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Anti-inflammatory and Pain-relieving Effects of Arnica Extract Hydrogel Patch in Carrageenan-Induced Inflammation and Hot Plate Pain Models

Sang Gil Lee[1], Eun Byul Lee [1], Tack Soo Nam[2], Sunho You[2], Dahye Im[2], Kyusun Kim[2], Bonseung Gu[2], Ga-young Nam[2], Hyerim Lee[2], Soon Jae Kwon[3], Yun Seok Kim[4] and Sang Geon Kim*[,3]

[1] *Center of Research and Development, A Pharma Inc, Goyang-si, Gyeonggi-do, 10326, Republic of Korea*
[2] *R&D Center, Wooshin Labottach, Digital-ro 288, Guro-gu, Seoul, 08390, Republic of Korea*
[3]*College of Pharmacy and Integrated Research Institute for Drug Development, Dongguk University-Seoul, Goyang-si, Kyeonggi-do 10326, Republic of Korea*
[4]*Department of Clinical Pharmacology and Therapeutics, College of Medicine, Seoul National University, Kwanak-gu, Seoul, 03080, Republic of Korea[4]*
* Correspondence sgkim@dongguk.edu; Tel: +8231-961-5218; Fax: +8231-961-5206

Abstract: *Arnica montana* (AM), which belongs to the daisy family Asteraceae, has a longstanding traditional use in Europe and North America for pain and inflammation treatment. This study investigates the inhibitory effects of '*Arnica montana* extract hydrogel patch (AHP)' on carrageenan-induced paw edema and hot plate-induced pain models. AHP exhibited transdermal permeability without the occurrence of issues like crystal precipitation. This study employed two animal model assessments using AHP, in comparison with Arnica Gel (AG), to evaluate anti-inflammatory and pain relief effects. AHP treatment for 2 days showed a decrease in paw edema thickness in mice as compared to vehicle or AG groups; Carrageenan-induced swelling increased maximally at 1 h with AHP group demonstrating a more reduction. Thus, AHP group exhibited a lower ratio of right/left paw thickness and a superior reduction in swelling, supportive of its ability to diminish edema. Histological analysis showed that AHP treatment reduced inflammatory cell infiltration. Consistently, the mRNA levels of inflammatory markers (Tnfa, Il1b, and Il6) were decreased to a greater extent than AG group. Particularly, Tnfa inhibition was better in AHP group, and the levels of Il1b and Il6 transcripts showed ~80% and 40% lower. Likewise, AHP reduced pain scores in a hot plate-induced rat model although AG failed to do so. Together, these results demonstrate that AHP has long-lasting inhibitory effects on fluid effusion and edema formation, production of inflammatory mediators and pain-sensation, supporting its anti-inflammatory and pain-relieving pharmacological effects.

Keywords: Arnica Patch, Edema, Anti-inflammatory, Inhibitory effects, Pain relief

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1. Introduction

Plant-derived medications seem to gain prominence due to their minimal side effects and cost-effectiveness. *Arnica Montana* (AM) is a flower belonging to the Asteraceae family and the Arnica genus. Traditionally, AM has a longstanding use in Europe and North America for treating pain and inflammation [1,2]. Various parts of AM including leaves, sepals, stems, flowers, roots, fruits, and branches have been employed with a focus on the whole plant. In European folk medicine, the flowers and rhizome are used as a universal remedy since ancient times. Nowadays, it has been claimed that AM may serve as a hemostatic agent for conditions such as angina, vasodilation, relief of vascular spasms, and treatment of bruises and piles.

Inflammation and pain, as a complex symptom affected by physiological, social, and psychological factors [3], contributes to health issues and adversely impacts patient's quality of life [3, 4]. Analgesics including narcotic analgesics, non-narcotic analgesics, and

analgesic adjuvants, show clinical efficacy in relieving pain. Nevertheless, their utilization is limited due to adverse effects [5, 6]. Pro-inflammatory cytokines, implicated in a variety of pathological conditions, play a role in inflammatory responses and pain sensitization [7]. Specifically, major cytokines such as TNFα, IL-1β, and IL-6 are closely linked to pathological processes and the consequential tissue damage [7]. Despite the above mentioned analgesic effects of AM, there is limited information available on its effect on pain in the context of the regulation of inflammatory mediators.

With a variety of pharmacological properties such as analgesic, antibacterial, and anti-inflammatory activities, several formulations comprising AM are now available as forms of gel, cream, liquid, and tablets [8, 9]. Unfortunately, however, oral administration is limited due to its cytotoxic nature, and thus topical administration, particularly in the form of gel or cream, is common [2]. Nonetheless, topical administration using gel or cream formulations faces limitations due to clothing and activities, potentially impacting drug penetration. When the component is applied as a dry patch, the adhesiveness is low because the surface is oily. Moreover, AM extract has the disadvantage of being weak against moisture.

Plasters are categorized into moist poultices called cataplasms and solid formulations known as plasters, both in the form of a dressing. Cataplasms, due to their nature, include a flexible layer separate from the adhesive layer [10]. Plasters, containing acrylic resins, for example, do not have moisture, presenting a disadvantage in terms of skin irritation compared to cataplasms [11]. To overcome the drawbacks of these traditional cataplasms and plasters, as well as cream formulations, there is ongoing research into hydrogel dressings as a transdermal absorption formulation. Herein, we report development of 'AM-containing hydrogel patch' and its pharmacological effects, based on our preliminary research outcomes that this formulation proposes a solution to the above problems and improves adhesion for stable penetration of the active components.

