Nanoemulsion improves the oral bioavailability of baicalin in rats: in vitro and in vivo evaluation

Abstract: Baicalin is one of the main bioactive flavone glucuronides derived as a medicinal herb from the dried roots of Scutellaria baicalensis Georgi, and it is widely used for the treatment of fever, inflammation, and other conditions. Due to baicalin’s poor solubility in water, its absolute bioavailability after oral administration is only 2.2%. The objective of this study was to develop a novel baicalin-loaded nanoemulsion to improve the oral bioavailability of baicalin. Based on the result of pseudoternary phase diagram, the nanoemulsion formulation consisting of soy-lecithin, tween-80, polyethylene glycol 400, isopropyl myristate, and water (1:2.1:5:3.75:8.25, w/w) was selected for further study. Baicalin-loaded nanoemulsions (BAN-1 and BAN-2) were prepared by internal or external drug addition and in vivo and in vitro evaluations were performed. The results showed that the mean droplet size, polydispersity index, and drug content of BAN-1 and BAN-2 were 91.2 ± 2.36 nm and 89.7 ± 3.05 nm, 0.313 ± 0.002 and 0.265 ± 0.001, and 98.56% ± 0.79% and 99.40% ± 0.51%, respectively. Transmission electron microscopy revealed spherical globules and confirmed droplet size analysis. After dilution 30-fold with water, the solubilization capacity of BAN-1 and BAN-2 did not change. In vitro release results showed sustained-release characteristics. BAN-1 formulation was stable for at least 6 months and was more stable than BAN-2. In rats, the area under the plasma drug concentration-time curve value of BAN-1 was 1.8-fold and 7-fold greater than those of BAN-2 and free baicalin suspension after oral administration at a dose of 100 mg/kg. In conclusion, these results demonstrated that the baicalin-loaded nanoemulsion formulation, in particular BAN-1, was very effective for improving the oral bioavailability of baicalin and exhibited great potential for future clinical application.

Keywords: nanoemulsion, baicalin, oral bioavailability, pharmacokinetics

Introduction

Baicalin (7-glucuronic acid 5, 6-dihydroxyflavone) is one of the main single active constituents isolated from the dried roots of Scutellaria baicalensis Georgi as a medicinal herb; its aglycone form is called baicalein (Figure 1). Current studies have shown various strong pharmacological properties of baicalin,1–5 such as antioxidative, antiviral, anti-inflammatory, antitumor, antiradical, antiproliferative, cardioprotective, and so on. However, it can be observed from the molecular structure of the currently marketed preparation of baicalin (Figure 1), including capsules and tablets, that both flavones and glucuronide can form intramolecular hydrogen bonds that result in poor solubility in water, which causes low oral bioavailability.6 In previous studies, baicalin was found to be moderately absorbed in the stomach and poorly absorbed in the small intestine and colon; in contrast, its aglycone moiety (baicalein; Figure 1)
Figure 1. Chemical structures of baicalein and baicalin.

was well absorbed in the gastrointestinal tract and then restored to baicalin in the body.4,7,8 After oral administration of baicalin and baicalein in rats, the absolute bioavailability was 2.2% and 27.8%, respectively.9 It was concluded that the oral bioavailability of baicalin was greatly improved after administration of baicalein. However, the baicalein content in Scutellaria baicalensis Georgi is very low; it is irrational to obtain baicalin by the extracting and purging process. In addition, baicalin is transformed to baicalein by enzyme hydrolysis and strong acid hydrolysis, which is costly and produces environmental pollution. Therefore, it is necessary to develop new drug dosages and new techniques for increasing baicalin’s solubility and dissolution rate and improving its oral bioavailability.

Nanoemulsions composed of oil, water, surfactant, and cosurfactant are thermodynamically stable and isotropically clear dispersion systems that are stabilized by an interfacial film of surfactant and cosurfactant.10 Nanoemulsions exhibit unique advantages and properties, such as reduced droplet size with larger surface area, improved drug solubility and dissolution rates, and enhanced drug absorption from the gastrointestinal tract by surfactant-induced mucosal permeability changes.11–19 Nanoemulsion formulations have been widely used to overcome problems related to the poor water solubility and low oral bioavailability of some drugs.20–26 Therefore, amongst the various drug delivery systems, nanoemulsions are considered an ideal alternative for the oral administration of drugs with poor water solubility, as they improve absorption and bioavailability. At present, the US Food and Drug Administration has approved nanoemulsions of compounds with poor water solubility, including cyclosporin (Neoral®), Gengraf®, saquinavir (Fortovase®), and ritonavir (Norvir®), for clinical use. However, to our knowledge, no related studies have demonstrated that nanoemulsion could improve the oral bioavailability of baicalin.

