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Short communication

Proliferative activity of a blend of *Echinacea angustifolia* and *Echinacea purpurea* root extracts in human vein epithelial, HeLa, and QBC-939 cell lines, but not in Beas-2b cell lines

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ABSTRACT

Echinacea is used for its immunostimulating properties and may have a role in modulating adverse immune effects of chemotherapy (i.e., use of 5-fluorouracil (5-FU); fluorouracil and its immunosuppressive effect). Patients may seek herbal remedies such as *Echinacea* (*Echinacea angustifolia* and *Echinacea purpurea*) for immune stimulation. *Echinacea* extracts have been prescribed to supplement cancer chemotherapy for their immune-supportive effects; however, the extracts may also influence tumourgenesis. Our study aimed to determine the proliferative effect of the ethanolic blend of *E. angustifolia* and *E. purpurea* on various cancer cervical and bile duct cell lines, including HELA and QBC-939. Various cancer cells (HeLa and QBC-939) and human vein epithelial cells (HUVEC) were treated with the *Echinacea* blend sample that was evaporated and reconstituted in Dimethyl sulfoxide (DMSO). As the extract concentration of *Echinacea* was increased from 12.5 μg/mL to 25 μg/mL, there was an increase in cell inhibition up to 100%, which then reduced to 90% over the next three concentrations, 50 μg/mL, 100 μg/mL, and 200 μg/mL, in HeLa cells; further inhibitory effects were observed in QBC-939 cells, from 9% inhibition at a concentration of 25 μg/mL up to 37.96% inhibition at 100 μg/mL concentration. Moreover, this is the first study to report the growth-promoting effects of this *Echinacea* blend in HUVEC, up to 800% at a dose concentration of 200 μg/mL. Previous studies have suggested that chicoric acid of *Echinacea* spp. is responsible for the increased cell growth. The results of this study show that the hydroethanolic extract of *Echinacea* herbal medicine promotes the growth of HeLa cells and QBC-939 cancer cell proliferation, and may interfere with cancer treatment (i.e., chemotherapy drugs such as 5-fluorouracil and Cisplatin (DDP)). However, the *Echinacea* blend shows potential in neurodegenerative diseases with growth-promoting effects in HUVEC. Further animal trials (*in vivo* effect) measuring dose toxicology are necessary to demonstrate the interaction of this blend with body and tumor growth, and also any positive synergistic or adverse interaction with chemotherapeutic drugs listed, so as to confirm the current observation and epithelial tissue growth or regeneration in a neurodegenerative disease model.

1. Introduction

Animal and clinical studies have shown *Echinacea* spp., in particular *Echinacea purpurea* and *Echinacea angustifolia*, to have pronounced immunomodulating effects during illness (i.e., common cold); increase circulating populations of total white blood cells, monocytes, neutrophils, and NK cells[1]; enhance macrophage activation[2]; and decrease formation of neoplasms.[3] Moreover, immune modulation such as T-cell cytokine response (interleukin-2 and interferon-γ) has been demonstrated using an acidic water-soluble extract from *E. purpurea* (L.) Moench.[4] Moreover, *Echinacea* has been shown to extend the lifespan of mice, as well as to be an effective cancer treatment.[5] This potential anticancer effect has been documented in AKR/J mice with thymic lymphoma consuming an oral preparation of *E. purpurea*, displaying significant suppression of lymphoma growth possibly via the suppression of cytokines

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(i.e., tumor necrosis factor-α and interleukin-12) and nonspecific immune response.[6] Further, in clinical trials of hepatocarcinoma with the use of cyclophosphamide (chemotherapeutic agent; guanine alkylating agent), *Echinacea* supplementation increased the number of NK cells and their activity.[7] In these cancer types and chemotherapy uses, *Echinacea* appears to act therapeutically.

