Percutaneous Absorption Enhancer Properties of Lavandula Angustifolia Essential Oil on Percutaneous Absorption of Naproxen Sodium from Topical Gel

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Research Article

Keywords: Lavandula angustifolia essential oil, Naproxen sodium, percutaneous absorption, permeation enhancer, Topical gel, Tail-flick test

Posted Date: April 19th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-344039/v1>

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Abstract

The naproxen bioavailability via percutaneous route is low, and several technologies have been used to overcome the problem. Although the permeation enhancer properties of natural essential oils have been reported, there is no study to show the effectiveness of *Lavandula angustifolia* essential oil on the percutaneous absorption of naproxen sodium from a topical gel to overcome poor percutaneous absorption of naproxen. To this end, the naproxen topical gel was formulated using Carbopol 940 (as a gelling agent), and several vehicles. The results showed a greater level of penetration into (222.19 ± 24.87 vs. $107.65 \pm 6.38 \mu\text{g}/\text{cm}^2$) and across (22.07 ± 4.42 vs. $13.14 \pm 2.87 \mu\text{g}/\text{cm}^2$) the skin layers for the formulation containing essential oil in comparison with the naproxen gel ($P < 0.05$). A significant antinociceptive property was observed in naproxen topical gel containing 0.5% essential oil in both the first and late phase of the formalin test. The effect was observed in the late phase of the tail-flick test too. In conclusion, the study demonstrated that *Lavandula angustifolia* essential oil significantly enhanced the percutaneous absorption of naproxen and the analgesic effects.

1. Introduction

The human skin is an acceptable and suitable route for topical, local, or systemic drug delivery. Nevertheless, diffusion via the skin is controlled by the stratum corneum, which causing a barrier for transdermal delivery of chemicals [1]. In order to achieve a better transdermal delivery, it is important to overcome skin barriers, particularly the stratum corneum [2]. Different strategies have been used and developed for enhancing percutaneous drug absorption. One of the acceptable and suitable strategies to increase chemicals delivered via the skin is the use of permeation enhancers [1, 3]. Permeation enhancer properties of natural essential oils have been reported in many studies [4-8]. The flowers and essential oils of *Lavandula angustifolia* (LA) known as "Ostokhoddous", are used in traditional medicines to treat several disorders and conditions. Many studies have reported antinociceptive and anti-inflammatory properties of different parts of LA [9]. The plant essential oil's major components are camphor, 1,8-cineole, and endo-borneol [10], which showed percutaneous enhancer effects in studies [11-13]. Naproxen sodium is an anti-inflammatory and antinociceptive drug that is used at an increasing rate in painful positions. The drug has high protein binding and may cause gastritis and peptic ulcer disease. The use of a percutaneous route could reduce the naproxen side effects. Nonetheless, the naproxen bioavailability via percutaneous route is low, and several technologies have been used to overcome the problem [14].

As there is no study on the percutaneous enhancing effects of *Lavandula angustifolia* essential oil (LAEO) on percutaneous absorption, the present study was aimed to investigate the percutaneous enhancing effects of LAEO on percutaneous absorption of naproxen sodium from a topical gel. In addition, anti-nociceptive and anti-inflammatory effects of the gel were measured in mice using tail-flick and formalin tests.

2. Materials And Methods

2.1 Materials

Naproxen sodium was purchased from Tehran Daroo pharmaceutical Co. (Tehran, Iran). PEG 400 and ethanol were purchased from Merck (Merck Co., Germany). Methylparaben was acquired from Sigma-Aldrich, and propylparaben was obtained from Acros Organics. Carbopol 934P was from BF Goodrich, (Cleveland, OH), and LAEO was obtained from Barij Essence, Tehran, Iran.

2.2 Gas chromatography-mass spectrometry (GC-MS) analysis

Perkin-Elmer 8500 equipped with a DB-5 capillary column (30 m $\times$ 0.25 mm; film thickness 0.25 mm) equivalent to USP *phase* G27 was used for GC analysis. The FID detector programmed at 60 °C for 5 min and then up to 220 °C at 4 °C/min. Helium in a constant flow rate (2 mL/min) was used as carrier gas, and the split ratio was 1:30. GC-MS analysis was done on Hewlett Packard 6890 series (Hewlett-Packard Enterprise, Palo Alto, CA) using electron energy of 70 eV with a scans time of 1 sec in split mode with 1:40 ratio. The acquisition mass range was 40-400 m/z. Column and carrier gas was set similar to the gas chromatographic analysis [15].

