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Enhanced Growth-inhibitory Effect of Microemulsified Curcumin Formulation in Human Prostate Cancer LNCaP Cells

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Authors' contributions

This work was carried out in collaboration between both authors. Author VD designed the study and conducted experiments, performed data analysis and wrote 1st first draft of manuscript. Author ROA provided experimental protocols, scientific oversight for the study and editorial input for the manuscript. Both authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

Aim: To assess the effect of curcumin microemulsified with non-ionic surfactant surfynol 465 W or dispersed using edible oils on prostate LNCaP cancer cell viability and glutathione status.
Methodology: LNCaP cells were treated for 72-144 hr with curcumin dissolved with fish or corn oil and microemulsified using non-ionic surfactant surfynol 465 W; alternatively LNCaP cells were treated with curcumin directly dispersed in fish or corn oil (0-50 µM) for 24 -72-144 hr. Cell viability was determined using resazurin (Vision blue™) fluorescence assay. Glutathione status was determined by monochlorobimane (MCB) assay.
Results: Treatment with 0-34 µM of microemulsified curcumin produced moderate cytotoxic effect

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on LNCaP cells, no 50% reduction of cell viability was observed graphically. However, when LNCaP cells were treated with curcumin dispersed with corn oil the concentration or 50% reduction of cell viability (IC50) was 12-45 μM. Similarly for cells treated with curcumin dispersed with fish oil, the IC50 was between 20-40 μM. Cytotoxic doses of curcumin dispersed with corn or fish oil increased GST status in cells by 272-656% (p =<0.01).

Conclusion: Microemulsified curcumin formulation prepared using fish or corn oil and surfynol 465 W surfactant had an inhibitory effect on viability of LNCaP cells as did direct dispersion of curcumin in fish or corn oil coupled with the ability for inducing intracellular GST status in LNCaP cells.

Keywords: Curcumin; phase II enzymes; prostate cancer cells (LNCaP); glutathione (GSH); glutathione-S-transferase (GST); monochlorobimane (MCB); resazurin; fish oil; corn oil.

1. INTRODUCTION

Prostate cancer is one of the most common cancers in males resulting in more than 307,000 deaths worldwide in 2012 [1]. In the UK, some 41,736 males were diagnosed with prostate cancer in 2011 out of which 10,793 males died [2] while in Unites States there were 29,720 deaths from 238,590 diagnosis in 2013 [3]. Cancer of the prostate is highly dependent upon the age with males over 50 year showing the highest incidence; 73% of deaths due to prostate cancer occurred above the age of 75 years [4]. Between the periods of 1999-2001 and 2008-2010 the European Age Standardized (AS) rate of incidences of prostate cancer increased by 22% [5,6].

Curcumin (diferuloylmethane) is obtained from the rhizome of the plant *Curcuma longa* (Turmeric). The naturally occurring spice is widely used as food and medicinal agent in Indian Ayurveda medicine and also Chinese medicine [7]. Recent investigations showed that curcumin acts as a chemopreventive agent for androgen sensitive (LNCaP cells) as well as androgen independent (DU145) prostate cancer cell lines [8]. Curcumin possess anti-proliferative activity and induces cell death by apoptosis [9,10]. Other investigations using omega-3/omega-6 polyunsaturated fatty acid found that these prevent the development and progression of prostate cancer [11]. However, there have been no specific investigations regarding the anti-proliferative property of curcumin in combination to fish oil (FO) or corn oil (CO).

Curcumin has low water-solubility. A variety of formulations, encapsulations, nano-formulations [12,13] have been adopted to increase site specific delivery of curcumin to cancer cells. The objectives for the present study were to examine the effect of two curcumin formulations on prostate cancer cell viability and GSH status. Curcumin microemulsified or directly dispersed with FO or CO were examined [14,15]. A combination of curcumin with CO or FO without surfactant was used as control.

