

## SYNERGISTIC ANTI-CANCER ACTIVITY OF CURCUMIN AND BIO-ENHANCERS COMBINATION AGAINST VARIOUS CANCER CELL LINES

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### ABSTRACT

**Objective:** Clinical utility of curcumin in the treatment of cancer is restricted due to multi-drug resistance and metabolism via glucuronidation and sulfation in the liver and intestine. To overcome these limitations, we have hypothesized to use curcumin in combination with natural bio-enhancers such as piperine, quercetin and silibinin, as these phytochemicals reverse the multi-drug resistance, inhibits the metabolism of curcumin and synergistically enhance the anti-cancer activity of curcumin.

**Methods:** Sulforhodamine B assay was used to evaluate the anti-cancer activity of curcumin-piperine-quercetin-silibinin combination in comparison with pure curcumin and positive control doxorubicin on Ovkar-3 (multi-drug resistance), HL60, HEPG2, HeLa, Colo205 and A549 cancer cell lines.

**Results:** Curcumin-piperine-quercetin-silibinin combination displayed enhanced anti-cancer activity on Ovkar-3, HL60, HEPG2, Colo205 and A549 cancer cell lines than the pure curcumin.

**Conclusion:** Enhanced anti-cancer activity of curcumin-piperine-quercetin-silibinin combination might be due to reversal of multi-drug resistance and synergistic enhancement of anti-cancer activity of curcumin by the bio-enhancers. The study concludes that the curcumin-piperine-quercetin-silibinin combination would significantly enhance the anti-cancer activity of curcumin in the treatment of various cancers. However, this pilot study data require further validation using molecular level studies and clinical trials.

**Keywords:** Anti-cancer, Curcumin, Doxorubicin, Piperine, Quercetin, Silibinin, SRB assay

### INTRODUCTION

Curcumin, a hydrophobic functional food polyphenol isolated from dried rhizomes of turmeric (*Curcuma longa* Linn), which is responsible for various pharmacological activities including anti-oxidant, anti-bacterial, anti-fungal, anti-viral, anti-inflammatory, anti-cancer and exhibits significant therapeutic potential in the treatment of arthritis, atherosclerosis, diabetes mellitus, fever, gastric ulcer, inflammatory bowel disease, lung diseases, malaria, multiple sclerosis, myocardial infarction, osteoporosis, pancreatitis, psoriasis, wound and cancer [1-7]. However, anti-cancer efficacy of curcumin is limited due to multi-drug resistance and metabolism via glucuronidation and sulfation [8-10]. To overcome these limitations, we have hypothesized to use curcumin in combination with natural bio-enhancers including piperine, quercetin and silibinin (Fig. 1).

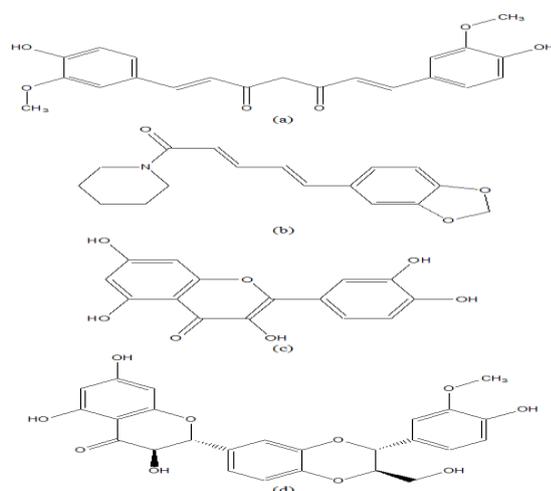


Fig. 1: Chemical structures of (a) Curcumin, (b) piperine, (c) quercetin, (d) silibinin

These phytochemicals has the ability to reverse the multi-drug resistance, minimize/prevent the metabolism of curcumin and

exhibit anti-cancer activity via multiple molecular targets, which in turn may synergistically enhance the anti-cancer activity of curcumin [10-15]. The present study was aimed to compare the anti-cancer activity of pure curcumin, curcumin-piperine-quercetin-silibinin combination and positive control doxorubicin using Sulforhodamine B (SRB) assay on Ovkar-3 (multi-drug resistance), HL60, HEPG2, HeLa, Colo205 and A549 cancer cell lines.