This research also explored the efficacy of the AM hydrogel patch formulation on the edema formation, production of inflammatory mediators, and pain sensation. Specifically, we sought to evaluate the effects of the AM hydrogel patch on the levels of inflammatory markers using the Carrageenan-induced inflammation model, examining its inhibitory effects on (1) edema formation, (2) inflammatory cell infiltration, and (3) inflammatory markers. Further, we utilized a rat model to assess its pain-relieving effect in comparison with a conventional Gel formulation. The outcomes of this study show the evidence that AM hydrogel patch has bona fide anti-inflammatory and pain-relieving effects and more intriguingly the hydrogel formulation exerted a convenient long-lasting biological effect compared to a gel-type formulation.

2. Materials and Methods

Preparation and manufacture of the AM extract hydrogel patch

The hydrogel patch pharmaceutical composition containing AM extract was developed, with the material composition summarized in Table 1 (Wooshinlabottach Co., Ltd., Seoul, Korea). The AM patch was designed to allow uniform distribution of high concentrations of active ingredients. The pH of the AM extract hydrogel patch ranged from 5.0 to 6.0, exhibiting transdermal permeability without issues such as crystal precipitation during manufacture and storage (Table 1).

Table 1. Material composition of arnica extract hydrogel patch

INCI Names	INCI monograph ID	Composition (%)
Aluminum Glycinate	104	0.11
Arnica Montana Extract	29326	1.00
Cellulose Gum	457	2.30
Disodium EDTA	894	0.09
Glycerin	1077	39.00

Polyacrylic Acid	2402	2.00
Polysorbate 80	2457	0.10
Tartaric Acid	3146	0.30
Titanium Dioxide	3217	0.20
Water	3342	55.8
Sodium Polyacrylate	6285	4.80
1,2-Hexanediol	16304	0.3

Arnica patches were manufactured as shown above. pH conditions are 5.0 to 6.0. (Wooshinlabottach Co., Ltd., Seoul, Korea)

Mouse experiments

The animal care and studies were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at Dongguk University (No. IACUC-2021-035-2). The C57BL/6 mice were purchased from Jabio (Suwon, Korea) housed at standard temperature (22±2℃) and humidity (50±5%) under a 12 h/12 h light/dark cycle, pathogen-free air, with food and water available ad libitum. Male mice at 8 weeks of age were used. To minimize environmental differences, mice were maintained for at least a week before each experiment.

Carrageenan-induced edema assays

Edema was induced using 1% Carrageenan. 50 μl of 1% Carrageenan in saline solution was injected into the right paw of the mouse and 50 μl of saline into the left paw. The left paw was injected with 50 μl of saline for mock treatment (For comparative purposes, the opposite paw was selected as the observation target, inducing swelling in the mouse's paw). The AM hydrogel patch (Wooshin Labottach Co., Ltd., Seoul, Korea) or AM gel (Bioron Co., Ltd., PA, USA) was applied 2-3 times daily depending on the characteristics of the test substance. The formulations were administered at 9:00 a.m. every day for 2 days, and they were applied immediately to enhance application stability. This step was repeated with an additional application at 1:00 p.m. (plus 6 p.m. for gels) (i.e., 2 or 3 times daily). The AM patch was replaced at 9 p.m. On day 3, the sizes of the edema were measured and photographed 1, 2 and 3 h time points after Carrageenan administration, and the final AM hydrogel patch or AM gel application; the morphological changes were observed hourly, which was terminated when changes were recognized.

Preparation of paw tissue samples

The mice were euthanized and sacrificed through cervical dislocation to obtain paw samples. The left paw tissues were removed from blood and fur from the skin. Kimwipes was used to eliminate moisture. Then, a razor blade was used to collect integumentary tissue from the swollen area of the mouse's paw and the opposite side which were subjected to immunohistochemisty and qRT-PCR assays for key inflammatory mediators.

Histopathology analysis

Mouse paw tissues were fixed in 10% formalin, embedded in paraffin, cut into sections, and mounted on slides. Paraffin-embedded colon tissue sections were stained with hematoxylin and eosin (H&E) using a commercial staining kit (ScyTek Laboratories, Logan, UT, USA) for tissue morphology.

RNA isolation and quantitative RT-PCR assays

Total RNA was extracted using Trizol (Invitrogen, Carlsbad, CA) and was reverse-transcribed. The resulting cDNA was amplified by qRT-PCR using LightCycler DNA Master SYBR Green-I Kit (Roche, Mannheim, Germany) according to the manufacturer's

instructions. Gapdh was used as a normalization control. The primer sequences used for qRT-PCR assays are listed in Supplementary Table 1.

