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Literature Review

# POTENCY OF LUTEOLIN WITH SOLID LIPID NANOPARTICLE (SLN)-POLYETHYLENE GLYCOL (PEG) MODIFICATION FOR ARTEMISININ-RESISTANT *PLASMODIUM FALCIPARUM* INFECTION

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***ABSTRACT***

*Falciparum malaria is still considered as one of the important global health problems and its causal agent (Plasmodium falciparum) is reported to be the third most common factor for contributing the number of deaths in the world. As we all know, Artemisinins are the most rapidly acting of currently available antimalarial drugs. Along with Artesunate, these two combining drugs, the so-called Artemisinin-based combination therapies (ACTs) has become the foundation of modern falciparum malaria treatment globally. Nowadays, however, there have been reports about intricate cases of resistance against Artemisinin in various Southeast Asian countries and it is predicted to spread over several other countries, including Indonesia. Therefore, adjuvant therapy is required along with first-line therapy administration to help eradicate both Artemisinin-sensitive and resistant P. falciparum. Luteolin in vitro has a prospective inhibitory activity (IC50<50 µg) in inhibiting the development of parasite's life cycle. Nonetheless, its poor bioavailability and pharmacokinetics restrict clinical application. The low bioavailability of luteolin requires encapsulation using solid lipid nanoparticle (SLN) and polyethylene glycol (PEG). SLN is useful for improving the bioavailability of luteolin in the body, whereas PEG is needed in order to prevent the destruction of luteolin-SLN substance by the reticuloendothelial system. Here in this literature review, we're trying to demonstrate the benefits, potential, way of constructions, pharmacokinetics, and pharmacodynamics of luteolin encapsulated with SLN with PEG modification. Thus, it is hoped that the results of this literature study may encourage further research in assisting the development of adjuvant therapy for cases of Artemisinin-resistant P. falciparum infection.*

**Keywords:** *luteolin, Plasmodium falciparum, Artemisinin resistance, SLN, PEG*

***ABSTRAK***

*Malaria falsiparum masih menjadi salah satu dari masalah kesehatan global penting dan agen kausalnya (Plasmodium falciparum) dilaporkan menempati posisi ketiga tersering dalam mengontribusi jumlah kematian di dunia. Seperti yang telah kita ketahui, Artemisinin adalah obat antimalaria yang memiliki onset kerja paling cepat yang dapat digunakan saat ini. Seiring dengan Artesunate, dua kombinasi obat ini, atau yang biasa disebut sebagai terapi kombinasi berbasis Artemisinin (ACTs) telah menjadi dasar pengobatan malaria falsiparum modern secara global. Namun, saat ini, hal ini diperparah dengan adanya laporan kasus resistensi terhadap Artemisinin di berbagai negara Asia Tenggara, serta diprediksi akan menyebar ke beberapa negara lainnya, termasuk Indonesia. Untuk itu, diperlukan terapi adjuvan selain terapi lini pertama untuk membantu eradikasi Plasmodium falciparum baik yang sensitif maupun resisten. Luteolin secara in vitro memiliki aktivitas inhibisi yang cukup prospektif (IC50<50 µg) dalam menghambat perkembangan hidup parasit. Meskipun demikian, bioavailabilitas yang buruk serta proses farmakokinetik yang kurang memuaskan membatasi penerapan klinis dari substansi ini. Rendahnya bioavailabilitas luteolin membutuhkan enkapsulasi menggunakan nanopartikel lipid padat (SLN) dan polietilen glikol (PEG). SLN bermanfaat untuk meningkatkan bioavailabilitas luteolin di dalam tubuh, sedangkan PEG bermanfaat untuk mencegah destruksi substansi luteolin-SLN oleh sistem retikuloendotelial. Di sini, dalam tinjauan literatur ini, kami mencoba untuk memaparkan manfaat, potensi, cara konstruksi, farmakokinetik, dan farmakodinamik luteolin yang dienkapsulasi*

*dengan SLN dengan modifikasi PEG. Dengan demikian, diharapkan hasil studi literatur ini dapat mendorong penelitian lebih lanjut dalam membantu pengembangan terapi adjuvan untuk kasus infeksi P. falciparum.*