### MATERIALS AND METHODS

#### Materials

Curcumin, Piperine, Quercetin, Silibinin and Sulphorhodamine B were obtained from Sigma Aldrich, India. Dulbecco's modified eagle medium (DMEM) and fetal bovine serum (FBS) were obtained from Himedia, India. Propanol and tris base were obtained from Merck, India.

#### In-vitro anti-cancer activity using SRB assay

The anti-cancer activity of pure curcumin, curcumin-piperine-quercetin-silibinin combination and positive control doxorubicin were assessed using SRB assay on human ovarian cancer cell (Ovkar-3; Multi-drug resistance), human leukaemia cell (HL60), human hepatoma cell (HEPG2), human cervix cancer cell (HeLa), human colon cancer cell (Colo205) and human lung cancer cell (A549) [16, 17]. Briefly, pure curcumin, curcumin-piperine-quercetin-silibinin combination and doxorubicin (positive control) were diluted with Dulbecco's Modified Eagle Medium (DMEM) supplemented with 2% inactivated fetal bovine serum (FBS) to obtain a stock solution of 5 mg/mL concentration, which was sterilized by filtration and finally centrifuged. Serial dilutions (10, 20, 40, 80 µg/mL) were made from the stock solution. About 0.1 mL of the diluted cell suspension (approximately 10,000 cells) was added to each well of the 96-well plate. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µL of different concentrations of test drugs were added. The plates were then incubated at 37°C for 3 days in 5% CO<sub>2</sub> atmosphere and microscopic examination was performed after every 24 hours interval. The cells were fixed using ice-cold trichloroacetic acid (TCA) for 1 hour at 4°C

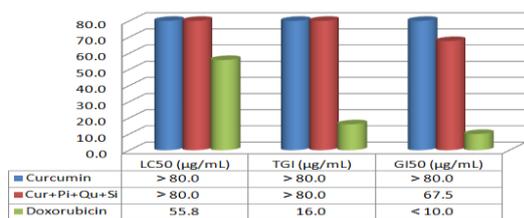
and then washed using distilled water to remove excess TCA and allowed to dry in the air. After 72 hours, the drug solutions in the wells were discarded and 50  $\mu\text{L}$  of SRB solution was added to each well and allowed to stain at room temperature for 30 minutes. The plate was washed with 1% v/v acetic acid to remove unbound dye and allowed to dry in the air. About 100  $\mu\text{L}$  of 10 mM unbuffered Tris Base (pH 10.5) was added to each well and the plates were gently shaken for 5 minutes on a shaker platform to extract the bound SRB. The absorbance was measured using a microplate reader (ELx800, Bio-Tek) at a wavelength of 492 nm. Parameters such as  $\text{GI}_{50}$  (Concentration of the drug that produces 50% inhibition of the cells), TGI (Concentration of the drug that produces total inhibition of the cells) and  $\text{LC}_{50}$  (Concentration of the drug that kills 50% of the cells) were calculated.

## RESULTS AND DISCUSSION

Pure curcumin, curcumin-piperine-quercetin-silibinin combination and doxorubicin were evaluated for anti-cancer activity using SRB assay on Ovkar-3, HL60, HEPG2, HeLa, Colo205 and A549 cancer cell lines and the results were displayed in Fig 2 to 7.

