

Development and evaluation of a Chinese herbal gel for analgesic and anti-inflammatory effects

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Abstract: This study aimed to develop a film-forming gel containing three Chinese herbal extracts, an extracted mixture of *Corydalis yanhusuo*, *Cynanchum paniculatum* and *Armadillidium vulgare* (Latreille) (MCCA), and evaluate its analgesic and anti-inflammatory activities. Using the Box Behnken Design, the optimal prescription for the MCCA gel was determined. The analgesic effects were tested through acid writhing and formalin pain models, while the rheumatoid arthritis model assessed the pain and anti-inflammatory effects. For the evaluation of the effect of MCCA gels on pro-inflammatory cytokines, as well, Elisa was used. Results showed that the MCCA Gel with 2% mint oil had the highest transdermal volume of $32.57 \pm 0.92 \mu\text{g}/\text{cm}^2$. High doses of MCCA gel were effective in suppressing pain, with a pain inhibition rate of 54.37% during the acetic acid peristaltic test that showed pronounced inhibition in the second phase of the formalin-induced analgesia test. In the rheumatoid arthritis model, the MCCA gel reduced inflammation scores in rat feet and decreased the expressions of four inflammatory factors in serum. Generally, The MCCA gel exhibits noticeable pain-relieving and anti-inflammatory properties with high penetration with the skin.

Keywords: *Corydalis yanhusuo*, *Armadillidium vulgare* (Latreille), *Cynanchum paniculatum*, film-forming gel, anti-inflammatory.

INTRODUCTION

Aches and pains, displeasing sensations, or mood experiences stemming from tangible or underlying organisational lesions (Katole 2022; Raja 2020), has become one of the most serious clinical problems afflicting human beings. It is common in the course of various diseases, such as rheumatoid and osteoarthritis pain, cancer pain, trauma pain, and so on, which bring serious physiological pain to patients. Moreover, with the increase of obese people and elderly people, treating and managing such diseases have become a huge burden on society (Widenka 2021). Therefore, a safe and efficient analgesic preparation with small side effects is urgent to be developed.

Chemical analgesics mainly include NSAIDs as well as narcotic analgesics. NSAIDs, one of the most extensive prescription drugs in the world, are widely used for the relief of osteoarthritis, rheumatoid arthritis, multiple fevers, and various pain symptoms (Atkinson 2020). However, long-term use of NSAIDs can cause adverse reactions. On the one hand, It can cause detrimental impact on the gastronomic intestinal tract, which may include feelings of nauseousness, vomiting and even upper gastrointestinal injury like perforation, ulcers and bleeding; on the other hand, it can cause adverse effects on the cardiovascular system, including elevated blood pressure, arterial thrombosis (Kalita 2021).

Corydalis yanhusuo (Cy), extracts would have been employed as analgesics in traditional Chinese medicine for centuries. The study established several pain models, they demonstrated that the extract of Cy have been found as an effective alleviator of chronic, infectious and debilitating conditions while not promoting any tolerability.

Armadillidium vulgare (Latreille) (Av), it extract serves as a remedy for conditions such as wound injuries, painful wounds, cutaneous warts, inflammation of the mouth, and soreness associated with cancer (Li 2020). The number of crepitations in the acetic acid-induced crepitations was markedly reduced in mice treated with Av administered orally in the acetic acid-induced crepitations test. In the second phase of the formalin-induced nociceptive study, paw withdrawal was inhibited and the present investigation revealed a strong anti-inflammatory effect in all these models of inflammation.

