EFFECT OF CURCUMIN

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ARTICLE TYPE

The Influence of Surface Charge on The Antiviral Effect of Curcumin Loaded in Nanocarrier System

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Abstract: ***Background*** **:** Curcumin is a well-documented bioactive compound present in *Curcuma sp*., a tropical medicinal plant. This substance exhibits broad spectrum biological activities including antivirus.

Objective : This study aims to produce curcumin nanoemulsion with different surface charge (curcumin (+) nanoemulsion and curcumin (-) nanoemulsion) and to evaluate its physical characteristics, *in vitro* cell cytotoxicity and antiviral activity against dengue virus (DENV) 1 and 2.

Method : Two forms of nanoemulsion were prepared which were differed from their surface charge through spontaneous procedure resulting similar characteristics except the zeta potential value. Cytotoxicity was determined using RT-PCR method in A549 cell line and anti-DENV properties were determined by calculation of inhibitory concentration 50 (IC_{50}) value.

Results : The positively charge of curcumin-loaded nanoemulsion showed better effect in reducing the viral replication represented by lower IC_{50} value. In addition, DENV-1 was more sensitive and responsive to curcumin as compared to DENV-2.

Conclusion : Positively surface charge of curcumin-loaded nanoemulsion improves the antiviral effect of the curcumin suggesting a promising approach for alternative treatment for dengue virus infection.

Keywords: curcumin, dengue virus, nanoemulsion, surface charge, antiviral activity

1. INTRODUCTION

Dengue is the most prevalent arthropod-borne viral disease of human, a member of the *Flaviviridae* family which is transmitted between human individuals through *Aedes sp*. mosquitoes's bites. Dengue virus (DENV) can cause an endemic called dengue fever [1]. DENV can be differentiated into four serotypes: DENV-1, DENV-2, DENV-3, and DENV-4 [2]. The transmission of DENV to a susceptible human host can cause infections with broad effects, ranging from dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS), with changes in hemostasis and vascular permeability [3,4]. The cases of dengue infection in Indonesia on 2017 was 204,171, mostly found in West Java (10,016 cases), East Java (7,838 cases), and Central Java (7,400 cases) [5].

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Although DENV infection causes severe effects, currently there are no recommended antiviral therapies [6,7]. The only available vaccine was developed by Sanofi Pasteur in 2015, however offers limited use for population below nine years old [7].

Curcumin is an isolate from Indonesia's natural ingredients, *Curcuma sp*. This bioactive compound has been reported to show several activities such as antibacterial, antifungal, antiviral, antioxidant, antiamoeba, anti-inflammatory, antifertility, antidiabetic, and anticancer [8,9]. In particular to dengue virus, curcumin can effectively reduce the replication of DENV-2 [10] particles in cells (BHK-21) and can modify the life cycle of DENV [9,11]. One mechanism that allows curcumin to inhibit DENV-2 replication is related to the changes in cytoskeleton and cell apoptosis. Curcumin inhibits the replication and synthesis complex of DENV-2 RNA by decreasing the regulation of the ubiquitin proteasome system (UPS) [12,13], which is one of the main pathway for protein degradation [11,14]. Despites the potential use of curcumin, it has various weaknesses such as low bioavailability and low therapeutic effect, there are low solubility in water, high metabolism in the body, and high systemic elimination, which all limit its clinical application [15]. Numerous approach have been taken to improve the effectivity of curcumin *in vivo*, including nano-based encapsulation systems, one of them is nanoemulsion [16]. Using nanoemulsion, physicochemical stability and shelf life of curcumin were enhanced [17].

