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by eljavenio@gmail.com 1

Submission date: 02-Jan-2023 09:23PM (UTC-0800)

Submission ID: 1988123872

File name: 6.pdf (821.83K)

Word count: 6419

Character count: 34495

Liposomal Formulation of Snakehead Fish (*Ophiocephalus striatus*) Powder and Toxicity Study in Zebrafish (*Danio rerio*) Model

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Article Info

Article History:

Received: 19 January 2019

Revised: 1 April 2019

Accepted: 17 April 2019

ePublished: 30 June 2019

Keywords:

-Liposome
-Powder
-Resistant cell
-Snakehead fish
-Toxicity effect
-Zebrafish

ABSTRACT

Background: Snakehead fish (*Ophiocephalus striatus*) is a freshwater fish that is utilized as anti-inflammatory and anticancer drug. The aim of this study was to determine the toxicity effect of snakehead fish powder (SFP), formulate it into liposome and in vitro study using sensitive and resistant breast cancer cells.

Methods: Dried powder of snakehead fish was made using the atomizer then made a test solution which was divided into 7 treatment groups in different concentrations. They were exposed to zebrafish embryos then observed for 72 h post fertilization (hpf). After acquiring the half maximal inhibitory concentration (IC₅₀) and lethal concentration (LC₅₀) of SFP, these concentrations were used to formulate SFP into liposome by extrusion method. SFP-liposomes were characterized and stable tested. Afterwards, SFP-liposomes were evaluated in vitro using sensitive and resistant breast cancer cells.

Results: The maximum allowed toxicant concentration of SFP was 0.0543 mg/mL meaning slight toxic symptoms, IC₅₀ = 0.0945 mg/mL showing the growth inhibition of zebrafish embryos, and LC₅₀ = 0.1549 mg/mL meaning very toxic category that has killed zebrafish embryos. The characterization results showed that size of SFP-liposome were 121 nm ± 0.29, polydispersity index 0.06 ± 0.02, zeta-potential -10.15 mV ± 0.36 and % entrapment efficiency (EE) 85.75% ± 2.24. Six weeks of stability study showed that size profile was stable at 25°C and 37°C. Moreover, SFP-liposomes inhibited breast cancer cell proliferation when evaluated with 4T1 and MDA-MB231-sensitive and resistant cells.

Conclusion: SFP has bioactive compounds based on toxicity effect and can be formulated into liposome as a promising nanonutraceutical formulation.

Introduction

The snakehead fish (*Ophiocephalus striatus*) is freshwater fish which is found in the Southeast Asian countries particularly Indonesia and is considered as a source of high protein and traditional remedy for some diseases. Snakehead is not only a healthy diet to eat and relish but is often used as medicine for treatment of various diseases.¹ It is one of the most common fish among the other freshwater fish having pharmacological benefits in treating wound and inflammation and also in boosting the immune system.²

The snakehead fish contains natural compounds such as albumin, amino acids, fatty acids, and minerals having many functions to prevent and cure diseases.^{2,3} This means that there are two inside components which are active pharmacologically i.e. hydrophilic compounds such as albumin, amino acids, and minerals and hydrophobic compounds such as fatty acids.³ All bioactive compounds in snakehead fish can give a good source of medicinal development. The major amino acids in its extract are glycine, alanine, lysine, aspartic acid, glutamic acid and proline. In addition, the major fatty

acids are docosahexaenoic acid, eicosapentaenoic acid, linoleic acid, and arachidonic acid.³⁻⁵

Inflammation is a non-explicit response of body tissues against destructive stimulus, injury, or infection to secure the body and promote the recovery process. Regarding this, a large group of disorders such as cancer, asthma, inflammatory bowel diseases, rheumatoid arthritis, allergies and sarcoidosis are originated from an inflammatory condition.⁶ Some researchers have studied about the benefits of snakehead fish for reducing inflammation. Local people believed that snakehead fish reduces pain and use it mostly for treating mothers after delivery and any post-operative pain,⁷ wound healing,^{5,8-9} anti-eczema,¹⁰ and platelet aggregation² are some well-known pharmacological properties of snakehead fish are demonstrated till now. Glycine is one of the amino acids which have the highest content in SFP. They have been shown many functions in the cell membrane for blood clotting⁵, wound healing and antinociceptive activities of snakehead fish.¹¹ Besides, four preliminary studies have reported that snakehead fish extract has potent anti-inflammatory property¹²⁻¹⁶ but to the best of our

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knowledge, no research was done to study the effect of snakehead fish aqueous extract in lipid base nanocarrier for inflammation and cancer.

