to unfolded proteins thereby suppressing possible aggregation. Hsp-70 directly inhibits apoptosis.

3) Thioredoxin – a stress-inducible antioxidant protein playing a cytoprotective role and being central metabolic regulators.

GRAPH C



The University of Catania researchers noted that there was a significant increase in Heme-Oxygenase-1 (HO-1), Heat Shock Protein (Hsp 70) and Thioredoxin in the total brain of Sprague Dawley rats supplemented with *Coriolus versicolor* and *Hericium erinaceus* when compared to control groups. The supplementation was an equivalent human dose of 3g per day[12-13].

***Coriolus versicolor* Supplementation in Meniere´s Disease**

Meniere's disease (MD) is a clinical syndrome affecting approximately 12 in every 1000 people world-wide[14]. It is characterised by episodes of spontaneous vertigo associated with fluctuating, low-to-medium frequencies sensorineural hearing loss (SNHL), tinnitus and aural fullness in one or both ears[15].

To date, the cause of MD remains largely unknown, although increasing evidence suggests that, as an oxidant disorder, oxidative stress, immunomodulation and neuroinflammation may be central to its pathogenesis[16]. At present, there is no cure for this distressing neurodegenerative condition.

In 2018 and 2019, researchers in the University of Catania enrolled 40 patients with Meniere's disease of which 22 were supplemented with *Coriolus versicolor* biomass (3 g/day-6 tablets per day-3 tablets in morning and 3 tablets in evening) over two months and 18 participants were part of the control group [17].

By supplementing Meniere´s Disease (MD) patients with *Coriolus versicolor* biomass (3g/day) for two months one can test the possibility that mushroom supplementation, in a clinical setting, can reverse oxidative damage, and conceivably affect beneficially the clinical course of Meniere's disease.

The researchers lead by Professors Luigi Maiolino and Vittorio Calabrese at the University of Catania tested the hypothesis that neurotoxic insult represents a critical primary mediator operating in Meniere´s Disease pathogenesis, reflected by quantitative increases of markers of oxidative stress and cellular stress response in the peripheral blood of human patients. (see Table 1)

Changes in systemic oxidative stress		Changes in cellular stress response
Carbonyl groups		Heat shock protein (Hsp70)
4-Hydroxynonenal (HNE)		Heme oxygenase-1 (OH-1)
Lipoxin A4		Thioredoxin levels
Isoprostane PF2		Sirtuin-1
Isoprostane PGF2		GSH and γ-GC ligase
11-dhydro TXB2		

1. Changes in systemic oxidative stress status

The effect of *Coriolus versicolor* supplementation on systemic oxidative status in MD was assessed by measuring differences in levels of oxidative stress biomarkers, anti-inflammatory and pro-inflammatory eicosanoids in Coriolus-treated and untreated patients.

Biomarkers of oxidative stress

Oxidative stress in tissue leads to the formation of carbonyl groups in amino acid residues of proteins, and to peroxidation of lipids, which produces 4-hydroxynoneneal (HNE) from arachidonic acid or other unsaturated fatty acids[18].

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Carbonyl Groups and 4-hydroxynoneneal (HNE)

Protein carbonylation is generally irreversible, and leads to production of potentially harmful protein aggregates, causing cellular dysfunction and loss of viability. HNE formation is associated with various toxic effects, primarily apoptosis. The levels of both these markers can be used as a measure of oxidative stress. Additionally, it is possible to indirectly estimate the presence of reactive oxygen species (ROS) by measuring enhanced intensity of the normal ultraweak luminescence (UWL) emitted by all living cells [18].

The effect of Coriolus on systemic oxidative status was assessed by measuring plasma carbonyl groups and HNE, and plasma and lymphocyte UWL in both patient groups at the end of the 2-month trial. Each of these markers of oxidative stress was significantly lower in the Coriolus-treated vs untreated patients. In fact, UWL in treated patients was similar to control samples taken from healthy volunteers (Fig. 1).

