

## Conference Paper

# Potency of Solid Lipid Nanoparticle (SLN)-Modified Luteolin-based Polyethylene Glycol (PEG) on *Allium fistulosum* as Innovation Therapy of Malaria Falciparum

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In 2013, 198 million cases of malaria were reported globally and 584,000 of them died. As much as 78% of cases occurred in children under five years of age. Indonesia is a has the second highest malaria incidence rate after India in the Asian region. Severe malaria can be characterized by the presence of severe anaemia, hyperparasitemia or cerebral malaria. Severe anaemia due to malaria or severe malaria anaemia (SMA) often occurs in children who suffer from falciparum malaria. SMA occurs due to a decrease in COX-2-PGE2, caused by phagocytosis of PfHz (*Plasmodium falciparum*-derived Hemozoin) by monocytes, macrophages and neutrophils. PfHz is a crystalline compound formed from the aggregation of heme hosts. Fe<sup>2+</sup> is one of the constituents of heme, luteolin can bind Fe<sup>2+</sup> so that the bond between luteolin and Fe<sup>2+</sup> in heme prevents the formation of PfHz, so that severe anaemia can be prevented. Like other naturally occurring active compounds, luteolin has low bioavailability in the body so it is encapsulated using Solid Lipid Nanoparticles (SLN) and Polyethylene Glycol (PEG). SLN is useful for increasing the bioavailability of luteolin in the body, while PEG is useful for preventing the destruction of luteolin-SLN by RES. The modified construction process includes the following steps: (1) luteolin extraction from *Allium fistulosum* and (2) luteolin encapsulation using SLN-PEG. The potential dose to be administered orally to humans is 2.43–8.11 µg/kg body weight.

**Keywords:** luteolin, Polyethylene Glycol, severe malaria anaemia, Solid Lipid Nanoparticles

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## 1. Introduction

Malaria is still one of the main infectious diseases in the world. In paediatric infections, malaria ranks third after pneumonia and diarrhoea as a disease with high mortality [1]. In

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2013 there were 198 million cases of malaria globally and 584,000 of them died. 78% of cases occurred in children under five years of age [2]. Indonesia is a country with the second highest malaria incidence after India in the Asian region [3]. Most deaths in malaria cases are caused by *Plasmodium falciparum* infection. Clinically severe malaria can be characterized by the presence of severe anaemia, metabolic acidosis, respiratory distress, hyperparasitemia, acute renal failure, hypoglycaemia, or cerebral malaria [4].

Severe anaemia due to malaria or severe malaria anaemia (SMA) is a complication that most often occurs in children. SMA can occur as a result of lysis of infected or uninfected erythrocytes and or by suppression of erythropoiesis. Erythropoiesis suppression which is the main cause of SMA occurs through the following mechanisms. *Plasmodium* requires host haemoglobin (Hb) to meet its nutritional and energy needs. This process produces heme which is toxic to *Plasmodium* itself, whereas *Plasmodium* cannot oxygenate heme, so heme is aggregated to form a crystal structure called PfHz (*Plasmodium falciparum*-derived Hemozoin). This process is assisted by the enzyme heme peroxidase [5]. Monocytes, macrophages and neutrophils phagocyte erythrocytes containing PfHz as well as free PfHz which have broken off from their schizonts [4]. Phagocytosis PfHz can reduce COX-2 expression which results in decreased PGE<sub>2</sub>. The COX-2-PGE<sub>2</sub> pathway plays an important role in the erythropoiesis process, COX-2 plays a role in erythroid maturation, while PGE<sub>2</sub> plays a role in preventing erythrocyte deformity. Thus, decreased COX-2-PGE<sub>2</sub> causes suppression of erythropoiesis resulting in SMA in children infected with *Plasmodium falciparum* [6].

Luteolin was a type of flavonoid that was found mostly in leeks (*Allium fistulosum*) [7]. Luteolin was able to bind to Fe<sup>2+</sup> so that this can be used to answer the above problems [8]. The bond between luteolin and heme containing Fe<sup>2+</sup> will result in the formation of PfHz so that the next mechanism will not occur and SMA can be avoided. Based on this proposed mechanism, the Luteolin compound from *Allium Fistulosum* has the potential effect to prevent SMA in paediatrics malaria.

Like other naturally occurring active compounds, luteolin has low stability and bioavailability, so the method of delivery must be considered. Solid Lipid Nanoparticles (SLN), a drug delivery method with submicron particles, is an opportunity that can overcome this weakness. SLN as luteolin encapsulation will increase the biocompatibility and bioavailability of these compounds [9], however, due to the hydrophobic nature of SLN, this encapsulation will be easily phagocytosed by macrophages because it will be recognized as foreign body and will then be destroyed by the Reticulo Endothelial System (RES). Modification using Polyethylene Glycol (PEG) is able to answer this problem. PEG is able to modify the hydrophobic part of the particle surface so that it

suppresses the bonding process with the opsonin factor which causes destruction by RES [10].