2. MATERIALS AND METHODS

2.1 Materials

Curcumin (>98% pure), fish oil (FO), corn oil (CO), surfactant (Surfynol 465), resazurin (Vision blue ^{TM})., dimethyl sulfoxide (DMSO) and related chemicals were obtained from Sigma-Aldrich, UK and stored at temperature -20°C. Human prostate cancer cell lines (LNCaP) were obtained from American type cell culture collection. Foetal bovine serum (FBS), RPMI1640, penicillin-streptomycin solution, trypsin 1X, and phosphate buffer saline (PBS) tablets were from GIBCO® Laboratories (Invitrogen Ltd, UK).

2.2 Instruments

Nucleo counter (model NC-3000, ChemoMetec, Denmark) was utilized for counting prostate cancer cells. Fluostar Omega Instrument (BMG-Lab-Tech, Germany) was used for fluorimetric assays. Incubator, 37°C temperature, 5% CO_2 (LEEC, UK) was used for *in vitro* incubation and culture. All cell culture operations were performed in a laminar flow cabinet (Air flow service, UK). Other equipment included a microscope (Olympus, Japan) and a Sanyo centrifuge (Max speed-4700, RPM/4964 RCF, Sanyo, UK).

2.3 Methods

2.3.1 Cell culture

Prostate cancer cell lines LNCaP were cultured using RPMI 1640 supplemented with FBS (10%), penicillin-streptomycin (1%). The cell culture

flasks and 96-well micro plates were incubated at 37°C in a 5% CO_2 atmosphere. Cells were trypsinized, counted using a Nucleo-Counter, and seeded (10,000 cells/ well) in 96-microwell plates with 50 µl of culture medium overnight at 37°C to allow attachment. Cells were treated with curcumin swollen micelles 0-100 µM (50 µl) for 72 hr and 144 hr at 37°C. Secondly, cells were treated with curcumin dispersed within edible oil for 24 hr, 72 hr or 144 hr respectively at 37°C; the edible oils were taken as positive control for the study.

2.3.2 Preparation of curcumin formulations

Swollen micelles were prepared from an aqueous solution of Surfynol-465 W (solution A) and curcumin dispersed on edible oil (solution B) as described for other phytochemicals [15]. Solution A comprised of 5% (w/w) solution of surfactant Surfynol-465 W dispersed in water. Solution B consisted of curcumin dispersed in edible oil (5 mg/ 10 ml) for 30 minutes and centrifuged (3000 x g) to remove undissolved solid. Swollen micelles were prepared by adding 5 ml of solution B to 95 ml of solution A with gentle stirring for 10-20 minutes. The resulting swollen micelles, containing 68 µM final curcumin concentrations and 5% oil fraction, were filtered-sterilized through 0.2 micron filters and diluted to various concentrations with RPMI 1640 for cytotoxicity testing.

Curcumin dispersions in edible oils were prepared as described for solution B and filter-sterilized. Cells were treated with oils directly for anticancer testing.

2.3.3 Determination of cell viability

Cell viability was determined using resazurin fluorescence assay [16] with modification. Growth medium and curcumin formulations were removed by washing the cells 4 times with ice-cold PBS (100 µl) Thereafter, 5 µl resazurin was added to each well on 96 microwell plate for assay. The plates were incubated for 2 hr at 37°C. Fluorescence readings were measured at excitation wavelength of 530-570 nm and emission wavelength of 590-620 nm.

2.3.4 Determination of intracellular GSH/ GST status

The monochlorobimane (MCB) assay was used to assess GST/GSH status in LNCaP cell lines [17]. After treatment with curcumin formulations, cells were washed four times with ice-cold PBS (100 µl) and 5 µl MCB was added to each well of 96 microwell plate followed by incubation for 2 hr (37°C). Fluorescence readings were measured using an excitation wavelength of 360 nm and an emission wavelength of 535 nm.

2.3.5 Statistical procedures

Data were expressed as mean±SEM of three independent experiments. One way ANOVA (SPSS) test was performed to identify significant statistical differences between treatment groups. $P<0.05$ were considered to be statistically significant.

