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Antiinflammatory and Antihyperlipidemic Activities of Root Extracts of *Hemidesmus indicus*: *In vitro* and *In vivo* Studies

Choudhury SS and Tetali SD*

Department of Plant Sciences, University of Hyderabad, Hyderabad, India

Abstract

Background: *Hemidesmus indicus* (HI) (Asclepiadaceae), commonly called as Indian Sarsaparilla is traditionally used for blood purification and to treat cardiovascular diseases. The present study is aimed to investigate the (i) anti-inflammatory, (ii) anti-hyperlipidemic, effect of alcoholic and water extracts of HI roots (HIAE and HIWE) using LPS/PMA induced human monocytic (THP-1) cells and high fat diet fed male Wistar rats respectively.

Methods: The phytochemical analysis of HIAE and HIWE was done using LC-Q-TOF-MS. Cytotoxicity of the plant extracts was tested by MTT assay. The anti-inflammatory effect of alcoholic and water extracts of HI roots was assayed using lipopolysaccharide (LPS) or phorbol-12-myristate-13-acetate (PMA) induced THP-1 cells. The study was performed by assaying the following markers: tumour necrosis factor alpha (TNF-α) secretion by ELISA, gene expression of proinflammatory markers by q-RT-PCR, translocation of nuclear factor kappaB (NF-κB) by confocol microscopy. Macrophage differentiation markers were also studied by q-RT-PCR. Further anti-hyperlipidemic effect was studied by testing serum lipid profile in high fat diet fed male Wistar rats.

Results: Dosage of the plant extracts limited based on cytotoxicity, there was no cell death at 1 mg dwt/mL of HIAE and HIWE. The plant extracts significantly inhibiting the gene expression of LPS induced proinflammatory markers (TNF-α, IL-6 and MIP-1α) and also secretion of TNF-α protein. The extracts also inhibited the translocation of NF-κB-p65 to the nuclei in LPS induced THP-1 cells. Extracts inhibited PMA induced differentiation of monocytes to macrophages, gene transcripts of CD14 and TLR2, however, there was no significant effect on TLR4 transcripts. Further, the study demonstrated anti-hyperlipidemic activity of HI root extracts (at 200 mg/kg body weight/day), treatments not only inhibited high fat diet induced serum lipid markers (total triglycerides, total cholesterol, LDL-cholesterol but also maintained good cholesterol, HDL.

Conclusion: The present study for the first time demonstrated anti-inflammatory (*In vitro*) and anti-hyperlipidemic (*In vivo*) effect of HI roots extracts supporting its traditional use for cardioprotective benefits.

Keywords: Inflammation; *Hemidesmus indicus*; THP-1 cells; TNF-α; Wistar rats

Abbreviations

AE: Alcoholic Extract; HDL-cholesterol: High-Density Lipoprotein Cholesterol; HFD: High Fat Diet; LDL-cholesterol: Low-Density Lipoprotein Cholesterol; PMA: Phorbol-12-Myristate-13-Acetate; TG: Tri Glycerides; TC: Total Cholesterol; WE: Eater Extract

Background

Hemidesmus indicus (L) R.Br. belongs to family Asclepiadaceae; (phylum: Magnoliophyta; order: Gentianales), commonly named as Indian Sarsaparilla. It has been used in folk medicine and Ayurvedic system of medicine to treat against the diseases of inflammation and other blood related diseases [1-2]. The dried roots of these plants are well known in Indian pharmacopoeia as the drug 'Anantmul', a common tonic prescribed for blood purification and other physiological disorders. Several scientific reports have been made on pharmacological benefits of *H. indicus* roots, such as hypoglycemic, antioxidant and cardioprotective properties using animal models [3-5]. Polyherbal formulations containing *H. indicus* (HI) shown to have hypolipidaemic activity using animal models [6-7], it also confirmed protective effect of HI in CCl4 induced alteration of lipid profile in

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Correspondence:
Sarada D. Tetali, Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Professor C. R. Rao Road, Gachibowli, Hyderabad 500046, India, Tel: +91-40-23134512; Fax: +91-40-23010120; E-mail: sdtsl@uohyd.ernet.in

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Table 1: LC-Q-TOF profile in negative and positive ion mode of HIAE and HIWE.

