

Anti-HIV substances of natural origin

An updated account

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Abstract

Human Immunodeficiency Virus (HIV) infection targets and destroys a specific type of white blood cells (T_4 Lymphocytes), leading to the development of Acquired Immuno Deficiency Syndrome (AIDS). With the recent advances in biochemistry and pathophysiology of HIV the complete life cycle of HIV and its pathogenesis is well understood and therefore, the anti-HIV agents can be classified on the basis of their role in replicative cycle of HIV virion. A number of synthetic, semisynthetic as well as drugs of natural origin are reported to be active against HIV. In this paper, various drugs of plant and mineral origin have been thoroughly discussed including their classification based upon their mechanisms of action. Since, more than 90% of HIV infected individuals live in developing countries where easy access to expensive and synthetic drugs is scarce and hence natural substance shall be proved to be a boon to mankind in continuing battle against AIDS.

Keywords: AIDS, Anti-HIV, CD4, Natural Anti-HIV agents.

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Introduction

In the nineteenth century, humans lost their fear of God and acquired a fear of microbes, whereas in the twentieth century, the anonymously acclaimed fear of microbes has been superseded by the fear of HIV i.e. AIDS. The Acquired Immuno Deficiency Syndrome (AIDS) is characterized by abnormal host defense mechanisms that predisposes to infections with opportunistic organisms or the occurrence of Kaposi's sarcoma or B cell lymphoma alongwith profound decrease in the number of CD4+T cells. A variety of opportunistic pathogens and concerns can kill AIDS patients¹. As an estimate, 70 million people worldwide were infected

with HIV since 1980 when it was recognized as an emerging disease. Now a days, nearly 16000 new infections occur everyday or 11 persons are infected every minute in the world. About 4 million children have been infected since the appearance of the virus first time. Around 5 million people worldwide were infected (with HIV) in 2003 alone, out of which, about 0.7 million were children, as a result of transmission during pregnancy and childbirth or from breastfeeding². More than 20 million people have died from AIDS, 3 million in 2003 alone. One fifth of infected people live in Asian countries including India. A total number of AIDS cases in this country has been reported as 86028 by the end of August 2004, including 7799 new cases of AIDS³.

Chemotherapy for AIDS has progressed steadily in the past decade with the advent of some HIV reverse transcriptase inhibitors, protease inhibitors and their combination. On the R & D front, researchers are trying to develop new medicines and vaccines, all of which are either in human trials or awaiting approvals. Preventive AIDS vaccines face a number of challenges due to the existence of various strains which continue to mutate⁴.

Many plant-based medicines are being used worldwide in the treatment of AIDS, but no scientific evidence is available to support their use. Non-selectivity of natural products against one or more types of viruses is another problem. The World Health Organization (WHO) has highlighted the importance of evaluating such remedies especially plant species possessing anti-HIV activity.

Drugs from natural origin are being used by about 80% of the world population primarily in the developing countries due to their safety, efficacy, cultural acceptability, lesser side effects and most particularly their cost effectiveness and easy accessibility⁵.

There is an increased use of herbal medicines in western countries also since synthetic agents can exert more

unwanted side effects when used too often indiscriminately and irrationally⁶.

Mechanism based classification of anti-HIV agents of natural origin

With better understanding of life cycle of HIV, it is possible to identify the sites at which natural products can be targeted in order to act as HIV inhibitor⁷⁻⁸. Thus, the natural products with anti-HIV property can be classified according to their mechanisms of action in relation to their inhibitory effect on some specific stages in the normal life cycle of HIV virion⁹⁻¹⁰. Schematic diagram of HIV virion is shown in Fig.1 and its life cycle in Fig.2.

1. Drugs affecting adsorption and penetration

Cells having CD4 receptors are susceptible to HIV infection as virus attachment depends on interaction between glycoprotein (gp-120) present on virus coat and CD4 receptor. Several natural products have been reported, which inhibit this binding and thus reduce infectivity of HIV.

1.1 Phenolic compounds: *Detarium microcarpum* Guill. & Perr. (Family: *Caesalpinaceae*) contains a flavonoid, (-) epicatechin-3-O-gallate which blocks the binding of gp-120 to CD4 (-). It is very common to other tannins and is also an inhibitor of HIV Reverse Transcriptase. Although the inhibition is not specific and hence not responsible for selective anti-HIV activity¹¹.

