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RESEARCH ARTICLE

EFFECT OF BLENDED FORMULATIONS OF SELECTED BIOLOGICAL IMMUNOMODULATORS ON 'ABSOLUTE CD_4' COUNT IN HIV 1 INFECTED INDIVIDUALS FOLLOWING PHASE – II HUMAN CLINICAL TRIALS

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ABSTRACT

Human clinical trials were conducted for the assessment of effect of selected potential biological immunomodulators on 'Absolute CD_4' count of 32 HIV reactive patients. Following clearance of 'Institutional Ethics Committee', effect of blended formulations of *Chlorophylum borivilianum, Withania somnifera, Wagatia spicata Dalz., Picrorrhiza kurroa, Spilanthes paniculata* Wall. ex. DC.were studied over the period of eleven months in each participant. Readings of 'Absolute CD_4' counts were recorded using 'Flow Cytometry'. Statistical analysis was carried out using 'Split Plot' analysis (Mixed Design Test) followed by 'Bartlett's test for Sphericity' and 'Levene's test for homogenecity of variance'. At the end of phase – II clinical trials, statistically significant rise in the 'Absolute CD_4' count was noted.

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INTRODUCTION

Immunostimulants, immunosuppressants and adjuvants are the three broad categories of immunomodulators. Diseases like Cancer, AIDS are characterized by dysfunction or deficiency of one or several components of immune system. This deficiency or dysfunction can be restored by the use of immune enhancing compounds called immunostimulants. 'Absolute CD_4' cells are the key cells which regulates various vital aspects of immune system. As these cells are the target for HIV, respective patients develop immunodeficiency (Levy, Jay A., 2007). India has an antecedent of using plant cell derivatives for treatment of diseases. Plants are the precious sources of immunomodulators which can be effectively used for control of diseases and disorders. Many research scholars gave experimental evidences for immunomodulating features of plant derivatives. Plant metabolites have been studied in details for their chemical and potential chemotherapeutic properties. Many plants are yet to be explored for their potential immunomodulating properties. Although the branch of 'Allopathy' still insists on the use of active and known chemicals for treatment of diseases and disorders, plant metabolites are known to exhibit potential synergistic activities for treatment of diseases. Many plants have been studied for potential anti-HIV properties, these include*Ancistrocladus abbreviates* (Manfredi *et al*., 1991), *Anogeissus acuminat e* (Batra Amla *et al*., 2002), *Ancistrocladus korupensis* (Boyd *et al*., 1994), *Areca catechu* (Kusumoto *et al*., 1995), *Arnebia euchronia* (Kashiwada *et al*., 1995), *Annona squamosa* (Wu *et al*., 1996), *Aspalanthus lineraris* (Eapen and George, 1998), *Astragalus membranaceus* (Shi and Peng 2003; Weber R. *et al*., 1999), *Aqularia agallocha* (Weber *et al*., 1999), *Angelica sinensis* (Weber *et al*., 1999), *Atractylodes macrocephala* (Weber *et al*., 1999), *Amomum villosum* (Weber *et al*., 1999), *Brucea javancia* (Okano *et al*., 1996), *Allium sativum* (Maroyi Alfred, 2014 ; Silprasit *et al*., 2011), *Artemisia afra* (Lubbe *et al*., 2012), *Artemisia annua* (Lubbe *et al*., 2012), *Aspilia*

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pluriseta (Cos *et al*., 2002), *Albizia amara* (Mar *et al*., 1991), *Azadirachta indica* (Rukanya *et al*., 2002), *Argemone mexicana* (Chang *et al*., 2003), *Buchnavia capitata* (John *et al*., 1992), *Bersama abyssinica* (Asres *et al*., 2001), *C. teysmanii* (Cragg *et al*., 1996), *Camellia sinensis* (Hashimoto *et al*., 1996), *Calophyllus spp.* (Eapen and George, 1998), *Crinum macowani* (Klos *et al*., 2009), *Cucurbita maxima* (Daly *et al*., 1999), *Carica papaya* (Rashed *et al*., 2013), *Cleome gynandra* (Mnzava and Chigumira Ngwerume, 2003), *Combretum hartmannianum* (Kisangau *et al*., 2011), *Croton lechleri* (Liu *et al*., 2009), *Citrus limon* (Lackman-Smith *et al*., 2010), *Dodonaea viscose* (Asres *et al*., 2001), *Stepherdia argentea* (Yoshida *et al*., 1996). In the present study, blended formulations of *Chlorophylum borivilianum, Withania somnifera, Wagatia spicata Dalz., Picrorrhiza kurroa, Spilanthes paniculata* Wall. ex. DC. were administered in HIV 1 infected individuals through oral route and was continued for anaverage period of 11 months. At the end of study, statistically significant rise in the 'Absolute CD_4'count was recorded.

