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Original Article

In vitro anticancer activity of dietary bioagent (isothiocyanates) on HepG2 and B16F10 cell lines: a comparative study

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Abstract: *In vitro* anticancer screening of different isothiocyanates (ITCs) [Allyl isothiocyanate (AITC), Phenylethyl isothiocyanate (PEITC) and Sulphoraphane (SUL)], derived from naturally occurring *Eruca sativa* seed oil (SO) against HepG2 human liver carcinoma cell line and B16F10 mice melanoma cell line have been carried out using MTT (Methyl Thiazole Tetrazolium), TBE (Trypan Blue Exclusion) and SRB (Sulphorodamine-B) bioassays. Combination of three ITCs Allyl isothiocyanate (AITC), Phenylethyl isothiocyanate (PEITC), Sulphoraphane (SUL) isolated from seed oil in ratio of 1:1:1 exhibited maximum percentage inhibition of 97.12%, 96.34% and 96.56% by HepG2 while 96.32%, 95.34% and 96.43% inhibition by B16F10 cell line for MTT, TBE and SRB respectively against reference drug doxorubicin at concentration 50 µM. Percent inhibition has been found to be more pronounced in isothiocyanates combination compared to its naturally occurring *E. sativa* seed oil in both the cell lines. Combination also displayed significant antimelanoma activity (IC_{50} value 18.51 µM) against B16F10 mice melanoma cell line and anticarcinoma activity (IC_{50} value 16.38 µM) against HepG2 human liver carcinoma cell line.

Keywords*:* Carcinoma, *Eruca sativa,* Isothiocyanates, Antimelanoma

Introduction

Cancer, a complex disease caused by oxidative damage which is a severe problem now a day. Majority of dietary supplements and foodstuffs are believed to have preventive effects on chronic diseases due to their radical scavenging or antioxidant property. Herbs are known to produce a wide range of secondary metabolites such as alkaloids, terpenoids, polyacetylenes, flavonoids, quinines, phenyl propionates etc. and proved for their useful medicinal properties. Epidemiological studies states that certain cruciferous vegetables consumption reduces various types of cancer due to its enriched content of isothiocyanates derived from Glucosinolates [1; 2]. Isothiocyanates are components of certain plants and vegetables (Family: *Cruciferae*) that have selective biological activities and functions against carcinogenesis [3]. Among various isothiocyanates, Allyl isothiocyanate, Phenylethyl isothiocyanate and Sulforaphane, are dietary compounds, found in cruciferous vegetables such as cauliflower, broccoli, and Brussels sprouts.

E. sativa, (Miller) is the most representative species of annual herb, which originated in the Mediterranean region (4), now is widely distributed all over the world, particularly in India (5), where the seeds are used for the production of a traditional spicy (taramira) oil. *E. sativa* oil is brought to the attention of the scientific community for various pharmacological properties viz. antidiabetic, antirenal-failure, antigastro-intestinal indigestion and antineurotoxic activity (6).

In continuation of our work (7; 8; 9; 10), on screening of Indian medicinal plants for various pharmacological activities, we have recently shown that *E. sativa* is a relevant source of antimicrobial activity (11). Various isothiocyanates have been isolated from *E. sativa* seed oil (12). Encouraging results on the above lines have inspired us to explore the *in vitro* effect of combination of three isothiocyanates (Allyl isothiocyanate, Phenylethyl isothiocyanate and Sulphoraphane) for anticancer activity against HepG2 human liver carcinoma cell line and B16F10 mice melanoma cell line along with its comparison *E. sativa* seed oil.

Materials and Method

Chemicals and reagents:

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Dulbecco's modified eagle's medium (DMEM), fetal bovine serum (FBS), glutamine, penicillin and streptomycin were purchased from Gibco BRL, USA. Trypan blue dye, MTT [3-(4, 5- dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium] dye and doxorubicin (Trade name: Adriamycin) were obtained from Sigma-Aldrich Chemical Company (UK). Allyl isothiocyanate, Phenylethyl isothiocyanate and Sulphoraphane were used as test samples. Analytical standards were purchased from Fluka-Sigma-Aldrich. Double distilled water was used throughout the experiment. All other chemicals and solvents used were of highest purity commercially available (Sigma–Fluka–Aldrich) and were of analytical grade.