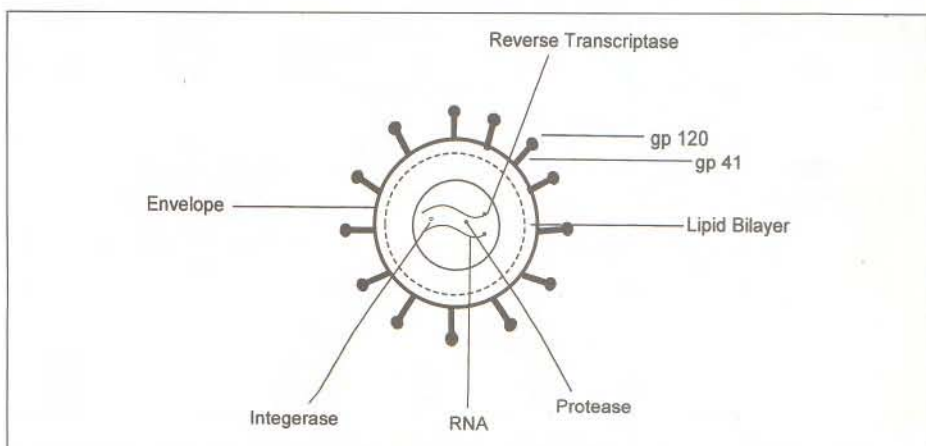


Fig. 1 : Cross-section diagram of HIV virion

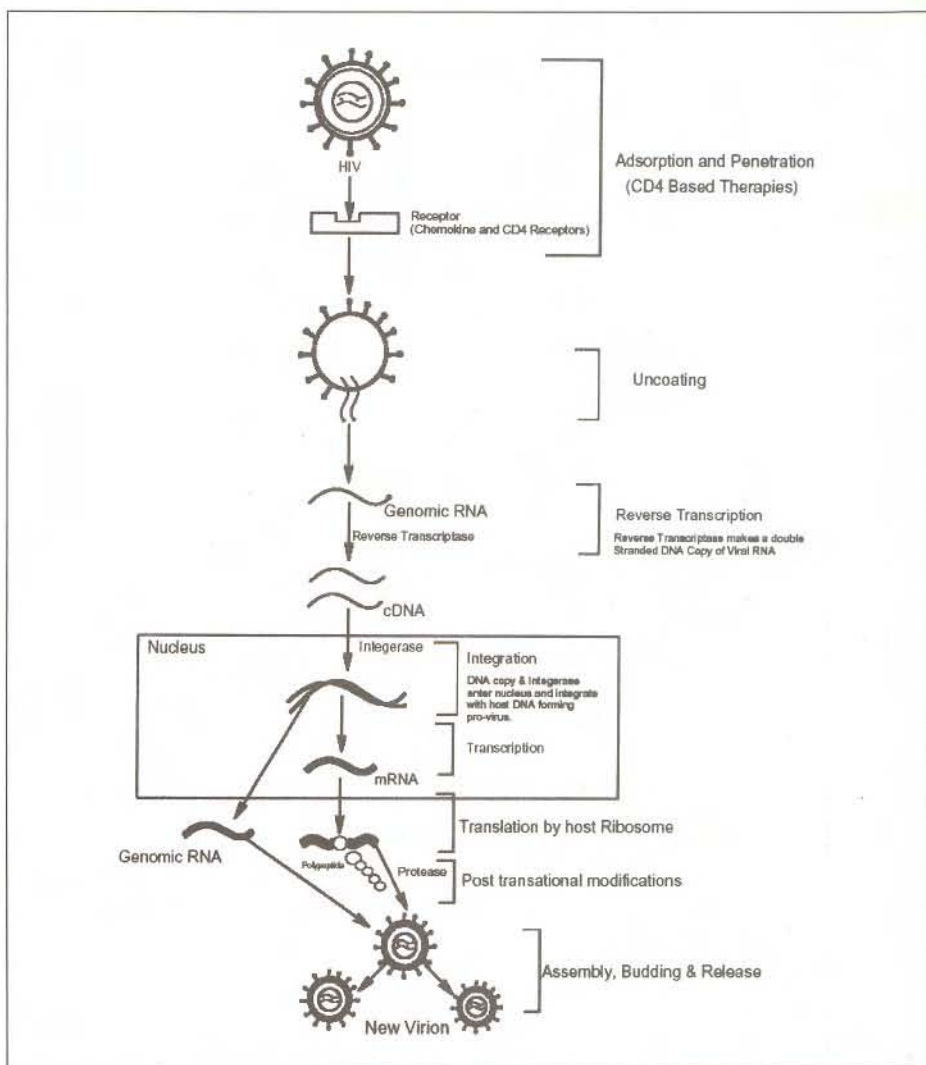
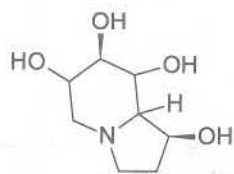


