

POTENTIAL ANTITUMOR ACTIVITY OF CURCUMIN WITH RETINYLESTER MICRO-CAPSULED FORMULA

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Abstract

Background: The Bioavailability, efficacy and disease resistance of chemotherapy are the limitations of cancer treatment. This study was designed to formulate a microcapsules containing curcumin and retinylester as core material and tested the anti-cancer activity against different cancer cell lines.

Materials and methods: This study involved the design and formulation of microcapsules containing curcumin and retinylester as (core materials) embedded in dextrin/gelatin as wall materials. It was examined by Fourier-transform infrared spectroscopy FTIR and tested against HeLa and HepG2 cancer cell lines.

Results: Analysis of microcapsule by FTIR showed the characteristic band (2000cm^{-1} ~ 3800cm^{-1}) that is indicated mainly from OH^- , $-\text{CH}_3$ and double bond. These bond stretching showed spectral bands peak 2975cm^{-1} and 2670cm^{-1} , 2836cm^{-1} respectively. The anticancer activity of these microcapsules on HeLa and HepG2 showed inhibition percent 87% and 85% respectively and non-toxic effect on normal cells.

Conclusion: Micro capsulated form of curcumin-retinylester showed an efficient medicinal mixture displayed a significant effect to HeLa and HepG2 cells which is very promising effective in the treatment of cancer.

Key words: Curcumin –Retinylester- Microcapsules-Anticancer

Introduction

Curcuminoids are a functional foods related to phenolic phytochemicals class usually produced from the rhizomes of turmeric (*Curcuma longa*) spice (Chamras et al., 2002). It was reported to have a number of beneficial biological activities as anti-inflammatory, antitumor, antimicrobial, gastric ulcers healing, irritable and pancreatic injury relieve (Serini et al., 2009). Retinyl ester is the precursor of vitamin A that exert different biological activity such as vision in dark, stimulate immune response, maintenance of epithelial tissue and differentiation (Spencer et al., 2009, Kimura et al., 2007 and Li et al., 2013). However, curcumin and retinylester bioavailability is important is in drug delivery to target tissues to increase their efficacy and stability which will be used in pharmaceutical purposes. Micro capsulation is a new biotechnology to protect the nutrient from oxidation and loss its biological activity (D'Eliseo et al., 2012). This micro capsulation are resistance to enzymatic degradation and oxidation, and enhanced its bioavailability to target tissue (Kokura et al., 2012 and Sauer et al., 2007). Many of the anticancer drugs that are widely clinically used today have high risks of adverse side effects and poor specificity and selectivity against tumors. Hence, an urgent need to encounter this problem by improving the efficacy, selectiveness and safety of the anti-tumor agents which can be done by modification with specific motifs by elevating the affinity for tumor cells and reach the biological targets (Siddiqui et al., 2011). The rational of this study was to produce microcapsules of curcumin and retinylester as core material and dextrin as wall material characterization and evaluate the anti-proliferative and pro-apoptotic effects on HeLa and HepG2.

Materials and Methods

Preparation of microcapsules

The curcumin and retinylester were purchased from GNC company, Jeddah, Saudi Arabia, curcumin (purity 99.6 %) and retinylester (purity 99.4 %). It was mixed in a ratio of 1:1.

The core material (curcumin /retinylester; 1:1 w/w), one ml of tween 80 as emulsifier was added and mixed well using sonicator for 10 min. Then, wall material (casein/gelatin, 4:1 ratio) was mixed at 39 °C for 16 hours. Particle size was measured by size analyzer and characterized using a PerkinElmer spectrum 100 FT-IR spectrometer.

Assessment of Antitumor activity of microcapsules

Cell culture and treatment

This study was carried out on cancer cell lines (HeLa and HepG2), it was obtained from KAU. Cell lines was maintained in a humidified incubator with 5% CO₂ at 37°C and grown in Dulbecco's Modified Eagle's Medium (DMEM)(UFC biotech, Riyadh, KSA) .The media were supplemented with 10% fetal bovine serum (FBS) (Gibco U.S.A) and 1% penicillin-streptomycin antibiotics (100 units/ml) (Gibco U.S.A). A 100 mM solution of studied compounds was prepared in 100% DMSO (*Dimethyl sulfoxide*, Gibco U.S.A) or water. From the stock solution, appropriate working concentrations were prepared in the culture media where the final concentration of DMSO was less than 0.1% in both untreated and treated cells.

Cell viability assay

The cell Proliferation assay test was based on the break of a tetrazolium salt (WST-1) by dehydrogenase in mitochondria to form formazan in viable cells. The higher the number of viable cells, the higher formazan produced following the addition of WST-1. It can be used also for detecting cytotoxicity.