No	Compound name/class of compound	Mol formula/Exact mass	Types of extracts	Abundance		RT min	Reference
				-ESI	+ESI		
1	Chlorogenic acid (ester of caffeic acid)	$C_{16}H_{18}O_9$ (354.31)	HIAE	6211	10844	9.14	Mass bank
			HIWE	962	ND	9.36	
2	Lupeol acetate (Triterpenoid)	$C_{32}H_{52}O_2$ (468.75)	HIAE	13688	48472	9.96	[26]
			HIWE	5137	15668	9.38	
3	Hexatriacontane (saturated aliphatic hydrocarbon)	$CH_3(CH2)_{34}CH_3$ 506.97	HIAE	8638	22761	10.68	[27]
			HIWE	9175	ND	10.32	
4	4-terpineol (monoterpene alcohol)	$C_{19}H_{18}O$ 262.14	HIAE	ND	ND	NND	Mass bank
			HIWE	17206	33185	11.82	
5	Nerolidol (sesquiterpene)	$C_{15}H_{26}O$ (222.37)	HIAE	ND	45402	12.87	Mass bank
			HIWE	ND	26896	13.11	
6	2-hydroxy-4-methoxy benzoic acid (aromatic acid)	$C_8H_8O_4$ 168.05	HIAE	150170	ND	12.92	Mass bank
			HIWE	ND	ND	NND	
7	Hemidesmin 1 (coumarinolignoid)	$C_{21}H_{20}O_9$ (416.11)	HIAE	ND	16715	13.62	[28]
			HIWE	ND	6937	13.64	
8	Hemidesmin 2 (coumarinolignoid)	$C_{20}H_{18}O_8$ (386.1)	HIAE	ND	19521	13.86	[29]
			HIWE	19564	ND	13.21	
9	Hemidesminine (coumarinolignoid)	$C_{23}H_{22}O_8$ (424.12)	HIAE	ND	15676	16.78	[30]
			HIWE	ND	18386	16.47	
10	β-sitosterol (phytosterol)	$C_{29}H_{50}O$ (414.71)	HIAE	ND	161233	18.23	Mass bank
			HIWE	ND	144272	17.84	
11	Emidine (pregnane glycosides)	$C_{39}H_{64}O_{12}$ 724.4	HIAE	4984	ND	18.31	[31]
			HIWE	ND	ND	NND	
12	Desinine (glycoside)	$C_{37}H_{58}O_{12}$ 694.37	HIAE	ND	13292	19.14	[32]
			HWE	ND	ND	NND	
13	Rutin (glycoside)	$C_{27}H_{30}O_{16}$ (610)	HIAE	ND	69084	21.98	Mass bank
			HIWE	ND	ND	NND	
14	Lupanone (triterpene)	$C_{30}H_{48}O_4$ 472.33	HIAE	14237	ND	22.16	[33]
			HIWE	ND	ND	NND	
15	Hemidine (pregnane glycoside)	$C_{27}H_{44}O_6$ (464)	HIAE	ND	30174	23.08	[34]
			HIWE	ND	ND	ND	
16	Heminine (pregnanae glycoside)	$C_{34}H_{56}O_9$ (608.44)	HIAE	16573	20555	25.19	[34]
			HIWE	ND	ND	NND	

blood serum of rats [8].

In view of the role of activated monocytes and macrophages in the pathophysiology of atherosclerosis, the present study was designed to investigate the effect of HI-root extracts (HIAE/HIWE) on LPS and/or PMA induced activation of human monocytic (THP-1) cells and also on lipid profile of high fat diet fed rats. Increased level of lipid markers i.e hyperlipidemia in serum is associated with increased risk of cardiovascular diseases (CVD), atherosclerosis [9]. Atherosclerosis is developed due to complexed interaction between activated blood monocytes and vascular endothelium due to infection or hyperlipidemia, mediated by the release of inflammatory cytokines [9]. High fat diet promotes hyperlipidemia, eventually leads to atherosclerotic plaque formation [10].

Therefore, in the present study, high fat fed rats have been used as experimental models for testing hypolipidaemic effect of HI-root extracts.

Methods

Chemicals and reagents

Power SYBR green PCR master mix was obtained from Applied Biosystems (Carlsbad, CA, USA), BD OptEIA human TNF-α ELISA kit was purchased from BD Biosciences (USA), lipid profile kits were from ERBA diagnostic Mannheim GmbH (Germany), iScript cDNA synthesis kit from Bio-Rad (CA, USA) and nuclear factor kappaB (NF-κB-p65) polycolonal rabbit antibody from Thermo Scientific, (USA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrezolium bromide (MTT) from Merck, alexa fluor 594 goat anti-rabbit IgG (H+L), fetal bovine serum (FBS), pen-strep, prolonged gold antifade reagent with 4',6-diamidino-2-phenylindole (DAPI), RPMI 1640, trizol reagent were purchased from Invitrogen (Germany). Bovine serum albumin (BSA), lipopolysaccharide-*Escherchia coli* (LPS), paraformaldehyde,

Table 2: Effect of HI-root extracts on high fat diet induced lipid profile.

S. No	parameters	TC (mg/dL)		TG (mg/dL)		LDL-C (mg/dL)		HDL-C (mg/dL)	
		-HFD	+HFD	-HFD	+HFD	-HFD	+HFD	-HFD	+HFD
1	Control group	58.7 ± 3.34 (100%)	108.9 ± 2.13(185%)	68.1 ± 3.56 (100%)	127.7 ± 9.2 (187%)	26.7± 4.51 (100%)	64.1± 8.15 (240%)	69.3 ± 5.9 (100%)	53.6 ± 2.3 (77%)
2	HIAE	64.1 ± 3.62 (109%)	79.5 ± 5.26 (135%)	64.2 ± 7.53 (94%)	73.2 ± 8.69 (107%)	34.4 ± 9.96 (128%)	49.9 ± 9.17 (186%)	64.6 ± 4.67 (93%)	62.8 ±13.6 (91%)
3	HIWE	65.6 ± 4.58 (111%)	78.4 ± 5.19 (134%)	55.4 ± 6.45 (81%)	54.3 ± 8.47 (80%)	37.8 ± 3.51 (141%)	43.1 ± 8.25(161%)	71.4 ± 4.95(103%)	72.8 ± 6.4(101%)

Normal and HFD diet fed male Wistar rats were put on HIAE/HIWE (200 mg/kg/day) for 30 days. At the end of 30 days treatment, lipid markers of serum sample wasanalyzed by microplate method. Data is expressed as mean ± SD (n= 7 animals).Serum total cholesterol (TC); triglycerides (TG); LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C).