2.3 Preparation of gels

The details of formulation compositions showed in Table 1. Carbopol (5% w/w) was dispersed in water and kept 24h to prepare the plain gel. Naproxen sodium solved in PEG400 at 40 °C by a heater stirrer (phase 1) and other components solved in ethanol at room temperature (phase 2). Two phases were mixed at room temperature and then was mixed with carbopol 5% gel under propeller homogenizer at 400 rpm (unneutralized Carbopol gel) [16].

2.4 Viscosity measurement and rheological behavior study

The viscosity was measured at different speeds (5, 10, 20, 50, 100 rpm) at 25°C using spindle S5 by Brookfield viscometer (Brookfield, DV-II +, USA) [17].

2.5 Physical Evaluation

2.5.1 Organoleptic Characteristics.

The formulations were assessed for physical appearance, color, and phase separation by visual observation. Homogeneity and texture were evaluated by sensations between the fingers, and on the skin.

The consistency (determines its "feel" and "body" and judge proper consistency) and presence of particles was also evaluated [18].

2.5.2. Spreadability.

Spreadability was measured by spreading the diameter of the sample (1g) on a premarked circle (2 cm diameter) between two glass plates (n=3). The weight 500 g applied to the upper plate for 5 minutes [19].

$$\% \text{ spreadability} = (A2 / A1) \times 100, (1)$$

A1 = 2 cm and A2 = after spreading

2.5.2 pH Values.

The gel (1 gram) was dispersed in deionized water (25 mL), and the pH was measured (n=3) by a pH meter (Mettler-Toledo Ingold Inc., Billerica, MA) [20].

2.6 Physical Stability

The formulation in triplicate was submitted to stability tests with tubes of 30 grams. The formulation was kept under three different temperatures for 6 months (4, 25, and 40 °C). At the temperature of 40 °C the humidity was selected at 75% in climate chamber HPPeco (Mettmert) [18].

2.6.1 Freeze-Thaw Cycle.

The formulations for 12 days remained in two different temperatures (4 °C and 40 °C) for a period of 24 hours in six cycles [18].

2.6.2 Centrifugation Test.

10 g of the sample was added in a test tube and was subjected to 3000 rpm for 30 minutes at 20 °C (3-30K, Sigma, Germany) [18].

2.7 Animals

To do tail-flick and formalin studies, four groups were selected: (1) naproxen-essential oil gel, (2) naproxen gel, (3) essential oil gel (placebo), and (4) base gel (control). For percutaneous studies, only two first groups were selected. The research method was acceptable with the Ethical Guidelines for Investigations in Laboratory Animals. In the studies, male Swiss–Webster mice weighing 25–30 g and

male Wistar rats (weighing 120-150 g) were used [19]. This study was approved and supervised by the ethics committee of Baqiyatallah University of Medical Sciences (No. IR.BMSU.REC.1398.403).

2.8 Tail flick test

The tail-flick studies were done with a tail-flick instrument (Model TF-1435; Technic Azma; Tabriz, Iran). 0.5 gram of the sample was rubbed 50 times on the animals' tails (n=5 in each group). The heat was applied on proximal 2 cm of the tail base. The delay in pain responses were considered as an indication of nociception. Ten seconds was selected as maximal exposure time to avoid tissue damage. The study was done every 5 minutes until 1 hour [19].

2.9 Formalin test

To start the test, the formulations were applied topically to the plantar surfaces of the left hind paws (n=5 in each group) by rubbing 50 times. Animals were placed in an observation chamber, immediately after injection of formalin 2.5 % (50 μ L) under the dorsal surface of the left hind paw, and the time spent on licking, shaking, and biting of the injected paw was measured and considered as an indication of pain. The early and the last phase was from 0 to 5 min and between 15 to 60 min after the injection, respectively [19].

2.10 *In vitro* skin permeation study

Male Wistar rats were anesthetized by injecting ketamine and xylazine 87 and 13 mg /kg, respectively. To remove abdominal skin, chloroform was used to kill rats after 48 h of the shaved time. The subcutaneous skin fat was cleaned, and then it was placed in contact with a normal saline solution (0.9%) for 24 h.

