NEEM EXTRACT: A POTENTIAL HERBAL REMEDY FOR TREATING HIV

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INTRODUCTION
AIDS is a condition characterized by the development of life-threatening opportunistic infection or malignancies in a patient with severe depression of the T-cell mediated immune system caused by infection with human immune deficiency virus (HIV). There are currently over 34 million people worldwide infected with human immune deficiency virus (HIV) with 15,000 new patients infected each day. India has an estimated 5.2 million HIV-infected people. The threat to their life is not from the virus alone. Opportunistic infections (OIs) and associated complications account for a considerable proportion of such mortality. The breakdown of body immune system is the hallmark of HIV infection. Infections which are rarely seen in those with normal immune systems are deadly to those with HIV. The effect of HIV on the immune system is monitored by measuring the CD4 (T-helper) lymphocyte count in the blood. Depletion of CD4 cell count is a hallmark of disease progression in AIDS. OIs are caused by various pathogenic microorganisms such as bacteria, fungi, virus and parasites. Many of the antibiotics used in management of bacterial infections are experiencing increased resistance posing enormous public health concern. Medicinal plants are part and parcel of human science from the dawn of civilization. In India they form the backbone of several indigenous traditional medicines. Medicinal plants are various plants used in herbalism and thought by some to have medicinal properties. Plants have a great potential for producing new drugs for human benefit.

HISTORY
Traditional systems of medicine like Ayurveda, Unani, Homeopathy and Siddha solely rely on phytopharmaceuticals that are obtained from selected medicinal plants (herbs) based on traditional knowledge gained over a period of time and expertise by the because of the wide spread belief that ‘herbal medicine’ is safer than costly synthetic drugs which possesses side effects. Neem is used in traditional medicine as a source of many therapeutic agents in the Indian culture and grows well in the tropical countries. Its twigs provide a chewing stick and are widely used in the Indian sub-continent (Alma's, K., and Ansallaf, T.R., 1995). The chemical constituents contain many biologically active compounds that can be extracted from Neem, including alkaloids, lavonoids, triterpenoids, phenolic compounds, carotenoids, steroids and ketones, biologically most active compound is azadirachtin, it is actually a mixture of seven isomeric compounds labelled as azadirachtin A-G and azadirachtin E is more effective (Verkerk and Wright, 1993). Other compounds that have a biological activity are salannin, volatile oils, meliantriol and nimbin (Anonymous, 1992).

The Sanskrit name ‘nimba’ comes from the term ‘nimbati swasthyamdadati’ which means ‘to give good health’. The benefits of neem are listed in ancient documents ‘Charak-Samhita’ and ‘Susruta-Samhita’, which form the foundation of the Indian system of natural treatment, Ayurveda. It is commonly called ‘Indian lilac’ or ‘Margosa’ and belongs to the family Meliaceae. The Persian name of neem is ‘Azad Darakhth-E- Hind’ which means ‘Free tree of India’. Neem is considered to be a part of India’s genetic diversity. Neem tree is the most researched tree in the world and is said to be the most promising tree of 21st century. It has great potential in the fields of pest management, environment protection and medicine. Neem is a natural source of insecticides, pesticides and agrochemicals.

Where did HIV come from?
The most recent presentation on the origin of HIV was presented at the 6th Conference on Retroviruses and Opportunistic Infections (Chicago, January 1999). At that conference, research was presented that suggested that HIV had “crossed over” into the human population from a particular species of chimpanzees, probably through blood contact that occurred during hunting and field dressing of the animals. The CDC states that the findings presented at this conference provide the strongest evidence to date that HIV-1 originated in non-
human primates. The research findings were featured in the February 4, 1999 issue of the journal, Nature.

We know that the virus has existed in the United States, Haiti and Africa since at least 1977-1978. In 1979, rare types of pneumonia, cancer and other illnesses were being reported by doctors in Los Angeles and New York. The common thread was that these conditions were not usually found in persons with healthy immune systems. In 1982 the Centers for Disease Control and Prevention (CDC) officially named the condition AIDS (Acquired Immune Deficiency Syndrome). In 1984 the virus responsible for weakening the immune system was identified as HIV (Human Immunodeficiency Virus).