3. RESULTS

3.1 Effect of Curcumin Swollen Micelles on Cell Viability

The effect of curcumin swollen micelles on the viability of LNCaP was determined by the resazurin assay. Fig. 1 shows that FO curcumin microemulsions inhibited LNCaP cells at 34µM resulting in 73% and 61% original cell viability after 72 hr and 144hrs. By comparison, the cell viability was reduced to 61% and 68% of original value after treatment with curcumin microemulsified with CO respectively. In the assay no 50% reductions of cell viability (IC_{50}) was estimated graphically at any concentration.

3.2 Effect of Curcumin Dissolved in Edible oils on Cell Viability

The effect of curcumin dissolved in edible oils on LNCaP cell viability after 24, 72 and 144 hr treatment is shown in Fig. 2.The concentrations of curcumin leading to 50% reductions of cell viability (IC_{50}) were estimated graphically with treatment time of 24, 72 and 144 hr respectively (Table 1). Both FO and CO were also found to be cytotoxic to LNCaP cells. Curcumin dissolved in FO (Curc.FO) was consistently more cytotoxic for LNCaP cells with 72 hr treatment. However, CO-curcumin was more cytotoxic at longer time-frames for testing (144 hrs).

3.3 Cellular GST/GSH Status of LNCaP through MCB Assay

The effect of curcumin dissolved in edible oil on cellular GST/GSH status of LNCaP, is shown in (Fig. 3). The results show changes in GST/GSGH status normalized for cell number. Readings obtained after the MCB assay were

divided with the readings obtained after the resazurin assay to obtain a normalized graph. As shown in (Fig. 3) treatment with FO, CO and curcumin dissolved with edible oils increased % GST/GSH status adjusted to % cell viability at treatment time of 72 hr.

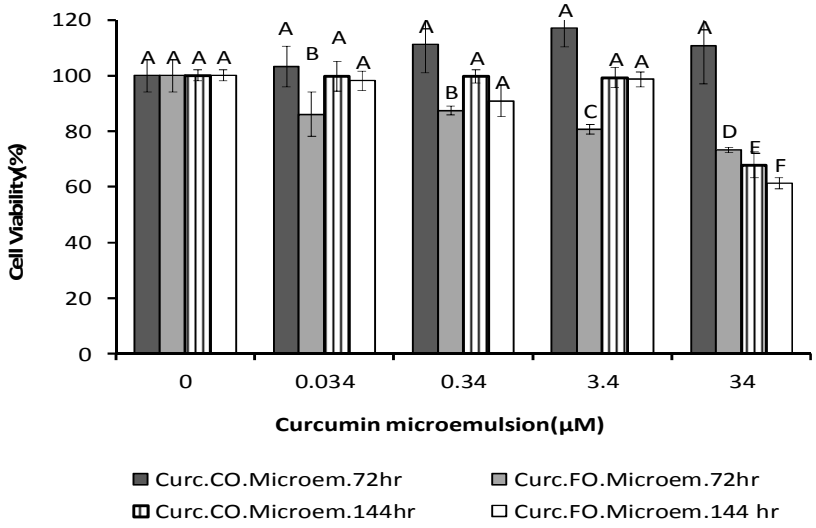

Fig. 1. Effect on LNCaP cell viability of curcumin microemulsified with Surfynol (465 W) and corn oil (Cur. CO. Microem) or fish oil (Curc. FO. Microem). Cells were treated with different concentrations of swollen curcumin micelles (0-34μM) for 72 hours and 144 hr and viability was determined using the resazurin assay. The results are percentage of fluorescence relative to media control cells (100%).Bars (means± SEM) with different letters are significantly different (P<0.05) from control

