

RESEARCH ARTICLE

Caffeic Acid Phenethyl Ester Loaded Poly (ϵ -caprolactone) Nanoparticles for Improved Anticancer Efficacy: Formulation Development, Characterization and *in Vitro* Cytotoxicity Study

Harshad S. Kapare ¹, Lohidasan Sathiyarayanan ^{2*}, Arulmozhi S. ³, Kakasaheb R. Mahadik ²

¹ Department of Quality Assurance Techniques, Poona College of Pharmacy, Bharati Vidyapeeth University, Erandwane, Pune, India.

² Department of Pharmaceutical Chemistry, Poona College of Pharmacy, Bharati Vidyapeeth University, Erandwane, Pune, India.

³ Department of Pharmacology, Poona College of Pharmacy, Bharati Vidyapeeth University, Erandwane, Pune, India.

ARTICLE INFO

Article History:

Received 04 Aug 2020

Accepted 14 Oct 2020

Published 01 Nov 2020

Keywords:

Design of experiment

Sulforhodamine B (SRB)

Assay

Nanotechnology

Anticancer

ABSTRACT

Cytotoxic potential of caffeic acid phenethyl ester (CAPE) which is an active constituent of bee propolis is well proven. The therapeutic efficacy of CAPE is affected due to poor solubility and bioavailability. In present study CAPE loaded Poly (ϵ -caprolactone) nanoparticles formulation (denoted as CPL) was designed and investigated with the aims of enhancement of solubility, anticancer efficacy and to achieve desired characteristics. Design of experiment approach is implemented in formulation development. Developed formulations were evaluated in detail for nanoparticle characterization and *in vitro* cytotoxicity study. Developed nanoparticles showed particle size and encapsulation efficiency of 187 ± 2 - 220 ± 2 nm and 64.37 ± 1.20 - $74.80 \pm 1.45\%$ respectively. Desirable characteristics in terms of drug content, *in vitro* release, surface morphology were observed. Total growth inhibition concentration was observed to be decreased by 40.87% for developed formulation as compared to CAPE in MCF-7 cells and 23.73% in human colon cancer cells HT-29. The study proven that the developed CPL exhibited improved solubility, sustained drug release, enhanced *in vitro* cytotoxicity in MCF-7 and HT-29 cell lines in comparison with pure CAPE. Thus the proposed system may be served as a useful tool for cancer treatment.

How to cite this article

Kapare HS, Sathiyarayanan L, Arulmozhi S, Mahadik KR. Caffeic Acid Phenethyl Ester Loaded Poly (ϵ -caprolactone) Nanoparticles for Improved Anticancer Efficacy: Formulation Development, Characterization and *in Vitro* Cytotoxicity Study. *Nanomed Res J*, 2020; 5(4): 324-331. DOI: 10.22034/nmrj.2020.04.003

INTRODUCTION

Caffeic acid phenethyl ester (CAPE), a well proven and reported chemical constituent of propolis is chemically polyphenolic compound[1]. CAPE has antitumor activity on various types of cancer cells with different mechanisms including inhibition of the NF- κ B and increased activity of caspase-3 or caspase-7[2]. Formal normal cells CAPE has been reported as a harmless but it inhibits the growth of various types of cancer cells[3,4]. The potent antitumor nature of CAPE

has been reported on cancer cells through NF- κ B inhibition [5,6]. But it is highly lipophilic in nature and thus it shows poor water solubility. CAPE is reported to get hydrolysis by an esterase plasma enzymes which leads to rapid clearance and short half-life[7]. The overall effect gets results in lower bioavailability[8]. Solubility parameter is a key factor which determines drug dissolution, absorption and bioavailability. It directly affects the biological effects. For improvement of drug solubility various approaches have been reported which includes solid

* Corresponding Author Email: pharmsathiya@gmail.com

dispersions, cyclodextrin complex formation or chemical modifications however there are various limitations associated with these approaches[9]. Applications of nanotechnology in drug delivery are also established for solubility enhancement, drug targeting and to overcome other limitations associated with conventional approaches. Various biodegradable polymers have been reported in drug delivery applications to achieve improved solubility, drug targeting by enhanced permeability and retention and prolonging the biological activity[10,11]. Among those poly (ϵ -caprolactone) (PCL) is one of the biodegradable polymer known for pharmaceutical application in various drug delivery systems[12-14]. PCL has applicability in formulation development of pharmaceuticals because of its various advantages such as biocompatibility, biodegradability, high permeability and 6-hydroxycaproic acid is a biodegradation end product of PCL which is neutral and not disturb normal pH balance[15,16].