Fig. 2 : Schematic diagram of replicative cycle of HIV virion with the sites of action of natural substances

1.2 Polysaccharides: Heparin, carrageenan, dextran sulfate and compounds of sulfated polysaccharides obtained from species of algae (Family: *Gigartaceae* and *Solieriaceae*) inhibit HIV replication *in vitro* by blocking the absorption of virus particles to cell through a selective action.

In addition to inhibiting HIV replication, these sulfated polysaccharides inhibit the formation of giant cells or syncytia, arising as a result of the fusion of HIV infected cells with uninfected CD4+ Cell. Various sulfated polysaccharides obtained from seaweeds like *Nothogenia fastigiata*, *Aghardhiella tenra* inhibit virus adsorption process¹²⁻¹⁴.

1.3 Glyco-alkaloids/sugar alkaloids: Group of sugar alkaloids known as sugar analogue alkaloids act by impairing the binding between CD4 and gp-120 thus interfering the synthesis of glycoproteins. Example is castanospermine, an indolizidine alkaloid found in *Castanospermum australe* A. Cunn. (Family: *Fabaceae*). Castanospermine inhibits the enzyme α -glucosidase. Interestingly, 1-deoxynojirimycin, a sugar alkaloid of the piperidine type found in *Morus* sp. (Mulberry) and also in culture



Castanospermine

filtrates of *Bacillus* and *Streptomyces* species, acts in similar fashion. *In vitro* studies have shown that castanospermine acts synergistically with zidovudine against HIV-1 and HIV-2 without any increase in toxicity. It also inhibits formation of syncytia¹⁵⁻¹⁶.

1.4 *Pseudomonas* exotoxin:

Isolation of hybrid protein consisting of human recombinant CD4 combined with toxins such as pseudomonas exotoxin produces anti-HIV agents as a result of interaction between CD4-T and gp-120. Only cells expressing gp-120 are targeted by CD4, which deliver the lethal toxin to cells containing HIV¹⁷.

1.5 Protein: Cynovirin-N, a protein from cyanobacteria (blue green algae) irreversibly inactivates HIV and also aborts cell-to-cell fusion and transmission of HIV. Cynovirin-N prevents HIV-1 from infecting host cell by interfering with glycoprotein gp-20, which is present on HIV-1 envelope and the receptors on cell infected by HIV-1^{11,18}.

2. Drugs affecting reverse transcription and integration

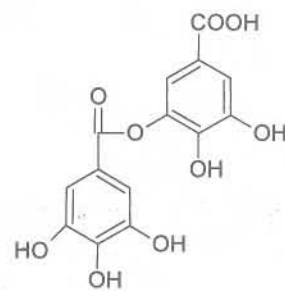
After penetration of HIV into CD4+ cells, the single stranded viral RNA is transcribed into double strand by virus specific reverse transcriptase. HIV RT has three distinct catalytic functions. It performs an RNA dependent DNA polymerase activity, utilizes the viral RNA strand as template and synthesizes a complimentary strand of DNA. As soon as

this DNA strand is formed, activity residing in the RNAase H domain of p66 serves to digest the RNA template. It also acts as DNA dependent DNA polymerase to complete the synthesis of the double stranded proviral DNA¹⁸.

Several flavonoids such as amento flavone, quercetin and scutellarin have been shown to inhibit the RT of some RNA tumour viruses as well as HIV, while some others were found to be strong inhibitors of mammalian DNA polymerase including baicalein, quarcetagenin and myricetin¹⁹.