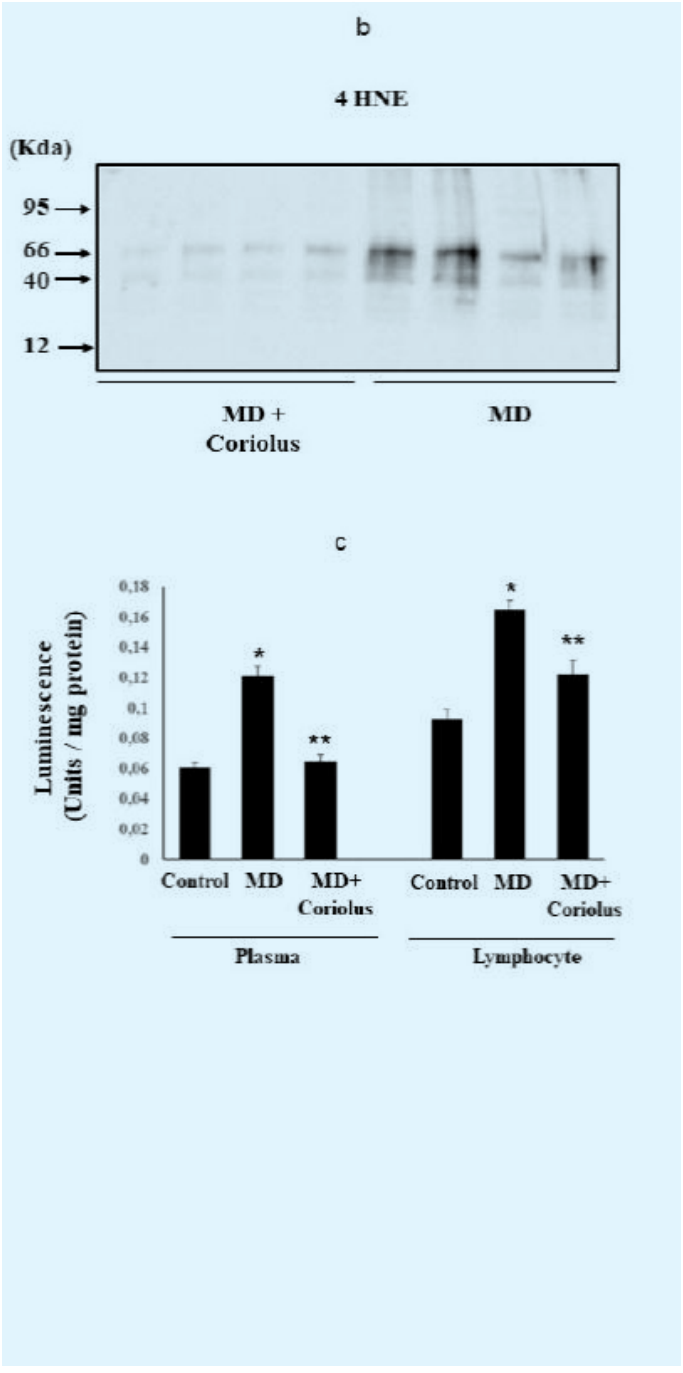
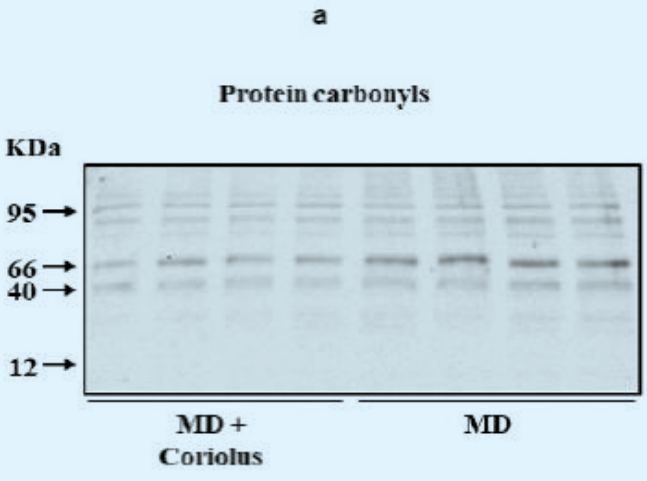


Fig. 1: Western blots (subsequently quantified by immunoassay – (data not shown) of plasma protein carbonyls (a) and HNE (b), and plasma and lymphocyte UWL (c) in Coriolus-treated and un-treated MD patients (healthy volunteer control samples were included for UWL analysis). *p < 0.05 vs control; **p < 0.05 vs untreated MD.

