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

by Netty Ino Ischak

Submission date: 30-Jun-2023 03:13PM (UTC+0800)
Submission ID: 2124668610
File name: Pharm-25-145.pdf (821.83K)
Word count: 6419
Character count: 34495

Pharmaceutical Sciences
June 2019, 25(2), 145-153
doi: 10.15171/PS.2019.22
https://ps.tbzmed.ac.ir/

Research Article

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

Robert Tungadi[1*], Wiwin Susanti Abdulkadir[1], Netty Ino Ischak[2], Bayu R Rahim[1]

[1]*Department of Pharmacy, Faculty of Sport and Health, State University of Gorontalo, Indonesia.*
[2]*Department of Chemistry, Faculty of Mathematic and Natural Sciences, State University of Gorontalo, Indonesia.*

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 (IC_{50}) and lethal concentration (LC_{50}) 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, IC_{50} = 0.0945 mg/mL showing the growth inhibition of zebrafish embryos, and LC_{50} = 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 aggregation[2] 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 clothing[5], wound healing and antinociceptive activities of snakehead fish.[11] Besides, four preliminary studies have reported that snakehead fish extract has potent anti-inflammatory property[12-16] but to the best of our

***Corresponding Author:** Robert Tungadi, E-mail: robert.tungadi@ung.ac.id*
©2019 The Authors. This is an open access article and applies the Creative Commons Attribution (CC BY), which permits unrestricted use, distribution and reproduction in any medium, as long as the original authors and source are cited. No permission is required from the authors or the publishers.

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 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 drugs[20] and simplify site-specific drug delivery to tumor tissues[21], 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 undertaken 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*-(carbonyl-methoxy-PEG2,000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine (DSPE-PEG$_{2000}$) were purchased from Lipoid Gmbh, Germany. Cholesterol (CHOL), potassium dihydrogen phosphate, disodium hydrogen phosphate, sodium chloride, chloroform, ethanol were purchased from Sigma Aldrich, Germany. XTT (2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-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 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 (IC_{50}) and lethal concentration (LC_{50}).

Liposomal preparation and SFP loading into liposomes

SFP-loaded liposomes (SFP-Liposome) and control liposomes (C-liposomes) were prepared with DPPC, cholesterol, DSPE-PEG$_{2000}$, and SFP (based on IC_{50} and LC_{50}) 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 minimalize 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-sensitve 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% CO_2 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 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)

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 IC_{50} was gained 0.0945 mg/mL which can be seen in Figure 4 and produced a sigmoid curve with a value of $R^2 = 0.9989$.

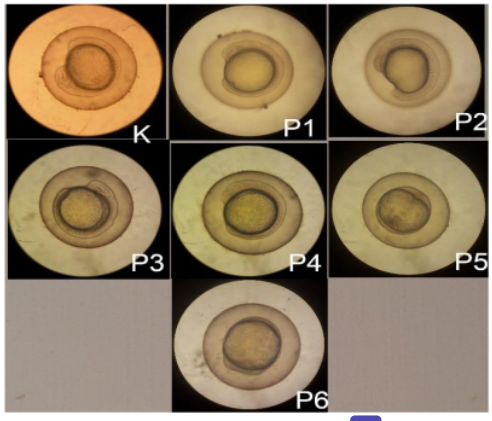

Figure 1. The observation of zebrafish embryo at 24 hpf. hpf: hours post fertilization; K: control group; P1: 0.0312 mg/mL of SFP; P2: 0.0625 mg/mL; P3: 0.125 mg/mL; P4: 0.25 mg/mL; P5: 0.5 mg/mL; P6: 1.0 mg/mL.