Density of 10⁴ cells/well for HeLa, HepG2 and Baby Hamster Kidney fibroblasts BHK cells were maintained and seeded on 96-multiwell plates. The cells were grown till the exponential phase and exposed to different concentrations of prepared microencapsulated (10, 20 and 40 µM) for several time periods (24, 48 and 72 h). Cell proliferation rate was evaluated through a rapid colorimetric cell proliferation assay using WST-1 reagent (Sigma Aldrich, USA). After incubation for the above mentioned duration, 10 µL of WST-1 solution was added and incubated for an additional 1 h at 37°C. Finally, the absorbance was read at 450 nm with a ELx800™ micro plate ELISA reader (Biotek, USA) .Thymoquinone was used as a positive control. The percentage of cell viability was calculated by assuming control (untreated) samples as 100 % viable (Cockbain et al., 2012). The IC₅₀ (50% inhibitory concentration) values were calculated from the dose-response curves.

Apoptosis assay

Apoptosis was a good indicator of cancer recovery. HeLa and HepG2 cells were poured in a 96-well plate at a density of 10⁴ cells /well and incubated for 24 h. After treating the cells with different doses of microcapsules for several time periods, Annexin V/7AAD was determined according to manufacturer's protocol. Briefly, 100 µl of Annexin reagent staining solution (containing annexin V fluorescein and 7AAD) was added and incubated at room temperature for 20 min in dark. The forward and side scatter was recorded at 10,000 events and the subsequent percentage of the early and the late apoptotic cells was analyzed using the Guava Benchtop Flow Cytometer and the results plotted using the In Cyte™ software (Millipore®, Billerica, MA).

Statistical analysis

Results were expressed as mean ± SE. Student's *t* test was carried out for statistical analysis for the group comparison between the pairs. One way analysis of variance (ANOVA) was performed using SPSS version 15

Results and Discussion

FTIR analysis of microencapsulated

Results in Fig. 1 described FTIR analysis of prepared microcapsule. It showed the characteristic band (2000cm⁻¹ ~ 3800cm⁻¹) is mainly seen from "OH", -CH₃ and double bonds. These bonds stretching showed spectral bands peak 2975cm⁻¹ and 2670cm⁻¹, 2836cm⁻¹ respectively. The wall materials mainly contain these bonds and so the characteristic peaks were found in the wall materials as well as in microcapsule. Curcumin contains two - OH and two -CH₃ groups at side chain while retinylester contain four double bonds, displayed strong absorption peak at 2836cm⁻¹. This spectral absorption is attributed to OH⁻ bond stretching vibration but -CH₃ bond absorption was strongest in the microcapsule that is caused by the core material. In addition spectral bands, 2836cm⁻¹ was found that correspond to the retinyl group due to four double bonds. Epidemiological studies reported that, a vitamin A is inversely related to cancer development. Vitamin A showed a decrease the incidence of chemically induced tumor in animals [Wen et al., 2003]. In addition, vitamin A inhibited the development of some types of tumors, including breast, lung and prostatic cancers (Harris et al., 2013).

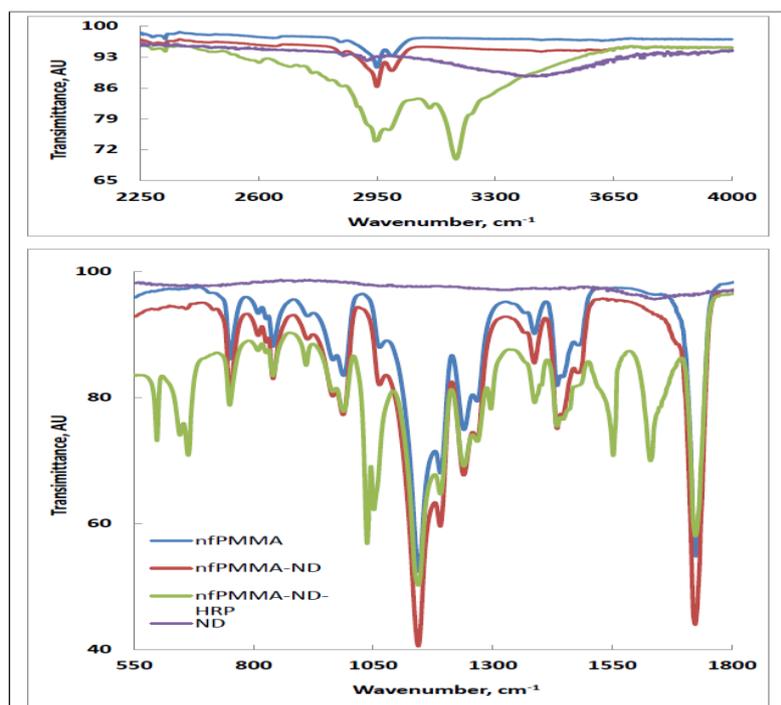


Figure 1: FTIR analysis of microencapsulated for of curcumin and retinylester.

