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Effect of Immunomodulating and Antiviral Agent of Medicinal Mushrooms (Immune Assist 24/7™) on CD4+ T-Lymphocyte Counts of HIV-Infected Patients

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ABSTRACT: Immune enhancement through the use of natural products is a potentially valuable therapeutic modality in HIV-infected people, especially those who are not good candidates for aggressive ARV therapy. One such immune enhancement, a medicinal mushroom product from the United States, is Immune Assist 24/7™. In this study the effect of Immune Assist 24/7™, which is a naturally derived immune-modulating and antiviral agent, on CD4+ T-lymphocyte counts was evaluated in 8 HIV-infected patients at the Sunyani Regional Hospital (Ghana). The subjects were administered three tablets of 800 mg Immune Assist 24/7™ once daily (2.4 g/day) and peripheral blood samples were drawn at baseline, day 30, and day 60, and the CD4+ count measured. The study revealed that Immune Assist 24/7™, used as a sole therapeutic agent without additional ARV drugs, significantly increased CD4+ T-lymphocyte populations in all of the patients. In one patient, the CD4+ T-lymphocyte count went from 4 at the baseline, to 170 cells in 60 days, representing an increase of more than 4000%. In another patient, the CD4+ count went from 88 to 470 cells within the same period. Even in the patients with the highest CD4+ counts of around 800, there was a significant elevation in the CD4+ count noted. This study did not deal with the effect of Immune Assist 24/7™ on other immune parameters such as CD3+ T-lymphocyte count, natural killer cells count, or viral load among HIV-infected patients. These initial results are promising, and indicate the potential value of further evaluating the effects of Immune Assist 24/7™ on other immune parameters and viral load among HIV patients, administered either as a sole therapeutic agent, as an adjuvant with standard ARV therapy, or in comparison with standard ARV therapy alone.

KEY WORDS: medicinal mushrooms, immunomodulating activity, antiviral, HIV-infected patients, CD4+ T-lymphocyte, natural killer cells, *Lentinus edodes, Grifola frondosa, Ganoderma lucidum, Trametes versicolor, Agaricus brasiliensis,* Immune Assist 24/7™

ABBREVIATIONS: AIDS, acquired immune deficiency syndrome or acquired immunodeficiency syndrome; **ARV,** antiretroviral; **cART,** combination antiretroviral therapy; **CD4,** co-receptor that assists the T cell receptor; **EGCG,** epigallocatechin gallate; **ELISA,** enzyme-linked immunosorbent assay; **HIV,** human immunodeficiency virus; **NK cells,** natural killer cells; **TCR,** T cell receptor

I. INTRODUCTION

Since its identification in 1993 the human immunodeficiency virus (HIV), the causative agent for acquired immune deficiency syndrome or acquired immunodeficiency syndrome (AIDS), has spread globally reaching pandemic proportions with over 20 million people killed so far. Sub-Saharan Africa remains the region most heavily affected by HIV. In 2008, sub-Saharan Africa accounted for 67% of HIV infections worldwide, 68% of new HIV infections among adults, and 91% of new HIV infections among children. The region also accounted for 72% of the world's AIDS-related deaths in 2008.[1] The epidemic continues to have an enormous impact on households, communities, businesses, public services and national

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economies in the region. Despite significant gains that have been achieved in recent times through treatment scale-up, sub-Saharan Africa's epidemic continues to outpace the response. Consequently, there is a critical need for new preventive and therapeutic options to mitigate the epidemic's impact.

Antiretroviral (ARV) treatment in HIV-infected patients remains the only efficient way to control viral infection and represents a success of modern medicine resulting in a high reduction of AIDS mortality and HIV-associated morbidity up to now. However, current treatments are complex and require long-term administration with undesired consequences, namely drug resistance and acute and long-term toxicity. Eliminating these problems is a driving force behind research on new therapies. The most optimal therapeutic solution is an effective and safe immune-based therapy that could regulate the immune response of the host in a more clinically favorable manner.