### *In-vitro* anti-cancer activity on Ovkar-3 cell line using SRB assay

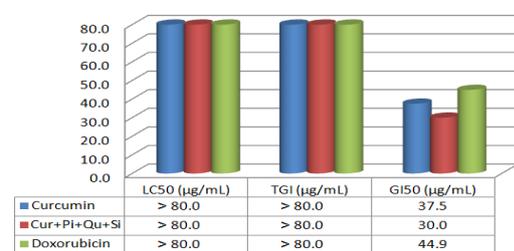
Curcumin displayed poor anti-cancer activity, whereas curcumin-piperine-quercetin-silibinin combination displayed enhanced growth inhibition ( $\text{GI}_{50}$ : 67.5  $\mu\text{g}/\text{mL}$ ; Fig. 2) than the pure curcumin, which might be due to reversal of multi-drug resistance by modulating ATP-binding cassette transporter proteins including P-glycoprotein and multi-drug resistance protein, which in turn have increased the accumulation of therapeutic concentration of curcumin and natural bio-enhancers in the cancer cells. However, positive control doxorubicin displayed significant anti-cancer activity than the curcumin and curcumin-piperine-quercetin-silibinin combination.



**Fig. 2:** *In-vitro* anti-cancer activity of curcumin, curcumin-piperine-quercetin-silibinin combination and doxorubicin on Ovkar-3 cancer cell line

### *In-vitro* anti-cancer activity on HL60 cell line using SRB assay

Curcumin displayed significant growth inhibition ( $\text{GI}_{50}$ : 37.5  $\mu\text{g}/\text{mL}$ ; Fig. 3), whereas curcumin-piperine-quercetin-silibinin combination displayed enhanced growth inhibition ( $\text{GI}_{50}$ : 30.0  $\mu\text{g}/\text{mL}$ ; Fig. 3) than the pure curcumin and positive control doxorubicin, which might be due to synergistic anti-cancer activity by the bio-enhancers.

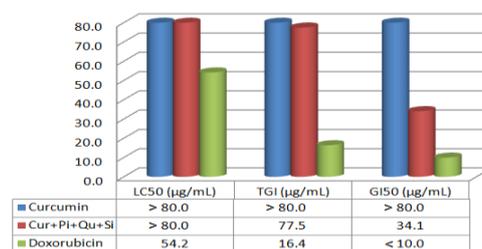


**Fig. 3:** *In-vitro* anti-cancer activity of curcumin, curcumin-piperine-quercetin-silibinin combination and doxorubicin on HL60 cancer cell line

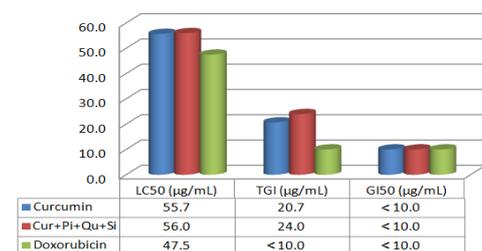
### *In-vitro* anti-cancer activity on HEPG2 cell line using SRB assay

Curcumin displayed poor anti-cancer activity, whereas curcumin-piperine-quercetin-silibinin combination displayed enhanced growth inhibition (TGI: 77.5  $\mu\text{g}/\text{mL}$  &  $\text{GI}_{50}$ : 30.0

$\mu\text{g}/\text{mL}$ ; Fig. 4) than the pure curcumin, which might be due to synergistic anti-cancer activity by the bio-enhancers. However, positive control doxorubicin displayed significant anti-cancer activity than the curcumin and curcumin-piperine-quercetin-silibinin combination.



**Fig. 4:** *In-vitro* anti-cancer activity of curcumin, curcumin-piperine-quercetin-silibinin combination and doxorubicin on HEPG2 cell line



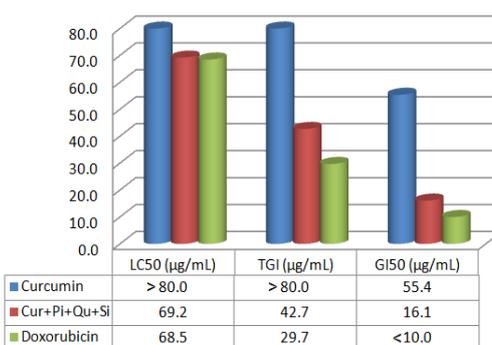
**Fig. 5:** *In-vitro* anti-cancer activity of curcumin, curcumin-piperine-quercetin-silibinin combination and doxorubicin on HeLa cell line

