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Curcumin and its analogues: A potential natural compound against HIV infection and AIDS

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Abstract

No safe and effective cure currently exists for human immunodeficiency virus (HIV). However, antiretroviral therapy can prolong the lives of HIV patients and lowers the secondary infections. Natural compounds, which are considered as pleiotropic molecules, could be useful against HIV. Curcumin, a yellow pigment present in the spice turmeric (Curcuma longa), can be used for the treatment of several diseases including HIV-AIDS, because of its antioxidant, anti-inflammatory, anticancer, antiviral, and antibacterial nature. In this review we summarized that how curcumin and its analogues inhibit infection and replication of viral gene and prevent the multiplicity of HIV. They exhibit inhibitor of HIV protease and integrase. Curcumin also inhibits Tat transactivation of HIV1-LTR genome, inflammatory molecules (interleukins, TNF-α, NF-κB, COX-2) and HIV associated various kinases including tyrosine kinase, PAK1, MAPK, PKC, cdk and others. In addition, curcumin enhances the effect of conventional therapeutic drugs and minimizes their side effects.

Keywords: Curcumin; HIV-AIDS; Antiretroviral therapy; Natural compounds; HIV protease; HIV integrase.
1. Introduction

The human immunodeficiency virus (HIV), a lentivirus, belongs to the subgroup of retrovirus. Infection of HIV causes destruction of the immune cells, which results in susceptibility to a wide range of infections and diseases. The most advanced stage of HIV infection is Acquired Immunodeficiency Syndrome (AIDS), which takes long time (2 to 15 years) to develop \textsuperscript{1}. Genetically, HIV contains single-stranded RNA that converted into double-stranded DNA by a virally encoded reverse transcriptase in host cells. Subsequently, viral DNA is imported into the cell nucleus and integrated into the cellular DNA by a virally encoded integrase and host co-factors \textsuperscript{2}. Once integrated, the virus may become latent, produce new RNA genomes and viral proteins that are packaged and released from the cell and begin new replication cycle. Basically, two types of HIV have been characterized: HIV-1 and HIV-2, where HIV-1 is more virulent and infective than HIV-2 \textsuperscript{3}.

HIV is one of the major global public health issues, claimed more than 39 million cases of infection. Globally, 1.5 million people died due to HIV infections, and 2.1 million people were newly infected with HIV in 2013. There were approximately 35 million people living with HIV at the end of 2013 worldwide. Sub-Saharan Africa is the most affected region all over the world, where 24.7 million people living with HIV in 2013. It also account for almost 70\% of the new HIV infections (WHO, 2014). There are many biomedical interventions with the strongest evidence of efficacy for different infectious disease but still there is no cure for HIV infection. Accumulated evidences suggest that spread of AIDS can be impeded by taking strong effort on prevention of mother-to-child transmission, HIV testing and counseling, medical male circumcision, and focus on key populations in which HIV infection is concentrated \textsuperscript{4}. However,
antiretroviral (ARV) drugs are being used to control the viral transmission. Globally, 12.9 million HIV patients are receiving antiretroviral therapy (ART) in 2013 (WHO, 2014).

Recent estimates of the global incidence of disease suggest that communicable infectious diseases account for \(\approx 19\%\) of global deaths. Focus on infectious diseases remains necessary to prevent their global spread or recrudescence. This deterrence of infectious diseases could also contribute to increase in economic development and health equity worldwide. As far the concern of HIV-AIDS, ART is being considered to be the best medication. However, how best to use this ART for individual health and for population-based prevention, is not well defined. Whether immediate therapy upon early diagnosis would confer the greatest benefit is also not very clear. Therefore, the use of alternate therapeutic agents is warranted. Mother Nature has provided a variety of natural compounds those have been traditionally used against several diseases and disorders including infectious diseases. Curcumin is one of them, which is considered to be safe, nontoxic, cost-effective and easily available. In the current review, we will discuss the role of curcumin and their analogues against HIV infection and AIDS.