Life Cycle of HIV

![Image of HIV Life Cycle]

**Stage 1: Viral Binding**
On the surface membrane of all living cells are complex protein structures that may serve as “receptors.” A receptor is often compared to a lock into which a specific key or “ligand” will fit. HIV binds to at least two specific receptors on the host cell: the primary receptor, called the CD4+, and a secondary receptor, a chemokine co-receptor, such as CXCR4 or CCR5, as described earlier. HIV infection of a lymphocyte begins with attachment of the virus, via it’s, to the cell membrane through both of these “ligand-receptor” interactions. Tight attachment of the viral particle to receptors on the cell’s membrane activates other proteins that enable viral fusion with the cell membrane.

**Stage 2: Entry and Uncoating**
Once the virus has fused with the host cell, the viral core and its associated RNA enter the cell. In order for the genetic material of the virus to reproduce, the coating that surrounds the RNA, or nucleocapsid, must be dissolved. A partial uncoating of the nucleocapsid occurs, resulting in the release of viral RNA into the cytoplasm of the host cell.

**Stage 3: Reverse Transcription**
Conversion of the viral genetic material (RNA) to DNA occurs through the action of an enzyme-reverse transcriptase—that HIV produces. Reverse transcriptase reads the sequence of viral RNA that enters the host cell and transcribes the sequence into a complementary DNA sequence, which can then use the cellular machinery to make viral proteins and additional copies of viral RNA. Without this process, the virus cannot replicate.

The process of reverse transcription is unique to retroviruses as a result of their reverse transcriptase; thus, multiple nucleoside reverse transcriptase inhibitors (NRTIs) and nonnucleoside reverse transcriptase inhibitors (NNRTIs) have been developed for use as ARVs to treat HIV infection. These ARVs are not as effective in treating HIV-2 infection and disease, however, because of differences in the HIV-2 reverse transcriptase. Current ARVs also suffer from the drawback that a single nucleotide mutation within the pol gene can yield a virus resistant to the ARV. In the case of HIV, the process of reverse transcription is error-prone; thus, a small number of mutations are introduced in the HIV genome each time it replicates. This error-prone process results in the extreme heterogeneity of HIV. At the cellular level, the viruses produced after each round of replication are not identical to the original infecting virions. This variation of HIV within an individual and between HIV isolates from distinct geographic regions has a profound impact on the diagnosis and treatment of HIV, as well as the design and development of potential HIV vaccines.

Stage 4: Integration into Host Chromosomal DNA
During this stage, viral DNA is randomly inserted into the host cell DNA by the viral enzyme integrase. This stage of the HIV lifecycle has enabled the design and development of a new class of ARVs known as integrase inhibitors; several are still in the testing phase and none is in clinical use currently. Once the viral DNA is integrated into the host genetic material, it can remain there in a latent state for many years. The ability of HIV to persist in this latent state poses a major barrier to eradicating or curing HIV.

Stage 5: Synthesis of Viral DNA
Upon activation of infected cells, viral DNA is transcribed along with the host DNA into messenger RNA (mRNA). The mRNA codes for the production of viral proteins and enzymes. The new viral RNA also serves as the genetic material for the next generation of viruses. Once produced, the viral mRNA is transported out of the nucleus and into the cytoplasm of the host cell.

Stage 6: Translation and Production of Viral Proteins
Translation of viral mRNA results in the production of polypeptide sequences. Each section of the mRNA corresponds to a protein or enzyme that serves as a building block used to construct new HIV particles.

Stage 7: Assembly of Virus and Budding from the Host Cell
This stage of the viral infection is the formation of a new virus particle, or virion, which is preceded by the assembly of functional viral proteins such as the envelope and core proteins and necessary viral enzymes (reverse transcriptase, protease, and integrase). Viral polypeptides must be cleaved into smaller parts by the viral protease enzyme. Inhibitors of this viral protease, termed protease inhibitors, block the ability of the protease to cleave the viral polypeptide into functional enzymes or proteins; thus, protease inhibitors interfere with the production of new HIV particles, although they do not prevent infection of the cell in the first place. When viral RNA and associated proteins are packaged and released from the cell surface as viral particles, they take with them a small portion of the cellular membrane that also contains viral surface proteins.