Design of experiments is an important approach implementing many formulation scientist to study effects of critical variables on the product. Nanoparticle formulations can be developed using DoE approach to study the interactions between formulation variables and reduce number of experiments. Factorial design method used to optimize the nanoparticle formulation [9, 7].

With aiming of anticancer drug delivery of CAPE some nanotechnology based attempts have been reported to overcome its limitations. However these studies have not attempted effects of formulation variables, detailed characterization of formulations, *in vitro* anticancer activity and biosafety performance[1, 18-20].

Prompted by the above facts, the present study has been designed to develop CAPE loaded PCL

nanoparticle formulation (denoted as CPL) to investigate its formulation characteristics and *in vitro* anticancer activity.

MATERIALS AND METHODS

Material

CAPE (> 98%), PCL (poly- ϵ -caprolactone, mol weight 65,000 Da), Pluronic® F-68 (PF-68) and dialysis bag were purchased from Sigma-Aldrich, Bangalore, India). Sodium hydroxide, potassium dihydrogen phosphate and acetone GR were procured from Merck India. All other pharmaceutical grade chemical and reagents used in study.

Methods

Preparation of CAPE loaded polymeric nanoparticle formulation

The nanoparticle formulation batches were prepared by solvent displacement method reported by Bothiraja et al., 2011. Briefly, PCL and Pluronic® F-68 were first dissolved in 3 ml of acetone in which the CAPE was dissolved as per composition shown in Table 1. This phase (organic) was injected into water phase (10 ml) 10 ml/min with magnetic stirring at 2000 rpm for 1 h at 40°C. The polymer aggregates were removed by membrane filtration. The concentrations of PCL and Pluronic® F-68 (PF 68) were optimized to obtain stable suspension[10].

Formulation characterization

The particle size (PS) of developed formulations was measured with by laser diffraction technique and expressed in terms of d (0.9) μ m value.

The CAPE concentration as drug content (DC) and encapsulation efficiency (EE) of the formulated batches were measured using previously developed and validated HPLC method.² The nanoparticle

Table1: The effect of various formulations (with different amounts of surfactant and polymer) on particle size and encapsulation efficiency by 3² factorial design with coded levels and actual amount values of variables.

Batches CPL	Coded levels (X1, X2)	Amt. of PCL (mg; X1)	Amt of PF 68 (mg; X2)	Drug Content (%)	Nanoparticle size (nm; Y1)	Encapsulation efficiency (%; Y2)
F1	+1, +1	62.50	100.00	90.15± 0.91	220± 2	64.37± 1.20
F2	+1, 0	62.50	75.00	90.13± 0.76	209± 2	67.73± 1.45
F3	+1,-1	62.50	50.00	90.83± 0.91	209± 3	67.83± 1.45
F4	0, +1	50.00	100.00	95.42± 0.76	199± 3	69.42± 1.80
F5	0, 0	50.00	75.00	95.61± 0.48	197± 3	70.61± 1.80
F6	0, -1	50.00	50.00	96.80± 0.48	191± 2	74.80± 1.45
F7	-1, +1	37.50	100.00	95.62± 0.91	190± 3	70.62± 1.45
F8	-1, 0	37.50	75.00	95.12± 0.76	188± 2	68.62± 1.20
F9	-1, -1	37.50	50.00	94.66± 0.91	187± 2	67.66± 1.45

Values were presented as mean ± SD (n = 3).

solution was suitably diluted with alcohol prior to determination. Encapsulation efficiency was measured by centrifugation at 12,000 rpm for 2 h at 4°C followed by HPLC analysis.

DoE study

DoE study was implemented for optimization of nanoparticle formulation. 3² (3 levels, 2 factors) factorial design was used, thus a total number of 9 batches were prepared. PS and EE values of formulated batches were measured. PS and EE were selected as dependent variables while the amount of PCL and amount of PF 68 were selected as independent variables.

Optimization by 3² factorial design

Trial batches were performed as preliminary experiments that helped in understanding the effects of variables on characteristics of polymeric nanoparticles. The amount of PCL (X1) and PF 68 (X2) concentration were identified as key factors in determining the characteristics of polymeric nanoparticles. Thus, a 3² factorial design was selected for optimization process while studying the effect of independent variables on PS and EE as responses.

DSC and FTIR study

DSC thermograms of vacuum evaporated samples of CAPE, PCL and CPL were recorded with 5 mg sample at heating rate of 10°C/min under a nitrogen atmosphere (flow rate 50 ml/min) using DSC 821e. KBr dispersion technique was used for FTIR study. FTIR spectra of CAPE, PCL and CPL were recorded on a FTIR spectrophotometer (JASCO FTIR-8400).

