Research Article

Evaluation of Immunity-Enhancing Effects of Melaleuca Alternifolia Concentrate (MAC) in Healthy Subjects

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ABSTRACT

Background: Melaleuca Alternifolia concentrate (MAC) is an essential oil extracted from a native Australian plant *Melaleuca Alternifolia. M. Alternifolia* has anti-inflammatory, antimicrobial properties and antioxidant properties

Aim: To evaluate the immunity-enhancing effects of 2 doses of MAC (150 mg and 300 mg) and compare it with a placebo and to evaluate and compare the safety of MAC versus placebo

Methodology: This study was a randomized, double-blind, placebo-controlled study that enrolled male or female healthy subjects ≥ 40 years of age. 120 subjects were randomized in a 1:1:1 manner to either of the three treatment groups namely MAC 150 mg od, MAC 300 mg od or placebo 1 od. The primary efficacy end points were CD4 T lymphocyte count, CD8 T lymphocyte count, CD 19-B lymphocyte count and NK cells.

Results: After 4 weeks of daily administration of 150 mg per day or 300 mg per day of MAC, the CD4 and CD8 levels increased till week 4. The levels of NK increased every week as compared to baseline

Conclusion: Melaleuca Alternifolia Concentrate (MAC) has immunostimulant effects. MAC stimulates T cells, B cells and upregulates NAK. MAC can have a vital role to play in maintaining good health by improving immunity.

Keywords: Melaleuca Alternifolia Concentrate, MAC, Immunomodulatory

Introduction

The cellular and humoral immune systems are mediated by T cells, B cells, Natural killer cells [1]. The $CD4^+$ The cells play an important role in establishing and maximizing the immune response [2]. $CD4^+$ T cells play a major role in mediating immune response through the secretion of specific cytokines [3].

Melaleuca Alternifolia Concentrate (MAC) is an essential oil extracted from a native Australian plant *Melaleuca Alternifolia. M. Alternifolia* is widely available over the counter in Australia, Europe, and North America and is marketed as a remedy for various ailments [4]. *M. Alternifolia* has anti-inflammatory, antimicrobial properties and antioxidant properties [4].

In-vitro studies have investigated the immunomodulatory effects of MAC. The parameters studied were cytokine production and protein expression in murine and human macrophage -like myeloid leukemic cell lines (Table 1) [5, 6].

The study by Low et al, demonstrated the immunomodulatory effects of MAC. Increasing doses of MAC increased peripheral blood immune cells.MAC increases $CD3^+$T cells, $CD4^+$ and $CD8^+$ lymphocytes [6].

Aim

1. To evaluate the immunity-enhancing effects of 2 doses of MAC (150 mg and 300 mg) and compare it with a placebo.

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2. To evaluate and compare the safety of MAC versus placebo.

Methodology

This study was a randomized, double-blind, placebo-controlled study that enrolled male or female healthy subjects ≥ 40 years of age. The inclusion criteria included subjects who were able to communicate with the investigators and were willing to provide written informed consent. The exclusion criteria included subjects participating in another clinical trial, subjects who were febrile 2 days prior to screening, and patients with any significant medical disorder which could have a confounding effect on the study outcomes. subjects taking any medication like immuno-suppressants, and subjects who regularly exercise. Pregnant and lactating women were excluded from the study. Subjects who exercised regularly or were athletes were also excluded from the study. 120 subjects were randomized in a 1:1:1 manner to either of the three treatment groups namely MAC 150mg od, MAC 300mg od or placebo 1 od. The primary efficacy end points were CD4 T lymphocyte count, CD8 T lymphocyte count, CD 19-B lymphocyte count and NK cells. The safety end points included haematology (Hb, WBC count, differential WBC count, RBC count, platelet count, haematocrit level), liver function test (AST level and ALT level), renal function test (serum urea and creatinine), fasting blood glucose. Adverse effects and related symptoms were studied. Efficacy was evaluated at week 0, week 1, week 2, week 3, week 4, week 8 and week 10. Safety end points were assessed at baseline, week 8, week 10 after initiation of treatment. The study was conducted in accordance with the principles of Good Clinical Practice (GCP). Ethics committee approval was taken from the institutional ethics committee before start of the study.

Results

One hundred and twenty subjects were enrolled in the study after screening 208 subjects.86 females and 34 males have included in the study. 117 subjects completed the study. Four patients dropped out of the study.

■ CD4 levels and CD8 levels

After 4 weeks of daily administration of 150mg per day or 300mg per day of MAC, the CD4 and CD8 levels increased till week 4 (Table 1). The levels of NK increased every week as compared to baseline (Table 2).