During cancer chemotherapy, some chemotherapeutic medications [5-fluorouracil (5-FU)/levamisole (LMS)] may reduce immune response; thus, search for an immune-supportive medication is required (i.e., levamisole).[8] Some patients demand a "natural cure," as *Echinacea* has been used in wound healing to treat glucocorticoid-mediated immune suppression during injury,[9] and a polysaccharide fraction isolated from *E. purpurea* (EPS-EPO VIIa) has been shown to reduce chemotherapy-induced leukopenia. [10] Thus, it could be assumed that it also has benefits in cancer chemotherapy, which causes immune suppression (i.e., toxic to hematopoiesis), partial anticancer effect, and other cancer indications. Currently, *Echinacea* in a multiherbal blend has been used to effectively treat gastrointestinal mucositis, a common complication of chemotherapy, reducing the disruption of chemotherapy cycles[11] and demonstrating an anti-inflammatory action as well. Further, in human neuroglioma cells, a CO_2 root extract of *E. angustifolia* DC., in particular the alkamides, showed a COX-2 mRNA stimulatory effect but did not stimulate prostaglandin synthesis, and thus may inhibit COX-2-dependent PGE2 formation where there is inflammation,[12] such as at the site of tumor burden or systemic inflammation during cancer or elevated cytokine profile, a hallmark of cancer.

In cancer, increased cytokine profiles are present, especially with the use of doxorubicin (with cyclophosphamide or cyclophosphamide) plus 5-FU, which may cause cognitive inhibition[13] or epithelial damage. The cytokine modulation by *Echinacea* extracts may be related to a reduction in tissue inflammation and can be of benefit in neurodegenerative diseases. Epithelial retinal pigment cells have been transplanted into patients with neurodegenerative disorders, e.g., Parkinson disease, and have shown to have a therapeutic effect.[14] Thus, the proliferation of epithelial cells during neurodegenerative diseases, such as Parkinson or Alzheimer's disease, may have a possible role in their treatment. Moreover, epithelial tissue surrounds the entire cardiovascular system, and its regeneration after cardiac failure, or arteriosclerosis, would also be of benefit, as would be the regeneration of epithelial cells during cervical or lung carcinoma (i.e., bronchial epithelium).

Interestingly, the action and mechanism of *Echinacea* and its phytochemicals vary between not only different diseases, but also different types of carcinomas. By contrast, evidence also exists that *Echinacea*, i.e., chicoric acid component, interferes with cancer cell growth and has been shown to proliferate and not inhibit HeLa cell growth.[15] Moreover, some phytochemicals in *Echinacea pallida* [(8Z,11Z)-pentadeca-8,11-dien-2-one] have been shown to exert a cytotoxic effect on human T-cell leukemia cancer lines (Jurkat and HL-60)[16]; cytotoxic effects were also exerted by polyacetylenes and polyenes in human pancreatic MIA PaCa-2 and colonic COLO320 cancer cell lines,[17] when conjoined with the assumed immunostimulatory effect of *Echinacea* spp., indicating that it is an ideal candidate for adjunct therapy during cancer.

A possible concern of using herbal medicines in cancer therapy (chemotherapy) is that a number of herbs and even foods are able to upregulate or inhibit P-glycoprotein (cytochrome P450). P-glycoprotein is responsible for exporting xenotoxins including pharmaceutical medicines, i.e., chemotherapeutic products, from the cell. *Echinacea* is a documented inhibitor of cytochrome P450 (CYP) 3A4 inhibitor *in vitro* and interacts with anticancer drugs such as etoposide (a P450 CYP3A4 substrate), causing thrombocytopenic epidose (i.e., hemolytic anemia).[18] Further, a multiherbal mixture (Sambucus Force) containing *E. purpurea* and *Sambucus nigra* caused a weak CYP3A4 inhibition[19] and CYP3A4 inhibition by *E. purpurea*, showing a weak inhibition potential towards CYP3A4-mediated *in vitro* metabolism.[20] Further, in human interstitial tissues (Caco-2 cells), *E. purpurea* has been shown to have a dose-dependent effect on digoxin flux from P-glycoprotein.[21] In particular, pentadeca-(8 Z,13 Z)-dien-11-yn-2-one, a phytochemical, extracted from *E. pallida* was shown to reduce PgP activity.[22] By contrast, a study on the effect of *E. purpurea* on inducing CYP3A4 showed that the pharmacokinetics of the CYP3A4 substrate docetaxel remained unchanged in cancer patients administered 135 mg of docetaxel (60-minute intravenous infusion) and taking 1 mL of *E. purpurea* extract three times daily (t.i.d.; 60 drops total), resulting in a nonsignificant change in either the mean area under the plasma concentration–time curve for docetaxel or the elimination half-life.[23] Lastly, the efficacy of *Echinacea* spp. are in some instances contradictory, i.e., immunostimulating effects, as the product is susceptible to adulteration and also sale of unstandardized extracts of *Echinacea*. More research is needed to analyze the efficacy of standardized and quality of commercial supplies of *Echinacea* hydroethanolic extracts.