Surface morphology and Zeta potential

Surface morphology of developed formulations was studied using Transmission electron microscopy (Zeiss EM 109) with carbon film covered copper grid technique. The zeta potential was measured with Zetasizer at a temperature of 25°C.

In vitro drug release study

in vitro drug release study was carried out in phosphate buffer (pH 7.4) as drug release medium with dialysis bag technique at 37 ± 0.5 °C with continuous magnetic stirring at 100 rpm. At predetermined time intervals sample was withdrawn maintaining sink condition. The samples were analyzed for CAPE content

by developed and validated HPLC method. The percent cumulative release of CAPE was plotted against time. Pure CAPE release was analyzed in a similar manner[21].

In vitro cytotoxicity

In vitro cytotoxic activity of CAPE and CPL was carried out on MCF-7 and HT-9 cell line using sulforhodamine B (SRB) assay. The study performed as per the procedure described by Bothiraja et al, 2013 at Tata Memorial Center, Advanced Centre for Treatment Research and Education in Cancer (ACTREC) Mumbai, India. CPL was diluted in distilled water so as to obtain CAPE in the concentration ranges of 10, 20, 40 and 80µg/ml which were analyzed for cytotoxicity using SRB assay. Absorbance was measured on an ELISA plate reader at a wavelength of 540 nm with 640 nm reference wavelength[21, 22].

Hemolysis study

The hemolysis study on CAPE and CPL were performed using the method reported by love et al., 2012. Distilled water taken as 100% hemolysis control and NaCl solution as the non hemolysis control. CAPE and CPL samples corresponding to concentrations of 0.25, 0.5, 0.75, 1 and 1.5 mg/ml of formulations were taken for study. Absorbance of supernatants was determined at 453 nm with UV-VIS spectrophotometer[23].

Stability study

CPL was transferred in glass vial and subjected to short term stability study at 4 °C in refrigerated condition. Optimized nanoparticles were analyzed for the change in particle size and encapsulation efficiency up to 180 days.

RESULTS AND DISCUSSION

Nanotechnology in drug delivery systems has proven for enhanced efficacy and safety of various drugs over the past few decades[24-26]. In this study, CAPE loaded polymeric nanoparticles have been investigated to improve its solubility, *in vitro* anticancer efficacy. The effect of the CAPE loaded polymeric nanoparticles composition on PS and EE was studied by DoE approach.

PS, DC and EE

The mean CPL nanoparticles size was obtained in the range of 187 ± 2 to 220 ± 5 and strongly

affected by the selected variables. The DC and EE were obtained in range of 90.13–96.80 % and 64.37–74.80 %, respectively for CPL (Table 1). A good fit r^2 for PS = 0.9284 and EE = 0.8134 for CPL was observed for the particle size and encapsulation efficiency the independent variables. Particle size affects in site specific drug delivery. In case very small particles also particle uptake by non targeted cells decreases and premature clearance occurs by the phagocytic system[27].

Optimization of CPL nanoparticles by 3² factorial design

Particle size and encapsulation efficiency are the key factors considered in nanoparticle formulation development. During the preliminary study, assessment of the concentrations of polymers and surfactants were done for obtaining non-sedimenting, non-aggregating nanoparticles. After preliminary studies, optimization was done by 3² factorial design to determine final proportions of polymer and surfactant (Table 1). CAPE content was kept constant for each batch. Batches were formulated as per 3² factorial design and responses shown in Table 1, Table 2 A&B. Multiple regression analysis performed with Design Expert® software and data summarized in Table 2 A&B. It was observed that positive coefficients of the terms X1 and X2 indicated a favorable effect on the mean particle size and EE with the PCL and PF68. The PCL had a linear effect on the PS and curvilinear effect on EE while PF 68 had linear effects on both PS and EE as seen in surface plot (Fig. 1).

Based on the results of the factorial design, the

solution for optimum batch selection with highest desirability of 0.852 was obtained with F6 for CPL so F6 batch was selected as an optimized formulation and further evaluated for various parameters.

Zeta potential

Zeta potential is essential parameter gives information about surface charges which has direct impact on colloidal stability. The CPL showed negative zeta potential -23.01 ± 0.45 mV that might be observed due to presence of PF 68 on surface. Desirable values of zeta potential obtained considering the colloidal stability of developed formulation.

Differential scanning calorimetry and Fourier transform-infrared spectroscopy (FTIR)

Study was performed for the free CAPE, PCL and CPL (Fig. 2A) in order to determine the molecular state of the CAPE. A sharp melting transition of free CAPE was observed at 127.48 °C with ΔH 38.01 J/g shows transit crystallinity. In CPL thermogram, the free CAPE peak was disappeared which indicates molecular dispersion of CAPE inside polymeric nanoparticles.

