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Development and Evaluation of polyherbal formulation as vitality and immunity booster

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ABSTRACT

Background and Aims: Elderly subjects have a dysregulation of immune response mainly due to the changes in cell - mediated immunity. Due to their weakened immune response, the elderly are at increased risk of infection and related complications. In Unani medicine *Tiryaq wabai* was used for the prevention of epidemic diseases during outbreaks, but it has not been explored scientifically so far. The study was aimed to evaluate the immune-stimulating effect of *Tiryaq wabai* in elderly.

Materials and Methods: *Tiryaq wabai* was given to test group 500 mg orally thrice in a week for 45 days. Placebo was given orally to the control group at a dose of 500 mg thrice in a week for 45 days. Response was assessed by total leucocyte count (TLC), lymphocyte percentage, absolute lymphocyte count (ALC), CD4 and CD8 count. The results were analyzed statistically using Graph Pad InStat 3.

Results: The test drug showed statistically significant increase in TLC ($P < 0.001$), lymphocyte percentage ($P < 0.001$), ALC ($P < 0.001$), CD4 count ($P < 0.001$) in comparison to control group, but increase in CD8 count was not statistically significant. No major adverse effect was observed throughout the study.

Conclusion: The findings outlined above indicate immune- stimulating activity of *Tiryaq wabai* and supports its use in conditions where immunostimulant is required and thus is suggestive of therapeutic usefulness.

KEY WORDS: Aging, immune-senescence, T-lymphocytes, Unani medicine

INTRODUCTION

Aging is a process of bodily change with time, leading to increased susceptibility to disease, and ultimately to death.[1-3] This is reflected in the rise in age specific death rates. The process of aging is attributed mostly to the

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excessive oxidants produced in the body. [4] Reactive oxidant species and immune dysfunction are major causes of age-related diseases, [5] immune-senescence, attributed partly to the reduction in number of T-lymphocytes and loss of their functions. Such loss increases the prevalence of infectious diseases in the elderly. [6,7] The maintenance of antioxidant and immune fitness is a rational approach to preventive health care. The importance of disease prevention is recognized by Unani medicine through experience accumulated over centuries. *Tiryaq wabai* is a well-documented and well-known drug in Unani system of medicine for its wide use for prophylaxis during epidemics of cholera, plague and other epidemic diseases. *Tiryaq wabai* was used by Avicenna and Galen in healthy persons as well as in patients during epidemics. [8,9] *Tiryaq wabai* was selected for immune-stimulating study for two reasons. First, Unani descriptions were correlated with the immune-stimulating activity, therapeutically *Tiryaq wabai* is indicated for prevention of epidemic diseases during epidemics. The second basis for its selection were scientific reports regarding immune modulating, and antioxidant activity or related pharmacological actions and therapeutic effects of the ingredients of this formulation. *Tiryaq wabai* consists of three ingredients *Sibr* (*Aloe barbadensis*), *Zaafran* (*Crocus sativus*) and *Mur* (*Commiphora myrrh*) in the ratio of 2:1:1. Antioxidant and immune-stimulating effect of *A. barbadensis,* [10-12] *C. myrrha*[13] and *C. sativus* [14] has already been established in animal models.

MATERIALS AND METHODS

This study was a randomized placebo-controlled trial designed to evaluate the immunostimulating effect of *Tiryaq wabai* in elderly persons. After obtaining informed consent, 30 immunocompromised elderly persons were selected and randomly assigned into two groups, 20 in test and 10 in the control group. [13] Test group was treated with *Tiryaq wabai* 500 mg 3 times a week

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for 45 days, while in the placebo group, roasted wheat flour was given in the dose of 500 mg 3 times a week for 45 days. Persons of either sex of ≥ 60 years with two or more signs of immunodeficiency like (i) history of recurrent infection, (ii) unexplained weight loss, (iii) persistent diarrhea, (iv) persistent thrush in mouth, (v) 2 or more months on oral antibiotics with little effect were included in the study. Persons below the age of 60 years, receiving immunosuppressive drugs, uncontrolled diabetes and hypertension, chronic obstructed pulmonary disease, ischemic heart disease were excluded from the study. The subjects were strictly advised to stick to the usual dietary habits until the completion of the study. The response was measured by the assessment of total leucocyte count (TLC) every 15th day up to 45 days [14]

RESULTS

Characteristics of the subjects before the start of the trial have been demonstrated. Study population comprised of 73.33% males and 26.66% females. About 63.34% subjects had mixed dietary habits, whereas 36.66% were vegetarians. [15]

In this study, immune-stimulating effect of *Tiryaq wabai* was evaluated. The response was measured by the assessment of TLC every 15th day , lymphocyte percentage, ALC, CD4 count and CD8 count before and after treatment. TLC, lymphocyte percentage, ALC and CD4 count showed a significant increase after treatment with respect to 0-day test and 45th day control CD8 count also showed increase, but the difference was not statistically significant. [16]

DISCUSSION

Age associated changes in cell mediated immunity strongly depend on thymic functions. [17] As an individual ages, the thymus undergoes a progressive involution and the output of new cells fall significantly. This results in decreased concentration of naïve T-cells in peripheral blood and lymph nodes. In the elderly, there is a decrease in the diversity and functional