***Kata kunci:*** *luteolin, Plasmodium falciparum, resistensi Artemisinin, SLN, PEG*

## INTRODUCTION

Malaria falciparum, one of the life-threatening diseases caused by *Plasmodium falciparum* is still one of the top priority health problems in Indonesia as well as around the world. In fact, malaria falciparum is one of the most common cases of infection in tropical countries such as Indonesia with a prevalence of 86.4%.[1] Currently, the management of *P. falciparum* infection is a combination of drugs that one of its components is Artemisinin or any of its derivatives (Artesunate, Artemether, Dihydroartemisinin). The combination of these drugs is also commonly referred as Artemisinin-based combination therapy (ACT). However, several studies in 2010 state that in some populations, Artemisinin substances that enter into the ring stages of parasites do not directly kill the parasites intracellularly. Instead, they only stay at their dormancy stage.[2,3] In addition, a study called Tracking Resistance to Artemisinin Collaboration (TRAC) in 7 Asian countries show that there is a decrease in the clearance of *Plasmodium* parasite with Artemisinin therapy in several Southeast Asian countries, such as Cambodia, Myanmar, Thailand as well as Vietnam, and is predicted to spread to other countries including ours, Indonesia.[4] Therefore, it is necessary to develop additional therapies to improve the effectiveness of primary therapy in eradicating the *P. falciparum* parasite.

One of the bioactive components that can be used as an adjuvant therapy in accordance with eradicating Artemisinin-resistant *P. falciparum* is luteolin. Luteolin is a polyphenol-containing flavonoid that is widely found in a variety of plants, especially on scallion (*Allium fistulosum*). From that information, we can deduce that luteolin can easily be utilized to fathom malaria problems in Indonesia.[5] Luteolin has a role in inhibiting the process of fatty acid synthesis in parasites, knowing that this whole synthesis process is very important in order to form new organelles and biomembranes. Thus, this can result in termination of the parasite's life cycle.[6] In vitro studies also show that luteolin is able to inhibit the development of young trophozoite (ring stage) so that the intraerythrocytic parasite cycle can be cut off.[7]

Unfortunately, just like the other natural active compounds, luteolin has low stability and bioavailability in human body so that its delivery method must be carefully considered. Solid Lipid Nanoparticle (SLN), a drug delivery method with submicron particle size is a solution to overcome these weaknesses. SLN as a luteolin encapsulation will increase the biocompatibility and bioavailability of the compound. However, due to the hydrophobic nature of SLN, this encapsulation will easily be phagocytosed by the reticuloendothelial system as a result of being recognized as foreign body by macrophages.[8] Therefore, further modification is required to eliminate the opsonization properties. According to some studies, the use of Poly Ethylene Glycol (PEG) modification agent was found to be successful in altering the hydrophobic part of the SLN particle surface. Thus, it is desirable that the bonding process by opsonin factor causing destruction by the reticuloendothelial system can be inhibited.[9]

Based on these information, the authors offer the idea of luteolin utilization and optimized by PEG modified SLN delivery method as the idea of developing additional therapies in improving the effectiveness of primary therapy to eliminate the *P. falciparum* parasite.

## FALLING ARTEMISININ SUSCEPTIBILITY IN SEVERAL SOUTHEAST ASIAN COUNTRIES

Based on several reports of published studies, poor therapeutic responses to Artemisinins have occurred for the last few years in Southeast Asia. The studies of Teuscher et al[10] and Witkowski et al[11] in 2010 state that some populations, the substance of Artemisinin that enters the ring stage do not directly kill intracellular parasites, but only in the dormancy stage. Therefore, parasites can grow sustainably. This is one of the hypothesises that is thought to be one mechanism of Artemisinin resistance against *Plasmodium falciparum* parasite. It is also suspected to be the cause of recrudescence in falciparum malaria. Hien et al[12] and Kyaw et al[13] 's study of Tracking Resistance to Artemisinin Collaboration (TRAC) is showed that there is a decrease in the clearance of *Plasmodium* parasite with Artemisinin therapy in some Southeast Asian countries, especially in Cambodia and in Myanmar.