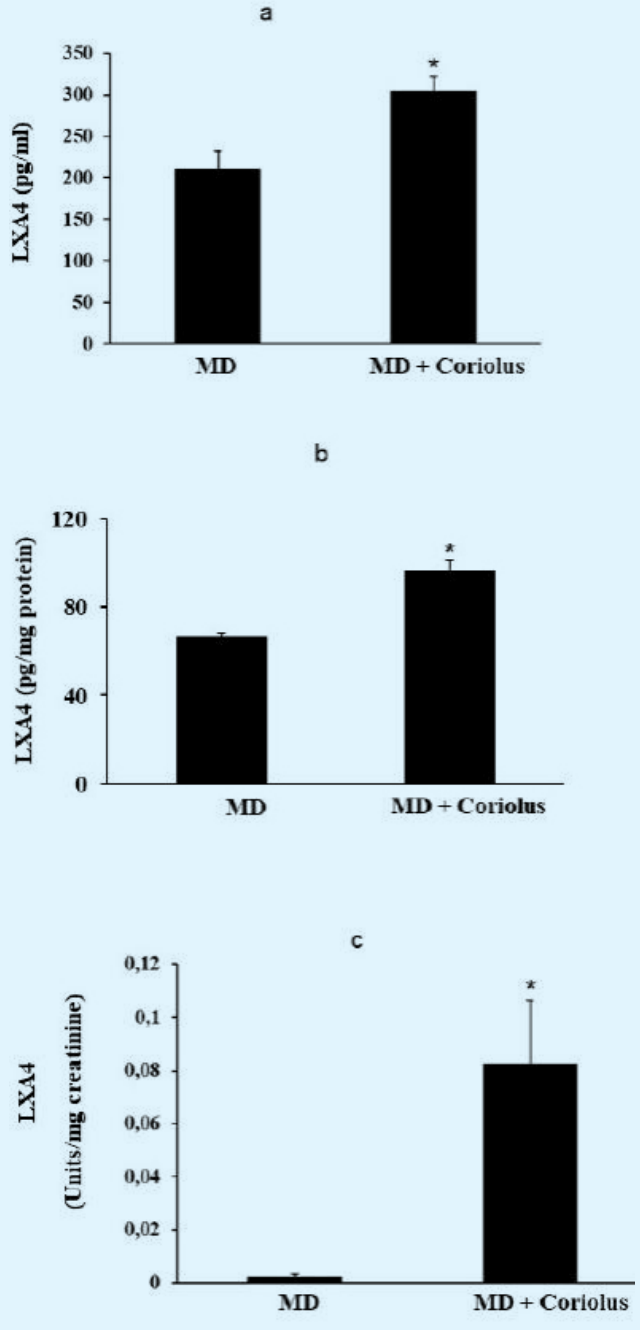


Endogenous anti-inflammatory eicosanoids: Lipoxin A4

Lipoxin A4 (LXA4) is an endogenously produced eicosanoid. It inhibits neutrophil recruitment and activation, reduces many cell responses evoked by pathogens and pro-inflammatory cytokines, and blocks the generation of pro-inflammatory cytokines and toxic compounds, including ROS. LX4A thereby promotes resolution of inflammation, acting as an endogenous 'braking signal' in the inflammatory process [12].

At the end of the 2-month trial, levels of LXA4 in plasma, lymphocytes and urine was significantly higher in patients treated with Coriolus. (Fig 2)

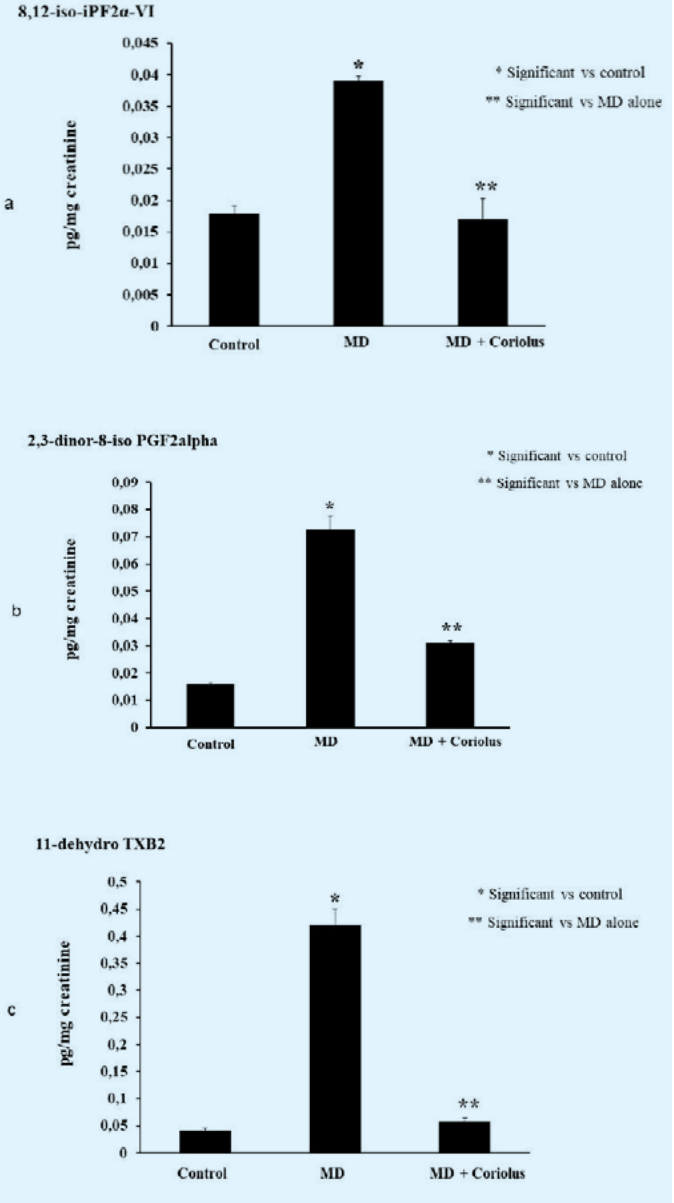
Fig. 2: Levels of LXA4 in the plasma (a), lymphocytes (b) and urine (c) of treated and untreated MD patients (estimated by HPLC + mass spectrometry). *p < 0.05 vs untreated MD.