Fig. 2B shows FTIR spectra of drug, polymer and formulation. FT-IR spectrum of free CAPE showed specific bands obtained at 3482 cm^{-1} and 3333 cm^{-1} of -OH stretching and at 3061.44 cm^{-1} of C-H stretch. The strong and narrow peaks also observed at 1683 cm^{-1} , 1602 cm^{-1} , and 1184 cm^{-1} of C=O, C=C, and C-O. In case of PCL the specific bands at 2940 cm^{-1} cm^{-1} of C-H hydroxyl groups asymmetric stretching and 2860 of C-H hydroxyl groups symmetric stretching were observed. Also bands at 1722 cm^{-1} (C=O stretching vibrations of the ester carbonyl group), 1238 cm^{-1} (C-O-C

Table 2 A: The results of multiple regression analysis of the obtained data

Sr. no	Final Equation in Terms of Coded Factors		
	EE	PS	
1	+68.38	+183.06	=
2	+32.21	+48.67	* X1
3	+0.73	+7.33	* X2
4	-12.84		* X1X2
5	-60.88		* X1 ²
6	+0.52		* X2 ²

Table 2 B: Optimized batch selection with desirability index.

Number	PLGA	PVA	EE	PS	Desirability
CPL	55.286	50.000	72.051	191.121	0.852

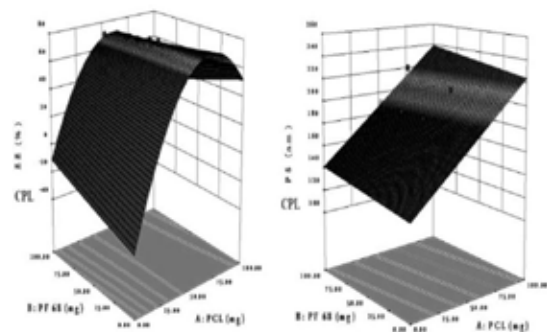


Fig. 1: Response surface plot showing effects of amount of PCL and PF 68 (independent variables) on dependent variables PS and EE for CPL

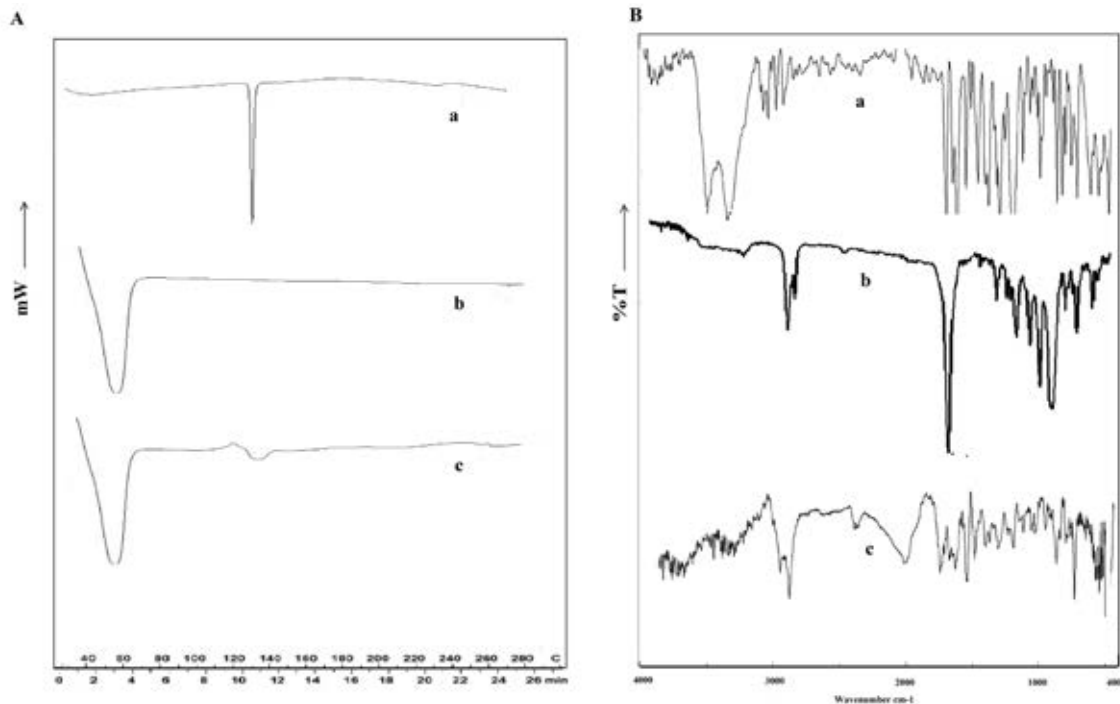


Fig. 2 A&B: (A) Comparative DSC thermo grams of CAPE (a), PCL (b), CPL (c) and (B) Comparative FTIR spectra of pure CAPE (a), PCL (b), and CPL (c).