2. Curcumin: a diverse natural compound

Curcumin (diferuloylmethane) is an active ingredient (nutraceuticals) of a spice turmeric (\textit{Curcuma longa}). It has been used as traditional medicine from ancient time, especially in Asian countries. Curcumin is a highly pleiotropic molecule with anti-inflammatory, antioxidant, chemopreventive, chemosensitization, and radiosensitization activities. Based on its safety and efficacy, U.S. Food and Drug Administration has approved curcumin as a “generally regarded as safe” (GRAS) compound in human clinical trials. Extensive research over the past few decades
has shown that it can directly interact with numerous signaling biomolecules, and has therapeutic potential against a wide range of inflammatory and infectious diseases. Effects of curcumin have been evaluated in the patients with various diseases including cancer, arthritis, cardiovascular disease, neurodegenerative diseases, irritable bowel disease, oral lichen planus, vitiligo, psoriasis, atherosclerosis, diabetes, renal disease, and infectious disease including HIV-AIDS. A large number of in vitro and in vivo studies suggest that curcumin exhibits potential against wide varieties of infectious diseases. Curcumin has been found to suppress the infection and activity of several human viruses such as human cytomegalovirus, and hepatitis C virus infection. Kundu et al showed that curcumin is also capable of eradicating Helicobacter pylori infection in mice as well as in human gastric epithelial (AGS) cells. In addition, in vitro and animals studies suggest that curcumin inhibits infection of several other pathogenic bacteria including Salmonella Typhimurium, Vibrio vulnificus, Neisseria gonorrhea and Trypanosoma cruzi. Besides these, curcumin was found to inhibit the growth and activity of human parasites. In a study, curcumin has shown its antimalarial activity. In a cerebral malaria model of mice, curcumin have shown potent activity against Plasmodium berghei and prevented cerebral malaria and delayed death of animals. This inhibition of infection further protects from infectious disease associated with pathogenic microbes. This article, intends to document the potentials of curcumin against HIV infection and AIDS that have been reported in both in vitro and in vivo studies.

There are several mechanism have been proposed through which curcumin protects against microbial infection. The most potent considered mechanisms are its anti-inflammatory and antioxidant properties. Numerous evidence have indicated that curcumin has ability to modulate multiple signaling molecules such as inflammatory cytokines.
(TNF-α, -β), pro-inflammatory transcription factors [nuclear factor-kappaB (NF-κB), signal transducer and activator of transcription (STAT)-3], pro-inflammatory enzymes [cyclooxygenase (COX)-2, 5-lipoxygenase (LOX), 12-LOX, matrix metalloproteinases (MMPs)], interleukin (IL)-1, IL-2, IL-6, IL-12), chemokines (monocyte chemo-attractant protein 1, IL-8), vascular endothelial growth factor (VEGF), adhesion molecules [intercellular adhesion molecule (ICAM-1), vascular cell adhesion molecule (VCAM)-1, endothelial-leukocyte adhesion molecule (ELAM)-1, and TWIST], prostaglandin E2, and transforming growth factor (TGF)-β.

Curcumin also induce antioxidant level by increasing GST, glutathione (GSH), HO-1, Nrf2 and decreasing malondialdehyde (MDA), ROS both in vitro and in vivo.

3. Preventive and therapeutic role of curcumin and its analogues in HIV infection and AIDS

Curcumin is a highly pleiotropic molecule with antimicrobial, insecticidal, larvicidal, antimitogenic, radioprotector, and anticancer properties. It exhibits anti-inflammatory, antioxidant, chemopreventive, chemosensitization, and radiosensitization activities. Numerous studies have shown that curcumin has potential against proinflammatory diseases including cancer, neurodegenerative diseases, depression, diabetes, obesity, atherosclerosis and HIV-AIDS. It also exhibits potential against HIV-AIDS associated secondary disorders (Fig 1). At the molecular level, this molecule has been shown to modulate numerous cell-signaling pathways (Fig 2). Here we describe the role and multiple targets of curcumin, which can impede the infectious and multiplicity properties of HIV.