As traditional Chinese medicine, *Cynanchum paniculatum* (Cp) relieves rheumatic arthralgia, lumbago, and pain due to traumatic injuries (Zhou 2020). The typical pharmacological active ingredient, phenol, is the chemical compound most abundant in the volatile oil of Cp, the extract has a variety of anti-inflammatory activities to treat conditions such as arthritis and soft tissue damage (Wang 2020). Ethyl acetate extract (20, 40mg/kg) has an obvious inhibitory effect on the licking time of formalin-induced inflammation mice but has no inhibitory effect on the early stage. In addition, an oral dose of 40 mg/kg ethyl

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acetate extract significantly reduced paw swelling in rats with arachidonic acid-induced paw swelling and the effect was related to COX and lipoxygenase (LOX) pathways (Chen 2020). Paeonpo reduces IL-1 β -induced chondrocyte apoptosis by inhibiting the Wnt/ β -catenin signaling pathway. Expenditure of inflammatory factors and associated MMPs, and promotes expression of SOX-9 and Col-II proteins, thus protecting against IL-1 β -induced chondrocytes damage (Wu 2021).

MATERIALS AND METHODS

Materials

Standard tetrahydropalmatine, ammonia water, Anhydrous ethanol, Phosphate, Triethylamine, Methanol, Carbomer, CMC-Na, Gelatin, Sodium alginate, Glycerol, Polyvinyl alcohol, PVP, HPMC, Ethyl hydroxybenzoate, Propylene glycol, Azone, NMP, Mint oil, Phosphate, Triethylamine and Glacial acetic acid (AR, Shantou Xilong Science Co., Ltd., China); Complete Freund's adjuvant (Chondrex, USA); Diclofenac Diethylamine emulgel (GlaxoSmithKline, UK); Elisa kit (IL-1 β), Elisa kit (IL-6), Elisa kit (TNF- α) and Elisa kit (PGE2) (AR, Shanghai Yuanye Biotechnology Co., Ltd., China); SD rats, BALB/c mice. All animal experiments have been approved by the Animal Ethics Committee of the Ministry of Health of China (Number of permit: SY201909003).

Preparation of MCCA gel

After obtaining mixture from *Cy*, *Av* and *Cp* (MCCA), the selective components of the herbs and adjuvants were subjected to single-factor and Box-Behnken Design (BBD) optimization experiments depending on the dosing rules of the traditional Chinese medicine prescriptions, resulting in the production of MCCA gel, the procedure of which is shown in fig. 1 (Weremfo 2023). One of the main components of MCCA extract, corydalis B, was selected as a marker for investigation and the results showed that the content of tetrahydropalmatine in the gel was higher than 160 μ g/g.

Optimization of MCCA Gel preparation method

In vitro skin permeation test

An in-vitro skin permeation test of SD rats was carried out to study the permeation effect of active components of MCCA gel. And appropriate penetration enhancers were selected to improve the permeation effect. After the selected healthy male SD rats were treated with euthanasia, the rat hair from the abdomen was removed and the skin fat and subcutaneous tissue were evenly scraped off. The isolated skin was flushed repeatedly with saline and then fixed between the supply chamber and the receiving chamber, with the cuticle upward, and dermis downward. With ethanol: normal saline = 5:1 as the receiving solution, the stirring speed of the transdermal absorber was set to 300 r/min, and the water bath temperature was set to 37 \pm 1 $^{\circ}$ C. 0.2g MCCA gel was

evenly applied to the skin surface in vitro. After the gel formed the film, 5.0 mL receiving solution was added to the receiving chamber. After clearing the bubble, 1.0mL receiving solution at 1h, 2h, 4h, 6h, 8h, 10h, 12h and 24h was taken out respectively. And the same volume of fresh receiving solution preheated to 37.0 $^{\circ}$ C was immediately added at the sampling port, and the receiving solution at each time point was filtered over a 0.45 μ m filter membrane. The corydalis B content was determined by HPLC. The cumulative transdermal volume Q (μ m \cdot cm $^{-2}$) per unit area of corydalis B was calculated by formula. With Q as the horizontal coordinate and t as the vertical coordinate, the Q - t curve was drawn. The effects of propanediol, oleic acid, Azone, NMP and mint oil were investigated.

