Research Title:

Highly Effective Unani Anti-HIV Drug, proven by DNA-PCR Antigen Test.

Research Paper Presented at:

WORLDS AIDS CONFERENCE
1-3 DEC 2006, TIANJIN, CHINA

Theme: Take Action, Stop AIDS!

Released by:

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In the Session Ten : **Development of Natural Products and TCM as Anti-HIV Drugs**

Place: VIP Meeting Room, 2F, Tianjin Tianbao International Hotel

Time: 08:30-12:00, Dec. 3, 2006

In the Presence of:

Dr. Nafis Sadik, (UN Special Envoy for HIV/AIDS in Asia & Pacific)
Dr. Luc Montagnier, (Pioneer in HIV/AIDS Disvoeries)
Dr. Avram Hershko, (Nobel Prize Laureate in Chemistry in 2004)
Dr. Rulf M. Zinkernagel, (1996 Nobel Prize Laureate in Medicine)
Dr. Richard J. Roberts (The Nobel Prize in Medicine 1993)
Professor Yunzhen Cao, Clinical Director
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Vaccine and Prevention Research Program Division,
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Molecular Targets Development Program£¬NCI
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ABSTRACT: -

The major goal of this research paper is to bring to light the actions of Unani Anti-HIV drug against HIV, through the observations made under a controlled condition with the help of superior facilities. Another, important goal of this paper is to highlight how this Unani anti-HIV drug has an edge over the other ART drug presently in use for the treatment of HIV/AIDS.

The major purpose of the entire effort is to develop an effective therapy for the HIV patients so that they can be safeguarded against symptomatic problems, opportunistic infections, AIDS related complex and other socio-economic concerns leading to poor quality life & early death.

At the same time it also aims to evolve the effective therapy to be very practical, cost effective and safe. And finally this paper projects a significant breakthrough in HIV Treatment, observed in the patients treated with this Unani drug providing an insight over the prospective of HIV research in developing an effective drug.

ORGANIZATIOIN INVOLVED:-

1) **Hootone Remedies** : An ISO GMP Certified Pharmaceutical concern manufacturing Unani Anti-HIV drug HOO-IMM PLUS.

2) **HAKH Medical Foundation** : The Chain of clinic providing multi-speciality Ayurvedic - Unani treatment for HIV infected patients.

3) **Hooone Life Sciences** : **Fully** equipped Molecular Research Lab providing Molecular Diagnostic Services.

INTRODUCTION: -

The paper focus on the research & development, finding's & observations of the Unani Anti-HIV drug HOO-IMM PLUS against HIV/ AIDS and thus emphasize on the following issues: -

1) Development of the Unani Anti-HIV Drug.
2) Invitro Lab Studies.
3) Invivo human clinical Studies.
4) Phenomenal change in DNA-PCR Test.
5) A major breakthrough in HIV Treatment: Present Antigen Vs Present Antibody Test.
6) A major finding, an insight for HIV research:Past Antibody Vs Present Antibody Test.
7) An Edge over the other Anti-HIV drug.
8) Felicitation and Appreciation of the therapy.

1) Development of the Unani Anti-HIV Drug

The formulation of the drug is made according to the Unani system of Medicine and approved from FDA (Food and Drug Administration), Mumbai, India. And the compositions of the formulation are purely herbal, standardized according to the Pharmacopoeia of Herbal Extracts.
The R&D of this particular drug initially started as a treatment for the asymptomatic and symptomatic HIV associated diseases and AIDS related complex. And later with the considerable improvement in the patients noted in response to the therapy more scientific and controlled studies were undertaken with the help of modern molecular diagnostic services from reputed and a well-equipped laboratory 'Reliance Life Science.
And the records of the tests were well maintained in the form of soft and hard copies representing R&D data pertaining to the treatment.
The formulation has undergone scientific studies for the two most important tools for analyzing the effectiveness of the drug; they are a) Invitro Studies and b) Invivo Studies. Also the formulation has successfully gone through a toxicity test and certified for free from heavy metals, steroid and animal origins, lead, arsenic, mercury etc.

