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STATUS OF HIV-1 PROVIRAL DNA WITH THE TREATMENT OF POLY PHYTOCHEMICAL MOLECULES.

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ABSTRACT

The use of Highly Active Anti Retroviral Therapy (HAART) in Human Immunodeficiency Virus (HIV) infected patients can often significantly reduce the viral RNA to undetectable levels in plasma in both adults and children. One of the current challenges in the suppression or eradication of HIV-1 proviral DNA in latently infected CD4 lymphocytes and other reservoirs, which can replenish and revive viral infection upon activation. The phytochemicals which are present in Medicinal Herbs have been reported to have anti-hiv properties. Some medicinal plants have been reported to have immunostimulant properties. The ideal antiviral drug regimen would be one that induces strong and persistent suppression of viral replication, gives prolonged immunologic and clinical benefits without toxicity, can be administered at frequent dosage intervals, is affordable and easy to store, and can thus benefit the greatest number of HIV infected people, including those in developing countries. A 30 year old female, a symptomatic HIV-1 positive patient was given with the polyherbal regimen containing anti-HIV properties, anti-viral properties and immunostimulant properties for about more than three years. The qualitative proviral DNA, quantitative RT-PCR, quantitative p24 antigen assay, qualitative antibody assays , CD4 cells enumeration, CD8 cells enumeration were done before the treatment, in-between the treatment, after the treatment. The results were observed, recorded and interpreted. During the treatment the viral load gradually declined and finally came to less than the detectable limit after the attainment of undetectable level of viral load the proviral DNA also vanished. The CD4 and CD8 cells gradually increased. The infectivity was tested in PBMC culture before and after the treatment. The infectivity was present before the treatment and absent after the treatment.

From the interpretations of the data the treatment has the significant effect towards the HIV-1 proviral clearance.

KEYWORDS

phytochemicals, proviral DNA, HIV-1, Herbal treatment

INTRODUCTION

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AIDS results from infection with the human immunodeficiency virus (HIV), and nearly 40 million adults and children worldwide live with HIV/AIDS[1]. The opportunistic infections characteristic of AIDS results from the progressive depletion of $CD4^{+}$ T lymphocytes in infected individuals[2]. During the acute phase, virus levels increase rapidly with a concomitant loss of $CD4^{+}$ T cells. As cellular and humoral antiviral immune responses take effect, virus levels decreases and $CD4^{+}$ T-cell numbers temporarily recover. During the subsequent phase of clinical latency, $CD4^{+}$ T-cell numbers slowly, but inexorably, decline. Finally, as immune system is exhausted by HIV, clinical immunodeficiency and lethal infections occur[3].

The tropism of HIV-1 is governed by the distribution of cellular proteins that the virus requires to gain access to the cell's interior. Hot on the heels of the discovery of HIV-1[4-6] came studies demonstrating that CD4 is a receptor for the virus[7,8]. Over a decade later, the chemokine receptors CCR5 and CXCR4 were identified as co receptors for HIV-1[9-13]. The distribution of these receptors permits infection of not only $CD4^{+}$ T cells, but also cells that are central to antigen presentation, including macrophages and dendritic cells (DCs). HIV-1 seems to have adapted to the unique biological characteristics of these types of cells. As a consequence, HIV-1 may exist in various replication states and tissue compartments, which would allow it to persist even in the face of host immune responses and highly suppressive antiretroviral regimens. Understanding these adaptations is important if we are to design strategies to restrict viral replication and pathogenicity.

In the course of effective immune response, first the virion is internalized by phagocytosis or endocytosis by the Antigen Presenting cells (APCs) like macrophages, dentritic cells etc and presents its antigen protein along with cell membrane protein associated molecules called HLA or MHC molecules. The CD4 cells recognize this MHC associated antigen and produces the cytokines. This cytokines increases the B cell and T cell activities, thus increases the humoral and cell mediated immune response.