In the last 4 decades, naturally derived immune enhancement compounds have been exploited as therapeutically useful agents for infectious diseases.[2,3] Examples of such immune enhancement compounds include Lentinan® from *Lentinus edodes* fruit-bodies, Schizophyllan from *Schizophyllum commune* mycelial broth, PSK and PSP from mycelial cultures of *Trametes versicolor*, Grifron-D from fruit-bodies of *Grifola frondosa*, and other polysaccharides and heteropolysaccharides from *Cordyceps sinensis*.[2] Increasingly, several of these compounds are now used extensively in Japan, Korea, and China and now also in the USA as sole therapeutic agents or adjuncts to standard radio- and chemotherapy because of their potent immune-modulating anti-tumor and antiviral activities. While most of these clinical studies have used individual immune enhancement compounds, some recent studies from Japan have shown that extract mixtures of these immune enhancement compounds, when taken as a supplement, have shown beneficial effects on the quality of life of severely ill patients.[2,4]

Immune Assist 24/7™ is one such newly developed immune enhancement and antiviral protection product. It is made in the United States from over 200 different hetero-polysaccharide immune modulator compounds that are extracted and purified from six different species of medicinal mushrooms, including beta-glucan compounds similar to Lentinan from *Lentinus edodes*; PSK and PSP from *Trametes versicolor*; Ganoderan A, B, C and D from *Ganoderma lucidum*; 1–3, 1–6-β-glucans from *Agaricus brasiliensis*; cyclomannans and beta-mannans, and other polysaccharides from hybridized *Cordyceps sinensis*; and Grifolan and protein bound 1–3, 1–4-β-glucans from *Grifola frondosa.* It also contains decaffeinated EGCG derived from green tea for its antioxidant and antiviral properties.[5] Immune Assist 24/7™ has been approved since 2004 in Ghana as a functional food by the Ministry of Health, and has so far been used by over 30,000 individuals for various clinical conditions including bacterial and viral infections.

Immune Assist 24/7™ works to address illness through (i) selective viral binding inhibition, (ii) increased systemic immune response, and (iii) inhibition of viral replication. While the selective viral binding inhibition is attributed to its epigallocatechin gallate content,[6] its immunomodulation activity is believed to be mediated through a two-part protein-bound hetero-polysaccharide complex made of beta bound glucose units, with varying degrees of side branching composed of different 5- and 6-carbon sugars.[7] The viral replication inhibition, however, is attributed to the presence of dozens of low dose, direct acting antiretroviral compounds of 3' deoxy-nucleoside and hydroxyl ethyl adenosine analog class.[8]

Sunyani Regional Hospital, one of the largest public antiretroviral treatment centers in the Brong Ahafo Region of Ghana, has adopted Immune Assist 24/7™ for the stabilization of the immune system of their patients with immune dysfunction diseases. Preliminary clinical studies indicated that Immune Assist 24/7™ can decrease the incidence of opportunistic infections, reverse AIDS-associated wasting, and help to achieve better clinical response. Our study was aimed at evaluating the effect of Immune Assist 24/7™ on CD4+ T-lymphocyte counts of HIV-infected patients.

II. MATERIALS AND METHODS

A. Medicinal Mushroom Material

Immune Assist 24/7™ is a naturally derived immune-modulating, antiviral agent, and dietary supplement manufactured by Aloha Medicinals Inc. (USA). It is a combination of multiple polysaccharide and heteropolysaccharide structures derived from several species of mushrooms long known for their medicinal properties: *Lentinus edodes, Grifola frondosa, Ganoderma lucidum, Trametes versicolor, Cordyceps sinensis,* and *Agaricus brasiliensis*. These immune-active polysaccharides include various beta-glucan structures, which have been shown to be active in enhancing immune response.[9] It also contains direct acting antiviral compounds of the nucleoside reverse transcriptase inhibitor class derived from hybridized *Cordyceps sinensis.* In addition to these medicinal mushroom-derived compounds, it contains purified epigallocatechin gallate (EGCG) in a timed-release matrix that allows it to bypass the stomach acid and maximize systemic absorption. EGCG has been implicated in viral binding inhibition against the HIV virus.[6]

B. Patients and Experimental Design

Eight HIV-infected patients, aged 20 to 51 years, were recruited from Sunyani Regional Hospital. The diagnosis of HIV infection was established by standard ELISA test and further confirmed by Western blot analysis. Three of the patients were in advanced clinical stage of HIV infection with baseline CD4+ count range of 4 to 88 cells/μL, while the remaining four had baseline CD4+ count range of 640 to 800 cells/μL. Informed consent was obtained from each volunteer who participated in the trial.

C. Treatment Regimens

None of the patients had received antiretroviral therapy prior to the trial. After initial screening, qualifying patients were administered with three tablets of 800 mg Immune Assist 24/7™ once daily (2.4 g/day).

D. CD4 Measurements

The samples of peripheral blood of the patients were analyzed by flow cytometry using fluorescent isothiocyanate-anti-CD4+ antibody against surface antigen of CD4+ T-lymphocyte. Assays were carried out at study entry, and after 30 and 60 days on Immune Assist 24/7™. The absolute CD4+ T-lymphocyte count was assessed. Analysis was carried out within 4 hours of blood collection.