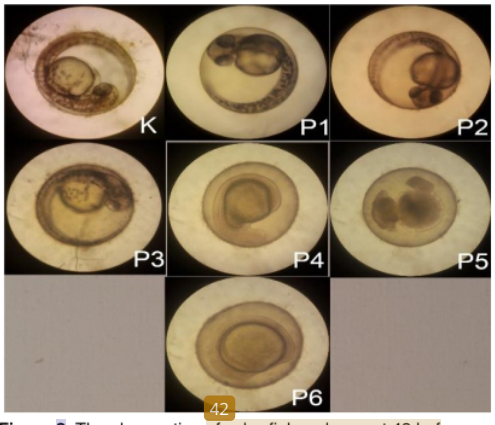

Figure 2. The observation of zebrafish embryos at 48 hpf.

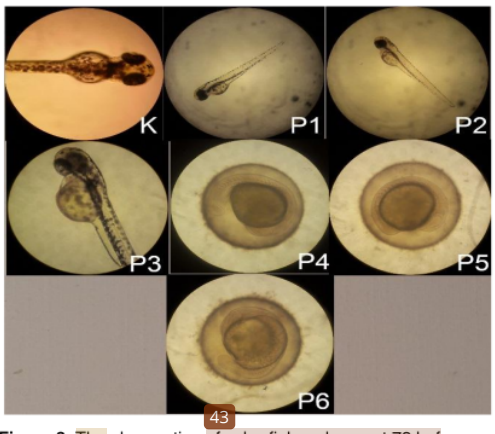

Figure 3. The observation of zebrafish embryos at 72 hpf.

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]

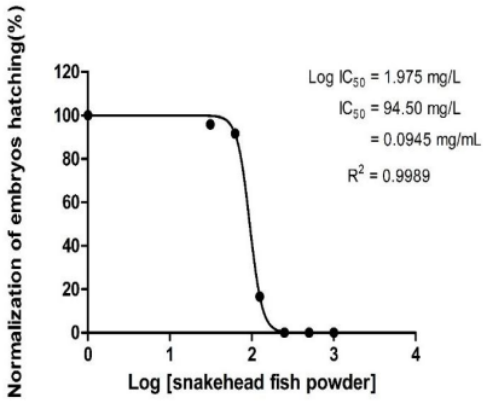

Figure 4. The IC_{50} of SFP.

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%±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 Formulaton	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)

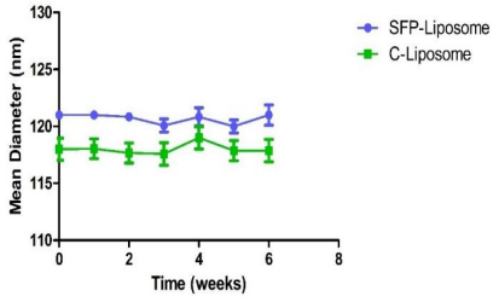

Figure 5. Colloidal stability of SfP-loaded liposome and control liposome for the mean size.

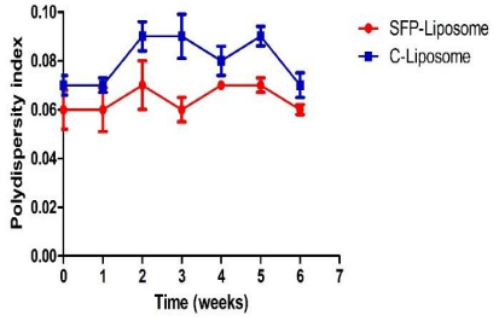

Figure 6. Colloidal stability of SfP-loaded liposome and control liposome for polydispersity index.

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 6 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 medicine[32] 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, IC_{50}, and LC_{50} 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-PEG$_{2000}$ 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.al[41], 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).

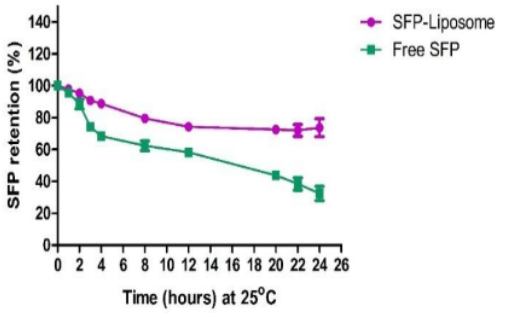

Figure 7. Stability of SFP-loaded liposome and free-SFP at 25°C.