Hot plate pain scoring test in rats

7-week-old male SD rats were purchased from Orientbio (Seongnam, Korea) and housed at standard temperature (22±2℃) and humidity (50±5%) under a 12 h/12 h light/dark cycle in Wooshin Labotach Co. The treatment doses of AM hydrogel patch and AM gel were calculated based on the body surface area of rats compared to humans. A dose of 0.5 g, equivalent to 35 mg of active ingredient, was established for the AM gel, whereas that of AM patch was done to have a dose of 3.46 cm^2, equivalent to 3.46 mg. The rats were randomly assigned to four groups: AM patch 3.46 mg for 1 h (patch 1 h; n = 8), AM patch 3.46 mg for 2 h (patch 2 h; n = 8), AM gel 35 mg for 1 h (gel 1 h; n = 8) and AM gel 35 mg for 2 h (gel 2 h; n=8). The formulations were applied on the left thigh of the animals. The formulations were removed 1 or 2 h afterward, and a hot plate test was done to evaluate pain response. All animal experiments were approved by Wooshin Labottach Co., Ltd., Ethical Committee for Animal Experimentation (Approval number; WS23002, WS23003).

Analgesic activity was assessed by the hot plate test, with the plate surface set at 55°C, and the rat's movement confined within an acrylic cylinder. Rats subjected to administration of test substances were individually placed on the plate and observed for abnormal behaviors such as licking the hind paw, stamping, and jumping over a duration of 1 min. A pain score was assigned: 1, licking and stamping; and 2, jumping. If no response occurred within 30 seconds, the rat was removed from the hot plate to prevent heat-related injury. Between each test, the hot plate surface was cleaned with 70% ethanol. The control value represents the mean pain score of vehicle-treated animals within each group. The pain score ratio compares the pain scores between the control group and the treatment group.

The hot plate test is a rapid and effective method for measuring acute thermal pain. However, it must take into account more complex behavioral characteristics compared to other pain assays. The latency time can be influenced not only by the analgesic effect but also by the rodent's genotype or learning through repeated measurements [12, 13]. Therefore, the experiment was performed only once per subject to minimize the impact of behavioral changes due to learning. The study did not consider the maximum and minimum values for data reliability.

Statistical analysis

The significance of differences among groups was analyzed by one-way analysis of variance (ANOVA) with Tukey's multiple comparison test using GraphPad Prism version 7.03 (GraphPad Software Inc., San Diego, CA, USA). The data was shown as the mean ± standard error of the mean (SEM). Statistical significance was set at $p < 0.05$.

3. Results

AM extract hydrogel patch formulation and patch preparation process

The formulation in this study utilized a hydrogel cataplasm, where water is the main component (Fig. 1A and B). This ensures excellent adhesiveness and allows the penetration of a significant amount of active ingredients for an extended period. As part of the patch preparation process, one portion of *Arnica montana* mother tincture was diluted with nine portions of water to prepare *Arnica montana* 1X. For the water solution, polyacrylic acid was dissolved in water, followed by the addition of *Arnica montana* 1X and tartaric acid, which were mixed thoroughly. The glycerol paste was prepared by

mixing glycerol, 1,2-hexanediol, polysorbate 80, titanium dioxide, aluminum glycinate, carmellose sodium, and sodium polyacrylate. This paste was then combined with the water solution to form a hydrogel. The hydrogel was spread between nonwoven fabric and a PET film, laminated, and cut into patches measuring 10 cm by 12 cm. These patches were aged for 24 h and stored in aluminum foil pouches. Finally, the product was analyzed to ensure it met specifications, including the identification of chlorogenic acid (Fig. 1C).