Optimization of the preparation prescription of MCCA Gel by Box Behnken Design

Taking the adjuvants like CMC-Na, PVP, and penetration enhancer content as the factors for investigation, and the comprehensive score of MCCA gel performance as the evaluation index. The calculation formula which A is 40/cumulative transdermal volume of a maximum unit * cumulative transdermal volume per unit area, B is appearance score, and C is minimum film-forming time * 30/film-forming time was as follows: $Y=A+B+C$

Optimization of MCCA Gel preparation method

Acetic acid writhing test

First of all, there were 32 mice divided into four groups ($n=8$) for the acetic acid-induced writhing test. After 3 days of feeding, an area of 2cm \times 3cm was shaved on the back of the mice with an animal hair eliminator. After uniform administration was given, the mice were fixed with pressure-sensitive adhesive tape. After 3 days of continuous administration, and after 1h of the last administration, the intraperitoneal injection of 0.6% acetic acid solution was performed with 10 mg/kg of injection volume. Then the writhing times of the mice within 15 min were observed. The inhibition rate was formulated in which A_1 represents the average writhing times in the control group and A_2 represents the average writhing times in the administration group.

$$\text{Inhibition rate} = (A_1 - A_2) \div A_1 \times 100\%$$

Formalin experiment

A formalin experiment was performed on mice according to the same group administration method. After 1h of formalin administration, 20 μ L 1% formalin solution was injected into right posterior feet of the mice. The time for licking right posterior feet during 0~5 min and 15~30 min was recorded.

Rheumatoid arthritis experiment

A rheumatoid arthritis model of male SD rats was constructed by complete Freund's adjuvant (Zhou 2016). Specifically, 0.02 mL (10 mg/mL) complete Freund's

adjuvant was administered to the left posterior feet of the male SD rats (Hao 2019). Seven days later, an area of 4 cm*4 cm on the back of the male SD rats was shaved. Before the construction of the model, weighing, measuring, grading and photographing of the left posterior feet thickness were conducted daily. After 10 days of the administration, the orbital blood collection of SD rats was performed. After adding 1% heparin sodium, an anticoagulant, 5000 rpm/min centrifugal treatment was carried out. The supernatant was collected to determine the expression of inflammatory factors by Elisa kit.

STATISTICAL ANALYSIS

All experiments were triplicated. Results are expressed as mean standard deviation. Design Expert 8.0.6 software, SPSS version 16.0 and GraphPad (Prism 8, California, USA), respectively. One-way ANOVA of variance was adopted to determine the variability of the findings, with a significance level of $P<0.05$.

RESULTS

In vitro skin permeation test

The *in-vitro* transdermal influence of corydalis B against time is shown in fig. 2. Different kinds and concentrations of penetration enhancers had different penetration promotion effects on MCCA gel. The cumulative transdermal volume of corydalis B in all groups was increased and higher than that in the non-penetration enhancer group. Compared with different kinds of penetration enhancers, 2% mint oil had the best penetration promotion effect, with 24h cumulative transdermal volume of $32.57\pm0.92 \mu\text{g}/\text{cm}^2$. In addition, we inspected the influence of the combined skin penetration enhancers and as shown in fig. 3, the cumulative 24-hour per unit area transdermal volume was higher in all penetration enhancement groups than in the non-penetration reinforcement groups. The 1% mint oil + 1% NMP group had the best penetration promotion effect, with 24 h cumulative transdermal volume per unit area of $31.12\pm1.21\mu\text{g}/\text{cm}^2$. Therefore, 2% mint oil had the best penetration promotion effect compared to others, the reason may be that it destroys the lipid structure between keratinocytes and promotes the expansion of the epidermal cell gap, thus reducing the blocking effect of skin on foreign drugs (Zhao 2022).