Infection by HIV is characterized by several effects on the host immune system. B cells decline in number and function[14], and, because of the toxicity of HIV antigens, cytokine regulation is distorted causing a decrease in CD4+ T-cells[15]. There is a distinct interplay between HIV and the immune defenses. Typical non-progressors (those who have been infected with HIV but do not show symptoms) display several responses that are different than those of progressors. Non-progressors show more TH1-type cytokines like IL-2 and IFN-γ and an elevated response by CD4+ T-cells and cytotoxic CD8+ T-cells towards HIV is observed. Additionally, there is an increased synthesis of chemokines also. The HIV virus encounters these defenses by varying antigenic sites (preventing an effective immune response and overwhelming the immune system) and by reducing MHC on the surface of cells, and reducing the number of CD8+ T-cells.

The recent studies reveals that the levels of cytokines present in body fluids are useful for understanding pathogenesis and as diagnostic and prognostic indicators in many diseases, including those induced by human immunodeficiency virus (HIV) infection[16]. The IFN-γ has antiviral property towards HIV also, if with greater yield by the secreting cells it can completely eradicate the virus from body including the proviral DNA possessing cells by apoptosis mechanism. Besides antiviral

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molecules, some phytochemical molecules present in some herbs has induced greater yield of IFN-γ to the level of eradication of virus, it is dose dependant[17]. We could eradicate HIV, including proviral DNA possessing cells by combining both the properties of antiviral and IFN-γ induction with phytochemical molecules which are present in some herbs.

MATERIALS AND METHODS

I selected nine medicinal plants, having antiviral and immunostimulant property were selected which are traditionally used in Indian System of Medicine.

1.*Curcuma longa* has antiviral property[18]
2.*Coriandrum sativum* has antiviral property[19]
3.*Glycyrrhiza rhiza* has antiviral property[20]
4.*Ocimum sanctum* has IFN-γ inducing property[21]
5.*Piper longum* has IFN-γ inducing property[21]
6.*Phylanthus emblica* has IFN-γ inducing property[21]
7.*Phylanthus niruri* has IFN-γ inducing property[21]
8.*Withania Somnifera* has IFN-γ inducing property[21]
9.*Eclipta alba* has IFN-γ inducing property[21]

The above plant's parts are extracted in water, alcohol, benzene, chloroform and acetic acid. The extracts were mixed together and the LD50 was calculated and the minimum therapeutic dose was arrived at the dose rate of 18 grams (i.e Maximum Therapeutic dose =1/20 of LD50 dose in kilo weight basis, minimum therapeutic dose is equal to 1/10 of maximum therapeutic dose) in divided doses thrice a day and the extracts were filled in six 1gram capsules and administered 6 capsules thrice a day.

The author previously has had an unpublished data regarding LD50 dosage for this regimen since this seven herbal extracts were cumulatively employed in some other study in 90s and was done as follows.

Five groups of rats, mean weight of each rat was 250 grams, for acute 6 animals (first group), sub chronic 8 animals (second group) and for chronic 30 (3x10 i.e.1st,2nd and 3rd sub group) animals were taken. The acute toxicological study towards Lethal dose 50 (LD50) carried out for 7 days, sub chronic for 2 months and chronic for 6 months. The initial drug dose was designed with reference to the quantity used in different kinds of works mentioned in various literatures and was taken 1% of the body weight of the animal that is 2.5 gram randomly as initial dose for all, a brief washout period was given for 3 days then the dose was gradually, step by step increased up to 6% of the body weight of the animal that is 15 gram in chronic study group. In chronic study group every month the drug dose was increased 1% of the body weight after a brief washout period, in first sub group the treatment was up to 2 months, in second sub group the treatment was up to 4 months, in third sub group the treatment was up to 6 months. An observation was made towards at which dose of the drug 50% of animals are being died in study group along with clinical pathology perturbations, mild clinical signs, body weight loss and histopathological changes[28].