In the present study, a novel nanoemulsion formulation was developed to enhance oral absorption and bioavailability of baicalin. The optimized nanoemulsion formulation was obtained according to the results of ternary phase diagram experiment. Afterward, both baicalin-loaded nanoemulsions, BAN-1 and BAN-2, were prepared by internal and external addition of the drug, respectively. The physicochemical properties and pharmacokinetics were investigated in comparison with a free baicalin suspension (baicalin control), which was baicalin suspended in 0.5% sodium carboxymethyl cellulose solution.

Materials and methods

Materials

Baicalin (>98.0%) was purchased from Mianyang Dongfangyuan Bio-Technology Co, Ltd (Sichuan, People’s Republic of China). Rutin (internal standard) was obtained from Chengdu Mansite Pharmaceutical Co, Ltd (Sichuan, People’s Republic of China). Isopropyl myristate (IPM), glyceryl monoleate, isopropyl palmitate (IPP), soy-lecithin, tween-80, polyethylene glycol 400 (PEG400), propylene glycol, and ethanol were obtained from Luzhou Juhe Chemical Co, Ltd (Sichuan, People’s Republic of China). All other chemicals and reagents used in this study were of analytical or high-performance liquid chromatography (HPLC) grade.

Animals

Male Sprague Dawley rats weighing between 150–180 g were obtained from the Laboratory Animal Center of Luzhou Medical College (Sichuan, People’s Republic of China). All animal experimental procedures were approved by the Luzhou Medical College Animal Ethical Experimentation Committee. The rats were housed at a temperature of 20°C ± 2°C, a relative humidity of 50%–60%, and 12-hour light–dark cycles. Free access to food and water was allowed. All the animals were fasted overnight with free access to water prior to the experiment.

Solubility study

The saturation solubility of baicalin in oil phases, surfactant, and cosurfactant was investigated in this study. Briefly, an excess amount of baicalin was added into sealed tubes of each vehicle described above and then was carried out by nitrogen-filled protection followed by vortex mixing.
Preparation of baicalin nanoemulsions

According to the results of solubility studies and the nanoemulsion regions in the pseudoternary phase diagram, in combination with safety, the nanoemulsion formulation composed of soy-lecithin, tween-80, PEG400, IPM, and water (1:2:1:1:1:3.75:8.25, w/w) was selected and developed into baicalin-loaded nanoemulsions. Baicalin was first dissolved in PEG400 and mixed with soy-lecithin, tween-80, and IPM, after which the required amount of water was added and the solution stirred to obtain a clear and transparent liquid, i.e., baicalin-loaded nanoemulsion (BAN-1). The resulting nanoemulsions were filled with nitrogen gas and tightly sealed and stored at room temperature.

In addition, baicalin was dissolved in the final nanoemulsion formulations to obtain baicalin-loaded nanoemulsions (BAN-2).

Characteristics of baicalin-loaded nanoemulsions

Transmission electron microscopy

The morphologies of the baicalin-loaded nanoemulsions were examined by transmission electron microscopy (TEM) (JEM-1400; JEOL Ltd, Tokyo, Japan). Prior to the analysis, the sample was diluted in distilled water (1:50) and a sample drop was placed on a copper grid. After the samples were dried, they were stained with saturated solution of uranyl acetate and investigated.

Droplet size measurement

The droplet size and polydispersity index (PDI) of the nanoemulsions were measured by Malvern ZEN3600 (Malvern Instruments Ltd, Worcestershire, UK). The sample was prepared by diluting the baicalin-loaded nanoemulsions with distilled water (50-fold, v/v).

Changes in baicalin solubility in nanoemulsions after dilution

To investigate the solubilization capacity of baicalin in the diluted nanoemulsions, BAN-1 and BAN-2 containing 7.5 mg/mL baicalin were diluted 30 times with normal saline at 37°C. The samples (100 µL) were withdrawn at various time points over 24 hours and centrifuged at 10,000 rpm for 10 minutes to remove the precipitated baicalin, if any. The concentration of baicalin in nanoemulsions was determined by using HPLC, as described above, after appropriate dilution with methanol.

