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Research article

HUMAN IMMUNODEFICIENCY VIRUS (HIV-1) REVERSE TRANSCRIPTASE INHIBITORY ACTIVITY OF *ECLIPTA ALBA* (L) LEAVES EXTRACTS

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ABSTRACT: Highly active anti-retroviral therapy (HAART) is the current HIV/AIDS treatment modality. Despite the fact that HAART is very effective in suppressing HIV-1 replication and reducing the mortality of HIV/AIDS patients, it has become increasingly clear that HAART does not offer an ultimate cure to HIV/AIDS. The high cost of the HAART regimen has impeded its delivery to over 90% of the HIV/AIDS population in the world. This reality has urgently called for the need to develop inexpensive alternative anti-HIV/AIDS therapy. This need has further manifested by recent clinical trial failures in anti-HIV-1 vaccines and microbicides. . In the current study, we characterized a panel of extracts of traditional medicinal plants for their activities against HIV-1 replication. The aim of the present study was to evaluate the invitro anti- HIV activity of *Eclipta alba* plant extracts. Extracts were prepared from dried fruits in n-hexane, ethyl acetate and n butanol. Peripheral Blood Mononuclear Cells (PBMCs) isolated from healthy donors by ficoll-hypaque density gradient centrifugation method. A toxicity study was performed on all crude extracts by MTT assay using PBMCs isolated from whole blood. HIV-1 RT inhibition activity of the all solvent extracts of *Eclipta alba* was determined by a RetrsoSys HIV-1 RT activity kit (Innovagen, Sweden). The aerial parts of *Eclipta alba* extracts are shows anti-HIV-1 activity and this plant has great potential for developing useful drugs.

Key words: HIV, *Eclipta alba*, PBMCs, HIV-1 RT, Cytotoxicity.

INTRODUCTION

Since the discovery of the human immunodeficiency virus as the causative agent of AIDS New chemical entities with such activity may be identified through a variety of approaches, one of them being the screening of natural products. Plant substances are especially explored due to their amazing structural diversity and their broad range of biological activities. Several plant extracts have been shown to possess activity against HIV by inhibiting various viral enzymes (Vermani and Garg, 2002). Medicinal plants as potential sources of new active agents not only combine the advantage of being relatively non-toxic and hence more tolerable than rationally designed drugs, but also represent an affordable and valuable source of pharmacologically active substances that can be made sufficiently available through cultivation (King & Rewers,1993) Nature has been a source of medicinal treatments for thousands of years, and plant-based systems continue to play an essential role in the primary health care (Budka *et al.,* 1995; Van Everbroeck *et al.,* 2000; Brown *et al.,* 2004). It is estimated that 25 to 50% of all current pharmaceuticals are derived from plants (Cowan, 1999). In fact, it is now believed that plant based systems contribute 90% of the newly discovered pharmaceuticals. The aim of the present study was to evaluate the *invitro* anti-HIV activity of *Eclipta alba* plant extracts.

MATERIALS AND METHODS

The aerial parts of the *Eclipta alba* were collected and left at room temperature for two weeks to dry, then ground into powder and extraction with soxhlet techniques with methanol. Obtaining methanolic crude extracts of *Eclipta alba* were then fractionated successively using solvents of increasing polarity, such as, *n*-hexane (HX), carbon tetrachloride (CT), and chloroform (CF) and aqueous fractions (AQ). All the four fractions (HXF, CTF, CFF and AQF) were evaporated to dryness by using rotary evaporator at low temperature (39^0C).