The aim of this study was to observe whether the use of a hydroethanolic root extract of *Echinacea* blend of *E. purpurea* and *E. angustifolia*, which is indicated for its various actions (i.e., immune modulation), would interact with cancer cells grown in both HeLa and QBC-939 cell lines and/or promote the growth of epithelial tissue [i.e., human vein epithelial cells (HUVEC)] and show potential for *in vivo* epithelial proliferation. Furthermore, the study aimed to gain preliminary evidence to proceed with an animal study to confirm either proliferation of tumor growth or that the observed effect is a misrepresentation due to the use of a cell line (i.e., postabsorptive modification of phytochemicals in the *Echinacea* blend) would render them an immune stimulatory effect rather than tumor proliferative effect. Also, the activity of the *Echinacea* blend was tested using a noncancerous human epithelial vein cell line "HUVEC" to identify if it has a role in the treatment of neurodegenerative diseases.

2. Materials and methods

2.1. Cell lines, chemicals, and biochemicals

HeLa (cervical cancer cell), QBC-939 (cholangiocarcinoma), Beas-2b (lung/bronchial epithelial), and HUVEC cell lines were kindly donated by Qiao Yao from the Yunnan Tumour Hospital, Yunnan, China. *Echinacea* blend hydroethanolic extract (Mediherb brand) was purchased from Integria Health Care (Warwick, QLD, Australia). Purity was assessed by the Integria research group led by Professor Kerry Bone via high-performance liquid chromatography (HPLC). The 5-FU injection was made by Tianjin Jing Yao Animo Acid Co., Ltd (Tianjin, P.R. China). Each bottle contains 250 mg 5-FU in 10 mL of DMSO. Cisplatin (DDP) is manufactured by Qilu Pharmaceutical (Hainan) Co., Ltd (Hainan, P.R. China), and diluted 250 mg in 10 mL. Both are 99.9% in purity. DMSO, MTT, Dulbecco's Modified Eagle Medium: Nutrient Mixture F -12 (DMEM/F12), 10% Fetal bovine serum (FBS), and 100 u/mL Penicillin/Streptomycin Solution (P/S) were purchased from Sigma-Aldrich. The assays were performed according to the manufacturer's instructions.

2.2. Plant material, extraction, and mass spectrometry–HPLC

One commercial *Echinacea* blend preparation was purchased from Integria Health Care (Mediherb brand). The sample composition was as follows: 40% *E. angustifolia* (1:2 root extract)/60% *E. purpurea* (fresh plant extract) contained in a final concentration of 50% ethanolic extract. The batch number was B155842, with the expiry in March 2015.

The blend had been analyzed previously for its phytochemical composition by Matthias et al,[24] but the sample was reanalyzed.

Phytochemical analysis was conducted by the Southern Plant Laboratory, Southern Cross University, NSW, Australia. Briefly, the mass spectrometry–HPLC was conducted as follows: the sample was injected through a Phenomenex Luna C18 column (100 mm × 4.6 mm ID; 3 μm particle size). The mobile phase used consisted of water with 0.005% triflouroacetic acid (TFA) and acetonitrile (ACN) with 0.05% TFA. The total running time was 29 minutes, with a gradient elution from 10% water (0.05% TFA):90% ACN (0.05% TFA) to 5% water (0.05% TFA):95% ACN (0.05% TFA) over the first 19 minutes and the remaining time for column re-equilibration.

The standardized extract contained the following identified phytochemicals: caftaric acid, echinacoside, chicoric acid, and alkylamides. The hydroethanolic extract (100 mL) was evaporated at room temperature until only solid sediment remained (refer to Fig. 1).