Table 1. IC₅₀ values (μM) of the microcapsules against tumor cells and the normal cells are presented as ± S.E.M.

Conc .of microcap (uM)	HeLa	Hep2G
10	32.30 ± 3.1 ^a	52.31 ± 4.4 ^a
20	19.15 ± 1.5 ^a	57.38 ± 3.1 ^a
40	21.20 ± 2.7 ^a	29.27 ± 0.5 ^a
Thymoquinone (40 uM)	16.75 ± 6.3	14.51 ± 2.7

Experiment was done triplicate (a): $p < 0.05$.

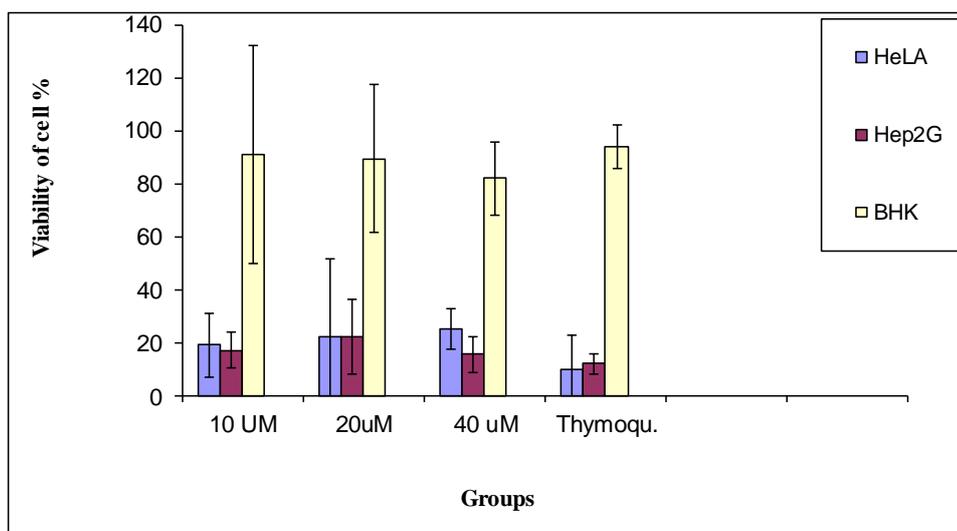


Figure 2. Antiproliferation activity of different concentrations of Microcapsules.

Anti-proliferation activity of Microcapsules

To determine the selectivity of microcapsules, at a concentration of 10 μM , significantly caused cell proliferation inhibition of HeLa cells by about 91% ($p < 0.001$). However, increasing the concentration to 20 μM induced a cell proliferation inhibition in HeLa and Hep2G by about 85% and 87% ($p < 0.001$) respectively. The cell viability at the highest concentration of 40 μM were found to be 23.3 %, 33 %, respectively for a time incubation of 24 h respectively (fig 2).

These results showed the combined effects of curcumin and retinylester on treating HeLa. Previous study found that inhibitory effect of retinylester on HepG2 and HeLa can be enhanced with the addition of curcumin. The mechanism has two aspects. Firstly, curcumin may reduce the oxidation reaction. Secondly, curcumin, itself has obvious effect of inhibiting tumor. Haast et al. (2014) demonstrated that curcumin can make tumor cells sensitive, especially human colon carcinoma cells. When different concentration of these microcapsules were used, the inhibitory rate was decreased by 20.587%, 22.6% respectively. The synergistic anticancer effects fall decreased slowly with decreasing concentration of microcapsulation (Williams et al., 2011 and Pot et al., 2008). However, the inhibition rate of the mixture will no longer increase that may be associated with low substrate concentration (Brown et al., 2010 and Thiebaut et al., 2009). Hence, for the longer antitumor activity of the capsule it is advised to increase the concentration of retinylester in the treatment therapy.

Detection of apoptosis

Finally, to confirm the intimacy of compounds **1**, **7** and **8**, apoptosis mechanism in HeLa and HepG2 was investigated. Annexin V-7AAD staining of the HeLa cells which were treated with increasing concentrations 10,20,40 μM for 24 h was detected. Increasing the concentrations was associated with an increase in the number of apoptotic cells. It was found that, began to significantly trigger apoptosis at 10 μM . While at a concentration of 40 μM , the percentage of the annexin V positive cells was higher and approximately consistent with the value detected in cell viability assays

Conclusion

The prepared microencapsulation of curcumin-retinylester enhance the bioavailability and showed synergistic effect on HeLa and Hep2 G cells with significantly higher anti-cancer activity. These combination of curcumin-retinylester considered as promising chemotherapy in treatment of some cancer types.

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Conflict of Interest: The Author declares that there is no conflict of interest.

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