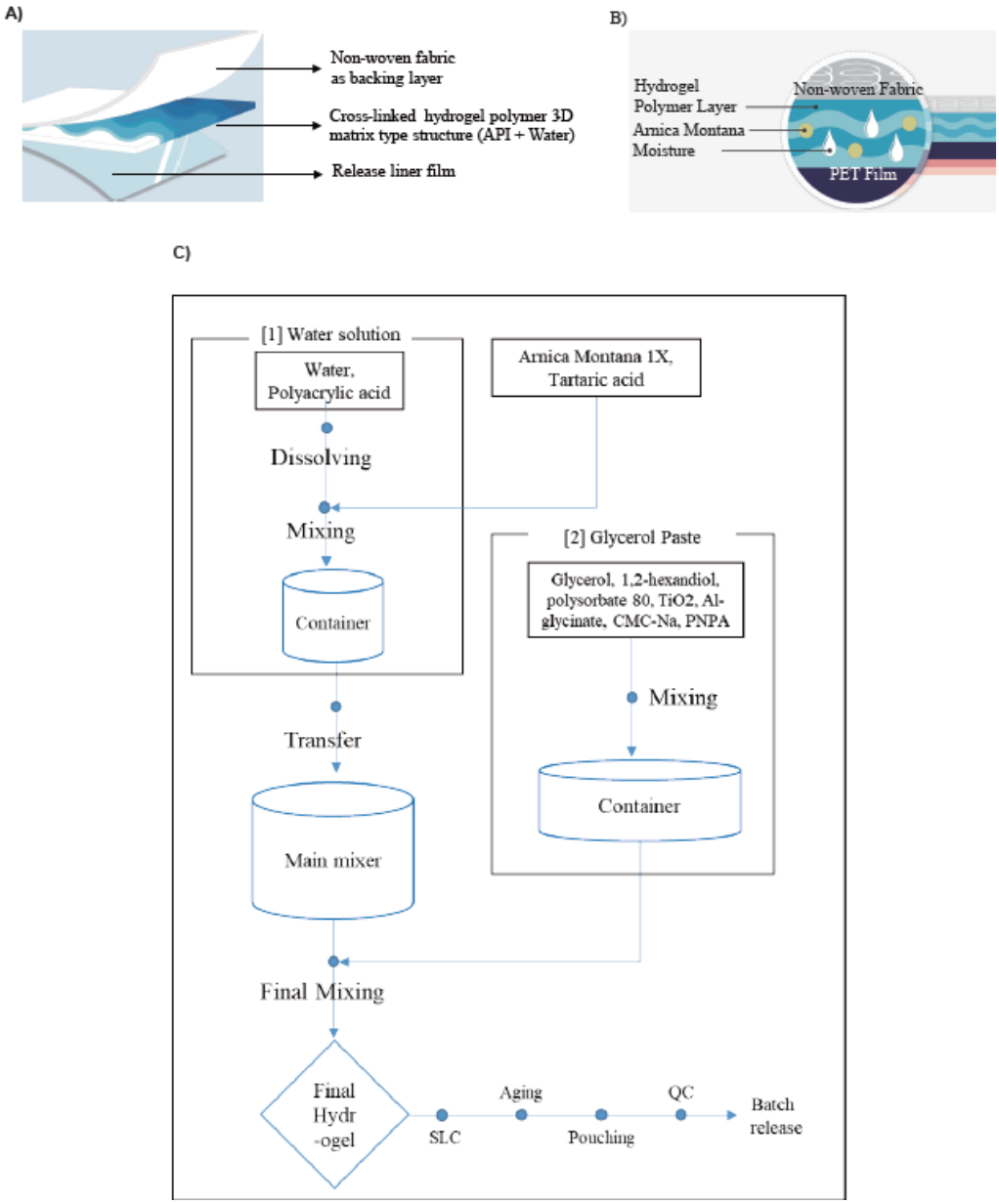

Figure 1. Schemes depicting the layers of AM extract hydrogel patch and formulation process chart

(A) A schematic illustrating the layers of the AM extract hydrogel patch.

(B) An active component layer depicting hydrated hydrogel polymer and an active ingredient.

(C) A patch preparation process flow.

Inhibition of carrageenan-induced edema formation

As an effort to assess the pharmacological effect of the newly developed AM hydrogel patch on inflammation, we employed Carrageenan-induced paw edema model. To assess its biological effect, we injected Carrageenan injection to mouse paws, we first examined morphology. The size of the fluid effusion and swelling elicited by Carrageenan increased at the 1 h time point and then gradually decreased afterward (Fig. 2A). When comparing the thickness of the right paw induced by Carrageenan, the AM Patch group treated for the first 2 days showed a decrease in the thickness of the initial paw edema induced by Carrageenan administration, at the third day, compared to the vehicle group. Moreover, it was confirmed that the AM Patch group had a more significant reducing effect than the AM Gel treatment group (Fig. 2B).

When compared not only based on the thickness difference in the right paw induced by simple edema but also by comparing the ratio with the left paw treated with saline, the AM Patch group exhibited a lower ratio in the size of the swelling than either vehicle or AM Gel treatment groups. Please note that the reason for the decrease in the right/left ratio at the 1 h time point was attributed to the temporary increase in thickness due to saline administration in the left paw, causing an increase not related to swelling but to the volume of saline (Fig. 2C).

A similar method of re-analysis based on the difference in thickness between the left and right paws showed consistent results. In the final assay stage (i.e., the 3 h time point on the third day), the left/right paw thickness difference was similar for vehicle and AM Gel treatment groups, whereas the AM Patch treatment group showed a statistically significant, compared to the AM Gel treatment group ($p=0.012$). Fluid effusion due to edema formation was confirmed by measuring the thickness of the paw. As previously mentioned, the size of edema induced over the long term was the lowest in the AM Patch group. Furthermore, regarding the changes in short-term paw edema on the third day, the AM Patch group showed a higher effectiveness compared to the AM Gel group (Fig. 2D).