In vitro release studies

The in vitro release of baicalin from nanoemulsions compared with drug suspensions was studied using a dialysis bag method (molecular weight cut-off [MWCO] 8,000–14,000) in 400 mL of artificial gastric juice (0.1 M HCl, pH 1.1) and phosphate-buffered saline (PBS, pH 6.8) as a release medium. Three mL of freshly prepared BAN-1, BAN-2, and free baicalin suspension as control (baicalin suspended in 0.5% sodium carboxymethyl cellulose solution) containing baicalin 7.5 mg/mL was placed into the dialysis bags and tightly sealed. The test bags were soaked in release medium at a stirring rate of 100 rpm at 37°C ± 0.5°C. At predetermined intervals, 1 mL of the release medium was withdrawn and replaced with an equal volume of fresh medium. The samples were analyzed by HPLC using a spectrophotometric detector with a 278 nm detection wavelength.
time points, 0.2 mL of sample solution was taken; meanwhile, the same volume of fresh release medium at 37°C ± 0.5°C was added to maintain the same volume. The sample solution was centrifuged at 10,000 rpm for 10 minutes and the supernatant liquid was measured by using HPLC, as described in the “Solubility study” section.

Stability studies
In accordance with the Technical Standard of Drug Stability Test (Chinese Pharmacopoeia 2010, appendix XIX C), stability studies were carried out for baicalin-loaded nanoemulsions (BAN-1 and BAN-2). For the accelerate stability test, samples were filled in amber-colored containers with nitrogen gas protection and stored at 40°C ± 2°C, RH 75% ± 5% for 6 months. Samples were withdrawn at time intervals of 1, 2, 3, and 6 months. After that, the samples were centrifuged at 10,000 rpm for 10 minutes to remove the precipitated baicalin, if any. The baicalin content in the supernatant liquid was determined by using HPLC, as described above. Changes in appearance from centrifugation and drug content were chosen as markers for stability evaluation in this study.

In vivo pharmacokinetic evaluations
Animal experiment
The rats used in this study were randomly divided into three main groups (BAN-1, BAN-2, and free baicalin suspension groups, n = 10 per group). Rats fasted overnight prior to the experiment, with free access to water. BAN-1 (7.5 mg/mL), BAN-2 (7.5 mg/mL), and free baicalin suspension (7.5 mg/mL) were administered to rats by oral gavage at a dose equivalent to 100 mg/kg of baicalin. Blood samples (0.25 mL each) were collected at 5 minutes, 15 minutes, 30 minutes, 60 minutes, 120 minutes, 150 minutes, 180 minutes, 300 minutes, 480 minutes, 720 minutes, and 1,440 minutes after oral administration. Plasma samples were separated immediately by centrifugation at 4,000 rpm for 5 minutes and stored at −20°C for further analysis.

Sample extraction
Baicalin was extracted from the plasma samples by a liquid–liquid extraction method. Briefly, 50 μL of rutin solution (internal standard) and 600 μL of methanol were added to 100 μL of rat plasma samples in turn, vortexed for 3 minutes, and then treated by sonication at room temperature for 10 minutes. After centrifugation at 8,000 rpm for 5 minutes, the supernatant was collected and evaporated to dryness at 40°C under nitrogen. The residue was reconstituted with 200 μL of mobile phase and centrifuged at 12,000 rpm for 5 minutes, after which 20 μL of the clear supernatant was injected into the HPLC system for analysis.

Data analysis
According to the data of plasma drug concentration-time, we calculated the main pharmacokinetic parameters, including the area under the plasma drug concentration-time curve (AUC0–t), the time to reach the maximum plasma drug concentration (tmax), the maximum plasma drug concentration (Cmax), the elimination half-life (t1/2), and the mean residence time (MRT), by noncompartmental modeling using a software program, DAS 2.0 (Mathematical Pharmacology Professional Committee of China, People’s Republic of China).

The results were represented as mean ± standard deviation, and differences between the pharmacokinetic data of BAN-1, BAN-2 and free baicalin suspension as control were evaluated using a two-tailed t-test. Statistical significance was set at P < 0.05.