The skin was used between the Franz cells halves (with an area of 3.8 cm²), and the dermis faced the receiver medium. Ethanol 50% was used as receiver fluid at 32 $\pm$ 0.5 °C (approximate normal skin conditions) and stirred at 150 rpm throughout the study. 1g (equal to 10000 μ g naproxen) of formulations (f3 and f4) were spread out uniformly on the skin surface as donor compartment. 4 ml of the fluid were withdrawn at predetermined times (2, 6, 8, 10, and 24 h) and fresh medium was replaced immediately after withdrawing the sample. The skin was removed and washed at the end of the study and cut into small pieces, and put in a tube for 24 h in contact with 15 ml ethanol 50% and then sonicated for 1 h with bath sonicator. The fluid was filtered through a filter paper (Whatman filter paper grade 591) *and selected for analysis*. All samples were subject to filtration by a syringe filter (0.22 μ m) and were analyzed for naproxen content [16].

2.11 HPLC

HPLC waters 2695 equipped with the Prodigy™ 5 µm ODS-3 100 Å, LC Column 125 x 4.6 mm, Ea was used in permeation study to detect naproxen at 230 nm. The mobile phase was 40:20:40 acetonitrile, methanol, and acetic acid (1% v/v). The flow rate was 0.7 ml/min, and the naproxen peak retention time was 6 minutes [14].

2.12 Statistical analysis

To measure differences between groups, ANOVA followed by the Newman–Keuls test was used and p-value equal to 0.05 was considered significant.

3. Results

In the preliminary stage, several formulations were prepared with different solvents and gelling agents, and the best formulation (F4) was chosen based on the physical stability as main naproxen sodium gel 1% w/w containing essential oil 0.5% w/w Table 1.

The formulation has a smooth texture with a clear and transparent appearance, homogeneous with the odor of LA. The characteristics remained constant for 6 months, and there was no difference in the aspect of the formulation before and after a freeze-thaw cycle. The pH values of the formulation were found to be 5.7 ± 0.02 . The spreadability percent of the formulation was 210%. Table 3 shows the rheological behavior of the formulation at different speeds. Figure 1 showed the rheological behavior of the formulation. The viscosity decreases as the shear rate increases (shear thinning). Based on the GC-MS analysis, 1,8-cineol (22.3%), Linalool (11.2%), camphor (7.9%), β -pinene (5.8%), α -terpineol (4.9%), α -pinene (4.6%), Terpinen-4-ol (4.2%), borneol (4.0%) were the major constituents of the essential oil; these compounds were also identified in Table 2. Figure 2 shows the permeated (transdermal delivery) amount of naproxen through the skin from both gels. Figure 3 shows the cumulative percentage of plots of naproxen penetrated (dermal delivery) and permeated after 24 h (transdermal delivery) for both gels. The amount of naproxen permeated through the skin and penetrated to skin layers was higher for F4. The concentration of naproxen quantified in the receptor chamber was $22.07 \pm 4.42 \mu\text{g}/\text{cm}^2$ for the formulation containing essential oil and was $13.14 \pm 2.87 \mu\text{g}/\text{cm}^2$ for naproxen gel. The amount of naproxen deposited in the skin for the formulation containing essential oil was $222.19 \pm 24.87 \mu\text{g}/\text{cm}^2$, and $107.65 \pm 6.38 \mu\text{g}/\text{cm}^2$ for naproxen gel. The tail-flick study showed more analgesic effect after 45 min for the naproxen sodium formulation, which containing LAEO ($p < 0.05$) than other formulations (Figure 4). The formalin test results showed the analgesic effect of formulations containing naproxen (f3 and f4) compared with the vehicle Figures 5 and 6. However, the analgesic effect of formulation containing LAEO was more. *In vitro* percutaneous absorption was performed, to compare the efficacy of naproxen-essential oil gel (as a sample) and standard naproxen gel (as control).