MATERIALSAND METHODS

Sources of the study

Chlorophylum borivilianum, Withania somnifera were collected from Pavan Agro Farm, Pune, *Wagatia spicata Dalz.* was collected from the hilly regions of Malakapur (Dist. Kolhapur), *Picrorrhiza kurroa* was collected from licensed ayurvedic supplier and *Splianthes paniculata* Wall. ex. DC. was collected from the hilly regions of Mulshi (Pune). All plant derivatives were authenticated by 'Botanical Survey of India'. These plant derivatives form one of the sources for the study. HIV positive patients, who voluntarily participated, were selected for the study. Clinical trials were conducted under the supervision of registered medical physician as approved by 'Institutional Ethics Committee, Bharati Vidyapeeth Deemed University, Pune.'

Human clinical trials

Using selected plants, phase-I study was conducted in 15 HIV infected individuals and 5 healthy individuals. Blended doses of selected biological immunomodulators were administered through oral route. The morning dose was blended as *Wagatia spicata Dalz.* (crude stem powder) – 100 mg, *Picrorrhiza kurroa* (crude root powder) – 50 mg. The evening dose was blended as *Withania somnifera* (crude root powder) – 25 mg, *Wagatia spicata Dalz.* (crude stem powder) – 50 mg, *Splianthes paniculata* Wall. ex. DC. (crude root powder) – 20 mg, *Chlorophylum borivilianum* (crude root powder) – 30 mg. under the supervision of registered medical practitioner, therapy regime was continued for 60 days. Patients were observed for drug toxicity and other parameters like pyrexia, hypersensitivity, body weight and secondary infections. Observing no side effects, phase-II clinical trials were conducted as per 'Cosmetic Amendment Bill LV II of 2007' and guidelines of WHO. With the written consent, effect of selected biological immunomodulators was studied in 18 HIV reactive males and 14 HIV reactive females (age group 20-47). Above mentioned blended doses were administered through oral route and continued for an average period of 11 months. Record of initial and final 'Absolute CD_4' count was kept by using 'Beckman Coulter Flow Cytometry' (version CXP 2.2).

OBSERVATION AND RESULTS

Following phase-I clinical trials, without developing adverse reactions,given doses of biological immunomodulators were well tolerated by male and female human participants. No signs and symptoms of illness other than originally described before starting of the therapy were observed for the period of 60 days. Following phase-II clinical trials, significant rise in the 'Absolute CD_4' count was observed as shown in Table no.1. Statistical analysis was carried out using 'Split Plot analysis (Mixed Design Test)' followed by 'Bartlett's test of Sphericity' ($p < 0.001$) and 'Levene's test for homogenecity of variance.' Test within the subject showed that selected biological immunomodulators have significant impact on 'Absolute CD_4' count [Wilk's Lambda = 0.215, F (2,29) = 52.98, p = 0.000]. Partial Eta squared = 0.785. Univariate test for 'Absolute CD_4' count indicated Greenhouse Geisser = 279084.084, f [1,30] = 33.016, p value = 0.000. Since p value is less than 0.05, it is concluded that there is significant difference between 'Absolute CD_4' counts before and after treatment. Simultaneously, interaction effect for 'Absolute CD_4' count was studied. Interaction effect for CD_4 is zero. From the table, it can be seen that for CD_4 count, Greenhouse Geisser = 29353.33, f [1,30] = 3.473, p value = 0.072. Since p value is <0.05, it is concluded that, the impact of biological immunomodulators on CD_4 count is same for men and women.

