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Full Length Research Paper

Immunomodulatory Potential of Herbal Formulations Containing Seeds of *Nigella sativa* Linn.

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ABSTRACT

Immunostimulation counteracts immunosuppression, thereby playing a role in re-establishing body equilibrium. Plants with immunostimulatory activities are used as an alternative therapy for a variety of diseases, including impaired immune responses derived from infections, physical and psychological stresses, alcoholism, and environmental damages. Several lines of evidences have suggested that *Nigella sativa* displays a remarkable array of biochemical, immunological, and pharmacological actions. The present study investigated different formulations of *N. sativa* seed used as alternative medicines for chronic and immunosuppressive conditions in Nigeria. Four different formulations of *N. sativa* seed as a major component were analyzed for their immunomodulatory properties. Freshly isolated peripheral blood mononuclear cells were cultured in the presence of test samples as well as the T-cell mitogen phytohaemagglutinin. Cell viability and proliferation were determined using trypan blue dye exclusion method. The proliferative activity of test samples reached the peak at 72 h after incubation, and the best activity was achieved with 4,000-fold dilution of the original samples. The effect was sustained even after sample removal, and 2.2- to 3.6-fold increase of viable cell number was observed at the end of the experiment. These results suggest that the formulations have potential for enhancing cellular immunity alongside other therapeutic functions

Keywords; T-lymphocyte stimulation, *Nigella sativa*, chronic immunosuppressive conditions, immunomodulatory activity, peripheral blood mononuclear cells

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INTRODUCTION

Plants are an abundant source of potential new drugs, therefore they are referred to as a store of new chemical entities. They have a variety of biological activities, including the modulation of immune systems. Currently, there has been a growing interest in their clinical use, in particular, as therapeutic modalities (Newman *et al.*, 2003). The inability of the populace in developing countries to access high-cost modern medicines has resulted in the use of alternative therapy (Calixto, 2000). Medicinal plants have been employed in different countries to improve immunological disorders. The activities of plants to enhance host defense mechanisms and cure diseases are based on the concept of Ayurveda and other cultural and medicinal practices (Atal *et al.*, 1986). A number of plants and their extracts have been examined for their immunomodulatory activity. Immunostimulants may exert non-specific protection against pathogens and constitute

an alternative to chemotherapy, which is attributed to the non-antigenic and functional stimulation of granulocytes, macrophages, complements, and natural killer (NK) cells (Wagner *et al.*, 2003). Therefore, they are considered to be useful agents for counteracting immunosuppressive conditions, such as physical and psychological stress, environmental damages, malnutrition, and long-term treatment with immunosuppressive drugs.

Nigella sativa, a family of Ranunculaceae, is rapidly emerging as an alternative medicine for various diseases because of its wide spectrum of pharmacological potential (Ahmad *et al.*, 2013). Some studies have demonstrated the effects of *N. sativa* seed extracts on the immune systems. These include anti-oxidant and anti-tumor properties, stimulation of bone marrow and B cells, and activation or inhibition of enzymes and gene expression (Ahmad *et al.*, 2013). The use of *N. sativa* seed for treatment of various diseases, especially HIV/AIDS, viral hepatitis, and cancer, is

becoming popular in Nigeria (personal communication, 2014). According to herbal medicine practitioners, *N. sativa* seed promotes health conditions by re-establishing body equilibrium and modulating host defense mechanisms (Talmate *et al.*, 2014).

The improvement of symptoms in various disorders, including viral infections, has been reported. These include a significant reduction in tumour size and metastasis (Salim, 2010), anti-allergic responses (El Mezayen *et al.*, 2006), amelioration of ulcer and liver reperfusion injury (Yildiz *et al.*, 2008; Nikakhlagh *et al.*, 2011), reversal and recovery from kidney nephrotoxicity (Sayed-Ahmed and Nagi, 2007; Hadjzadeh *et al.*, 2012) and prophylactic effect on asthmatic airways (Boskabady *et al.*, 2007). Also, Wistar rats exposed to *Nigella sativa* treatment had improvement in cognitive deficits (Perveen *et al.*, 2008), reduction of anxiety and epileptic seizures (Perveen *et al.*, 2009) while persons living with HIV had significant increase in CD4 count followed by sero-reversion in six individuals (Onifade *et al.*, 2011; Onifade *et al.*, 2013). Thus, it seems important to clarify the immunomodulatory activity of these agents to explain their efficacy in immunological disorders. In the present study, we investigated the effect of different formulations of *N. sativa* on the proliferation of human peripheral blood mononuclear cells (PBMCs) *in vitro*.