These viral proteins then become the “envelope” of the new viral particles. As described earlier, these envelope proteins then bind to the receptors on other immune cells, thereby facilitating continued infection. If this process of viral replication occurs in CD4+ lymphocytes in a progressive and uncontrolled manner, HIV will eventually destroy them and progressively deplete their numbers. These infected CD4+ cells may also become functionally defective and inefficient in executing their central immunoregulatory functions. An additional consequence of CD4+ cell depletion is the development of opportunistic infections or malignancies that would otherwise not occur in immunocompetent individuals, as the CD4+ cell count is depleted to less than 200 cells/mm3. It is well documented that HIV can be cytotoxic to infected CD4+ lymphocytes. This immune-mediated cytotoxic effect probably involves inhibition of T-cell regeneration in the thymus. For example, T-cell proliferative responses to HIV are quickly lost and the repertoire of antigen recognition diminishes with time. The use of ARVs has provided evidence, however, that some of these immune cells, particularly CD4+ T-lymphocytes, can be reconstituted and become functionally effective once more. It has been suggested that Africans may have an activated immune system because of chronic exposure or infection with other pathogens, resulting in an unusual susceptibility to HIV infection, which may play a significant role in a more rapid progression of AIDS.

Stage 8: Maturation
The final step in the viral lifecycle, maturation, is required in order for the virus to become infectious. Shortly after budding from the host cell, the protease enzymes in the new viral particle become active and cleave the polypeptides into their appropriate functional subunits, or proteins and enzymes. This processing step
results in the generation of a mature and infectious virion.

**Pathophysiology**

![Pathophysiology of HIV/AIDS](image)

The virus enters the body there is a period of rapid viral replication, leading to an abundance of virus in the peripheral blood. During primary infection, the level of HIV may reach several million virus particles per milliliter of blood. This response is accompanied by a marked drop in the number of circulating CD4+ T cells. The acute viremia is almost invariably associated with activation of CD8+ T cells, which kill HIV-infected cells, and subsequently with antibody production, or seroconversion. The CD8+ T cell response is thought to be important in controlling virus levels, which peak and then decline, as the CD4+ T cell counts recover. A good CD8+ T cell response has been linked to slower disease progression and a better prognosis, though it does not eliminate the virus.

Ultimately, HIV causes AIDS by depleting CD4+ T cells. This weakens the immune system and allows opportunistic infections. T cells are essential to the immune response and without them; the body cannot fight infections or kill cancerous cells. The mechanism of CD4+ T cell depletion differs in the acute and chronic phases. During the acute phase, HIV-induced cell lysis and killing of infected cells by cytotoxic T cells accounts for CD4+ T cell depletion, although apoptosis may also be a factor. During the chronic phase, the consequences of generalized immune activation coupled with the gradual loss of the ability of the immune system to generate new T cells appear to account for the slow decline in CD4+ T cell numbers.

HIV seeks out and destroys CCR5 expressing CD4+ T cells during acute infection. A vigorous immune response eventually controls the infection and initiates the clinically latent phase. CD4+ T cells in mucosal tissues remain particularly affected. Continuous HIV replication causes a state of generalized immune activation persisting throughout the chronic phase. Immune activation, which is reflected by the increased activation state of immune cells and release of pro-inflammatory cytokines, results from the activity of several HIV gene products and the immune response to...
ongoing HIV replication. It is also linked to the breakdown of the immune surveillance system of the gastrointestinal mucosal barrier caused by the depletion of mucosal CD4+ T cells during the acute phase of disease.

How is HIV Transmitted?
HIV can be transmitted from an infected person to another through:
- Blood (including menstrual blood)
- Semen
- Vaginal secretions
- Breast milk

Activities That Allow HIV Transmission
- Unprotected sexual contact
- Direct blood contact, including injection drug needles, blood transfusions, accidents in health care settings or certain blood products.

- Mother to baby (before or during birth, or through breast milk)

Figure no 3: transmission of HIV.

Sexual intercourse (vaginal and anal)
In the genitals and the rectum, HIV may infect the mucous membranes directly or enter through cuts and sores caused during intercourse (many of which would be unnoticed). Vaginal and anal intercourse is a high-risk practice.

Oral sex (mouth-penis, mouth-vagina)
The mouth is an inhospitable environment for HIV (in semen, vaginal fluid or blood), meaning the risk of HIV transmission through the throat, gums, and oral membranes is lower than through vaginal or anal membranes. There are however, documented cases where HIV was transmitted orally, so we can't say that getting HIV-infected semen, vaginal fluid or blood in the mouth is without risk. However, oral sex is considered a low risk practice.

