LIPOSOM FORMULATION AS A THYMOQUINON NANO-CARRIER TO INCREASED THE ANTICANCER ACTIVITY

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Abstract

Background. *The use of liposom in drug delivery system have been developed rapidly in order to increase terapeutics effect of anticancer.*

Objective. *This research was aimed to find an optimal condition of thymoquinon liposom formulation which have a good characteristic of liposom i.e. liposom size, polidispersibility index (PI), and potential zeta, which can increase a terapeutics effect.*

Methods. *The formulation method of thymoquinon liposom was Mozafari methode which had been modified. In this research the concentration of phospholipid and thymoquinon had been optimized. Characterization of liposom used the Particle Sizer Analysis (PSA) for determination the size of liposom and polidispersibility index, Zeta Sizer Analysis for determination the potential zeta of liposom, and Transmition Electron Microscopy (TEM) for describe the morphology of liposom.*

Outcome measured. *liposom size, polidispersibility index (PI), and potential zeta*

Results. *The result showed that the formulation of thymoquinon liposom had 80,8 nm in liposom size (a good liposom have 10-100 nm in size), 0,348 in polidispersibility index (PI) (a good PI is under 0,5), and -15,34 in potential zeta (its related to the liposom stability). From the TEM analysis could be seen the morphology of thymoquinon liposom.*

Conclusion. *From this result could be conclude that thymoquinon liposom has a good characteristic so it can be developed as a potent anticancer agent.*

Keywords *thymoquinon, liposom, nano-carrier, anticancer*

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INTRODUCTION

Liposomes have long been recognized as drug delivery vehicle for cancer therapy (Khan, 2010; Khan et al., 2008; Wang et al., 2008; Allen and Cullis, 2004). They can accommodate both hydrophilic and hydrophobic drugs by storing them either in their internal aqueous core or their phospholipid bilayer, respectively. Liposomes are generated from phospholipids, that makes them an ideal candidates for drug delivery systems because they are nontoxic, biocompatible, biodegradable and nonimunogenic (Wang et al., 2009; Washington et al., 2001). Liposomal treatment has been shown capability to reduce some of the traditional side effects associated with chemotherapy when compared to unencapsulated drugs. An important physical aspect associated with the clinical successes of liposomese-based drugs is the size of the nanocarrier (Ian MacLachlan, 2007).

Thymoquinon is the main bioactive component of the volatile oil of the black seed (*Nigella sativa*, Linn). Previous studies reported that thymoquinone exhibited inhibitory effects on cell proliferation of many types of cancer cell lines and can induce cells apoptosis (Gali-Muhtasib et al., 2006; Yi et al., 2008; Ivankovic, 2006; El-Mahdy, 2005).

Cancer cell can effluxs the drug or another substances which entered to it, so the maximum therapy cannot be achieved, but nanoparticle can entered to the cancer cells easily (Yuan et al., 2010). To solve this problem, preparation of nanocarrier as drug delivery system have been developed. In this research thymoquinon as an active ingredient had been formulated to nanoliposomes which have good characteristic i.e. liposomes size, polidispersibility index (PI), and potential zeta, which can increase a terapeutics effect as anticancer. The formulation method of thymoquinon liposomes was *Mozafari methode* which had been modified. In this research the concentration of phospholipid and thymoquinon had been optimized.

METHODS

1. Thymoquinon Nanoliposomes Formulation (Mozafari et al., 2008)

Formula optimization had been done with two factor were optimized, phosphatidilcholine concentration (9,5 mg/mL and 19,5 mg/mL) and thymoquinon concentration (5 mg/mL dan 10 mg/mL). Formula 1 (F1) with 9,5 mg/mL fosfatidilkolin and 5 mg/mL thymoquinon, and Formula 3 (F3) with 19,5 mg/mL fosfatidilkolin and 10 mg/mL thymoquinon. The characteristic of liposomes thymoquinon had been measured, that were the size of liposomes, polidispersibility index, potensial zeta, and morphological of thymoquinon liposomes.

Lipid phase had been made with mixture the soya phosphatidilcholine with cholesterol 1:1, on the *hotplate stirrer* (e.g. RET basic IKAMAG 1 Safety Control, IKA), the speed was 1000 rpm, and the temperature was 40^{o}C during 45-60 minutes, under the nitrogen circulation. Then glyserol was be added (the final concentration of glycerol was 3% v/v). Thymoquinon was be added after the temperature was decrease, and the condition was under the nitrogen circulation until 1 hour to get a stabil nanoliposomes.