Endogenous pro-inflammatory eicosanoids

At the end of the study period, urinary levels of pro-inflammatory eicosanoids – isoprostane PGF2 alpha-VI; 2,3 isoprostane PGF2 alpha; and 11-dehydro TXB2 – were significantly lower in the Coriolus-treated patients.

Fig.3: Levels of pro-inflammatory eicosanoids in urine: isoprostane PGF2 alpha-VI (a), 2,3 isoprostane PGF2 alpha (b) and 11-dehydro TXB2 (c) (estimated by HPLC + mass spectrometry). *Significant vs control; **significant vs untreated MD.



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Summary

In Coriolus-treated patients (compared with untreated):

- Oxidative stress biomarkers were lower
- Anti-inflammatory LXA4 levels were higher
- Pro-inflammatory eicosanoid levels were lower

2. Heightened cellular stress responses

Vitagenes are genes that encode proteins whose function is to preserve cell survival under conditions of stress. Vitagenes encompass: vital heat shock proteins (Hsp70 and heme oxygenase-1 [HO-1]); sirtuin-1 (SIRT1); thioredoxin; and γ-glutamylcysteine ligase (γ-GC ligase)[12][19]. The effect of Coriolus supplementation on cellular stress response in MD patients over 2 months was assessed by comparing the levels of each of these biomarkers in both treated and untreated groups at the end of the study.

Heat shock proteins

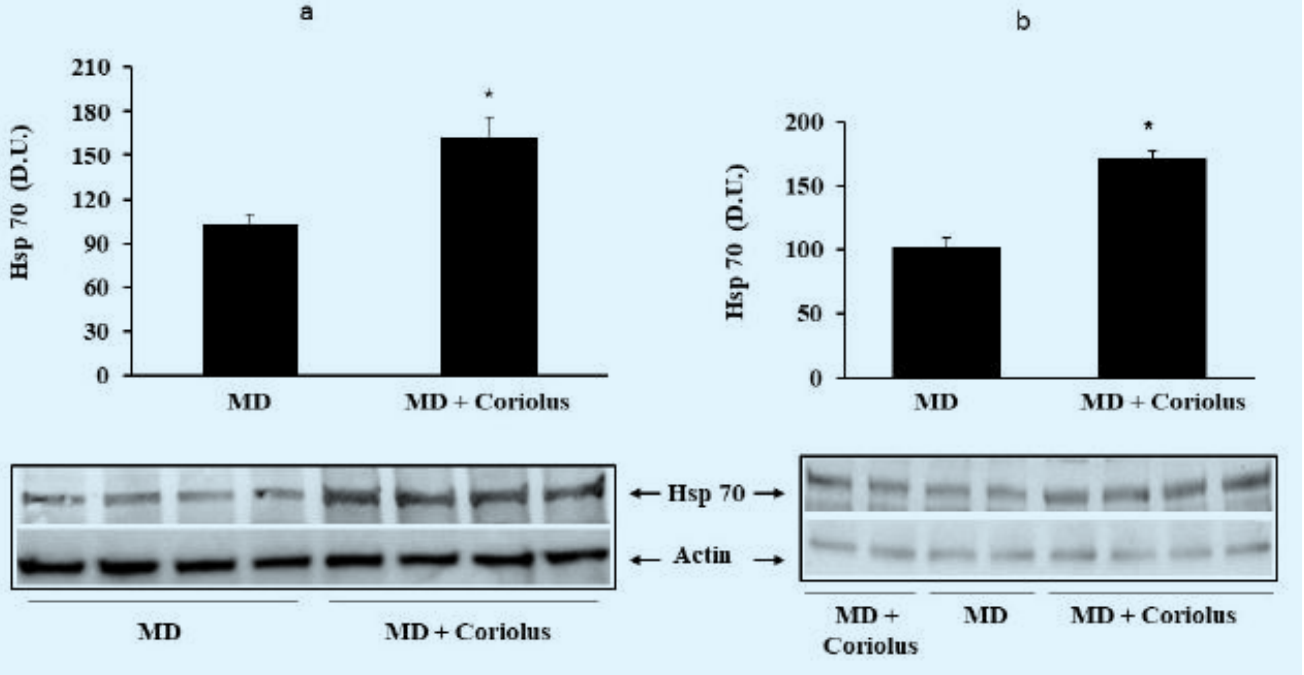
Evolutionarily, heat shock proteins (HSPs) are a highly conserved family of proteins that play a critical role in guiding both the initial folding of nascent proteins and the subsequent refolding of partially denatured structures, thus conferring protection to cells against stressful environments[20]. HSPs are produced in response to thermal or oxidative stress. They include Hsp70 and HO-1, both of which can act as markers of response to thermal and/or oxidative stress.