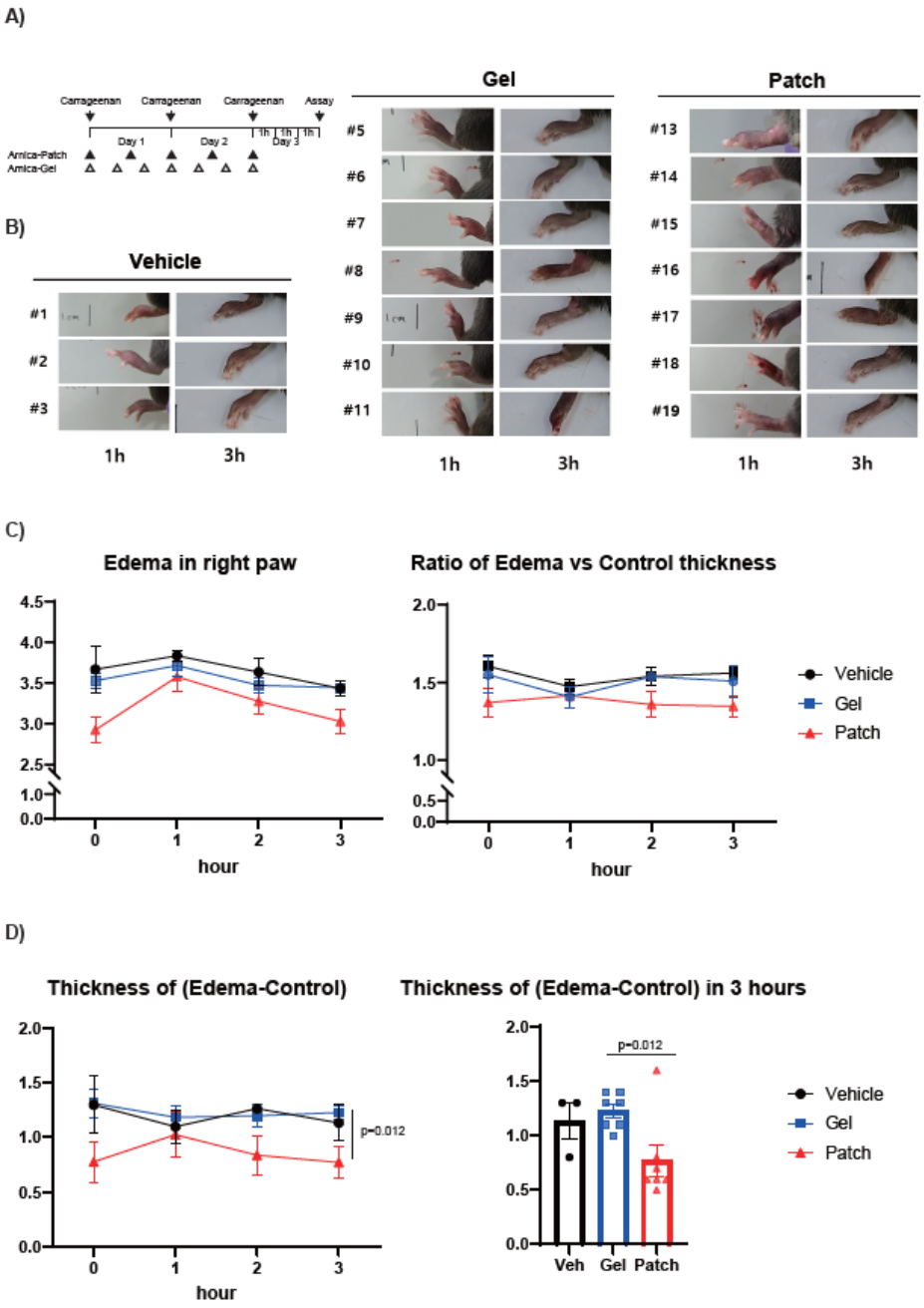

Figure 2. Inhibitory effects of AM extract hydrogel patch on carrageenan-induced edema formation in mice

(A) Experimental scheme.

(B) Appearances of paw edema in each group.

(C) Left: Thickness of the right paw induced with Carrageenan for edema formation. Right: Ratio of the thickness between the right paw induced with Carrageenan and the left paw treated with Saline (Control).

(D) Left: Time-dependent difference in thickness between the right paw induced with Carrageenan and the left paw treated with saline (Control). Right: Difference in

thickness at the 3-h time point between the right paw induced with Carrageenan and the left paw treated with saline (Control).

Group 1 (n=3), Carrageenan + Saline (Vehicle); Group 2 (n=7), Carrageenan + Arnica Gel (Gel); and Group 3 (n=7), Carrageenan + Arnica Patch (Patch).

For C and D, values were expressed as mean ◎ SEM (*P < 0.05, **P < 0.01). Statistical significance was tested via two-tailed Student's t-tests.

Hematoxylin and eosin analyses of paw edema tissue

In the histological examination (H&E staining) of the paw sections, Carrageenan injection resulted in edema formation, as evidenced by a wide and mild tissue composition, overall the microscopic visual fields. Consistently, a significant increase in neutrophils infiltration was evident in the Carrageenan treatment group. Notably, the AM gel treatment group displayed suppression in this effect, demonstrating a clear inhibition of inflammatory cell infiltration from blood vessels. In this group, the tissue composition became denser with the efficacy of reducing inflammatory cell infiltration being more pronounced, compared to the other groups (Fig. 3).

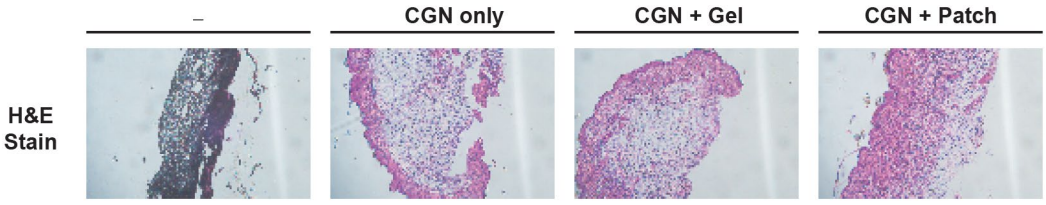

Figure 3. Representative H&E staining images of paw edema tissue in the carrageenan-induced inflammation animal model

