Transdermal Absorption of Sclareol, an Active Ingredient in Clary Sage Oil: A Complementary and Alternative Medicine for Menopausal Symptoms

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Abstract: Clary sage oil is commonly used in complementary and alternative medicine to treat menopausal symptoms. However, whether sclareol, which is considered to have estrogen-like effects, can be absorbed transdermally remains unknown. Therefore, in this study, we applied sclareol dissolved in jojoba oil to the dorsal skin of male Hos-HR-1 mice 30 min before blood sampling. We examined whether sclareol can be detected in the plasma because of transdermal absorption using gas chromatography-mass spectrometry analysis. The concentration of sclareol in plasma and liver samples calculated based on the measured sample concentration and the recovery rate was 0.36 ± 0.08 and 1.69 ± 0.32 ppm, respectively. Furthermore, there was a significant positive correlation between the plasma sclareol concentration and hepatic homogenate sclareol concentration. Our findings indicate that sclareol is absorbed transdermally and accumulates in the liver. Moreover, the lack of change in plasma blood urea nitrogen, aspartate aminotransferase, and alanine aminotransferase levels among the three groups indicates that there was no hepatic or renal damage due to transdermal absorption of sclareol in Hos-HR-1 mice. However, further validation in humans is required because the thickness of the dermis and the number of pores significantly differ between mice and humans.

Keywords: complementary and alternative medicine; menopausal symptoms; clary sage oil; sclareol; transdermal absorption; liver and kidney damage

1. Introduction

Menopause is the stage when ovarian hormone production declines. During this stage, estrogen levels in the blood fluctuate and decline, causing menopausal symptoms such as autonomic dysfunction, hot flashes, and vaginal discomfort. Although menopausal hormone therapy (MHT) effectively treats menopausal symptoms, it is associated with adverse effects such as venous thromboembolism, coronary heart disease, stroke [1–3], and dementia [4,5]. Therefore, many women in various countries and regions use complementary and alternative medicine as a safer treatment option for menopausal symptoms [6–8]. In Japan, Kampo medicine and soy isoflavones are complementary and alternative medicines for patients with contraindications or an aversion to MHT [9].

It has been reported that aromatherapy massage using clary sage oil effectively relieves menstrual pain and amenorrhea [10,11]. The essential oils used in aromatherapy massage are diluted in vegetable oils such as jojoba, sweet almond, and macadamia nut oils to various concentrations. According to a 2019 survey conducted by a Japanese academic organization, jojoba oil is the most widely used for diluting essential oils among aromatherapists [12]. Jojoba oil is a wax ester that differs in composition from common vegetable oils rich in triglycerides [13].

Clary sage oil is characterized by the presence of sclareol, an estrogen-like chemical that is considered an active ingredient of this essential oil. In Japan, clary sage oil is used in aromatherapy massage to treat menstrual irregularities, premenstrual syndrome, and menopausal symptoms. However, there have been no reports on the transdermal...
absorption of sclareol. In this study, we aimed to elucidate whether sclareol can be absorbed transdermally and whether it has any adverse effects on the liver and kidneys.

2. Materials and Methods

2.1. Chemicals and Reagents

Analytical grade sclareol (CAS No. 515-03-7) was purchased from Sigma-Aldrich (St. Louis, MO, USA; Lot. STBG9905). The chemical structure of sclareol is shown in Figure 1. Jojoba oil from *Simmondsia chinensis* was obtained from Kenso-Igakusya (Yamanashi, Japan; Lot. GL15A). According to the package insert, the fatty acids in the jojoba oil used in this study included eicosenoic acid (content 73.4%, molecular weight [MW] 310.51 g/mol), erucic acid (content 14.7%, MW 338.57 g/mol), and oleic acid (content 8.3%, MW 282.47 g/mol). HPLC-grade methanol was purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan) and used as a solvent. Ultrapure water was produced using a Millipore Direct-Q® water purification system (MilliporeSigma, Burlington, MA, USA).