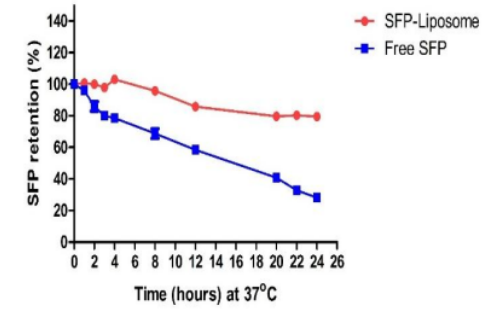

Figure 8. Stability of SFP-loaded liposome and Free-SFP at 37°C.

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 drug 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 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]

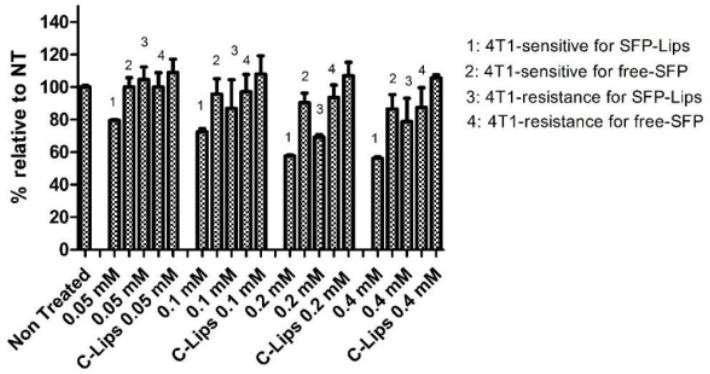

Figure 9. Effect of SFP-loaded liposomes and control liposomes on tumor cell proliferation (4T1-sensitive and resistant cells).

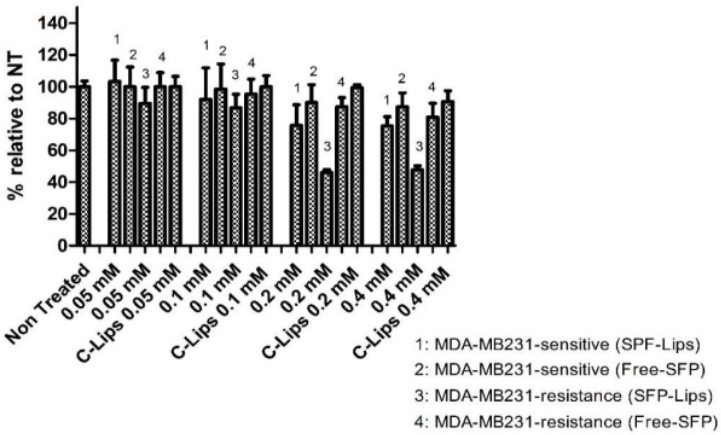

Figure 10. Effect of SFP-loaded liposomes and control liposomes on tumor cell proliferation (MDA-MB231-sensitive and resistant cells).

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 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.

References

1. Rahman MA, Arshad A, Amin SMN. Growth and production performance of threatened snakehead fish, *Channa striatus* (Bloch), at different stocking densities in earthen ponds. Aquac Res. 2012;43(2):297-302. doi:10.1111/j.1365-2109.2011.02830.x
2. Mat Jais AM. Pharmacognosy and pharmacology of Haruan (*Channa striatus*), a medicinal fish with

