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*An in vitro proliferation study using the commercial nutraceutical mix
(Metabolic Cell-Support™) on non-stimulated human lymphocytes*

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Abstract: The combining of nutraceuticals to synergistically enhance efficacy has a particular application in immune stimulation where a number of activation pathways are available. Many new products have utilised this principle to create pharmaceutical grade products using known bioactive compounds from medicinal herbs and fungi. Using the individual compounds however does remove the holistic approach to natural therapies and may lead to the exclusion of important components of the original herb that may play a role in clinical outcomes. A recently developed Australian product, Metabolic Cell-Support™ (MC-S), has been formulated using a pharmacological approach to natural therapies, using a highly standardised multi polysaccharide enriched mix of medicinal mushrooms and a herb aimed at improving immune function. To demonstrate this preparation activates immune function, an *in vitro* study of peripheral blood lymphocyte proliferation was undertaken at varying doses of MC-S. **Conclusion: This study does demonstrate that within a broad range of doses, MC-S significantly enhances white blood cell (immune cell) proliferation, indicating that as a commercial product it stimulates immune cell activity.**

Introduction:

Traditional medicine has long utilized the medicinal properties of mushrooms and herbs for healing and health. Scientific validation of the clinical efficacy of these natural therapies has resulted in a commercial approach to delivery, including the pharmaceutical preparation of active ingredients into both capsules and tablets. Unfortunately this approach has in some circumstances resulted in products that when compared to their natural sources have lost functionality (Borchers 2004). This study examines a commercially available immune stimulate, Metabolic Cell-Support™ (MC-S), to determine whether its herbal based formulation retains immunological activity comparable to what is observed for its individual natural ingredients. Metabolic Cell-Support (MC-S) is composed of three medicinal mushrooms, *Ganoderma Lucidum*, *Lentinus edodes* (mycelia) and *Coriolus versicolor*; a herb, *Astragalus membranaceus* (from the root) and vitamin C. Individually all these components have demonstrated immune stimulating potential, but using combinations of these have not been extensively tested. It is known that a range of active compounds found in herbs can stimulate the human immune system. As these compounds can vary in bioactivity and biospecificity, it may be possible to utilize a range of the compounds to symbiotically stimulate the immune system leading to a more efficacious response (Smith 2002). The formulation of MC-S has been based upon this principle. *Ganoderma Lucidum* (*Lingzhi* or *Reishi*) has long been regarded in traditional Chinese medicine for enhancing body disease resistance and consolidating the constitution of patients (Lin ZB 2005). Studies on the polysaccharide extracts of *Ganoderma Lucidum* have demonstrated mitogenicity and activation of immune effector cells such as macrophages, natural killer cells and T-lymphocytes (Goa and Zhou 2001), stimulating the production of cytokines interferon (INF), interleukins (IL) and Tumor necrosis factor (TNF). *Lentinus edodes* (*Shiitake mushroom*) is a common eatable mushroom. The two extracts: LEM extract (from *Lentinus edodes mycelium*) and Lentinan, are both proven immuno-modulators augmenting activation and proliferation of peripheral mononuclear cells (Aoki 1984, Hobbs 2000). *Coriolus versicolor* contains two bioactive polysaccharides that are chemically similar; polysaccharide-peptide (PSP) and polysaccharide-K or Krestin (PSK).

These only differ by the presence of fucose on PSK and rhamnose and arabinose in PSP (Smith 2002). Both are potent immunomodulating activating T-cells, antigen-presenting cells, monocytes and macrophages, and inducing INF gamma and IL2 production (Tzianabos, 2000). Finally, extracted polysaccharides and saponins from *Astragalus membranaceous* have been shown both *in vitro* and *in vivo* to stimulate NK-cell activity and PBL proliferation (Sun 1983). The objective of this study was to examine the effect of varying doses of MC-S on the *in vitro* proliferation of non-stimulated human peripheral blood lymphocytes (PBL's) as a measure of immune stimulating potential.

Materials and Methods:

Metabolic Cell- Support (MC-S):

MC-S (without vitamin C) (Metabolic Research Pty Ltd, Newcastle, Australia) was used in powdered form. The vitamin C was excluded from the *in vitro* testing due to its acidity and potential inhibitory effect on the tissue culture. Prior to testing a stock solution of MC-S was prepared in sterile water ($100000\mu\text{g mL}^{-1}$). The test material was then prepared by diluting this stock to a final concentration of $781.25\mu\text{g mL}^{-1}$ in AIM-V serum free medium (Gibco, Invitrogen, Carlsbad California USA) and sterile filtering through a 0.22 micron filter (Sartorius , Hannover Germany). Stimulating efficacy of this test material was studied using 1:4 dilutions to a minimum concentration of $0.0004\mu\text{g mL}^{-1}$ in AIM-V serum free medium.