MATERIALS AND METHODS

***Nigella sativa* formulations:** The formulations contain *Nigella* seed powder as the main constituent. The formulations were purchased from an herbal practitioner in Nigeria. The test samples were filtered for sterilization using 0.22 µm filter and were further examined for mycoplasma DNA with the MycoSEQ mycoplasma real-time PCR detection kit (Life technologies, USA), using the Applied Biosystems 7500 Real-Time PCR system. The formulations were coded as follows: CA (anticancer preparation), HV (anti-human immunodeficiency virus preparation), HP (anti-hepatitis preparation), and HB (anti-hepatitis B virus preparation).

Isolation of human peripheral blood mononuclear cells: Fifty milliliters (50mL) of blood was collected from a healthy human donor, who is HIV and HBV/HCV negative, into a bottle containing the anti-coagulant heparin. Blood was then mixed with an equal volume of culture medium. Ten milliliters (10mL) of the mixture was overlaid on 5 ml of Ficoll-Paque plus (Sigma-Aldrich, USA) in centrifuge tubes. The tubes were spun at 2,000 rpm for 30 min at room temperature. The peripheral blood mononuclear cell (PBMC) layer separated by density gradient centrifugation was collected after aspirating the plasma (Boyum, 1968). The PBMCs were washed with phosphate buffered saline (PBS) 3 times at 2,000 rpm for 10 min for the first wash and 5 min for subsequent washes. Then, the viable cell number was counted after staining with trypan blue (Warren, 2015) under an inverted microscope.

Viability and proliferation of PBMCs after stimulation: Isolated PBMCs were cultured at 1×10^6 cells/ml in a microtiter plate in the presence of different formulations of *N. sativa* seed (test samples). The test samples were diluted at

400- to 4,000-folds of the original samples, while phytohaemagglutinin-P (PHA-P) was used as a positive control of T-cell proliferation. The cells were incubated at 37°C and examined for their viability and proliferation by trypan blue exclusion method every 24 h for 3 days.

After 3 days of incubation with the test samples, the cells were collected into a centrifuge tube and centrifuged at 1,500 rpm for 5 min at room temperature. Supernatants containing the test samples or PHA-P were removed, and cells resuspended in 1 ml of growth medium (RPMI-1640 with 20% fetal bovine serum and interleukin-2) were assessed for viability by trypan blue exclusion method. Thereafter, cells were transferred to a flask and incubated for further 4 days, during which cells viability and proliferation determination was also done. The experiments were conducted twice to confirm reproducibility of the results

RESULTS

Proliferation of PBMCs during stimulation: Viable cell number was determined by the dye exclusion method every 24 h for 3 days. The cell number was found to increase from the initial count (1×10^6 cells/mL) and reached the peak after 3 days of incubation with the test samples. HV had the highest stimulatory effect on PBMCs. Its 400- and 4,000-fold dilutions increased the cell number up to 4×10^6 and 4.75×10^6 cells/ml, respectively (Fig. 1). This was followed by HB and CA, while the HP and PHA-P had the weakest effect. On the overall, the 4,000-fold dilution of the test samples gave better results than the 400-fold dilutions.

Proliferation of PBMCs after stimulation: After removal of the test samples on day 3, PBMCs were resuspended in culture medium and further incubated. The viable cell number was determined every 24 h for 4 days. The highest viable cell number (2.7×10^6 cells/ml) was obtained for the cells stimulated with PHA-P followed by CA (Fig. 2), while those of other preparations were modest (1.2 – 1.9×10^6 cells/ml). The viable cell number at the end of culture was compared with the initial number to obtain a fold increase between the pre-stimulation and post-stimulation phases. Again, the highest increase in viable cell number was achieved with PHA-P followed by HP (4,000-fold dilution) and HB (400-fold dilution) (Fig. 3).

DISCUSSION

The role of plants in drug discovery and development is becoming important due to their proven effectiveness in disease treatment. Some plants are known to have activities against various diseases and support host defenses. Therefore, people particularly in low resource settings tend to achieve better health conditions with medicinal plants, thereby indicating the need for investigating the biological properties of medicinal plants as front-line drugs for clinical use (Newman *et al.*, 2013).