Based on this explanation, the aim of this review to construct the process of Luteolin encapsulated by PEG modified SLN, to explain the administration process and the potential dosage, and also to review the pharmacokinetic of using luteolin from *Allium Fistulosum* extract by delivery using PEG-modified SLN.

## 2. Material and Method

This article is based on a literature review (library research) of various literature whose validity has been tested and supports the writing analysis. The data obtained are then analysed descriptively and analytically to produce scientific studies that can be developed and applied on an ongoing basis.

Data obtained through the Google Scholar search engine using the keywords “Malaria”, “Falciparum”, “luteolin”, “SLN”, “PEG” or a combination of several of them. References taken are trusted journals from 2001-2015. The analysis was carried out after various data were collected. The data were then selected to obtain relevant data and in accordance with the writing analysis. The analysis technique used was an argumentative descriptive analysis describing the potential of luteolin in the *Allium fistulosum* with PEG-modified SLN carriers as an innovation in falciparum malaria therapy. After the analysis-synthesis process, general conclusions are drawn based on the analysis.

## 3. Results

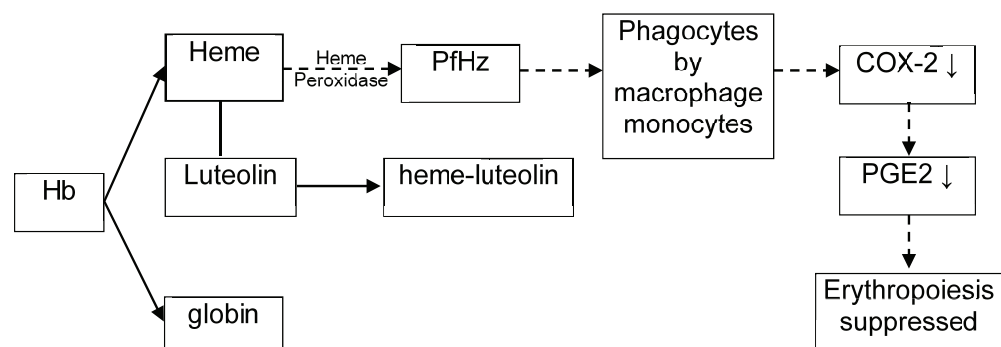
A total of 21 papers published from 2001-2015 were included in this paper. The data obtained including *Int J Biol Sci*, *J Mic Inf*, *J Agric Food Chem*, *J Nanopart Res*, *Int J Nanomedicine*, *Nucl. Acids Res*, *Indian J Pharm Sci*, etc. All of the data were collected from studies that performed Malaria, luteolin, SLN, and PEG experiments already fulfilled the inclusion criteria.

## 4. Discussion

#### 4.1. Mechanism of action of PEG modification SLN-Related Luteolin in Malaria Falciparum

Hemozoin is a brown crystalline structure formed in the Plasmodium vacuole as a product of haemoglobin (Hb) catabolism. Plasmodium requires hb host to fulfil its nutritional and energy needs. The process of Hb catabolism will produce waste in the form of heme which is toxic to plasmodium itself. Plasmodium cannot excrete heme in the free form and cannot oxygenate heme so that the heme is aggregated to form a crystal structure using the heme polymerase enzyme and produces a compound called PfHz [5]. When schizonts are finished replicating in host erythrocytes, erythrocytes will undergo lysis and release PfHz along with merozoites. PfHz will be phagocytosed by monocytes / macrophages and neutrophils. This phagocytosis process results in the release of a number of inflammatory mediators. This results in a decrease in COX-2 expression which consequently also decreases PGE<sub>2</sub> expression. The COX-2-PGE<sub>2</sub> pathway plays an important role in the erythropoiesis process, COX-2 plays a role in erythroid maturation, while PGE<sub>2</sub> plays a role in preventing erythrocyte deformity. Thus decreased COX-2-PGE<sub>2</sub> leads to suppression of erythropoiesis resulting in SMA [6].

Luteolin has the ability to bind Fe<sup>2+</sup>, heme is a structure consisting of Fe<sup>2+</sup> in the central, planar and porphyrin ring [18] . When administered and reaches circulation, luteolin will bind Fe<sup>2+</sup> to heme so that heme cannot aggregate to form PfHz. If PfHz is not formed then further processing will not occur so complications in the form of severe anaemia can be prevented. The mechanism of action of luteolin can be seen in Figure 1 below.



