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Pharmacological activity of Curcumin-Bio-Enhancer loaded polymeric loaded nanoparticles: An invitro cell line study

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ABSTRACT

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 used Curcumin and Bio-Enhancer (piperine, quercetin and silibinin) loaded polymeric loaded nanoparticles. Sulforhodamine B assay was used to evaluate the anti-cancer activity of Curcumin and Bio-Enhancer (piperine, quercetin and silibinin) loaded polymeric loaded nanoparticles in comparison with pure curcumin and positive control doxorubicin on Ovkar-3, HL60 and HEPG2 cancer cell line. Curcumin and Bio-Enhancer (piperine, quercetin and silibinin) loaded polymeric loaded nanoparticles displayed enhanced anti-cancer activity on Ovkar-3, HL60 and HEPG2 cancer cell lines than the pure curcumin. Enhanced anti-cancer activity of Curcumin and Bio-Enhancer (piperine, quercetin and silibinin) loaded polymeric loaded nanoparticles 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 and Bio-Enhancer (piperine, quercetin and silibinin) loaded polymeric loaded nanoparticles would significantly enhance the anti-cancer activity of curcumin in the treatment of various cancers. However, this pilot study data requires further validation using molecular level studies and clinical trials.

INTRODUCTION

Curcumin, a hydrophobic functional food polyphenol isolated from dried rhizomes of turmeric (Curcuma longa Linn), which is responsible for various pharmacological activities including antioxidant, 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. However, anti-cancer efficacy of curcumin is limited due to multi-drug resistance and metabolism via glucuronidation and sulfation. To overcome these limitations, we have hypothesized to use Curcumin and Bio-Enhancer (piperine, quercetin and silibinin) loaded polymeric loaded nanoparticles. These phytochemicals have 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. The present study was aimed to compare the anticancer activity of pure curcumin, Curcumin and Bio-Enhancer (piperine, quercetin and silibinin) loaded polymeric loaded nanoparticles and positive control doxorubicin using Sulforhodamine B (SRB) assay on Ovkar-3, HL60 and HEPG2 cancer cell line. [1-5]

The present study was aimed to compare the anticancer activity of pure curcumin, Curcumin and Bio-Enhancer (piperine, quercetin and silibinin) loaded polymeric loaded nanoparticles and positive control doxorubicin using Sulforhodamine B (SRB) assay on Ovkar-3, HL60 and HEPG2 cancer cell line. [6-10]

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. [11-15]

In-vitro anti-cancer activity using SRB assay

The anti-cancer activity of pure curcumin, Curcumin and Bio-Enhancer (piperine, quercetin and silibinin) loaded polymeric loaded nanoparticles 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). Briefly, pure curcumin, Curcumin and Bio-Enhancer (piperine, quercetin and silibinin) loaded polymeric loaded nanoparticle 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% CO2 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 µL 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 µL 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 GI50 (Concentration of the drug that produces 50% inhibition of the cells), TGI (Concentration of the drug that produces total inhibition of the cells) and LC50 (Concentration of the drug that kills 50% of the cells) were calculated.

Reference for In-vitro anti-cancer activity using SRB assay

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RESULTS AND DISCUSSION

Pure curcumin, Curcumin and Bio-Enhancer (piperine, quercetin and silibinin) loaded polymeric loaded nanoparticles and doxorubicin were evaluated for anti-cancer activity using SRB assay on Ovkar-3, HL60, HEPG2 cancer cell lines and the results were displayed below tables

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

Curcumin displayed poor anti-cancer activity, whereas Curcumin and Bio-Enhancer (piperine, quercetin and silibinin) loaded polymeric loaded

nanoparticles displayed enhanced growth inhibition than the pure curcumin, which might be due to reversal of multi-drug resistance by modulating ATP-binding cassette transporter proteins including Pglycoprotein 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 and Bio-Enhancer (piperine, quercetin and silibinin) loaded polymeric loaded nanoparticles.

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

Curcumin and Bio-Enhancer (piperine, quercetin and silibinin) loaded polymeric loaded nanoparticles displayed enhanced growth inhibition than the pure curcumin and positive control doxorubicin, which might be due to synergistic anti-cancer activity by the bio-enhancers.

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

Curcumin displayed poor anti-cancer activity, whereas Curcumin and Bio-Enhancer (piperine, quercetin and silibinin) loaded polymeric loaded nanoparticles displayed enhanced growth inhibition 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 and Bio-Enhancer (piperine, quercetin and silibinin) loaded polymeric loaded nanoparticles.

Samples	**Ovkar-3 cancer cell line**		
	LC50 (µg/mL)	**TGI (µg/mL)**	**GI50 (µg/mL)**
Curcumin	>80.0	>80.0	>80.0
Curcumin and Bio-Enhancer (piperine, quercetin and silibinin) loaded polymeric loaded nanoparticles	65.0	62.0	66.0
Doxorubicin	50.0	20	11
HL60 cell line			
	LC50 (µg/mL)	**TGI (µg/mL)**	**GI50 (µg/mL)**
Curcumin	>80.0	>80.0	40.0
Curcumin and Bio-Enhancer (piperine, quercetin and silibinin) loaded polymeric loaded nanoparticles	>80.0	>80.0	30.0
Doxorubicin	>80.0	>80.0	40
HEPG2 cell line			
	LC50 (µg/mL)	**TGI (µg/mL)**	**GI50 (µg/mL)**
Curcumin	>80.0	>80.0	>80.0
Curcumin and Bio-Enhancer (piperine, quercetin and silibinin) loaded polymeric loaded nanoparticles	>80.0	70.0	30.0
Doxorubicin	50.0	20.0	<10

CONCLUSIONS

In the present study, we have compared the anti-cancer activity of pure curcumin, Curcumin and Bio-Enhancer (piperine, quercetin and silibinin) loaded polymeric loaded nanoparticles and positive control doxorubicin. Pure curcumin displayed poor anti-cancer activity in multi-drug resistance Ovkar-3 cell line, HEPG2 cell line but Curcumin and Bio-Enhancer (piperine, quercetin and silibinin) loaded polymeric loaded nanoparticles have shown enhanced growth inhibition in these cancer cell lines. Whereas, pure curcumin displayed moderate anti-cancer activity in HL60 cell line but Curcumin and Bio-Enhancer (piperine, quercetin and silibinin) loaded polymeric loaded nanoparticles have shown enhanced growth inhibition in these cancer cell lines.

Enhanced anticancer activity of Curcumin and Bio-Enhancer (piperine, quercetin and silibinin)

loaded polymeric loaded nanoparticles 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 Ovkar-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 and Bio-Enhancer (piperine, quercetin and silibinin) loaded polymeric loaded nanoparticles 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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