Results and discussion
Solubility studies
For nanoemulsion formulations, oil phase is an important ingredient that can solubilize lipophilic drugs and enhance the amount of lipophilic drug transported through the intestinal lymphatic system. Therefore, the solubility of drug in oil phase is crucial for the development of nanoemulsion formulations. The solubilities of baicalin in oil phase, including glyceryl monooleate, IPM, and IPP, were investigated (Figure 2). Among these oil phases, the highest solubilization capacity was observed in IPM (0.0138 ± 0.0036 mg/mL), followed by glyceryl monooleate (0.0054 ± 0.0009 mg/mL) and IPP.
was expressed. In addition, baicalin was dissolved in the final nanoemulsion formulations to obtain baicalin-loaded nanoemulsions (BAN-2). Nanoemulsions with a fixed concentration of 7.5 mg/mL of baicalin were prepared and used in the following studies.

Characteristics of nanoemulsions

The droplet size of nanoemulsions is an important factor because it influences drug-release behavior and stability. The PDI ranged from 0.0 to 1.0, representing the uniformity of droplet size. The closer to zero the PDI value, the more homogeneous the droplets are. In the current study, droplet size, PDI, and drug content of baicalin-loaded nanoemulsions are shown in Table 1. The results showed that the mean droplet size of BAN-1 and BAN-2 measured by Malvern ZEN3600 was less than 100 nm with a maximum PDI of 0.313, which demonstrated that the particle size distribution of baicalin-loaded nanoemulsions was homogeneous. The drug content of BAN-1 and BAN-2 was 98.56% ± 0.79% and 99.40% ± 0.51%, respectively, which meets the requirement of Chinese Pharmacopoeia (2010 edition, part II).

To examine morphology and confirm droplet size analysis, TEM was used to investigate the baicalin-loaded nanoemulsions in this study. TEM images of BAN-1 and BAN-2 are shown in Figure 4 and show that spherical nanoemulsion globules had a diameter of less than 100 nm, in accordance with the results obtained by Malvern ZEN3600.

In general, when nanoemulsions containing poor water-soluble drug are diluted with water in the gastrointestinal tract after oral administration, they can result in drug precipitation. Therefore, it is necessary to investigate solubilization capacity via dilution with water (Figure 5). Figure 5 indicates that the baicalin-loaded nanoemulsions showed satisfactory solubilization capacity for at least 24 hours, suggesting that both BAN-1 and BAN-2 would not cause drug precipitation upon contact with the water solution in the gastrointestinal tract.

In vitro release studies

To simulate drug-release behaviors in the gastrointestinal tract, the in vitro release of baicalin-loaded nanoemulsions (BAN-1 and BAN-2) was performed in 0.1 M HCl (pH 1.1) and PBS (pH 6.8) as release medium (Figure 6). According to the in vitro release profiles of BAN-1 and BAN-2, the accumulative release amount of baicalin from the nanoemulsions in PBS was much higher than it was in 0.1 M HCl, which could be attributed to the higher solubility of baicalin at high pH values. A similar release behavior was observed.
Table 1 Droplet size, polydispersity index and drug content of baicalin-loaded nanoemulsions

<table>
<thead>
<tr>
<th>Samples</th>
<th>Droplet size (nm)</th>
<th>Polydispersity index</th>
<th>Drug content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAN-1</td>
<td>91.2 ± 2.36</td>
<td>0.313 ± 0.002</td>
<td>98.56 ± 0.79</td>
</tr>
<tr>
<td>BAN-2</td>
<td>89.7 ± 3.05</td>
<td>0.265 ± 0.001</td>
<td>99.40 ± 0.51</td>
</tr>
</tbody>
</table>

Note: Data are expressed as mean ± standard deviation (n = 3).

Abbreviations: BAN-1, baicalin-loaded nanoemulsion created by dissolution of baicalin in PEG400 and mixing with soy-lecithin, tween-80, IPM, and water; BAN-2, baicalin-loaded nanoemulsion created by dissolution of baicalin in the final nanoemulsion formulations.

