HIV-1 Reverse Transcriptase Inhibitor and HIV-1 Integrase Inhibitory Effects of Combinational drug of New Upgraded HOOIMM PLUS; A combinational phyto-based drug for multi target HIV.

Background: Reverse transcriptase (RT) enzyme plays a pivotal role in the early steps of HIV-1 life cycle and facilitates the conversion of the viral RNA genome into a viral DNA. Integrase (IN) is an essential enzyme for HIV replication that catalyzes insertion of the reverse transcribed viral DNA into the human chromosomal DNA. The pivotal role of Reverse transcriptase (RT) and Integrase (IN) in the HIV-1 life cycle made it a druggable target for the HIV treatment.

Highly active anti-retroviral therapy (HAART) is a HIV/AIDS treatment method that combines three or more antiviral drugs that inhibit the activities of viral reverse transcriptase and Integrase that includes 5 FDA-approved INSTIs, but these exhibit side effects, and induce a resistance effect; making the treatment a virologic failure.
Early Integrase inhibitors (INIs) mainly serve as integrase strand transfer inhibitors (INSTI) that disrupt strand transfer by binding the catalytic core domain of Integrase (IN). However, mutations of Integrase (IN) can confer resistance to INSTI. Therefore, non-catalytic integrase inhibitors (NCINI) have been developed as next-generation INIs, but none of the drug in Highly active anti-retroviral therapy HAART combination or other has ever licensed till date as Non-catalytic integrase inhibitors (NCINI).

Purpose: To overcome this, the current study focuses on discovering Dual Inhibitory effect of phyto based Anti HIV drug HOOIMM plus with *in vitro* enzyme assay against HIV-1 Reverse transcriptase (RT) & HIV-1 Integrase (IN) (Non catalytic integrase activity).

Methods : In this study, we evaluated and compared the activity of Reverse transcriptase inhibitor (RTI) and Non catalytic Integrase inhibitor (NCINI) according to the manufacturer protocol and analysis method. Antiviral activity was compared using two different control drug, ARVs (EFA) (NNRTI) for reverse transcriptase inhibitor and ARVs (EVG) (INSTI) for Integrase inhibitor. Control drug was taken as per its mol wt and HOOIMM PLUS (300mg) reconstituted in 5 ml DMSO to give 100 mM concentration and serially diluted with molecular grade water to achieve 100 µM of drug with 0.1% DMSO. Additionally, to analyze dual inhibition activity against HIV-1 Reverse transcriptase (RT) and 3′-processing/ Non Catalytic integrase (NCINI) were performed with modified protocol provided by Xpressbio. The kit used for the above test are :

1) HIV-1 reverse transcriptase Assay (Xpress Bio, USA)
2) HIV-1 integrase Assay (Xpress Bio, USA)
   (Non Catalytic integrase inhibitor)
3) Alere Rt-2100T (Abbott)

Results: With an in vitro enzyme assay, combinational plant extracts of HOOIMM PLUS showed an in vitro percentage inhibition against HIV-1 Reverse transcriptase (RT) of 96.96% and 97.28% against HIV-1 Integrase (IN) (3' processing/Non catalytic integrase) and at a standard concentration of 10 µM (6µg/mL). The proposed natural candidates showed excellent in vitro inhibitory efficacy against HIV-1 RT and HIV-1 IN, exhibiting the potential drug to serve as standalone drug against HIV/AIDS.

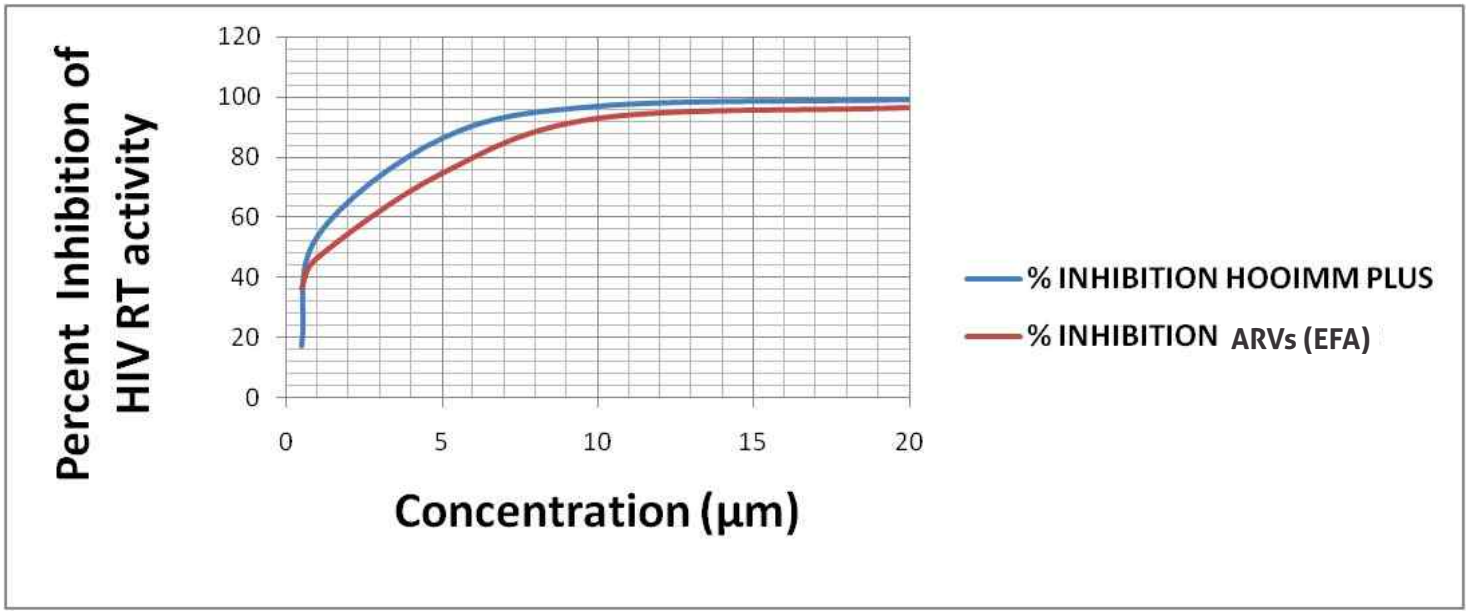
Conclusion: In our unrelenting study on the anti-HIV activity of compounds present in HOOIMM Plus, we report the HIV-1 Reverse transcriptase (HIV-1 RT) & HIV-1 integrase (HIV-1 IN) inhibitory effects of whole drug combination of HOOIMM plus whose different compounds were earlier reported in various life science journal and their interaction against HIV-1 Reverse transcriptase (RT) and HIV-1 integrase (IN) were determined through molecular docking and simulation. An in silico study confirms the crucial interactions of compounds (1) Betulinic acid, (2) glycyrrhizin, (3) calanolide, (4) chebulagic acid and (5) repandusinic acid against HIV-1 RT and (6) Gallic acid, (7) curcumin and (8) Map30 against HIV-1 IN, were reported. In this present study, involves an in vitro enzyme assay, combinational plant extracts of HOOIMM plus showed an in vitro percentage inhibition against HIV-1 RT of 96.96% and 97.28% against HIV-1 IN (3' processing/Non catalytic integrase) at a standard concentration of 10 µM (6 µg/mL). However, Drug content of HOOIMM plus shown to be more promising candidates exploring the Dual inhibitory action against multiple targets of HIV. Additional to HIV-1 RT inhibition, in vitro enzyme assay confirms the dual inhibitory action of drug against HIV-1 IN (3' processing/Non catalytic integrase).