Composition of the Unani Anti-HIV drugs: -

HOO-IMM PLUS (A)

1) Saffron (Zafran) Pg292* - Restorative, builds up body's defence mechanism thereby strengthening the immunity system considerably.
2) Orchis Latifolia (Salab Misri) Pg147* - Tonics for blood, vital energy and vital essence.
3) Pipper Longum (Phil Phil Daras) Pg 119* - Strengthens up spleen, lymphatic glands. Activates the endocrine glands of the body.
4) Peganum Harmala (Hurmul) Pg 61* Increases level of cellular immunity; inhibits replication of viral activities.
5) Pipper Nigrum (Kali Miri) Pg 336* - A Pulmonary tonic, important in long term strengthening of the lungs.
6) Withania Somnifera (Asgandh) Pg 64* - A Nervine tonic, helps relieve associated psychological tension or anxiety.
7) Sida Cordifolia (Beej Band) Pg 140* - A Diuretic, relieves the congestive nature accompanied by water retention.
8) Pearl (Moti) Pg345* - Increases amount of calcium, iodine and phosphorus in the body.

HOO-IMM PLUS (B)

1) Pistachia lentiscum (Masthagi) Pg339* - Tonics for blood, vital energy and vital essence.
2) Hollerrhena Antidysenterica (Inderjo Talkh) Pg79* - Anti-diarrheal relieving dysenteric symptoms.
3) Emblica Officinalis (Amla) Pg 49* - Strengthens up lymphatic glands by promoting lymphocyte transformation.
4) Arista Lochia (spp) (Zaravant) Pg 140* - An Anti-microbial, helps the body control and clear bacterial infection.
5) Zingiber Officinalis (Zanjabeel) Pg 55* - An Expectorant remedy, helps to maintain a minimum build up of sputum in the lungs.
6) Pistachia Vera Linn (Gul Pista) Pg122* - Enhance renal functions.
7) Punica Granatum Linn (Gul Anar) Pg75* - Prevents degeneration of renal tubes & nephrons.

HOO-IMM PLUS (C)

1) Santalum Album (Sandal) Pg 166* -

Enhances new cells regeneration, cleans up dead cells & prevents pus formation.

2) Similex China Linn (Chob Chini) Pg167* - Accelerates protein synthesis, thus stimulating cell regeneration.
3) Piper Cubeba (Kabab Chini) Pg 263* - Accelerates protein synthesis, thus stimulating cell regeneration.
4) Swertia Charata (Chiraita Shirin) Pg160*- Detoxify the impure blood.
5) Borage Officinalis (Gav Zuban) Pg296* - Increases level of cellular immunity; inhibits growth of tumor cells.
6) Centaurea Behen (Behman Surkh) Pg108* - Anti-neoplastic, prevents development & maturation of malignant cells.
7) Lavendula Steochas (Istukhudus) Pg63*- Strengthens up spleen, lymphatic and endocrine glands.

HOO-IMM PLUS (D)

1) Delphinium Denudatum (Jadhwar) Pg 359*- An Anti-microbial, helps the body control and clear bacterial infection.
2) Planta Goovata (Isabgol) Pg62* - Promote bowel movement through stimulation of bile production in the liver.
3) Chena Podium Album (Bathwa) Pg 93* - Circulatory Stimulant, promotes the circulation of blood and thus oxygen availability for vascular resistance,
4) Artemisia Absinthium (Afzantheen) Pg 66*- A Vascular Tonic, helps nourish the tissue of the arteries and veins. Prevents against hardening of fibrous muscles of the Heart.
5) Arista Lochia (spp) (Zaravant) Pg 207* - An An Anti-spasmodic, eases the muscle spasms, cramps that are the immediate cause of pain.
6) Terminalia Arjun (Arjun) Pg 57* - Cardiac & Vascular Tonic, supports the tissue of the cardiovascular system, maintaining flexibility and tones affected vessels.
7) Cinnamomum Zeyanicum (Dalchini) Pg181* - This drug rectifies all mucus membrane in the body after reducing the virus.

HOO-IMM PLUS (E).

1) Swertia Charata (Chiraita Shirin) Pg160*- Detoxify the impure blood. Antipyretic subsides persisting fever
2) Kawal Gatta (Nelumbu Nucifera) Pg283* - Relieves congested & catarrhal conditions of liver and controls secretion of bile.
3) Meba Azadrach (Bakain) Pg 100* - Anti-tumor immuno-surveillant system, intensifies phagocytosis of reticulo-endothelial systems.
4) Rubia Cordifolia (Majeeth) Pg 335*- Accelerates protein synthesis, thus stimulating cell regeneration.
5) Valerina Officinalis (Balched) Pg 91* - Hepato-protective, detoxifying the liver with the deleterious effects of daily encounters with air-water and food-borne toxins.
6) Chena Podium Album (Bathwa) Pg 93* - Circulatory Stimulant, promotes the circulation of blood and thus oxygen availability for vascular resistance,
7) Berberis Vulgaris (Dar Hald) Pg 182* - Anti-microbial, facilitates the body ridding itself of pathogens present reducing inflammation and its resulting symptoms.