For parenteral administration of drugs that might be targeted to specific cells, small particulate carriers are required. The other formulations, such as emulsion, micro emulsion, and micelles, have all been investigated, but by far the most widely studied approach makes use of liposomes formulation from natural resources when lipids or phospholipids are dispersed in an aqueous solution. Liposomes can trap both hydrophobic and hydrophilic compounds, avoid decomposition of the entrapped combination, and release the entrapped drugs at assigned targets.¹⁷⁻¹⁹ Because of their biocompatibility, biodegradability, low toxicity, and capability to encapsulate both hydrophilic and lipophilic drugs²⁰ and simplify site-specific drug delivery to tumor tissues²¹, liposomes have been utilized as an investigational system and commercially as a drug delivery system. Many studies have been conducted on liposomes with the goal of decreasing drug toxicity and targeting specific cells.²²

²⁴ The current study was to undertake determination of toxicity effect of snakehead fish extract using zebrafish model and formulated it into liposome and in vitro study using sensitive and resistant breast cancer cells.

Materials and Methods

Materials

Snakehead fish powder (SFP) containing aqueous extract was gained from Royal Medica, Pharmaceutical Company (Makassar, Indonesia). Zebrafish were gained from the traditional market in Gorontalo. 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and *N*-(carboxyl-methoxy-PEG2,000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine (DSPE-PEG₂₀₀₀) were purchased from Lipoid GmbH, Germany. Cholesterol (CHOL), potassium dihydrogen phosphate, disodium hydrogen phosphate, sodium chloride, chloroform, methanol were purchased from Sigma Aldrich, Germany. XTT (2,3-Bis-(2-Methoxy-4-Nitro-5-Sulphophenyl)-2H-Tetrazolium-5-Carboxanilide) purchased from Merck (Darmstadt, Germany). The materials used for the cell cultures including Dulbecco's modified Eagle's medium (DMEM and RPMI), fetal bovine serum (FBS), phosphate buffer saline (PBS) and trypsin-EDTA 0.25% all purchased from GIBCO (Invitrogen, Germany). 4T1 and MDM-AB231 cell line were obtained from Medical Biology Research Center at LIPI (Indonesia).

The experimental protocols were approved by Institutional Animal Ethics Committee as per guidelines of the health ethics committee, Faculty of Medicine, Hasanuddin University, Indonesia Government with registration number UH 12180095.

Maintenance and Spawning of Zebrafish (*Danio rerio*)

Zebrafish were kept in spawning aquariums measuring 35cm × 22cm × 26cm and filled with purified water as much as 4/5 parts of the aquarium with a temperature

setting of 27 ± 1°C using 14 hours bright cycle lighting and 10 hours dark. Spawning was done by placing male and female fish in a ratio of 2: 1, namely 20 male zebrafish and 10 female zebrafish. Oxygenated and spawning trap were installed at the bottom of the aquarium and fed 3 times a day with dry flakes of tetramine and artemia.

Making the test solution

Making test solutions in each concentration by dissolving 100 mg in 100 mL distilled water as a stock solution (1 mg/mL). Afterwards, the stock solution was diluted with distilled water to obtain solution concentration of 0.03125, 0.0625, 0.125, 0.25, and 0.5 mg/mL.

Toxicity test with the ZFET method (Zebra Fish Embryo Test)

Zebrafish embryos were exposed to test solutions that have previously been made in different concentrations. Then 1 mL test solution and 1 zebrafish embryo were put in each well. After that, zebrafish embryos were observed for hatchability after 72 hours and calculated maximum allowed toxicant concentration (MATC), inhibitory concentration (IC₅₀) and lethal concentration (LC₅₀).

