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In vitro and in vivo Characterization of New Formulations of St. John’s Wort Extract with Improved Pharmacokinetics and Anti-nociceptive Effect

Junya HATANAKA1,2, Yukiko SHINME1, Kazuki KURIYAMA1, Atsushi UCHIDA1, Keitatsu KOU3, Shinya UCHIDA1, Shizuo YAMADA1 and Satomi ONOUE1,*

1Department of Pharmacokinetics and Pharmacodynamics and Global Center of Excellence (COE) Program, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan
2Functional Foods Laboratory, Yokohama Oils and Fats Industry Co., Ltd., Yokohama, Japan
3Laboratory of Electron Microscopy, Showa University School of Medicine, Tokyo, Japan

Summary: The main purpose of the present study was to develop a novel formulation of St. John’s Wort (SJW) extract with the aim of improving its pharmacokinetics and anti-nociceptive effect. Several formulations of SJW were prepared, including cyclodextrin inclusion (SJW-CD), solid dispersion (SJW-SD), dry-emulsion (SJW-DE), and nano-emulsion (SJW-NE). Physicochemical properties of SJW formulations were characterized with a focus on the morphology, dissolution behavior, colloidal properties, and dispersion stability in water. Although all the SJW formulations and SJW extract itself exhibited fine dissolution behavior in water, SJW extract and most formulations tended to cream, aggregate, or flocculate after dispersion in distilled water. In contrast, there were no significant changes in appearance and particle size of the SJW-NE for at least a few weeks, suggesting that SJW-NE was the most stable form as a carrier of SJW in the present study. After oral administration of the SJW-NE formulation (5.2 mg hyperforin/kg) in mice, higher hyperforin exposure in plasma (1188±41 nM·h) and the brain (52.9±1.6 pmol/g tissue·h) was observed with 2.8- and 1.3-fold increases of the area under the concentration curve from 0 to 6 hours (AUC0-6) compared to those of the SJW extract (417±41 nM·h in plasma and 41.6±1.5 pmol/g tissue·h in the brain). In the formalin test for scoring properties of the first and second phases of the pain response in mice, single oral administration of SJW-NE significantly reduced the nociceptive response compared with SJW extract. From these findings, the NE approach might be efficacious in improving the oral bioavailability and anti-nociceptive effect of SJW extract.

Keywords: St. John’s Wort; nano-emulsion; absorption; anti-nociceptive effect; formalin test

Introduction

Hypericum perforatum L., alternatively known as St. John’s Wort (SJW), is a well-known medical plant and is included in the monograph of the German Commission E and numerous pharmacopoeias for anti-depressive and antiviral human application.1) Alcoholic extracts of SJW, having a favorable side-effect profile, are mainly used for the treatment of mild-to-moderate forms of depression as an alternative to classic anti-depressants.2) In addition to the anti-depression effect, anti-inflammatory and anti-nociceptive effects of SJW extract have also been reported.3-5) The potential of SJW extract as an alternative with favorable side effects to non-steroidal anti-inflammatory drugs (NSAIDs) for arthritis has been reported.6) Recently, studies have been carried out to identify components responsible for the anti-nociceptive activity of SJW.7,8) In these studies, the main mechanisms of SJW extract in anti-nociception were presumed to be inhibition of protein kinase C (PKC) phosphorylation and involvement in the opioid-dependent pathway of hypericin and hyperforin. Thus, daily intake of SJW extract seems to be promising for chronic pain

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*To whom correspondence should be addressed: Satomi ONOUE, Department of Pharmacokinetics and Pharmacodynamics and Global Center of Excellence (COE) Program, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan. Tel. +81-54-264-5633, Fax. +81-54-264-5635, E-mail: onoue@u-shizuoka-ken.ac.jp