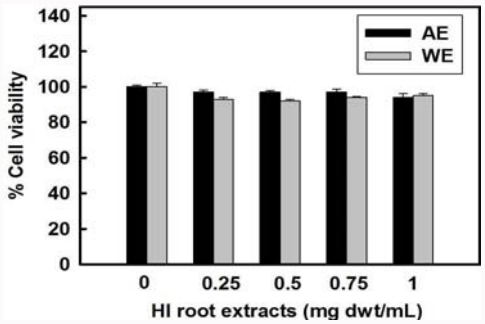

Figure 1: Cytotoxic effect of HI root extracts on human monocytic (THP-1) cells.
The cells were incubated with different concentrations of HIAE and HIWE for 24 h at 37◦C and 5% CO_2. Media without cells but with respective concentrations of plant extracts were used as appropriate blanks and the cell viability was examined by MTT assay. Vehicle control was limited to <1%. Experiments were performed at least in triplicate and the results were expressed as the mean ± S.D, n=6.

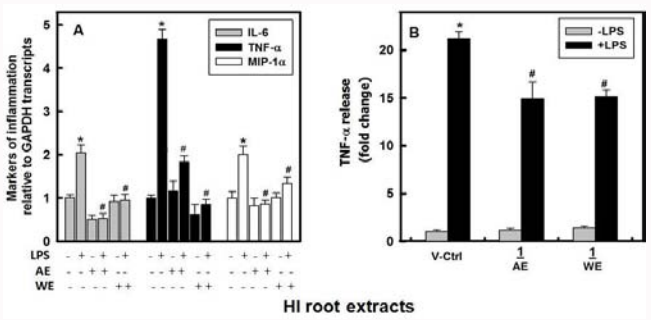

Figure 2: Effect of HI-root extracts on proinflammatory markers of LPS induced THP-1cells.
A) Effect of HI-root extracts (1 mg dwt/mL) on gene expression of IL-6; TNF-α and MIP-1α in control *vs* LPS induced THP-1 cells.
B) Effect of HI root extracts on LPS-mediated TNF-α secretion in THP-1 cells. Cells were seeded at 5x10^5 cells/mL. Data represents fold change in TNF-α release into the media by the cells treated with LPS (0.5 µg/mL for 3h) compared to untreated cells. HIAE and HIWE added to the cells 12h prior to incubation with LPS. Vehicle volume (alcohol or water) did not exceed 1% and had no effect. Experiments were performed at least in triplicate and the results are expressed as the mean ± S.D. *p<0.001 for comparison between Cells+LPS *vs* cells-LPS. #p<0.001 compared between cells treated with LPS in the presence of plant extracts *vs* in their absence.

phorbol-12-myristate-13-acetate (PMA), trypan blue and DEPC treated water were purchased from Sigma-Aldrich (Germany).

Plant material collection and preparation of HI-root extracts

The roots of the *H. indicus* (voucher # 46808) were obtained from UoH campus, authenticated by Dr. K. Venkata Ratnam and were deposited in the Department of Botany, Sri Krishanadevaraya University Herbarium (SKU), Anantapur, India. The collected roots were allowed to shade dry for at least 8 to 10 days and stored at room temperature in the dark until further use.

Alcoholic and water extracts from HI roots were prepared [11] using 1 g of dried fine root powder extracts stored until further use at 4°C not exceeding more than 3 days. The alcoholic and water extracts yielded 3.7% (w/w) and 2.9% (w/w) respectively. The effect of respective vehicle concentrations were tested in all sets of cell based experiments.

ESI-LC-MS/MS analysis

A mass spectrometry analysis of HIAE and HIWE was conducted using high performance liquid chromatography coupled to 6520 accurate quadrupole time of flight mass spectrometer (HPLC-Q-TOF-MS) (Agilent Santa Clara, CA). Metabolites of the extracts were separated using HPLC reverse phase column. Solvent A and solvent B of mobile phase were ammonium formate (1mM) with formic acid (0.1% v/v) and acetonotrile with formic acid (0.1% v/v) respectively [12]. ESI parameters: both on negative and positive ion mode; mass range 100-1700; spray voltage 4 KV; gas temperature 325°C; gas flow 10 L/min; Nebulizer 40 psi.

Cell culture and treatments

The human monocytic (THP-1) cells were obtained from National Centre for Cell Sciences, India. The cells were cultured at 37°C in 5% CO2 in RPMI media supplemented with 10% fetal bovine serum (FBS).