2. Characterization of Thymoquinon Nanoliposomes (Wang *et al.*, 2009)

a. Measurement of liposomes size and liposomes size distribution (*polidispersibility index*) used the *Particle* Size Analyzer (PSA) and for measured the potential zeta was used *Nano Series ZS Zetasizer* (Malvern Instruments Ltd, Worcestershire, UK)

b. Determination the liposomes morphology used the *negative staining* Transmission Electron Microscopy (TEM).

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RESULT AND DISCUSION

The result showed that the 1 Formula (F1) had 175 nm in liposomes size, with 0,356 in polidispersibility index, and the potential zeta was -12,58. The liposomes size distribution can be seen in figure 1.

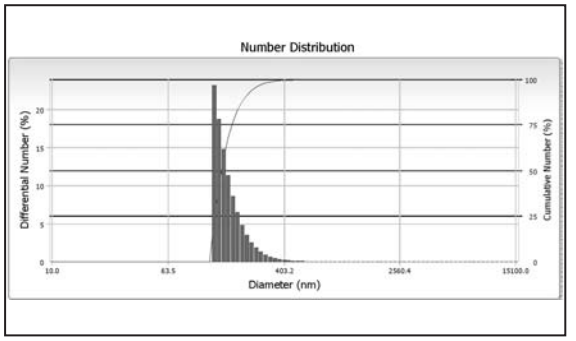

Figure 1. Liposomes size distribution for F1

Formula 3 (F3) showed that the liposomes size was 80,8 nm, polidispersibility index was 0,348, and potential zeta was -15,34. The liposomes size distribution can be seen in figure 2.

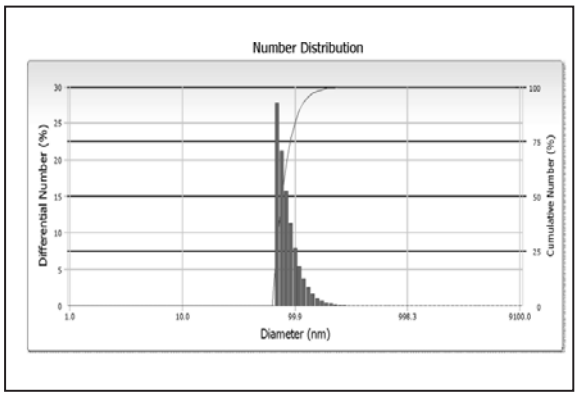

Figure 2. Liposomes size distribution for F3

The comparation between F1 and F3 showed that liposomes size of F3 was smaller than F1, that is 80,8 nm (< 100 nm), so F1 was better to be developed as anticancer than F3. Liposomes with 10-100 nm in size have better to be accumulated in cancer cell. In fact, previous studies have shown that liposomes which have size over from 100 nm are removed fast from circulation. These systems remain in circulation long enough such that they can be accumulated within tumor tissue at levels great enough to have the intended cytotoxic effect. An important physical aspect associated with the clinical successes of liposomes-based drugs is the overall size of the nanocarrier (Wang et al.,2009 ; Zauner et al.,2001).

Polydispersibility index (PI) describe the size distribution of liposomes. PI < 0,5 is called good, liposomes size distribution is homogeneous. Besides that the potential zeta is an important factor because its related with liposomes stability. The higher of potential zeta (negative or positive) showed the better stability of liposom (Couvreur et al., 2002; Parida, 2011).

From the TEM determination we can see the result in figure 3 and 4.

From the figure above, we can see the morphologic of the thymoquinon liposomes, its

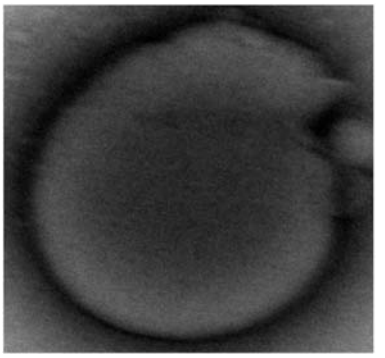

Figure 3. Result from F1 TEM determination

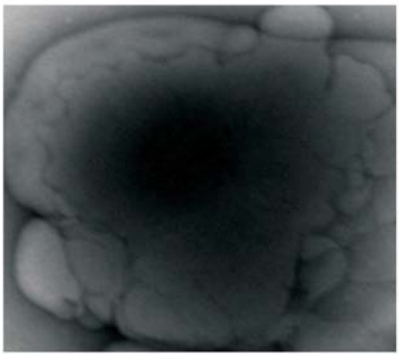

Figure 4. Result from F3 TEM determination

described the lamellarities of liposomes, and the drug can be entrapped into the liposomes.