Hsp70: Thermal and oxidative stresses can cause proteins to 'unfold', which can eventually lead to them clumping together as protein aggregates. Hsp70 binds to unfolded proteins and, in doing so, reduces the potential for aggregation.

HO-1: This is a transcription product of an Nrf2*-regulated gene that plays a critical role in the prevention of vascular inflammation, especially in atherogenesis[21].

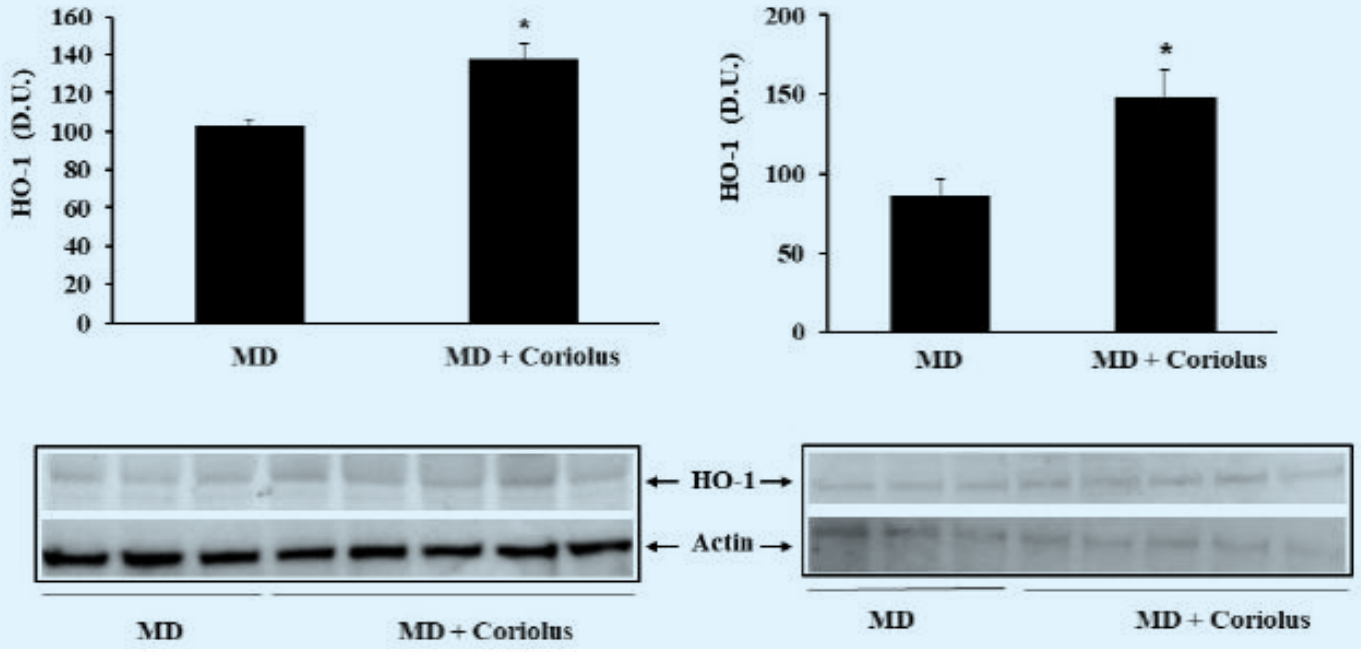
The extent to which Coriolus-treated and untreated patients were able to respond to oxidative stress was assessed by measuring differences between the two groups in up-regulation of the inducible isoform of Hsp70 and HO-1 in lymphocytes and plasma. The levels of both of these HSPs were greater in treated patients, indicating that they were responding more effectively than untreated patients (Fig. 4 and 5).

Fig. 4: Levels of oxidative stress measured as up-regulation of the inducible isoform of Hsp 70 in lymphocytes (a) and plasma (b) (estimated by Western blot + immunoassay). *p < 0.05 vs untreated MD.



*Nuclear factor E2-related factor 2 (Nrf2) is a transcription factor that coordinates the basal and stress-inducible activation of a vast array of cytoprotective genes. *p < 0.05 vs untreated MD.