Previous findings have indicated that constituents of *N. sativa* enhance cellular immunity while down-regulate humoral immunity. Therefore, the present study has focused

on determining the stimulatory effect of the therapeutic formulations on T-cell proliferation

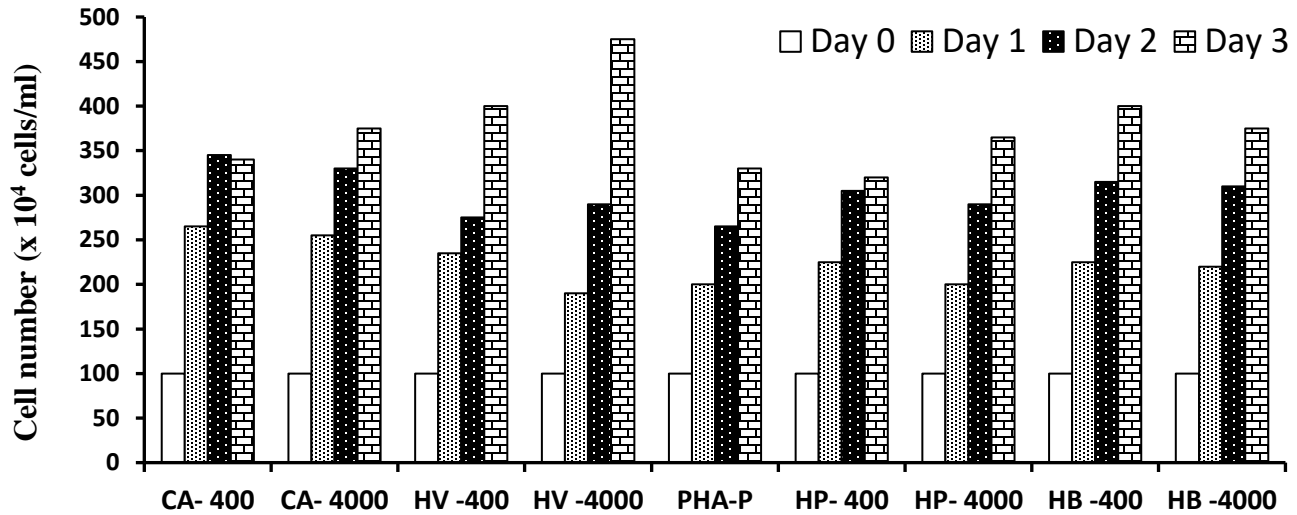


Figure 1:
Peripheral blood mononuclear cell proliferation during treatment with *N. sativa* seed formulations