Nanocarriers offer potential promise in targeted drug delivery to a particular sites by manipulating the physicochemical properties, including the surface modification. One of the most important characteristics of nanocarriers is surface charge [18]. Nanocarriers with positive surface charge or cationic nanocarriers interact more with cell membranes which is negatively charged, as compared with the neutral or negatively charged ones, due to favourable electrostatic interaction [19]. Nowadays, cationic polymers nanocarriers showed superior properties including strong interaction with DNA and the ability to provide oriented bonds with proteins [20]. Based on several studies, cationic nanocarriers show better results such as positively charged polymeric nanoparticles with polyethylene glycol, which showed a favourable distribution and higher bioavailability in Caco-2 cells *in vitro* and small intestinal epithelial cells *in vivo* [21]. In the same way, the positively charged magnetic nanoparticles provides better internalization process in human breast cancer cells [22], as well as the positively charged silver nanoparticles which showed highest bactericidal activity against *Staphylococcus aureus*, *Staphylococcus mutans* and *Streptococcus pyogenes* [23].

Previously, we successfully developed curcumin nanoemulsion using Cremophor RH 40 and PEG 400 with the average droplet size of 40.85 ± 0.919 nm. Although, this nano system suppressed the growth of DENV, there was still no significant difference compared to the curcumin solution in DMSO. This might be happened because of the negative value of the droplet surface (-7.039 ± 0.532 mV) [16]. Therefore, in present study we added didodesyldymethylammonium bromide (DDAB) to change the surface charge into positive [24,25], to enhance the virucidal effect of the curcumin on A549 cell-infected DENV-1 and DENV-2. It has been reported that the cationic surface charge using DDAB demonstrates better penetration across intestinal epithelium due to electrostatic interaction with the negatively charged cell membranes. Thereby, it will be increasing the retention time of the drug on the cell surface and then the oral bioavailability [26,27].

2. MATERIALS AND METHOD (FOR RESEARCH ARTICLES ONLY)

2.1 Materials

The human alveolar epithelial cell line A549 was obtained from the culture collection at Eijkman Institute for Molecular Biology (Indonesia). RPMI-1640 medium, Minimum Essential Medium (MEM) medium, fetal bovine serum (FBS), Antibiotic-Antimycotic, Dulbecco's Phosphate Buffer Saline (DPBS) and Trypsin-EDTA were purchased from Gibco-Thermo Scientific. Two DENV strains were isolated from clinical isolates and propagated in Vero cells. DENV-1 JMB-034-P2 was isolated from dengue patients in Jambi (Indonesia) and DENV-2 SUB-011-P4 was isolated from patients in Surabaya (Indonesia). Curcumin (98.2% purity) was obtained from PT. Combiphar (Indonesia). Castor Oil and polyethylene glycol 400 were obtained from PT Brataco, Bekasi, Indonesia and Cremophor RH 40 was obtained from Clariant Iberica Production, Banten, Indonesia. Didodecyldimethylammonium bromide (DDAB) (98% purity) was purchased from Sigma-aldrich, St. Louis, USA. QIAamp Viral Mini Kits was obtained from Qiagen, Hilden, Germany. Superscript III reverse transcriptase was obtained from Invitrogen-Life Technologies, Carlsbad, CA, and PowerUp™ SYBR™ Green Master Mix was obtained from Applied Biosystems, CA.

2.2 Curcumin-Loaded Emulsion

Curcumin was loaded in the nanoemulsion system containing castor oil, Cremophor RH 40, and PEG 400. Castor oil (oil phase), cremophor RH 40 (surfactant), and PEG 400 (co-surfactant) were mixed with the ratio of 1:8:1 using a magnetic stirrer (Thermo Scientific) at 100 rpm for 2 hours to form the homogenous oil phase. Further, the oil phase was sonicated for 60 minutes in a sonicator bath. Subsequently, aquabidest was added to the oil phase with the ratio of 5:1 and then stirred to make a clear and homogeneous curcumin nanoemulsion. Further, we mention this preparation as curcumin (-) nanoemulsion. As a comparison, curcumin solution was prepared in DMSO with same concentration.