The decrease in the parasitic clearance rate is associated with the interaction of many kinds of genotypes. One of the most widely accepted theory is that there's an area on the 13$^{th}$ chromosome of *P. falciparum* which is strongly associated with decreased in vivo parasite clearance. The mutated genes, now commonly known as K13, encode proteins that contain the "kelch" motive, a figure that resembles six-bladed structure. Mutations occurring in the K13 gene will cause impaired function of ubiquitin pathway so that the apoptotic process does not occur and the parasite will escape the Artemisinin's mechanism of actions.[14,15]

## LUTEOLIN: CHEMICAL STRUCTURE AND ITS POTENTIAL SOURCES

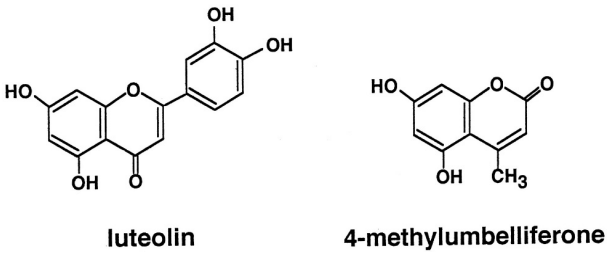


**Figure 1.** The chemical structure of luteolin. As a flavonoid compound, luteolin has a basic structure comprising two phenyl groups and is interconnected with a triple carbon group.[16]

Luteolin or sometimes called as 3', 4', 5,7-tetrahydroxyflavone, is a flavonoid of plants that are often utilized for its pharmacological activities as herbal medicine ingredients (as seen in Figure 1).[16] In addition, luteolin is often used as an anticarcinogenic, antithrombotic, antiallergic, antidiabetic, antiobesitic, immune-enhancer, and also for its antimalarial effect.[16] One of the potential plants that contain luteolin is scallion (*Allium fistulosum*). This is because the luteolin contained in scallion is considered highest when compared with other tropical plants, reaching 391 mg/kg.[5]

## MECHANISM OF LUTEOLIN AS AN ANTIMALARIAL AGENT

As mentioned earlier, the presence of resistance from Artemisinin drug requires an adjuvant therapy agent with different working mechanisms to help eradicate the *P. falciparum* parasite. The results showed that luteolin works by targeting apicoplast in *Plasmodium falciparum*, an organelle analogous to plastids which function in a wide range of intracellular activities including fatty acid synthesis. The synthesis of fatty acids is very important for *Plasmodium falciparum* parasites. This is due to the role of essential fatty acids in the parasitic life cycle, which is an important component in the process of synthesizing new organelles. The process of synthesizing the new organelles in question takes place in one of its life cycle's process, which is when the parasites are turning the ring stage into a schizon (a collection of merozoites) or into a gametocyte. Fatty acids are also known to be important components in the biomembranes synthesis of *Plasmodium falciparum*.[6,7]

A study from the last five years conducted by Qidwai et al[17] showed that luteolin has a different way of eradication mechanism from previous malaria medicines. Luteolin works specifically on the regulation of fatty acid synthesis of *Plasmodium falciparum* primarily in the inhibition of three of the four key enzymes, namely FabG, FabZ, and FabI (Fab=Fatty acid biosynthesis). Fab is one of the

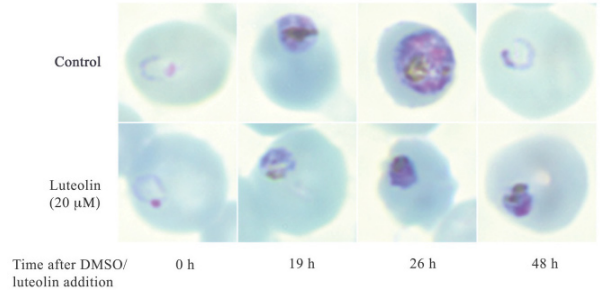


**Figure 2.** The effect of luteolin on the intraerythrocytic growth of *P. falciparum*.

enzymes that play an important role in the process of fatty acid synthesis of *Plasmodium falciparum* especially in the process of fatty acid elongation into a functional structure for the parasite.[17] Based on these findings, it is hoped that luteolin can be the latest breakthrough in initiating the development of adjuvant therapy of Artemisinin-resistant *Plasmodium falciparum*.