Selection of a patient

I selected a 30 year old asymptomatic female HIV-1 positive patient. Informed consent from the subject and Institutional Ethics Committee approval obtained for this study. Before starting the treatment the proviral DNA, Viral load, HIV-1 antibodies, P24 antigen assay, CD4, CD8 cell counts and Interferon- γ (IFN-γ) levels were recorded and all the parameters were recorded every six months once during the treatment. The patient's serum and

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blood cells (PBMC) were demonstrated in cell culture for the presence of virus before starting the treatment, during the treatment and after the treatment. One normal blood donor, same age, same sex without clinical complaints or signs of disease and negative for anti-HIV-1/2 antibodies was included in this study as control. Both control and patient were monitored for routine laboratory values such as hemoglobin, liver or renal function tests, the patient also monitored for any adverse effects during treatment. Both were monitored for other illness and co-infections for entire study period.

Detection of HIV-1 Proviral DNA by PCR

DNA extraction and purification of PBMCs, Lymphnode biopsy for HIV-1 DNA detection

Pheripheral blood mononuclear cells (PBMC) were isolated from whole blood by Ficoll-Paque gradient separation (Amersham Pharmacia). Cell pellets, corresponding to 5x 10^6 PBMC (Tryphan blue exclusion technique was followed with haemocytometer). Cell pellet was lysed with lysis buffer, DNA was extracted and purified from pellet by DNAeasy tissue kit (Qiagen) following the manufacturers' instructions.

The inguinal lymph node biopsy was taken by a surgeon, to the lymph node biopsy material 1X Phosphate buffer saline was added,triturated, mixed well and centrifuged at 2500 rpm, the pellet was taken for DNA extraction. Cell pellet was lysed with lysis buffer and DNA from lymph was extracted by using QIAGEN DNA extraction kit and using the sequences of the selected HIV-1 *gag* forward primer BK1F and the reverse primer BK1R were respectively: 5'-GTA ATA CCC ATG TTT TCA GCA TTA TC-3' and 5'-TCT GGC CTG GTG CAA TAG G-3'[22] both for blood and lymph and standard Polymerase Chain Reaction Protocol was followed. PCR was performed with 50-µl reaction mixes with the above primer pair. Reactions (50µl) were performed for 30 cycles in thermal cycler with the following cyclic timings: 30 s at 94^0C, 30 s at 55^0C and 1 min at 72^0C.The products were analysed by electrophoresis on 1.2% agarose gel. The (Applied Bioscience) Thermal cycler was used for thermal cycling. The (Bio rad)UV Trans illuminator was used to detect the HIV-1 proviral DNA in the Agarose gel.

The samples were also send to Reliance life science laboratory, Mumbai and assay was performed in The National HIV Repository Center, Bangkok for counter check up.

HIV-1 (RT-PCR) Quantitative assay

The HIV-1 viral load was done with Roche COBAS AMPLICOR version 1.5 Ultra sensitive method using Roche kits and compared with real time PCR (Roche) with its kits.

The plasma Viral RNA was purified by using QIAamp® Viral RNA Mini Kit and its protocol before going for Amplicor.

The AMPLICOR HIV-1 MONITOR Test is based on five major processes: specimen preparation, reverse transcription (RT) of target RNA to generate cDNA, PCR amplification of target cDNA using HIV-1 specific complimentary primers, hybridization of the amplified products to oligonucleotide probes specific to the target(s), and detection of the probe – bound amplified products by colorimetric determinations. The test permits the reverse transcription and amplification of HIV-1 and

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Quantitation Standard (QS) RNA to occur simultaneously. The processed specimens are added to the amplification mixture in reaction tubes in which both reverse transcription and PCR amplification occurs. The downstream or antisense primer (SKCC1B) and the upstream or sense primer (SK145) are biotinylated at the 5' ends. The Master Mix reagent contains a biotinylated primer pair specific for HIV-1 and QS target nucleic acid and has been developed to yield equivalent quantification of HIV-1. The quantitation of HIV-1 viral RNA is performed using a Quantitation Standard (QS). The QS is a noninfectious RNA transcript that contains the identical primer binding sites as the HIV-1 target and a unique probe binding region that allows QS amplicon to be distinguished from HIV-1 amplicon. The QS is incorporated into each individual specimen at a known copy number and is carried through the specimen preparation, reverse transcription, PCR amplification, hybridization, and detection steps along with the HIV-1 target and is amplified along with the HIV-1 target. HIV-1 RNA levels in the test specimens are determined by comparing the absorbance of the specimen to the absorbance obtained for the QS. Therefore, the QS compensates for any effects of inhibition and controls for the amplification process to allow the accurate quantitation of each specimen.