2.3 Curcumin Cationic Nanoemulsion

Curcumin was loaded in the nanoemulsion system containing castor oil, Cremophor RH 40, and PEG 400. Castor oil (oil phase), cremophor RH 40 (surfactant), and PEG 400 (co-surfactant) were mixed with the ratio of 1:8:1 using a magnetic stirrer (Thermo Scientific) at 100 rpm for 2 hours to form the homogenous oil phase. On another vial, DDAB was dissolved in aquabidest and stirred for 3 hours. Further, the oil phase was sonicated for 60 minutes in a sonicator bath. Subsequently, DDAB solution was added to

the oil phase with the ratio of 5:1 and then stirred to make a clear and homogeneous curcumin nanoemulsion. Further, we mention this preparation as curcumin (+) nanoemulsion.

2.4 Evaluation and Characterization of Curcumin Nanoemulsion

The particle size and polydispersity index of both curcumin nanoemulsion were determined using Delsa™ Nano C Particle Analyzer, Beckman Coulter. Zeta potential value of both curcumin nanoemulsion was measured by NanoPartica SZ-100, Horiba, Japan. The morphology of curcumin nanoemulsion was observed using negative staining transmission electron microscope JEM-1010, JEOL, Japan. Briefly, 10 µL of sample was dropped on a 400 mesh cryo-TEM grid and allowed to dry for 30 seconds before being stained with 10 µL of uranyl acetate (2%). Grid was allowed to dry for 5 minutes and place into the transmission electron microscope at Eijkman Institute for Molecular Biology, to inspect the morphology of curcumin nanoemulsion.

The calculation of curcumin content in nanoemulsions was performed by a direct method. Both curcumin nanoemulsion were centrifuged at 14,000 rpm for 20 min and 5 mL of DMSO was added to 10 µL of supernatant, to extract the curcumin. Curcumin concentration in DMSO was measured using a UV-Visible spectrophotometer (Beckman DU 7000). A calibration curve was generated with concentrations in the range of 1.5 - 5 ppm ($R^2 = 0.995$).

The curcumin content in the nanoemulsion was calculated using the following equation:

%LC = Measured amount of curcumin entrapped in nanoemulsion x (Total amount of curcumin applied in preparing nanoemulsion)-1 x 100%

2.5 Cell Viability Assay

MTT assay was done using Vybrant® MTT Cell Proliferation Assay Kit (V-13154) according to manufacturer's instructions. Twelve mM MTT stock solution was prepared by dissolving MTT powder in Dulbecco's Phosphate Buffered Saline (DPBS). Then, 10 mL of 0.01 M HCl was added to one tube containing 1 g of SDS. A549 cells ($1x10^5$ cells/well) was prepared in 96-well plate. Plates were incubated at 37°C with 5% CO_2 for 16-24 hours. Samples were diluted with various concentrations in the range of 6.25-200 µg/mL for (-) nanoemulsion, 0.25-4 µg/mL for (+) nanoemulsion, and 0.1% $^{v}/_{v}$ blank nanoemulsion and (+) nanoemulsion. Medium from the wells were removed and replace with 100 μL sample/well. Plates were incubated at 37°C with 5% CO_2 for 48 hours. Following the incubation period, the supernatant was removed from each well and replenished with 100 µL of fresh medium followed by the addition of 10 µL of 12 mM MTT solution and the plates were incubated at 37°C with 5% CO_2 for 2 hours. Then, 100 μL of the SDS-HCl solution was added to each well and plates were incubated for 4-18 hours. Absorbance was recorded at 570 nm using Multiskan™ FC Microplate Photometer (Thermo Fisher Scientific, CA. USA).

2.6 Study The Antiviral Effect of Curcumin Nanoemulsion

A549 cells were seeded 2 x 10^5 cells/well in 96-well plate and incubated overnight. The cells were subjected to infection with DENV-1 and DENV-2 (2 x 10^5 PFU/well) serotypes with multiplicity of infection (m.o.i) value of 1, and with the presence of both curcumin nanoemulsion at various concentrations. The supernatants were harvested 48 hours post-treatment and transferred to 1.5 mL tube prior to be stored in the –80°C. The total viral RNAs were extracted from 50 samples using QIAamp Viral Mini Kits (Qiagen, Hilden, Germany) according to manufacturer's instructions. Viral RNA binds specifically to the QIAamp silica membrane, and pure viral RNA is eluted in buffer provided with kit.