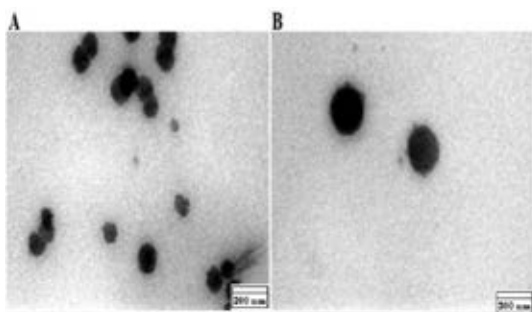


Fig. 3: Transmission electron microscopy images of CPL (A&B)

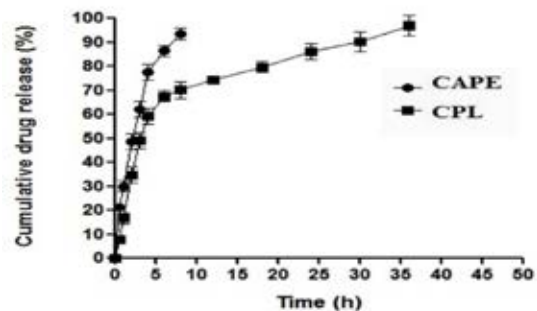


Fig. 4: In vitro release study of pure CAPE and CAPE from CPL

asymmetric stretching) and 1160 cm^{-1} (C-O-C symmetric stretching) were observed. FTIR spectra of CPL, band intensity of CAPE at 3482 cm^{-1} , 3333 cm^{-1} and 1602 cm^{-1} were significantly reduced gives confirmation of CAPE encapsulation.

Transmission electron microscopy (TEM) of nanoparticles

Surface morphology of the CPL was studied TEM from which it can be observed that the nanoparticles were spherical in shape and freely dispersed (Fig. 3). Larger size nanoparticles were observed in TEM than that tested by laser diffraction that may be due to low melting points of PF 68 ($\sim 55\text{ }^\circ\text{C}$) and PCL ($\sim 60\text{ }^\circ\text{C}$), may undergoes

the melting and expansion in TEM.

In vitro release study

Fig. 4 revealed that CAPE showed 92.39% release in 6 h. However, CAPE release from CPL showed initial burst release (19.00%) within the first 1 h and further sustained release up to 36 h.

Drug release from nanoparticle occurs through erosion, diffusion and degradation mechanisms. For developed system any or all three mechanisms may be contributed. The initial burst release may be because of drug adsorbed on the surface of the nanoparticles or dissolved in medium, while a sustained release could be caused by diffusion of the drug. For development of desired

Table 3: TGI and GI₅₀ values of CAPE, CPL and ADR on MCF-7 breast cancer and HT-29 colon cancer cell line.

Samples	MCF-7 cell line TGI (µg/ml)	MCF-7 cell line GI ₅₀ (µg/ml)	HT-29 cell line TGI (µg/ml)	HT-29 cell line GI ₅₀ (µg/ml)
CAPE	46.00±0.020	12.1±0.010	47.20±0.015	20.10±0.010
CPL	27.20±0.010	< 10	36.00±0.010	12.00±0.010
ADR	< 10	< 10	< 10	< 10

Values were presented as mean ± SD (n = 3).

TGI- Concentration of drug that produce total inhibition of cells, GI₅₀- Concentration of drug that produces 50% inhibition of cells,

ADR- Adriamycin (Positive control)

formulation, sustained release of entrapped drug from nanoparticles is an important parameter, as it maintains constant amount of drug persistently at site of action.

In vitro anticancer activity

The *in vitro* anticancer activity of CPL was performed on MCF-7 and HT-29 cells. The observations showed in Table 3, Fig. 5 indicated that CPL displayed superior anticancer activity than CAPE on MCF-7 cells. The TGI value for CPL was observed 27.20±0.010 µg/ml while for CAPE it was 46.00±0.020 µg/ml. In case of HT-29 cells lines Table 3, Fig. 6 indicated that CPL displayed superior anticancer activity than CAPE. The TGI for CPL was observed 36.00±0.010 µg/ml and for CAPE 47.20±0.015% µg/ml.