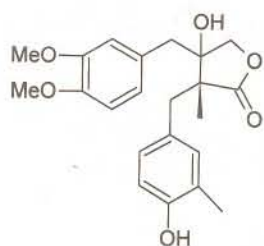
A number of natural products have been reported to interact with reverse transcriptase. Potential anti-HIV agents must inhibit viral enzyme but not interfering with mammalian DNA polymerase activity²⁰.

A number of tannins¹¹ are reported to have inhibitory effect on HIV RT. Digallic acid¹² is a potent inhibitor of HIV RT which also affects DNA polymerase. Tetragalloyl quinic acid obtained from commercial tannins at anti-viral concentration has been found to be potent inhibitors of reverse transcription. However, in some cases it is not sufficient to inhibit HIV replication. A macrocyclic ellagitannin such as oenothin B isolated from *Oenothera erythrosepala* Borbas (Family: *Onagraceae*) inhibits both the viral absorption and reverse transcription²¹.



Digallic acid

Some lignanoides obtained from *Ipomoea cairica* (Linn.) Sweet (Family: *Convolvulaceae*) namely (–) Arctigenin and (–) Trachelogenin were identified as important inhibitors of HIV replication. Evidences suggest that these lignanoides are prodrugs, which become active integrase inhibitors following metabolism of cells to yield 3-O-demethyl derivatives²².

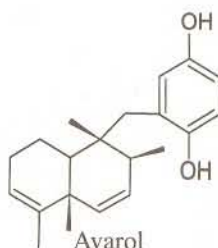


Arctigenin

Calanolide A, a coumarin derivative, isolated from a tropical tree *Calophyllum lanigerum* Miq. (Family: *Clusiaceae*) is a potent inhibitor of DNA polymerase activity of HIV-1 RT but has no effect on RNA H activity¹¹. This compound protects cells from cytopathic effects of Zidovudine resistant strains of HIV 1 but inactive against HIV 2.

Recently, certain novel compounds from marine resources have shown transcription inhibitory action. Avarol, a sesquiterpene hydroquinone and its quinone derivative Avarone have been reported to inhibit HIV replication *in vitro*. Several Avarol derivatives with prominent HIV RT inhibitory activities have been obtained from some sponge species from Red Sea. The natural marine substance illimaquinone is targeted for RNAase H function of reverse transcriptase¹⁸. The most potent compounds Avarone E and F have inhibited

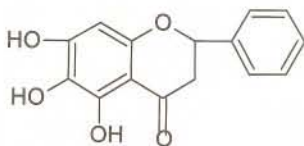
all the RT enzyme activities while other derivatives did not show potent inhibition against one or more RT function²³.



Avarol

Among other natural substances having good anti-HIV activity include psychrine, phloroglucinol derivatives, calanolides²⁰, curcumin (diferuloyl methane) from turmeric, dicaffeoyl quinic acid, dicaffeoyl artaric acid and L-chicoric acid. A number of fungal metabolites are also reported to have prominent HIV integrase inhibitory activity, e.g. equisetin phomasetin, oteromycin and integraric acid²⁴.

A Chinese herbal medicine, *Scutellaria baicalensis* Georgi (Family: *Lamiaceae*) and its isolated compounds baicalein and baicalin inhibit infectivity and replication of HIV. Baicalein has shown remarkable anti-HIV activity, through inhibition of reverse transcriptase²⁵.



Baicalein

3. Drugs affecting transcription and translation (replication)

Papaverine, an alkaloid obtained from opium/poppy, *Papaver somniferum* Linn. (Family: *Papaveraceae*) is

reported to inhibit HIV replication *in vitro*. It has been found that production of HIV proteins is markedly reduced. However, all proteins were not affected to the same extent, showing some degree of selectivity. Conversely, morphine present in opium has been shown to promote growth of HIV in peripheral blood mononuclear cells and evidences show that it also activates latent HIV infection in some tissues such as brain²⁴.