Dengue viral RNAs were measured using a two-step RT-PCR reaction method. In the reverse transcriptation (RT) step, the total RNA were reverse-transcribed into cDNAs by reverse transcription-polymerase chain reaction (RT-PCR; SimpliAmp™ Thermal Cycler, Applied Biosystems, CA) using Superscript III reverse transcriptase (RT; Invitrogen-Life Technologies, Carlsbad, CA) and pan-dengue primers (reverse) for all serotypes of DENV. In the PCR step, PCR products are synthesized from cDNA samples using the PowerUp™ SYBR™ Green Master Mix (Applied Biosystems, CA) with generic pan-dengue primers (forward and reverse) for all serotypes of DENV. The PCR reactions were allowed to run on an ABI 7500 fast real-time PCR machine (Applied Biosystems, CA) with thermal cycle settings according to manufacturer's recommendation.

3. RESULTS AND DISCUSSION (FOR RESEARCH ARTICLES ONLY)

We successfully produced curcumin (+) nanoemulsion and curcumin (-) nanoemulsion with transparent visual appearance (Fig. **1**), simply indicating the droplet size was smaller than the wavelengths of visible light. As shown in table **1**, the droplets size of curcumin (+) nanoemulsion was bigger than that of curcumin (-) nanoemulsion, confirming the influence of the cationic surfactant. Both curcumin nanoemulsions have uniform size with the polydispersity index value was less than 0.5 [28].

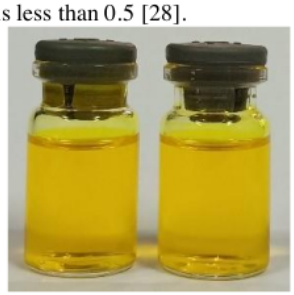

Fig.1. Visual appearance of curcumin (-) nanoemulsion (left) and curcumin (+) nanoemulsion (right).

As depicted in Fig.**1**, there is no different physical appearance between two preparations of the nanoemulsion and both systems show good physical stability. Unlike other colloidal systems, the physical stability of nanoemulsion

does not depend on zeta potential value as presented in table **1**. This was due to the high concentration of surfactant located on the surface of oil droplets which will avoid the attractive force among the droplets [17]. Cremophor RH40 is a polyoxyl 40 hydrogenated castor oil and it contains a fatty acid ester that contributed to the negative charge of curcumin nanoemulsion as previously reported [17]. However, the addition of DDAB to the nanoemulsion system changed the surface charge into positive and concentration dependence. When substrate with negative surface charges (droplet of nanoemulsion) was placed in an aqueous solution contains didodecyldimethylammonium bromide (DDAB), the cationic surfactant with two hydrocarbon chains could be assembled into the biomembrane-like tail-to-tail double-layer structure on the solid surface with the positively charged head groups toward outside, making the surface charge reverse from negative to positive [24]. As seen in table **1**, curcumin was almost completely encapsulated in both nanoemulsion systems accounted to >90%. Thus, all data listed in table **1** are very promising results for this successful self-emulsification technique, which will contribute to provide good rate and extent of drug release, absorption and then the bioavailability of curcumin [17].

Table 1. The physical parameter of curcumin-loaded nanocarrier system.

Parameter	Curcumin (+) Nanoemulsion	Curcumin (-) Nanoemulsion
Particle size (nm) (average ± SD; n=3)	28.17 ± 4.02	24.8 ± 2.5
Polydispersity index (average ± SD; n=3)	0.447 ± 0.027	0.189 ± 0.385
Zeta Potential (mV) (average ± SD; n=3)	+2.2 ± 0.17	-4.3 ± 0.5
Loading Capacity (%) (average ± SD; n=3)	99.43 ± 0.501	93.745 ± 0.948

The morphology of curcumin (+) nanoemulsion and curcumin (-) nanoemulsion were analyzed using transmission electron microscope (TEM). It confirms the spherical shape of both nanoemulsions with uniform size and nearly monodisperse system (Fig. **2**). Visualization techniques of nanoemulsion using TEM imaging is likely the most powerful and accurate technique to determine a specimen's morphology, purity and particle size distribution [17]. As seen in figure **2**, curcumin in (+) nanoemulsion demonstrates bigger droplet size, confirming the measurement using PSA technique as before mentioned.