Box-Behnken design assay

The preparation prescription of MCCA gel was optimized by Design Expert 8.0.6 software. The design table is shown in table 1. According to the design prescription, MCCA gel was prepared and comprehensively scored. A 3D response surface map and 2D contour map are shown in fig. 4. The *in vitro* transdermal test was carried out to investigate the 24h cumulative transdermal volume and score it. The results showed that A (CMC-Na) and C

(Mint oil) had a significant effect on the comprehensive score of MCCA gel ($P<0.05$) and PVP had a non-significant effect on the comprehensive score of MCCA gel ($P>0.05$), AB, BC ($P<0.05$), with the determination coefficient of $R^2=98.84\%$ and the adjusted determination coefficient of $R^2_{\text{Adj}}=97.35\%$. The above data showed that the model had good accuracy. The simulation lack of fit was $P=0.1910>0.05$, with no significant difference, which indicated that the model had a good fitting degree. The fitting equation was: $Y=89.98-1.85A-0.28B-4.04C-7.56AB-1.84AC-2.46BC-13.57A^2-11.96B^2-8.69C^2$. It showed that there was the highest response value on the response surface, that is, there was a maximum value in the comprehensive score of preparation, which was predicted to be 90.50. Optimal prescription: CMC-Na=1.94%, PVP=1.53%, Mint oil=1.77%. The verification of the optimal prescription found that the average comprehensive score was 89.44, $RSD=1.44\%$, and relative error=1.17%. It showed that the model can predict the optimal prescription of MCCA gel. The gel is prepared as a brown viscous semi-solid preparation, as shown in fig. 5. After applying the gel for about 160s, the film will be formed, as shown in fig. 6.

Pharmacodynamics

The results of the acetic acid writhing test are shown in fig. 7. Compared with the control group, the writhing times in the high-dose MCCA gel group were significantly suppressed. The pain inhibition rate, as shown in table 2, also reached 47.24% and 54.37%, respectively. The inhibition rate in the high-dose MCCA gel group was higher than that in the positive control group. The pain reaction induced by formalin was divided into two stages in the formalin experiment. 0~5 min was the first stage when the pain was caused by stimulating local nerve endings, and 15~30 min was the second stage when the pain was caused by the release of inflammatory mediators, as shown in fig. 8. Compared with the control group, MCCA gel had an obvious inhibitory effect on the pain caused by the release of inflammatory mediators and had no obvious effect on the pain caused by stimulating local nerve endings.

The measurements of feet thickness of SD rats are shown in fig. 9. After 7 days after modeling, the left posterior feet thickness of rats applied with blank gel increased, indicating that the inflammation and swelling were uncontrolled. Compared with the model group, the therapeutic effect of the Diclofenac Diethylamine positive group was significant. The MCCA gel had an inflammatory inhibition effect in the low- and high-dose MCCA gel groups and the therapeutic effect of the high-dose MCCA gel group was significant. The results of the body weight change of rats were shown in fig. 10. The body weight of rats in each group increased steadily, indicating that there was no obvious toxicity in the dosage preparation.