Conclusion

From the formula optimization between F1 and F3, it could be concluded that F3 is the better formula than F1, because F3 has smaller size of liposomes (80,8 nm) than F1 (175 nm), and the potential zeta of F3 (-15,34) is more negative than F1 (-12,58), so thymoquinon liposomes of F3 can be developed as a good anticancer which has capability to increase the anticancer activity.

REFERENCES

Allen T.M. and Cullis,P.R., 2004, Drug Delivery Systems: entering the mainstream, *Science*, **303** (5665) : 1818–1822.

Couvreur, P., Barratt, G., Fattal, E., Legrand, P.dan Vauthier, C., 2002, Nanocapsule Technology: a Review,*Crit. Rev. Ther. Drug Carrier Syst*,, **19**:99-134.

El-Mahdy, M.A., 2005, Thymoquinone Induces Apoptosis through Activation of Caspase-8 and Mitochondrial Events in P53-Null Myeloblastic Leukemia HL-60 Cells,*International Journal of Cancer*, 117(3):409-417.

Gali-Muhtasib, H., A. Roessner, and R. Schneider-Stock, 2006.,Thymoquinone: A promising anti-cancer drug from natural sources*, The International Journal of Biochemistry & Cell Biology*, **38**(8): p. 1249-1253.

Ian MacLachlan, 2007, Liposomesal Formulations for Nucleic Acid Delivery, Taylor & Francis Group, LLC, chapter 9, page 239.

Ivankovic, S., 2006.,The Antitumor Activity of Thymoquinone and Thymohydroquinone *In Vitro And In Vivo. Experimental Oncology*, 28(3):220-224.

Khan,D.R., 2010, The Use of Nanocarriers for Drug Delivery in Cancer Therapy, *Journal of Cancer Science and Therapy*, **2** (3): 58–62.

Khan,D.R., Rezler,E.M,.Lauer-Fields,J., and Fields,G.B, 2008, Effects of Drug Hydrophobicity on Liposomesal Stability, *Chemical Biology and Drug Design*, **71**(1): 3–7.

Mozafari,M.R., Johnson, C., Hatziantoniou, S., and Demetzos,C., 2008, Nanoliposomeses and Their Applications in Food Nanotechnology,*Journal of Liposomese Research*, 18:309–327.

Parida,P.K., 2011,Natural Polymer Based (Alginate And Chitosan) Microparticles for Oral Drug Delivery,sit.Anonim, 1985, Zeta Potential of Colloids in Water and Waste Water, *ASTM Standard D 4187-82*, American Society for Testing and Materials.

Wang, X., Wang, Y., Chen, Z., Shin, D.M., 2009, Advances of Cancer Therapy by Nanotechnology, Review Article,*Cancer Res Treat*, **41**(1):1-11.

Wang,X., Yang, L., Chen, Z., and Shin, D.M., 2008, Application of Nanotechnology in Cancer Therapy and Imaging, *CA Cancer Journal for Clinicians*, **58** (2) : 97–110.

Washington, N., Washington, C., dan Wilson, C. G.,2001, PhysiologicalPharmaceutics: Barriers to Drug Absorption, ed. 2, Taylor & Francis, London.

Yi, T., Cho, S.G., Yi, Z., Pang, X., Rodriguez, M., Wang, Y., Sethi, G., Aggarwal, B.B., dan Liu, M., 2008, Thymoquinone Inhibits Tumor Angiogenesis and TumorGrowth Through Suppressing AKT and ExtracellularSignal-Regulated Kinase Signaling Pathways, *Mol Cancer Ther*,**7**(7).

Yuan, Z., Chen, D., Zhang, S., dan Zheng, Z., 2010, Preparation, Characterization and Evaluation of Docetaxel-Loaded, Folate-Conjugated PEG-Liposomeses,

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The Phamaceutical Society of Japan, **130**(10) 1353-1359.

Zauner W., Farrow N.A., dan Haines A.M., 2001, *In Vitro* Uptake of Polystyrene Microspheres: Effect of Particle Size, Cell Line and Cell Density, *J. Control. Release*, **71**: 39- 51.