3.1 Curcumin as viral growth inhibitor
Curcumin has been associated with antimicrobial activities including antiviral, antifungal and antibacterial activities\textsuperscript{16,17}. Curcumin present in a polyherbal cream (Basant) has been shown to prevent the entry of HIV-1(IIIB) virus into HeLa-based P4-CCR5 cells\textsuperscript{18}, indicating its anti-HIV properties. Besides natural form of curcumin, its analogues and derivatives also displayed growth inhibition of HIV. In a study, a truncated curcumin analogue, C5-curcuminoid 2a exhibited high-potency anti-HIV\textsuperscript{19}. Other derivative of curcumin such as curcumin bioconjugates, di-O-decanoyl curcumin and 4-O-ethyl-O-gamma-folyl curcumin have shown potent antiviral property against a wide range of viruses, like HIV, HSV, VSV and many others\textsuperscript{20}. These studies prove the anti-HIV potential of curcumin in natural as well in derivative form.

3.2 Curcumin as HIV protease inhibitor

Numerous studies have revealed that curcumin is potent protease inhibitor\textsuperscript{21-23}. It inhibits HIV-1 (IC50; 100 µM) and HIV-2 (IC50; 250 µM) proteases that could contribute its anti-HIV properties. However, when curcumin forms complex with boron decreases the IC50 value to 6 µM. The improved affinity of the boron complexes could be due to binding of the orthogonal domains of the inhibitor in intersecting sites within the substrate-binding cavity of the enzyme\textsuperscript{24}. Other study showed that curcumin binds to the active sites of HIV protease. In a protease docking study, it has been observed that curcumin structure fitted well to the active site, interacting with residues Asp25, Asp29, Asp30, Gly27', Asp29', and Asp30' of HIV protease. Thus, symmetrical structure of curcumin seems to play an important role for binding to the protease protein. These results suggested that extensive hydrogen bonding promoted by the o-hydroxyl and/or keto–enol structures are important for both integrase and protease inhibitory actions\textsuperscript{25}. Besides curcumin, its analogue (E)-2-(3,4-dimethoxybenzylidene)-6-((E)-3-(3,4-
dimethoxyphenyl)-acryloyl)cyclohexanone (2e) was also found to be the strongest human HIV-1 protease inhibitor \textit{in vitro} among the tested compounds \textsuperscript{26}. Thus study indicates that the presence of specific structural feature of curcumin allows proteins binds to it.

3.3 Curcumin as HIV integrase inhibitor

The HIV-1 integrase is a multidomain enzyme needed for the integration of a double-stranded DNA of the viral genome into a host chromosome and for further replication. Targeting HIV integrase could be one of the potential approaches for the treatment of HIV. Curcumin has shown as an inhibitor of HIV-1 integrase \textsuperscript{27}. In purified HIV-1 integrase, curcumin inhibited it with IC50; 40 µM by interacting with the integrase catalytic core. Further energy minimization studies revealed that curcumin’s anti-integrase activity was associated with intramolecular stacking of two phenyl rings that bring the hydroxyl groups into close proximity \textsuperscript{28}. Vajragupta et al \textsuperscript{25} showed that curcumin preferentially binds to HIV-1 integrase. The curcumin-binding site is formed in residues Asp64, His67, Thr66, Glu92, Thr93, Asp116, Ser119, Asn120, and Lys159. They also showed that curcumin links the catalytic residues adjacent to Asp116 and Asp64, and close to divalent metal Mg2+ ion. The keto-enol form of curcumin and only one side of the terminal o-hydroxyl has tight binding to the integrase active site \textsuperscript{25}. This inhibitory activity of curcumin by binding different enzymes and proteins including HIV integrase directs its potential against HIV treatment.