The samples were also send to Reliance life science laboratory, Mumbai and the same assay was performed by the author in "The National HIV Repository Center", Bangkok for counter check up.

Real time RT-PCR

HIV-1 virions were quantified using primers either to Gag or to the 5′ end of HIV-RNA. For detection, dual-labeled fluorescent probes with a fluorescein (FAM) moiety at the 5′ ends and a tetramethylrhodamine (TAMRA) moiety at the 3′ end were used. HIV-Gag was measured using primers skcc1b for cDNA synthesis, primers ts5′gag (upstream; 5′-*CAAGCAGCCATGCAAATGTTAAAAGA*-3′), boe2 (downstream), and boe3 (probe) for amplification and detection. HIV-5′ RNA was measured using primer mf86 (5′-*CCACACTGACTAAAAGGGTCTGAGGGATCT*-3′), cr1(5′-*TCTCTGGCTAACAGGGAACCCACTGCTT*-3′), cr2 (5′-*TGACTAAAAGGGTCTGAGGGATCTCTAGTTACCAG*-3′), and mf74tq (FAM-5′-*AGCACTCAAGGCAAGCTTTATTGAGGC*-3′-TAMRA). PrP mRNA was measured using PCR primers as previously described and using a fluorescent probe prpe2+tq (FAM-5′-*CAACCGAGCTGAAGCATTCTGCCT*-3′-TAMRA). PCR was performed as described previously in a single-tube system (Qiagen 1-step RT-PCR) with an additional "hot-start" using Ampliwax (Applied Biosystems, Foster City, California, United States) to separate cDNA synthesis and PCR amplification steps. cDNA synthesis and subsequent amplification were performed in duplicate in a real-time thermocycler (Roche)

CD4 and CD8 Counts

The enumeration of CD4 and CD8 cells were done on fully automated two Laser BD FACS Calibur Flowcytometer U.S.A using BD four colour

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CD45/CD3/CD4/CD8 reagents by the commercial laboratory, Thyrocare Technologies Ltd, Mumbai.

Estimation of IFN-γ

The serum interferon gamma was estimated by ELISA method by Span Diagnostics, USA ELISA Reader and BD Bioscience kits were used, the protocol was followed as per the manufacturers recommendations. I collected 8 ml of blood in dry tube, serum was separated, aliquoted, and stored at -70°C. Initially 96-well microtitre plate was sensitized with determined anti-interferon gamma monoclonal antibody. Next 200µl of test, positive control, and negative control sera were added (dilution 1:2) they were incubated at 37°C for periods ranging from 30 to 60 minutes. Four washes were performed with detergent solution containing 2-chloroacetamide (0.1%). This was repeated until the phase preceding substrate addition. Later, biotin marked plates received streptovidine-peroxidase. After incubation, a substrate of hydrogen peroxide (0.02%) and tetramethylbenzione (2%) was added. Reaction was interrupted at room temperature with 2N sulfuric acid. Results were evaluated by reading optical density (OD) in ELISA reader at 450nm and was calculated from a standard curve[29,30]. This assay was performed in Naval Institute of Molecular Research in Namakkal, Tamilnadu.

Estimation of P24 Antigen assay

This was done with signal amplification technology[23] with ELISA Reader with BIOMERIEUX kits.