Although SJW extract has attracted attention because of its pharmacological potential, its active components might be difficult to apply for pain treatment. Leaves and flowers of this plant contain flavonoidic compounds, anthraquinone constituents containing hypercin, and acylchloroglucinol substances containing hyperforin.1 Issachi and coworker showed that a commercial sample of dried extract contained 12.7% flavonoids, 4.2% hyperforin, and 0.3% hypercin, and they also reported the solubility of components in SJW extract: 353 µg/mL for flavonoids, 6.9 µg/mL for I3,II8-biapigenin, <0.1 µg/mL for hyperforin, and 10.4 µg/mL for hypercin.10 These physicochemical properties might be the cause of the unsuitable dispersion stability in water, possibly leading to low bioavailability issues. Recently, various solubilization techniques such as emulsification, cyclodextrin inclusion, and solid dispersion have been applied to overcome these difficulties for plant extracts such as silymarin,11 quercetin,12 puerarin,13 and curcumin.14,15 A limited number of trials have also been attempted regarding the pharmacokinetics and anti-depressant effects of SJW extract using pharmaceutical technology.10,16 However, the impact of formulation design on the physicochemical properties, pharmacokinetics, and anti-nociceptive effect of SJW extract has not been elucidated.

The main purpose of the present study was to develop a novel formulation of SJW extract with the aim of improving pharmacokinetics and pharmacodynamics. In the present study, four new formulations of SJW extract were prepared using various solubilization techniques (Table 1). The physicochemical properties of the novel formulations were characterized with a focus on morphology, dissolution behavior, colloidal properties based on particle size and zeta-potential, and dispersion stability in water. The pharmacokinetic behavior of hyperforin after oral administration of SJW samples in mice was determined for comparison. In addition, the anti-nociceptive effects of SJW extract and the novel formulation were evaluated after single oral administration.

### Materials and Methods

**Chemicals:** St. John’s Wort extract (SJW), Hypericum perforatum dry extract 0.3%ET, and hyperforin dicyclohexylammonium (DCHA), was kindly supplied by Indena (Milan, Italy). According to the specification, Hypericum perforatum dry extract 0.3%ET is extracted with ethanol and contains more than 0.3% hypercin and 3.2% hyperforin. Medium chain triglyceride (MCT) was purchased from Kao Corporation (Tokyo, Japan), decaglyceryl monooleate was from Taiyo Kagaku Co., Ltd. (Osaka, Japan), soybean lecithin was from Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan), hydroxypropylcellulose (HPC, HPC-SSL grade) was from Nippon Soda Co., Ltd. (Tokyo, Japan), γ-cyclodextrin was from Cyclochem Co., Ltd. (Kobe, Japan), glycerol was from Sakamoto Yakuhin Kogyo Co., Ltd. (Osaka, Japan), formalin was from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and other reagents used in the study were commercial products of analytical grade.

**Preparation:**

**Cyclodextrin inclusion (SJW-CD)**

One gram of SJW extract was dissolved in 100 mL ethanol, and 19 g γ-CD was added to the SJW alcoholic solution. The mixture was dried using a rotary evaporator at 40°C under reduced pressure.

**Solid dispersion (SJW-SD)**

One gram of SJW extract was dissolved in 25 mL ethanol, and 19 g HPC-SSL was dissolved in 75 mL deionized water separately. The SJW alcoholic solution was added to HPC-SSL aqueous solution with stirring, and the mixture was dried using a rotary atomizer spray-dryer (L-8i type spray-dryer; Ohkawara Kakuhki, Kanagawa, Japan) under the following conditions: inlet temperature, 150°C; outlet temperature, 80°C; rotation speed of atomizer disc, 33,000 revolutions per minute (rpm); and feeding rate of the emulsion mixture, 30 mL/min.

**Nano-emulsion (SJW-NE)**

The NE formulation was prepared using a mecha-chemical method as reported previously.17 Briefly, the lipid phase, composed of SJW and MCT, and the aqueous phase, composed of glycerol, deionized water, and emulsifying agents such as decaglycerol monooleate and soybean lecithin, were heated to 80°C, separately. The lipid phase was added to the aqueous phase using a T.K. Robomix (PRIMIX Corporation, Osaka, Japan) at 9,000 rpm for 15 min.