Inhibition of inflammatory transcript marker levels

Subsequently, RT-PCR assays were done on the inflammatory markers for *Tnfa, Il1b, and Il6* using the right paw of the mice. Notably, the AM Patch group showed statistically significant ~40% lower *Tnfa* levels compared to the Gel group. However, there was no significant difference in *Tnfa* levels between vehicle group (i.e., Carrageenan only) and the AM Gel treatment group. The expression levels of *Il1b* were significantly lower by more than 60% in both the Arnica Gel and Arnica Patch groups compared to the Carrageenan-only group (Fig. 4). Again, the AM Patch treatment group demonstrated superior effectiveness, showing an approximately 80% inhibition. By the same token, the AM Patch treatment group exhibited statistically significant 40% lower expression in *Il6* compared to vehicle-treated control. However, no significant difference existed between vehicle and the AM Gel group. The paw of the saline vehicle group, where Carrageenan was not administered, did not induce swelling, and thus the inflammatory markers were almost non-existent.

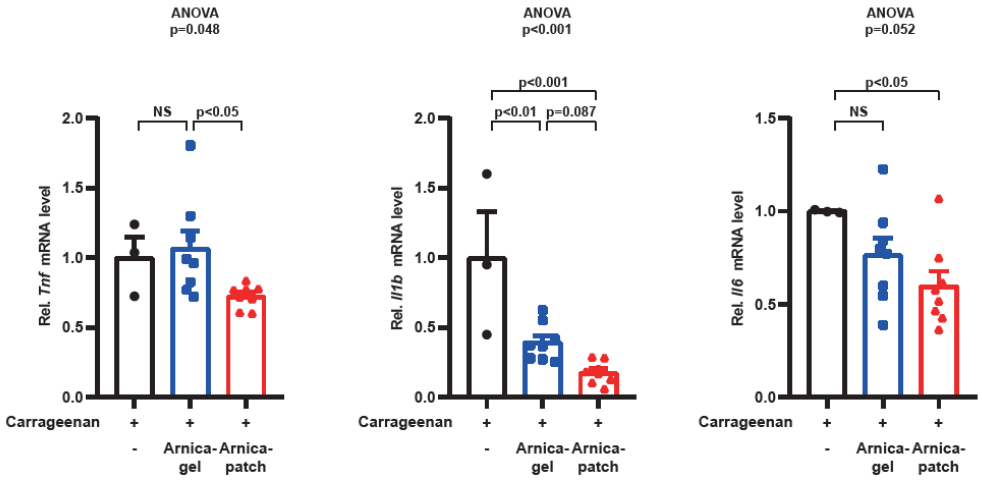

Figure 4. Inhibitory efficacy of AM extract hydrogel patch on inflammatory markers within paw edema in carrageenan-induced inflammation mouse model

RT-PCR assays were conducted for Tnfa(TNF), Il1b, and Il6 mRNA levels in the tissue of the right paw induced with Carrageenan. Group 1 (n=3), Carrageenan + saline (-); Group 2 (n=7), Carrageenan + Arnica gel (Gel); and Group 3 (n=8), Carrageenan + AM extract hydrogel patch (Patch). Statistical significance was tested via one-way ANOVA coupled with Bonferroni's method or the LSD multiple comparison procedure when appropriate. Values were expressed as mean ⊚ SEM (*P < 0.05, **P < 0.01).

Pain relief effect of AM Patch in hot plate test

Having identified the *bona fide* anti-inflammatory effects of the AM Patch, we were lastly examined its pain-relieving effect, as monitored by Hot plate test in rats. One or two hours after the AM patch or the AM gel administration, the hot plate test was done to assess pain scores in the control and the administration groups. As expected, the AM hydrogel patch treatments significantly reduced pain scores at either 1 or 2 h after treatment. However, the AM Gel treatment group failed to show significant changes as compared to control (Fig. 5).

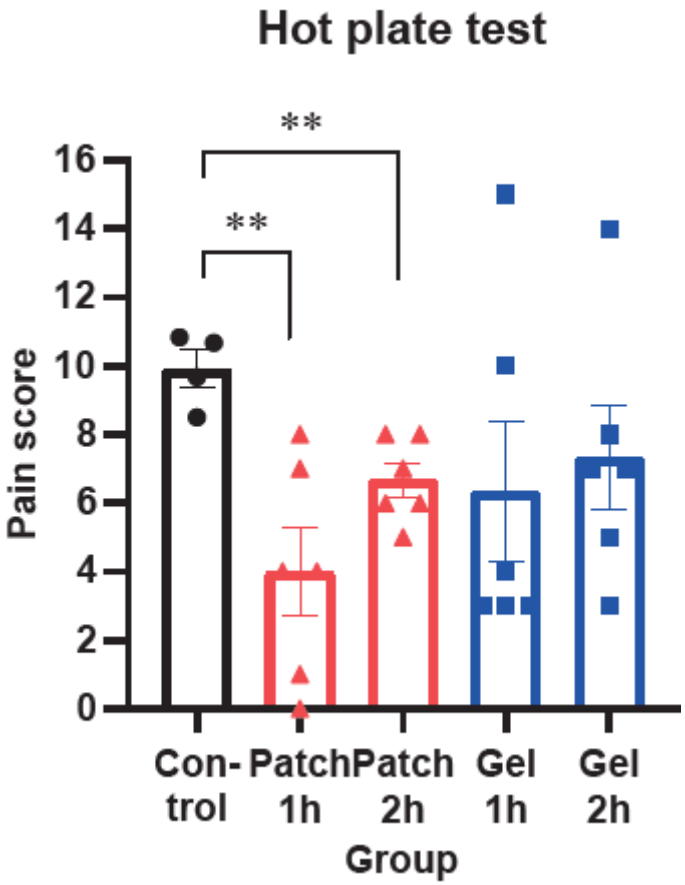

Figure 5. Pain relief effect of AM extract hydrogel patch in hot plate test using rats