The p24 antigen assay modified with a booster step is based on heat-denatured immune complex dissociation p24 antigen and amplified signal of ELISA with biotinyl tyramide to increase the lower limit of p24 antigen detection. The assay was performed according to the manufacturer's instructions integrating the ELAST ELISA amplification system (Perkin-Elmer Life Sciences,Inc., Boston, Mass.) procedure into the Vironostika HIV-1 antigen assay before the addition of the chromogenic substrate. The plasma was diluted with 0.5% Triton X-100 to a concentration of 1:6. The diluted plasma was heated at100°C for 5 min. A known concentration p24 antigen that varied between 0 to 80pg/ml was used as a positive control. All samples (100 µl in each well) were incubated at 37°C for 60 min. After washing with diluted phosphate-buffered saline (PBS), 100 µl of anti-HIV-1 (human) conjugate labeled with horse radish peroxidase (HRP) was added to each well, and incubated at 37°C for 60 min. The streptavidin-HRP (S-HRP) conjugate was used at a dilution of 1:500. After washing, 100 µl of the biotinyl tyramide working solution from the ELASTELISA amplification system was added to each well and incubated at room temperature for 60 min. Then, 100 µl of diluted S-HRP from the ELAST ELISA amplification system was diluted to 1:500 using 1% bovine serum albumin-PBSTween20 (1% BSA-PBS-T) which was added to each well after the washing step, and the plates were incubated at room temperature for 30 min. Then, 100 µl of tetramethylbenzidine substrate in urea peroxide solution was added to each well after a washing step, and the plates were incubated at room temperature for 30min. Finally, the color reaction was stopped by adding 100 µl of 1M sulfuric acid. The absorbance of each well was read at 450 nm within 15 min by the ELISA reader.

Eight negative controls and six positive controls, ranging from 0.5 to 80 pg/mlin serial dilution, were tested in each qualitative and quantitative assay. The cutoff value, the optical density (OD) at 450 nm, for each test was the sum of

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the means of the absorbance of eight negative controls plus 3 standard deviations[28]. Samples with an absorbance value greater than or equal to the cutoff value were considered positive for HIV-1 p24 antigen. Samples with an absorbance value less than the cutoff value were considered negative. Samples with protein concentrations above the assay range were subjected to repeat testing by dilution plasma. A standard curve was generated, from which OD values of the unknown specimens are interpolated to determine their concentration. The standard curve is constructed using a linear graph plotting the concentration of the HIV-24antigen that OD values converted onto log10 p24 antigen (in femto grams per milliliter) on the *y* axis versus known values of viral load (in log10 copies per milliliter) on the *x* axis. The controls must be included for each assay.

This assay was performed in Naval Institute of Molecular Research in Namakkal, Tamilnadu as well as in "The National HIV Repository Center", Bangkok

HIV-1 co-culture

The PBMC was separated from infected patients and co- cultured with healthy donors PBMC. In Peripheral Blood Monocyte Separation (PBMC) 5-10 ml of EDTA blood sample was taken, centrifuged at 400 g for about 10 minutes to collect plasma. I diluted the cell pellet with equal volume of 1X sterile Phosphate Buffer Saline (PBS) and I over layered Ficoll Hypaque (Density =1.077) to the cell pellet at the ratio of blood : Ficoll =1:3 in 15 ml centrifuge tube, centrifuged at 1000g for 15-25 minutes without break. I collected the cell PBMC fraction (Interface) and transferred into the new centrifuge tube containing 9ml 1X PBS, centrifuged at 1000g for 10 minutes for washing, washed three times finally transferred to 1X RPMI medium at the cell density of 1-3x10^6 cells /ml and supplemented with 10% Fetal Bovine Serum (Sigma),10-20 U/ml IL-2 (Sigma) also added in the culture. Equal number 3 days Phytohaemagglutinin (PHA) activated healthy donor PBMC along with same quantity of FBS and IL-2 in 1000 ml of RPMI medium, incubated at 37^0C with 5%CO2. In day 7, the supernatant was subjected for presence of P24 antigen and presence of virus. The cells were tested for HIV-1 proviral DNA.

