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[Research article]

Studies on Anti-HIV activity and Cytotoxicity of *Wrightia tomentosa* Leaf

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ABSTRACT

Wrightia tomentosa leaf extracts and its isolated compounds (isatin, indirubin and indigotin) have been studied for inhibition of HIV-1 and -2 replication in MT-4 cells. Cytotoxicity was also investigated in uninfected MT-4 cells (C-type Adult T Leukemia cells). All the extracts and isolated compounds exhibited cytotoxicity in MT-4 cells (CC_{50}: 8-117 µg/ml) and Indirubin displayed marked cytostatic properties in MT-4 cells (CC_{50}:8.32±3.83 µg/ ml).

Keywords: *Wrightia tomentosa, anti*-HIV activity, Indirubin, Cytotoxicity

INTRODUCTION

Acquired Immunodeficiency Syndrome (AIDS) is a life threatening and debilitating disease condition caused by a Retrovirus, Human Immunodeficiency Virus (HIV). Three different classes of chemotherapeutic agents are generally combined to block the replication of HIV type 1 (HIV-1) and to prevent any development of resistance, namely, reverse transcriptase inhibitors (RTI), protease inhibitors (PRI), and fusion inhibitors. This widespread triple combination therapy is referred to as highly active antiretroviral therapy (HAART)[1]. HAART effectively inhibits HIV replication to such an extent that the virus becomes undetectable in the blood. However, it fails to eradicate viruses that have integrated in the host genome or that persist in cellular and anatomical "reservoirs." In addition, prolonged drug exposure leads to HIV drug resistance, thus reducing patients' therapeutical options[2]. The above considerations and the toxicity of a number of antiretroviral agents have prompted the discovery of anti-HIV drugs from natural sources.

Wrightia tomentosa Roem & Schult, family: Apocynaceae, is an important medicinal plant used

in the Indian system of medicine for the treatment of variety of diseases[3] and *Wrightia* species were possess analgesic[4], anti-fertility[5] cytotoxic[6], hemostasis[7], anti-ulcer activity[8] and anti-HIV activity[1]. Review of literature indicated that the antiviral activity of *Wrightia tomentosa* against HIV has been less explored. Present work is to study the anti-HIV activity of various extracts and isolated compounds of *Wrightia tomentosa* leaf. Cytotoxicity was also investigated in mock-infected or uninfected MT-4 cells (C-type Adult T Leukemia cells).

MATERIALS AND METHODS

Extraction

Leaves of *Wrightia tomentosa* (Apocynaceae) were collected from collected from the hills of Yercaud forest, Salem district of Tamilnadu, India and identified[10] and were dried in shade, subjected to hot continuous percolation using ether, chloroform, methanol, ethanol. Ether (EWT), chloroform (CWT), ethanol (ETWT), methanol (MWT), of *Wrightia tomentosa* were concentrated by distillation and dried under vacuum.

Isolation of compounds by Column chromatography

The crude extract (3g) of *Wrightia tomentosa* obtained using the solvent chloroform was subjected to column chromatography. The extract was mixed thoroughly with silica gel. Column was packed with silica gel (60-120mesh, 60g, dia 3cm X 50h) using hexane as solvent. It was followed by adding the admixture to the top of the column, and the column was continuously flooded by adding solvent in an increasing order of polarity. The solvent strength was gradually increased from 0 – 100%. Ethyl acetate in hexane and the various fractions were collected. The pure fractions were eluted in 15% and 20% EtOAc in hexane and from this, the compounds isatin, indigotin and indirubin were isolated.

Anti-HIV Activity and Cytotoxicity assay

The extracts and isolated compounds were tested for anti-HIV activity for inhibition of replication of HIV-1(III_B) and HIV-2(ROD) in MT-4 cells[11]. The cells were grown and maintained in RPMI 1640 medium supplemented with 10% heat–inactivated fetal calf serum (FCS), 2 mM-glutamine, 0.1% Sodium bicarbonate and 20µg / ml gentamicin (culture medium). HIV-1 (HTLV-IIIB/LAI) strain and HIV-2 ($LAV\text{-}2_{ROD}$) strains were used in the experiment. The virus strains were propagated in MT-4 cells, and the titer of virus stock was determined in MT-4 cells and stored at -70°C until further use. Inhibitory effects of the compounds on HIV-1 and HIV-2 replication were determined by inhibition of virus-induced cytopathic effect in MT-4 cells, which was subsequently confirmed by MTT assay. Briefly, 50µl of HIV-1 and HIV-2 (100-300 $CCID_{50}$) were added to flat-bottomed MT–4 cells ($6x10^5$cells/ml). After 5 days of incubation, at 37°C the number of viable cells was determined by the 3 - (4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) method. Cytotoxicity of the compounds for mock-infected MT-4 cells was also assessed by the MTT method. Anti-HIV activity and cytotoxicity of standard antiviral drug AZT were also determined by a similar method. The results of assays for anti-HIV activity and cytotoxicity on MT-4 cells are presented in Table-1

RESULTS AND DISCUSSION

Different leaf extracts and isolated compounds (isatin, indirubin and Indigotin) were investigated for antiviral activity against HIV-1 & -2 in MT-4 cells. Cytotoxicity was also studied against mock-infected MT-4 cells (C-type Adult T Leukemia cells) by MTT assay. All the extracts and isolated compounds exhibited cytotoxic properties in MT-4 cells (CC50: 8-117 µg/ml) and Indirubin displayed marked cytostatic properties at 8.32±3.83 µg / ml in MT-4 cells.(Table-1).

Indole derivatives such as isatin and its dimers indirubin, indigotin is considered as the principal active constituents of *Wrightia tomentosa*, which are attributed for its wide spectrum biological activity[12]. Indirubin-3'-monoxime, a derivative of indirubin isolated from Chinese anti-leukemia drug *Wrightia tinctoria* had been shown to inhibit HIV-1 replication[13] and *Isatis tinctoria* another isatin containing plant had been reported to having anti-HIV activity[14]. This results of the present study clearly indicated cytotoxic property of *Wrightia tomentosa* owing to the presence of indirubin.

Table 1:Anti-HIV activity and cytotoxicity of *wrightia tomentosa*

Extracts	Strain	IC_{50}[a] (µg/ml)	CC_{50}[b] (µg/ml)	Maximum Protection
CWT	IIIB	>95.33	95.33±2.74	33
	ROD	>95.33	95.33±2.74	16
ETWT	IIIB	>93.53	93.53±2.69	26
	ROD	>93.53	93.53±2.69	16
EWT	IIIB	>69.35	69.35±2.22	2
	ROD	>69.35	69.35±2.22	3
MWT	IIIB	>117	≥117	46
	ROD	>117	≥117	15
WT-IS	IIIB	>76.63	76.63±6.80	4
	ROD	>76.63	76.63±6.80	8
WT-Indirubin	IIIB	>8.32	8.32±3.83	17
	ROD	>8.32	8.32±3.83	2
WT-Indigotin	IIIB	>71.63	71.63±5.87	6
	ROD	>71.63	71.63±5.87	2
AZT (STD)	IIIB	0.0015±0.0002	>25	96
Zidovudine	ROD	0.0016±0.0003	>25	76

[a]Effective concentration of compound, achieving 50% protection of MT-4 cells against the cytopathic effect of HIV. [b]50% Cytotoxic concentration of compound, required to reduce the viability of mock infected MT-4 cells by 50%. HIV 1-(HTLV IIIB): HIV-2 (ROD)

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