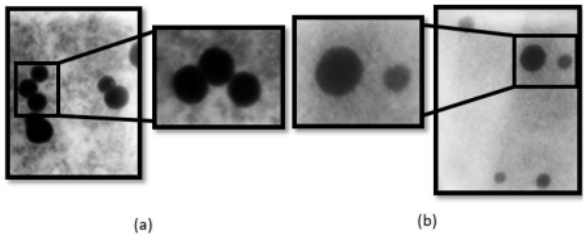

Fig.(2). Cryo-TEM photographic of (a) curcumin (-) nanoemulsion) and (b) curcumin (+) nanoemulsion. Magnification 30.000x.

We also did comparison on the cytotoxicity and viral inhibitory characteristics of curcumin (+) nanoemulsion and curcumin (-) nanoemulsion, to understand the effect of surface charge. The CC_{50} value represents the concentration of an active substance that can induce 50% reduction of the cell population. Based on our study, the CC_{50} value of curcumin in the form of (+) nanoemulsion was smaller than in the (-) nanoemulsion (Table **2**). It concludes that (+) nanoemulsion has higher toxicity effect to A549 cell lines.

Table 2. The in vitro characteristics of curcumin (+) nanoemulsion and curcumin (-) nanoemulsion in A549 cell lines as determined by CC_{50} and IC_{50} values.

Parameters	Curcumin (+) Nanoemulsion	Curcumin (-) Nanoemulsion
Cell cytotoxicity/CC_{50} (μg/mL)	2.1	12.42
IC_{50} of challenge virus (μg/mL)		
DENV-1	0.777	1.688
DENV-2	1.764	4.809

A significant decrease in cell viability was observed after treated with both curcumin (+) nanoemulsion and curcumin (-) nanoemulsion, respectively at the concentration of 4 μg/mL and 25 μg/mL. A lower cell viability was observed in curcumin (+) nanoemulsion-treated cells than in curcumin (-) nanoemulsion and there was significant impact observed on cell viability treated with vehicle control of curcumin (+) nanoemulsion. The use of surfactants in the nanoemulsion was known to be toxic to cultured cells at a certain concentration [29], so that the vehicle control of curcumin (+) nanoemulsion was also cytotoxic to a certain concentration limit. Obviously, the DDAB-generated (+) nanoemulsion was more cytotoxic. DDAB is a cationic surfactant that can cause death the cancer cells, including A549 cells, through inducing the caspase pathway or by creating pores in the cancer cell membrane [30]. In other site, DDAB can also increase the cellular uptake of curcumin [25], which was potential to promote cancer cells apoptosis [31]. Figure **3** shows the cell viability as a function of curcumin concentration in the nanoemulsion system. As

seen, more than 80% cells viable was observed when they treated with 2 μg/mL and 10 μg/mL of curcumin respectively loaded in (+).

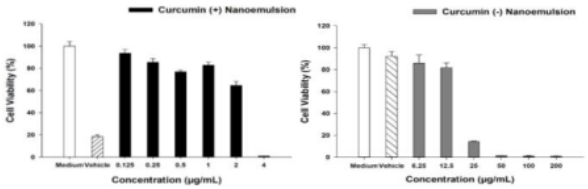

Fig.(3). A549 cell viability treated with different concentration of curcumin (+) nanoemulsion and curcumin (-) nanoemulsion.