This assay was performed in Naval Institute of Molecular Research in Namakkal, Tamilnadu as well as in "The National HIV Repository Center", Bangkok

Detection of HIV-1 antibody

The HIV-1 antibodies were detected using ELISA method and Western-Blot method using J.Mitra & Co. Ltd. Kits as per manufacturer's recommandations. This assay was performed in Naval Institute of Molecular Research in Namakkal, Tamilnadu.

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TABLE 1

LD ***50***

ACUTE (6 animal)			SUBCHRONIC (8 ANIMALS)			CHRONIC (3x10=30 animals)		
DAYS	DOSE	MORTALITY %	DAYS	DOSE	MORTALITY %	MONTHS	DOSE	MORTALITY %

CALCULATION OF THE DRUG REGIMEN

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1ST DAY	1% of bw (2.5 gms)	Nil	1-30 days	1% of bw (2.5 gms)	Nil	1st month	1% of bw (2.5 gms)	First sub group treated up to two months, nil mortality.
2nd DAY		Nil				2nd month		
3rd DAY		Nil						
4th DAY		Nil				3rd month	2% of bw (5 gms)	Second subgroup treated up to four months,15% mortality.
5th DAY		Nil	30 -60 days		Nil	4th month	3% of bw (7.5 gms)	
6th DAY		Nil		2% of bw (5 gms)				
7th DAY		Nil				5th month	4% of bw (10 gms)	Third subgroup treated up to six months, 50% mortality.
						6th month	5% of bw (12.5 gms)	
							6% of bw (15 gms)	

** bw –body weight*

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RESULTS

The LD50 dose (Table 1) in rat for about 250 gms weight is 15gms, arrived 60gms for one kilogram and as such applied for average weight of 60 kg human beings as 3600 gms and 1/20th of LD50 dose was calculated to arrive maximum therapeutic dose i.e 180 grams again calculated to arrive minimum therapeutic dose i.e 180/10 =18 gms for average human being (This is being followed in feed additives in the field successfully, data not presented).The amplicor HIV-1 RNA monitor Test v1.5 quantitates viral load by utilizing a second target sequence (QS) that is added to the amplification mixture at a known concentration. The QS is a noninfectious 233 nucleotide in vitro transcribed RNA molecule with primer binding region identical to those of the HIV-1 target sequence. The QS, therefore, contains SK145 and SKCC1B primer binding sites and generates a product of the same length (155 bases) and base composition as the HIV-1 target. The probe binding region of the QS was modified to differentiate QS-specific amplicon from HIV-1 target amplicon.The optical density in each well of the plate is proportional to the amount of HIV-1 or QS amplicon in the well, and the total optical density is proportional to the amount of HIV-1 or QS RNA, respectively, input into each reverse transcription/PCR amplification reaction. The amount HIV-1 RNA in each specimen is calculated from the ratio of the total optical density for the HIV-1 specific well to the total optical density for the QS-specific well and the input number of QS RNA molecules using the following equation:

$$\frac{\text{Total HIV-1 OD}}{\text{Total QS OD}} \times \text{Input QS copies per PCR reaction} \times 40 = \text{HIV-1 RNA copies/ml}$$

After 42 months of treatment with herbal extracted molecules, the viral load has come from 54000 copies/ ml of serum to an undetectable level and there is no significant difference in viral load level between two laboratories and two test methods result. Likewise there is no significant difference in assay results carried out in different laboratories. The proviral DNA disappeared both in PBMC (Fig 1) and Lymphnode (Electropherogram not given). The CD4 cells increased from 360 cells/µl of blood to 990 cells/µl of blood in the duration of treatment about 42 months. The CD8 cells also increased from 830 cells/µl to 1160 cells /µl (Table 2). The control showed the normal CD4 and CD8 range all the times (Data not shown) The P24 antigen in the blood has decreased from 2700 fg/ml to undetectable level.

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Table2:

Showing different parameters and co-culture results before and during the treatment.