Rats were subjected to hot-plate test, and pain scores were assessed 1 h or 2 h after AM extract hydrogel patch (Patch) or Arnica gel (Gel) administration. The pain score ratio was obtained from those from the control group and the treatment groups (n = 6 rats per group). Values were expressed as mean ⊚ SEM (**p < 0.01 vs. Control).

In the cross-sectional H&E staining of the paw induced by Carrageenan (CGN), overall tissue organization was characterized by a significant increase in the infiltration of inflammatory cells (neutrophils) in the Carrageenan-only group. This phenomenon was suppressed in the AM extract hydrogel patch group (Patch), indicating an inhibition of inflammatory cell infiltration. According to the tissue image analysis of AM extract hydrogel patch group, the tissue structure becomes denser, demonstrating a pronounced efficacy in reducing inflammatory cell infiltration compared to other groups.

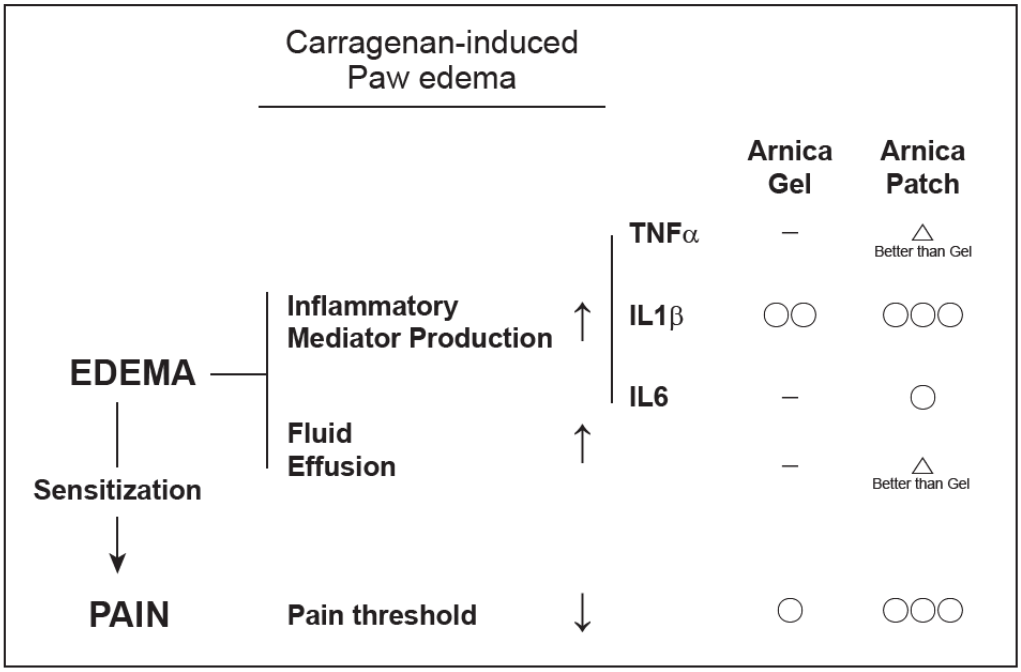

Figure 6. A diagram illustrating the anti-inflammatory and pain-relieving effects of AM extract hydrogel patch (Arnica Patch) and Arnica Gel

4. Discussion

This study systematically explored the anti-inflammatory and analgesic potential of a hydrogel patch containing *Arnica montana* (AM) extract, emphasizing its superiority over a gel formulation through various pharmacological, histological, and molecular evaluations. The findings clearly demonstrate that the hydrogel patch offers distinct advantages in reducing inflammation, alleviating pain, and providing sustained therapeutic effects.

The carrageenan-induced paw edema model, a well-established method for assessing acute inflammation, highlighted the hydrogel patch's enhanced efficacy. The patch significantly reduced paw thickness and inflammatory cell infiltration compared to the gel. These outcomes were supported by molecular analyses showing robust suppression of key inflammatory cytokines, including Tnfa, Il1b, and Il6. The downregulation of Il6, in particular, was unique to the hydrogel patch and not observed with the gel formulation, suggesting a mechanistic advantage that underpins its superior anti-inflammatory properties. Histological findings corroborated these results, revealing reduced tissue damage and inflammatory cell recruitment in the hydrogel patch-treated group. This sustained anti-inflammatory effect can be attributed to the patch's prolonged delivery mechanism, which ensures a steady release of active ingredients over time, unlike the gel formulation, which may lose efficacy due to faster absorption or evaporation.