Results and microscopic images showed that in Fig. 5 A cells were appeared with high content and

density which are of normal control group of MCF-7. In Fig. 5 B is of positive control group treated with Adriamycin, Fig. C & D were images of CAPE and CPL treated group respectively and appearing to be less dense and rounded.

Similarly Fig. 6 A cells were appeared with high content and density which are of normal control group of HT-29. In Fig. 6 B is of positive control group treated with Adriamycin, Fig. C & D were images of CAPE and CPL treated group respectively and appearing to be less dense and rounded.

The enhanced anticancer efficacy may be because higher cellular uptake of CPL with phagocytosis/ fusion which resulted in the EPR of the cell membrane to CAPE that allows sufficient drug concentration inside the cells. Therefore, CPL might serve as good carrier to improve *in vitro* anticancer activity of CAPE. The lower anticancer activity of free CAPE in solution may be because

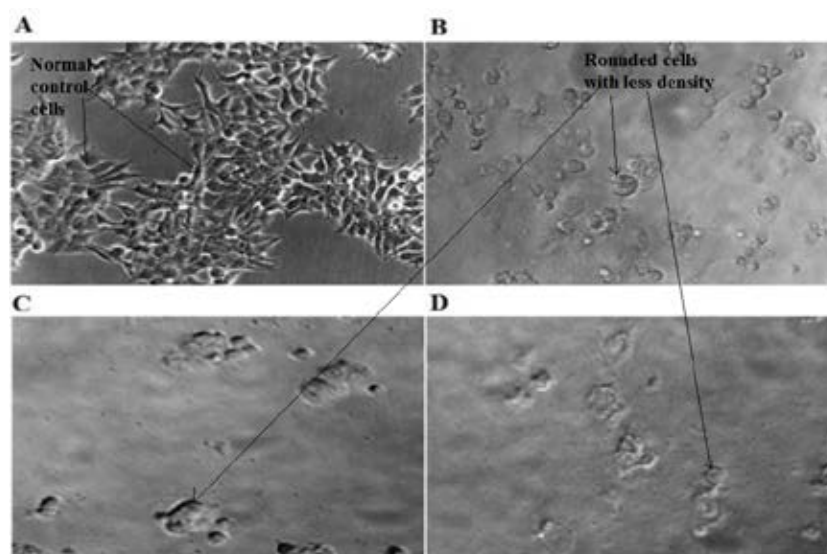


Fig. 5: In vitro cytotoxicity study on MCF-7 Cell lines (A) Normal control (MCF-7 cell line) (B) Positive control (ADR) (C) CAPE Treated (D) CPL Treated

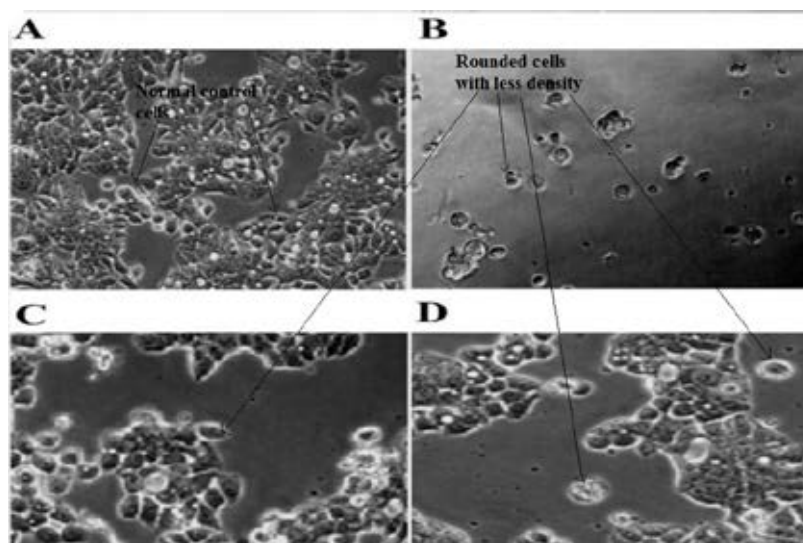


Fig. 6: In vitro cytotoxicity study on HT-29 Cell lines (A) Normal control (MCF-7 cell line) (B) Positive control (ADR) (C) CAPE Treated (D) CPL Treated

of P-glycoprotein pumps efflux. The GI50 value for CPL was observed less than 10 µg/ml indicates anticancer active on MCF-7 whereas it was 12 ± 0.010 µg/ml for HT-29 cells. From the values obtain it can be concluded that the CPL showed better anticancer potential on MCF-7. Similarly on both cell lines CAPE showed better anticancer potential on MCF-7 breast cancer cell line. Also CPL showed enhanced cytotoxicity on both cell lines as compare to CAPE. The reason for enhanced cytotoxicity may be attributed to increased solubility, enhanced permeability and retention (EPR) effect because of reduction in particle size and developed formulation as a nanocarrier.