Cell viability with various concentrations of plant extracts was assayed using MTT reagent [11]. All the experiments were performed at a cell density of 5 x10^5 cells/mL. An acute inflammatory stimulus was given to the cells by incubating with 0.5 µg/mL of LPS for 3h at 37°C in 5% CO2. In order to stimulate the differentiation of THP-1 cells to macrophages, cells were treated with PMA (5 ng/mL) for 48 h at 37°C in 5% CO2 [13]. The images of the cells were taken under inverted microscope.

Transcript analysis

After respective treatments with LPS or PMA, in the presence or absence of plant extracts, total RNA from the cells was isolated using trizol reagent (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions. Subsequently, one microgram of RNA was used for c-DNA synthesis (Bio-Rad synthesis kits). Then the transcripts were quantitated by real time PCR using specific set of primers.

Quantification of TNF-α

Cells were incubated with or without plant extracts (1 mg dwt/mL) at 37 °C for 12h. After LPS treatment, cells were centrifuged and

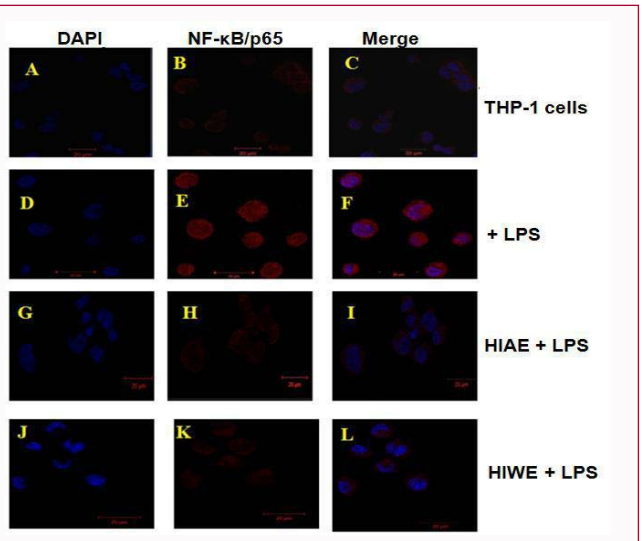

Figure 3: Effect of HI-root extracts on subcellular localization of NF-κB-p65 in LPS induced THP-1 cells.
Confocal microscopic images demonstrating the effect HI-root extracts on LPS-induced subcellular localization of NF-κB-p65 in THP-1cells. Images with vehicle control 0.5% (alcohol) cells are shown in panel 1. Vehicle control of water also had no effect (not shown). NF-κB translocation was analyzed by staining with NF-κB-p65, red (A); nucleus with DAPI, blue (B); merged image of NF-κB-p65 and DAPI (C). THP-1 cells treated with LPS (0.5 µg/mL for 3h) shown in 2nd panel (D to F); cells pretreated with HIAE and HIWE (1 mg dwt/mL) and then treated with LPS shown in 3rd (G to I) and 4th panels (J to L) respectively.

collected supernatants were stored at -80 ˚C until further use. TNF-α present in the supernatants was determined using TNF-α ELISA kit as per the manufacturer's protocol.

Nuclear translocation of nuclear factor kappaB (NF-κB)

The NF-κB-p65 in THP-1 cells was detected by indirect immunofluorescence assay using confocal microscopy [14]. After the respective treatments of plant extracts, the cells were fixed (4% paraformaldehyde) and blocked with 3% BSA. Subsequently polyclonal antibodies against NF-κB-p65 (1:250) were applied for 12h followed by 3h incubation with anti-rabbit IgG conjugated to Alexa fluor 594 antibody (1:250). The nuclei were visualized by staining with DAPI and subsequently subjected to fluorescence microscopy.

Experimental design of animals

Male Wistar rats of 126 ± 9.3 g average body weight were obtained from National Institute of Nutrition, Hyderabad and used throughout the study. The animals were maintained in the animal house on University's campus as per the CPCSEA guidelines and regulation. The animals were kept in individual cages and allowed to acclimatize for a week. The study was approved by the Institutional Animal Ethics Committee Institutional Animal Ethics Committee approved as per the guidelines of Committee for the Purpose of Control and Supervision on Experiments, New Delhi, India (IAEC/CPCSEA, LS/IAEC/SDT/11/02). Food and water were supplied *ad-libitum* continuously. Hyperlipidemia was induced feeding high fat diet (HFD) of Amul butter 2 g/day for 30 days. Rats were randomly assigned to six groups of 7 animals each. Group I: normal diet (for 30 days), group II: HFD, group III: HIAE (200 mg/kg body weight/day), group IV: HIAE (200 mg/kg body weight/day) + HFD, group V: HIWE (200 mg/kg body weight/day) and group VI: HIWE (200 mg/kg body weight/day) + HFD. At the end of 30 days, the rats were fasted overnight and sacrificed. Blood sample was obtained by cardiac puncture of anaesthetized rats. Serum was separated from the blood and subsequently analyzed for total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol using commercially available kits (Erba Diagnostics Germany). Weight of the rats was recorded before and after respective treatment periods.

Statistical analysis

To analyze the obtained data, Sigma Plot 11 software was used. All the data obtained was analysed by one way analysis of variance (ANOVA) test using statistical package for the Life Sciences (SPLS version 11). Data obtained from all the experiments was expressed as mean ± S.D of three individual experiments. *P*-values lesser than 0.001 were considered as statistically significant.