An innovative feature of the hydrogel patch is its dual-action sensory effect. Unlike traditional cryotherapy, which relies on actual temperature changes to reduce tissue damage, the hydrogel patch provides a perceived cooling sensation coupled with an exothermic effect mediated by nerve receptor activation. This mechanism, akin to the action of vanillyl butyl ether (VBE), enhances the patch's analgesic properties by targeting nerve endings directly. This sensory feedback not only improves user experience but also augments pain management by providing a soothing and warming sensation, which could be particularly beneficial for patients suffering from musculoskeletal or neuropathic pain. The absence of actual temperature changes further reduces the risk of cold burns or discomfort, making the patch suitable for prolonged use.

In the hot plate-induced pain model, the hydrogel patch demonstrated superior analgesic effects compared to the gel formulation. The patch provided sustained pain relief, likely due to its controlled-release mechanism and superior skin adhesion, which allow for consistent delivery of active compounds. By contrast, the gel formulation failed to maintain comparable pain relief, underscoring the limitations of traditional formulations in delivering prolonged therapeutic effects. This finding is particularly important for clinical scenarios requiring long-term pain management, where a consistent therapeutic effect is critical for patient comfort and recovery.

The study also addressed several limitations associated with traditional topical therapies, such as skin irritation, residual solvent toxicity, and unreacted monomers. The hydrogel patch, formulated with partially neutralized polyacrylic acid and biocompatible crosslinking agents, mitigates these risks while offering a safe and user-friendly application. Furthermore, its design ensures minimal interference with daily activities, enhancing patient compliance and satisfaction. The patch adheres well to the skin, provides sustained relief, and avoids the drying or cracking often associated with conventional gels or plasters.

An additional advantage of the hydrogel patch is its potential for customization. The formulation could be expanded to include other pharmacologically active compounds, such as ketoprofen, ibuprofen, diclofenac, or lidocaine, to further enhance its therapeutic scope. This adaptability positions the hydrogel patch as a versatile platform for addressing a wide range of inflammatory and pain-related conditions. Incorporating such compounds could also broaden its applications in treating chronic conditions like arthritis or neuropathic pain, where a combination of anti-inflammatory and analgesic effects is often required.

The findings of this study align with the long-standing use of *Arnica montana* in traditional medicine, where it has been utilized for its anti-inflammatory and analgesic properties. However, the hydrogel patch formulation overcomes many limitations of traditional applications, such as variability in dosage and absorption. The modern formulation ensures controlled release, consistency in therapeutic outcomes, and reduced risk of adverse effects, such as skin irritation or systemic toxicity.

In conclusion, the *Arnica montana* hydrogel patch offers a novel and effective approach to managing inflammation and pain, combining traditional herbal medicine with modern pharmaceutical technology. Its ability to provide sustained relief, coupled with its safety and user-friendly design, addresses key challenges in topical therapy. These findings not only validate the potential of *Arnica montana* in modern medicine but also pave the way for further development of advanced topical formulations tailored to diverse clinical needs.

5. Conclusions

Overall, our findings highlight the significant therapeutic potential of the *Arnica montana* hydrogel patch as a modern and effective approach to managing inflammation and pain. Compared to the conventional gel formulation, the hydrogel patch demonstrated superior efficacy in reducing carrageenan-induced paw edema, suppressing inflammatory mediators, and alleviating pain in preclinical models. The robust inhibition of key cytokines such as Tnfa, Il1b, and Il6, along with reduced inflammatory cell infiltration, underscores the patch's potent anti-inflammatory properties.

The hydrogel patch's innovative dual-action sensory effect, delivering both cooling and perceived exothermic sensations, enhances its therapeutic utility by improving user experience and augmenting pain relief. Its controlled-release mechanism ensures sustained delivery of active ingredients, making it particularly suitable for conditions requiring prolonged therapeutic action. Moreover, the patch formulation successfully addresses limitations of traditional topical treatments, such as skin irritation, toxicity from residual solvents, and poor adhesion, offering a safer and more convenient option for patients.

Our findings also suggest that the hydrogel patch has potential for further optimization by incorporating additional active pharmaceutical ingredients, such as ketoprofen, ibuprofen, diclofenac, or lidocaine, to broaden its applications in pain and inflammation management. This adaptability positions the hydrogel patch as a versatile platform capable of addressing diverse clinical needs, from acute injuries to chronic inflammatory conditions.

In summary, our findings demonstrate that the *Arnica montana* hydrogel patch represents an advanced therapeutic modality that combines the traditional benefits of herbal medicine with modern pharmaceutical technology. Its ability to provide sustained and effective relief, coupled with its safety and patient-friendly design, underscores its promise as a valuable addition to the field of topical anti-inflammatory and analgesic therapies. Further research and clinical development are warranted to fully realize its potential in broader medical contexts.

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Institutional Review Board Statement: The animal care and studies were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at Dongguk University (No. IACUC-2021-035-2) and Wooshin Labottach Co., Ltd., Ethical Committee for Animal Experimentation (Approval number; WS23002, WS23003).

I**nformed Consent Statement:** Not applicable.

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Conflicts of Interest: The authors have declared that SGK serves as a consultant for the Wooshin Labottach Co. company, and that TSN, SY, DI, KK, BG, and GN hold stocks for Wooshin Labottach Co.

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