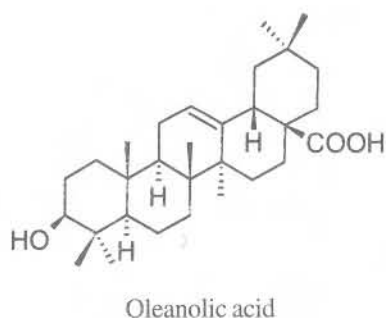


Papaverine

Oleanolic acid has been identified as anti-HIV principle which is obtained from several plants including *Rosa woodsii* Lindl. (leaves), *Prosopis glandulosa* Torr. (leaves and twigs), *Phoradendron juniperinum* A. Gray (whole plant), *Syzygium claviflorum* (Roxb.) Wall. ex Cowan & Cowan (leaves), *Hyptis capitata* Jacq. (whole plant) and *Ternstroemia gymnanthera* (Wight & Arn.) Sprague (aerial parts)²⁶. Natural triterpenoids such as oleanolic acid and pomolic acid have also shown HIV replication inhibition²⁷.

A number of compounds would be able to inhibit HIV 1 gene expression at transcription level. The flavonoid chrysin (through inhibition of kinase II), the antibacterial peptide melittin (from bees venom and cecropin) and betulinic acid, a triterpene isolated from *Syzygium claviflorum* have been

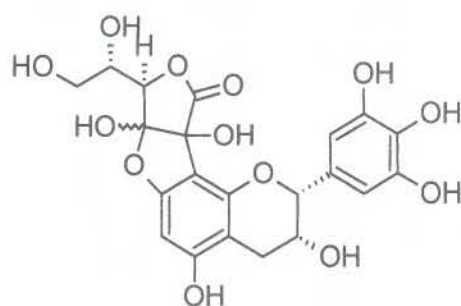
reported to be active against HIV replication, whereas structurally related plautoic acid is less active than betulinic acid. Betulin, which is less potent than betulinic acid, has yielded extremely potent compounds after minor chemical modifications²⁷.



Suksdorfin is a pyranocoumarin derivative isolated from fruits of *Lomatium suksdorfii* (S. Wats.) Coult & Rose (Family: *Apiaceae*) and *Angelica morii* Hayata (Family: *Apiaceae*) has been found to be active in inhibiting HIV replication in the T cell line, H9. Mechanism of action studies on these coumarin lead derivatives has revealed that they do not inactivate virus, block viral entry, alter cellular metabolism, regulate (enhance or suppress) integrated HIV in chronically infected cells, or block viral budding. These compounds do suppress viral

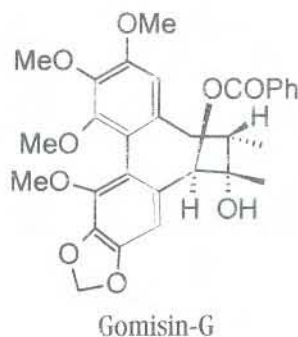
replication in HIV infected, T cells and monocyte/macrophages^{24,28}.

Out of 38 polyphenols isolated from tea, 8-C-ascorbyl (-)-epigallocatechin and theasinensin-D are the most potent inhibitors of HIV replication. Shinjulatone C, isolated from *Ailanthus altissima* (Mill.) Swingle is the most active quassinoid inhibiting HIV replication²⁹.

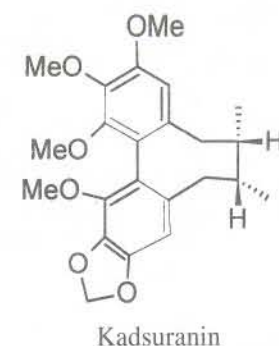
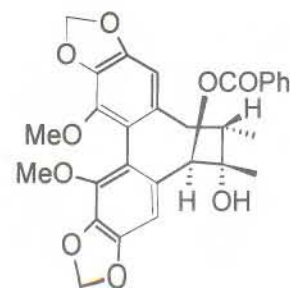


4. Drugs affecting replication

Seven of the twelve known lignans isolated from *Kadsura interior* A. C. Smith (Family: *Schisandraceae*) inhibited HIV replication out of which, Gomisin-G was the most potent³⁰.



Schisantherin-D, Kadsuranin and Schisandrin-C were also found to be quite active in inhibiting the replication of HIV.