Table 1. Effect of biological immunomodulators on 'Absolute CD_4' count in HIV 1 infected individuals

Patient's Id No.	Absolute CD_4 count	
	Initial	Final
M 1	358	381
F 2	509	630
M 3	419	459
M 4	483	576
F 5	335	582
F 6	162	444
M 7	75	110
F 8	384	1040
M 9	60	171
M 10	75	104
F 11	106	580
F 12	423	534
M 13	528	629
M 14	319	469
F 15	167	320
M 16	285	446
M 17	621	671
F 18	348	432
M 19	73	193
F 20	265	396
M 21	83	179
M 22	367	492
F 23	117	110
M 24	354	374
F 25	69	227
M 26	184	340
F 27	413	408
M 28	258	361
F 29	79	60
M 30	415	560
F 31	89	171
M 32	169	230

DISCUSSION

AIDS is a pandemic disease and the fear of HIV infection has affected the major global population than that of clinical infection of the virus. Due to this, many people have started believing that, HIV is one of the deadliest infections that have

so far been invented. At the end of June 2015, about 36.9 million people were living with HIV (www.unaids.org Report 2015). Since 2000, about 25 million people have died of AIDS related complications. In the present study, five different plants were selected as potential biological immunomodulators. *Withania somnifera* has been utilized in Ayurveda as a base of different medicines. Seena *et al*. (1993) reported anticancer effect of *Withania somnifera* [Agarwal S. S and Y.K.Singh (1999)]. Effect on cytokine secretion of the same plant was studied by Duley *et al*. (1997). Antimalignant properties of Withaferin isolated from *Withania somnifera* were reported by Devi *et al*. (1995). Immunomodulating properties of *Picrorrhiza kurroa* were reported bt Atal *et al*. (1986). DTH response of *Picrorrhiza kurroa* was studied by Sharma *et al*. (1994). *Chlorophylum borivilianum* is a popular ingredient of many ayruvedic medicines. Triveni (2003) has described herb as excellent rejuvenator. Mayank Thakur *et al*. (2006) have reported the effect of the plant on cellular immunity. Ballal *et al*. (2015) documented immunomodulating activity of *Wagatia spicata Dalz.* Activation of macrophages is experimental animals by *Spilanthes acmella* was reported by Savadi R.V. (2010). No other documentation consolidated the use of *Spilanthes paniculata* Wall. ex. DC. as an immunomodulator. Previous studies as described were conducted by selecting an individual plant or herb. Present study differs by focusing on synergistic action of selected biological immunomodulator than that of single one. As T lymphocyte is a prime target of HIV, their response decides the rate of disease progression. Clinical progression of HIV infection reported decline in 'Absolute CD_4' cells at the rate of 25 to 60 cells/µl/year (Lang *et al*., 1989). Differentiation and maturation of microphages and macrophages requires cytokines secreted by CD_4 cells (eg: GM – CSF). Activation of human macrophages and subsequent distribution of virally infected cells requires ɣ - interferons produced by CD_4 cells. Even the formation of 'Antibody forming B lymphocytes' requires cytokines produced by CD_4 cells (Abbas, Lichtman and Pillai 2014, Nandini Shetty 2014, Peter J. Delves *et al*. 2011). Thus, CD_4cells indirectly regulates the humoral immune response including secondary immune response. During HIV infection as the count of CD_4 cells declines, many functional aspects of cellular immunity and humoral immunity get badly hampered to the extent to develop immunocomprised state. Due to the synergistic action of the selected biological immunomodulators, claimed immunostimulant properties were observed in terms of elevated 'Absolute CD_4' count.

Conclusion

As a part of phase – I clinical trials, blended formulation of *Wagatia spicata Dalz.* and *Picrorrhiza kurroa* was well tolerated by human beings. Similarly, blended formulation of *Withania somnifera, Wagatia spicata Dalz., Splithantes paniculata* Wall. ex. DCand *Chlorophylum borivilianum* was well tolerated by human participants. As a part of phase – II clinical trials, immunomodulating properties of selected biological immunomodulators were studied. Administration of blended formulations of biological immunomodulators as described above increased the count of 'Absolute CD_4' cells in HIV 1 infected individuals.

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