PARA METERS	BEFORE TREATMENT	T 6 MTH	T 12 MTH	T18MTH	T24 MTH	T30 MTH	T36 MTH	T42MTH
PROVIRAL DNA in PBMC, PROVIRAL DNA DNA in Lymphnode	present	present	present	present	present	present	absent	Absent Absent
VIRAL LOAD. (copies/ml).	54000	45000	27000	18000	9000	Not detectable	Not detectable	Not detectable
CD4 (cells/µl)	360	450	540	630	720	810	900	990
CD8 (cells/µl)	830	880	910	1000	1030	1080	1090	1160
P24 (fg/ml)	2700	1800	900	540	270	90	Nil	Nil
IFN-γ pg/ml	9	19	27	45	54	90	108	118
HIV CO-CULTURE	P24,virus, proviral DNA present	P24,virus, Pro viral DNA present	P24,virus, proviral present	P24,virus , proviral DNA present	P24,virus, proviral DNA present	P24,virus, proviral DNA present	P24,virus absent. Pro DNA present	P24,virus, proviral DNA absent
ANTI HIV ANTIBODIES	+	+	+	+	+	+	+	+

MTH*-Month

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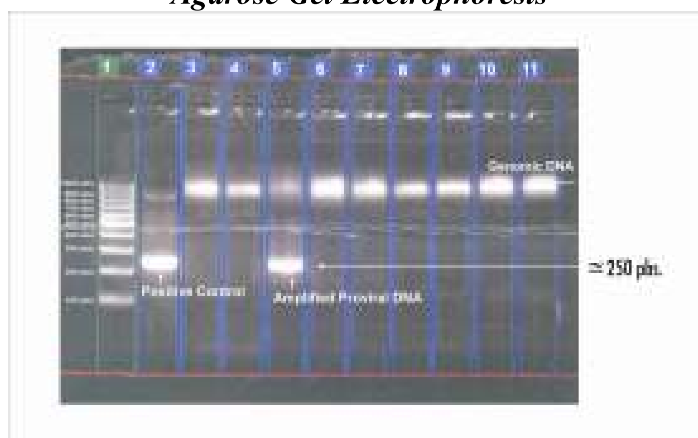
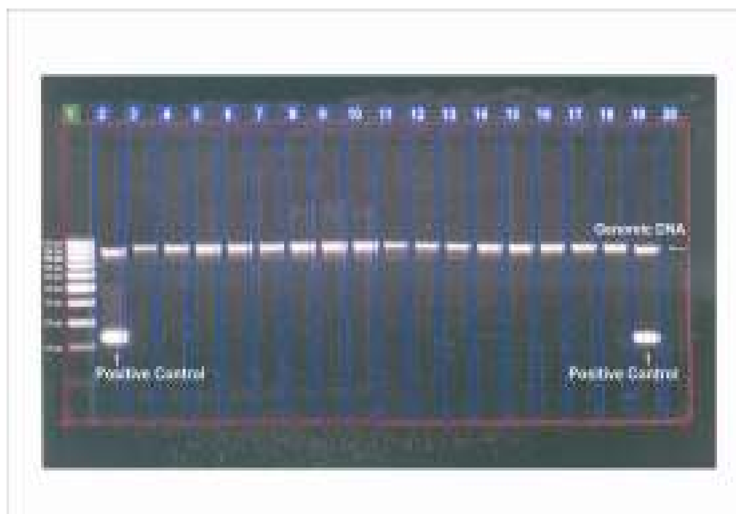
Figure 1 a
Agarose Gel Electrophoresis



This is the picture shows the presence of Proviral DNA beforer treatment in PBMC. Lane No.1: 100bp Molecular weight marker. 2:Positive Control. Lane: 5 Our Study Sample. Lanes 3,4,6,7,8,9,10 and 11:: Negative Controls. :

Figure 1 b
Agarose Gel Electrophoresis



This is the picture shows the absence of Proviral DNA after treatment in PBMC. Lane No.1: 100bp Molecular weight marker. Lane 2 and 19:Positive Control. .Lane 5: Our Study Sample. Lanes 3,4,6,7,8,9,10,11,12,13,14, 15, 16,17 and 18: Negative Controls.