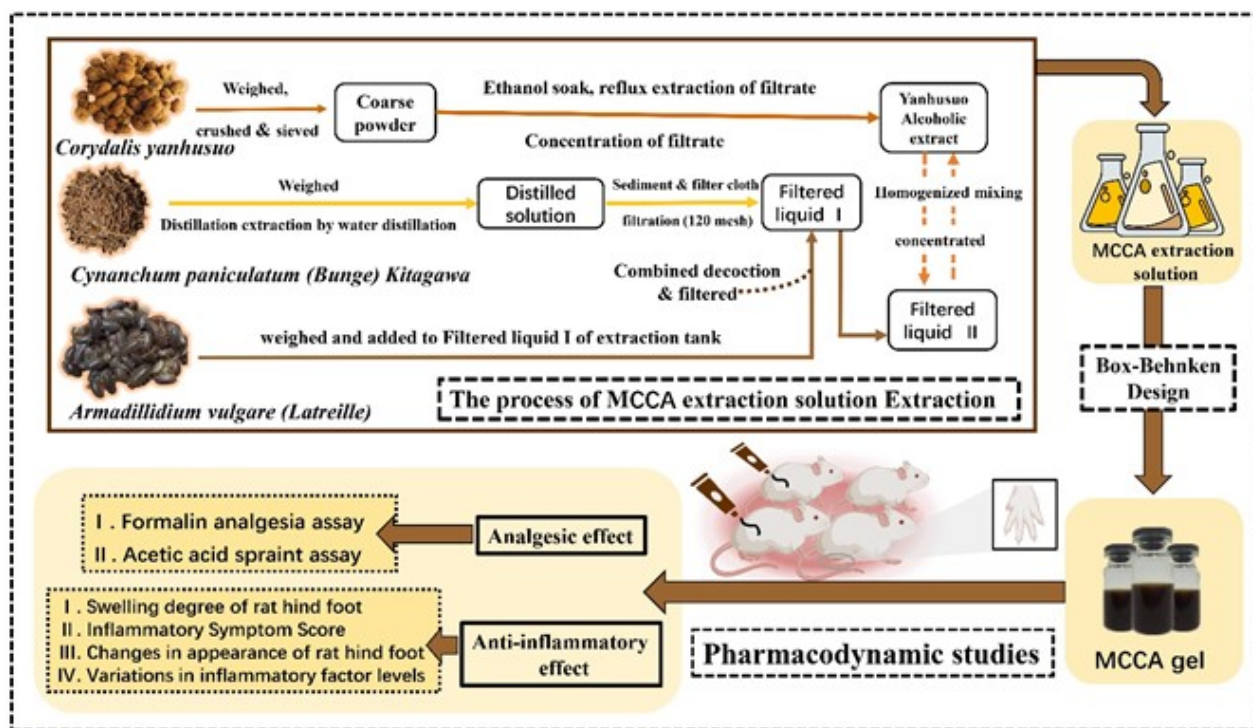


Fig. 1: The procedure of MCCA Gel

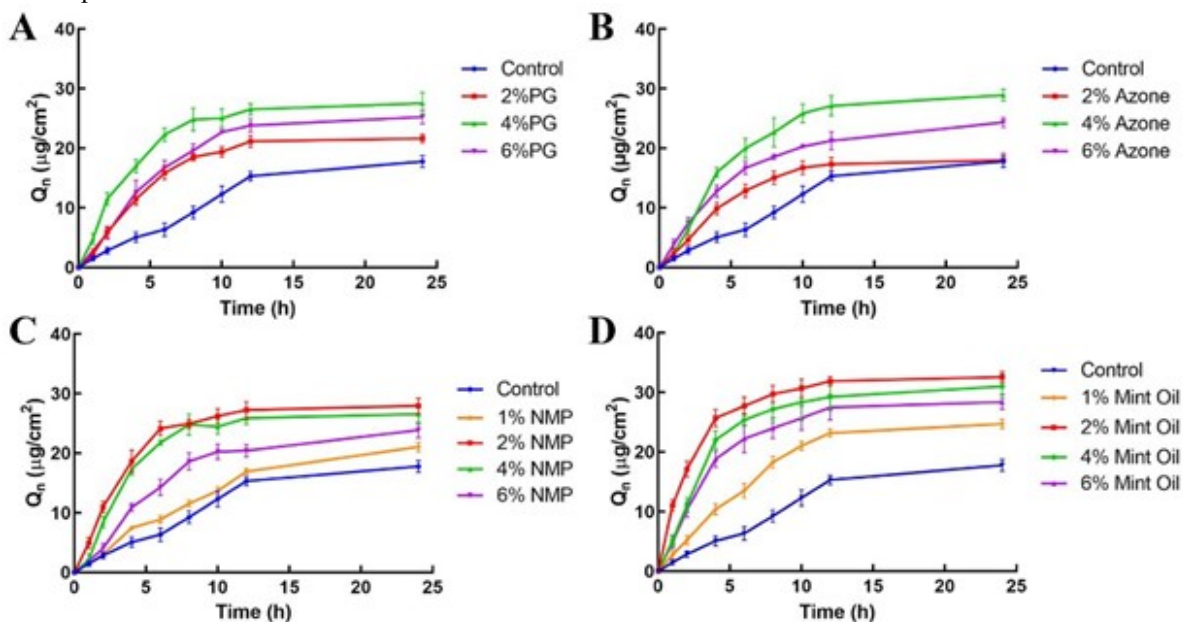
Fig. 2: *In vitro* transdermal curves of corydalis B (A) different concentrations of PG (B) different concentrations of Azone (C) different concentrations of NMP (D) different concentrations of Mint Oil.