wound healing properties. Bol. Latinoam. Caribe Plant. Med. Aromaticas. 2007;6(3):52-60.
3. Tungadi R, Susanty W, Wicita P, Pido E. Transdermal delivery of snakehead fish (*Ophiocephalus striatus*) nanoemulgel containing hydrophobic powder for burn wound. Pharm Sci. 2018;24(4):313-23. doi:10.15171/PS.2018.45
4. Zuraini A, Somchit MH, Solihah MH, Goh YM, Arifah AK, Zakaria MS, et al. Fatty acid and amino acid composition of three local Malaysian *Channa spp*. fish. Food Chem. 2006;97(4):674-8. doi:10.1016/j.foodchem.2005.04.031
5. Guidoni M, de Christo Scherer MM, Figueira MM, Schmitt EFP, de Almeida LC, Scherer R, et al. Fatty acid composition of vegetable oil blend and in vitro effects of pharmacotherapeutical skin care applications. Braz J Med Biol Res. 2019;52(2):e8209. doi:10.1590/1414-431X20188209
6. Krishnaraju AV, Rao CB, Dodda S, Krishanu S, Golakoti T. Anti-inflammatory activity of *Vitex leucoxylon* L. bark extracts against freund's complete adjuvant induced arthritis in Sprague dawley rat. Am J Infect Dis. 2009;5(2):68-73. doi:10.3844/ajidsp.2009.68.73
7. Mohamad I, Abu Bakar S, Md Tohid SF, Mat Jais AM. *Channa striatus* cream down-regulates tumour necrosis factor (TNF)-alpha gene expression and alleviates chronic-like dermatitis in mouse model. J Ethnopharmacol. 2016;194:469-74. doi:10.1016 /j.jep.2016.10.033
8. Tungadi R, Attamimi F, Sabu EF, Nugraha E. The acceleration of wound healing of snakehead fish cream towards rabbit's skin wound histopathologically. Jurnal Ilmu Kefarmasian Indonesia. 2011; 9(2):91-97.
9. Baie SH, Sheikh KA. The wound healing properties of *Channa striatus* cetrimide cream wound contraction and glycosaminoglycan measurement. J Ethnopharmacol. 2000;73(1-2):15-30. doi:10.1016/s0378-8741(00)00253-1
10. Mohd SM, Abdul Manan MJ. Therapeutic potential of the haruan (*Channa striatus*): from food to medicinal uses. Malays J Nutr. 2012;18(1):125-36.
11. Zakaria ZA, Mat Jais AM, Somchit MN, Sulaiman MR, Fatimah CA. Report on some of the physical properties of bioactive compounds responsible for the *Channa striatus* fillet extract antinociceptive activity. J Biol Sci. 2006;6(4):680-6. doi:10.3923/jbs.2006.680.686
12. Somchit MN, Solihah MH, Israf DA, Ahmad Z, Arifah AK, Mat Jais AM. Anti-inflammatory activity of *Channa striatus*, *Channa micropellets* and *Channa lucius* extracts: chronic inflammatory modulation. J Orient Pharm Exp Med. 2004;4:91-4.
13. Mohd Hassan S. Anti-inflammatory and antinociceptive properties of three local *Channa* species crude extracts. University of Putra Malaysia; 2005.