In a quantitative structure–activity relationship (QSAR) study using multiple linear regressions, curcumin derivatives also showed as HIV-1 integrase inhibitors \textsuperscript{29}. Other curcumin analogues, dicafeoyl methane and rosmarinic acid, have found to inhibit activities of integrase with IC50 values below 10 µM \textsuperscript{30}. Thus the results of docking, CoMFA, and pharmacophore
mapping give structural insights as well as important binding features of curcumin and its derivatives as HIV-1 integrase inhibitors which can provide guidance for the rational design of novel HIV-1 integrase inhibitors \(^{29}\). These studies indicate that curcumin and its analogues bind with numerous molecules. The interaction of curcumin with these signaling molecules is through numerous amino acids.

### 3.4 Curcumin as an inhibitor of HIV genome expression

Expression of HIV-1 gene depends on Tat and Rev viral regulatory proteins. Tat stimulates transcription elongation while Rev is required for the transport from the nucleus to the cytoplasm of the unspliced and incompletely spliced mRNAs that encode the structural proteins of the virus \(^{31}\). Thus, Tat protein plays a critical role in the pathogenesis of AIDS because of its ability to infect other normal cells. Curcumin has been found potential in the inhibition of Tat protein \(^{32-34}\).

In a study, curcumin at 10 to 100 nM inhibited Tat transactivation of HIV1-LTR by 70 to 80% in HeLa cells \(^{34}\). Other study also showed that curcumin is potent and selective inhibitors of HIV-1 LTR-directed gene expression, as well as inhibitor of p24 antigen production in cells, either acutely or chronically infected with HIV-1 \(^{35}\). Curcumin found to inhibit UV-activated HIV-LTR gene expression in HIVcat/HeLa cells. Mechanistically, curcumin modulates UV activation of HIV-LTR gene expression through the inhibition of NF-κB activation \(^{36}\). Besides NF-κB, AMPK and HDAC1 pathways also involve in curcumin-induced inhibition of Tat-regulated transcription \(^{32}\). It has been also reported that curcumin inhibits proliferation of HIV by the inhibition of HIV-Tat protein acetylation by p300 in SupT1 cells. Thus, non-toxic curcumin may serve as a lead compound in combinatorial HIV therapeutics \(^{33}\).
Besides these, curcumin derivatives also showed a significant inhibition of Tat-transactivation \(^{34}\). Curcumin-loaded apotransferrin nanoparticles completely blocks the synthesis of viral cDNA in the gag region and inhibition of HIV-1 replication. These nanoparticles enter to cells through transferrin-mediated endocytosis and nano-curcumin releases significant quantities of drug gradually over a fairly long period \(^{37}\). Because of this potent activity, nano-curcumin could be the one of the valuable agent against HIV.

### 3.5 Curcumin as inhibitor of HIV-associated kinases

Numerous kinases have been reported to involve in infectivity and multiplication of HIV. Activation of protein kinase C (PKC) helps in replication of HIV-1. Tat protein of HIV has shown to induce IL-10 production via a PKC-dependent pathway \(^{38}\). Studies showed that curcumin selectively inhibits PKC in different cell types \(^{39},^{40}\). HIV-1 Tat also increases endothelial solute permeability through tyrosine kinase and mitogen-activated protein kinase-dependent pathways, which further contribute to HIV-1 infection \(^{41}\). However, these tyrosine kinase and mitogen-activated protein kinase are inhibited by curcumin \(^{42},^{43}\). Oncogenic kinase PAK1 is also found to be responsible for AIDS \(^{44}\) and curcumin blocks PAK1 without affecting normal cell growth \(^{45}\). Besides these, several other kinases including ferritin and creatine kinase \(^{46}\), Src kinase Hck \(^{47}\), cyclin-dependent kinase \(^{48}\), cAMP-dependent protein kinase \(^{49}\) and others, have found to involve in HIV infection and host cell dysregulation. Curcumin potentially modulates all these kinases \(^{7},^{40},^{50}\), which further laid curcumin in prevention and treatment of not only HIV-AIDS rather several other infectious and chronic diseases.