**Dry-emulsion (SJW-DE)**

Decaglycerol monooleate, soybean lecithin, and HPC-SSL were dissolved in deionized water at 80°C. MCT and SJW

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### Table 1. Composition of SJW extract-loaded formulations

<table>
<thead>
<tr>
<th>Formulations</th>
<th>SJW extract</th>
<th>γ-CD</th>
<th>HPC-SSL</th>
<th>MCT</th>
<th>Decaglycerol monooleate</th>
<th>Lecithin</th>
<th>Glycerol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>SJW-CD</td>
<td>5</td>
<td>95</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>SJW-SD</td>
<td>5</td>
<td>—</td>
<td>95</td>
<td>15</td>
<td>12</td>
<td>3</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>SJW-NE</td>
<td>5</td>
<td>—</td>
<td>—</td>
<td>15</td>
<td>12</td>
<td>3</td>
<td>50</td>
<td>15</td>
</tr>
</tbody>
</table>

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extract were added to the aqueous phase and processed using a T.K. Robomix at 9,000 rpm for 15 min. The obtained emulsion was dried using a spray-dryer as described for SJW-SD above.

*Scanning electron microscopy (SEM)*

The surface observation of powder formulations was carried out using a scanning electron microscope (SEM, TOPCON SM350, Topcon, Tokyo, Japan). Powder samples were manually dispersed on an aluminum stub with a thin self-adhered carbon film. The samples were made electrically conductive by coating in a vacuum (13 Pa) with gold (5 nm/min) using an ion coater (IB-3 ion coater, Eiko, Tokyo, Japan) for 120 s at 6 mA. SEM images were taken at an excitation voltage of 15 kV.

*Dynamic light scattering (DLS) analysis*

The particle size and zeta-potential of droplets in water were determined with an ELS-Z analyzer (measurement range 0.6–7,000 nm; Otsuka Electronics, Osaka, Japan) by the dynamic light scattering method. Prior to measurement, 0.5 g of each formulation was diluted with 50 mL distilled water and dispersed homogeneously. All measurements were performed at 25 °C at a measurement angle of 160°. Particle size distribution, which was calculated by histogram analysis of scattering intensity, was evaluated as cumulative values at 10% (d10), 50% (d50), and 90% (d90), representing the 10th, 50th, and 90th percentiles of the particle size distribution. The zeta-potential was calculated using the Smoluchowski equation from electrophoresis mobility and electric field strength.

*Laser diffraction (LD) analysis*

Particle size based on LD analysis was determined using a SALD-2100 analyzer (measurement range 0.03–1,000 µm; Shimadzu, Kyoto, Japan) equipped with a flow cell unit. Deionized water was used as the dispersant. Maximal particle size was calculated by histogram analysis of volume distribution at a cumulative value of 99% (d99), representing the 99th percentile of the particle size distribution.

*Transmission electron microscopy (TEM)*

Each sample was diluted with deionized water and placed on a carbon-coated Formvar 200 mesh nickel grid. The sample was allowed to stand for 15–30 s, and then any excess solution was removed by blotting. The samples were negatively stained with 2% (w/v) uranyl acetate and allowed to dry. They were then visualized under an H-7600 transmission electron microscope (Hitachi, Japan) operating at 75 kV.

*Dissolution profile*

Dissolution tests were carried out for 30 min in 100 mL distilled water with constant stirring of 200 rpm in a beaker at 37 °C. Ten milligrams of the SJW extract or 200 mg of each SJW formulation was weighed in a beaker. Five-hundred-microliter samples were collected at the indicated periods (2.5, 5, 10, 15, 20, and 30 min) and centrifuged at 15,000 g to remove insoluble materials. For determination of the dissolved SJW in the supernatant, 350 µL methanol was added to 350 µL supernatant, and absorbance at 350 nm of each supernatant was measured using a Safire microplate reader (Tecan, Männedorf, Switzerland).

*Dispersion stability*

In order to estimate dispersion stability in water, SJW extract and formulations corresponding to 150 mg SJW extract were dispersed in 50 mL deionized water. Ease of dispersal and the temporal appearance change over a period of 1 month in terms of creaming and precipitation were noted.