14. Rahman MA, Molla MHR, Sarker MK, Chowdhury SH, Shaikh MM. Snakehead fish (*Channa striata*) and its biochemical properties for therapeutics and health benefits. SF J Biotechnol Biomed Eng. 2018;1(1):1-5.
15. Mohd SM, Abdul Manan MJ. Therapeutic potential of the haruan (*Channa striatus*): from food to medicinal uses. Malays J Nutr. 2012;18(1):125-36.
16. Ab Wahab SZ, Abdul Kadir A, Nik Hussain N, Omar J, Yunus R, Baie S, et al. The effect of *Channa striatus* (Haruan) extract on pain and wound healing of post-lower segment caesarean section women. Evid Based Complement Alternat Med. 2015;2015:849647. doi:10.1155/2015/849647
17. Atrooz OM. Effects of alkylresorcinolic lipids obtained from acetonic extract of Jordanian wheat grains on liposome properties. Int J Biol Chem. 2011;5(5):314-21. doi:10.3923/ijbc.2011.314.321
18. Benech RO, Kheadr EE, Laridi R, Lacroix C, Fliss I. Inhibition of listeria innocua in cheddar cheese by addition of nisin Z in liposome or by in situ production in mixed culture. Appl Environ Microbial. 2002;68(8):3683-90. doi:10.1128/aem.68.8.3683-3690.2002
19. Shehata T, Ogawara K, Higaki K, Kimura T. Prolongation of residence time of liposome by surface-modification with mixture of hydrophilic polymers. Int J Pharm. 2008;359(1-2):272-9. doi:10.1016/j.ipharm.2008.04.004
20. Johnson MJ, Semple SC, Klimuk SK, Ansell S, Maurer N, Cullis PR. Characterization of the drug retention and pharmacokinetic properties of liposomal nanoparticles containing dihydrosphingomyelin. Biochem Biophys Acta Biomembr. 2007;1768(5):1121-7. doi:10.1016/j. bbamem.2007.01.019
21. Hafheinz RD, Gnad-Vogt SU, Beyer U, Hochhaus A. Liposomal encapsulated anti-cancer drugs. Anticancer Drugs. 2005;16(7):691-707. doi:10.1097/01.cad.0000167902.53039.5a
22. Omri A, Suntres ZE, Shek PN. Enhanced activity of liposomal polymyxin B against *Pseudomonas aeruginosa* in a rat model of lung infection. Biochem Pharmacol. 2002;64(9):1407-13. doi:10.1016/s0006-2952(02)01346-1
23. Schiffelers RM, Storm G, Bakker-woudenberg IA. Host factors influencing the preferential localization of sterically stabilized liposomes in *Klebsiella pneumonia*-infected rat lung tissue. Pharm Res. 2001;18(6):780-7.
24. Stano P, Bufali S, Pisano C, Bucci F, Barbarino M, Santaniello M, et al. Novel camptothecin analoque (gimatecan) containing liposomes prepared by the ethanol injection method. J Liposome Res. 2004;14(1-2):87-109. doi:10.1081/LPR-120039794
25. Lee SH, Kang JW, Lin T, Lee JF, Jin DI. Teratogenic potential of antiepileptic drugs in the zebrafish model. BioMed Res Int. 2013;2013:1-6. doi:10.1155/2013/726478