2.3. Determination of proliferative or inhibitory effects of a commercial Echinacea blend extract on HeLa cells, QBC-939 cells, Beas-2b cells, and HUVEC

The *Echinacea* extract was applied to HeLa cells, QBC-939 cells, Beas-2b cells, or HUVEC to determine either an inhibitory or a proliferative activity. The *Echinacea* blend extract was dissolved in DMSO at a concentration of 12.5 μg/mL, 25 μg/mL, 50 μg/mL, 100 μg/mL, or 200 μg/mL. A 10-mL volume of growth media was added to the herbal extract, and the cell suspension was centrifuged at 3000 g for 10 minutes. The supernatant was poured off and resuspended in 10 mL of media. Cells were counted on a hemacytometer and diluted accordingly, based on the number of cells needed for the assay. Cells were seeded at 30,000 cells per well. Twenty-four hours later, following cell seeding in 96-well plates, the medium was removed from the well and 0.2 mL of a new medium was added (DMEM/F12, 10% FBS, 100 u/mL P/S). The plate was incubated for a further 72 hours, and then the medium was removed from the well, and 0.2 mL of another new medium containing 10% MTT (5 mg/mL) was added (cell staining). The plate was incubated for another 4 hours, and then the MTT was removed and 0.2 mL DMSO was added. The plate was shaken in the dark for 10 minutes, and then the optical density was recorded using a reader at 490 nm. Controls for the assay included DMSO, cells alone, and DPP alone. The cell growth was plotted against *Echinacea* concentration and compared with the cell growth in doxorubicin alone.

2.4. Statistical analysis

Once the data were collected and processed, the data sets were tested with 1-way analysis of variance (ANOVA) using SPSS