As seen in Figure 2, Giemsa-stained smears were prepared at 0, 19, 26, and 48 hours after the addition of luteolin (20 μM) dissolved in DMSO (dimethyl sulfoxide) or an equal volume of DMSO (control; 0.025% DMSO) to infected erythrocytes. While control parasites developed into mature trophozoites (26 hours) that subsequently gave rise to daughter parasites (48 hours), the growth of luteolin-treated parasites was arrested at the young trophozoite stage. Luteolin-treated parasites did not give rise to daughter parasites, resulting in a reduced parasitemia compared to the control at the 48 hours.[7]

Giemsa-stained smears were prepared at 0, 19, 26 and 48 h after the addition of luteolin (20 μM) dissolved in DMSO or an equal volume of DMSO (control; 0.025% DMSO) to 7G8-infected erythrocytes (1% parasitemia, 2% hematocrit). While control parasites developed into mature trophozoites (26 h) that subsequently gave rise to daughter parasites (48 h), the growth of luteolin-treated parasites was arrested at the young trophozoite stage. Luteolin-treated parasites did not give rise to daughter parasites, resulting in a reduced parasitemia compared to the control at the 48 h time point.[7]

## PHARMACOKINETIC OF LUTEOLIN

When luteolin is administered orally, it will be recognized by the body as a substrate for the conjugation and hydrolysis process by various enzymes that present in the small intestine. The conjugation process will convert luteolin into glucuronide and sulphate derivatives, thereby facilitating its absorption and excretion through the gallbladder and bile secretion. The absorption process of luteolin in the form of glucuronide will undergo further process by the microbiota of the small intestine, thereby altering the chemical properties of glucoronide to the aglycone compound. Aglycone will experience several

catabolism phases into a low molecular weight compound so that it can be readily absorbed by the small intestinal villi. Meanwhile, a group of components that has not been absorbed by the small intestine will lead to the large intestine and undergo further modification by the normal flora of the colon.[18]

Unfortunately, those several metabolic processes cause incomplete absorption of luteolin by the human digestive system. Thus, this results in the low bioavailability of luteolin in the human body.[18]

## THE ENCAPSULATION OF SOLID LIPID NANOPARTICLE (SLN) WITH POLYETHYLENE GLYCOL (PEG) MODIFICATION

Although bioflavonoids like luteolin have a wide variety range of health benefits and potentials in vitro, studies have shown that the role of bioflavonoids in vivo is somewhat ineffective, even has no benefit at all. This is due to the poor lipid solubility properties of luteolin and the unsuitable molecular size of the absorption, leading to poor absorption and bioavailability levels of bioflavonoids. Nevertheless, the demand for bioflavonoid use as an herbal treatment is reported to have increased worldwide due to the lack of its side effects and better therapeutic effects in compare with modern medicine. Thus, a drug delivery medium may be required to improve the bioavailability of the herbs.[19]

One of the lipid-based drug delivery media that has been recognized for its effectiveness in improving drug bioavailability is solid lipid nanoparticle (SLN). SLN was introduced in 1991 and is a lipid-based medium of a colloidal system used as an alternative in drug delivery processes. SLN is a submicron particle, having a size from 50 nm to 1000 nm and is made from modified lipid materials so that it has a solid form at the room temperature. Compared to other lipid-based drug delivery media, SLN has several advantages, among others, such as SLN-based materials have good biocompatibility with the human body, easily measured and sterilized, drug release can be controlled with components bioactive in SLN, has a large drug capacity, has proven to be the best lipid-based drug delivery substance in enhancing the bioavailability of the encapsulated drug, and has a high degree of stability in the human body.[20]

In general, the process of preparing encapsulation with the SLN method is divided into two, namely the heat homogenization technique and the cold homogenization technique. The cold homogenization technique is judged to have less weakness when compared with the heat homogenization technique. Whereas, the technique of heat homogenization can cause various kinds of damage to the medical substance, such as degradation of drugs due to high temperature.[20]