26. Berghmans S, Jette C, Langenau D, Hsu K, Stewart R, Look T, et al. Making waves in cancer research: new models in the zebrafish. Biotechniques. 2005;39(2):227-37. doi:10.2144/05392RV02
27. Westerfield M. The Zebrafish Book: A Guide for the laboratory use of Zebrafish *(Danio rerio)*. 4th ed. Eugene: Oregon University Press;2000.
28. Anderson JE, Goetz CM, McLaughlin JL, Suffness M. A blind comparison of simple bench top bioassay and human tumor cell cytotoxities as antitumor prescreens. Phytochem Anal. 1991;2(3):107-111. doi:10.1002/pca.2800020303
29. Arcanjo DDR, Albuquerque ACM, Melo-Neto B, Santana LCLR., Medeiros MGF, Citó AMGL. Bioactivity evaluation against *Artemia salina* Leach of medicinal plants used in Brazilian Northeastern folk medicine. Braz J Biol. 2012;72(3):505-9. doi:10.1590/s1519-69842012000300013
30. Wei OY, Xavier R, Marimuthu U. Screening of antibacterial activity of mucus extract of snakehead fish, *Channa striatus*(Bloch). Eur Rev Med Pharmacol Sci. 2010;14(8):675-81.
31. Mustafa A, Widodo MA, Kristianto Y. Albumin and zinc content of snakehead fish (*Channa striata*) extract and its role in health. Int J Sci Technol. 2012;1(2):1-8.
32. Bunel V, Ouedraogo M, Nguyen AT, Stevigny C, Duez P. Methods applied to the in vitro primary toxicology testing of natural products:state of the art, strengths, and limits. Planta Med. 2014;80(14):1210-26. doi:10.1055/s-0033-1360273
33. Akbarzadeh F, Khoshgard K, Hosseinzadeh L, Arkan E, Rezazadeh D. Investigating the cytotoxicity of folate-conjugated bismuth oxide nanoparticles on kB and A549 cell lines. Adv Pharm Bull. 2018;8(4):627-35. doi:10.15171/apb.2018.071
34. Olney JW, Labruyere J, Wang G, Wozniak DF, Price MT, Sesma MA. NMDA antagonist neurotoxicity: mechanism and prevention. Science. 1991;254(5037):1515-8. doi:10.1126/science.1835799
35. Wang S, Liu K, Wang X, He Q, Chen X. Toxic effects of celastrol on embryonic development of Zebrafish (*Danio rerio*). Drug Chem Toxicol. 2011;34(1):61-5. doi:10.3109/01480545.2010.494664
36. Ulhaq M, Carisson G, Orn S, Norrgren L. Comparison of developmental toxicity of seven perfluoroalkyl acids to Zebrafish embryos. Environ Toxicol Pharmacol. 2013;36(2):423-6. doi:10.1016/j.etap.2013.05.004
37. Krasnici S, Werner A, Eichhorn ME, Schmitt-Sody M, Pahernik SA, Sauer B, et al. Effect of the surface charge of liposomes on their uptake by angiogenic tumor vessels. Int J Cancer. 2003;105(4):561-7. doi:10.1002/ijc.11108
38. Li HJ, Du JZ, Du XJ, Xu CF, Sun CY, Wang HX, et al. Stimuli-responsive clustered nanoparticles for improved tumor penetration and therapeutic efficacy. Proc Natl Acad Sci USA. 2016;113(15):4164-9. doi:10.1073/pnas.1522080113
39. Somchit MN, Solihah MH, Israf DA, Zuraini A, Arifah AK, Mat Jais AM. Effects of three local Malaysian *Channa* spp. fish on chronic inflammation. J Oriental Pharm Exp Med 2004;5:91-4. doi:10.3742/OPEM.2004.4.2.091
40. Zakaria ZA, Kumar GH, Jais AM, Sulaiman MR, Somchit MN. Anti-nociceptive, anti-inflammatory and antipyretic properties of *Channa striatus* fillet aqueous and lipid-based extracts in rats. Methods Find Exp Clin Pharmacol. 2008;30(5):355-62. doi:10.1358/mf.2008.30.5.1186084
41. Sara AA, Bo S, Alice CC, Youn JK, Yoon Y. Release kinetics study of poorly water-soluble drugs from nanoparticles: Are we doing it right? Mol Pharm. 2015;12(3):997-1003. doi:10.1021/mp500817h
42. He Y, Zhang L, Song C. Luteinizing hormone-releasing hormone receptor-mediated delivery of mitoxantrone using LHRH analogs modified with PEGylated liposomes. Int J Nanomedicine. 2010;5:697-705. doi:10.2147/IJN.s12129
43. Mignogna C, Staibano S, Altieri V, De Rosa G, Pannone G, Santoro A, et al. Prognostic significance of multidrug-resistance protein (MDR-1) in renal clear cell carcinomas: a five year follow-up analysis. BMC Cancer. 2006;6(1):293. doi:10.1186/1471-2407-6-293
44. Ambudkar SV, Kim IW, Sauna ZE. The power of the pump: mechanisms of action of P-glycoprotein (ABCB1). Eur J Pharm Sci. 2006;27(5):392-400. doi:10.1016/i.ejps.2005.10.010
45. Svaton M, Zemanova M, Skrickova J, Jakubikova L, Kolek V, Kultan J, et al. Chronic inflammation as a potential predictive factor of nivolumab therapy in non-small cell lung cancer. Anticancer Res. 2018;38(12):6771-82. doi:10.21873/anticanres.13048
46. Naganuma M, Tahara K, Hasegawa S, Fukuda A, Sasaoka S, Hatahira H, et al. Adverse event profiles of solvent-based and nanoparticle albumin-bound paclitaxel formulations using the food and drug administration adverse event reporting system. SAGE Open Med. 2019;7:1-7. doi:10.1177/2050312119836011
47. Mahmud M, Piwoni A, Filiczak N, Janicka M, Gubernator J. Long-Circulating curcumin-loaded liposome formulations with high incorporation efficiency, stability and anticancer activity towards pancreatic adenocarcinoma cell lines in vitro. PLoS One. 2016;11(12): e0167787. doi:10.1371/journal.pone.0167787