Liposomal preparation and SFP loading into liposomes

SFP-loaded liposomes (SFP-Liposome) and control liposomes (C-liposomes) were prepared with DPPC, cholesterol, DSPE-PEG₂₀₀₀, and SFP (based on IC₅₀ and LC₅₀) in molar ratios indicated in Table 1. Total lipid (TL) concentration of one batch of formulation was 20 mM. Liposomes were prepared using lipid film/hydration. Lipids without SFP, were dissolved in chloroform and methanol (1:1 v/v, total 10 mL) in a round-bottom flask, and a lipid film was prepared by rotary evaporation (Büchi Labortechnik AG, Flawil, Switzerland) for 1 hours. Subsequently, the lipid film was hydrated with 10 mL PBS containing SFP (pH 7.4) to form lipid dispersion. To minimize the size of the lipid dispersion and to create uniform-size liposomes, the lipid particles were reduced by multiple sequential extrusion steps using a Lipex extruder (Northern Lipids, Burnaby, BC, Canada) through polycarbonate membranes (Nuclepore, Pleasanton, CA, USA) with final filters of pore size 100 nm. Afterward, SFP-liposome was done purification to remove free SFP using Tangential Filtration Flow 100 kDa (Pall Cooperation, USA) for 2 hours.

Characterization of SFP-liposomes

Determination of the mean diameter size, polydispersity and zeta potential

SFP-loaded liposomes were diluted to a total lipid concentration (0.1 mM in 100 mM PBS) before measuring the mean diameter size, polydispersity (PDI) and zeta potential. All determinations were recorded at room temperature (25°C) using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK).

Table 1. Characteristic of the test sample

Treatment group	Unit	KK	P1	P2	P3	P4	P5	P6
Age of embryo	hpf	5.25	5.25	5.25	5.25	5.25	5.25	5.25
Number of embryos per group	embryo	24	24	24	24	24	24	24
Number of embryo per well	embryo	1	1	1	1	1	1	1
Length of observation	hour	72	72	72	72	72	72	72
Concentration of SFP	mg/mL	-	0.03125	0.0625	0.125	0.25	0.5	1.0
Volume of SFP per well	mL	1	1	1	1	1	1	1

KK: control group; P1-P6: treatment group; hpf: hours post fertilization.

Measurement of Albumin loading into liposome in powder and encapsulation efficiency

The SFP-liposomes containing albumin were disrupted by the treatment of them with 2% Triton®X-100 at 75°C for 5 min. The concentration of albumin in the liposomes was quantified by HPLC (Agilent) on wavelength 280-400 nm.

Stability of SFP-loaded liposomes

Liposome colloidal stability under storage conditions was studied by monitoring their mean size and size distribution with DLS every week for 6 weeks upon storage in PBS at 4°C.

Besides that, the stability studies of the selected SFP-loaded liposome formulations were performed at 25°C and 37°C to see SFP retention into liposome for 24 hours. SFP-liposome (5 mL) was done dialysis method using float-A-lyzer 300 kDa (Sigma Aldrich) and tween 80 as outer medium were collected at 25°C and 37°C at different intervals (0, 1, 2, 3, 4, 8, 12, 20, 22 and 24 hours). The concentrations of SFP containing albumin and the lipid were measured in the collected liposomal samples fraction and compared with the initial concentration of albumin and lipids, as described above for the determination of EE (%).

Determination of in vitro cytotoxicity (XTT Assay)

Cytotoxicity was determined using the XTT assay. In brief, 4T1 and MDA-MB231-sensitive and resistant cells were seeded in triplicate into 96-well culture plates at 150 cells/well and incubated at 37°C in a humidified atmosphere and 5% CO₂ overnight. Cells, in triplicate wells, were treated in a dose concentration-dependent manner with free SFP, free liposome and SFP-loaded liposome in different concentrations (0.05, 0.1, 0.2, 0.4 mM), and incubated for 4 hours. Subsequently, the medium was carefully removed from the wells, followed by addition of 50 µL of dye solution (0.5 mg/mL of XTT salt in culture medium) and incubated at 37°C for 4 hours. The absorbance of the samples was calorimetrically measured at 500 nm with a reference wavelength of 630 nm on a microplate reader UVM 340 (Biogenet, Poland). The untreated control was normalized to 100% for each assay, and treatments were expressed as the percentage of control.