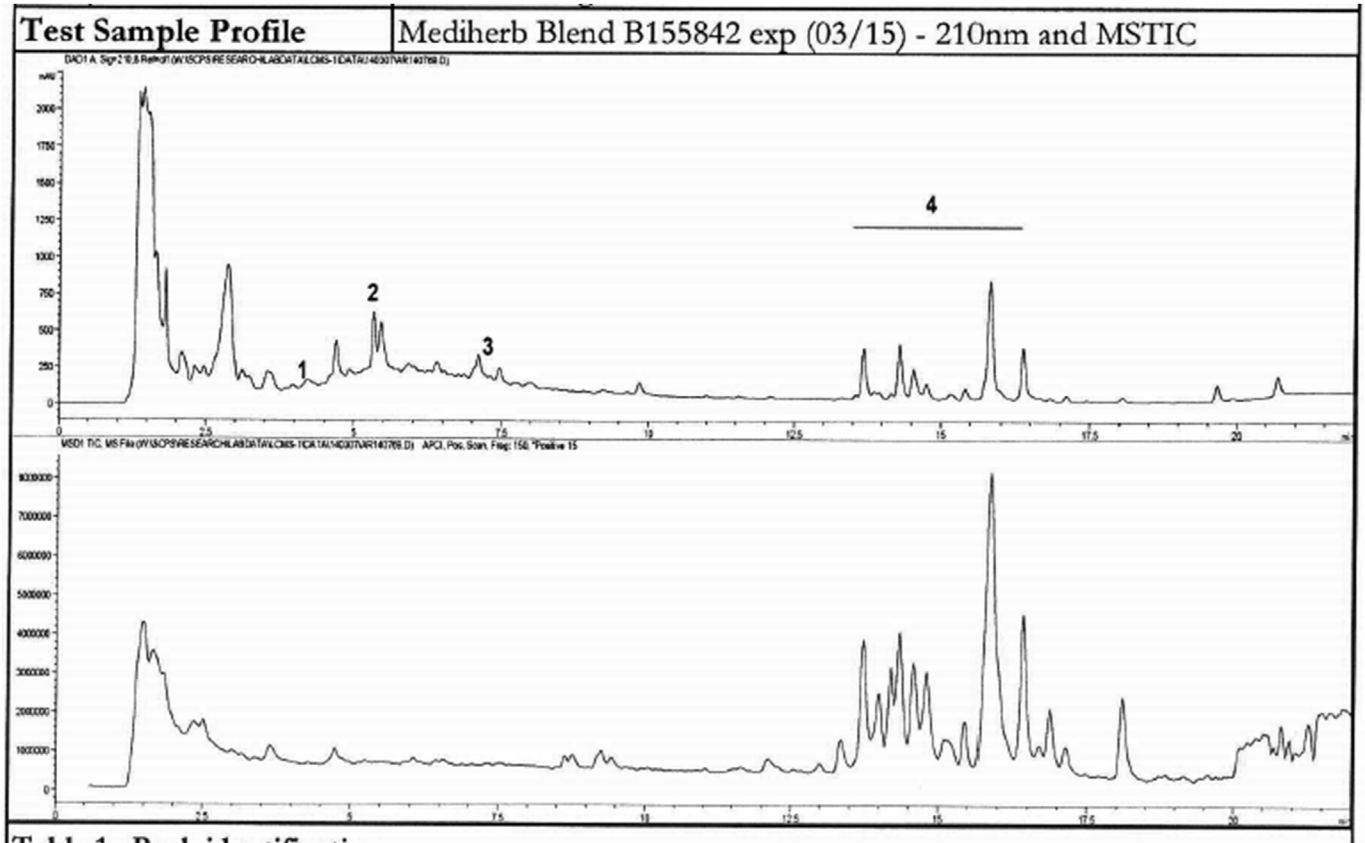

Table 1. Peak identification

Peak #	RT (min)	Fragment ions [M+H]	Compound (tentative or possible) ID (MW)
1	4.2	-	caftaric acid
2	5.4	-	echinacoside
3	7.3	-	chicoric acid
4	13.5-16.5	248, 246, 232, 286	alkylamides (major peak, m/z 248 echinacein)

Fig. 1. Test sample profile.

12.0 for Windows to $p = 0.05$. Results were calculated as the mean ± standard error of the mean, using $n = 5$, and then converted into either percent proliferation or inhibition of the DMSO control. Student *t* test was used for statistical analyses.

3. Results

Treatment of HeLa cells with *Echinacea* showed a concentration-dependent trend from 12.5 μg/mL to 50 μg/mL, followed by plateaus (see Table 1), whereas at 12.5 μg/mL and 25 μg/mL, there was a slight growth suppression in QBC-939 cells, but a steady suppression at 50 μg/mL, 100 μg/mL, and 200μg/mL (Fig. 2).

This observation was also noted in case of human epithelial vein cell line "HUVEC", but with an eight-fold magnitude increase, as was seen in the HeLa cells (Fig. 2), whereas no real growth inhibition or proliferative effect was seen in Beas-2b cells (Table 1).

4. Discussion

In this study, a commercially available *Echinacea* blend extract conferred growth-proliferative effects in HeLa cells, QBE-939 cells, and HUVEC *in vitro*. Previously, the proliferative activity of *Echinacea* extracts has been investigated in a number of studies using cancer cell lines, but not epithelial cells. These cancer cells included human colon cancer cells (Caco-2 and HCT-116), with the *E. purpurea* flower extract displaying a cytotoxic effect, due to the major phytochemical chicoric acid (main constituent in Fig. 1) that reduces telomerase activity; induction of apoptosis via DNA fragmentation, activation of caspase-9, cleavage of PARP, and down-regulation of β-catenin[25]; and thus mitochondrial signaling of apoptosis. The growth-promoting effect in HeLa cells by *Echinacea* root extract has been attributed to cynarine and chicoric acid *in vitro*, as evidenced previously by Huntimer et al,[15] confirming the mechanism of effect observed in this current study.

Further elaboration on the phytochemicals present in *E. angustifolia* and *E. purpurea* blend reveals a high relative concentration of chicoric acid in this commercial blend. Another phytochemical is caftaric acid, which belongs to a class of chemicals known as cinnamates (hydroxycinnamic acids). It has been isolated from *Vitis coignetiae* Pulliat, which has been shown to display antimutagenic effect toward dimethylbenzo[a]anthracene-induced mutagenesis in mice and inhibit the induction of inflammation by 12-O-tetradecanoylphorbol-13-acetate in mice.[26] Moreover, in another study, echinacoside (dose: 1 μg/mL, 10 μg/mL, or 100 μg/mL) was shown to be protective against tumor necrosis factor-α-induced apoptosis in human neuroblastoma (SHSY5Y) cells, which was associated with a correlation of increasing concentration of the antiapoptotic protein "Bcl2."[27] By contrast, in MCF-7 cells, chicoric acid showed a proliferative effect in both HeLa and MCF-7 cells,[15] rather than an apoptotic effect, reconfirming in this study the proliferation effect observed in HeLa cells.