### *In-vitro* anti-cancer activity on HeLa cell line using SRB assay

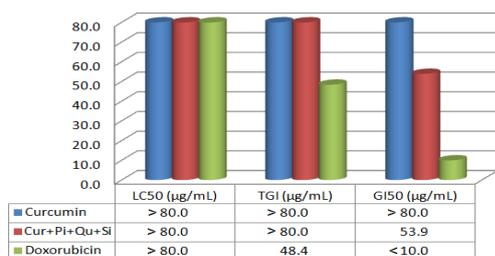
Curcumin and curcumin-piperine-quercetin-silibinin combination displayed comparable cytotoxicity and growth inhibition with positive control doxorubicin (Fig. 5). However, curcumin-piperine-quercetin-silibinin combination has not shown enhanced cytotoxicity and growth inhibition than the pure curcumin.

### *In-vitro* anti-cancer activity on Colo205 cell line using SRB assay

Curcumin displayed poor anti-cancer activity, whereas curcumin-piperine-quercetin-silibinin combination displayed enhanced cytotoxicity and growth inhibition ( $\text{LC}_{50}$ : 69.2  $\mu\text{g}/\text{mL}$ ; TGI: 42.7  $\mu\text{g}/\text{mL}$  &  $\text{GI}_{50}$ : 16.1  $\mu\text{g}/\text{mL}$ ; Fig. 6) than the pure curcumin, which might be due to synergistic anti-cancer activity by the bio-enhancers. However, curcumin-piperine-quercetin-silibinin combination has shown comparable anti-cancer activity with doxorubicin.



**Fig. 6:** *In-vitro* anti-cancer activity of curcumin, curcumin-piperine-quercetin-silibinin combination and doxorubicin on Colo205 cell line



**Fig. 7: In-vitro anti-cancer activity of curcumin, curcumin-piperine-quercetin-silibinin combination and doxorubicin on A549 cell line**

#### **In-vitro anti-cancer activity on A549 cell line using SRB assay**

Curcumin displayed poor anti-cancer activity, whereas curcumin-piperine-quercetin-silibinin combination displayed enhanced growth inhibition (GI50: 53.9 µg/mL; Fig. 7) than the pure curcumin, which might be due synergistic anti-cancer activity by the bio-enhancers. However, positive control doxorubicin displayed significant growth inhibition than the curcumin and curcumin-piperine-quercetin-silibinin combination.

#### **CONCLUSIONS**

In the present study, we have compared the anti-cancer activity of pure curcumin, curcumin-piperine-quercetin-silibinin combination and positive control doxorubicin. Pure curcumin displayed poor anti-cancer activity in multi-drug resistance Ovar-3 cell line, HEPG2 cell line, and A549 cell line but curcumin-piperine-quercetin-silibinin combination have shown enhanced growth inhibition in these cancer cell lines. Whereas, pure curcumin displayed moderate anti-cancer activity in HL60 cell line and Colo205 cell line but curcumin-piperine-quercetin-silibinin combination have shown enhanced growth inhibition in these cancer cell lines. Enhanced anti-cancer activity of curcumin-piperine-quercetin-silibinin combination might be due to reversal of multi-drug resistance by modulating ATP-binding cassette transporter proteins including P-glycoprotein and multi-drug resistance protein, which in turn have increased the accumulation of therapeutic concentration of curcumin and natural bio-enhancers in the Ovar-3 cell line cancer cells and synergistic anti-cancer activity by the bio-enhancers in other cancer cell lines. The study concludes that the curcumin-piperine-quercetin-silibinin combination would significantly enhance the anti-cancer activity of curcumin in the treatment of various cancers. However, this pilot study data require further validation using molecular level studies and clinical trials.

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