Sharing injection needles
An injection needle can pass blood directly from one person's bloodstream to another. It is a very efficient way to transmit a blood-borne virus. Sharing needles is considered a high-risk practice.

Mother to Child
It is possible for an HIV-infected mother to pass the virus directly before or during birth, or through breast milk. Breast milk contains HIV, and while small amounts of breast milk do not pose significant threat of infection to adults, it is a viable means of transmission to infants.

The following "bodily fluids" are NOT infectious
- Saliva
• Tears  • Sweat

Progression of AIDS

There are three major stages.

➢ The 1st stage is the acute infection period.
In this stage, which can last for several weeks, the virus is establishing itself in the body. Up to 70% of people newly infected experience “flu-like” symptoms (fevers, chills, night sweats, rashes). After experiencing these initial flu-like symptoms, people resume feeling and looking normal. During this initial stage the virus makes it way to the lymph nodes where it actively replicates itself. This burst of rapid HIV replication usually lasts two months. At this stage, the person has a very high viral load. Detection and formation of antibodies take roughly 6 to 12 weeks and the person will not test positive for HIV antibodies. During the period of early infection, a person has the greatest chance of passing HIV infection to others.

➢ The 2nd stage is the clinical latency period.
This stage has two phases: (1) the asymptomatic stage and (2) the early-and medium-stage of HIV symptomatic disease. During the asymptomatic stage, people feel normal and can feel well for many years. During this time the only indication of HIV infection is through testing or the presence of swollen lymph glands. During this time, even though, the person feels great –the virus is slowly dismantling their immune system and this can go on for longer than 10 years.

Early and Medium Stage HIV Symptomatic Disease is when people being experiencing mild HIV disease symptoms such as skin rashes, fatigue, night sweats, slight weight loss, mouth ulcers, and fungal skin and nail infections. It can take 5 to 7 years for the mild symptoms to appear. As the disease progresses, typical problems include chronic oral or vaginal thrush, recurrent herpes blisters, ongoing fevers, persistent diarrhea, and significant weight loss.

➢ The 3rd stage is late-stage HIV disease also known as AIDS.
AIDS, the T-cell count drops down to 200 or less, and opportunistic infections develop. These are infections and illnesses that the normal immune system can suppress or present. According to the CDC, an AIDS diagnosis includes an HIV infection, a T-cell CD4 count under 200 cells/mm3 of blood, and 1 or more OIs. Having AIDS is not an imminent death sentence. With treatment it may be possible to keep HIV from progressing to AIDS.

Ecology of Neem
Neem is drought resistant and can tolerate almost any length of high temperature. Typically this tree is grown in sub-arid to sub-humid areas, where the annual rainfall varies between 400 mm to 1200 mm. This tree thrives in any types of soil, however, best grown in well drained deep sandy soil. This tree cannot stay alive below 4°C temperatures. At this temperature, leaf shedding is confirmed in addition to premature death. Neem has impressive and far-reaching pesticide properties. Recently, neem has been suggested as an effective infertility agent in controlling populations of rodents, such as rats. It has also been shown to be effective in controlling food borne pathogens; making it a potential agent against food spoilage bacteria.6 Neem tree has various usages in a variety of sectors of which its medicinal usagesare the foremost.

**Cultivation Collection of Neem**

Neem is a large tree growing about 25 m in height with semi-straight to straight trunk, 3 m in girth and spreading branches forming a broad crown. A neem tree normally starts fruiting after 3-5 years. In about 10 years it becomes fully productive. From the tenth year onwards it can produce up to 50 Kg of fruits annually. The plant is reported to live up to two centuries. The tree has adaptability to a wide range of climatic, topographic and edaphic factors. It grows well in dry, stony shallow soils and even on soils having hard calcareous or clay pan, at a shallow depth. Neem tree requires little water and plenty of sunlight. The tree grows naturally in areas where the rainfall is in the range of 450 to 1200 mm. However, it has been introduced successfully even in areas where the rainfall is as low as 150 to 250 mm. Neem grows on altitudes up to 1500 m. It can grow well in wide temperature range of 00C to 490C. It cannot survive water-logged areas and poorly drained soils. The pH range for the growth of neem tree lies in between 4 to 10. It grows on almost all types of soil including clayey, saline and alkaline soil, but does well on black cotton soils and deep well drained soil with good sub-soil water. Neem trees have the ability to neutralize acidic soils by a unique property of calcium mining.

