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Inhibition of pulmonary metastasis by *Emilia sonchifolia* (L.) DC: An *in vivo* experimental study

Gilcy George K, Girija Kuttan*

Department of Immunology, Amala Cancer Research Centre (Affiliated to the University of Calicut), Thrissur 680 555, Kerala State, India

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ABSTRACT

Background: *Emilia sonchifolia* (L.) DC is a widely distributed medicinal herb used mainly in the indigenous Ayurvedic system of medicine in India. This plant is one among the ten sacred plants of Kerala state in India, collectively known as Dasapushpam.
Purpose: To assess the therapeutic efficacy of this well-known medicinal plant in a catastrophic complication like metastatic cancer progression. This study further aimed to scientifically validate the traditional medicinal use of this sacred plant.
Study design: Highly metastatic B16F10 melanoma will spontaneously metastasize in C57BL/6 mice and is accepted as a useful murine model for the study on metastasis. Three different experimental modalities of prophylactic, simultaneous and after tumour development were used for data accumulation and analysis.
Methods: Whole plant genuine extract of *E. sonchifolia* (25 mg/kg bodyweight) was administered intraperitoneally to C57BL/6 mice. Animals were sacrificed on 21st day after tumour induction and the lung tumour nodules were counted. Various lung and serum biochemical parameters along with major cytokine levels were recorded. Survival rate was monitored. Histopathology of the lung tissue and expression studies of the major genes involved in metastasis was also carried out.
Results: *E. sonchifolia* significantly inhibited pulmonary tumour formation and increased the life span of animals. Lung collagen hydroxyproline, uronic acid, hexosamine, serum sialic acid, γ-glutamyl transpeptidase, vascular endothelial growth factor (VEGF), granulocyte monocyte colony-stimulating factor and other cytokine levels were significantly lowered in the treated group of animals. Histopathological analysis was also correlated with these findings. *E. sonchifolia* down regulated the expression of matrix metalloproteinases; extracellular signal-regulated kinases and VEGF at the same time up regulated the expression of tissue inhibitor of matrix metalloproteinases.
Conclusion: Previous studies on *E. sonchifolia* proved its significant biological properties including antitumour, anti-inflammatory and antioxidant activities. Present report is so far the first study to demonstrate the anti-metastatic potential of this medicinal herb justifying its conventional use in the traditional medicine.

Introduction

One of the major causes of death in cancer patients is due to the ability of tumour cells to metastasize. Metastasis is a complex process that requires malignant cells to leave the primary tumour and proliferate at a distant site. The incidence of serious side effects limits the therapeutic application of cancer treatment using chemotherapeutic agents and ionizing radiations. Therefore, additional therapeutic approaches to eliminate these limitations must be established. Many traditional cancer therapies can improve key aspects of anti-cancer immunity by inducing tumour cell death in a way that is immunostimulatory or by modulating tumour-induced immunosuppression (Nowak et al. 2003). Agents that are able to block metastatic process of tumour cells have wide potential as anticancer agents therefore, it is essential to search for novel antimetastatic agents with minimum side effects. Hence there is an incitement to find out newer drugs with less toxic effects to prevent metastasis.

Emilia sonchifolia (L.) DC (*E. sonchifolia*), the Lilac tassel flower belonging to the family Asteraceae with the local name of

Abbreviations: Erk, extracellular signal-regulated kinase; GGT, gamma glutamyl transpeptidase; GM-CSF, granulocyte monocyte colony-stimulating factor; IL-1β, interleukin-1β; IL-6, interleukin-6; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of matrix metalloproteinase; TNF –α, tumour necrosis factor-α; VEGF, vascular endothelial growth factor.

* Corresponding author. Tel.: +91 487 2307968; fax: +91 487 2307968.
E-mail address: immunologyacrc@gmail.com (G. Kuttan).

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Muyalchevian in Kerala state of India, is an edible plant used in the Ayurvedic system of medicine for the treatment of gastropathy, diarrhoea, ophthalmia, nyctalopia, cuts and wounds, intermittent fevers, pharyngodyma and asthma (Nair and Chopra 1996). This plant is used in the folklore medicine for treating tumour and inflammation (Shylesh et al. 2005). Previous studies conducted on this plant revealed its anti inflammatory (Nworu et al. 2012) and anti tumour (Shylesh and Padikkala 2000) properties. There are reports on *E. sonchifolia* that evince its protective effect on oxidative stress and modulation of selenite cataract (Lija et al. 2006) and antinociceptive effect (Couto et al. 2011). Most recently the immunomodulatory effect was reported from our laboratory (Gilcy and Kuttan 2015). Studies on the apoptotic activity of this plant on cancer cells (Lan et al. 2012) further proved its anticancer potential. The present study was designed to investigate the antimetastatic activity of *E. sonchifolia,* with already proven antitumour activity.