Table 1: BBD design table about MCCA gel

Factor	Levels		
	Low (-1)	Medium (0)	High (+1)
CMC-Na	1.0%	2.0%	3.0%
PVP	0.5%	1.5%	2.5%
Mint Oil	1.0%	2.0%	3.0%

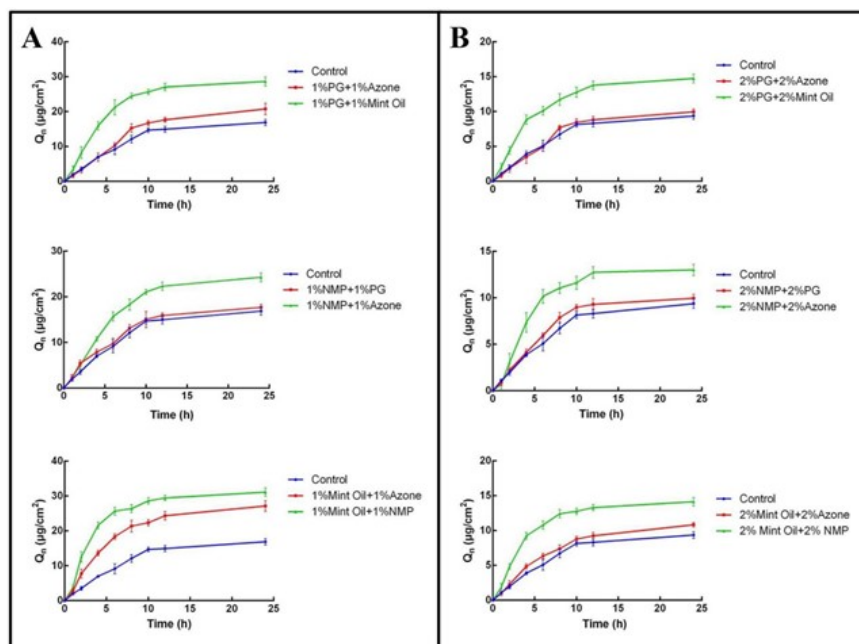


Fig. 3: *In vitro* transdermal curves of corydalis B (A With 2% Complex, skin penetration enhancer (n=3), B With 4% Complex skin penetration enhancer)

