In vivo evaluation of luteolin-loaded phytosome

Cysilia K Hindarto, Silvia Surini, Fadlina C Saputri and Candra Irawan

Abstract

Luteolin exhibits antioxidant, antimicrobial, and antiinflammatory activities. However, it shows poor oral bioavailability due to its low lipid-solubility. The aim of this study was to improve absorption of luteolin in the gastrointestinal tract. Luteolin was formulated into luteolin-loaded phytosome (LLP) by thin film hydration method. In vivo evaluations were then performed to see plasma levels of luteolin in rats given oral LLP suspension and compared with those in the control group given pure luteolin suspension. Round-shaped LLPs were confirmed with average particle size 105.3 nm, PDI 0.735, zeta potential -34.4 mV, and entrapment efficiency 91.12%. In vivo studies showed a 3.54-fold increase in plasma level (AUC = 5426 µg.min/mL) of luteolin compared with those in control group. Phytosomes formulation successfully increased the absorption of luteolin hence it can serve as a promising delivery system for drugs with low lipids solubility.

Keywords: Absorption, in vivo, luteolin, phytosome, plasma concentration

Introduction

Luteolin (5, 7, 3’, 4’-tetrahydroxiflavones) is an active compound found in most herbs and food plants. Many researches have reported that luteolin exhibits antioxidant, antimicrobial, antiinflammatory, anticancer, and antimutagenic activities, and also has effect on cardiovascular disease by decreasing cholesterol level and blood pressure [1, 2]. However, it shows poor oral bioavailability due to its low lipid-solubility and metabolism in gastrointestinal tract [3, 4]. Many have claimed that phytosome formulation is effective to improve absorption and oral bioavailability of low lipid-solubility active compounds [5, 6, 7]. The objective of this study was to improve absorption of luteolin in the gastro-intestinal tract.

Materials and Methods

Materials

All chemical reagents were of analytical grade and used without further purification unless otherwise stated. Luteolin (purity grade = 98% HPLC) was purchased from Acetar Biotec (Cina); L-a-phosphatidicholine (soybean phosphatidicholine, P 3644) was purchased from Sigma (United State of America); Methanol (LiChrosolv®), dimethylsulfoxide (DMSO), acetic acid glacial, and dichloromethane were purchased from Merck (Germany). All solutions used in high performance liquid chromatography (HPLC LC-20AD, Shimadzu, Japan) analysis were filtered and degassed using a 0.22 µm membrane filter with a filtration system. Male Sprague-Dawley rats, weighing 200-250 g, were obtained from Faculty of Veterinary, Bogor Agriculture Institute laboratory animal center (UPHL FKH IPB). The rats were kept in an environmentally controlled room (temperature 25-30 °C, relative humidity 80-90%) for 14 days prior to experiments. All animal procedures were conducted in accordance with all appropriate regulatory standards according to the protocol approved by the ethics committee of Faculty of Veterinary, Bogor Agriculture.

Preparation and Characterization of LLP (Luteolin-Loaded Phytosome)

The LLPs were prepared by thin film hydration methods [9]. Briefly, 60 mg luteolin (LU) were dissolved in 70 mL of methanol, while 180 mg soybean phosphatidicholine (SPC) were dissolved in 20 mL dichloromethane. The two solution were transferred to a glass round bottom flask and refluxed (4h, 60 °C) resulting a clear yellow solution. Solvents then removed by vacuum evaporator (Rotavapor® R II, Buchi, Germany) at 337 mbar, 40°C, to produce the thin film which was hydrated with 200 mL of distilled water. The entrapment efficiency and luteolin content in LLP were then determined using UV-visible spectroscopy (350 nm). The average particle size, polydispersity index (PDI) and zeta potential...
of the luteolin-loaded phytosomes (LLPs) were determined by DLS using Malvern Zetasizer (Malvern Instruments, UK). The 2D surface morphology of the LLPs was visualized by TEM (JEM 2100, Japan).

**In vivo Evaluation of Luteolin-loaded Phytosome (LLP)**

In vivo evaluation of LLP was carried out based on methods describe in previous studies with modification [1, 9]. The rats were fasted overnight (8 h) before experiments with free access to water and randomly divided into two groups (n=6). The free luteolin suspension and LLP suspension were administrated to rats by oral gavage at dose of 20 mg/kg. 0.3-0.5 mL of blood was collected into tubes (with EDTA) at 0, 10, 20, 30, 60, 90, 120, 180, and 240 minutes after administration. Then plasma was separated immediately by centrifugation at 10,000 rpm for 10 minutes and stored at -20°C for analysis.

