

## Anticancer and anti-inflammatory activities of extracts of *Merremia emerginata*

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### ABSTRACT

Anticancer and Anti-inflammatory activities of Hexane(IA), Ethyl acetate(IB) and Methanol(IC) extracts of *Merremia emerginata* were studied by testing the inhibitory activity against cancer cell proliferation and pro-inflammatory cytokine TNF  $\alpha$ . Three solvent extracts were tested on cell proliferation of different cancer cell lines like DU-145 (Human Prostate Carcinoma), KB (Mouth; Carcinoma), A549 (Human; Lung; Carcinoma) and MIA-PaCa2 (Human; Pancreas; Carcinoma). Ethyl acetate extract of *M. emerginata* was found to be showed inhibitory effect on KB, DU-145, A549 and MIA-PaCa<sub>2</sub> cell lines followed by methanol and hexane extracts. The results showed that ethyl acetate extract has anti-inflammatory activity even at 5.986 $\mu$ g/ml and had a significant cytotoxic activity on different cells. The extracts of *M. emerginata* were screened to inhibit the TNF  $\alpha$  secretion in LPS induced THP-1 cells. Among three extracts, ethyl acetate extract exhibited good activity against TNF  $\alpha$  production and the half maximal inhibitory concentration (IC<sub>50</sub>) was 5.9  $\mu$ g/ml followed by IC.

**Key words:** *Merremia emerginata*, anticancer and anti-inflammatory activity.

### INTRODUCTION

Natural Products, especially plants, have been used for the treatment of various diseases for thousands of years. Terrestrial plants have been used as medicines in Egypt, China, India and Greece from ancient time and an impressive number of modern drugs have been developed<sup>1</sup> from them. According to the World Health Organization, about three quarters of the world's population currently uses herbs and other forms of traditional medicine to treat diseases<sup>2</sup>. The plant *Merremia emerginata*, commonly known as Elikajemudu<sup>3</sup> belongs to *Convolvulaceae* family (or Morning Glory family). In India, it is mainly found Chennai and in some places of Andhra Pradesh<sup>4</sup>. Its therapeutic uses<sup>5</sup> are, whole plant is useful in cough, headache, neuralgia and rheumatism; leaves powder used as snuff during epileptic seizures; juice

acts as purgative, given internally for headache and migraine; as an ear-drop in cases of abscess; root and powdered leaves mixed with floor and water applied externally for swelling.

The areas of cancer and infectious diseases have a leading position in utilization of medicinal plants as a source of drug discovery. Among FDA approved anticancer and anti-infectious preparations drugs of natural origin have a share of 60% and 75% respectively. In many countries, cancer is the second leading cause of death after heart diseases<sup>6</sup>. The estimated worldwide incidence of different carcinomas is about 10 million; half of these are in developed countries<sup>7</sup>. Among the cancer patients in the USA, the use of complementary and alternative medicine was represented mainly by plants, ranges<sup>8</sup> between 30-75%. In response to inflammatory stimulation,

secretion of pro-inflammatory cytokines from macrophages or monocytes is essential criteria. Among pro-inflammatory cytokines, TNF  $\alpha$  play a vital role in the inflammation process. Tumor necrosis factor  $\alpha$  (TNF  $\alpha$ ) is a pleotropic cytokine produced by the immune system. Subsequent studies established that TNF  $\alpha$  is a key mediator of inflammation. Activated monocytes, macrophages, fibroblasts, and T cells release numerous cytokines like TNF  $\alpha$  and interleukin-1<sup>9</sup>. Anticancer and anti-inflammatory herbal extracts can be screened by testing the inhibitory potential against different cancer cell proliferation and inflammatory mediators like TNF  $\alpha$ . The aim of this study was to evaluate the anticancer and anti-inflammatory activities of different crude solvent extracts isolated from *M. emerginata*.

## MATERIALS AND METHODS

### Plant material

The plant material was collected from the Botanical garden of Acharya Nagarjuna University, Nagarjunanagar in the month of August 2008 and authenticated by the Department of Botany.

### Preparation of Extracts

The air dried finely powdered plant material (1kg) was transferred into a silica gel column and chromatographed different solvents starting with hexane, ethyl acetate and methanol. The elutions were collected individually and dried in vacuum to get extracts of hexane (IA), ethyl acetate (IB) and methanol (IC).

### Biological activity studies

#### Anticancer activities of the crude extracts

The inhibitory activity of the extracts was tested on cell proliferation of DU-145 (Human Prostate Carcinoma), KB (Mouth; Carcinoma), A549 (Human; Lung; Carcinoma) and MIA-PaCa2 (Human; Pancreas; Carcinoma) using MTT assay<sup>10</sup>. The cell lines were obtained from National Centre for Cell Science (NCCS), Pune (India) and cultivated in Dulbecco's modified Eagle's red medium (DMEM) (Sigma Life Science, USA) containing 10% fetal bovine serum (FBS). The cells (2000 cells per well) were seeded in a 96-well micro plate containing 100  $\mu$ l of DMEM+10% FBS medium per well and incubated at 37°C with 5% CO<sub>2</sub>. The cells were

treated different concentration of compounds (0-50  $\mu$ g/ml) up to 72 h. Controls were maintained with 0.5% DMSO. After 72 h treatment, 5 $\mu$ l of MTT reagent (R&D Systems, USA) along with 45  $\mu$ l of DMEM white medium (Sigma Life Science, USA) without FBS was added to each well and plates were incubated at 37°C with 5% CO<sub>2</sub> for 4 h. Thereafter, 50  $\mu$ l of solubilization buffer (R&D Systems, USA) was added to each well to dissolve the colored formazan crystals produced by the reduction of MTT. After 24 h, the optical density was measured at 550 nm using micro plate reader (Bio-Rad, USA). The same procedure was followed for other cell lines used in the present investigation.