Cell culture:

HepG2 human liver carcinoma and B16F10 mice melanoma cells were purchased from National Centre for Cell Sciences, Pune, India. These cells (1×10^5) were maintained in DMEM medium supplemented with antibiotics, L-glutamine (2 mM) and 10% fetal calf serum. HepG2 human liver carcinoma and B16F10 mice melanoma cells were considered for *in vitro* cytotoxic studies on the basis of their metastatic nature. The cells were grown in the following conditions: CO_2 (5%) atmosphere in high humidity (95%) at 37^0C in a CO_2 incubator. Each batch of cells was assessed for cell cytotoxicity by TBE (Trypan Blue Exclusion), MTT (Methyl Thiazole Tetrazolium) and SRB (Sulphorodamine-B) assays.

Trypan blue exclusion assay:

Cells (1×10^5/plate) were incubated in poly-l-lysine precoated tissue culture petri plates with complete medium (MEM medium with fetal bovine serum) and allowed to adhere for 24 h in CO_2 incubator at 37 °C. The medium was replaced with incomplete medium (MEM medium without fetal bovine serum) containing serial dilution series of test samples (3 - 50 µM) separately for 24 h in CO_2 incubator at 37 °C. The medium was removed from the wells by aspiration and trypsinized to obtain the cell suspension. Trypan blue dye (0.4%; 0.1 ml) was mixed with cell suspension, 15 min prior to completion of incubation period (13). At the end of incubation period, the number of viable cells (unstained) was counted using a haemocytometer. Viability was expressed as a percentage of control number of cells excluding trypan blue dye.

Methyl thiazole tetrazolium assay:

MTT assay depends on the mitochondrial enzyme reduction of tetrazolium dye to determine cell viability. Briefly, the cells were plated at a density of 1×10^5 cells/well into 96 well plates and allowed to adhere for 24 h in CO_2 incubator at 37 °C. The medium was replaced with the serum free medium containing different concentrations of test samples (3 - 50 µM) again for 24 h in CO_2 incubator at 37°C. MTT dye (20µl; 5 mg/ml) was added to each well 4 h prior to completion of incubation period. The medium was removed by adding 200 µl of DMSO to each well and incubated for further 10 min (14). The absorbance was read at 550 nm using ELISA reader (Synergy HT, Biotech, USA). The average values were determined from triplicate. Percent inhibition was calculated by using the formula: (C−T)/C×100, where C=Absorbance of control, T=Absorbance of Treatment. The IC_{50} values of test compound were compared with standard drug (15).

Sulphorhodamine-B Assay:

Cells ($1x10^5$/well) were seeded in poly-l-lysine precoated flat bottomed 96 well tissue culture plates and allowed to adhere for 48 h in CO_2 incubator at 37^0C. The medium was replaced with the complete medium containing different concentrations of test samples (3 - 50 µM) separately again for 24 h in CO_2 incubator at 37^0C. The plates were stained with sulphorhodamine B solution (0.4% in water) in dark for 30 min and then excess dye was washed out with acetic acid (1%) (16).The unwashed dye was eluted with tris-buffer and quantified at 570 nm using a microplate reader (Synergy HT, Biotech, USA).

Statistical analysis:

All experimental data were given as mean ± SD. Statistical analysis was carried out using the one-way analysis of variances (ANOVA). Post Dunnett test was applied between control, reference drug and test samples using Graph Pad Prism software. Probability values were found to be less than 0.05.

Results and Discussion

***In vitro* Cytotoxicity:**

A combinational study of three different Isothiocyanates: Allyl isothiocyanate, Phenylethyl isothiocyanate and Sulphoraphane were screened for their *in*

vitro cytotoxic activity against HepG2 human liver carcinoma and B16F10 mice melanoma cell lines. Inhibitory concentration (IC_{50}) was evaluated for all the three native phytoproducts with respect to vehicle control and standard drug (doxorubicin). Based on (IC_{50}) values, different combinations of the three phytoproducts were prepared and monitored for their cytotoxic effect against both the cell lines.

Cytotoxicity in terms of percent inhibition was monitored after 48 h incubations by MTT, TBE and SRB assays. Current assays for measuring cytotoxicity are based on alterations of plasma membrane permeability and the consequent release of components into the supernatant or the uptake of dyes. In case of trypan blue exclusion assay, the dead cells uptake the dye while the viable cells are excluded. In MTT assay, dead cells are unable to metabolize yellow tetrazolium salt while viable cells metabolize yellow tetrazolium salt into purple formazan crystal and sulphorhodamine B is an anionic aminoxanthene dye that forms an electrostatic complex with the basic amino acid residues of proteins under moderately acidic conditions, which provides a sensitive linear response. Three (MTT, TBE and SRB) bioassays are likely to provide real picture of percent inhibition of phytoproducts and hence selected for the present study.