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

ORIGINALITY REPORT

19%	13%	14%	8%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Kunjachan, Sijumon, Błażej Rychlik, Gert Storm, Fabian Kiessling, and Twan Lammers. "Multidrug resistance: Physiological principles and nanomedical solutions", Advanced Drug Delivery Reviews, 2013. Publication	1%
2	Samin Abbaszadeh, Alireza Garjani, Hossein Nazemiyeh, Sepideh Ayadi, Majid Mohajer Milani, Hamid Soraya. "Hydroalcoholic Extract from Rhizomes of Cynodon dactylon Improve Hemodynamic and Electrocardiogram Parameters in Myocardial Infarction in Rats", Pharmaceutical Sciences, 2019 Publication	1%
3	van der Meel, Roy, Sabrina Oliveira, Isil Altintas, Raimond Heukers, Ebel H. E. Pieters, Paul M. P. van Bergen en Henegouwen, Gert Storm, Wim E. Hennink, Robbert J. Kok, and Raymond M. Schiffelers. "Inhibition of Tumor Growth by Targeted Anti-EGFR/IGF-1R	1%

Nanobullets Depends on Efficient Blocking of Cell Survival Pathways", Molecular Pharmaceutics, 2013.
Publication

4	dergipark.org.tr Internet Source	1 %
5	Submitted to October University for Modern Sciences and Arts (MSA) Student Paper	1 %
6	Jun Zhang, Huali Liang, Hui Yao, Zhenpeng Qiu, Xinyan Chen, Xixi Hu, Junjie Hu, Guohua Zheng. " The preparation, characterization of Lupeol PEGylated liposome and its functional evaluation as well as pharmacokinetics in rats ", Drug Development and Industrial Pharmacy, 2019 Publication	1 %
7	Submitted to Our Lady of Fatima University Student Paper	1 %
8	www.vibgyorpublishers.org Internet Source	1 %
9	www.teknoscienze.com Internet Source	1 %
10	Sara A. Abouelmagd, Bo Sun, Alice C. Chang, Youn Jin Ku, Yoon Yeo. "Release Kinetics Study of Poorly Water-Soluble Drugs from	1 %

Nanoparticles: Are We Doing It Right?", Molecular Pharmaceutics, 2015
Publication

11	Kristina Borochova, Katarzyna Niespodziana, Margarete Focke-Tejkl, Gerhard Hofer, Walter Keller, Rudolf Valenta. "Dissociation of the respiratory syncytial virus F protein-specific human IgG, IgA and IgM response", Scientific Reports, 2021 Publication	1 %
12	P V Asharani. "Toxicity of silver nanoparticles in zebrafish models", Nanotechnology, 06/25/2008 Publication	1 %
13	Heba Mohamed Fahmy. "In vitro study of the cytotoxicity of thymoquinone/curcumin fluorescent liposomes", Naunyn-Schmiedeberg's Archives of Pharmacology, 2019 Publication	<1 %
14	Submitted to Universitas Diponegoro Student Paper	<1 %
15	www.ieese.org Internet Source	<1 %
16	www.e-journals.in Internet Source	<1 %