The heat homogenization technique is carried out at temperatures above the melting point of the lipid (± 30°C to 48°C), so it can be said that the homogenization process is a process for the emulsion. First, the substance of the drug to be encapsulated is mixed with the melted lipids. Subsequently, there will be a dispersion process from the lipid phase to a hot mixture called a liquid-surfactant. At this stage, a fast but regular stirring process is required to produce a mixture called pre-emulsion. Then, once pre-emulsion formation has created, a high pressure homogenization process is performed. This homogenization process aims to make the mixture blended in very well for the purpose of producing two phase heterogeneous substances called colloids. The colloids are further cooled at room temperature (20°C to 25°C) in order to make the solidification process into a drug that has been encapsulated by SLN.[20]

In the cold homogenization technique, the initial process of cold homogenization techniques is not much different from the high-temperature techniques. The process starts with mixing the desired substance with melted lipids. After that, a process of reducing the size of the substance is done to break down the fat globules into some smaller fat particles. Thereafter, the mixture was suspended with water and followed by high pressure homogenization. When the process is complete, a drug product is encapsulated by SLN.[20]

Although SLN has good biocompatibility, some studies show that SLN is often recognized as a foreign antigen in the human body. This is caused by hydrophobic molecules that act as opsonin factors of macrophages so that SLN tends to be phagocytosed by various components present in the reticuloendothelial system. To prevent the occurrence of this phagocytosis and to keep the level of the drug in blood plasma remained high, the surface of the drug needs to be modified furthermore using polyethylene glycol (PEG). PEG works by decreasing the immunological properties of the SLN's hydrophobic molecule like bovine serum albumin in order to avoid immunological reactions between protein-breaking enzymes, such as superoxide dismutase, arginase, and asparginase.[21]

PEG also has dual molecule properties (hydrophilic and hydrophobic surfaces) that can bind to SLN hydrophobic part (Figure 3). The outer part of PEG (hydrophilic) serves to conceal the SLN hydrophobic molecules that are

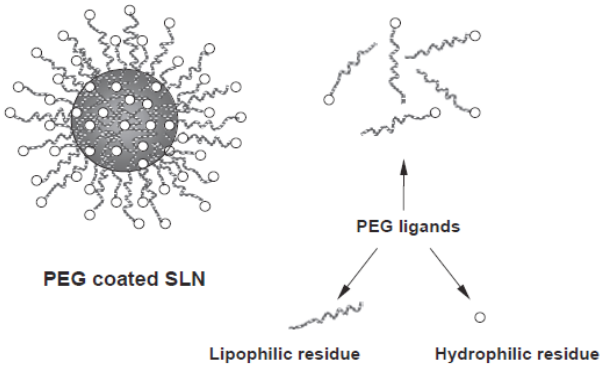


**Figure 3.** Representation scheme of SLN modified with PEG and the molecular structure of PEG.[9]

believed to be the opsonin factor of the macrophages so that in this case, PEG acts indirectly to protect SLN from the phagocytic process.[21]

## BIOPHARMACEUTICAL CHARACTERIZATION INFORMATION

### The Construction Process of This Product

The SLN-encapsulated luteolin construction process with further PEG modification is divided into various stages. The first stage is to extract luteolin from *Allium fistulosum*. Some studies recommend that if you want to extract compounds that have limited solubility in a solvent, such as bioflavonoids, you need to use a distillation-based extraction method called Soxhlet extraction. The process of luteolin extraction by this method lasted for 8 hours and resulted in a concentrated solution containing 174.76 µg/mL luteolin (2 grams of dried *Allium fistulosum* equals to 174.76 µg/mL luteolin).[22,23]

Furthermore, luteolin encapsulation with SLN was prepared using a cold homogenization method. The liquid lipid phase was prepared using glyceryl monostearate (Tween 80) and soy lecithin aqueous chloroform solution in 1:1 ratio. The extracted luteolin solution was mixed with the lipid matrix and then reduced in size (micronization) using nitrogen liquid and high pressure homogenization. The solution phase is then combined using PEG-400 (polyethylene glycol) in water and heated until it reaches 80°C. Finally, the two phases were combined under mechanical agitation (for 6 minutes at an amplitude of 60%) and the solvent was evaporated by heating the mixture at a temperature of 80°C. The emulsion obtained after evaporation was then cooled to 0°C for 1 hour until it crystallized completely.[24,25]

### Pharmacokinetic of This Product

SLN can be administered by mouth, parenteral, and topical. However, luteolin as a flavonoid compound derivative exhibits better efficacy if administered orally or topically.[27] Based on these findings, the preferred administration method for this product is oral administration.