Fig. 5: Levels of anti-inflammatory response measured as up-regulation of the inducible isoform of HO-1

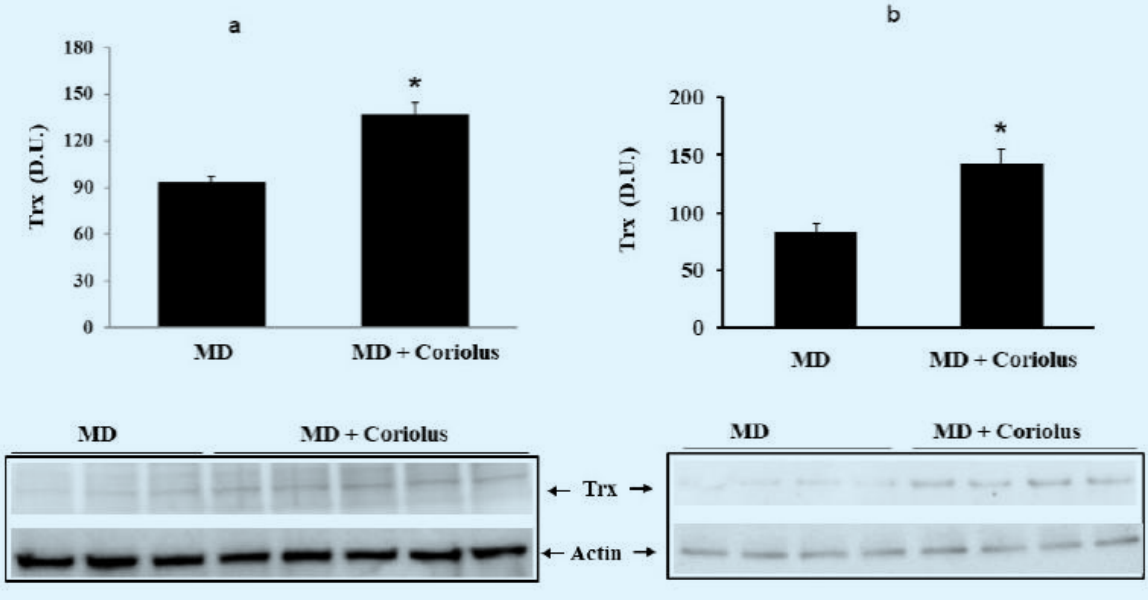


Thioredoxin

Thioredoxin is a class of small redox proteins that act as biological antioxidant by facilitating the reduction of other proteins, thus maintaining proper, functional, redox state[22].

In Coriolus-treated patients, there was significantly greater up-regulation of thioredoxin in lymphocytes and plasma compared with untreated patients (Fig. 6).

Fig. 6: Levels of thioredoxin proteins in lymphocytes (a) and plasma (b) (estimated by Western blot + immunoassay). *p < 0.05 vs untreated MD.



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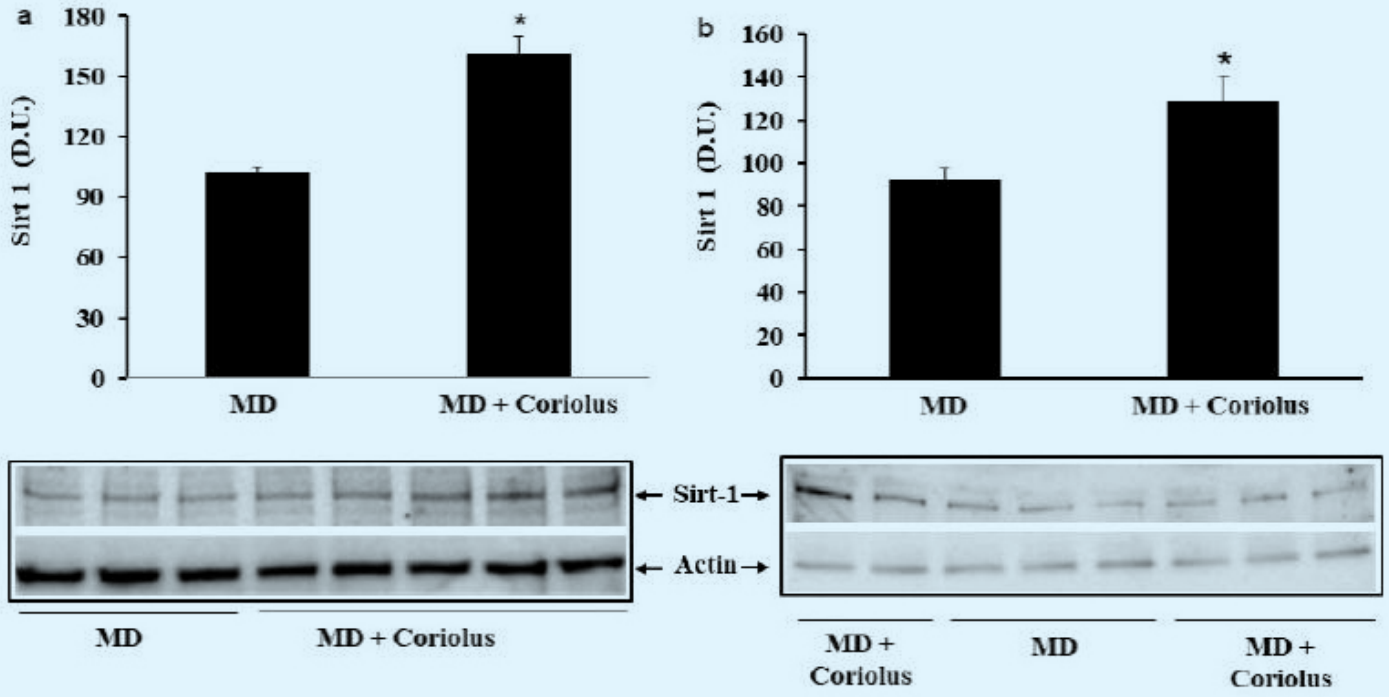
Sirtuin-1