17	Naser Soltani, Jamshid Manzoori, Mohammad Amjadi, Farzaneh Lotfipour, Abolghasem Jouyban. "Development and Validation of A Spectrofluorimetric Determination of Calf Thymus DNA Using a Terbium-Danofloxacin Probe", Pharmaceutical Sciences, 2016 Publication	<1 %
18	Submitted to University of College Cork Student Paper	<1 %
19	Submitted to Chung-Ang University Student Paper	<1 %
20	www.biorxiv.org Internet Source	<1 %
21	pubs.rsc.org Internet Source	<1 %
22	vital.seals.ac.za:8080 Internet Source	<1 %
23	www.jstage.jst.go.jp Internet Source	<1 %
24	Submitted to SUNY Cobleskill Student Paper	<1 %
25	jppres.com Internet Source	<1 %
26	cancerscienceandresearch.jacobspublishers.com Internet Source	<1 %

27	Konstantinos N. Kontogiannopoulos, Andreana N. Assimopoulou, Konstantinos Dimas, Vassilios P. Papageorgiou. "Shikonin-loaded liposomes as a new drug delivery system: Physicochemical characterization and in vitro cytotoxicity", European Journal of Lipid Science and Technology, 2011 Publication	<1 %
28	Diogo Matias, Marisa Nicolai, Lucília Saraiva, Rute Pinheiro et al. " Cytotoxic Activity of Royleanone Diterpenes from Benth ", ACS Omega, 2019 Publication	<1 %
29	Nobuhito Hamano, Roland Böttger, Suen Ern Lee, Yang Yang, Jayesh A. Kulkarni, Shell Ip, Pieter R. Cullis, Shyh-Dar Li. "Robust Microfluidic Technology and New Lipid Composition for Fabrication of Curcumin-Loaded Liposomes: Effect on the Anticancer Activity and Safety of Cisplatin", Molecular Pharmaceutics, 2019 Publication	<1 %
30	www.fisheriesjournal.com Internet Source	<1 %
31	David B. Fenske, Arcadio Chonn, Pieter R. Cullis. "Liposomal Nanomedicines: An Emerging Field", Toxicologic Pathology, 2008 Publication	<1 %

32	Hawiset. "Screening Neuropharmacological Activities of *Kaempferia parviflora* (Krachai Dam) in Healthy Adult Male Rats", American Journal of Applied Sciences, 2011 Publication	<1 %
33	Josimar O. Eloy, Amalia Ruiz, Felipe Tita de Lima, Raquel Petrilli et al. "EGFR-targeted immunoliposomes efficiently deliver docetaxel to prostate cancer cells", Colloids and Surfaces B: Biointerfaces, 2020 Publication	<1 %
34	Submitted to Universitas Andalas Student Paper	<1 %
35	cyberleninka.org Internet Source	<1 %
36	docplayer.net Internet Source	<1 %
37	mdpi-res.com Internet Source	<1 %
38	researchmgt.monash.edu Internet Source	<1 %
39	static.frontiersin.org Internet Source	<1 %
40	www.gov.br Internet Source	<1 %

41	www.tandfonline.com Internet Source	<1%
42	Manikandan Velayutham, Ajay Guru, Mariadhas Valan Arasu, Naif Abdullah Al-Dhabi et al. "GR15 peptide of S-adenosylmethionine synthase (SAMe) from Arthrospira platensis demonstrated antioxidant mechanism against H2O2 induced oxidative stress in in-vitro MDCK cells and in-vivo zebrafish larvae model", Journal of Biotechnology, 2021 Publication	<1%
43	Ram Manohar Basnet, Daniela Zizioli, Michela Guarienti, Dario Finazzi, Maurizio Memo. "Methylxanthines induce structural and functional alterations of the cardiac system in zebrafish embryos", BMC Pharmacology and Toxicology, 2017 Publication	<1%
44	publications.aston.ac.uk Internet Source	<1%

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		