Results

Characteristics of test sample

The samples used in this study were snakehead fish that have been made in the form of dried extracts (powder) using an atomizer with a pressure of 1 atm, -40°C which

1 resulted in dry powder with a composition of 30.2% albumin and amino acids.³

The experimental animals used in this study were zebrafish embryos aged 5.25 hours after fertilization (hpf), because at that age it was the same as the period of implantation in mammals.²⁵ The choice of zebrafish as a test animal has several advantages such as embryogenesis is fast, transparent and 75% of its DNA sequences are similar to humans.²⁶ Meanwhile, Westerfield stated that the relatively smaller zebrafish body shape provides distinct advantages because it does not require a large treatment site in use as a test animal if compared with other fish species such as trout and other fish.²⁷ The characteristics of test sample can be seen in Table 1.

Toxicity test with the ZFET method (Zebra Fish Embryo Test)

1 SFP was divided into 7 treatment groups in different concentrations i.e. 0.03125, 0.0625, 0.125, 0.25, 0.5, 1.0 mg/mL and negative control group and different observation time at 24, 48, and 72 hpf (Table 1). The SFP-treated embryos showed dose-dependent toxicity under laboratory conditions. The observation at 24 hpf, all groups showed no growth of embryos (Figure 1) but can be seen at 48 hpf, group 1 (0.0312 mg/mL), group 2 (0.0625 mg/mL) and group 3 (0.125 mg/mL) had shown embryos growth which were the same as control group (no SFP) (Figure 2). Moreover, observation at 72 hpf, the embryos on group 1-3 and control group had hatched into seed (Figure 3). Whereas, treatment groups of 4 – 6 (0.25, 0.5, 1.0 mg/mL) did not show the embryos growth on all observation time. This means that toxicity effects have started affecting the embryos development at 48 hours incubation (0.125 mg/mL). This toxicity effect can be characterized by bent and twisted notochord, accumulation of blood in the blood vessels near the tail, low heart rate, pericardial edema and degeneration of body parts. The toxic symptoms were observed at 24 hpf and became more pronounced by 72 hpf.

Based on the calculation of the MATC value was obtained 0.05434 mg/mL which derived from the inhibitory concentration value of 50% zebrafish embryo hatchability and the lowest concentration of SFP that had given results of death or toxic symptoms in small quantities. This means that this concentration had shown mild toxic symptoms. It is accordance with the MATC function which is the maximum permissible threshold of concentration (pollutant) and safe for the development of fish life. Moreover, the calculation of IC₅₀ was gained 0.0945 mg/mL which can be seen in Figure 4 and produced a sigmoid curve with a value of R² = 0.9989.

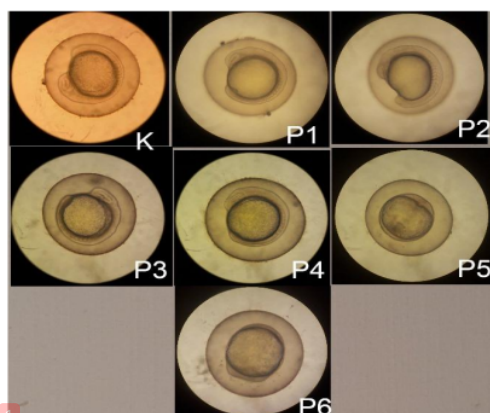


Figure 1. The observation of zebrafish embryos at 24 hpf. hpf: hours post fertilization; K: control group; P1: 0.0312 mg/mL of SFP; P2: 0.0625 mg/mL; P3: 0.125 mg/mL; P4: 0.25 mg/mL; P5: 0.5 mg/mL; P6: 1.0 mg/mL.

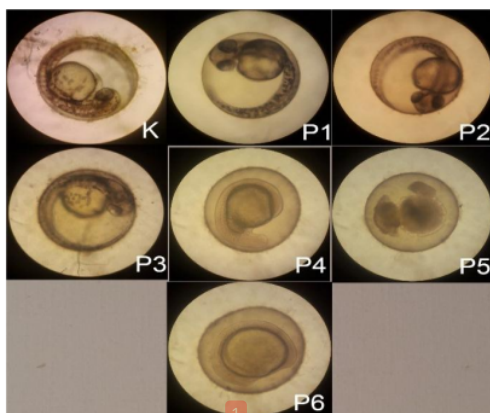


Figure 2. The observation of zebrafish embryos at 48 hpf.