Table 1: Effect of MAC on CD4 and CD8 cells

	Mean difference	n	MAC 150 mg Mean % Difference	n	MAC 300 mg Mean % Difference	n	Placebo Mean % Difference CD4
CD4	W1-w0	40	4.8%*	36	3.4%*	40	5.2%*
	W2-w0	40	4.7%*	36	0.6%**	40	0.7%*
	W4-w0	40	5.1%*	36	1.8%	40	3.8%*
CD8	W1-w0	14	16.2%*	10	21.8%*	14	6.3%*
	W2-w0	0	N/A	0	N/A	0	N/A
	W4-w0	28	14.1%*	25	8.1%*	29	5.2%*

N/A: Not available; *: Positive increase from baseline

Table 2: The levels of NK increased every week as compared to baseline

	Mean difference	n	MAC 150 mg Mean % Difference	n	MAC 300 mg Mean % Difference	n	Placebo Mean % Difference CD4
NK cell	W1-w0	40	20..2%*	36	28.2%*	40	17.3%*
	W2-w0	40	24.5%*	36	25.4%**	40	11.4%*
	W4-w0	40	28.4%*	36	31.5%	40	3.8%*

*: Positive increase from baseline

Discussion

B cells originate from bone marrow hematopoietic stem cells and, after maturation, leave the bone marrow. They are capable of expressing a unique antigen-binding receptor on their membrane. Unlike T cells, B cells can recognize antigens directly, without the need for APCs, due to the presence of unique antibodies expressed on their cell surface. The B cells produce antibodies against foreign antigens. Under certain circumstances, B cells can also act as APCs [7].

When activated by foreign antigens to which they have an appropriate antigen-specific receptor, B cells undergo proliferation and differentiate into antibody-secreting plasma cells or memory B cells. Memory B cells are considered to be "long-lived" survivors of infections in the past which continue to express antigen-binding receptors. On re-exposure to the same antigen, these cells can be called upon to respond quickly. They produce antibodies and eliminate the antigen. B cells play a major role in the humoral or antibody-mediated immune response (as opposed to the cell-mediated immune response, which is governed primarily by T cells) [7-9].

Antibodies have a critical role to play in controlling virus proliferation during the acute phase of infection. But, they cannot eliminate a virus once infection has occurred. After an infection is established, cell-mediated immune mechanisms play a role in host defence against most intracellular pathogens [8, 9].

Cell-mediated immunity does not involve antibodies, but rather protects an organism through:

- The activation of antigen-specific cytotoxic T cells that induce apoptosis of cells displaying foreign antigens or derived peptides on their surface, such as virus-infected cells, cells with intracellular bacteria, and cancer cells displaying tumour antigens;
- The activation of macrophages and NK cells, enabling them to destroy intracellular pathogens; and the stimulation of cytokine (such as IFNγ) production that further mediates the effective immune response [8].

The stimulation of cytokine (such as IFNγ) production plays a role in further mediation of the effective immune response [8].

Cell-mediated immunity is directed primarily at microbes that survive in phagocytes as well as those that infect non-phagocytic cells. This type of immunity is most effective in eliminating virus-infected cells and cancer cells, but can also participate in defending against fungi, protozoa, cancers, and intracellular bacteria. Cell-mediated immunity also plays a major role in transplant rejection [8].

Cytokines are small protein interact with receptors and act as signalling molecules involved in the inflammatory pathway and can be either pro-inflammatory (IL-1 beta, GM-CSF, IFN-gamma, TNF-alpha) or anti-inflammatory (IL-4, IL-10, and IL-13). Macrophages are important players in the immune response. They increase the production of pro-inflammatory cytokines. Modulating the actions of macrophages can be an important approach for modulating immune reactions [10]. Cytokines are involved in the induction and effector phases of all immune and inflammatory responses. Cytokines represent tools and targets for modulating immune responses [10].

The "good" effects of cytokines include stimulation of the immune system to mount a defence against foreign pathogens or countering tumors, and reduction of an immune response, in patients with multiple sclerosis to reduce neuronal inflammation. Cytokines may be "bad" if their upregulation is associated with inflammatory diseases, rheumatoid arthritis, asthma and Crohn's disease. Therapeutic modulation of cytokine expression needs to consider which cytokines must be upregulated and which cytokines need to be inhibited [10].

Melaleuca Alternifolia Concentrate (MAC) is an essential oil which is extracted from a native Australian plant *Melaleuca Alternifolia* [4]. It is marketed widely as an over the counter remedy for various ailments in Australia, Europe, and North America [4]. *M. Alternifolia* has anti-inflammatory, antimicrobial properties, and antioxidant properties, the water-soluble components of tea tree oil can inhibit the production of pro-inflammatory mediators by activated human monocytes [8]. The water-soluble components of tea tree oil can suppress pro-inflammatory mediator production by activated human monocytes [11].

The water-soluble components of tea tree oil have the ability to suppress pro-inflammatory mediator production by activated human monocytes [11]. The components such as terpinen-4-ol and alpha-terpineol of tea tree

oil have been demonstrated to suppress the production of inflammatory mediators in in vitro studies where LPS-stimulated human macrophages were treated tea tree oil. This effect was attributed to y interference with the NF-kB, p38 or ERK MAPK pathways [12].

The current study proved the immunomodulatory effects of *Melaleuca Alternifolia* Concentrate (MAC). The effects last for up to 4 weeks after treatment initiation. After 4 weeks of daily administration of 150 mg per day or 300 mg per day of MAC, the CD4 and CD8 levels increased till week 4. In the current study, the levels of NK increased every week as compared to baseline.

Conclusion

Melaleuca Alternifolia Concentrate (MAC) has immunostimulant effects. MAC stimulates T cells, B cells and upregulates NAK .MAC can have a vital role to play in maintaining good health by improving immunity.

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