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Peripheral Blood Mononuclear Cells (PBMCs) were collected from the blood of healthy volunteers, by ficol-Hypaque density gradient centrifugation method. The samples were diluted at 1:1 ratio with PBS, layered onto HISEP media (Himedia, Mumbai) at a volume ratio of 3:1 and centrifuged at 1,000 x g for 30 min. During the centrifugation the PBMCs moved from the plasma and were suspended in the density gradient, The PBMCs layer was removed and then washed twice with PBS. The supernatant was then removed and the cells were resuspended in RPMI 1640 medium supplemented with 1 mM L-glutamine, 100 units/ml penicillin and 100 µg/ml streptomycin, 10% inactivated FBS, and adjusted to pH 7.2 by the addition of 15 mM HEPES. The PBMC cell density used in the cytotoxicity study was 1 x 10^5 cells/ well of the 96-well tissue culture plate.

Cell viability was determined by the MTT 3-(4, 5dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide) test method. Briefly, MTT (5 mg/ml) was dissolved in PBS. PBMC Cells were cultured in 96-well plates (1.0 x 10^4 cells/ well) containing 100 µl medium prior to treatment with four fractions of selected plants at 37°C for 24 h. After that, 100 µl fresh medium containing various concentrations (0.02, 0.04, 0.09, 0.18, 0.37, 0.75 and 1.5 mg/ml) of fractional extracts were added to each well, and incubated for another 48 h. Diluted fractional extracts solutions were freshly prepared in DMSO, The metabolic activity of each well was determined by the 3-(4,5 dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) assay and compared to those of untreated cells.

The HIV reverse transcriptase enzyme inhibition due to each fraction was determined using HIV RT inhibition assay by using of RetrsoSys HIV-1 RT activity kit (Innovagen, Sweden). Briefly, the diluted extract fraction are then added to a plate with reaction mixture. After 30 minutes of preincubation at 33°C, the reaction is started by the addition of a standardised amount of RT. The RT will now incorporate BrdUMP depending on the level of inhibition. The reaction is stopped by washing the plate. The product is quantified by the addition of the RT Product Tracer which binds to the incorporated BrdUMP. After removing excess tracer the amount of bound tracer is determined by an alkaline phosphatase / pNPP colour reaction. Plot the percentage of residual RT activity against the concentrations of the substance dilutions for each of the tested substances. AZT (Azidothymidine/Zidovudine) was used as positive control. The inhibitory effect of each substance is expressed as an IC_{50} value and is determined with the aid of the obtained graph. The percentage inhibition of HIV-1 RT was calculates as,

Inhibition (%) = [(A control-A sample) / A control] x 100.

For statistical analysis, the results of anti-HIV-1 RT activity were expressed as means ± SD of three determinations. The IC_{50} values were calculated using the Microsoft Excel program. Results were considered significant if the p-values were less than 0.05.

RESULTS AND DISCUSSION

The yield of methanol crude extract of *Eclipta alba* was 82 (16.4%) g. The percentage yield of these fractions of the methanolic extract of *Eclipta alba* were showed in the Table-1. The CTF fractions obtained highest yield (2.2%) when compared to other fractions. 0.8% yield obtained in HXF which is lowest.

S.No	Fractions	Yield (%)
1	HXF	0.8
2	CTF	2.2
3	CFF	1.5
4	AQF	1.2

After cells were treated with different fractions of *Eclipta alba* at various concentrations for 48 h, the cytotoxic effects were investigated using the MTT assay. Cytotoxicity of each extract fraction was determined by an inhibitory concentration at 50% growth (IC_{50}). The IC_{50} values of HXF, CTF, CFF and AQF fractions of *E. alba* on PBMCs were 1.0±0.02, >100, 1.4±0.12 and >100 mg/ml respectively. HXF and CFF fractions of *Eclipta alba* were cytotoxic (below 50% cell viability) at 1.5 mg/ml concentration in PBMC cells. AQF and CTF fraction are non-cytotoxic even at the concentration of 1.5 mg/ml.