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integrity of both CD4 and CD8 T-cell subsets, which contribute to decreased ability to respond adequately to repeated infection. Production and maintenance of the diverse peripheral T-cell repertoire are critical to the normal function of the immune system.[18]

Table 1: Characteristics of the subjects

Characteristics	Test group (%)	Control group (%)
Age	67.1±7.80	64.3±4.620
Weight (kg) (mean±SD)	60.25±4.482	63.2±6.125
Height (cm) (mean±SD)	164.55±6.525	166.1±4.932
Gender		
Male	14 (46.66)	8 (26.66)
Female	6 (20)	2 (6.66)
Food habits		
Mixed diet	11 (36.66)	8 (26.66)
Vegetarian	9 (30)	2 (6.66)

Animal studies have shown that *A. barbadensis* increases the proliferation of T-lymphocytes in involuted thymus. Gonzalez *et al*. 1990 found that newly weaned mice treated with 30 mg water extract of *Aloe* subcutaneously showed greater proliferation of T-lymphocytes at thymus cortex that delayed fat infiltration of the involution at that level.[19] Reynolds and Dweck 1999 and Turner 2004 found immune modulating activities of the polysaccharides in *A. barbadensis* and suggest that these effects occur via activation of macrophage cells to generate nitric oxide, secrete cytokines and present cell

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surface markers. Acemannan, a polysaccharide stimulates antigenic response of human lymphocytes as well as the formation of all types of leukocytes from both spleen and bone marrow in irradiated mice. Lectin found in *Aloe* acts as mitogenic factor to stimulate lymphocyte proliferation and growth. Lectin enables the cells to recognize foreign cells and to stimulate macrophages for endocytosis.[20]

Said found that *C. myrrh* significantly increases all types of leucocytes during different stages of healing. On microscopic examination of blood smear from *C. myrrh* treated rats with skin injury, Said found an elevated count of middle-sized lymphocytes and neutrophils with well-defined nuclear lobules and rich granular cytoplasm. Furthermore, in spleen [21]

In this study, increase in TLC, lymphocyte percentage, absolute lymphocytes and CD4 cell count after the use of *Tiryaq wabai* is supported by animal studies showing an increase in all types of leucocytes after the use of *C. myrrh* and *A. barbadensis* in separate studies. *Aloe* also increases proliferation of lymphocytes at thymus cortex and stimulates macrophages for endocytosis. *C. sativus* increases DTH reaction in mice as mentioned above. DTH reactions are mediated by T-cells and monocyte/macrophages rather than by antibodies. Delayed hypersensitivity is a major mechanism of defense against various intracellular pathogens including Mycobacteria, fungi and certain parasites. [22]

Leukocytes are cells of the immune system involved in defending the body against both infectious disease and foreign materials. Neutrophils, basophils, and eosinophils are granulocyte leucocytes; these cells contain granules in their cytoplasm. These granules are membrane-bound enzymes that act primarily in the digestion of endocytosed particles. Neutrophils/polymorphonuclear defend against bacterial or fungal infection and other very small inflammatory processes that are usually first responders to microbial infection. Basophils are

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chiefly responsible for allergic and antigen response by releasing the chemical histamine causing vasodilatation. Eosinophils deal with parasitic infections. These are also the predominant inflammatory cells in allergic reactions.[21] Monocyte and macrophages are vital to the regulation of immune responses and the development of inflammation; Macrophages function in both nonspecific defense (innate immunity) as well as specific defense mechanisms (adaptive immunity) of vertebrate animals. Their role is to phagocytose, or engulf and then digest cellular debris and pathogens, either as stationary or as mobile cells by producing powerful chemical substances (monokines) including enzymes, complement proteins, and regulatory factors such as interleukin-1. They also stimulate lymphocytes and other immune cells to respond to pathogens. They are specialized phagocytic cells that attack foreign substances, infectious microbes and cancer cells through destruction and ingestion.[22] Increased proliferation of T-lymphocytes at thymus cortex results in an increased number of naïve CD4 T-cells in peripheral blood. CD4 T-cells respond to newly encountered antigens and once activated, provide help to cognate B-cells. These interactions are essential to germinal center formation and high affinity antibody generation.[23] CD4 T-cells (helper T-cells) coordinate the immune response and play a central role in immune protection. They help B-cells to make antibodies, to induce macrophages to develop enhanced microbicidal activity, to recruit neutrophils, eosinophils, and basophils to the sites of infection and inflammation, and through production of cytokines and chemokines and to orchestrate the full panoply of immune responses.[24] Safety parameters were found within normal range before and after treatment in both groups. Thus, our drug may be considered to be safe. This study suggests that *Tiryaq wabai* has immunostimulating capabilities and provides direct evidence for the immunostimulating effects of *Tiryaq wabai* in humans. [23]

The overall effect of the test drug was found quite encouraging in the treatment of immunocompromised elderly persons. No clinically significant side effects

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were observed in the test group and overall compliance to the treatment was found excellent. The findings outlined above indicate immune-stimulating activity of Unani test drug *Tiryaq wabai* and suggest its use in conditions where immune-stimulation is required and thus is suggestive of its therapeutic usefulness. Such plant based immune-stimulant may have application in the treatment of immunodeficiency diseases, allergic manifestation, and combinational therapy with antibiotics and as a vaccine adjuvant. [24]

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