Results

Metabolite profiling of HIAE and HIWE by LC-MS/MS

In the present study, LC-Q-TOFMS was employed for metabolite fingerprinting of the plant extracts. Negative ion and positive ion modes of LC-QTOF-MS detected 7 and 12 compounds in HIAE; 4 and 7 compounds in HIWE respectively. Their abudance and RT values in minutes are shown in (Table 1). The detected compounds belong to pregnane glycoside, terpenoid or coumarinolignoid. Chlorogenic acid, lupeol acetate, hexatriacontane, nerolidol, hemidesmin 1, hemidesmin 2, hemidesminine, β-sitosterol were commonly detected in both HIAE and HIWE. 2-hydroxy-4-methoxy benzoic acid, emidine, lupanone, heminine, desinine, rutin, hemidine were exclusively detected in HIAE, while 4-terpineol was detected only in HIWE.

Effect of HIAE and HIWE on LPS induced proinflammatory gene expression

The effect of the plant extracts on LPS induced gene expression of inflammatory markers was tested at transcriptional level. The transcript analysis by real-time quantitative PCR showed that LPS stimulated significantly the expression of IL-6 (2 fold), TNF-α (4.7 fold) and macrophage inflammatory protein (MIP-1α) (2 fold) as shown in (Figure 2A). Such stimulation of proinflammatory gene expression was markedly inhibited in the cells pretreated with HIAE/HIWE (1 mg dwt/mL).

However, TNF-α has been considered as potent proinflammatory cytokine secreted by activated monocytes and promotes the progression of atherosclerosis. As shown in (Figure 2B), LPS stimulated THP-1 cells secreted 22 fold more amount of TNF-α compared to control unstimulated cells. Pretreatment of cells with 1 mg dwt/mL of HIAE brought down secretion to 14 fold and HIWE to 15 fold respectively. Therefore, rest of the cell based assays were performed with cells pretreated with plant extracts of 1 mg dwt/mL. Toxicity of plant extracts, if any, on THP-1 cells was determined by MTT assay as shown in (Figure 1). No cell death was observed up to 1 mg dwt/mL of HIAE and HIWE. Vehicle solvents (alcohol or water of <1%) had no effect on cell viability.

Effect of HIAE and HIWE on the subcellular localization of NF-kB-p65

In order to further elucidate, the molecular mechanism by which the plant extracts modulate gene expression in THP-1 cells, the effect of HIAE/HIWE on transcriptional factor NF-κB was investigated. The transcription factor NF-κB plays a crucial role in the regulation of gene expression of several proinflammatory markers including TNF-α. Results presented in the (Figure 3) clearly indicated that

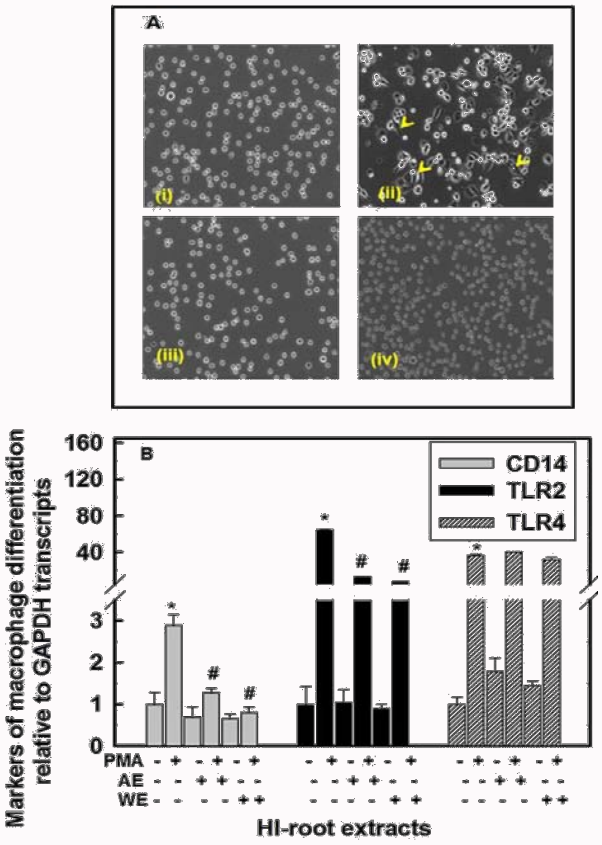

Figure 4: Effect of HI root extracts on PMA induced THP-1 cells.
A) Effect of HI root extracts on PMA induced macrophage differentiation on THP-1 cells. Cells were seeded at 5 x 10^5 cells/well. Differentiation of monocytes to macrophages were activated by PMA (5 ng/mL) for 48 h. Cells were pretreated with HIAE and HIWE for 12 h then challenged with PMA for 48 h. (i) THP-1 cells (ii) THP-1 cells + PMA (5 ng/mL); (iii) THP-1 cells + HIAE (1 mg dwt/mL) + PMA; (iv) THP-1 cells + HIWE (1 mg dwt/mL) + PMA. In figure (ii) arrow marks clearly indicates the THP-1 derived elongated, flattened, thread like differentiated macrophage cells in the presence of PMA after 48 h treatment. **B)** Effect of HI-root extracts on PMA induced CD14, TLR2 and TLR4 mRNA transcripts in THP-1cells, transcript levels of CD14, TLR2 and TLR4 were determined by RT-PCR as a measure of THP-1 cell differentiation. Vehicle control solvent (alcohol/water) were limited to < 1%. Data shown are mean value of 3 independent experiments ± S.D. *p<0.001, for comparison between treated (cells + PMA) and untreated groups (cells - PMA). #p<0.001 compared between cells treated with PMA in the presence of HIAE and HIWE *vs* in their absence.