*Animals and drug administration*

Male ICR mice (Japan SLC Inc., Shizuoka, Japan) weighing 25 ± 5 g were housed two per cage in the laboratory with free access to food and water; the mice were maintained on a 12-h dark/light cycle in a room with controlled temperature (24 ± 1 °C) and humidity (55 ± 5%). All procedures used in the present study were conducted according to the guidelines approved by the Institutional Animal Care and Ethics Committee of the University of Shizuoka. Prior to administration, each sample was dispersed in distilled water. Mice received SJW extract or SJW-NE (5.2 mg hyperforin/kg body weight) orally. Blood and brain were collected at 0.5, 1, 3, and 6 h after oral administration. For estimation of oral bioavailability, other mice were given SJW extract intravenously and their blood was collected at 5, 10, 20, 30, and 60 min. Blood samples were centrifuged at 10,000 g for 10 min at 4 °C to prepare plasma samples, which were kept below −80 °C until analysis.

*Plasma and brain concentrations of hyperforin*

Hyperforin concentrations were determined by the internal standard method using HPLC-ECD (HPLC, high-performance liquid chromatography; ECD, electron chemical detector). Briefly, 300 µL acetonitrile, 50 µL internal standard (20 µg/mL retinyl acetate), and 200 µL of 10 mM KH₂PO₄ buffer (pH 3.0) were added to 200 µL plasma and centrifuged at 8000 g for 10 min at 4 °C. The supernatant was added to 1 mL purified water for solid-phase extraction. The brain was homogenized with acetonitrile and 10 mM KH₂PO₄ buffer (pH 3.0), and 1 mL of the obtained homogenate was mixed with 50 µL internal standard (20 µg/mL retinyl acetate) and centrifuged at 8000 g for 10 min at 4 °C. The supernatant was added to 700 µL purified water for solid-phase extraction. Prior to extraction, a solid-phase cartridge (Oasis HLB, Waters, Milford, MA) was pretreated with 1 mL methanol and 1 mL KH₂PO₄ buffer. The sample was added to the cartridge and they were washed with 2 mL KH₂PO₄ buffer and eluted with 2 mL methanol. After drying with nitrogen gas at 40 °C, the residue was reconstituted with 100 µL mobile phase, consisting of acetonitrile and 10 mM phosphate buffer (pH 4.0) (4/1, v/v), and analyzed using a Shimadzu Prominence HPLC system (Shimadzu, Kyoto, Japan), which included an LC-20A solvent delivery unit with high-pressure flow-line selection valves, an SIL-20A auto sampler, and a CTO-20A column oven, connected to LC solution software.
Hyperforin was detected using an ESA Coulochem III 5010 detector (ESA, Inc., Chelmsford, MA) operating at an applied voltage of 600 mV. A CAPCELL PAK C18 MGII column (particle size: 5 µm, column size: φ 3.0 × 150 mm, Shiseido, Tokyo, Japan) was used and was maintained at 40°C. Hyperforin DCHA, as the standard, and samples were separated using a mobile phase consisting of acetonitrile and 10 mM phosphate buffer (pH 4.0) (4/1, v/v). The flow rate was set at 0.5 mL/min.

**Anti-nociceptive effect**

In the formalin test, mice were placed in open Plexiglas observation chambers 1 h before injection of formalin. Formalin (20 ml of 2.5% solution in saline) was injected subcutaneously into the dorsal surface of the right hind paw of mice using a Hamilton microsyringe with a 30-gauge needle (Hamilton Company, Reno, NV), as previously described. Each mouse was immediately returned to the observation chamber after formalin injection. A mirror was placed behind the chamber to allow unhindered observation of the formalin-injection paw. The time spent licking or biting the injected paw, the nociceptive response, was measured with a stopwatch at 5 min intervals until 30 min post-formalin injection and was considered a quantitative indication of nociception. The total licking/biting time from 0–5 min was considered as the first phase, whereas the second phase was taken as the total licking/biting time from 10–30 min. SJW extract or SJW-NE was administered orally 0.5, 1, 3, or 6 h before formalin injection.

### Statistical analysis

For statistical comparisons, one-way analysis of variance (ANOVA) with pairwise comparison by Fisher’s least significant difference procedure was used. \( P < 0.05 \) was considered significant for all analyses.