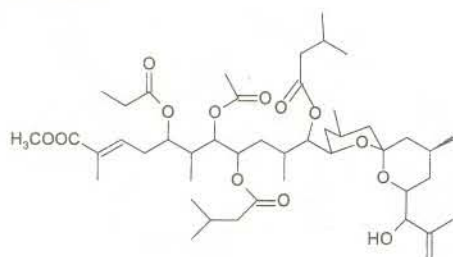


A new kaurane type diterpene lactone, neotripterifordin, isolated from roots of *Tripterygium wilfordii* Hook.f. (Family: *Celastraceae*), a poisonous liana found in southern China, has shown potent anti-HIV replication activity in H9 lymphocytes. Another kaurene diterpene: 16 β , 17-dihydroxy-ent-kauran-19-oic acid obtained from *Annona squamosa* Linn. has been identified as anti-HIV agent. Two known saponins Gleditsia saponin-C isolated from fruits of *Gleditsia japonica* Miq. (Family: *Caesalpiniaceae*) and Gymnocladus saponin-G obtained from fruits of *Gymnocladus chinensis* Baill. (Family: *Caesalpiniaceae*) have also been found to inhibit HIV replication^{29,31}.

5. Drugs affecting post translational modifications

Since HIV-protease is a highly specific enzyme and plays a key role in

replication, it may be an excellent target for anti-HIV agents. Though a number of synthetic tripeptides have been developed, their uses are limited due to bioavailability problems. A complex ester namely Didemnaketol of *Didenum* species possesses some HIV protease inhibiting property but its potential is limited due to low potency and instability. It is possible that natural sources may provide more promising HIV protease inhibitors in future³².

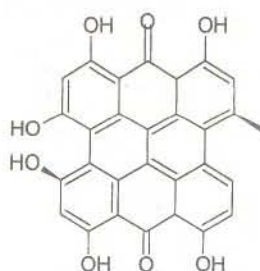


Didemnaketol

6. Drugs affecting viral assembly and viral release

The mannose specific lectins from plants, e.g. *Galanthus*, *Hippeastrum*, *Narcissus*, *Epipactis helleborine* (Linn.) Crantz (Family: *Orchidaceae*) and *Listera ovata* (Linn.) R. Br. (Family: *Orchidaceae*) and N-acetyl glucosamine specific lectin from *Urtica dioica* Linn. (Family: *Urticaceae*) can be targeted to inhibit virus cell fusion process¹¹. Podophyllotoxin is another important inhibitor of microtubule assembly³³. Two aromatic polycyclic diones hypericin and pseudohypericin found in plants of family *Hypericaceae*, have potent antiretroviral activity. Both compounds are highly effective in preventing viral-induced manifestations that follow infections with a variety of retroviruses both *in vivo* and

in vitro. Pseudohypericin and hypericin probably interfere with viral infection and/or viral spread by direct inactivation of the virus or by preventing virus shedding, budding, or assembly at the cell membrane³³.

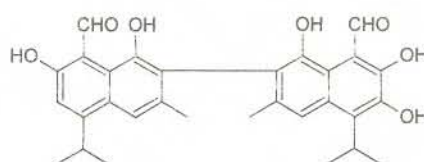


Hypericin

Studies have shown that hypericin and pseudohypericin probably interfere with viral assembly, release and viral stability after release. Although *Hypericum* species are responsible for phytotoxic effects in grazing animals, very little toxicity was seen in mice at doses sufficient to produce anti-viral effect³⁴.

7. Drugs with miscellaneous/undetermined modes of action

7.1 Gossypol: It is a polyphenolic bis-sesquiterpene present in cotton seed and had been used traditionally as a male contraceptive in China. In recent years it has been found that gossypol has weak activity against HIV. Out of two enantiomers available,



Gossypol

(-) isomer exhibited appreciable activity *in vitro* whereas (+) isomer was active against HIV in cytotoxic concentrations^{24,35}.

7.2 Prostratin: It is a non-tumour promoting, relatively polar, 12-deoxyphorbol ester, exhibits a potent anti-HIV activity against HIV 1. However, the anti-HIV mechanism of prostratin is not well understood. It is isolated from *Homalanthus nutans* Benth. & Hook. f. ex Drake (Family: *Euphorbiaceae*), a plant used in Samoan herbal medicine. It is also found to inhibit *de novo* HIV infection, probably by inducing down regulation of HIV receptor from surface of the target cells³⁶⁻³⁸.