Peripheral Blood Lymphocyte (PBL) Preparation:

Human whole blood was defibrinated using universal tubes containing 4mm glass beads and PBL's were collected by density gradient centrifugation separation. In brief, 20 mL of defibrinated blood, diluted 1:2 with Hanks buffered salt solution (HBSS), was overlaid on 15 mL of Ficoll-PaqueTM Plus (Amersham Biosciences Uppsala, Sweden)

and centrifuged (Sigma 3-16k Centrifuge, Sigma Laborzentrifugen Osterode, Germany) at 400g for 40 minutes at 20⁰C. Following centrifugation, the PBL rich interface was harvested and pelleted by centrifugation. The PBL harvest was then washed twice with Hanks buffered salt solution (HBSS) and resuspended in AIM-V serum free media at a final concentration of 3x10⁶ cells mL⁻¹.

Lymphocyte Proliferation Assay:

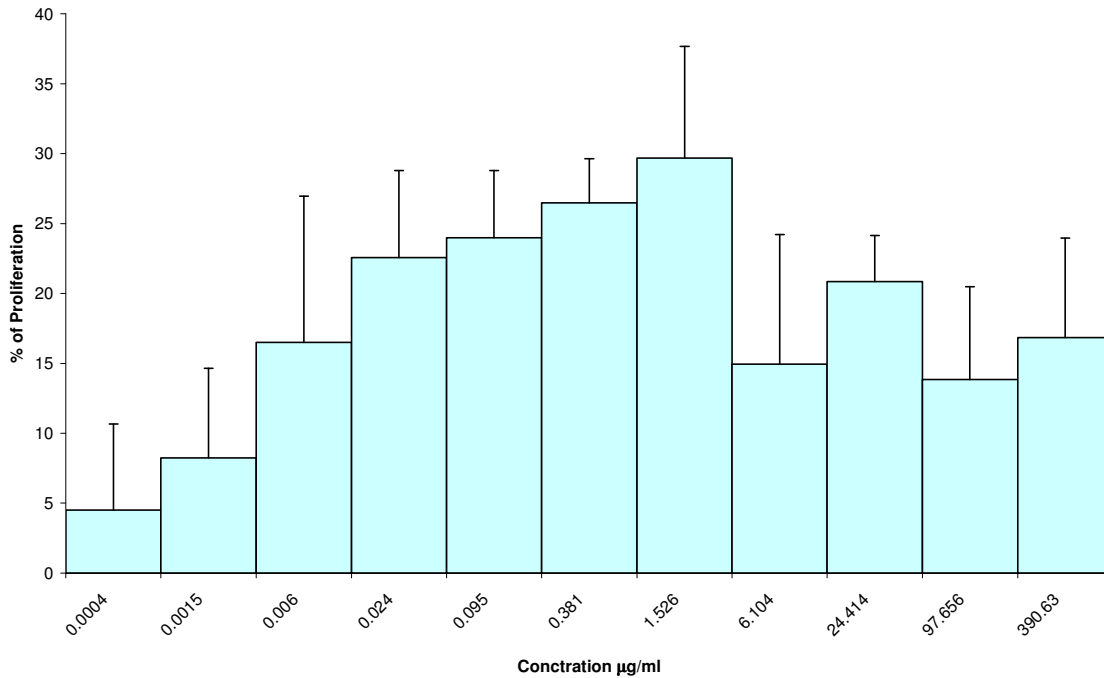
50µL of each dilution of MC-S test solution were added to six wells of a 96 well flat bottom culture plate (Nunc, Roskilde Denmark). To three wells of each dilution set, 50µL of PBL cell suspension was added, giving a final concentration of 1.5x10⁵ cells per well. In the remaining three wells for each dilution set was added 50µL of AIM-V serum free media as a background control. Controls for this assay were non-treated non-stimulated PBL's (negative control and base line) and non-treated Phytohemagglutinin (PHA) (Sigma, Sigma-Aldrich St Louis USA) stimulated PBL's 10µg ml⁻¹ (positive control). Plates were incubated at 37⁰C in a CO₂ incubator for 48 hours. Proliferation was measured using CellTiter 96 AQueous Non-Radioactive Cell proliferation assay kit (Promega Corporation, Madison Wisconsin USA) as per manufacturers' instructions with absorbance measured at 490nm using a Bio-Rad microplate reader model 680 (Bio-Rad Laboratories Hercules California USA). Absorbance data was calculated as a percentage of proliferation in comparison to non-treated/ non-stimulated control. Results were graphed as mean ± SD of 3 separate assays.

Statistical Analysis :

Statistical analysis was performed using T-test analysis for two samples assuming equal variances, two tailed using means on percentage of increase of each assay (Microsoft Excel). A p-value of less than 0.05 was considered to be significant.