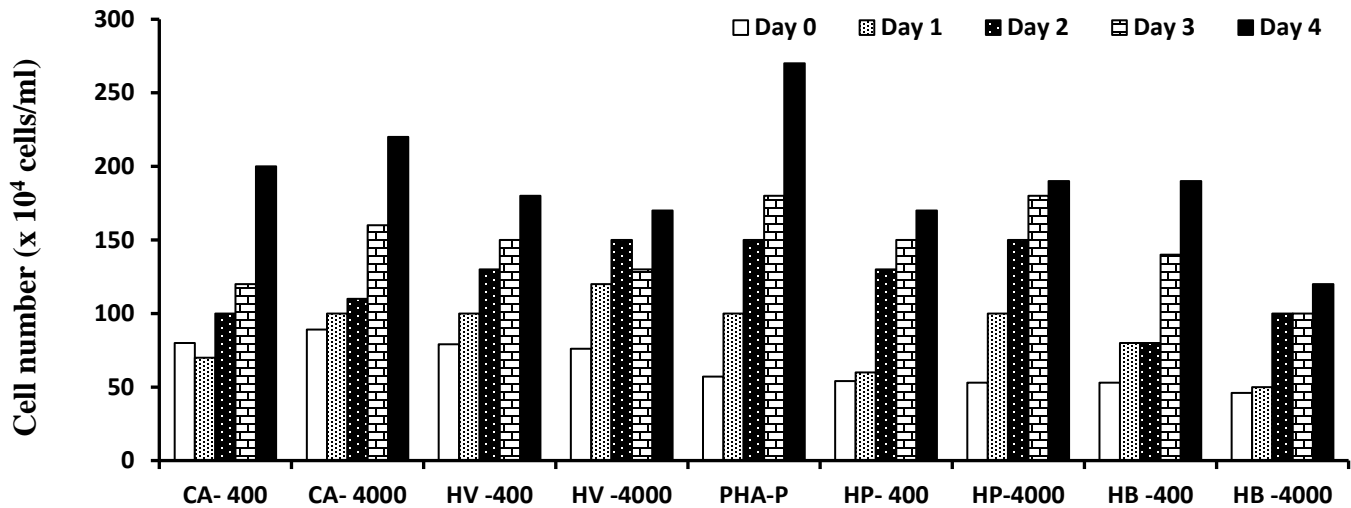


Fig. 2:
Peripheral blood mononuclear cell proliferation at post stimulation

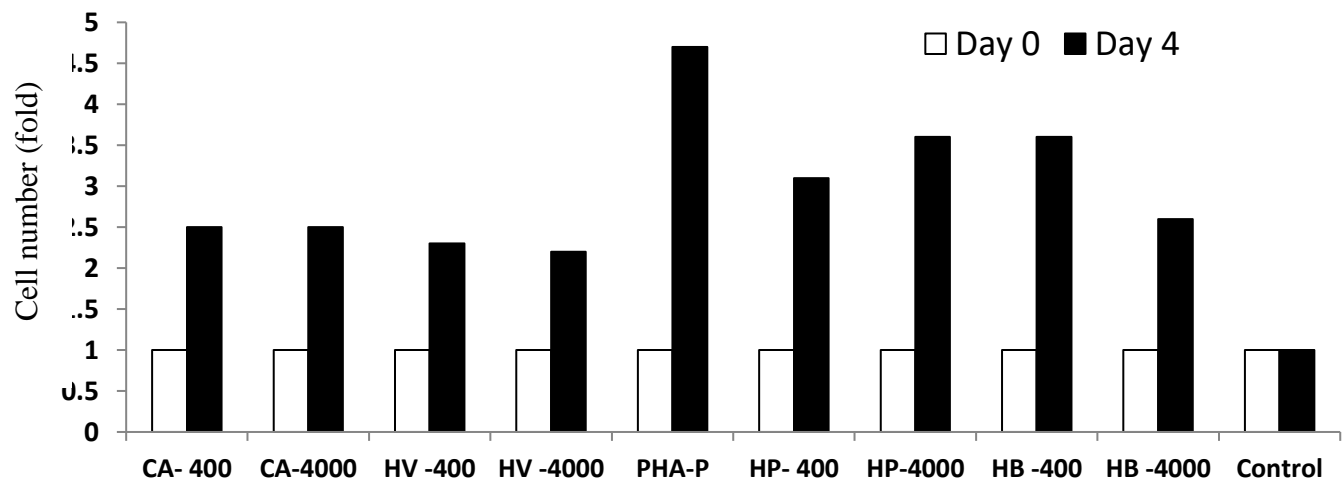


Fig. 3:
Fold increase in cell proliferation

Our result showed that the samples were capable of initiating lymphocyte proliferation, and this activity was sustained after stimulation with the samples. The enhancement of cell proliferation was the highest with the 4,000-fold dilutions. Furthermore, a similar effect continued after removal of the samples from culture medium. The immunomodulatory effect of *N. sativa* seeds and oil has been documented using in vitro and in vivo assays. Swamy and Tan reported an enhanced proliferative response of T cells to Concanavalin-A (Con A) in the presence of ethyl acetate and water fractions of *N. sativa* seed (Swamy and Tan, 2009). An in vivo study also demonstrated 55% and 30% increase in the CD4/CD8 ratio and natural killer (NK) cell functions, respectively, in individuals treated with *N. sativa* seed and oil for 4 weeks (Haq et al., 1999). Additionally, increased white blood cells, bone marrow cellularity, and restoration of resistance to lethal infections following immunosuppression by cyclophosphamide were observed in BALB/c mice treated with the methanolic extracts (Ghonime et al., 2011).

The present study showed that the four formulations of *N. sativa* seed had activity comparable to that of the T-cell mitogen PHA-P. The enhanced proliferative response of PBMCs was observed as 2.2 to 3.6-fold increase in cell number between the stimulation and post-stimulation phases, suggesting that the various formulations improved cellular immunity. This is consistent with the findings demonstrating their enhancing effect on T cell proliferation in previous studies. Two-fold increase in cytotoxic activity of splenic NK cells against tumor cells was observed in mice following 1 week oral administration of an aqueous extract of the seed (Abuharfeil et al., 2001). Furthermore, evidences for enhanced innate and cellular immunity was observed following oral administration of *N. sativa* oil to diabetic hamsters (Fararh et al., 2004). A supply of the seed as nutritional supplement aided immune responses among elderly individuals by altering the total amount and type of dietary lipids. Consequently, this ameliorated age-associated decline in T-cell functions (Wu et al., 1990). Furthermore, treatment with the oil significantly increased the delayed type hypersensitivity (DTH) in those exposed to the tetanus toxoid and T. mentagrophyte antigens (Christou et al., 1989). Taken together, the formulations of the *N. sativa* seed used as therapeutic agents for chronic disorders like HIV/AIDs, hepatitis, and cancer in Nigeria may have a stimulatory effect on T cell-mediated immune response alongside other therapeutic functions. Thus, the activity of *N. sativa* seed may play an important role in the regulation of diverse immune reactions involved in various chronic diseases, suggesting its prophylactic use for opportunistic infections and supportive treatment in cancer patients.

In conclusion, since *N. sativa* seed has an enhancing effect on T-cell proliferation, it should be further explored as a source of novel bioactive chemicals for immune modulation in chronic immunosuppressive conditions.

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