### Results and Discussion

**Morphology:** Representative SEM images of the SJW extract and powder formulations are shown in Figure 1. SJW extract and SJW-CD showed irregularly shaped particles. According to the manufacturer’s specifications, SJW is extracted by ethanol from the plant, dried, ground, and sieved in the production process, and SJW-CD was prepared by dissolving in ethanol and drying by evaporation. These production processes for SJW extract and SJW-CD, dissolving and evaporation, might lead to non-uniform shapes as observed on their SEM images, whereas SJW-SD and SJW-DE, prepared using a spray-dryer, exhibited a warped spherical form with sizes ranging from 10 to 100 µm, suggesting that the drying process gave similar-shaped powder formulations.

The particle size distribution in water is shown in Figure 2. More than 50% of particles of SJW extract in water were >7 µm, the upper limit of detection by DLS measurement; the maximal particle size using LD measurement, defined as \( d_{90} \), was estimated to be 77 ± 0.5 µm, suggesting that SJW extract was not sufficiently micronized in water. Particle size distributions of formulations at \( d_{10} \)–\( d_{90} \) in water were 180–5,400 nm for SJW-CD, 90–4,200 nm for SJW-SD, 200–6,600 nm for SJW-DE, and 120–320 nm for SJW-NE, respectively. The \( d_{90} \) values suggested the presence of coarse particles in dispersed powder formulations, whereas SJW-NE formed nano-sized fine particles by mixing with water. The shapes of micronized droplets in water were observed using TEM as shown in Figure 2. In both NE and DE, round particles were observed, suggesting that a micronized emulsion was formed in water by diluting the emulsion formulations.

**Dissolution behavior:** In order to estimate the wettability and dispersibility in water, dissolution tests of SJW samples were carried out (Fig. 3). SJW-SD exhibited the slowest profile, requiring more than 20 min to dissolve 70% of the sample, and the dissolution rate constant was estimated to be 4.8 ± 0.23 h\(^{-1}\). Other samples exhibited faster dissolution behavior, as evidenced by 70% release at 5 min. SJW extract was well dissolved in water with stirring, although it contained insoluble components. According to the flow process of SJW extract production, a small amount of sugar was added as an excipient prior to drying, and the presence of sugar on the dried powder surface might result in favorable wettability, leading to faster dissolution. All SJW formulations, except for SJW-SD, exhibited similar profiles to that of SJW extract, suggesting that excipients of these formulations did not affect the wettability of SJW extract. SJW-SD was mainly composed of HPC-SSL as a carrier of solid dispersion. HPC-SSL might form a hydration layer on the surface with high viscosity, leading to the delayed release and diffusion of SJW constituents. Although SJW-DE contained HPC-SSL as well as SJW-SD, it exhibited faster dissolution, suggesting that the proportion of HPC-SSL and other excipients might affect the dissolution rate of SJW formulations.

**Physicochemical stability:** High negative zeta-potential values allow the prediction of good colloidal stability due to the high-energy barrier between particles. The zeta-potentials of SJW extract and SJW-SD were similar at \(-11 ± 0.2 \) mV and \(-14 ± 0.7 \) mV, respectively. HPC-SSL, a matrix agent of SJW-SD, had no impact on the zeta-potential. The zeta-potential of SJW-CD was \(-24 ± 0.7 \) mV, \( \gamma \)-CD, a matrix agent in SJW-CD, had a slightly high negative charge. SJW-DE and SJW-NE had a substantially higher negative charge than others at \(-51 ± 0.1 \) mV and \(-46 ± 0.4 \) mV, respectively. Emulsion formulations could form micronized droplets in water by covering the oil-water surface with emulsifiers, attributed to the negative charge. Zeta-potential measurement indicated the higher electrostatic stability of SJW-DE and SJW-NE.

Practical physicochemical stability was estimated by comparing the appearance change in the diluted solutions of SJW extract and formulations in water. Although SJW extract could be dispersed into water by stirring, precipitation was observed within 60 min. SJW-CD and
SJW-DE developed a thin creaming layer on the upper surface of the solution, and SJW-SD showed precipitation at the bottom within 3 days after dilution. No appearance change was observed in diluted solution of SJW-NE for at least 2 weeks, and the mean particle size of SJW-NE after 2 weeks storage was found to be ca. 210 nm, suggesting higher colloidal stability of SJW-NE compared with other SJW formulations. From these findings, SJW-NE was thought to be the most suitable formulation, and further investigation of the pharmacokinetic behavior and anti-nociceptive effect was carried out with the focus on SJW-NE.