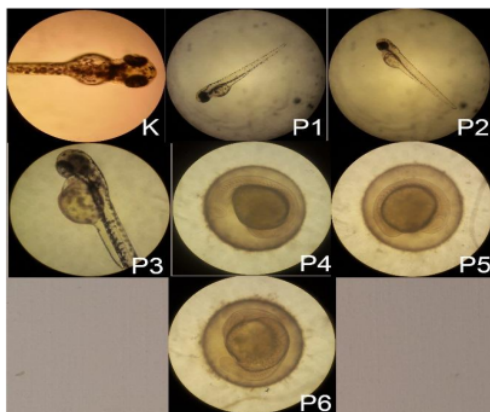


Figure 3. The observation of zebrafish embryos at 72 hpf.

This means that this concentration had been able to inhibit zebrafish embryos hatchability between 0.0625 and 0.125 mg/mL. The IC_{50} can be used as the basis for determining concentration in designing pharmaceutical dosage forms relating to the active compounds of SFP. Based on the calculation of LC_{50} using the probit analysis was obtained 0.154 mg/mL. This means the concentration gave a toxic effect killing 50% of zebrafish embryos and categorized as very toxic (0 - 0.25 mg/mL).²⁸ LC_{50} values less than 1.0 mg/mL can be said to have chemical compounds which is pharmacologically potential as bioactive compounds.²⁹

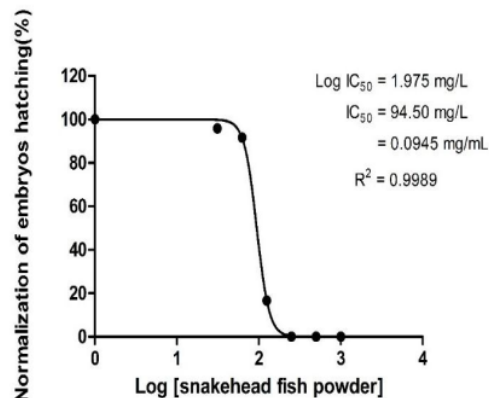


Figure 4. The IC_{50} of SFP.

Preparation and characterization of SFP-liposomes

The physicochemical characteristics of SFP-loaded PEGylated liposomes and control PEGylated liposomes (C-liposomes) are summarized in Table 2. Hydration of the lipid film with PBS resulted in a heterogeneous, opaque dispersion for both SFP-liposomes and C-liposomes.

After extrusion, both liposomal formulations were translucent and displayed comparable mean sizes around 121 nm and polydispersity < 0.1 (Table 2). The slightly negative-close to neutral ζ -potentials indicated that the negative charge of the SFP lipids was shielded by the PEG corona on the surface of liposomes (Table 2). The entrapment efficiency of albumin-loading content in SFP-liposomes was $85.75\% \pm 2.24\%$ after doing separation between albumin loaded and free albumin by tangential filtration flow (TFF) 100 kDa.

Stability of SFP-loaded liposomes

Changes in mean size of both liposomal formulations were minimal under liposome-storage conditions at 4°C over a period of 6 weeks (Figure 5). Liposome-size distribution was relatively narrow and remained < 0.1 on the PDI (Figure 6). In addition, there were no significant changes in particle size or PDI values between C-liposomes and SFP-liposomes in a stability study at 25°C and 37°C on physiological pH (pH 7.4).

Table 2. Characteristics of liposomes

Liposomal Formulation	Molar ratio SFP:DPPC:Chol DSPE-PEG ₂₀₀₀	Mean diameter nm	PDI	Zeta potential mV	SFP- entrapment Efficiency (%)
SFP-liposome	1.5:2.6:0.25:0.15	121 ± 0.29	0.06 ± 0.02	-10.15 ± 0.36	85.75±2.24
C-liposome	0:4.1:0.25:0.15	118 ± 0.98	0.07 ± 0.07	-12.86 ± 0.41	not applicable

Data presented as ratio or mean ± SD (n=3)

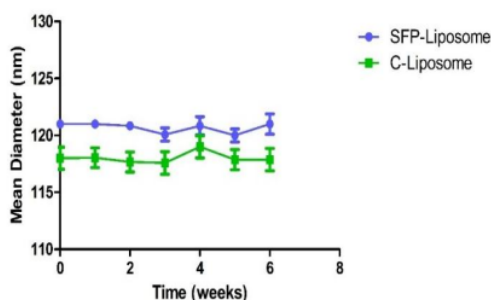


Figure 5. Colloidal stability of SFP-loaded liposome and control liposome for the mean size.