7.3 Anti-HIV Protein: Trichosanthin is a ribosome inactivating protein found in *Trichosanthes kirilowii* Maxim. (Family: *Cucurbitaceae*), shows selective reduction in level of viral proteins and RNA in HIV infected cells. Proteins, which inhibit HIV infection and replication *in vitro* have also been isolated from *Gelonium multiflorum* Juss. (Family: *Euphorbiaceae*) and the Carnation, *Dianthus caryophyllus* Linn.^{24,39} (Family: *Caryophyllaceae*).

8. Natural products of mineral origin

Colloidal silver solution kills a variety of pathogens, including HIV. Once the virus invades a cell in the body, the cell reverts back to the primitive type structure and uses an enzyme as its chemical lung, which is promptly crippled

by the presence of colloidal silver. As a result, the cell suffocates and dies, thus denying an opportunity of the virus to replicate. Colloidal silver kills not only the present virus form, but future forms as well. Because no matter how the virus mutates, it can not change the way of human cells response to invasion. Due to its catalytic nature, colloidal silver is not affected in the reaction and continue to kill other single celled pathogens nearby⁴⁰.

On the other hand, indiscriminate use of colloidal silver solutions has resulted in cases of argyria, a permanent blue-gray discoloration of the skin and deep tissues. According to the FDA rule, a colloidal silver product of any drug use will first have to be approved by FDA under the New Drug Application Procedures. The final rule classifies colloidal silver products as misbranded because adequate directions are not being written on its label for safe use of these drugs for intended purposes. They are also said "misbranded" when their labeling suggests falsely that there is a substantial scientific evidence to establish that the drugs are safe and effective for their intended uses⁴¹.

Some researchers believe that AIDS can be treated and even cured in some cases with hydrogen peroxide. The theory being that HIV and opportunistic pathogens are anaerobic and do not thrive when exposed to singlet oxygen supplied by the hydrogen peroxide followed by its decomposition into water and oxygen in the reactions: $\text{H}_2\text{O}_2 \rightarrow \text{H}_2\text{O} + \text{O}$. Singlet oxygen is the active microbicidal agent which kills, or severely inhibits anaerobic organisms^{42, 43}. Ranjbar and Holmes investigated the influence of H_2O_2 on HIV-1 infection of cell cultures. The CD4+HeLa

human epithelial carcinoma cell line clone pBKTRlac was infected with HIV-1MN being treated with 0.01-5mM H_2O_2 and the infectivity of virus was detected using three different analytical techniques. Treatment of HIV-1MN cell free virus particles with 0.01mM H_2O_2 resulted in a significant increase in virus infection. This effect was observed to be declined with increasing H_2O_2 concentrations from 0.05 to 0.1 mM. Further increases in H_2O_2 concentration up to 5 mM resulted in significant suppression in virus infection. These observations indicate that H_2O_2 may play a significant role in affecting the course of HIV infection⁴⁴.

Ozone inactivates extra-cellular HIV at non-cytotoxic concentrations and hence it has been used for the treatment for AIDS. When ozone is introduced into the blood, it reacts with red blood cells, producing hydrogen peroxide; its presence in the pharmacological concentrations in the blood is clearly a double-edged sword, causing as much harm as good. Antiviral effects of ozone include viral particle disruption, reverse transcriptase inactivation and/or a perturbation of the ability of the virus to bind to its receptor on target cells. Ozone treatment has been proved to be a mean to inactivate human retroviruses in human body fluids and blood product preparations^{45, 46}.

Conclusion

A number of synthetic drugs are likely to be launched in the market, in the new patent regime, they shall be affordable to western population. The third world countries, who are the major sufferers of this dreaded disease will have

to rely on the natural products due to lesser side effects, easy accessibility and low cost. Formulations based on plant products and minerals shall not only serve the mankind in fighting against this disease but can also be used in combination with synthetic drugs due to better compatibility.

There is an urgent need to focus as to, identify and develop such natural agents that selectively inhibit HIV virion at specific stages of life cycle. Combination of drugs acting on different stages of HIV virion life cycle will prove to be a boon in interrupting the replication of this virus and hence, combating against this disease. Acquired Immune Deficiency Syndrome is a nonspecific clinical reality that is merely an extreme manifestation of globally induced immune deficiency in human, thanks to modern therapeutics and welcome to natural drugs.

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