For free baicalin suspension. However, in both release mediums, the release of baicalin from nanoemulsions was much higher than that from the free baicalin suspension, which could result from the solubilizing effect of nanoemulsions. The accumulative release of BAN-1 and BAN-2 in PBS was 19.302% and 16.940%, respectively, within 48 hours, while that in 0.1 M HCl was 4.666% and 5.152%, respectively. These results may be attributed to the presence of a diffusion membrane composed of oil phase and oil–water interface.
Nanoemulsion improves oral bioavailability of baicalin in rats

Figure 4 Transmission electron microscopy of baicalin-loaded nanoemulsions with 50-fold dilution in distilled water.
Notes: (A) BAN-1, (B) BAN-2.
Abbreviations: BAN-1, baicalin-loaded nanoemulsion created by dissolution of baicalin in PEG400 and mixing with soy-lecithin, tween-80, IPM, and water; BAN-2, baicalin-loaded nanoemulsion created by dissolution of baicalin in the final nanoemulsion formulations.

Figure 5 Change of baicalin solubility in nanoemulsions after 30 times dilution with normal saline at 37°C.
Note: Data are expressed as mean ± standard deviation (n = 3).
Abbreviations: BAN-1, baicalin-loaded nanoemulsion created by dissolution of baicalin in PEG400 and mixing with soy-lecithin, tween-80, IPM, and water; BAN-2, baicalin-loaded nanoemulsion created by dissolution of baicalin in the final nanoemulsion formulations.

Figure 6 In vitro release profile of BAN-1 and BAN-2 and free baicalin suspension in 0.1 M HCl (pH 1.1) and PBS (pH 6.8) as release medium.
Note: Data are expressed as mean ± standard deviation (n = 3).
Abbreviations: BAN-1, baicalin-loaded nanoemulsion created by dissolution of baicalin in PEG400 and mixing with soy-lecithin, tween-80, IPM, and water; BAN-2, baicalin-loaded nanoemulsion created by dissolution of baicalin in the final nanoemulsion formulations; PBS, phosphate-buffered saline.

Stability studies
Stability studies were performed because stability is a crucial marker for quality evaluation of new drug dosage forms (Table 2). For the BAN-1 formulation, the results showed no significant difference (P > 0.05) in baicalin content and no appearance changes after 6 months, compared with initial samples at 0 months. For BAN-2, a significant difference (P < 0.05) in baicalin content and some precipitation was observed after 3 months. Therefore, it is suggested that BAN-1 formulation is stable for at least 6 months – more stable than BAN-2.

Pharmacokinetic behavior
To investigate whether a nanoemulsion carrier system could increase the oral bioavailability of baicalin, the plasma drug concentration in rats was determined by HPLC to evaluate the pharmacokinetic behavior of BAN-1 and BAN-2 in comparison with free baicalin suspension after oral administration at a dose of 100 mg/kg. In this study, the reverse-phase HPLC method was developed and validated for the determination of baicalin in rat plasma. The analysis method had good specificity (Figure 7). Good linearity was obtained between 0.1 µg/mL and 12.5 µg/mL for plasma (r > 0.999).
Table 2 Results of the accelerate stability test: difference in baicalin content over time compared with 0 months

<table>
<thead>
<tr>
<th>Items</th>
<th>Time (months)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAN-1 (%)</td>
<td>98.56 ± 0.79</td>
<td>98.15 ± 0.66</td>
<td>98.95 ± 0.84</td>
<td>97.76 ± 0.65</td>
<td>97.25 ± 0.38*</td>
<td></td>
</tr>
<tr>
<td>BAN-2 (%)</td>
<td>99.40 ± 0.51</td>
<td>96.24 ± 0.86*</td>
<td>95.31 ± 0.57*</td>
<td>90.19 ± 0.47†</td>
<td>85.23 ± 0.58</td>
<td></td>
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</tbody>
</table>

Note: Data are expressed as mean ± standard deviation (n = 3). *Not significant compared with 0 months, P > 0.05. †Significant compared with 0 months, P < 0.05.

Abbreviations: BAN-1, baicalin-loaded nanoemulsion created by dissolution of baicalin in Peg400 and mixing with soy-lecithin, tween-80, IPM, and water; BAN-2, baicalin-loaded nanoemulsion created by dissolution of baicalin in the final nanoemulsion formulations.
The intraday and interday assay of precision and accuracy for plasma samples ranged from 1.2% to 4.2% and from 92.5% to 95.2%, respectively. The extraction recoveries ranged from 80.2% to 81.7%.