**TAXONOMICAL CLASSIFICATION OF NEEM**

**Order:** Rutales  
**Suborder:** Rutinae  
**Family:** Meliaceae  
**Subfamily:** Melioidae  
**Genus:** Azadirachta  
**Species:** Indica  
**Latin:** Azadirachta indica

**Therapeutic properties**

The internal medicinal uses of Neem include malaria, tuberculosis, rheumatism, arthritis, jaundice and intestinal worms as well as skin diseases. The oil is NOT normally taken Internally - but as a decoction made from the leaves. The extract of Neem leaves has also demonstrated significant anti-diabetic potential. Neem also enhances the immune system – making it a possible substance of use for AIDS and cancer patients. It also helps to decrease blood sugar levels and may possibly be used to reduce the use of insulin by 30%-50% - making it a possible effective compound for diabetic patients. The extracts are also beneficial for heart diseases, hepatitis, fungal infection, malaria, psoriasis, and ulcers. Neem is used externally for ringworm, eczema, psoriasis, lice, fungal infection as well as for painful joints and muscles. The cosmetic use of Neem oil includes the fighting of acne and pimpls as well as improving skin elasticity.

In HIV/AIDS patients, a 12-week oral administration of acetone water neem leaf extract (IRAB) had a significant influence *in vivo* on CD4 cells (which HIV reduces) without any adverse effects in the patients. Of the 60 patients who completed treatment, 50 were completely laboratory-test compliant. The mean levels of CD4 cells increased by 159% in 50 patients, which is a major increase; the number of HIV/AIDS pathologies decreased from the 120 baseline to 5; and significant increases were experienced in body weight (12%), hemoglobin concentration (24%), and lymphocyte differential count (24%). IRAB is recommended as part of an HIV/AIDS drug treatment.

**Crude extract preparation**

The leaves of *A. indica* obtained in January 2007 from the University of Nigeria, Nsukka campus, were identified at the Herbarium, University of Nigeria, Nsukka, air_dried at room temperature and reduced to fine powder by milling. The resulting powder was subjected to extraction with 50% acetone. The hydroacetone extract was concentrated using a rotary evaporator and further dried under reduced pressure. For the tissue culture assays, the extract was dissolved in DMSO, diluted further with RPMI 1640 complete medium (supplemented with 10 % fetal calf serum (FCS), and 1% penicillin/streptomycin) (Gibco), and filter sterilized with a 0.20_nalgene syringe filter. The leaves of *A. indica* obtained in January 2007 from the University of Nigeria, Nsukka campus, were identified at the Herbarium, University of Nigeria, Nsukka, air_dried at room temperature and reduced to fine powder by milling. The resulting powder was subjected to extraction with 50% acetone. The hydroacetone extract was concentrated using a rotary evaporator and further dried under reduced pressure. For the tissue culture assays, the extract was dissolved in DMSO, diluted further with RPMI 1640 complete medium (supplemented with 10 % fetal calf serum (FCS), and 1% penicillin/streptomycin) (Gibco), and filter sterilized with a 0.20_nalgene syringe filter.

**Inhibitory effect of Neem on HIV**

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Parts of Plant</th>
<th>Uses</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Cake</td>
<td>Soil Manure &amp; additive, Animal fodder, Fertilizer</td>
</tr>
<tr>
<td>2</td>
<td>Seed, cake, oil</td>
<td>Plant Protectant, Commercial Pesticide, Medicine, Animal Care, Oil Extraction.</td>
</tr>
<tr>
<td>3</td>
<td>Twings</td>
<td>Dental Hygiene.</td>
</tr>
<tr>
<td>4</td>
<td>Wood</td>
<td>Fuel, Furniture, Construction Material</td>
</tr>
<tr>
<td>5</td>
<td>Roots &amp; Fruits</td>
<td>Oil Extraction, Medicine</td>
</tr>
</tbody>
</table>