**Figure 1:** Scheme of the mechanism of action of luteolin.

## 4.2. Construction process

The construction process of PEG modified SLN encapsulated luteolin is divided into several stages. The first step that must be done is luteolin extraction from *Allium fistulosum*. This extraction process is carried out by referring to previous research conducted by Loizo et al. who have extracted and isolated luteolin from *A. excelsa* leaves. One kilogram of dry powder of *Allium fistulosum* leaves was extracted with 70% methanol using a Soxhlet extractor and then evaporated. The residue was dissolved in one litre of aquadest then extracted with petroleum ether, dichloroform and ethyl acetate respectively. Each fraction was dried with anhydrous sodium sulphate and concentrated at lower pressure to produce a fraction of 29 g petroleum ether, 14 g chloroform and 10.5 g ethyl acetate. Then proceed with column chromatography method for ethyl acetate bioactive fraction using silica gel as adsorbent and elution was carried out with chloroform followed by methanol which enhanced polarity. Elution using  $\text{CHCl}_3$ : MeOH (98: 2) produces two fractions, namely fractions A and B, while a ratio of 95: 5 produces fraction C and a ratio of 90:10 produces fraction D. This elution and fractionation process is controlled using UV light and exposure to ammonia vapor. The AD fraction was then chromatographed using Sephadex LH-20 with aquadest to obtain 25 mg of pure luteolin [19].

The next step is luteolin encapsulation using PEG-modified SLN. This encapsulation was carried out using cold homogenization technique. Glycerol monostearate (GMS) and PEG<sub>2000</sub> – SA heated at 60°C. Acid stearic and luteolin are dissolved in 1 ml of ethanol and then the mixture is added to a mixture of GMS-PEG drop -by- drop. After removing ethanol.

The mixture is cooled by pouring it into liquid nitrogen until it forms a solid dispersion of microparticles. The microparticles were then suspended in the liquid phase using Polaxamer 188 (F<sub>68</sub>) 1% and 20% sugar, then homogenized at 18000 g for 30 minutes using a homogenizer followed by homogenization at 20000 psi using a high pressure homogenizer to produce the desired modification in solution form [20].

## 4.3. Potential dosage and administration mechanism

PEG modified SLN encapsulated luteolin will be administered at a dose of 2.43 - 8.11 µg/kgBW orally. Based on previous studies, it is known that to bind Fe<sup>2+</sup> tested in mice, 30-100 µg/kg of luteolin is required [8]. Dose conversion by calculating Human Equivalent Dose (HED) in units of µg/kg body weight. The dose in animals is multiplied

by the division between the Konstanta Michaelis (Km) of the animal divided by the Km of humans. The Km values for mice and for humans were 3 and 37, respectively (Figure 2) [21].

Formula for Dose Translation Based on BSA	
$HED \text{ (mg/kg)} = \text{Animal dose (mg/kg)} \text{ multiplied by } \frac{\text{Animal Km}}{\text{Human Km}}$	

**Figure 2:** Dosage conversion formula based on BSA.

Based on the above formula, the calculations are as follows:

$$HED = 30 \times \frac{3}{37} \text{ mg} = 2,43 \text{ mg/kg}$$

$$HED = 100 \times \frac{3}{37} \text{ mg} = 8,11 \text{ mg/kg}$$

Thus, the potential dose ranges for humans are 2.43 - 8.11  $\mu\text{g/kg}$  body weight for one administration.

SLN can be administered orally, parenterally, and transdermally [22]. Luteolin as a flavonoid derivative shows better efficacy when administered orally or topically [23]. Under these conditions, method of administration that luteolin is recommended to use the administration per oral. As well as the compounds of flavonoids that other, luteolin has low bioavailability in the body so encapsulation using SLN to overcome this problem. In addition, the advantages of using SLN are: (1) controlling the release of active compounds; (2) increase the stability of the compounds in the body; (3) it is easy to modify and (4) the cost is relatively affordable compared to other carriers [21], however, SLN which is hydrophobic will be easily opsonized by macrophages and destroyed by RES because it is recognized as a foreign object so it is necessary to add PEG. PEG is able to modify the hydrophobic side of SLN which ultimately prevents the destruction of compounds by RES.

#### 4.4. Pharmacokinetic review

Pharmacokinetic review includes absorption, distribution, metabolism, and excretion. Luteolin is well absorbed in the duodenum and jejunum [23]. The maximum concentration of luteolin is reached after 1-2 hours and luteolin remains in the plasma for several hours. Like other natural compounds, luteolin has low bioavailability in the body because it will undergo first pass metabolism in the RES organs so that it has low bioavailability in the body.

PEG-modified SLN encapsulation will increase luteolin adsorption in the duodenum and jejunum. The lipid-based formulation design can increase the efficiency of the release of active compounds and facilitate the formation of a dissolved phase for optimal absorption. Furthermore, the compound will be distributed systemically with high bioavailability. Modification of these compounds is distributed towards the target via blood circulation and encapsulation allows the stability of luteolin to be maintained during the distribution process [24]. The addition of solid SLN and PEG reduces the clearance of the RES and thus increases the half-life of the compound by up to 7 days [25]. Finally, the compounds will be eliminated effectively in the kidneys due to their biodegradable properties.

## 5. Conclusion

Based on previous exposure, Luteolin encapsulated by PEG modified SLN has the potential effect to be used as an innovative therapy for severe anaemia in falciparum malaria in children. To realize the ideas of this review, further research is needed regarding the potential dose confirmation and pharmacokinetics of PEG-modified SLN encapsulated luteolin for falciparum malaria in children.

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## Conflict of Interest

The authors declare that there is no conflict of interest.

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