The inhibitory effect of nanoemulsion against DENV was measured as a percentage of viable virus titre in the supernatant of treated cells to the viral titre in the control medium. In this study, we used co-treatment method in which cells were exposed to various concentrations of both curcumin nanoemulsions mixed with dengue virus at the same time. This method was used to observe the virucidal of both curcumin nanoemulsions when given along with the entry of the dengue virus. As seen from the data, the decrease in virus titre was directly proportional to the increasing concentration of the curcumin in both nanoemulsions system after 48 hours of incubation, indicating the virucidal properties on the dengue virus.

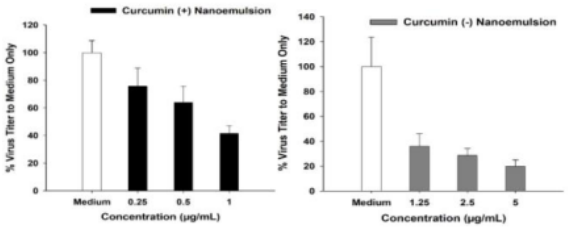

Fig.(4). Inhibitory effect of curcumin (+) nanoemulsion and curcumin (-) nanoemulsion to the replication of DENV-1 using co-treatment approach.

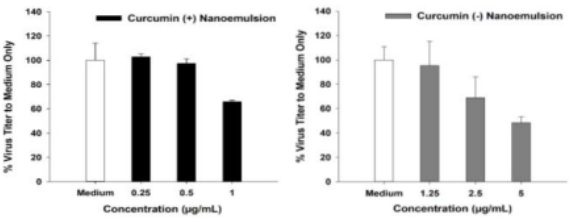

Fig.(5). Inhibitory effect of curcumin (+) nanoemulsion and curcumin (-) nanoemulsion to the replication of DENV-2 using co-treatment approach.

The viral titre values after incubation with curcumin (+) nanoemulsion and curcumin (-) nanoemulsion were calculated from RT-qPCR results. The viral titre of DENV-1 and DENV-2 decreased after treated with either curcumin (+) nanoemulsion and curcumin (-) nanoemulsion (Fig. **4** and Fig. **5**). Against DENV-1, curcumin (+) nanoemulsion's virucidal effect was lower than curcumin (-) nanoemulsion (Table **2**). This phenomena may happened because the use of cationic surfactant may affect the interaction of nanoparticles with negatively charged biological components and cell membranes, and thus determines the ultimate clinical application of nanoparticle drug delivery system [21, 22, 33]. Thereby, it will be increasing the retention time of the drug on the cell surface [26,27]. In contrast to DENV-2, although the decrease in viral titre was directly proportional to the increasing concentration of the curcumin, the provision of both curcumin (+) nanoemulsion and curcumin (-) nanoemulsion did not provide significant results when compared to the control (Fig. **5**). Several possibilities might explain this phenomena, such as the concentration was still too low or the faster replication rate of the DENV-2 than DENV-1. Overall, we address important clue i.e the curcumin (+) nanoemulsion was more powerful to show the antiviral effect on DENV at the *in vitro* model.

CONCLUSION

Curcumin (+) nanoemulsion was successfully developed with the particle size less than 50 nm and the system was nearly monodisperse, confirmed by the value of polidispersity index and morphology analysis using cryo-Transmission Electron Microscope. Addition of cationic surfactant results the nanoemulsion with zeta potential value was +2.2 ± 0.17 mV. This cationic nanoemulsion exhibits higher virucidal effect against DENV-1 and DENV-2 as compared with the (-) corresponding form. This finding emphasizes the important charge on the virucidal effect of the curcumin loaded in the nanoemulsion system, especially against DENV-1 and DENV-2 in the *in vitro* model.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not Applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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SUPPORTIVE/SUPPLEMENTARY MATERIAL

The data that support the findings of this study are availale from the corresponding author, [Prof. Dr. Heni Rachmawati], upon request.

REFERENCES

[1] Reynolds ES, Hart CE, Hermance ME, Brining DL, Thangamani S. An overview of animal models for arthropod-borne viruses. Comp Med 2017; 67(3): 232-241.

[2] Gubler DJ. Dengue and dengue hemorrhagic fever. Clin Microbiol Rev 1998; 11(3): 480–496.