2) Invitro Lab Studies

In invitro lab studies, this drug was put for Drug Sensitivity Test and Anti-retroviral Activity Test in the laboratory adhering to the standard protocol and the highest inhibition rate of 98% was recorded against the causative agent, HIV virus. The detail procedures undertaken in an invitro lab study are mentioned as under: -

Methods:

- Collect 20 ml Blood in an EDTA containing vial
- Mix it properly and keep at room temperature.
- Overlay this blood sample on 2 ml Histopaque solution in 25-ml sterile centrifuge tube.
- Centrifuge the tube at 1500 rpm for 20 min.
- Two layers will be formed after centrifugation.
- Separate out the upper layer of Histopaque with the help of sterile pipette and collect the lymphocyte ring in 2 ml sterile eppendorf tube.

- Microfuge it for 1min at the bottom of the eppendorf tube, lymphocytes sediment will be observed.
- Resuspend the sediment in 2-ml fresh RPMI-C Media.
- Prepare the serial dilutions of the drug of our interest or to be tested in the range of 1ug/ml-500 ug/ml.
- Add 500 ul of the lymphocyte culture with the help of sterile pipette / tips and add the appropriate dilution of the drug to the respective culture well along with control without addition of any drug.
- Incubate it in CO2 incubator at 37 degree Celsius (5% Co2).

Results:

1) After incubation, the P-24 ELISA test for the Control and for the Test were done.
2) Interpreted the results on the basis of difference in between the Control O.D and Test O.D and calculate the % Inhibition of the viral growth in Test in comparison with Control.
3) Example: - O.D of the Control always ranges from 0.2-0.4 while the O.D of Test ranges in between 0.004-0.008 that indicates the inhibition of the virus multiplication.

Conclusion: This Test is having lots of significance over the other available tests in the Market as it gives the results within 24 Hrs. It is very effective and economic too but requires skills of overlaying of the blood, preparation of Media, handling of the highly infectious viral lysates, result interpretation etc.

Basic principle of the Test: We are giving the identical conditions for the growth of Virus in control as well as in Test. As we are treating the Test sample with the drug and after incubation the P-24 ELISA give us core protein formation during incubation comparison with control

3) Invivo Clinical Studies

In the Invivo human clinical studies, this drugs were provided as a treatment to the HIV patients through HAKH Medical Foundation and the improvements in the patients physically as well as pathologically were recorded in proper, controlled conditions through standard procedure and all the blood test pertaining to HIV were undertaken through Molecular Diagnostic Services in association with Reliance Life Science and the reports & blood samples are maintained for more than 3-5 yrs for repeatability of the tests, if required.

The clinical observation of this drug revealed the following improvements in the patients: -

1) 7-8 folds decrease in HIV viral load counts within 5-6 weeks of the treatment.[7,8]
2) Viral load count decreased to less than detectable limit (<100) within 5-7 months of the treatment.[7,8]
3) Augment in CD4 count noted.
4) 4-6 pounds increment in weight within 2-4 months.
5) Relief from HIV symptoms such as fever, diarrhea etc.
6) Prophylactic against opportunistic infections.
7) No Drug Resistance observed & No STIs required.
8) No side-effects and drug interactions observed.

4) Phenomenal change in DNA-PCR Antigen Test

Patients those continued the treatment for 18-24 months' once attaining the viral load to less than detectable limit and adhering to the instruction strictly, had recorded HIV DNA-PCR Qualitative Antigen Test 'Not Detected' and the test interpreted the blood sample to be negative for the presence of HIV virus. And so the medications were completely stopped for almost one year and again undergone for HIV DNA-PCR Antigen test (Qualitative) and again recorded 'Not Detected'. The same test was repeated for 2nd, 3rd & 4th year after completely stopping the medications and the patients yet recorded 'Not Detected' for the test.

The method for the confirming the presence of HIV antigen is HIV DNA-PCR Antigen test (Qualitative) test by Nested PCR Technique.