#### Anti inflammatory activity

THP-1 (Monocyte; Human; Acute monocytic leukemia) cells were harvested from the 75 cm<sup>2</sup> flask and washed with phenol red free DM. Washed cells were resuspended in phenol red free DMEM containing 1% FBS and 200,000 cells in 500 $\mu$ l medium were plated in each well of 24-well plate. Cells were treated with different conc. of drugs for 2 hours. Then the cells were incubated with 100ng/ml of LPS (Lipopolysaccharide) for further 4 hours. Cells incubated with 0.1% DMSO were treated as the vehicle control. After 4 hours incubation, cell cultures were centrifuged at 3000g for 15 minutes at 4°C. Collect the supernatant and store at -80°C. Tumor Necrosis Factor  $\alpha$  (TNF- $\alpha$ ) determination by ELISA Pro-inflammatory cytokine, TNF- $\alpha$  was quantitatively measured in the cell culture supernatant by human TNF- $\alpha$  (R&D Systems, USA).

Briefly, 96-well microtiter plates (Corning, MA) were coated with anti-TNF- $\alpha$  capture antibody and the following blocking non-specific binding sites, the wells were reacted with cell free culture supernatants (clarified at 10,000 g/10min/4°C). Specifically bound cytokines were probed with TNF- $\alpha$  detection antibody and followed by Streptavidin-HRP conjugate. The color reaction was developed by 3,3',5,5'-tetramethyl benzidine in the presence of H<sub>2</sub>O<sub>2</sub>. Finally, the absorbance was read at 450 nm in an ELISA reader (Bio Rad, Hercules, CA). Standard wells contained known concentrations of Recombinant human TNF- $\alpha$ . Standard curves were plotting the O.D at respective concentrations of standard<sup>11</sup>.

## RESULTS AND DISCUSSION

Anticancer and anti-inflammatory (Inhibition of Human TNF-  $\alpha$ ) activity results of the extracts IA, IB and IC of *M. emerginata* were incorporated in Tables 1 and 2 respectively. The inhibitory activities of three extracts were studied on cell proliferation of KB, DU-145, A549 and MIA-PaCa<sub>2</sub> using MTT assay. Among the three different extracts, IB was found to be showed inhibitory effect on KB, DU-145, A549 and MIA-PaCa<sub>2</sub> cell lines followed by IC and IA (Table 1). The extract IB was exhibiting a promising inhibitory action on human lung cancer cell line (A549) followed by Mouth carcinoma (KB), pancreatic carcinoma (MIA-PaCa<sub>2</sub>)

and prostate carcinoma (DU-145). Plant derived agents are being used for the treatment of cancer. Several anticancer agents including taxol, vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, and etoposide derived from epipodophyllotoxin are in clinical use all over the world. A number of promising agents such as flavopiridol, roscovitine, combretastatin A-4, betulinic acid and silvestrol are in clinical or preclinical development<sup>12</sup>.

The extracts of *M. emerginata* were screened to inhibit the TNF  $\alpha$  secretion in LPS induced THP-1 cells. Among three extracts, ethyl acetate extract exhibited good activity against TNF

**Table 1: Anticancer activity of extracts of *Merremia emerginata***

Extract	IC <sub>50</sub> ( $\mu$ g/ml)			
	KB	DU-145	A549	MIA-PaCa2
IA	110	174.2	163.5	133
IB	37.2	69.4	28.5	51
IC	103.5	92.9	86.4	95.6

**Table 2: Anti-inflammatory activity of extracts of *Merremia emerginata***

Extract	IC50 ( $\mu$ g/ml)
IA	14% inhibition at 50 $\mu$ g/ml
IB	5.9
IC	36.4

$\alpha$  production and the half maximal inhibitory concentration (IC<sub>50</sub>) was 5.9  $\mu$ g/ml followed by IC. But the hexane extract was showing weak anticancer as well as anti-inflammatory properties when compared to other extracts. LPS is regarded as a potent activator for inflammatory response in monocytes and it activates MAP kinase enzymes and that led to enhanced production of TNF  $\alpha$ <sup>13</sup>. TNF  $\alpha$  serves as a key regulating factor of inflammatory diseases such as rheumatoid arthritis, Crohn's disease, psoriasis, asthma and

atherosclerosis, among others<sup>14</sup>. *In vitro* study of the three extracts, especially ethyl acetate extract from *M. emerginata* revealed a positive indication in anticancer and anti-inflammatory properties. Attempts are in progress to elucidate the bioactive compounds present in IB and their mechanism of action.

## CONCLUSIONS

The ethyl acetate extract has good anti-inflammatory activity at 5.986 $\mu$ g/ml and had a significant cytotoxic activity on different cells(KB, DU-145, A549 and MIA-PaCa<sub>2</sub> cells).

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