Hemolysis study

For *in vivo* application as a biomedicine it is necessary to evaluate the biosafety of developed formulation. The results of hemolysis study indicates that the hemolysis rate for up to 1.5 mg/ml concentration of CAPE and CPL were 2.5 and 2.6 % respectively which is within acceptable hemolysis rate[26] and safe for internal use.

Stability studies

No significant deviations in EE and PS were observed as compared to initial values ($P > 0.05$) of freshly prepared nanoparticle formulation over the period of six months during stability study which indicates the developed formulation was physically stable for at least 6 months.

CONCLUSION

Nanoparticulate formulation composed of PCL and PF-68 was developed for delivery of anticancer bioactive, CAPE. The developed formulation displayed desirable particle size, encapsulation efficiency and sustain release with better stability. CPL demonstrated better anticancer efficacy as compared to CAPE. CPL was observed with better *in vitro* cytotoxicity in both MCF-7 and HT-29 than its free form, which may leads to reduction in dose. Developed formulation can be further investigated as a dosage form for CAPE to enhance therapeutic efficacy in cancer chemotherapy.

ACKNOWLEDGEMENTS

All Authors are thankful to Advanced Centre of Treatment Research and Education in Cancer (ACTREC), Mumbai, India for *in vitro* cytotoxicity study. This work was supported by the University Grants Commission, Government of India [grant numbers UGC-MRP, F.No.42-699/SR 2013].

CONFLICT OF INTEREST

The authors report no conflicts of interest in this work.

REFERENCES

1. Kapare HS, Sathiyarayanan L. Nutritional and Therapeutic potential of Propolis: A Review. *Research Journal of Pharmacy and Technology*. 2020;13(7):3545.
2. Kapare H, Sathiyarayanan L, Arulmozhi S, Mahadik K. Standardization, chemical profiling, *in vitro* cytotoxic