Luteolin was extracted from the plasma by the liquid-liquid extraction method for HPLC analysis. Briefly, 0.2 mL 6% perchloric acid was added slowly into 2 mL plasma sample to precipitate protein completely. Luteolin was extracted with 3 mL of ethyl acetate by vortexing for 5 minutes. After centrifugation for 10 min at 3500 rpm, supernatant of organic phase were carefully transferred to another tube and evaporated to dryness in the vacuum desiccators under room temperature. The resulting residue was reconstituted in 250 µL of mobile phase, the mixture was centrifuged for 10 min at 5,000 rpm and supernatant was injected into HPLC apparatus.

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Table 1: Physicochemical properties of luteolin-loaded phytosome (LLP)

<table>
<thead>
<tr>
<th>Luteolin : Phosphatidylcholine Molar Ratio</th>
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<tr>
<td>Entrapment Efficiency (%)</td>
<td>91.12</td>
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<tr>
<td>Luteolin Content in 25 mg LLP (%)</td>
<td>22.22</td>
</tr>
<tr>
<td>Average Dn (d.nm)</td>
<td>105.3</td>
</tr>
<tr>
<td>Polidispersity Index (PDI)</td>
<td>0.735</td>
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<tr>
<td>Zeta Potential (mV)</td>
<td>-34.4</td>
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</table>

**Fig 1:** Morphology of LLPs

In vivo Evaluation of Luteolin-loaded Phytosome (LLP)

In vivo evaluation of luteolin-loaded phytosome (LLP) was carried out to examine whether the produced LLPs can significantly improve the absorption of luteolin in the gastrointestinal, by determining luteolin plasma concentration after oral administration. Luteolin plasma concentration in the group administered with oral LLP suspension was higher than those in control group that administered with oral free luteolin suspension (Figure 2). As shown in Table 2, prepared LLPs showed 3.54 times more bioavailability(AUC = 5426 μg.menit/mL) and 11.61 times Cmax compared to luteolin suspension. As naked luteolin concentration in plasma was significantly low due to low-lipid solubility and glucuronidation by UDP-glucuronosil transferase, the use of nanomaterial helps increase luteolin absorption [3, 4, 11, 12]. Thus it can be suggested that phytosome formulation protects luteolin from glucuronidation so that it can be found in higher level of plasma concentration. These data make a clue for supporting phytosome as a promising delivery system for the enhancement of oral administration of a poorly lipid-soluble drug.

![Table 1: Physicochemical properties of luteolin-loaded phytosome (LLP)](image)

![Fig 1: Morphology of LLPs](image)

![Fig 2: Luteolin plasma concentration after oral administration](image)
Table 2: Pharmacokinetic parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Luteolin Phytosome</th>
<th>Free Luteolin (Control)</th>
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<tr>
<td>Tmax (minutes)</td>
<td>20</td>
<td>60</td>
</tr>
<tr>
<td>Cmax (µg/mL)</td>
<td>300.831</td>
<td>25.918</td>
</tr>
<tr>
<td>AUC (µg.min/mL)</td>
<td>5426</td>
<td>1480.309</td>
</tr>
</tbody>
</table>

However, as shown in Figure 2, orally administered of luteolin-loaded phytosome was rapidly absorbed as well as rapidly eliminated. Luteolin concentration in plasma is decreased significantly to 62.39 µg/mL in 30 min after administration. This showed that phytosome formulation has short time duration of action. This phenomenon is corresponds with the negatively charged of LLPs as can be seen in Table 1. Previous studies stated that negatively charged nanoemulsion will rapidly eliminated and have higher reticuloendothelial uptake than those with neutral or positively charged.

Conclusion

A novel work in this study is to synthesize LLPs by thin film hydration methods. Round-shaped LLPs were confirmed with average particle size 105.3 nm, PDI 0.735, zeta hydration methods. Round-shaped LLPs were rapidly absorbed and significantly increased luteolin plasma concentration and bioavailability after oral administration (Cmax = 300.831 µg/mL, Tmax 20 minutes, and AUC 5426 µg.min/mL), hence it can serve as a promising delivery system for drugs with low lipids solubility.

Acknowledgements

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References