Pharmacokinetic reviews include the subject of absorption, distribution, metabolism, and excretion. SLN-like lipid properties result in a similar process of digesting lipid-containing materials. The SLN digestion process begins with the breakdown of fat through the process of hydrolysis into fatty acids and glycerol using lipase enzymes that are secreted by the chief cells in the stomach. After that, the breakdown product is carried to the duodenum. Furthermore, the fatty acid induces the pancreas to secrete the cholecystokinin enzyme to release the colipase enzyme to break the triglycerides into simpler form.[26]

Micelles, a colloidal substance that has a function to make the fat particles can be readily absorbed by the intestinal villi, will be absorbed by several mechanisms

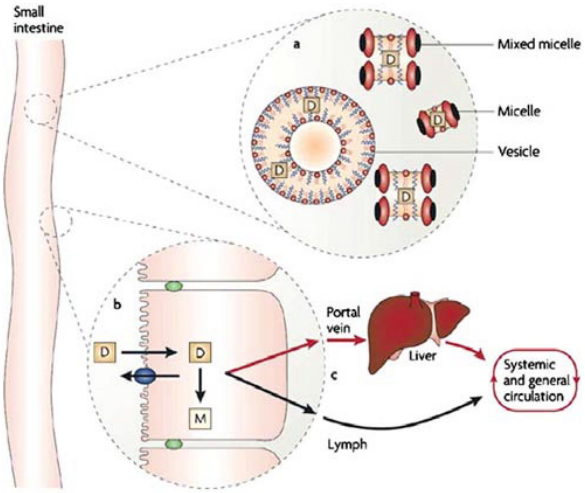


**Figure 4.** Various mechanisms of enhancement of drug bioavailability in the presence of lipid substance (SLN) within the drugs: (a) solubilization of drug in the intestinal fluid by formation of colloidal substances, vesicles, mixed micelles and micelles; (b) interference with enterocyte-based transport and metabolic processes, thereby potentially changing drug uptake, efflux, disposition, and the formation of metabolites (M) within the enterocyte; (c) by selective lymphatic uptake which reduces first-pass drug metabolism as intestinal lymph travels directly to the systemic circulation.[26]

such as the passive diffusion transport method, facilitated diffusion, and active transport through the enterocyte membrane. Once absorbed into cytosol, the carrier protein will bring the fatty acid into the smooth endoplasmic reticulum. In the smooth endoplasmic reticulum, fatty acids and glycerol will be brought to the Golgi apparatus and released by exocytosis to the extracellular space in the form of vesicles. Another important step is the absorption of the medicinal active ingredient (luteolin) through an intestine's intermediate lipoprotein called chylomicron. During the absorption phase, the drug molecule is usually metabolized with the enzyme cytochrome P450-3A4 present at the end of the small intestinal villi. Studies are showed that the presence of these enzymes increases the bioavailability of the drug if administered with lipids. This causes the lipophilic drugs tend to have a higher half-life when compared to hydrophilic drugs (Figure 4).[26]

The majority of drugs that administered orally have access to systemic circulation via portal circulatory system. However, highly lipophilic drugs such as PEG modified SLN will have access to systemic circulation through the lymphatic circulatory system. Thus, lipophilic drugs tend to have higher bioavailability in the human body because they do not pass through the first-pass metabolism presented in the liver. In contrast, the addition of PEG to the product reduced the drug clearance by the reticuloendothelial system so that the half-life of the drug would be increased.[26] This was supported by a study conducted by Das et al[28]

showing that the half-life of SLN encapsulated luteolin with PEG modification can reach seven days in circulation. The last pharmacokinetic aspect, excretion, shows that luteolin will be effectively eliminated in the kidneys due to its highly biodegradable nature.[26]

**Evidence-Based Studies Regarding Improvement of Drug's Bioavailability, Pharmacokinetics by Solid Lipid Nanoparticle (SLN) (In Vivo Studies)**