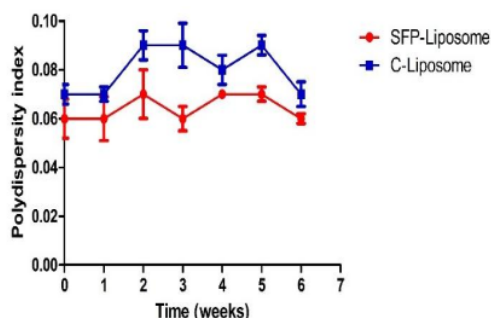


Figure 6. Colloidal stability of SFP-loaded liposome and control liposome for polydispersity index.

Determination of in vitro cytotoxicity (XTT Assay)

The cell lines utilized for the present study were chosen according to a review by Xavier *et al.* and Mustafa *et al.*, who abridged the available information as a guide for researchers to choose suitable cell lines for specific research needs.³⁰⁻³¹ SFP has anticancer activity against different cancers, including breast cancer, which has an especially high death rate. Taking the points of interest (anticancer activity) and trying to defeat the inconveniences (toxicity or solvency) is one methodology for researchers to enhance drug loading, bioavailability and activity, alongside diminishing cytotoxicity to normal cells, in order to cure proliferative diseases such as tumors.³¹ Taken together, the aforementioned advantages, liposomal formulations of liposomes and SFP showed a significantly increased cytotoxicity, which is probably due to better bioavailability. SFP-loaded liposomes showed a better cytotoxic activity compared with free SFP towards two breast cancer cell lines i.e. 4T1 and MDA-MB231-sensitive and resistance.

Discussion

Snakehead fish powder (aqueous extract) is a natural material having active compounds which can prevent and cure some diseases particularly inflammatory disorder such as cancer. Sometimes plants and animals extract usually are utilized as cancer treatment traditionally, although the efficacy of such traditional treatments should be cautiously evaluated. Because cancer is one of diseases which is a very specific and complex disease, seems to be poorly characterized in the terms of folklore and traditional medicine³² and certainly requires modern treatment modalities, based on surgery and chemotherapy. In spite of the fact that cytotoxicity is neither essential nor adequate for anticancer action, it is an action predictable with antitumor movement as it is delicate to each component required for cell survival or cell demise. The after effects of cytotoxicity screening, accordingly, could choose which materials are to be exposed to a decontamination procedure.³³ Therefore, this needs a concern relating to the toxicity effect of natural compounds.

Zebrafish model is one of toxicity tests to determine toxicity effect of materials which is derived from natural products because it is easy to be applied and utilized as important model for understanding the mechanisms of development and diseases such as cancer. Besides, the results of this test can be calculated the value of MATC, IC₅₀, and LC₅₀ from active compound materials referring to dose determination. No data of snakehead fish extract toxicity is shown by researchers. Therefore, it is important to study the toxicity effects from snakehead fish powder before doing liposomal formulation to obtain toxic concentration as the basis for determining concentration of liposomal formulation.

This is related to the content of SFP having bioactive compounds that is easily soluble in water, a protein consisting of albumin and amino acids. According to Olney *et al.* stated that there are several amino acids containing sulfur and acid groups (aspartate and glutamic acid) that can cause toxic effects on body tissues, especially affecting the development of nerves and body tissues, causing or damaging the growth of certain organs if consumed in excessive amounts.³⁴ Besides, the exposure of SFP concentration for 72 hours and high concentration caused albumin and amino acids to be easily penetrate the chorion and included in chorion which can inhibit embryonic growth and development. According to Wang *et al.* stated that an increase in exposure concentration will reduce the hatchability of zebrafish embryos by inhibiting embryo development into larvae to break chorion and hatch.³⁵ Moreover, the ability of the embryo to hatch is a physiological process which is characterized by chorion destruction and the chorionase

enzyme has the property of reducing chorion, so the chorion layer will become thin, soft and easily broken, and also affected by the embryo movement to break the chorion and followed by the body and head of the embryo.³⁶