Analysis Report :

1) HIV-1 Reverse Transcriptase Assay (Xpress Bio, USA)

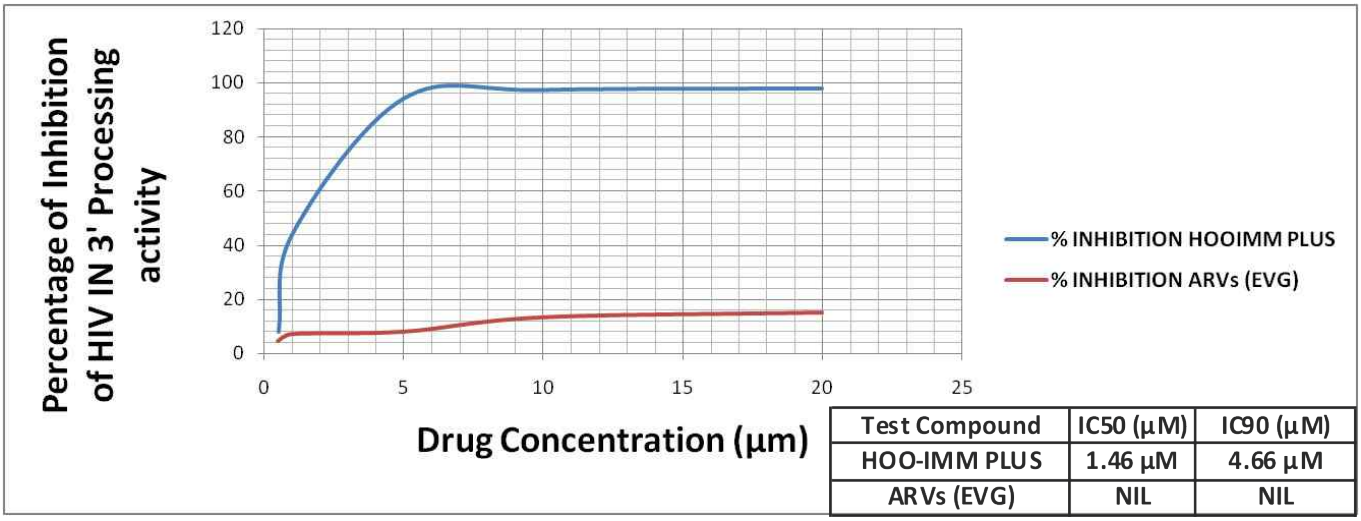
CONCENTRATION IN µM	% INHIBITION ARVs (EFA)	% INHIBITION HOOIMM PLUS
0.5	36.23	17.19
1	46.72	53.64
5	74.46	86.39
10	92.98	96.96
20	96.7	99.1

Test compound	IC50 (µM)	IC90 (µM)
HOO-IMM	0.950 µM	6.70 µM
ARVs (EFA)	1.472µM	9.19µM



2) HIV-1integrase Assay (Non Catalytic integrase inhibitor) (Xpress Bio, USA)

CONCENTRATION IN µM	% INHIBITION HOOIMM PLUS	% INHIBITION ARVs (EVG)
0.5	7.87	4.59
1	44.145	7.23
5	94.19	8.1
10	97.281	13.49
20	97.89	15.27



Test Compound	IC50 (µM)	IC90 (µM)
HOO-IMM PLUS	1.46 µM	4.66 µM
ARVs (EVG)	NIL	NIL

3) Dual inhibition of drug against Reverse Transcriptase and Integrase