### 3.6 Curcumin as inhibitor of HIV associated inflammation
Inflammation has been implicated in the pathogenesis of intracellular parasitic infectious disease including AIDS. Curcumin has been shown to inhibit proinflammatory cytokines TNF-α and IL-1, inflammatory transcription factors NF-κB and STAT3. In a cultured ts1-infected cells from the C1 astrocytic cells and ts1-infected mice that resembles HIV-AIDS, have found increased expression of COX-2 in the brainstem tissues. However, treatment of curcumin reversed the level of COX-2 in infected cells. Besides these, nano-curcumin also has shown to inhibit HIV-1 induced expression of inflammatory molecules Topo II α, IL-1β and COX-2. Nano-curcumin affects the HIV-1 induced inflammatory responses through pathways downstream or independent of TNF-α. Thus, these studies indicate that curcumin has anti-inflammatory properties in HIV-AIDS.

3.7 Curcumin prevents HIV-associated diarrhea

Diarrhea is the most common gastrointestinal symptom in patients with HIV. In outpatient studies, the prevalence of diarrhea ranged from 0.9 to 14%. Studies show that curcumin is useful in patients with HIV-associated diarrhea. In a study, 8 patients with HIV-associated diarrhea were given a mean daily dose of 1,862 mg of curcumin and followed for a mean of 41 weeks. They found a resolution of diarrhea and normalization of stool quality in a mean time of 13 ± 9.3 days. Mean number of bowel movements per day dropped from 7 ± 3.6 to 1.7 ± 0.5. Seven of eight patients had considerable weight gain on curcumin. Thus, it has been observed that curcumin has rapid and complete resolution of diarrhea, substantial weight gain, improvement in the reduction of bloating and abdominal pain. Thus, curcumin not only potent against HIV-AIDS but also other disorders associated with this disease.
3.8 Curcumin and HIV-associated cancer

There are several risk factors for cancers, and infection is one of them. Epstein-Barr virus (EBV) infection is responsible for development of B-cell lymphomas, which occur more frequently in immunodeficient states including HIV infection. In a study, curcumin has shown to block EBV-induced B cell immortalization. Curcumin inhibits the proliferation of EBV-transformed lymphoblastoid cell lines (LCL) via enhanced apoptosis. Curcumin also induces apoptosis in resting B-cell chronic lymphocytic leukemia (B-CLL) cells. Thus, it can be concluded that curcumin is an effective treatment for B-CLL and are of high clinical significance considering the growing population of patients with HIV infection.

3.9 Curcumin inhibits HIV-1-associated neurotoxicity

A wide range of neurological deficits has been reported in individuals suffering from HIV-1 infection. There are two neurotoxic proteins gp120 (a coat glycoprotein) and Tat (transactivation). These have been found to be associated with the AIDS virus. Curcumin is able to ameliorate HIV-1-associated neurotoxicity. Curcumin exerted a powerful inhibitory effect against HIV-1 gp120-induced neuronal damage through the reduction of ROS, TNF-α and MCP-1 and further inhibiting apoptosis of primary rat cortical neurons. Infection with the gp120 V3 loop can also cause HIV-1 associated neurocognitive disorders. However, curcumin inhibits gp120 V3 loop-induced neuronal damage by inhibiting the activation of L-type calcium currents, relieving intracellular Ca(2+) overload, promoting Bcl-2 expression, and inhibiting Bax activation.