The physicochemical characterization of liposomes, such as size, shape and charge are fundamental parameters to convey enhanced bio-distribution and prolonged pharmacokinetics of encapsulated cytotoxic drugs.³⁷⁻³⁸ All parameters showed a small homogenous size, low PDI index indicating good homogenization of liposomes, and no aggregation or fusion processes happening after liposome formulation. In some cases, the zeta-potential of liposomes was resolved and the tested liposomes were characterized by a low remaining negative charge, potentially derived from charged DSPE-PEG₂₀₀₀ molecules which as our own outcomes demonstrated that has no impact on such liposomes circulation time.³⁹⁻⁴⁰

SFP-loaded liposomes showed a desirable stability in size and PDI (Figure 5 and 6) and in EE % in both storage time at 4°C and 37°C, suggesting that liposomes could serve as a suitable carrier for SFP. Phospholipids with a higher phase-transition temperature, such as DPPC, with a phase transition temperature of almost 42°C, have higher membrane stability.

It can be seen that the stability of SFP-liposome at 25°C and 37°C kept stable for 24 hours using tween 80 as outer medium to keep sink condition (Figure 7 and 8).

Sara et.al⁴¹, stated that tween 80 is most recommendable among those tested in the stability because it is more stable than in FBS/PBS and does not need extra sample treatment for HPLC analysis, also produce a comparative discharge profile at that in FBS/PBS, which fulfilled a sink condition.

Besides that, SFP-liposome contained PEG chain having capability to interact with the liposome surface and can stabilize liposomes because of its repulsive barrier against other molecule surfaces.⁴² Taken together with these proof, we concluded that the PEG on the SFP-liposome surface, a compliance that would confer a higher stability to the system. Therefore, the SFP-liposome would be relied upon to diminish the interactions between liposome and blood cells or liposomes, bringing about a high blood compatibility and physicochemical stability for over 1 month.

Throughout the years, a wide range of techniques have been utilized to defeat multidrug resistance. Incredible desires have been related with the utilization of low-molecular-weight inhibitors, yet so far, the vast majority of these endeavors have been unsuccessful, because of low selectivity, innate toxicity and pharmacokinetic interactions with anticancer drugs.⁴³ Therefore, we utilized 2 types of cells such as sensitive and resistant cells to compare results of cytotoxicity assay and explore SFP might be new bioactive compounds for cancer and inflammation. As results, it can be seen SFP-loaded liposomes could inhibit cell proliferation at 0.4 mM both cells for incubation time 4 hours showing the viability cell 56.2% of 4T1-sensitive and 75.3% of MDA-MB231-

sensitive but for resistant cells, MDA-MB231 cell gave the viability cell lower than 4T1 cell i.e. 47.8% and 78.8%. Otherwise, Free-SFP and control-liposome did not show strong inhibition of both cells and only had the viability cell around 85%. This proved that SFP-loaded liposomes could give strong inhibition against both cells. Regarding this, liposome is one of lipid base nanocarriers which can deliver active compounds into cell continuously and also the stability of liposome (Figure 9 and 10).

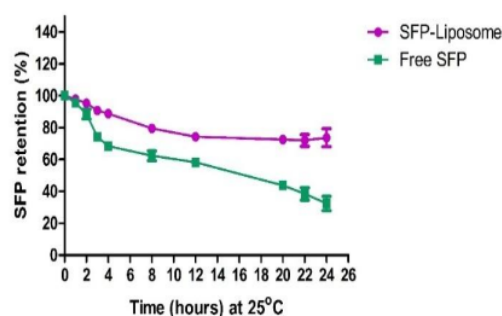


Figure 7. Stability of SFP-loaded liposome and free-SFP at 25°C.

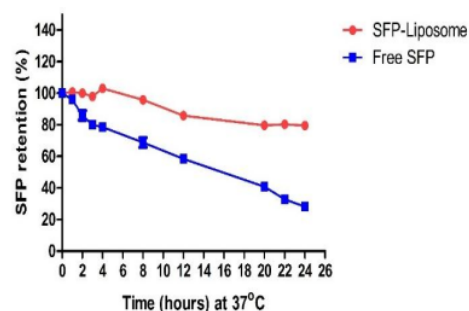


Figure 8. Stability of SFP-loaded liposome and Free-SFP at 37°C.