Technique involved for Pro-viral HIV DNA-PCR Antigen Test[2, 3, 4]

C-PCR is highly specific, sensitive for subtype-C strains. It is based on amplifying a region encompassing a long terminal repeat and gag in the first round, followed by two sets of nested primers; one amplifies multiple subtypes, while the other is specific to subtype C. C-PCR, is a multiplexing of two individual regions of the subtype- C virus, thus offering the advantage of reducing false negative results due to genetic diversity. The Leader/gag region is one of the most conserved regions of the virus. C-PCR, therefore, is highly sensitive to detect all infections by HIV-1 regardless of the subtype. The 232-bp Leader/ gag common HIV region was amplified from all the subtypes, while the 138-bp fragment specific to subtype C was restricted to subtype C. C-PCR conditions optimized to detect 1 to 10 copies of plasmid templates containing full genomes of single and/or multiple subtypes in individual and multiplex PCR.

5) A major breakthrough in HIV Treatment: (Present Antigen Vs Present Antibody Test)

A major breakthrough was recorded when the patients those had completed the treatment with this Unani Anti-HIV drug, undergone HIV Antigen & Antibody Test simultaneously. Their HIV antibody test showed presence of HIV antibodies while their Antigen test showed absence of HIV antigen. This study is detailed as under: -

Method: 6ml serum sample were drawn from the HIV patients who were earlier confirmed HIV positive through Western blot antibody test or HIV DNA-PCR Antigen test and later undergone complete treatment with Unani medicine HOO-IMM PLUS. The sample were cautiously handled as per the NABL guidelines and were sent to a renowned and reliable PCR Molecular diagnostic lab. 3 ml of the sample is to be allocated for Western Blot Antibody test and the other 3 ml for HIV DNA-PCR Antigen test.

Results: The above mentioned tests showed that the blood sample is reactive for Western blot Antibody test & Not Detected for HIV DNA-PCR Antigen test. This directly interprets that presently the person is negative for the presence of HIV antigen, though he still carries HIV antibodies authenticating his earlier infection with HIV virus.

Conclusion: It could be concluded that our body immune system creates cell mediated immunity response towards HIV antigen to produce antibodies that may persist for a long time, even after the antigen is completely eliminated from the body. The other best example is Mycobacterium Tuberculosis antibodies.

The human body responds to antigen in many different ways. These fall into two major categories:

Antibody-mediated immunity. Antibodies, dissolved in blood, lymph, and other body fluids bind the antigen and trigger a response to it. (This form of immunity is also called humoral immunity).

Cell-mediated immunity (CMI). T cells (lymphocytes) bind to the surface of other cells, display the antigen and trigger a response and may remain in the body for a long time. The response may involve a) other lymphocytes and b) any of the other white blood cells (leukocytes)

6. A major finding, an insight for HIV research: (Past Antibody Vs Present Antibody Test)

A major finding was uncovered when the patients earlier Western blot antibody test before starting the treatment was compared with the present western blot test after the treatment. The HIV-1 intermediate Gag precursors P31, P39 and matrix protein P17 was found to be absent in all the patients who had recorded 'Not Detected' in an HIV DNA-PCR antigen test after the treatment.

The matrix protein P17 MA also plays an important role in the HIV-1 viral life cycle. P17 is responsible for the transfer of viral nucleo capsids from the nuclei to plasma membranes, the site of virus assembly. Thus Gag precursor P39 & Matrix Protein P17 are responsible for a redirection of the assembly process to the membrane of the endoplasmic reticulum.

Thus indicating the absence in the process of new viral assembly of HIV virus in the body.

Western Blot Kit Used : Genelab HIV Blot 2.2 (US FDA Approved)[5,6]

7) An Edge over the other Anti-HIV drug.

No consideration over the following issues:-

A) When to start the therapy.
B) Consideration for pregnant women.
C) Affect on liver for co-infected hepatitis patients.
D) Side-effects & drugs interactions.
E) Multi drug resistance and STIs.
F) High cost for different combination.

8) Felicitation & Appreciation.

- Parliament debate on 25th Aug 2004. (Lok Sabha question No. 4112).
- All India Hakeem Ajmal Khan Award being conferred for "Invention of Unani Formula for HIV/AIDS" by Union Health Minister Dr. Anbumani Ramadoss on 28th Sept 2005.
- Global Award 2006 for "The only Anti-HIV Drug, proven by DNA-PCR Antigen Test" by Health Minister, New Delhi, Dr. Yoganand Shastri on 27th Dec 2006.
- Global Award 2006 for "Inventor of HIV/AIDS Medicines" by Health Minister New Delhi, Dr. Yoganand Shastri on 27th Dec 2006.
- 23rd International European Convention Award for Innovation & Technology in Medical Science on 19 Feb 2007.
- Ibnu Sina National Award 2007 from HoD of Central Council for Unani Research & Medicine (CCRUM), India held in Mumbai on 18th March 2007.

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