4. Discussion

Many studies have reported the anti-inflammatory and antinociceptive effects of different parts of LA [9, 21-23]. Silva et al. studied the anti-inflammatory and antinociceptive activity of LAEO and reported LAEO has an anti-edematogenic effect similar to dexamethasone. They also reported that LAEO has an antinociceptive activity similar to tramadol, and dose-dependent antioxidant activity [22]. Hussein et al. have assessed *Lavandula officinalis* extract analgesic and anti-inflammatory effects by formalin test where their effects were similar to the effects of morphine, dexamethasone, and indomethacin [23]. Cardia et al. have reported that topical application of LAEO had significant effects on acute inflammatory responses in different models. They also reported LAEO at below concentration of 10 µg/ml did not show *in vitro* cytotoxicity [10]. Hajhashemi et al. evaluated the anti-inflammatory and antinociceptive effects of essential oil, polyphenolic fraction, and hydroalcoholic extract of the leaves of LA [9]. Terpenes are one of the promising clinically acceptable enhancers (at a concentration between 1-5%) Due to their minimal systemic toxicity and dermal irritation with high enhancement activity [7]. 1,8-Cineole is a terpene that is characterized as a main constituent of several aromatic plants' essential oil [24] is a major part of LAEO, which could be the suitable potential of LAEO as an enhancer in naproxen sodium gel. These results have been reported by other researchers previously [10, 25-28]. The formulations were prepared with Carbopol 934 as the gelling agent at the concentration of 1% Table 1. pH, physical stability, spreadability, and organoleptic characteristics of formulations were examined. The results approved suitable properties of carbopol 940 as the gelling agent. Topical dermal or transdermal formulations were evaluated for skin penetration into and across the skin, respectively. Even though the human's skin can serve as a more valid model, most studies were performed on rat's skin [19]. Similarly, we used rat's skin as a model to evaluate the efficacy of LAEO. Figure 2 shows the permeated (transdermal delivery) amount of naproxen through the skin from both gels. Results show that after 8 h, naproxen permeated more from F4. This indicates that LAEO is suitable for enhancing transdermal delivery too. Figure 3 shows the cumulative percentage of plots of naproxen penetrated (dermal delivery) and permeated after 24 h (transdermal delivery) for both gels. The amount of naproxen permeated through the skin and penetrated to skin layers was higher for F4 ($p < 0.05$). Naproxen concentration measured in the receptor chamber was $22.07 \pm 4.42 \mu\text{g}/\text{cm}^2$ for the formulation containing essential oil and was $13.14 \pm 2.87 \mu\text{g}/\text{cm}^2$ for naproxen gel ($p < 0.05$). The deposited concentration of naproxen in the skin for the formulation containing essential oil was $222.19 \pm 24.87 \mu\text{g}/\text{cm}^2$, and $107.65 \pm 6.38 \mu\text{g}/\text{cm}^2$ for naproxen gel it was ($p < 0.05$). The results showed that the LAEO increases the drug permeation and also increases the accumulation of naproxen in the horny layer. Naproxen topical gel containing essential oil 0.5% induced a significant analgesic effect in the early phase of the formalin test. Moreover, this effect was increased in the late phase. The result of tail-flick test was consistent with the formalin test. Latency time in tail-flick test was increased in the group that received naproxen topical preparations with essential oil. As any significant analgesic and anti-inflammatory effect was not observed in both formalin and tail-flick tests for the gel, which have only the essential oil. The authors concluded that the increasing anti-inflammatory effects of naproxen topical gel containing 0.5% essential oil is due to the enhancement percutaneous absorption of the drug in the presence of essential oil, and the essential oil by itself has no anti-inflammatory effect. It is suggested the use of a higher concentration of LAEO could show more analgesic and anti-inflammatory activity because of both enhancement and antinociceptive effects of the essential oil. Low concentrations of

LAEO was selected, as the study aim was to evaluate the enhancement effects of LAEO. Okabe et al. studied the enhancement activity of cyclic monoterpenes on the transdermal absorption of indomethacin gel [29]. They reported that d-limonene could increase the percutaneous absorption of indomethacin. They also evaluated the d-limonene skin pretreatment, and observed no enhancement of d-limonene on skin barrier function, which suggest that d-limonene might have reversible alteration on the skin [29]. 1,8-Cineol, Linalool, and camphor, the major constituents of *Lavandula Officinalis* essential oil accelerate transepidermal absorption of naproxen sodium. Xie et al. evaluated the penetration-enhancing profile of (+)-camphor via *in vitro* percutaneous experiments. They reported that camphor could significantly increase the transdermal absorption of drugs with different hydrophobicity, but maximum penetration-enhancing efficiency was for hydrophilic drugs. They also reported relatively low skin irritation of camphor [13]. Monoterpenes are safe compounds as they have reversible effects on skin lipids and relatively low cutaneous irritation at concentrations (1–5%w/v). For formulations containing limonene, linalool, anethole, thymol, carvacrol, and menthol, no skin irritation in humans was reported [30].