**Pharmacokinetic behavior:** Involvement of hyperforin in anti-nociceptive activity via the opioid-dependent pathway and PKC has been reported. The hyperforin concentration in plasma and brain after single oral administration of SJW extract and SJW-NE to mice, and their pharmacokinetic parameters, are shown in Figure 4 and Table 3, respectively. The analytical method was validated partly according to ICH guideline Q2B Validation of Analytical Procedures: Methodology. The linearity of the standard was fine, with a correlation coefficient of 0.999 over the range 5 nM to 1 µM. The limit of quantification for hyperforin was estimated to be 1 nM on the basis of the signal-to-noise ratio. Three injections at 10 nM exhibited high reproducibility with a variation coefficient of less than 10%.

Administration of SJW-NE led to a significantly higher hyperforin plasma concentration than for the SJW extract, 285 ± 39 nM and 118 ± 45 nM at 0.5 h, 572 ± 95 nM and 192 ± 78 nM at 1 h, and 124 ± 34 nM and 38.7 ± 12 nM at 3 h, respectively. In the brain, administration of SJW-NE also led to a significantly higher hyperforin concentration (10.9 ± 1.9 pmol/g tissue) than for the SJW extract (4.4 ± 1.1 pmol/g tissue) at 1 h. The AUC_{0-6} after administration of SJW-NE and SJW extract was calculated to be 1188 ± 41 nM·h and 417 ± 41 nM·h in plasma, and 52.9 ± 1.6 pmol/g tissue·h and 41.9 ± 1.5 pmol/g tissue·h in the brain, respectively. Although there were no significant
differences in dissolution behavior between SJW extract and SJW-NE, there appeared to be 2.8-fold (plasma) and 1.3-fold (brain) significant enhancements in the absorption of hyperforin using the NE approach. The bioavailability of hyperforin also increased from 10% for SJW extract to 26% for SJW-NE. After dispersion in aqueous media, SJW extract alone tended to form aggregates and precipitates immediately; however, SJW-NE formed nano-sized fine droplets for at least 2 weeks. This might in part explain the data discrepancy between the dissolution and pharmacokinetic behaviors of SJW samples. Generally, NE delivery systems have higher solubilization capacity than simple suspensions, so NE approaches have been applied to several types of poorly-soluble drugs and nutraceuticals.\textsuperscript{19,20} DLS analysis showed that the NE formulation could be thoroughly dispersed as nano-sized fine droplets with favorable physicochemical stability and high negative charge. Theoretically, the gastrointestinal absorption of drugs can be enhanced by emulsification due to the increased surface area for direct contact with the intestinal mucosa.\textsuperscript{21} Hyperforin in SJW extract seemed to dissolve in the oily phase in the NE system because the predicted logP of hyperforin calculated using the ALOGPs program is highly lipophilic (6.32), as listed in the DrugBank database.\textsuperscript{22} Thus, the improved pharmacokinetic behavior of hyperforin using the NE approach might be partly attributed to the increased surface area of oil droplets dissolving hyperforin. From these findings, taken together with the physicochemical properties, the NE approach would likely be effective for developing a nano-emulsified formulation of SJW extract with improved systemic exposure of hyperforin.

**Anti-nociceptive effect:** Anti-depressant drugs such as tricyclics have been widely used in the treatment of patients with chronic pain as well as depression, and the reuptake of serotonin and noradrenaline may be responsible for their anti-nociceptive activity.\textsuperscript{23} The improvement of the opioidergic system in the anti-nociceptive mechanism has been demonstrated in mice.\textsuperscript{24} Recently, the anti-nociceptive activity of SJW extract has also been reported,\textsuperscript{5} and utilization of SJW extract for pain treatment with a favorable side-effect profile and gastroprotective effects is expected as an alternative to NSAIDs.\textsuperscript{25} In the present study, the anti-

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**Fig. 2.** Transmission electron microscopic images of SJW extract-loaded emulsion formulations in water, SJW-NE (A) and SJW-DE (B) Bars represent 500 nm.