SIRT1 is an enzyme that deacetylates proteins involved in cellular regulation. Activation of SIRT1 suppresses oxidative stress and confers protection against physiological and cognitive disturbance in old age[23]. In Coriolus-treated patients, there was significantly greater up-regulation of SIRT1 in lymphocytes and plasma compared with untreated patients (Fig. 7).

Fig. 7: Levels of Sirtuin-1 proteins in lymphocytes (a) and plasma (b) (estimated by Western blot + immunoassay). *p < 0.05 vs untreated MD.

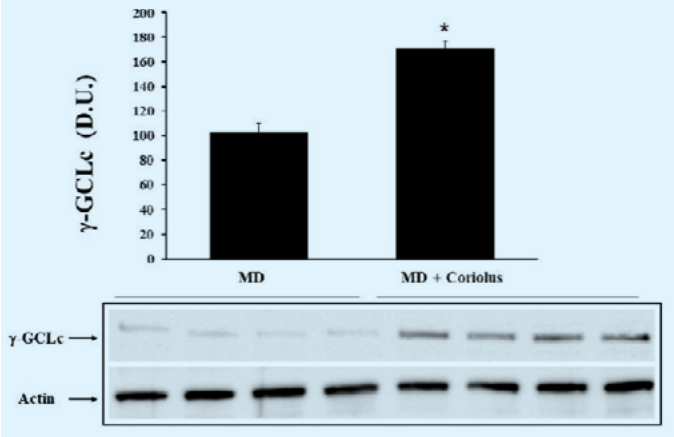


Glutathione (GSH) and Glutamate-cysteine ligase (GCL/γ-GC ligaseGlutathione is an antioxidant that can prevent damage to important cellular components caused by ROS.

GLC enzymatic function and activity is known to be involved in the vast majority of human diseases such as diabetes, Parkinson´s disease, Alzheimer´s disease and cancer. When GLC is impaired, this leads to decreased GSH biosynthesis, reduced cellular antioxidant capacity and the induction of oxidative stress. GSH concentration and γ-GC ligase activity in the central nervous system decline with age in association with increased oxidative stress[24].

In Coriolus-treated patients, lymphocyte levels of y-GC ligase were significantly higher than in untreated patients (Fig. 8), reflecting the expression of y-GC ligase in lymphocytes, plasma levels of GSH were significantly higher in Coriolus-treated patients. This corresponded to significantly lower oxidised glutathione (GSSG) levels and resulted in a significantly higher plasma GSH/GSSG ratio. (see Table 2 on next page)

Fig. 8: Levels of y-GC ligase in lymphocytes (estimated by Western blot + immunoassay). *p < 0.05 vs untreated MD. *p < 0.05 vs untreated MD



Summary

Coriolus-treated subjects had a more pronounced biological response to oxidative stress than untreated patients

Table 2: Levels of GSH and GSSG, and GSH/GSSG ratio in lymphocytes (measured by NADPH-dependent] GSSG reductase assay) of Coriolus-treated and untreated MD patients, and healthy volunteers (control).
*p < 0.05 vs control; **p < 0.05 vs untreated MD.