![Figure 1. Chemical structure of sclareol (MW 308.50).](image)

2.2. Animals

Sixteen male hairless mice (Hos-HR-1; 7 weeks old) were purchased from Hoshino Laboratory Animals, Inc. (Bando, Japan) through Japan SLC, Inc. (Hamamatsu, Japan). Five animals were housed together per cage (22.5 cm × 33.8 cm × 14 cm) under specific-pathogen-free conditions in our animal care facility. They were fed a standard commercial diet (Clea, Tokyo, Japan) and provided water *ad libitum* until just before euthanasia. The mice were housed at 22–24 °C with 50–60% relative humidity under a 12-h light–dark cycle. All of the mice were 8 weeks old and weighed 26.9–32.3 g (average = 29.8 g) at the start of the experiments.

All of the animals were cared for in accordance with the Guide for the Care and Use of Laboratory Animals [14] and law No. 105 and Notification No. 6 of the Japanese Government. All animal experiments were conducted with the approval of the Animal Experimentation Committee of Tokai University (protocol code 171096 and date of approval 24 October 2017; protocol code 181020 and date of approval 30 March 2018) and the Yokohama City University Animal Experiment Committee (protocol code F-A-15-026 and date of approval 30 March 2015).

2.3. Tissue and Blood Sample Collection

On the day of the experiment, all of the mice were randomly assigned to one of three groups, each with 4–6 animals: the naïve control group (*n* = 6), jojoba oil-administered group (*n* = 6), and sclareol-administered group (*n* = 4). In the jojoba oil-administered group, non-diluted jojoba oil (4 µL/g) was applied to the dorsal skin of the mice. In the sclareol-administered group, a solution of 4 µL/g sclareol dissolved in jojoba oil and adjusted to 266,666 ppm was applied to the dorsal skin of the mice. In this study, sclareol was uniformly dissolved in jojoba oil using an ultrasonic homogenizer (UH-50; SMT Co., Ltd., Tokyo, Japan) on the day of the experiment. The blood was collected via cardiac blood
sampling with the mice under isoflurane-induced deep anesthesia before and 30 min after application. To obtain the plasma, the blood was centrifuged (1118×g, 15 min, 4 °C). The plasma and liver samples were stored at −80 °C until analysis.

2.4. Sample Preparation for Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The pretreatment of the biological samples was performed to determine the sclareol concentration in both the plasma and liver samples using a GC-MS apparatus. The liver samples were homogenized in ice-cold methanol (eight times the volume) using an ultrasonic homogenizer (UH-50; SMT Co., LTD, Tokyo, Japan). To remove protein deposits, the plasma and liver homogenates were subjected to protein removal using cold methanol (eight times the volume), followed by size exclusion filtration to remove polymeric compounds using an Ultracel YM-10 membrane with a 10-kDa cut-off (MilliporeSigma, Burlington, MA, USA). The plasma and liver homogenates were dried using a Thermo SPD111V SpeedVac concentrator (Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, the residues of the plasma and liver homogenates were redissolved in methanol to 15- and 10-fold concentrations, respectively, and the samples were subjected to GC-MS analysis.

2.5. GC-MS Analysis

The GC-MS analysis of sclareol in the plasma and liver samples was performed using a GC-17A gas chromatograph (Shimadzu Co., Kyoto, Japan) coupled with a GC-MS-QP5050A mass spectrometer (Shimadzu Co.). A Stabilwax (Restek Co., Bellefonte, PA, USA) capillary column (30 m length × 0.25 mm ID × 0.25 μm film thickness) was used to separate sclareol. The oven temperature was initially held at 40 °C for 2 min, then increased to 240 °C at a rate of 7 °C/min, and maintained at 240 °C for 10 min. Helium (purity > 99.99%) was used as the carrier gas with an inlet pressure of 60.9 kPa and a flow rate of 1.4 mL/min. A split injector (split ratio of 1:30) at 250 °C was used to add the sample into a capillary column (injection volume: 1.0 μL). The interface temperature was set to 230 °C. Electron ionization at 70 eV was used for MS measurements. MS was performed in the full scan mode (m/z 45–500) and selected ion monitoring (SIM) mode, and the solvent delay time was set to 3 min. The interval time was set to 0.5 s in the scan mode and 0.2 s in the SIM mode. Before analyzing new samples, a blank sample was measured to ensure that there was no carry-over.