The only study on the efficacy of solid lipid nanoparticles in improving bioavailability and pharmacokinetics of a drug derived from a study that was conducted by Dang et al.[8] The drug luteolin was detected by a high-performance liquid chromatograph (HPLC) and the UV detector was operated at the 350 nm wavelength. Also, they used male Sprague–Dawley rats, weighing from 180 to 220 g and were kept in environmentally controlled room (temperature 25±2°C, humidity 60±5 %, 12/12h dark/light cycle) for 1 week prior to the experiments. They were given daily fresh diet with free access to water.[8]

Luteolin-SLN (LU–SLN) were prepared by slightly modification in hot-microemulsion ultrasonic technique. Briefly, soybean lecithin, Tween 80, and water were placed together in a beaker and heated to the lipid melting temperature. Glyceryl monostearate with luteolin was also melted at 75±2°C separately. The hot aqueous emulsifier mix was injected into the lipid melt containing luteolin drop by drop, under magnetic stirring to obtain a microemulsion. The obtained pre-emulsion was sonicated by an ultrasonic cell pulverizer for 20 minutes and cool it with ice bath immediately.[8]

The rats were fasted overnight before experiments with free access to water and randomly divided into two groups (n=6). The free luteolin suspension and LU–SLN were administrated to rats by oral gavage at a dose of 20 mg/kg. 0.3-0.5 mL of blood was collected into heparinized tubes at 0, 0.167, 0.333, 0.5, 0.667, 1, 2, 4, 6, 9, 12, 15, and 24 hours after administration. Then, plasma was separated immediately by centrifugation at 10,000 rpm for 10 minutes and stored at -20°C for analysis.[8]

Luteolin was extracted from the plasma by liquid–liquid extraction method for LC–MS/MS (liquid chromatography–mass spectrometry) analysis. Briefly, plasma sample (100 lL) was spiked with 500 lL MTBE (methyl tert-butyl ether) and 100 lL internal standard solution (diosmetin, 500 lg/L in methanol) by vortex mixing for 30 ss. After centrifugation at 10,000 rpm for 10 minutes at 4°C, the supernatant was transferred to a fresh tube, and evaporated to dryness under a nitrogen gas. The residue was dissolved in 100 lL methanol. The reconstituted extraction was thoroughly mixed and then centrifuged again at 5,000 rpm for 2 min at 4°C. Finally, the supernatant solution was injected into the tandem liquid chromatography-mass spectrometry system (Figure 5).[8]

Orally administered of LU–SLN was rapidly absorbed, as evidenced with less Tmax for LU–SLN than the pure suspension. The relative bioavailability of Luteolin was

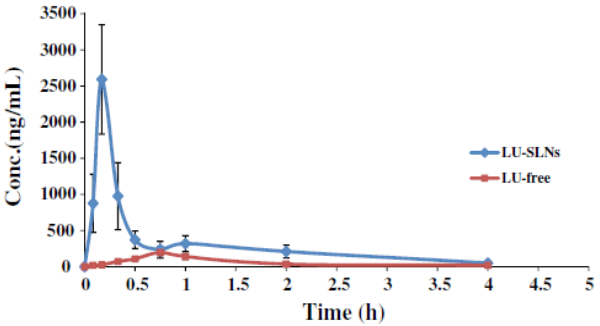


**Figure 5.** Mean plasma concentration–time profile of Luteolin with and without SLN after single dose oral administration of pure luteolin suspension to rats (20 mg/kg).[8]

improved (more than 4.89-fold) when incorporated into the SLNs. At the same time, distribution and clearance of luteolin with SLNs were decreased. These data make a clue for supporting SLNs are a promising delivery system for the enhancement of oral administration of a poorly water-soluble drug.[8]

**Human Effect Matrix**

The human effect matrix discusses the toxicology as well as the side effects that a drug can cause on the human body. However, no studies have been done to test the toxicity of luteolin-SLN-PEG against humans. Nevertheless, based on a review study conducted by Chen et al[29] regarding toxicology reviews from luteolin substance, it was mentioned that high doses of luteolin had no toxic effect. Therefore, luteolin is expressed as a GRAS (Generally Recognized as Safe) substance.

## SUMMARY

luteolin encapsulated by SLN with further PEG modification is highly potential to be used as an innovative adjuvant therapy for either Artemisinin-resistant or Artemisinin-sensitive *P. falciparum* parasite. However, preclinical studies in the pharmacology field need to be done to confirm the pharmacokinetics and the potential dose of this product.

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