[3] Martina BEE, Koraka P, Osterhaus ADME. Dengue virus pathogenesis: an integrated view. Clin Microbiol Rev 2009; 22(4): 564-581.

[4] Povoa TF, Alves AMB, Oliveir CAB, Nuovo GJ, Chagas VLA, Paes MV. The pathology of severe dengue in multiple organs of human fatal cases: histopathology , ultrastructure and virus replication. PLoS ONE 2014; 9(4): e83386.

[5] Indonesian Ministry of Health. The situation of dengue fever in Indonesia in 2017 [Situasi penyakit demam berdarah di Indonesia tahun 2017]. Indonesian Ministry of Health; [accessed 2020 Sept 22]. https://www.kemkes.go.id/resources/download/pusdatin/infodatin/InfoDatin-Situasi-Demam-Berdarah-Dengue.pdf

[6] Low JGH, Ooi EE, Vasudevan SG. Current status of dengue therapeutics research and development. J Infect Dis 2016; 215(S2): S96-S102.

[7] Dighe SN, Ekwudu O, Dua K, Chellappan DK, Katavic PL, Collet TA. Recent update on anti-dengue drug discovery. Eur J Med Chem 2019; 176: 431–455.

[8] Kumavat SD, Chaudhari YS, Borole P, Mishra P, Shenghani K, Duvvuri P. Degradation studies of curcumin. International journal of pharmacy review and research 2013; 3(2): 50-55.

[9] Padilla L, Rodriguez A, Gonzales MM, Juan C, Gallego G, Jhon C, Castano O. Inhibitory effects of curcumin on dengue virus type 2-infected cells in vitro. Arch Virol 2014; 159(3): 573-579.

[10] Balasubramanian A, Pilankatta R, Teramoto T, Sajith A M, Nwulia E, Kulkarni A, Padmanabhan R. Inhibition of dengue virus by curcuminoids. Antiviral Res 2019; 162: 71-78.

[11] Glickman MH, Ciechanover A. The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. Physiol Rev 2002; 82(2): 373-428.

[12] Chen TY, Chen DY, Wen HW, Ou JL, Chiou SS, Chen JM, Wong ML, Hsu WL. Inhibition of enveloped viruses infectivity by curcumin. PLoS ONE 2013; 8(5): e62482.

[13] Wang JL, Zhang JL, Chen W, Xu XF, Gao N, Fan DY, An, J. Roles of small GTPase Rac1 in the regulation of actin cytoskeleton during dengue virus infection. PLoS Negl Trop Di 2010;. 4(8): e809.

[14] Qin Y, Lin L, Chen Y, Wu S, Si X, Wu H, Zhai X, Wang Y, Tong L, Pan B. Curcumin inhibits the replication of enterovirus 71 in vitro. Acta Pharm Sin B 2014; 4(4): 284–294.

[15] Rachmawati H, Soraya IS, Kurniati NF, Rahma A. In vitro study on antihypertensive and antihypercholesterolemic effects of a curcumin nanoemulsion. Sci Pharm 2016; 84(1): 131–140.

[16] Nabila N, Suada NK, Denis D, Yohan B, Adi AC, Veterini AS, Anindya AL, Sasmono RT, Rachmawati H. Antiviral action of curcumin encapsulated in nanoemulsion against four serotypes of dengue virus. Pharm Nanotechnol 2020; 8(1): 54-62.

[17] Rachmawati H, Budiputra DK, Mauludin R. Curcumin nanoemulsion for transdermal application: formulation and evaluation. Drug Dev Ind Pharm 2014; 41(4): 560-66.

[18] Wei X, Shao B, He Z, Ye T, Luo M, Sang Y, Liang X, Wang W, Luo S, Yang S, et al. Cationic nanocarriers induce cell necrosis through impairment of Na + / K + -ATPase and cause subsequent inflammatory response. Cell Res 2015; 25(2): 237-253.