2.6. Preparation of Calibration Curves

For the standard stock solution, 10 mg of the sclareol standard was dissolved in methanol, and the volume was fixed at 10 mL (1000 ppm sclareol). The standard solutions for the calibration curves were prepared by diluting the standard stock solution with methanol to prepare solutions at four concentrations of 0.1–10 ppm. These standard solutions were measured in the SIM mode, and absolute calibration curves were prepared.

2.7. Plasma Biochemical Parameters

The plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN), and creatinine (CRE) levels were measured at Kotobiken Medical Laboratories (Tsukuba, Japan). The biochemical data were quantified using the BioMajesty™ autoanalyzer (JCA-BM8060; JEOL Ltd., Tokyo, Japan). The plasma ALT and AST levels were determined using the JSCC transferable method with L-Type ALT J2 and L-Type AST J2, respectively (FUJIFILM Wako Pure Chemical Corporation). The plasma ALP level was measured using the IFCC transferable method with L-Type ALP IFCC (FUJIFILM Wako Pure Chemical Corporation). The plasma BUN level was determined using the urease-GIDH method with L-Type UN-V (FUJIFILM Wako Pure Chemical Corporation). Plasma CRE level was determined using an enzymatic method with Determiner L CRE (Hitachi Chemical Diagnostics Systems Co., Ltd., Tokyo, Japan).
2.8. Statistical Analysis

The data are expressed as mean ± standard error (SE). Statistical analyses were performed using the SPSS 24.0 statistical software package (SPSS Japan Inc., Tokyo, Japan). Paired t-tests were used to compare the means between groups. A one-way analysis of variance and Tukey’s post-hoc test were used to compare means among the three groups. Correlation analysis was performed based on Pearson’s correlation coefficient (r). Statistical significance was set at p < 0.05.

3. Results

3.1. GC-MS Analysis of Sclareol Standard

The retention time of the sclareol standard analyzed in the scan mode was 39.8 min. The mass spectrum of sclareol (Figure 2) was compared with that of sclareol from the National Institute of Standards and Technology library, and the commonly identified ions at m/z 81, 109, and 290 were analyzed in the SIM mode (m/z 81 for quantification).

![Figure 2](image_url)

Figure 2. Mass chromatogram of sclareol obtained using GC-MS analysis in the full scan mode. The vertical axis shows the signal intensity, and the horizontal axis shows m/z. We analyzed (A) m/z 81, (B) m/z 109, and (C) m/z 290 in the GC-MS analysis as monitor ions.

3.2. Standard Curve for GC-MS Analysis

The four-point calibration curve constructed using the sclareol standard showed good linearity with $R^2 = 0.9990$ ($r = 0.9995$) in the concentration range of 0.1–10 ppm. The linear regression equation of the calibration curve was $y = 2.2 \times 10^{4}x + 6.4 \times 10^{1}$ (where y is the peak area, and x is the concentration of sclareol [ppm]), and the S/N of the peak at 0.1 ppm was 1.671.

3.3. GC-MS Analysis of Sclareol Concentration in Plasma and Liver after the Topical Application of Sclareol

The plasma and liver samples were analyzed in the SIM mode after treatment with sclareol for 30 min. The results of the quantitative analysis showed that in the naïve control and jojoba oil-administered groups, no peak was detected in the retention time of the sclareol standard in both plasma and liver (Table 1). In contrast, in the sclareol-administered group, peaks were observed at retention times similar to those of the sclareol standard, and all three monitor ions were detected (Figure 3B,C). In the sclareol-administered group, the sclareol concentration in the plasma and liver samples was $2.51 \pm 0.55$ and $7.93 \pm 1.50$ ppm, respectively. Consequently, the concentration of sclareol in the plasma and liver samples calculated based on the sample concentration and recovery rate (46.8%) and confirmed by spike and recovery tests was $0.36 \pm 0.08$ and $1.69 \pm 0.32$ ppm, respectively (Figure 3A). The sclareol concentration in the liver homogenate was approximately 4.7 times higher than that in the plasma.
Table 1. GC-MS analysis of sclareol concentrations in the plasma and liver.

| Parameters                      | Control (n = 6) | Jojoba (n = 6) | Sclareol (n = 4) |
|---------------------------------|-----------------|----------------|------------------|
| Plasma sclareol concentration (ppm) | N.D.            | N.D.           | 0.36 ± 0.08      |
| Liver sclareol concentration (ppm)  | N.D.            | N.D.           | 1.69 ± 0.32 *    |

Values are mean ± SE. N.D., not detectable. Control, naive control group (n = 6); Jojoba, jojoba oil-administered group (n = 6); Sclareol, sclareol-administered group (n = 4). * p < 0.05, compared with the plasma sclareol concentration in the sclareol-administered group.