the plant extracts inhibited LPS induced translocation of NF-κB in THP-1 cells. The confocal images of (Figures 3A-3C) (1st panel) showed that NF-κB-p65 was mostly sequestered in the cytoplasm of normal uninduced cells, whereas it was predominately located in the nuclei of the LPS stimulated cells (Figures 3D-3F). Such LPS dependent nuclear translocation of NF-κB-p65 was blocked in the cells pretreated with HIAE (Figures 3G-3I) and HIWE (Figures 3J-3L). These results demonstrated that HIAE and HIWE elicited anti-inflammatory activity in THP-1 cells by modulating subcellular localization of transcriptional factor NF-κB.

Effect of HIAE and HIWE on PMA induced macrophage differentiated markers

Human monocytic (THP-1) cells are suspension in nature and do not adhere to the plastic surfaces of the culture plates as shown in (Figure 4A(i)). Treatment with PMA (5 ng/mL) stimulated the differentiation of THP-1 cells into macrophages as indicated by their adherence to substratum of the culture dish (Figure 4A(ii)). The adherent cells showed morphological characteristics similar to macrophages. The effect of alcoholic and water extracts of HI roots on such differentiation process was tested by treating the cells with PMA in the presence or absence of plant extracts. Pretreatment with HIAE or HIWE (1 mg dwt/mL) significantly attenuated the process of differentiation in PMA challenged THP-1 cells as shown in (Figures 4A(iii,iv)).

Literature survey clearly demonstrates that differentiated monocytic cells compared to monocytic cells had an increased expression of CD14, TLR2 and TLR4 which are macrophage-specific antigens. Therefore PMA differentiated THP-1 cells were examined by quantitative real-time PCR to check the expression of selected genes CD14, TLR2 and TLR4 compared to untreated cells on the basis of fold changes. Results showed that PMA upregulated the expression of CD14 by 3 fold and toll like receptors (TLR2 and TLR4) by 64 and 37 fold respectively (Figure 4B). Such upregulation of the markers was markedly attenuated by HIAE/HIWE (1 mg dwt/mL). However, plant extract did not show much effect on TLR4 expression (Figure 4B).

Effect of HIAE and HIWE on HFD induced serum lipid profile in rats

Administration of HFD in rats shows significant increase in their serum lipid markers like total cholesterol:108 mg/dL(185%); triglyceride: 127 mg/dL (187%); and LDL-C: 64 mg/dL (240%) levels with respect to the baseline value of normal rats (Table 2) whereas serum HDL-cholesterol level has reduced to 53 mg/dL(77%). In the present study, co-treatment with HIAE or HIWE prevented such HFD induced increase in serum total cholesterol, LDL-cholesterol, triglycerides and decrease in HDL-cholesterol as shown in (Table 2).

Discussion

In recent years, increasing interest has been recognized in developing and developed countries for use of herbal products for maintaining primary health care [15]. Polyherbal drug containing HI-roots namely PHF-AROGH and RO7D are shown for their analgesic and antiulcer properties in animal models [16-17]. However, mechanism of action of HI-roots in offering anti-inflammatory property and its relevance to cardiac health remains unclear. To the best of our knowledge, this is the first attempt that has been made to understand the biochemical basis for anti-inflammatory and anti-hyperlipidemic activity using human cell culture and animal model.

In this study, acute inflammatory model i.e. LPS/PMA induced THP-1 cells were used to evaluate anti-inflammatory activity of HI-roots. TNF-α is a potent proinflammatory cytokine secreted by monocytes in the blood and plays a major role in developing many inflammatory diseases [18]. The markers studied IL-6 and MIP-1α play a significant role in inflammation associated with atherosclerotic lesion development and LPS activates the expression of selected markers via nuclear translocation of NF-κB pathway [19]. HI-root extracts significantly inhibited gene expression of above markers and also nuclear translocation of NF-κB in LPS stimulated THP-1 cells. These results suggest that the plant extracts regulate gene expression by modulating subcellular localization of NF-κB. Studies in *Apoe*$^{-/-}$/*Myd88*$^{-/-}$ double knockout mice demonstrated that CD14, TLR2 and TLR4 are strongly associated with inflammation and atherosclerosis development [20]. The extracts inhibited the expression of PMA induced cell differentiation markers CD14 and TLR2 as well as

morphological characteristics of macrophages.