	Plasma (nmol/mL)			Lymphocyte (nmol/mg. Protein)		
	Control	MD	MD+Coriolus	Control	MD	MD+Coriolus
Total GSH	16.7 ± 2.1	8.33 ± 3.0*	14.23 ± 2.4**	9.81 ± 0.8	5.3 ± 0.7*	7.3± 0.5**
GSH	15.62 ± 2.0	8.44 ± 1.7*	13.44 ±1.7**	9.58 ± 0.6	4.27 ± 0.4*	7.20 ± 0.5**
GSSG	0.138 ± 0.01	0.169 ± 0.01*	0.146 ± 0.01**	0.093 ± 0.01	0.118 ± 0.01**	0.096 ± 0.006**
Ratio GSH/GSSG	113.2 ± 11	56.9 ± 15*	92.05 ± 13**	96.5 ± 10	42.6 ± 7.9	75.0 ± 9.6**
* Significantly different from control (p<0.05). **Significant different from MD alone (p<0.05)						

Study findings

Improved symptom scores At the onset and end of the 2-month study, the researchers measured MD symptoms in both groups.

The psycho-emotional status of all patients was assessed using a Profile of Mood States (POMS) questionnaire.

Total mood disturbance was improved in patients treated with *Coriolus versicolor* biomass (3g/day) but remained unchanged in the untreated patients (Table 1). Improvement was seen in five of the six mood parameters measured: anger, confusion, depression, fatigue, and tension; only vigour was unchanged. All the parameters in the untreated patients remained unchanged [24].

Table 3: Profile of mood status in Coriolus-treated (A) and untreated (B) MD patients.
*Significant vs Group A pre-therapy scores.

	Pre-Therapy (T0)		Post-Therapy (T1)	
	Score		Score	
Group	**A**	**B**	**A**	**B**
Anger (0-48)	28	29	22	29
Confusion (0-28)	17	17	10	16
Depression (0-28)	41	39	25	37
Fatigue (0-28)	16	19	10	19
Tension (0-36)	31	29	13	28
Vigour (0-32)	19	17	19	16
Total Mood Disturbance (-32 to 200)	114 ± 9.8	116 ± 8.6	61 ± 6.11	113 ± 8.1

All patients completed a Tinnitus Handicap Inventory (THI) questionnaire to define the clinical grading of tinnitus. This measures changes in frequency range, average hearing loss in decibels, and verbal discrimination. Tinnitus in treated group showed significant improvement at the end of 2 months but was unchanged in the untreated group (Table 3).

Table 4: Tinnitus handicap inventory in Coriolus-MRL-treated (A) and untreated (B) MD patients. p <0.05 vs untreated MD

Tinnitus Handicap		Inventory	
Pre-Therapy Score		(T0) Post-Therapy Score	(T1)
Group A	Group B	Group A	Group B
74 ± 2.46	78±2.73	52 ±1.73*	74 ± 2.65
*significantly different vs. control untreated MD patients ($p<0.05$)			

Summary

Mood and tinnitus improved
in patients treated with Coriolus supplementation

CONCLUSION: Present results strongly indicate MD as an oxidant disorder, with the underlying pathology involving systemic oxidative stress and demonstrate that *Coriolus versicolor* biomass supplementation (3g/day) for at least two to six months may provide a useful means to amplify the body's response to oxidative challenge and cellular stress in Meniere's disease.

This improved stress response appears to translate into measurable symptom relief (reduction in tinnitus and improved mood). The finding offers the exciting possibility that nutritional supplementation with *Coriolus versicolor* biomass supplementation has potential as a modulator of the MD pathological process with significant reduction in symptom severity in affected patients. (Further studies are in process to further support these conclusions.)

Another conclusion, confirming other previous studies, reveals a proof of concept that mushroom-preparations do reduce oxidative stress and free-radical-induced cell damage; thereby reinforcing further research on the use of mushroom-preparation as a disease modifying therapy in other neurodegenerative conditions.

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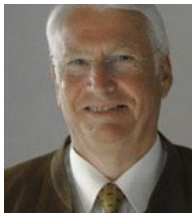
ARTICLE 1

Central Nervous System Profiling of *Hericium erinaceus* Biomass Powder by an Electropharmacogram Using Spectral Field Power in Conscious Freely Moving Rats



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ARTICLE 2

Targeting Neurogenesis with Mushroom Nutrition: A Mini Review

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ARTICLE 3

Mushroom Nutrition in Neurodegenerative Diseases

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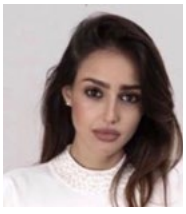

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Degrees in Molecular Biology and Biomolecular Chemistry at the University of Catania, Italy and was a research fellow at both the Institute of Human Virology of Maryland and at Kurukshetra University, India. She currently directs the Sicilian vaccination campaign against Covid 19 for Pfizer. Her research focuses on the role of free radicals and antioxidants in human disease, particularly neurodegenerative diseases. The results of her research have been reported in 24 papers published in high impact peer reviewed international journals.

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