III. RESULTS

The results of this 60-day HIV trial with 8 patients are shown in Table 1. After 30 days on Immune Assist 24/7™ without any antiretroviral drug, the absolute values of CD4+ lymphocytes per μL of blood increased significantly in all the patients. The patients who were in the advanced clinical stage, with baseline CD4+ count below 100 cells/μL, were the ones that benefited most from Immune Assist 24/7™ .

In the first patient (Table 1), the baseline CD4+ of the patient went from 4 to 170 cells/μL in 60 days, representing an increase of more than 4000%. Similarly, T-lymphocyte count in the other two patients in the advanced clinical stage of the infection increased significantly. While one of them had an increase from 6 to 150 cells/μL, an increase from 88 to 470 cells/μL was observed in the other.

Even in the healthier patients, there was still a significant elevation in CD4+ counts. An increase of about 44% was recorded in the patient with baseline CD4+ count of 640 cells/μL within the study period, while an average increase of about 50% was noticed in patients with baseline CD4+ count of about 800 cells/μL, except for the last patient (Table 1) who had a 140.5% increase in the CD4+ count. Interestingly, none of the 8 patients had a decrease in the CD4+ count within the study period.

TABLE 1. Results of 60-Day Immune Assist 24/7™ Trial with 8 HIV+ Patients Without Antiretroviral Drug

Patients	Age	Sex	Baseline CD4 cells/μL blood	30 days CD4 cells/μL blood	60 days CD4 cells/μL blood	CD4 change in 60 days
A	29	F	4	55	170	4150%
B	51	F	6	102	150	2400%
C	40	F	88	240	470	434%
D	30	F	640	720	920	44%
E	38	M	740	803	903	22%
F	44	M	760	949	1140	50%
G	45	M	800	1211	1234	54%
H	31	F	800	1813	1824	128%

IV. DISCUSSION

The introduction of combination antiretroviral therapy (cART) has substantially modified the natural history of HIV infection. At the beginning of the cART era, the focus was on HIV-associated mortality and morbidity, but as this objective was accomplished other issues emerged, including toxicity, resistance and compliance with treatment.[10] To overcome these issues, other new or enhanced therapeutic options are needed. We believe that immunonutrition therapy, which uses simple and harmless nutritional compounds to initiate immunobiological changes in the living body of the host in order to achieve a favorable clinical response, is an indispensable part of therapeutic strategies against HIV. Thus, the development of novel immune-based therapies is an urgent objective for anti-HIV drug discovery. In this study the effect of Immune Assist 24/7™, a mushroom-derived immunomodulator and natural antiviral dietary supplement, on the CD4+ count of HIV patients was investigated. The subjects were administered three tablets of 800 mg Immune Assist 24/7™ once a day (2400 mg/day). The results of this study revealed that Immune Assist 24/7™ significantly increased the CD4+ T-lymphocyte population of the patients. For instance, the baseline CD4+ count of one of the patients who was in the advanced clinical stage of the infection increased from 88 to 470 cells/μL within 60 days, showing clearly that Immune Assist 24/7™ has positively influenced the treatment outcome and disease progression in this patient. A similar study was carried out in Nigeria to assess the effect of the lamivudine, nevirapine, and stavudine combination on the CD4+ count of 37 HIV patients. The mean CD4+ cell count increased from 255 at baseline to 284, representing an increase of 11.37% at week 12, and then to 346, representing an increase of 35.68% at week 24.[11] Interestingly, the response observed in this study is comparatively higher than what is observed with this combination antiretroviral therapy. The high percentage increase seen in the patients, particularly those in the advanced clinical stage of the infection, and even in patients in a healthier state, demonstrates clearly that that Immune Assist 24/7™ could represent a possible dietary immunomodulatory treatment modality for HIV.

The study, however, has some limitations. For example, the sample size was small and the intervention period was not long enough to demonstrate the full spectrum of the potential effects of Immune Assist 24/7™. The positive results suggest the potential value of a future study to evaluate the effects of Immune Assist 24/7™ more elaborately and in larger populations, as well as to evaluate the long-term outcomes. Similarly, viral load is a predictor of HIV disease progression; its persistent elevation

in HIV-infected patients being indicative of poor prognosis.[12,13] While there were earlier indications in previous studies that Immune Assist 24/7™ may reduce the viral burden, viral load could not be monitored in our current study, mainly due to lack of equipment in the facilities where the studies were conducted. Viral load would be an important parameter to record in any future studies.

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