**Fig. 3.** Dissolution profiles of SJW samples prepared by different treatments in deionized water
SJW extract, □; SJW-NE, ○; SJW-DE, △; SJW-CD, ▽; and SJW-SD, ◆. Each bar represents the mean ± SE of 3 independent experiments.

**Table 3.** Pharmacokinetic parameters for plasma and brain after single oral administration of SJW extract or nano-emulsion

<table>
<thead>
<tr>
<th>Sample</th>
<th>Plasma</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\text{AUC}_{0\to\infty}$ (nM h)</td>
<td>$C_{\text{max}}$ (nM)</td>
</tr>
<tr>
<td>SJW extract</td>
<td>417 ± 41</td>
<td>192 ± 78</td>
</tr>
<tr>
<td>SJW-NE</td>
<td>1,188 ± 41** 572 ± 95*</td>
<td>52.9 ± 1.6** 11.2 ± 1.7</td>
</tr>
</tbody>
</table>

Each value is the mean ± SE of 6–8 experiments.
*P < 0.05, **P < 0.01 with respect to SJW extract.
nociceptive effect of SJW extract was evaluated by the formalin test after single oral administration of SJW samples. In this method, it is considered that the first phase of formalin-induced behavior reflects the direct activation of A delta and C afferent fibers, while the second phase reflects both ongoing peripheral sensory input and central sensitization. The nociceptive responses after formalin induction and single oral administration of SJW extract and SJW-NE are shown in Figure 5. In the first phase, the licking/biting time was 127 ± 2.0 s in the vehicle group. SJW extract administration significantly reduced the licking/biting time to 109 ± 2.8 s (14% reduction) at 3 h, and SJW-NE administration also significantly reduced the time to 108 ± 9.9 s (14% reduction) at 0.5 h, 100 ± 5.3 s (21% reduction) at 1 h, and 88.9 ± 4.7 s (30% reduction) at 3 h. The SJW-NE group exhibited a significantly lower response (18% lower) than the SJW extract group 3 h after administration. In the second phase, the licking/biting time was 219 ± 8.6 s in the vehicle group. SJW extract administration significantly reduced the time to 134 ± 9.0 s (37% reduction) 1 h after administration and 137 ± 12 s (39% reduction) at 3 h; SJW-NE administration also significantly reduced the time to 181 ± 3.8 s (17% reduction) at 0.5 h, 134 ± 8.2 s (39% reduction) at 1 h, and 135 ± 11 s (38% reduction) at 3 h. SJW-NE administration significantly reduced the time by 20% at 0.5 h, as well as achieving a significant reduction at 0.5 h in the first phase. The reduced nociceptive response after administration of SJW-NE was consistent with its higher plasma and brain concentration of hyperforin compared to that of the SJW extract. These observations might give helpful information for the development of nutraceutical preparations containing SJW extract with the aim of chronic pain treatment. However, since the active components of plant extracts tend to be more susceptible to light, heat, and the presence of oxygen in solution than in the solid state, the solubilization of plant extract also might lead to reduced stability of these compounds. Thus, a long-term stability test of active components using the NE formulation of SJW extract might also be necessary in the future. The NE approach would at least be useful for improving the anti-nociceptive effect of SJW extract, and further optimization of the NE system...
would be required for better colloidal stability and pharmacokinetic behavior, possibly leading to better clinical outcomes from SJW-based therapy.

Conclusions

In this investigation, novel formulations of SJW extract were prepared using four solubilization techniques and their physicochemical characteristics were established. The NE formulation exhibited nano-sized particles, a high negative charge, and the highest physicochemical stability in water of the four formulations. The impact of the NE approach in terms of pharmacokinetic and pharmacological effects of SJW extract were also demonstrated with regard to systemic exposure to hyperforin, an active component, and antinociceptive effects in mice using the formalin test. These results suggested that improved systemic exposure to hyperforin using the NE approach would potentiate the anti-nociceptive activity of SJW extract. The NE approach is a promising delivery option for SJW extract with the aim of chronic pain treatment.

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