Table No. 1: Parts of Neem with their uses
Parts of the *Azadirachta indica* tree have been used for medicine including claims of its anti-retroviral potential. Anti-HIV agent which could possibly inhibit the early stages of the HIV replicative cycle would be very useful in treating HIV-infection. Our results demonstrate that compared to the standard anti-HIV drug AZT, a hydroacetone extract of *A. indica* significantly (p <0.05) inhibited acute HIV-1 infection and cell-to-cell transmission, as assayed by syncytia formation. At 100 g/ml the extract completely inhibited syncytium formation but the anti-fusion activity of the extract was slightly lower when 50 _g/ml of extract were used. Formation of syncytium is mediated by interaction of viral glycoprotein gp120 with specific regions of the host cell CD4 receptor (Tang and Levy, 1990). Neem extract almost completely inhibited the fusion interaction between normal C8166 and HIV-1 chronically infected C8166 cells in tissue culture. The active principles in the extract possibly inhibited syncytia formation by binding to the viral gp120, thereby blocking the gp120-CD4 interaction, thereby inhibiting not only HIV binding to the CD4+ cells but also preventing HIV-induced syncytium (giant cell) formation. The anti-HIV effect of neem extract could not have been due to the toxic effect of the extracts on the C8166 cell line since cell viability studies showed that the tested concentrations were relatively non-toxic to the cells in tissue culture. Furthermore, the effect of the extract on HIV-1 replication in C8166 CD4+ cells was investigated *in vitro* by determining HIV-1 reverse transcriptase (RT) activity and p24 antigen expression levels. The extract significantly inhibited (p < 0.05) the biochemical activity of HIV reverse transcriptase in the culture supernatant. The potency was not significantly different (p >0.05) from that of the standard drug AZT. Since, the p24 antigen is one of the major capsid proteins expressed by matured HIV, decreased concentrations of the antigen suggests low levels of HIV particles and hence, antiretroviral activity. The level of expression of the p24 antigen was also significantly decreased (p < 0.05) in the culture supernatant of cells treated with neem extract. The reverse transcriptase inhibitory activities of the active principles present in the extract possible contributed to the decrease in HIV p24 antigen concentration. The inhibitory potential of the extract on HIV RT activity and p24 antigen expression were apparently not significantly different (p > 0.05) from that of AZT the standard anti-HIV drug, suggesting a very potent anti-HIV activity of the plant extract. The antiretroviral mechanism of this extract is however, not completely understood. The extract completely inhibited syncytia formation and the biochemical activity of HIV reverse transcriptase, resulting in a decreased p24 antigen expression with its activity not significantly different (p > 0.05) from that of AZT the standard anti-HIV drug. These inhibitory effects of the plant extracts were all dose-dependent and no cytotoxicity on uninfected target cells was detected over a 1,000-fold concentration from 0.1 to 100 _g/ml of the extracts. Mbah et al. (2007) postulated that neem extract was beneficial to HIV/AIDS patients though the mechanism of action was not known. This result therefore suggests a possible anti-retroviral mechanism of action of the active principle in neem extract using well-established bioassays.

The extract also significantly reduced (p < 0.05) the level of expression of the hyperimmune activation markers CD38 and CD69 in PHA activated lymphocytes, thereby indicating inhibitory effects. Prior to stimulation with PHA, only about 2.19% of the PBMCs expressed CD69 but after stimulation 41.18% of the PBMC expressed CD69 in the absence of the extract and in the presence of the extract only 35.32% of the PBMC expressed CD69.

The capability of the neem extract to downregulate the expression of these immune activation markers suggests immunomodulatory potential of the extract. The mechanism of action of the inhibitory effect of the extract is however not known. Neem extract may be blocking the PHA binding site or possibly inhibiting enzymes involved in hyperimmune activation such as the mitogen activated proteins kinases. The immunomodulatory potential observed in this study is consonance with previous reports on the immunomodulatory potentials of neem extract (Upadhyay et al. 1993). These findings are of particular interest given that neem appears to have anti-retroviral and immunomodulatory activities, raising the possibility of the extract modulating the hyperimmune activation observed in HIV/AIDS as well as reducing the viral load. Our present results warrant further investigation to ascertain the mechanism by which *A. indica* extract decreases HIV-1 replication *in vitro* and *in vivo*. Furthermore, studies need to be conducted in order to identify the active compound responsible for the anti-HIV activity.