From these results, it may be proposed that if cancer patients are also using herbal medicines (i.e., *Echinacea* spp.) with chemotherapy or anticancer drugs, the phytochemicals in the *Echinacea* spp., in this instance *E. angustifolia* and *E. purpurea*, may reduce the effectiveness of the anticancer drug and possibly the proliferative effects of *Echinacea* on the cancer cells, as observed in this study and the study of Huntimer et al.[15] This is the first publication to show a growth-promoting effect of an *Echinacea* blend hydroethanolic extract in QBC-939 cells, but more importantly in HUVEC, which can be studied further for treatment of neurodegenerative disease states. These findings suggest a role of *Echinacea* blend hydroethanolic extract in the regeneration of epithelial tissue, in particular the venous tissue. Organ damage during or after congestive heart failure, or due to carcinoma, may be remedied via the use of a hydroethanolic extract of an *Echinacea* blend. However, this publication reports only preliminary *in vitro* observation, and more in-depth research in animal models and also in humans is required to measure the levels of chicoric acid or its metabolites in plasma and to determine whether there is a cancer cell proliferative effect *in vivo* both in animals and in humans.

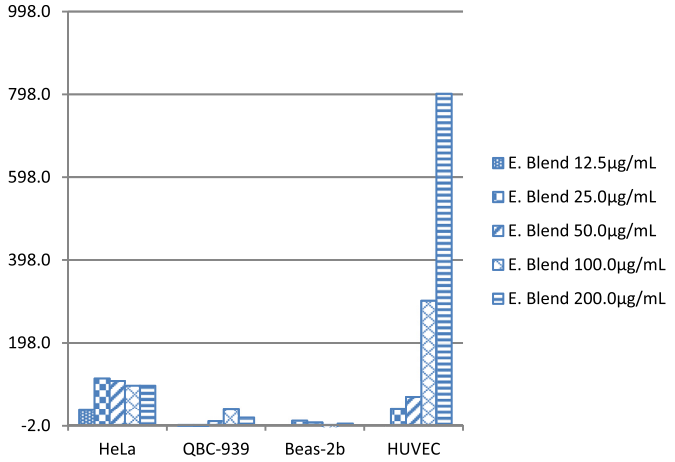

Fig. 2. Results of Table 2, displayed as proliferation or inhibitory effect of *Echinacea* blend on various cell growth. *Y*-axis = proliferation rate (%) (+value). Inhibition displayed as (−value) as a percent of DMSO control. *X*-axis = cell line type.

Table 1
Peak identification.

Peak no.	RT (min)	Fragment ions (M + H)	Compound ID (tentative or possible) (MW)
1	4.2	—	Caftaric acid
2	5.4	—	Echinacoside
3	7.3	—	Chicoric acid
4	13.5–16.5	248, 246, 232, 286	Alkylamides (major peak, *m/z* 248 echinacein)

ID = identification; RT = retention time.

5. Conclusion

This study has shown that an *in vitro* exposure of both HELA and QBC-939 cells to an *Echinacea* blend of *E. angustifolia* and *E. purpurea*, as a commercial extract, enhances the growth of both HELA and QBE-939 cells. The *Echinacea* blend also promotes the growth of HUVEC and may have a therapeutic role in neurodegenerative diseases. The effect of an *Echinacea* extract requires in-depth study *in vivo* in animal models to observe tumor growth/suppression and also its interference with anticancer drugs leading to proliferation of tumor cells. Conversely, the observation may be an artifact of *in vitro* exposure and may not take into account absorption or posthepatic modification of phytochemicals present in *Echinacea* and thus the physiological *in vivo* effects. A more promising observation is the stimulation of epithelial cells by the *Echinacea* extract, and encourages further research to examine the possible vein epithelial cell regeneration and regenerative effects of the presented *Echinacea* extract.

Conflicts of interest

All authors declare no conflicts of interest. Further, no author received any financial benefit from Mediherb (Integria Healthcare

Pty. Ltd), nor a provincial or national research fund in P.R. China or Australia.

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