Dietary cholesterol used as composition of HFD causes increase in serum cholesterol and decrease in HDL-cholesterol and it has been widely known that elevation of serum cholesterol and triglyceride can lead to atherosclerosis and coronary heart diseases and High levels of LDL cholesterol are deposited in the interior of blood vessels resulting in hardening of arteries and narrowing of the blood vessels [21]. There are few reports published on hypolipidaemic activity of polyherbal formulations containing *H. indicus* (HI) [6-8] or its callus cell culture extract using animal models [22]. In the present study, co-treatment of HI-root extracts significantly reduced serum lipid level in high fat diet fed rat model demonstrating hypolipidaemic activity of phytoconstituents of HI root extracts.

Furthermore, LC-MS analysis showed the presence of several bioactive secondary metabolites in HIAE and HIWE. Compounds namely 2-hydroxy-4-methoxy benzoic acid, β-sitosterol shown for their pharmacological activities [23,24]. Chlorogenic acid (37 µg/mL) showed for their LPS induced anti-inflammatory property in RAW264.7 cells [24]. In summary, the present study validated traditional use of HI roots for cardioprotective effect using THP-1 cells and Wistar rats and also showed several bioactive compounds can be extracted using solvents like alcohol and water extracts. *H. indicus* which can be grown widely in tropical and subtropical regions of the world, therefore, HI-roots can be considered as potential resource drug candidates for treating inflammatory diseases like atherosclerosis.

Conclusion

The present study highlights the effectiveness of HI root extracts in attenuating the proinflammatory and hyperlipidemic response. Critical cellular and molecular investigation of the present study demonstrates that alcoholic and water extracts of HI roots: (i) Had bioactive compounds known to have anti-inflammatory properties like pregnane glycoside, terpenoid, coumarinolignoid (ii) Can attenuate LPS induced activation of proinflammatory gene expression in THP-1 cells by inhibiting nuclear translocation of NF-κB; (iii) Can inhibit PMA induced monocyte to macrophage differentiation; (iv) Can significantly reduced the HFD fed hyperlipidemia in animal model. Thus the present study supports the anti-inflammatory and anti-hyperlipidaemic activity of HI-roots. Further studies using *in vivo* models like Apoe-/- atherosclerotic mice would help in validating use of *H. indicus* roots in treating cardiovascular diseases.

Author's Contribution

SSC involved in performing experiments, data analysis and writing the manuscript. SDT involved in inception of the project, raising funds required to perform the experiments, designing and directing of all the experiments, correction and evaluation of entire manuscript.

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References

1. Lakshman K, Shivaprasad HN, Jaiprakash B, Mohan S. Antiinflammatory and antipyretic activities of *Hemidsmus indicus* root extract. Afr. J. Tradit. Complement. Altern Med. 2006;3:90-4.
2. Chidrawar VR, Ushir YV, Sudarshan S, Patel KN, Patel NJ, Vadalia KR. Possible Role of Natural Nephroprotective; *Hemidesmus indicus* in Congestive Heart Failure. Phcog Res. 2009;1:367-74.
3. Murshed S, Rokeya B, Nahar N. Hypoglycemic and hypolipidemic effect of *Hemidesmus indicus* root on diabetic model rats. J Diabetes Res. 2005;39:15-23.
4. Saravanan N, Nalini N. Antioxidant effect of *Hemidesmus indicus* on ethanol-induced hepatotoxicity in rats. J Med Food. 2007;10(4):675-82.
5. Zarei M, Javarappa KK, Zarei M, Baker S. Cardioprotective effect of the root extract of *Hemidesmus indicus* against doxorubicin -induced oxidative stress in mice. Der Pharmacia Lettre. 2013;5:334-9.
6. Asokan BR, Jaikumar S, Ramaswamy S, Thirugnanasambandam P, Nirmala P. Anti-hyperlipidemic activity of a poly herbal formulation in experimental models. Pharmacologyonline. 2010;1:433-442.
7. Mary NK, Achuthan CR, Babu BH, Padikkala J. Antiatherogenic effect of Caps HT2, a herbal Ayurvedic medicine formulation. Phytomedicine. 2003;10:474-82.
8. Murali A, Ashok P, Madhavan V. Protection against CCl4 induced hepatotoxicity by pretreatment with methanol extract of Hemidesmus indicus var. pubescens leaf in Wistar rats. Int J Appl Res Nat Product. 2012;5 (2):5-13.
9. Ross R. Atheroscelerosis-an inflammatory disease. N. Engl. J. Med. 1999;340:115–26.
10. Warnholtz A, Mollnau H, Oelze M, Wendt M, Munzel T. Antioxidants and endothelial dysfunction in hyperlipidemia. Curr Hypertens Rep. 2001;3:53-60.
11. Kokkiripati PK, Kamsala RV, Bashyam L, Manthapuram N, Bitla P, Peddada V, et al. Stem-bark of Terminalia arjuna attenuates human monocytic (THP-1) and aortic endothelial cell activation. J. Ethnopharmacol. 2013;146:456-64.
12. Surveswaran S, Cai YZ, Xing J, Corke H, Sun M. Antioxidant properties and principal phenolic phytochemicals of Indian medicinal plants from Asclepiadoideae and Periplocoideae. Nat Prod Rep. 2010;24:206–21.
13. Tsuchiya S, Kobayashi Y, Goto Y, Okumura H, Nakae S, Konno T, et al. Induction of maturation in culture human monocytic leukemia cells by phorbol diester. Cancer Res. 1982;42:1530–6.
14. Jeong JW, Jin CY, Kim GY, Lee GD, Park C, Kim GD, et al., Anti-inflammatory effects of cordycepin via suppression of inflammatory mediators in BV2 microglial cells. Int Immunopharmacol. 2010;10:1580-6.
15. Pal SK1, Shukla Y. Herbal medicine: current status and the future. Asian Pac J Cancer Prev. 2003;4(4):281-8.
16. Mohan M, Gulecha VS, Aurangabadkar VM, Balaraman R, Austin A, Thirugnanasampathan S. Analgesic and antiinflammatory activity of a polyherbal formulation (PHFAROGH). Orient Pharm Exp Med. 2009;9:232-7.
17. Jaikumar S, Asokan BR, Sengottuvelu S, Ramaswamy S. Antiulcer activity