- effects, in vivo anticarcinogenic potential and biosafety profile of Indian propolis. *J Ayur Int Med.* 2017; 10: 81-87.
3. Ilhan A, Koltuksuz U, Ozen S, Uz E, Ciralik H, Akyol O. The effects of caffeic acid phenethyl ester (CAPE) on spinal cord ischemia/reperfusion injury in rabbits. *European Journal of Cardio-Thoracic Surgery.* 1999;16(4):458-63.
 4. Burke TR, Jr., Fesen M, Mazumder A, Yung J, Wang J, Carothers AM, et al. Hydroxylated Aromatic Inhibitors of HIV-1 Integrase. *Journal of Medicinal Chemistry.* 1995;38(21):4171-8.
 5. Wu J, Omene C, Karkoszka J, Bosland M, Eckard J, Klein CB, et al. Caffeic acid phenethyl ester (CAPE), derived from a honeybee product propolis, exhibits a diversity of anti-tumor effects in pre-clinical models of human breast cancer. *Cancer Letters.* 2011;308(1):43-53.
 6. Liao H-F, Chen Y-Y, Liu J-J, Hsu M-L, Shieh H-J, Liao H-J, et al. Inhibitory Effect of Caffeic Acid Phenethyl Ester on Angiogenesis, Tumor Invasion, and Metastasis. *Journal of Agricultural and Food Chemistry.* 2003;51(27):7907-12.
 7. Gao S, Hu M. Bioavailability Challenges Associated with Development of Anti-Cancer Phenolics. *Mini-Reviews in Medicinal Chemistry.* 2010;10(6):550-67.
 8. Weng Y-C, Chuang S-T, Lin Y-C, Chuang C-F, Chi T-C, Chiu H-L, et al. Caffeic Acid Phenylethyl Amide Protects against the Metabolic Consequences in Diabetes Mellitus Induced by Diet and Streptozocin. *Evidence-Based Complementary and Alternative Medicine.* 2012;2012:1-12.
 9. Kapare H, Sathiyarayanan L, Arulmozhi S, Mahadik K. Design and Development of Indian Propolis Loaded Poly (ϵ -Caprolactone) Nanoparticles For Improved Anticancer Efficacy, *International Journal of Pharmaceutical Research* 2017; 9 (3): 73-80.
 10. Chellampillai B, Pawar AP. Andrographolide, a novel bioactive phytoconstituent encapsulated in sustained release biodegradable nanoparticles. *International Journal of Nanotechnology.* 2011;8(8/9):764.
 11. Badran MM, Harisa GI, AlQahtani SA, Alanazi FK, Zoheir KMA. Pravastatin-loaded chitosan nanoparticles: Formulation, characterization and cytotoxicity studies. *Journal of Drug Delivery Science and Technology.* 2016;32:1-9.
 12. Han M, Diao, Fu, Hu, Jiang, Tsutsumi, et al. Doxorubicin-loaded PEG-PCL copolymer micelles enhance cytotoxicity and intracellular accumulation of doxorubicin in adriamycin-resistant tumor cells. *International Journal of Nanomedicine.* 2011:1955.
 13. Kumari A, Yadav SK, Yadav SC. Biodegradable polymeric nanoparticles based drug delivery systems. *Colloids and Surfaces B: Biointerfaces.* 2010;75(1):1-18.
 14. Mei L, Zeng, Cheng, Zheng, Song, Huang, et al. Novel docetaxel-loaded nanoparticles based on PCL-Tween 80 copolymer for cancer treatment. *International Journal of Nanomedicine.* 2011:2679.
 15. Dubey N, Varshney R, Shukla J, Ganeshpurkar A, Hazari PP, Bandopadhaya GP, et al. Synthesis and evaluation of biodegradable PCL/PEG nanoparticles for neuroendocrine tumor targeted delivery of somatostatin analog. *Drug Delivery.* 2012;19(3):132-42.
 16. Pitt G, Gratzl M, Kimmel G, Surles J, Sohindler A. Aliphatic polyesters II. The degradation of poly (DL-lactide), poly (ϵ -caprolactone), and their copolymers in vivo. *Biomaterials.* 1981;2(4):215-20.
 17. Akl MA, Kartal-Hodzic A, Oksanen T, Ismael HR, Afouna MM, Yliperttula M, et al. Factorial design formulation optimization and in vitro characterization of curcumin-loaded PLGA nanoparticles for colon delivery. *Journal of Drug Delivery Science and Technology.* 2016;32:10-20.
 18. Abamor ES. Antileishmanial activities of caffeic acid phenethyl ester loaded PLGA nanoparticles against *Leishmania infantum* promastigotes and amastigotes in vitro. *Asian Pacific Journal of Tropical Medicine.* 2017;10(1):25-34.
 19. Arasoglu T, Derman S, Mansuroglu B. Comparative evaluation of antibacterial activity of caffeic acid phenethyl ester and PLGA nanoparticle formulation by different methods. *Nanotechnology.* 2015;27(2):025103.
 20. Derman S. Caffeic Acid Phenethyl Ester Loaded PLGA Nanoparticles: Effect of Various Process Parameters on Reaction Yield, Encapsulation Efficiency, and Particle Size. *Journal of Nanomaterials.* 2015;2015:1-12.
 21. Bothiraja C, Kapare HS, Pawar AP, Shaikh KS. Development of plumbagin-loaded phospholipid-Tween®80 mixed micelles: formulation, optimization, effect on breast cancer cells and human blood/serum compatibility testing. *Therapeutic Delivery.* 2013;4(10):1247-59.
 22. Meng J, Xing J, Wang Y, Lu J, Zhao Y, Gao X, et al. Epigenetic modulation of human breast cancer by metallofullerenol nanoparticles: in vivo treatment and in vitro analysis. *Nanoscale.* 2011;3(11):4713.
 23. Kapare HS, L S, Arulmozhi, Kr M. Indian Propolis Loaded Folic Acid Conjugated PLGA Nanoparticles: Formulation Development, Characterization, In Vitro and In Vivo Anticancer Study. *Journal of Pharmaceutics & Drug Delivery Research.* 2017;06(01).
 24. Mattheolabakis G, Rigas B, Constantinides PP. Nanodelivery strategies in cancer chemotherapy: biological rationale and pharmaceutical perspectives. *Nanomedicine.* 2012;7(10):1577-90.
 25. Elizondo E, Veciana J, Ventosa N. Nanostructuring molecular materials as particles and vesicles for drug delivery, using compressed and supercritical fluids. *Nanomedicine.* 2012;7(9):1391-408.
 26. Bamrungsap S, Zhao Z, Chen T, Wang L, Li C, Fu T, et al. Nanotechnology in therapeutics: a focus on nanoparticles as a drug delivery system. *Nanomedicine.* 2012;7(8):1253-71.
 27. Brigger I, Dubernet C, Couvreur P. Nanoparticles in cancer therapy and diagnosis. *Advanced Drug Delivery Reviews.* 2002;54(5):631-51.