Results:

The exposure of non-stimulated PBL's to varying doses of MC-STM over a 48 hour period mediated a proliferation response at each dose. The peak of proliferation for these experiments occurred at 1.526 $\mu\text{g mL}^{-1}$ (Fig 1). Significant different proliferations were reported for the dose range of 0.024 $\mu\text{g mL}^{-1}$ to 390.63 $\mu\text{g mL}^{-1}$ (Table 1). Fig.1. Percentage increase in proliferation of PBL's when treated with MC-STM at varying doses after 48 hours of incubation. Results represent the mean of 3 experiments performed in triplicate. Table 1. Comparative analysis of PBL proliferation at each dose of MC-STM between treated samples and non-treated control. Results are representative of 3 experiments performed in triplicate.



Dose µg/ml	Percentage of Proliferation ± SD	p value
0.0004	4.5 ± 6.14	0.273
0.0015	8.23 ± 6.4	0.088
0.006	16.5 ± 10.44	0.051
0.024	22.57 ± 6.21	0.003
0.095	23.97 ± 4.8	0.0009
0.381	26.47 ± 3.17	0.0001
1.526	29.67 ± 8	0.003
6.104	14.93 ± 9	0.049
24.414	20.83 ± 3.31	0.0004
97.656	13.83 ± 6.64	0.022
390.63	16.83 ± 7.12	0.015

Discussion:

These experiments demonstrated that MC-S has a mitogenic effect on non-stimulated PBL's, exhibiting a statistically significant peak proliferation of $29.7 \pm 7.99\%$ at a dose of $1.526\mu\text{g/ml}$, when compared to resting PBL's ($p \leq 0.003$). These findings compare favorably to the results achieved for the individual medicinal components of MC-S shown in other studies, however as most research to date in this area has been directed specifically at concentrates of active compounds isolated from the whole fungi and plant it is not possible to make direct comparisons. In this study the concentration of the known active compounds would be relatively low, especially when you consider that 200kg of shiitake mushrooms yields only 31gram of Lentinan (Smith 2000). This makes the results of the current study more significant. Although the clinical efficacy of the individual components of MC-S has been long recognized and utilized in Asian countries, the Western world has only in recent years realized the potential of complementary medicines. Along side this realization is the acknowledgement of the need for scientific validation of potential clinical applications. In Australia the Therapeutic Good Association (TGA) does monitor claims made for complementary

therapies, but even so there is a gap that exists between accepted applications of whole or parts of plants and fungi, isolated active agents and combinations of both. When these products are turned into pharmaceutical preparations, once again their efficacy requires re-testing. Unfortunately many products are in the market without what could be considered adequate scientific review.

Conclusion:

This study does demonstrate that MC-S actively proliferates white blood cells *in vitro*, suggesting that as a commercial product it stimulates immune cell activity. Future clinical studies are likely to support this claim.

Disclaimer:

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References:

1. Smith JE. Rowan N. Sullivan R. 2002. Medicinal mushrooms: Their therapeutic properties and current medical usage with special emphasis on cancer treatments. Special report commissioned by Cancer Research U.K.
2. Kidd PM. 2000. The use of mushroom Glucans and Proteoglycans in Cancer Treatment. *Altern Med Rev* 5;1:4-27
3. Borchers AT. Keen CL. Gershwin ME. 2004 Mushrooms, Tumors and Immunity: An Update. *Exp Bio Med* 229; 5:393-406

4. Lull C. Wichers HJ. Savelkoul HFJ. 2005. Antiinflammatory and immunomodulating properties of fungal metabolites. *Mediators of Inflammation* 2; 63-80
5. Hsieh TC. Kunicki J. Darzynkiewicz Z. Wu JM. 2002. Effects of extracts of *Coriolus versicolor* (Im-Yunity™) on cell-cycle progression and Expression of interleukins-1b, -6 and -8 in promyelocytic HL-60 leukemic cells and mitogenically stimulated and non-stimulated human lymphocytes. *J Altern Complement Med* 8; 5:591-602
6. Lin Z. 2005. Cellular and molecular mechanisms of Immuno-modulation by *Ganoderma lucidum*. *J Pharmacol Sci* 99; 144-153
7. Sun Y. Hersh E. Lee S. McLaughlin M. Loo T. Mavligit G. Preliminary observations on the effects of the Chinese medicinal herbs *Astragalus membranaceus* and *Ligustrum lucidum* on lymphocyte blastogenic response. *J Biol Response Mod* 2; 227 - 37
8. Oka M. Hazama S. Suzsuki M. etal. 1996 In vitro and in vivo analysis of human leukocyte binding by the antitumor polysaccharide, lentinan. *Int J Immunopharmac* 18; 3: 211-216
9. Hobbs CH. 2000. Medicinal value of *Lentinus edodes* (Berk) Sing. (Agaricomycetidae) A literature review. *Int J Med Mushr* 2; 287-302
10. Monograph. 2003. *Astragalus membranaceus* *Altern Med Rev* 8;1:72-77