The plasma drug concentration-time curve is shown in Figure 8, and the main pharmacokinetic parameters are summarized in Table 3. A significant difference between the pharmacokinetic behavior of baicalin-loaded nanoemulsion and free baicalin suspension was observed (Figure 8). At each time point, the plasma drug concentration of BAN-1 and BAN-2 was much higher than that of free baicalin suspension. The peak concentration ($C_{\text{max}}$) of baicalin from BAN-1 and BAN-2 was $3.155 \pm 0.132$ mg/L and $4.625 \pm 0.203$ mg/L, respectively, which was about 3–4 times that of free baicalin suspension ($1.143 \pm 0.105$ mg/L) – a significant increase ($P < 0.05$). The AUC$_{(0-\infty)}$ value of baicalin in rats treated with BAN-1 or BAN-2 was $98.439 \pm 4.579$ mg/L*h and $54.443 \pm 3.879$ mg/L*h, respectively, which was improved more than 7 and 4 times than that of free baicalin suspension ($13.681 \pm 1.092$ mg/L*h) ($P < 0.05$). In addition, the $\text{MRT}_{(0-\infty)}$ and $t_{1/2}$ value of BAN-1 was about 3.5-fold greater than that of BAN-2 and the reference preparation. It was reported that the oral bioavailability of baicalin was enhanced approximately 2.6 times by solid lipid nanoparticles. However, in the present study, unexpected results demonstrated that the baicalin-loaded nanoemulsion BAN-1 was much more effective than nanoparticle, which could be attributed to enhanced permeability induced by surfactant and cosurfactant, uptake of BAN-1 in gastrointestinal tract, and the sustained-release of drug from BAN-1. In this study, the AUC$_{(0-\infty)}$ value of

![Figure 7](https://www.dovepress.com/)

**Figure 7** Representative HPLC chromatograms of baicalin and rutin in rat plasma determined by HPLC method.

**Notes:** (A) Blank plasma. (B) Blank plasma spiked with baicalin and rutin (internal standard). (C) Plasma samples collected 60 minutes after oral administration of baicalin-loaded nanoemulsions.

**Abbreviations:** HPLC, high-performance liquid chromatography; WVL, wavelength.

![Figure 8](https://www.dovepress.com/)

**Figure 8** Plasma concentration-time profiles of baicalin-loaded nanoemulsions in rats after oral administration of BAN-1, BAN-2, and BA control.

**Note:** Data are expressed as mean ± standard deviation (n = 3).

**Abbreviations:** BAN-1, baicalin-loaded nanoemulsion created by dissolution of baicalin in PEG400 and mixing with soy-lecithin, tween-80, IPM, and water; BAN-2, baicalin-loaded nanoemulsion created by dissolution of baicalin in the final nanoemulsion formulations; BA control, free baicalin suspension (baicalin suspended in 0.5% sodium carboxymethyl cellulose solution).
BAN-1 was 1.8-fold higher than that of BAN-2, which could have resulted from the longer $\text{MRT}_{(0\rightarrow\infty)}$ and $t_{1/2}$ values, in combination with being more stable.

**Conclusion**

A novel baicalin-loaded nanoemulsion formulation consisting of soy-lecithin, tween-80, PEG400, IPM, and water (1:2.1:5.3:75:8.25, w/w) was developed to improve the oral bioavailability of baicalin. Baicalin-loaded nanoemulsions (BAN-1 and BAN-2) were prepared by internal and external drug addition. No significant differences between BAN-1 and BAN-2 in droplet size, PDI, drug content, in vitro release behavior, and solubilization capacity via dilution with water were observed. However, BAN-1 is stable for at least 6 months, more stable than BAN-2. The $\text{AUC}_{(0\rightarrow\infty)}$ value of BAN-1 was 1.8-fold and 7.0-fold that of BAN-1 and free baicalin suspension after oral administration at a dose of 100 mg/kg in rats. To sum up, these results demonstrated that the baicalin-loaded nanoemulsion formulation, in particular BAN-1, was effective for improving the oral bioavailability of baicalin and exhibited great potential for future clinical application.

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**Disclosure**

The authors report no conflicts of interest in this work. The authors alone are responsible for the content and writing of the paper.

**References**