One of the more debilitating of the HIV-related syndromes is AIDS-related dementia (HAD). Dietary supplementation of curcumin has shown a potential therapeutic strategy for the
treatment and prevention of HAD. Intragastric infusion of curcumin in animals inhibited gp120 V3 peptide-induced impairment of spatial learning and memory. It also inhibited oxidative stress and neuronal injury. Other study in rats showed that treatment of curcumin improves learning and memory dysfunction induced by gp120, and thus overcome HIV associated dementia. This is evident by water maze test where curcumin groups had shorter escape latencies compared with those in gp120 treated group. Curcumin also decreases MoMuLV-ts1-induced dementia by inhibiting COX-2 level in central nervous system and thus exhibits neuroprotective. MoMuLV-ts1 is a mutant retrovirus that causes a neurodegeneration and immunodeficiency syndrome and resembles human HIV-AIDS. Thus, these reports suggest that curcumin is potent neuroprotective agent in HIV-AIDS patients.

3.10 Chemosensitizing effect of curcumin in anti-HIV drugs

Curcumin not only regulates infectivity of HIV, also enhances the efficacy of HIV-AIDS therapeutic drugs. In a study, curcumin has shown to enhance systemic exposure of saquinavir in rats, while it did not affect the intravenous pharmacokinetics of saquinavir. The AUC and Cmax of oral saquinavir increased by 3.8- and 2.7-folds, respectively in the presence of curcumin-loaded solid dispersion. Riva et al also reported that combination of indinavir with curcumin significantly reduces viral infectivity and viral relative infectivity when compared to the reduction produced by indinavir alone. Thus, the use of curcumin with indinavir could help to reduce HIV-1 production in persistently infected cells.

Besides these, curcumin also blocks HIV protease inhibitor ritonavir-induced vascular dysfunction. Ritonavir causes reductions of eNOS and nitric oxide release in arteries, which are restored by curcumin treatment. In addition, curcumin reversed the Ritonavir-induced superoxide
anion production in the vessels, thus curcumin exhibits cardioprotective in HIV-AIDS patients. Curcumin analogs, dicaffeoylmethane and rosmarinic acid also enhances the effects of described integrase inhibitor NSC 158393. Combining either curcumin analog with the NSC 158393 resulted synergistic inhibition of integrase activity.

3.11 Curcumin inhibits secondary infection in HIV patients

Besides these, it inhibits secondary infection in the AIDS patients. Curcumin was found to suppress the growth of Candida species in AIDS patients and prevents secondary infection. It has also observed that curcumin was much more efficient than fluconazole in inhibiting the adhesion of Candida species, particularly those strains isolated from the buccal mucosa of AIDS patients. This finding demonstrates that curcumin is a promising lead compound for therapeutical use in immunocompromised patients.

4. Conclusion

Curcumin has been used as traditional medicine against various ailments for thousands of years. Numerous preclinical studies suggest that curcumin is effective against cancer, neurodegenerative diseases, depression, diabetes, obesity, atherosclerosis and AIDS because of its mutitageting nature. Curcumin is effective in multiple steps of HIV infection and multiplication (Fig 3). Multiple studies over the past decade have also indicated that curcumin is safe, effective and bioavailable. In a clinical trial with HIV patients, although curcumin did not show significant effect on viral loads or CD4 counts, the participants reported better feeling while taking curcumin. Therefore, some more clinical trials of curcumin in the HIV-AIDS patients are needed to validate the existing clinical and preclinical observations. In spite of these
multiple inherent qualities curcumin has not yet been approved for human use. Poor bioavailability due to poor absorption, rapid metabolism, and rapid systemic elimination along with limited adverse effects have reported by some investigators those limit its therapeutic efficacy. 

Competing interests
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References


**Figure Legend:**

Fig 1: Effects of curcumin in HIV infection induced disorders.

Fig 2: List of molecules targeted by curcumin in prevention of HIV infection and development of disease.

Fig 3: Therapeutic targets of curcumin against infection and multiplication of HIV.
Fig. 1: Effects of curcumin in HIV infection induced disorders.
381x285mm (72 x 72 DPI)
Fig 2: List of molecules that curcumin targets in prevention of HIV infection and development of disease.

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