A perusal of the data shows the percent inhibition of AITC-76.41%, PEITC-62.52%, SUL-64.42%, AITC:PEITC-83.24%, PEITC:SUL-84.35%, SUL:AITC-82.34%, AITC:PEITC:SUL-96.32%, SO (2mg/ml)-67.35% for MTT assay; AITC-77.65%, PEITC-63.54%, SUL-64.68%, AITC:PEITC-83.64%, PEITC:SUL-85.67%, SUL:AITC-83.68%, AITC:PEITC:SUL-95.34%, SO (2mg/ml)-68.24% for TBE assay; AITC-77.85%, PEITC-63.42%, SUL-64.42%, AITC:PEITC-83.24%, PEITC:SUL-84.35%, SUL:AITC-83.47%, AITC:PEITC:SUL-96.43%, SO (2mg/ml)-67.34% for SRB assay at concentration of 50 µM by B16F10 mice melanoma cell line.

While for HepG2 human liver carcinoma cell line, maximum percent inhibition are as follows: AITC-77.25%, PEITC-65.38%, SUL-63.85%, AITC:PEITC-86.19%, PEITC:SUL-85.6%, SUL:AITC-84.39%, AITC:PEITC:SUL-97.12, SO (2mg/ml)- 68.46% for MTT assay; AITC-77.39%, PEITC-64.28%, SUL-63.49%, AITC:PEITC-84.75%, PEITC:SUL-84.39%, SUL:AITC-83.69%, AITC:PEITC:SUL-96.34, SO (2mg/ml)- 66.82% for TBE assay; AITC-77.85%, PEITC-63.42%, SUL-62.35%, AITC:PEITC-84.68%, PEITC:SUL-84.39%, SUL:AITC-83.57%, AITC:PEITC:SUL-96.56, SO (2mg/ml)- 65.92% for SRB assay at concentration of 50 µM.

Isothiocyanates and seed oil were screened against HepG2 human liver carcinoma and B16F10 mice melanoma cell line with increasing concentrations for 24 h by the MTT, TBE and SRB bioassays. A perusal of Figure 1 presents the data of percent inhibition of Doxorubicin, isothiocyanates (single and combination) and SO (2mg/ml) against B16F10 and HepG2 cell lines by the TBE, SRB and MTT bioassays.

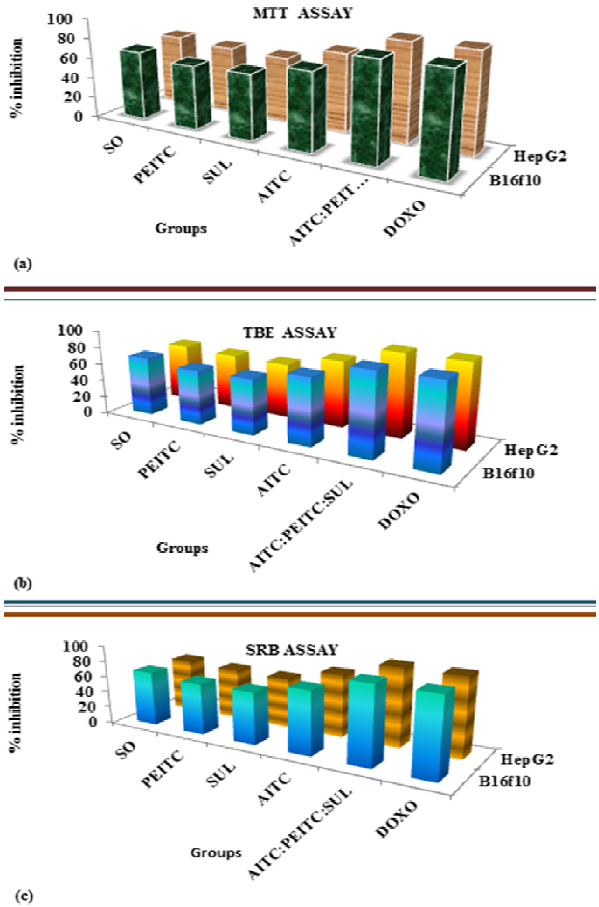

Fig. 1: *In vitro* cytotoxic effect of Doxorubicin, ITCs (single and combination) and Seed oil on HepG2 human liver carcinoma and B16F10 mice melanoma cell lines using (a) MTT, (b) TBE & (c) SRB bioassays. Each value is mean ± SD (n=3). $p < 0.01$ vs control. Control: 2 % dimethyl sulfoxide, Doxo: Doxorubicin, SO: Seed oil, AITC: Allyl isothiocyanate, PEITC: Phenylethyl isothiocyanate, SUL: Sulphoraphane.