of polyherbal formulation-RO7D on experimentally induced ulcer in rats. Pharmacologyonline. 2009;3:419-23.

18. Csiszar A, Smith K, Labinskyy N, Orosz Z, Rivera A, Ungvari Z. Resveratrol attenuates TNF-alpha-induced activation of coronary arterial endothelial cells: role of NF-kappa B inhibition. Am J Physiol Heart Circ Physiol. 2006;291:1694-1703.18.
19. Magni P, Ruscica M, Dozio E, Rizzi E, Beretta G, Maffei Facino R. Parthenolide inhibits the LPS-induced secretion of IL-6 and TNF-α and NF-κB nuclear translocation in BV-2 microglia. Phytother Res. 2012;26(9):1405-9.
20. Bjorkbacka H, Kunjathoor VV, Moore KJ, Koehn S, Ordija CM, Lee MA, et al. Reduced atherosclerosis in MyD88-null mice links elevated serum cholesterol levels to activation of innate immunity signaling pathways. Nat Med. 2004;10(4):416-21.
21. Freedman JE. High-fat diets and cardiovascular disease: are nutritional supplements useful? J Am Coll Cardiol. 2003;41(10):1750-2.
22. Bopanna, KN, Bhagyalakshmi N, Rathod SP, Balaraman R, Kkannan, J. Cell culture derived *hemidesmus lndicus* in the prevention of hypercholesterolemia in normal and hyperlipidemic rats. Indian J Pharmacol. 1997;29:105-9.
23. Alam MI, Gomes A. Viper venom induced inflammation and inhibition of free radicals formation by pure compound (2-hydroxy-4-methoxy benzoic acid) isolated and purified from Anantamul (*Hemidesmus indicus* R.Br) root extract. Toxicon. 1998;36:207-15.
24. Loizou S, Lekakis L, Chrousos GP, Moutsatsou P. ß-Sitosterol exhibits anti-inflammatory activity in human aortic endothelial cells. Mol Nutr Food Res. 2010;54: 551-8.
25. Shan JH, Fu J, Zhao Z, Kong X, Huang H, Luo L, et al. Chlorogenic acid inhibits lipopolysaccharide-induced cyclooxygenase-2 expression in RAW264.7 cells through suppressing NF-kappaB and JNK/AP-1 activation. Int Immunopharmacol. 2009;9:1042-8.
26. Nasser JA, Al-Fadhli AA. Chemical Study on stem bark of Etlingera hornstedia. Intl J Agri Crop Sci. 2009;1(1):21-3.
27. Ananthakrishnan TN, Senrayan R, Murugesan S, Annadurai RS. Kairomones of *Heliothis armigera* and *Corcyra cephalonica* and their influence on the parasitic potential of *Trichogramma chilonis* (Trichogrammatidae: Hymenoptera). J Biosci. 1991;16:111-9.
28. Das PC, Joshi PC, Mandal S, Das A, Chatterjee A. New Coumarinolignoids from *Hemidesmus indicus* R. Br. Indian J Chem. 1992; Sect B 31: 342-343.
29. Mandal S, Das PC, Joshi PC, Chatterjee A. Hemidesminine-A new coumarinolignoid from *Hemidesmus indicus* R. Br. Indian J Chem. 1991; Sect B 30:712-3.
30. Chandra R, Deepak D, Khare A. Pregnane glycosides from *Hemidesmus indicus*. Phytochemistry. 1994; 35:1545-8.
31. Oberai K, Khare MP, Khare A. Pregnane ester diglycoside from *Hemidesmus indicus*. Phyrochemistry. 1985;24:2395-7.
32. Ashim G, Bittu S, Prasad PB, Pranab G: Selenium dioxide oxidation of oxime derivative of lupanone and antimicrobial activity of the oxidized products. Res. J. Chem. Sci. 2013;3:64-8.
33. Prakash K, Sethi A, Deepak D, Khare A. Khare MP. Two pregnane glycosides from *Hemidesmus indicus*. Phytochemistry. 1991;30:297-9.
34. Sigler P, Saksena R, Deepak D, Khare A. C21 steroidal glycosides from *Hemidesmus indicus*. Phytochemistry. 2000;54(8):983-7.