3.4. Correlation between Plasma and Liver Homogenate Sclareol Concentrations

Pearson’s correlation coefficients were used to determine the presence of a correlation between the parameters of interindividual variability in sclareol biodistribution. As shown in Figure 4, a strong positive correlation was observed between the plasma sclareol concentration and liver homogenate sclareol concentration (r = 0.949, p = 0.026).

Figure 4. Correlation between plasma and liver homogenate sclareol concentrations 30 min after transdermal administration of sclareol (n = 4). There was a strong positive correlation between the plasma sclareol concentration and liver sclareol concentration (r = 0.949, p = 0.026). The correlation between these parameters was analyzed using Pearson’s correlation analysis with the SPSS 24.0 statistical software package. * p < 0.05.
3.5. Plasma Biochemical Parameters

Plasma AST, ALT, BUN, and CRE levels were measured to determine acute hepatic and renal damage caused by a high dose of sclareol. There was no significant difference in the plasma levels of BUN, AST, and ALT among the three groups (Figure 5A,C,D). On the contrary, the plasma level of CRE in the sclareol-administered group was lower than that in the jojoba oil-administered group (Figure 5B; \( p = 0.017 \)). In addition, the plasma level of ALP in the sclareol-administered group was significantly higher than that in the control group (Figure 5E; \( p = 0.014 \)).

![Figure 5](image.png)

**Figure 5.** Plasma levels of (A) BUN, blood urea nitrogen; (B) CRE, creatinine; (C) AST, aspartate aminotransferase; (D) ALT, alanine aminotransferase; and (E) ALP, alkaline phosphatase. Control, naïve control group (n = 6); Jojoba, jojoba oil-administered group (n = 6); Sclareol, sclareol-administered group (n = 4). Values are presented as the means ± SEs. A one-way analysis of variance and Tukey’s post-hoc test were performed using the SPSS 24.0 statistical software package to compare the means of the three groups. * \( p < 0.05 \).

3.6. Correlation between the Plasma/Liver Sclareol Concentrations and Plasma Biochemical Parameters

The plasma CRE level was significantly lower in the sclareol-administered group than in the jojoba oil-administered group (Figure 5B), and the plasma ALP level was significantly higher in the sclareol-administered group than in the naïve control group (Figure 5E). However, because the number of animals in the sclareol group was small (n = 4), Pearson’s correlation analysis was performed to determine whether these fluctuations correlated with the increased sclareol concentration in the plasma and liver homogenates (Table 2). The results showed no statistically significant correlation.
Table 2. Correlations among the plasma/liver sclareol concentrations and plasma biochemical parameters based on correlation coefficients in the sclareol-administered group.

|        | BUN | CRE | AST | ALT | ALP |
|--------|-----|-----|-----|-----|-----|
| Plasma | r   |     |     |     |     |
| sclareol | −0.369 | 0.041 | −0.945 | −0.638 | 0.011 |
| Liver  | r   |     |     |     |     |
| sclareol | −0.194 | 0.323 | −0.807 | −0.664 | 0.300 |

BUN, blood urea nitrogen; CRE, creatinine; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase. Correlations among these parameters were analyzed using Pearson's correlation analysis with the SPSS 24.0, statistical software package. n = 4.

4. Discussion

Clary sage oil has been reported to effectively relieve menstrual cramps, regulate the menstrual cycle [10,11], and improve symptoms (including hot flashes and night sweats) in menopausal women [15] because of its estrogen-like components and ester ingredients. Clary sage oil is commonly used in complementary and alternative medicine to treat these symptoms; however, it is not clear whether sclareol, which is considered to have estrogen-like effects, can be absorbed transdermally. This study addresses this question.