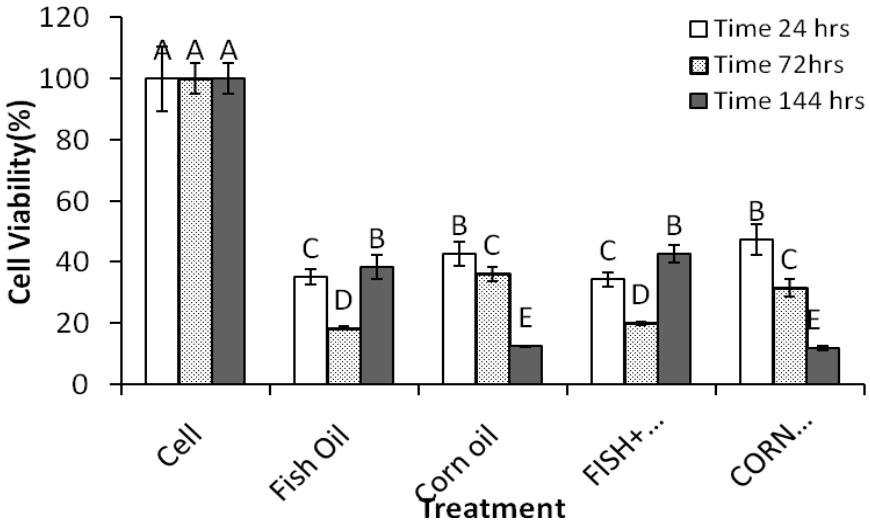

Fig. 2. Effect on LNCaP cell viability of curcumin dissolved with fish oil or corn oil (50μM). Cells were treated for 24 hr, 72 hr and 144 hr. Cell viability was determined by using the resazurin assay. The results are represented as percentage of fluorescence relative to control cells (100%).Bars with different letters are significantly different (P<0.05) from control. Quantified data are expressed as means± SEM. Bars (means ± SEM) with different letters are significantly different (P<0.05)

Table 1. Inhibition in prostate cancer cell (LNCaP) by curcumin dissolved with edible oils

	IC_{50}value (μM)*	
Time	**Curc-FO**	**Curc-CO**
24 hr.	34	45
72 hr.	20	31
144 hr.	42	12

**IC50 = concentrations of curcumin leading to 50% decline in cell viability. Curc.FO is curcumin in fish oil, Curc.CO = curcumin dissolved with corn oil*

4. DISCUSSION

Chemopreventive agents are believed to function via three mechanisms, (a) inhibition of phase I enzymes for activation of pro-carcinogens, (b) induction of phase II enzymes (e.g. GST) for cell detoxification and more recently (c)modulation of pathways for cancer cell survival, proliferation, cell death, metastasis and angiogenesis [18,19,20]. Curcumin was recently found to be cytotoxic towards prostate cancer cell line LNCaP [10,21] by regulating multiple pathways for cell survival and growth [22,23,24,25]. However, there have been no specific investigations regarding the anti-proliferative property of curcumin in combination to FO or CO with (out) surfactant. Recent research has also shown that GST is silenced in LNCaP and that loss of enzyme activity may be an important step in aetiology of prostate cancer. In this study we examined the effect on LNCaP cell viability of curcumin swollen micelles produced with Surfynol-465 W and curcumin directly dissolved in oil [11,14,15].

All formulations of curcumin were found to be cytotoxic towards LNCaP cells. Curcumin dissolved with CO and FO was cytotoxic with a similar IC_{50} value compared to curcumin dissolved with DMSO (10 to 20 μM) for 72-144 hr exposure time [10,21]. Apparently curcumin swollen micelles formed using Surfynol-465 W well less cytotoxic compared with dissolved curcumin however, the emulsions contained 5% oil fraction and higher curcumin exposure could be created by using higher volume fraction of oil. The cytotoxicity of FO alone towards LNCaP is consistent with previous research suggesting that omega-3 fatty acids exhibit anticancer effects compared to omega-6 fatty acids [11,26]. High levels of omega-3 fatty acids occur in FO whereas CO contains >50% omega-6 fatty acid in the form of linoleic acid. Surprisingly, Fig. 2 suggests that the combination of curcumin with FO or CO provided no additional increases in cytoxcity under the conditions of this study.