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IFN-γ:Calculation of Results

Calculated the mean absorbance for each set of duplicate standards, controls and samples. Subtract the mean zero standard absorbance (ie, plate background) from each.

Ploted the standard curve on log-log graph paper, with IFN-γ concentration on the x-axis and absorbance on the y-axis. Draw the best fit straight line through the standard points.

To determine the IFN-γ concentration of the unknowns, found the unknowns' mean absorbance value on the y-axis and drawn a horizontal line to the standard curve. At the point of intersection, drawn a vertical line to the x-axis and read the IFN-γ concentration. If samples were diluted, multiply the interpolated IFN-γ concentration by the dilution factor.

Computer-based curve-fitting statistical software was also employed. The IFN-γ has raised from 9pg/ml to 118pg/ml. The control showed only within the normal level all the times, no significant difference (Data not shown)

However, the haemogram, kidney function tests, liver function tests reveals almost normal in all the times both in control and patient (Data not shown) and no other illness and co-infections recorded for the entire study period.

DISCUSSIONS

In HIV co-culture before the treatment virus and P24 antigen were present and 36 th month, 42nd month results reveals that the proviral DNA, virus and P24 antigen were absent. But in all the time the HIV-1 antibodies were present. This has to be studied.

With the Cell-mediated immune response the host defenses that are mediated by antigen- specific T cells and various non-specific cells of the immune system. It protects against intracellular bacteria and viruses. CD8 T-cells or cytotoxic T- lymphocytes are white blood cells that find and kill infected cells in the body. The CD8 T-cell responses are important in controlling the HIV levels.

Interferons are several glycoprotein produced and secreted by certain cells that induce an antiviral state in other cells and also help to regulate the immune response. Interferon gamma (IFN-γ) is secreted by T_H1, T_C, NK cells. The IFN-γ inhibits viral replication, enhances macrophages activity and increases the expression of class I and class II MHC molecules[24].Interferon- mediated antiviral activity may involve several mechanisms. Due to HIV-1 infection there is sustained impaired activity in most of the cells including low level expression of MHC. The IFN-γ restores the expression of both I MHC and II MHC molecules, there by CTL activity against HIV-1 infected cells occurs[29].

Finally ,IFN-γ increases the activity of T_C cells, macrophages, and NK cells, all of which play a role in the immune response to viral infected cells. All nucleated cells express class I MHC molecules, if the cells are infected with HIV-1, if the cells possesses foreign DNA like HIV-1 proviral DNA that is altered self-cell. Each and every altered self-cell expresses class I MHC and HIV antigenic peptide complex, the CTL very well recognizes that type of cells and destroys that. By keep on increasing CTLs and its activity by IFN-γ with the induction of phytochemicals all the proviral gene

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possessing cells, viral infected cells could be destroyed.

Some phytochemical molecules have anti-HIV activity[25], the plants which were employed for this study namely *Curcuma longa ,Coriandrum sativum ,Glycyrrhiza rhiza*

Possesses antiviral activity but further study has to be carried out for isolation, characterization, structural identification and mechanism of action, how it acts as anti- HIV molecules. The Hyper Active Anti Retroviral Therapy (HAART) very often gets resistant to the HIV-1 mutants[26], like wise CTL mediated virus infected cell lysis also often failure due to CTL escape mutants[27].

In the case of above conclusions further research should be done to understand about CTL escape mutants and drug resistance to mutants with the response to this phytochemical therapy.

CONCLUSSION

From the above study the herbal extracts possesses antiviral compounds, interferon inducers and immunostimulating molecules. Further work has to be done what kind of molecules involved in this activity and also to be studied the molecular mechanisms to this activity.

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Conflict of Interest Statement

No competing interest declared.

Informed consent and IEC approval was obtained as per helsinky declaration. The work has been approved by the Institutional Ethics Committee, Naval Aids Research Center, Namakkal.

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