5. Conclusion

This experiment was aimed to evaluate the enhancing effects of LAEO on the transdermal absorption of naproxen sodium from a topical gel. In conclusion, this study demonstrated that LAEO significantly enhanced the percutaneous absorption of naproxen and its analgesic effects. The *Levandulla Officinalis* essential oil is a rich source of terpenoids, especially 1,8-Cineole which can enhance skin absorption. The formulation was examined for pH, spreadability, organoleptic characteristics, and physical stability. The formulation was stable over a period of 6 months at different temperatures. The results approved suitable properties of carbopol 940 as the gelling agent.

Declarations

Ethics approval and consent to participate:

Animal experiments were approved by the ethics committee of Baqiyatallah University of Medical Sciences (No. IR.BMSU.REC.1398.403). All animal studies were carried out under strict compliance with governmental and institutional protocols.

Consent for publication:

Not Applicable.

Availability of data and materials:

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests:

The authors declare that they have no competing interest.

Funding information:

Not Applicable.

Authors' contributions:

Maryam Iman: Conceptualization, review & editing, Project administration. Seyyed

Sohrab Rostamkaleei: Methodology, Investigation, Formal analysis, Writing - original draft. Ramin Ataee: Methodology, Writing - review & editing.

Acknowledgment

The authors are grateful for the support provided by the Baqiyatallah University of Medical Sciences, and Islamic Azad University-Ayatollah Amoli Branch.

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Tables

Table 1

Composition (w/w %) of various gels.

	Water	PEG 400	Eth*	Carbopol	P-p **	M-p***	LAO****	Nap*****
F ₁	28.8	60	10	1	0.02	0.18	0	0
F ₂	28.3	60	10	1	0.02	0.18	0.5	0
F ₃	27.8	60	10	1	0.02	0.18	0	1
F ₄	27.3	60	10	1	0.02	0.18	0.5	1
* Ethanol ** Propyl paraben *** Methyl paraben **** LA oil ***** Naproxen								

Table 2

The percentage composition of the total oil from LA.

RT (min)	Compound	Percentage (%)
7.74	Pinene-α	4.6
9.04	pinene-β	5.8
10.81	1,8-cineole	22.3
13.21	Linalool	11.1
14.65	Camphor	7.9
15.41	Borneol	4.0
16.04	ol-4-Terpinen	4.2
16.62	-terpineola	4.9

Table 3

Viscosity of the formulation at different speeds

Spindle number	speed (RPM)	F4	
		Torque (%)	Viscosity (cP)
S5	0.5	0.1	140000
S5	1	0.1	120000
S5	2.5	0.2	100000
S5	5	0.4	90000
S5	10	0.6	75000
S5	20	1.2	60000
S5	50	2.9	45000
S5	100	5.1	33000

Figures

Figure 1

Impact of the shear rate on gel formulations' viscosity.

Figure 2

The accumulated amount of naproxen permeated through the rat skin within 24 hours (data are shown as mean $\pm$ SD, $n = 3$; the ANOVA test followed by the Tukey test showed a significant impact of time and formulation on the permeation of naproxen $p < 0.05$). The sample represents 1 percent naproxen with 0.5 percent essential oil, and the standard formulation is 1 percent naproxen gel.

Figure 3

Amount of naproxen skin layer penetration (Dermal delivery) and permeation (transdermal). Error bars are standard deviation, the sample represents 1 percent naproxen with 0.5 percent essential oil, and 1 percent naproxen gel without LAEO is standard formulation; t-test was conducted between sample and standard. The difference was significant $p < 0.05$; $n = 3$.

Figure 4

Tail flick latency in different topical preparation. Values are presented as Mean $\pm$ SD (n=5 animals per group).

Figure 5

First phase (0-5 min) of formalin test in topical preparations. Data are presented as Mean $\pm$ SD (n=5 animals per group) mean $\pm$ SD (n=5 animals per group). $P < 0.05$ for the first phase (Tukey test).

Figure 6

Late phase (15-50 min) of formalin test in topical preparations. Data are presented as Mean $\pm$ SD (n=5 animals per group) mean $\pm$ SD (n=5 animals per group). $P < 0.05$ for the first phase (Tukey test).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [graphicalabstract.docx](#)