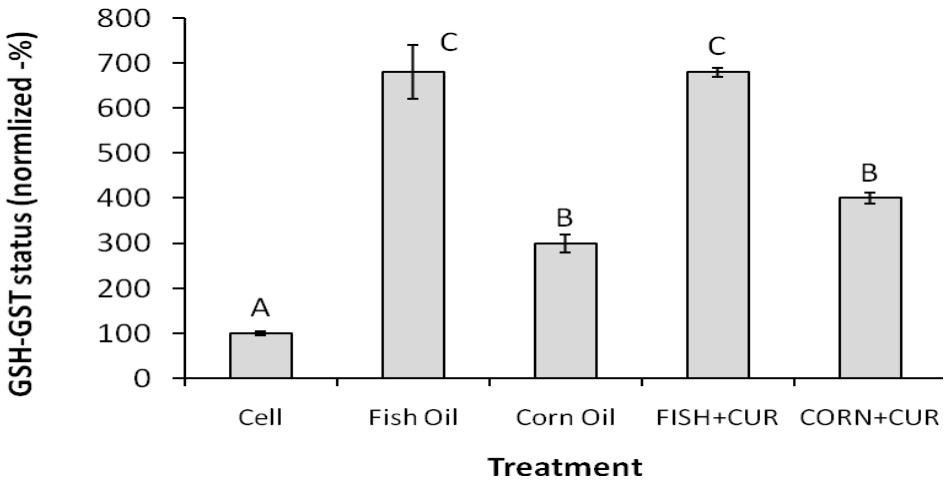

Fig. 3. Effect on GSH/GST status for LNCaP cells treated with fish oil, corn oil and curcumin dissolved in FO or CO for 72hr. Cell viability was determined by the resazurin assay. Intracellular GSH/GST status was determined by monochlorobimane (MCB) assay. The results are normalized for cell number represented as percentage of fluorescence relative to media control (100%).Bars (means ± SEM) with different letters are significantly different (P<0.05) from control

An increase of phase II enzyme activity is expected to be beneficial for healthy prostate tissue and lead to enhanced detoxification and cancer chemoprevention [27,28,29]. In context to established prostate cancer cells, over expression of phase II enzymes can contribute to increased resistance towards therapeutic agents [30]. In the present study, intracellular GST/GSH increased following FO, CO and combination of curcumin edible oil treatments. The GST/GSH activity increased by 656% by FO ($p = 0.004$), 272% by CO ($p<0.001$) whereas in case of curcumin combined with FO or CO the GSH/GST activity increased by 650% ($p < 0.001$) and 360% ($p<0.001$) respectively (Results Section: Fig. 3). Higher degrees of enzyme induction might be observed following the increase in treatment time but such data is expected to be less reliable owing to the low numbers of viable cells present in the system (Results Section: Fig. 2). This pattern of changes in phase II enzyme response is similar to those recently reported [27] for the effect of is othiocyanate and glutathione conjugates on breast cancer cells. These studies also reported that cancer cells were more resistant to phase II induction compared to healthy tissue.

5. CONCLUSION

Curcumin microemulsified using surfynol-465 W and fish or corn oil retained cytotoxicity towards LNCaP prostate cancer cells. However, curcumin dissolved with edible oil only was also cytotoxic and induced intracellular GST/GSH i.e. phase II enzyme activity in LNCaP prostate cancer cells. Currently, no previous investigations related to the effect of whole oils on LNCaP have been reported. The effect of whole oils and isolated fatty acids needs further investigations also in view of recent controversies related to the effect of dietary lipids on prostate cancer risk [31]. More research is needed also in order to improve understanding of possible underlying molecular mechanism.

CONSENT

Not applicable.

ETHICAL APPROVAL

LNCaP cells were cultured outside the human body; therefore the storage of cell lines does not require a human tissue authority (HTA) license. No ethical approval is required.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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