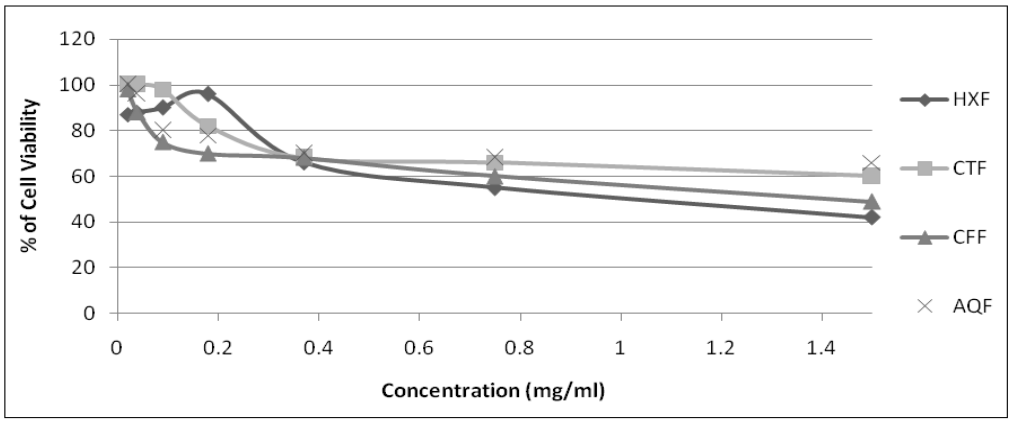

Fig 1: Effect of *E. alba* extract fractions on PBMC cells
(HXF=n-Hexane fraction, CTF=Carbon tetra chloride fraction, CFF=Chloroform fraction and AQF=Aqueous fraction)

The highest non-cytotoxic concentration of HXF, CTF, CFF and AQF were 0.18, 0.04, 0.02 and 0.02 mg/ml respectively. The CTF fraction was considered as the most effective fraction as compared to other fractions while the CFF fraction was the minor effective fraction.

Inhibition of HIV-RT by *E. alba* plant different fractions at different concentrations were presented in Fig-2. *Eclipta alba* HXF fraction at 1 mg/ml concentration shows highest (75%) HIV-RT inhibition compare to other fractions at different concentrations. At the concentration of 0.0015 mg/ml all fractions shows lower HIV-RT inhibition. At all concentration the control drug AZT shows more than 50% HIV-RT inhibition. After analysing the results from this study we stated that, *E. alba* shows more than 50% HIV-RT inhibition at the concentrations from 0.25 to 1 mg/ml in all fractions.

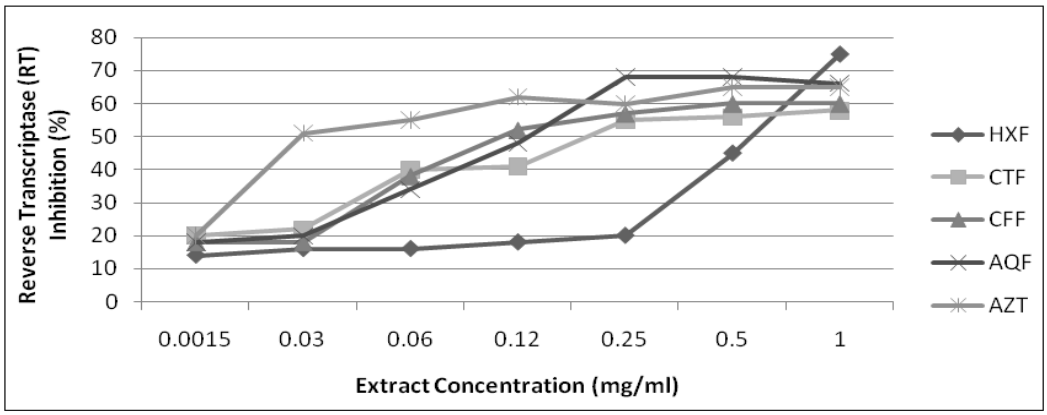

Fig 2: Inhibition of HIV-RT by *E. alba* plant different fractions at different Concentrations (HXF=n-Hexane fraction, CTF=Carbon tetra chloride fraction, CFF=Chloroform fraction, AQF=Aqueous fraction)

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