Percent inhibition resulting from TBE, SRB and MTT bioassays demonstrated that seed oil and isothiocyanates exhibit cytotoxicity (antimelanoma and anticarcinoma activity) against B16F10 mice melanoma and HepG2 human liver carcinoma cell line. Seed oil and isothiocyanates showed pronounced efficacy on B16F10 cell line ($p < 0.05$). However, combination of isothiocyanates showed more pronounced efficacy (maximum inhibition 97.12%) at the concentration 50 µM at 24 h compared to *E sativa* seed oil against HepG2 human liver carcinoma cell line ($p < 0.05$) compared to B16F10 mice melanoma cell line (maximum inhibition 96.43%).

Conclusion

The present piece of work demonstrates that the combination of three isothiocyanates isolated from *E. sativa* seed oil shows anticarcinoma activity, simultaneously having encouraging antimelanoma activity compare to its naturally occurring seed oil. It is recommended to understand specific cellular, molecular and genetic mechanisms of combinational effects of these phytoproducts that contribute cancer growth and progression.

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References

1. Gasper AV, Traka M, Bacon JR, Consuming broccoli does not induce genes associated with xenobiotic metabolism and cell cycle control in human gastric mucosa. Journal of Nutrition, 2007, 137,1718-24.

2. Meyer M, Adam ST, Comparison of glucosinolate levels in commercial broccoli and red cabbage from conventional and ecological farming. European Food Research and Technology, 2008, 226, 1429- 37.

3. Park EJ, Pezzuto JM, Botanicals in cancer chemoprevention. Cancer Metastasis Reviews. 2002, 21, 231–55.

4. Steven JT, Jackson & Singletary KW, Sulforaphane: a naturally occurring mammary carcinoma mitotic inhibitor which disrupts tubulin polymerization. Carcinogenesis, 2004, 25, 219-227.

5. Park EJ & Pezzuto JM, Botanicals in cancer chemoprevention. Cancer and Metastasis Reviews. 2002, 21,231 –255.

6. Kataya HAH & Hamza AA, Red cabbage (*Brassica oleracea*) ameliorates diabetic nephropathy in rats. Evidence-Based Complementary and Alternative Medicine. 2008, 5(3),281-287.

7. Ojeswi BK, Khoobchandani M, Hazra DK & Srivastava MM, *In vitro* antibreast cancer efficacy of two indigenous plants on human cancer cell line MCF-7. National Academy Science Letters. 2009, 32,105-109.

8. Khoobchandani M, Ojeswi BK, Sharma B & Srivastava MM, *Chenopodium album* preventive progression of cell growth and enhancement in cell toxicity in human breast cancer cell lines. Oxidative Medicine and cellular longevity. 2009, 2(3), 160-165.

9. Ojeswi BK, Khoobchandani M, Hazra DK & Srivastava MM, Protective effect of *Thuja occidentalis* against DMBA-induced breast cancer with reference to oxidative stress. Human and Experimental Toxicology. 2010, 29(5), 369-375.

10. Khoobchandani M, Bansal P, Medhe S, Ganesh N & Srivastava MM, Antioxidant and antimutagenic activities of isothiocyanates rich seed oil of *Eruca sativa* plant chemistry of phytopotentials: Health, Energy and Environmental Perspectives. DOI: 10.1007/978-3-642-23394-4_10, Springer-Verlag, Berlin, Heidelberg, 2012, 47-51.

11. Khoobchandani M, Ojeswi BK, Ganesh N, Srivastava MM, Gabbanini S, Matera R, Iori R & Valgimigli L, Antimicrobial properties and analytical profile of traditional *Eruca sativa* seed oil: Comparison with various aerial and root plant extracts. Food Chemistry. 2010, 120, 217-224.

12. Khoobchandani M, Ganesh N, Gabbanini S, Valgimigli L & Srivastava MM, Phytochemical potential of *Eruca sativa* for inhibition of melanoma tumor growth. Fitoterapia. 2011, 82, 647-653.

13. Frieauff W, Hartman A & Suter W, Automatic analysis of slides processed in the Comet assay. Mutagenesis. 2001, 16, 133-137.

14. Lee SE, Ju EM & Kim JH, Antioxidant activity of extracts from *Euryale ferox* seed. Experimental and Molecular Medicine. 2002, 34(2), 100–106.

15. Kumar V & Yadav SK, Plant-mediated synthesis of silver and gold nanoparticles and their applications. Journal of Chemical Technology & Biotechnology. 2009, 84, 151-157.

16. Choi EH, Chang HJ, Cho JY & Chun HS, Cytoprotective effect of anthocyanins against doxorubicin induced toxicity in H9c2 cardiomyocytes in relation to their antioxidant activities. Food and Chemical Toxicology. 2007, 45, 1873-1881.

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