[19] Forest V, Pourchez J. Preferential binding of positive nanoparticles on cell membranes is due to electrostatic interactions : a too simplistic explanation that does not take into account the nanoparticle protein corona. Mater Sci Eng C Mater Biol Appl 2017; 70(1): 889-896.

[20] Farshbaf M, Davaran S, Zarebkohan A, Annabi N, Akbarzadeh A, Salehi R. Significant role of cationic polymers in drug delivery systems. Artif Cells Nanomed Biotechnol 2017; 46(8): 1872-1891.

[21] Du XJ, Wang JL, Iqbal S, Li HJ, Cao ZT, Wang YC, Du JZ, Wang J. The effect of surface charge on oral absorption of polymeric nanoparticles. Biomater Sci 2018; 6(3): 642-650.

[22] Osaka T, Nakanishi T, Shanmugam S, Takahama S, Zhang H. Effect of surface charge of magnetite nanoparticles on their internalization into breast cancer and umbilical vein endothelial cells. Colloids Surf B Biointerface 2009; 71(2): 325-330.

[23] Abbaszadegan A, Ghahramani Y, Gholami A, Hemmateenejad B, Dorostkar S, Nabavizadeh M, Sharghi H. The effect of charge at the surface of silver nanoparticles on antimicrobial activity against gram-positive and gram-negative bacteria : a preliminary study. J Nanomater 2015; 2015: 720654.

[24] Hu Y, Sun H, Hu N. Assembly of layer-by-layer films of electroactive hemoglobin and surfactant didodecyldimethylammonium bromide. J Colloid Interface Sci 2007; 314(1): 131-140.

[25] Zhao T, Chen H, Yang L, Jin H, Li Z, Han L, Lu F, Xu Z. DDAB-modified TPGS-b-(PCL-ran-PGA) nnoparticles as oral anticancer drug carrier for lung cancer chemotherapy. World Scientific Publishing Company 2013; 8(2): 1350014.

[26] Song C, Labhasetwar V, Cui X, Underwood T, Levy RJ. Arterial uptake of biodegradable nanoparticles for intravascular local drug delivery: results with an acute dog model. J Control Release 1998; 54(2): 201-211.

[27] Madishetty S, Syed MA, Kandadi P, Veerabrahma K. Cationic Diclofenac Lipid Nanoemulsion for

Improved Oral Bioavailability: Preparation , Characterization and In Vivo Evaluation. Int J Pharm Sci Nanotech 2015; 8(2): 2874-2880.

[28] Wu L, Zhang J, Watanabe W. Physical and chemical stability of drug nanoparticles. Adv Drug Deliv Rev 2011; 63(6): 456–469.

[29] Moghadamtousi SZ, Kadir HA, Hassandarvish P, Tajik H, Abubakar S, Zandi K. A review on antibacterial, antiviral, and antifungal activity of curcumin. BioMed Res Int 2014; 2014: 186864.

[30] Kusumoto K, Ishikawa T. Didodecyldimethylammonium bromide (DDAB) induces caspase-mediated apoptosis in human leukemia HL-60 cells. J Control Release 2010; 147(2): 246-252.

[31] Yao Q, Lin M, Wang Y, Lai Y, Hu J, Fu T, Wang L, Lin S, Chen L, Guo Y. Curcumin induces the apoptosis of A549 cells via oxidative stress and MAPK signaling pathways. Int J Mol Med 2015; 36(4): 1118–1126.

[32] Chen Q, Lu G, Wang Y, Xu Y, Zheng Y, Yan L, Jiang Z, Yang L, Zhan J, Wu Y, et al. Cytoskeleton disorganization during apoptosis induced by curcumin in A549 lung adenocarcinoma cells. Planta Med 2009; 75(8): 808-813.

[33] Silva AM, Gomes CM, Coutinho TE, Fangueiro JF, Lopez ES, Pashirova TN, Andreani T, Souto EB. Soft cationic nanoparticles for drug delivery : production and cytotoxicity of solid lipid nanoparticles (SLNs). Appl Sci 2019; 2019(9): 4438.

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