Materials and methods

Plant material

The authenticated whole plants, including roots and areal parts of *E. sonchifolia* (L.) DC collected locally was obtained from Amala Ayurveda Pharmacy, Thrissur district, Kerala state, India in July 2011, and the voucher specimen is deposited at the herbarium of Amala Cancer Research Centre (Voucher No. 108/ACRC). The plant name has been checked with http://www.theplantlist.org and the original publication was the contribution to the botany of India by Robert Wight (International plant names index (IPNI) with id 203080-1). Standardized whole plant genuine extract of *E. sonchifolia* (L.) DC was used for the study in accordance to the European Medicines Agency (EMA) guidelines. The whole plants of *E. sonchifolia* including roots stem and leave were dried at 45 °C, then crushed and coarsely powdered. Approximately 100 g of the whole plant powder was extracted with 500 ml, 70% v/v methanol in a soxhlet apparatus for 24 h. After extraction the solvent was evaporated to dryness at 42 °C under reduced pressure using rotary evaporator. The yield of the dried whole plant genuine extract of *E. sonchifolia* was 17% (w/w) with a drug extract ratio of 100 g of the initial whole plant powder; 17 g of the whole plant genuine extract. The composition of the extract was analysed using Saturn 2200 (Varian, Inc., CA, USA) GC/MS system equipped with fused silica capillary column (30 m x 0.25 mm x 0.25 μm) coupled to a mass selective detector, operated in EI mode (70 eV). Helium was the carrier gas at flow rate of 1 ml/min. The injector and detector temperature were maintained at 250 °C and 300 °C respectively. The oven temperature was programmed from 100 to 150 °C at 4 °C/min and then held at 270 °C for 20 min. Sample volume 1 μl was injected with 1:20 split ratio. Scan interval was 0.5 s with mass range, m/z 40–600. Standardization of the genuine *E. sonchifolia* whole plant extract with unknown composition and the identification of the marker compounds (Gilcy and Kuttan 2015) was made by comparing retention indices and mass spectra with those in the literature, as well as by computerized matching of the acquired mass spectra with those stored in the NIST and Wiley mass spectral library and other published mass spectra.

Animals, cell line and experimental protocol

Healthy adult male C57BL/6 mice (6–8 weeks old) weighing 25–28 g were accommodated in individual ventilated cages fed with normal mice chow and water *ad libitum*. All the animal experiments were conducted in accordance with the internationally accepted principles for laboratory animal use and care and carried out with the prior approval of the Institutional Animal Ethics Committee (IAEC) and were conducted strictly adhering to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) constituted by the Animal Welfare Division of Government of India (Sanction No. 149/1999/CPCSEA).

B16F-10 melanoma cell line was obtained from the National Centre for Cell Sciences, India.

Intraperitoneal route of drug administration is the commonly used route in small animals like mice because of the greater bioavailability, to reduce the chance of degradation by gastric juice and it offers a convenient alternative model to the intravenous administration of anticancer drugs in humans. Moreover the intraperitoneal administration of this immunomodulatory herbal extract (Gilcy and Kuttan 2015) with fast absorption to the vasculature has a direct effect on the cancer cells. The whole plant genuine *E. sonchifolia* methanolic extract in different concentrations (200, 100, 50, 25 mg/kg body wt.) were administrated intraperitoneally in mice for 14 days and observed for mortality, behavioural changes, and change in body weight. On 15th day, all the animals were sacrificed by cervical dislocation and selected organs such as liver, spleen, thymus, kidney, and lungs were dissected out and weights were recorded. Blood was collected by heart puncture; serum separated and was used for the analysis of hepatic and renal functions. Liver function markers, such as alkaline phosphatase (ALP), glutamate pyruvate transaminase (GPT), and kidney function markers such as creatinine and blood urea nitrogen were determined.

For animal experiments 25 mg/kg of the whole plant genuine extract was resuspended in 1% gum acacia and administered intraperitoneally (ip) in 3 different modalities as follows:

(I) Prophylactically with tumour induction: animals were treated with 10 consecutive doses prior to B16F10 tumour cell administration.
(II) Simultaneously with tumour induction: given to animals simultaneously with B16F10 metastatic tumour cells for 10 consecutive days.
(III) After tumour development: administration was done 7 days after B16F10 melanoma induction for 10 consecutive days.

Apparatus and chemicals

Thermal cycler (MJ Research, USA), Gel documentation system (Vilber Lourmat, France) and ELISA plate reader (ThermoLabsystems, USA) were used for the study. Hydroxyproline, glucuronic acid lactone and oligonucleotide primer sequences were purchased from Sigma Aldrich (Bangalore, India). *N*-Acetyl neuraminic acid was purchased from Sisco Research Laboratory (Mumbai, India). Specific quantitative sandwich ELISA kits for mouse IL-1β, IL-6, TNF-α and GMCSF were obtained from Pierce Biotechnology, Rock ford, IL, USA. ELISA kit for VEGF and TIMP was purchased from R&D Systems (Minneapolis, Minn., USA). All other chemicals used were of analytical reagent grade.

Determination of antimetastatic activity of E. sonchifolia

B16F10 melanoma cells (10^6 cells/animal) were injected through the lateral tail vein of C57BL/6 mice which serve as the metastatic tumour model.

Lung tumour nodule formation and rate of survival

C57BL/6 mice were divided into 4 groups (16 animals/ group). Pulmonary metastasis was induced to all the animals as described above. Group I animals were kept as untreated metastatic tumour bearing control. Treatment in Group II animals were done prophylactically, in group III animals simultaneously and in group