Udeinya et al (2004) first reported antiretroviral activity by IRAB on patients with HIV/AIDS in a phase I clinical trial. In another study by Mbah et al (2007) that lasted 12 weeks, IRAB was administered (1g daily) to 60 patients infected with HIV-1 & 11. The results showed a significant increase from the baseline mean CD4+ cell count of 266cells/ul by week 12. Mean body weight and hemoglobin concentration were also significantly increased. Opportunistic infections and other HIV/AIDS related conditions were reportedly completely resolved in most patients by week 12. Also, the erythrocyte sedimentation rate (ESR) was reported to have been significantly decreased from a baseline mean value of 64mm/hr to 16mm/hr. Anyaehie (2009) compared the anti-retroviral properties of IRAB with that of highly active antiretroviral therapy (HAART) and results are consistent with Mbah et al (2007) after 16 weeks of therapy. However, Anyaehie (2009) documented a more significant increase in CD4+ cells and elimination of skin rashes among patients on HAART compared to patients on IRAB. Mbah et al (2007) and Anyaehie (2009) agree that there was a significant improvement in
patients’ condition with respect to presenting symptoms and physical examination findings. These reports of correction of CD4+ cell counts and improvement in patient’s clinical profile may indeed relate to corresponding reduction in viral activity and led to a presumptive conclusion that the fractionated neem leaf extract (IRAB) is safe and increases CD4+ Cell Levels in HIV/AIDS patients, a conclusion the work of Anyaehie (2008) further supported.

**EFFECT OF NEEM EXTRACT**

**Effect of neem extract on syncytia formation**

The ability of the extract to block HIV-1 entry was investigated. *A. indica* mediated a substantial anti-HIV-1 effect in infected C8166 T cells. The extract inhibited acute HIV-1 infection and cell-to-cell transmission, as shown in the syncytia formation assay. A dose-dependent inhibition of syncytia formation was observed, with the best activity at a concentration of 100 g/ml.

**Effect of neem extract on HIV-1 reverse transcriptase activity**

The crude extract of *A. indica* exhibited strong HIV-1 RT inhibitory activity. At concentration of 50 and 100 g/ml of the extract significantly reduced (p < 0.05) the polymerase activity of the recombinant HIV-1 RT with the peak inhibition of 92.4% at 100 g/ml. Zidovudine (AZT), a nucleoside analogue inhibitor of the HIV-1 enzyme reverse transcriptase, blocked viral replication as expected.

**Effect of neem extract on HIV-1 p24 antigen expression**

The extract significantly inhibited (p < 0.05) HIV-1 replication in C8166 cell line infected with HIV-Bru (N+/E+/GFP). In the presence of AZT and 50 and 100 g/ml of the extract in the culture supernatant, there were no detectable levels of p24 antigen in the culture supernatant.

**Inhibition of Phytohaemagglutinin A (PHA)-induced activation**

Flow cytometry was used to investigate the effect of the neem extracts on PHA-induced hyperimmune activation in PBMCs. The extracts significantly (p < 0.05) reduced the level of expression of the immune activation markers CD38 and CD69 in PHA-activated lymphocytes, thereby suggesting suppression of hyperimmune activation of human lymphocytes.

**Side effects or interactions**

Neem leaf extracts appear to be very safe at recommended intake levels with no significant reports of problems. Also, use of IRAB has consistently reported no adverse effects either as anti-malaria (Udeinya et al, 2006 and 2008) or an anti-retroviral agent (Mbah et al, 2007 and Anyaehie 2009). Water extracts of neem leaf have been shown to decrease blood levels of chloroquine in rabbits (Nwafor et al, 2003) but this has not been investigated with IRAB. The use in pregnancy has also not been evaluated and thus is not yet recommended. At the time of writing, there were no well-known drug interactions with IRAB.

**CONCLUSION**

Many of the existing synthetic drugs cause various side effects. Hence, plant compounds based drug development could be useful in meeting this demand for newer drugs with minimal side effect. It is possible that Neem may take a role as an adjuvant to the use of antibiotics or as a replacement of current antibiotics to treat the viral infections. Fractionated acetone/water neem leaf extract is the only drug reported to have activities against HIV/AIDS. The present study showed the effectiveness of Neem plant part extract with chloroform and acetone as a solvent against the most common infection associated with AIDS. Due to high cost of medicines most of the people cannot get such anti viral drugs but the neem extract is easy to prepare and it doesn't required the high cost therefore it is necessary to know the medicinal value of neem and their antiviral activity and used to treat AIDS.

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