We examined whether sclareol could be detected in the plasma owing to transdermal absorption by applying sclareol dissolved in jojoba oil to mouse dorsal skin 30 min before blood sampling. Blood was sampled 30 min after the topical application of sclareol because previous studies have found that the peak blood concentrations of essential oil components (i.e., linalool and hinokitiol) are reached 20–30 min after the start of transdermal absorption in both mice and humans [16,17]. In this study, sclareol was detected in the blood 30 min after sclareol application (Figure 3B,C), and the sclareol concentration in the liver homogenate was significantly higher than that in the plasma (Figure 3A). Furthermore, there was a significant positive correlation between plasma sclareol concentration and liver homogenate sclareol concentration (Figure 4). These results suggest that sclareol was absorbed transdermally and accumulated in the liver. In the GC-MS analysis, the reliability of quantification was ensured by confirming that all three monitored ions (m/z 81, 109 and 290; Figure 2) were detected at the retention time of the sclareol standard (Figure 3B,C).

As the molecular weight of sclareol is less than 500 Da, we expected that it would be absorbed transdermally [18]. However, as the concentration of sclareol in the blood after transdermal absorption was expected to be very low [19], a high concentration of sclareol dissolved in jojoba oil was used. The rationale for the sclareol concentration used was based on a previous study in which we applied clary sage oil to ddY mice and examined whether sclareol could be detected in plasma 30 min after application using GC-MS [19]. In that experiment, we could not detect any peak of sclareol. However, as the concentration of sclareol in clary sage oil was 0.68% [19], we could not exclude the possibility of a lower limit of detection. In general, the lower limit of detection is the injection volume at which the signal-to-noise ratio (S/N) is 2 or 3. In our experimental system, the S/N of 0.1 ppm sclareol was 1.67. Therefore, in this study, the lower limit of detection of sclareol was assumed to be approximately 0.1 ppm, and the dosage was calculated considering the recovery rate and concentration during pretreatment.

The two major routes of eliminating xenobiotics absorbed into the body are through the liver and kidneys [20]. In other words, they are the most frequent target organs of drug toxicity. In general, fat-soluble xenobiotics are metabolized in the liver, converted to more polar structures, and excreted through the kidneys. The metabolic pathway of sclareol in the body remains unknown. However, as sclareol is insoluble in water, it is likely that it is first metabolized in the liver to a more polar substance. This hypothesis is supported by the fact that sclareol and its metabolites were not detected in the urine after intravenous administration in rats [21]. The in vivo clearance of sclareol may have been slow because it was deposited in tissues [22]. As our results suggested that transdermally absorbed sclareol may have accumulated in the liver (Figure 3A), we quantified hepatic sclareol and...
measured biochemical data to determine whether liver or kidney damage was induced. In this study, the sclareol-administered group had significantly increased plasma ALP level compared with that in the naïve control group (Figure 5E) and significantly decreased plasma CRE level compared with that in the jojoba oil-administered group (Figure 5B). Therefore, we performed Pearson’s correlation analysis to determine whether these changes correlated with increased sclareol concentration in the plasma and liver homogenates and found no significant correlation (Table 2). These results suggest that changes in the plasma ALP and plasma CRE levels do not have a clear association with sclareol administration. As the plasma ALP level increases to more than twice the upper limit of normal in drug-induced liver injury with biliary stasis, the levels in the sclareol-administered group can be considered mildly elevated with a negligible physiological effect. Furthermore, a lack of change in plasma concentrations of BUN (Figure 5A), AST (Figure 5C), and ALT (Figure 5D) indicated that a plasma sclareol level of approximately 0.36 ppm does not induce hepatic or renal damage. The amount of sclareol in clary sage oil used in aromatherapy is less than the amount of sclareol administered to the mice in this experiment. Therefore, the risk of drug-induced hepatic and renal injuries from dermal absorption of sclareol appears to be low. However, further validation in humans is required because the thickness of the dermis and the number of pores significantly differ between mice and humans.

5. Conclusions

In conclusion, our findings suggest that sclareol is absorbed transdermally and subsequently accumulates in the liver. In addition, the amount of clary sage oil normally used in aromatherapy is unlikely to induce liver and kidney damage caused by the active ingredient sclareol. Further studies are needed to clarify the safety and distribution of sclareol in humans after transdermal absorption.

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