The interesting result of MDA-MB231-resistant cell showed the best inhibition compared to 4T1-resistant cell. This can be related to SFP-loaded liposome which is able to deliver high concentrations of chemotherapeutic drugs and/or multidrug resistance (MDR) inhibitors to cells. In resistant cells, however, most of these rapidly internalized small molecules were rapidly sensed by MDR proteins and efflux out of the cells.⁴⁴

This ATP-dependent efflux mechanism is a prominent feature of multidrug resistant cancer cells. Moreover, SFP content albumin and amino acids which are able to accelerate the process of tissue formation. Albumin also has some functions as binding and transport substance, osmotic pressure regulation, inhibition of platelet formation and anti-thrombosis, increasing cell permeability, and as antioxidants.⁴⁵⁻⁴⁶

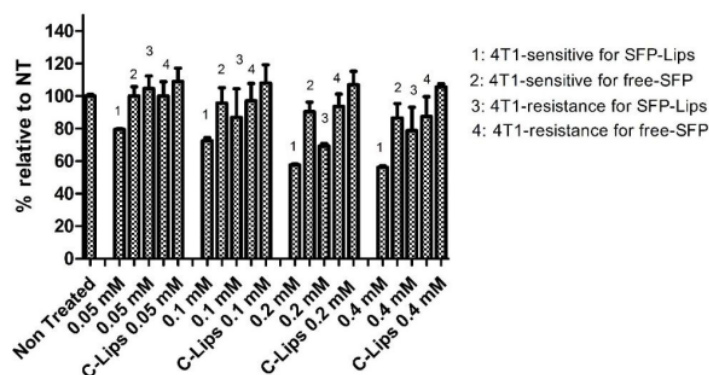


Figure 9. Effect of SFP-loaded liposomes and control liposomes on tumor cell proliferation (4T1-sensitive and resistant cells).

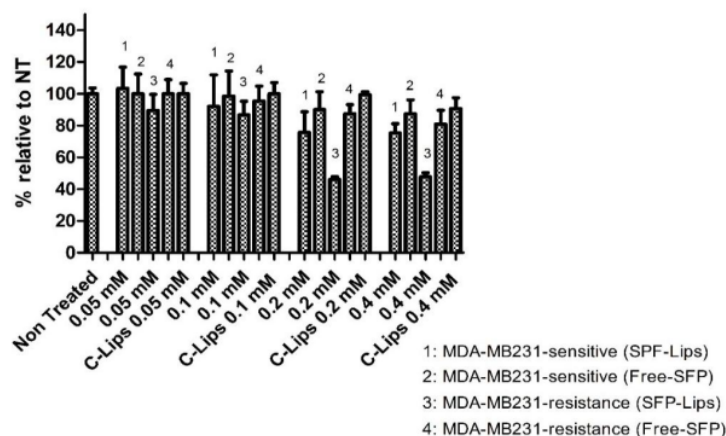


Figure 10. Effect of SFP-loaded liposomes and control liposomes on tumor cell proliferation (MDA-MB231-sensitive and resistant cells).

Various approaches have been attempted to encapsulate SFP within liposomes to gain a good pharmacokinetics profiles. Therefore, our goal was to establish a new stable, rigid formulation of SFP with excellent in plasma retention ability and a formulation having excellent anticancer properties. Those liposomal formulations might be able to reach breast cancer if injected repetitively several times using combination with other chemotherapeutics, preferably in the form of nanocarriers.⁴⁷ Therefore, our studies are the first step in demonstrating the utility of a new approach to breast cancer therapy involving long-circulating SFP-containing liposomes which might be able to not only increase the bioavailability of the therapeutic agent, but also to have a sufficiently long biological retention time to enable the accumulation of the liposomes in cancer tissue by the EPR effect.

Conclusion

Snakehead fish powder containing hydrophilic powder has bioactive compounds potentially based on the toxicity test and can be formulated into liposome as a promising

nanonutraceutical formulation for intravenous delivery of fish particularly cancer and inflammatory disorders.

Acknowledgments

The authors are thankful to Indonesia endowment fund for education and the ministry of research, technology, and higher education of Indonesia which have funded this research. Authors would like to thank Dr. Mansyur for providing snakehead fish powder and also all authors as a team in this project.

Conflict of interests

The authors claim that there is no conflict of interest.

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