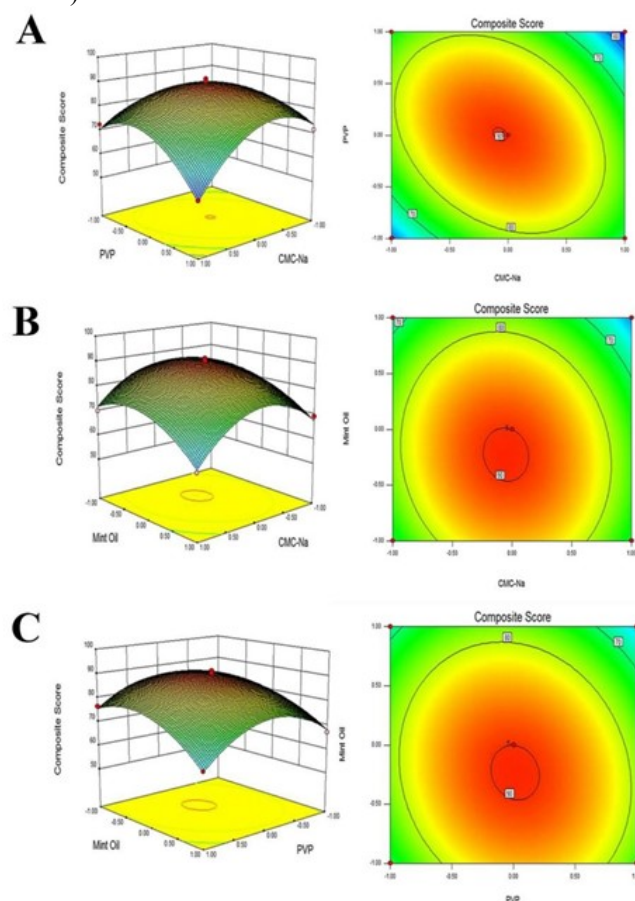


Fig. 4: (A)Effect of the Interaction of CMC-Na and PVP on the Comprehensive Score of MCCA gel (B) Effect of the Interaction of CMC-Na and Mint Oil on the Comprehensive Score of MCCA gel (C) Effect of the Interaction of PVP and Mint Oil on the Comprehensive Score of MCCA gel and Mint Oil on the Comprehensive Score of MCCA gel.

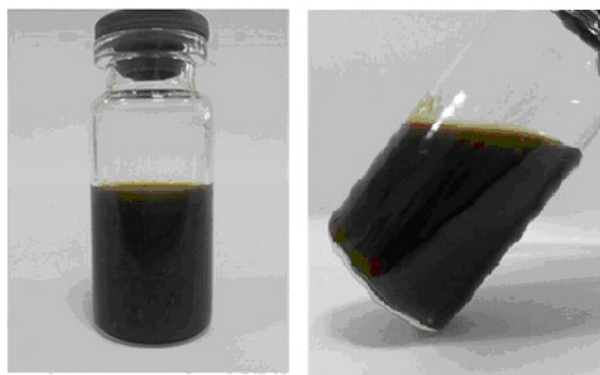


Fig. 5: The appearance of MCCA gel

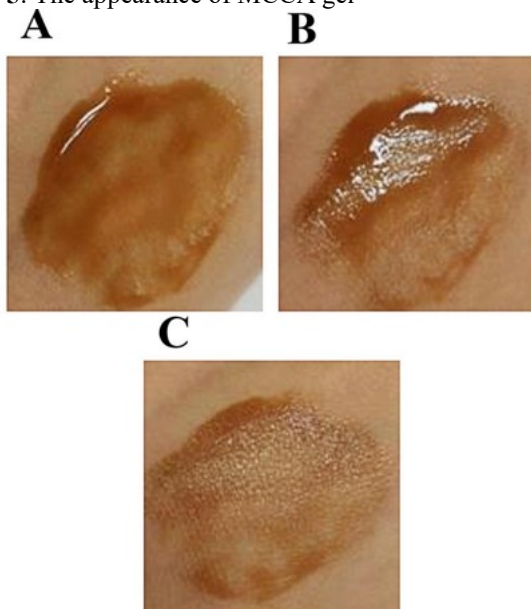


Fig. 6: Formability of MCCA gel (A) After evenly applying (B) After the 60s of application (C) After 160s of application.

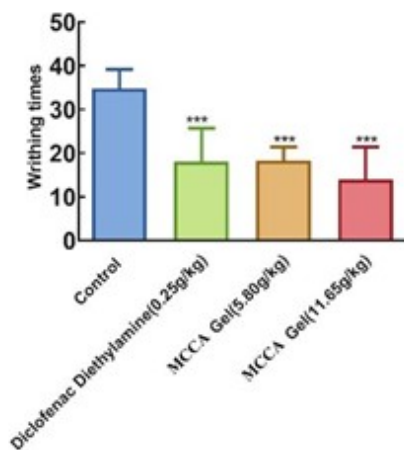


Fig. 7: Number of writhing times (n=8, *P<0.05, **P<0.01, ***P<0.001, Diclofenac Diethylamine, MCCA gel (5.80g/kg), MCCA gel (11.65g/kg) separately compared with Control.

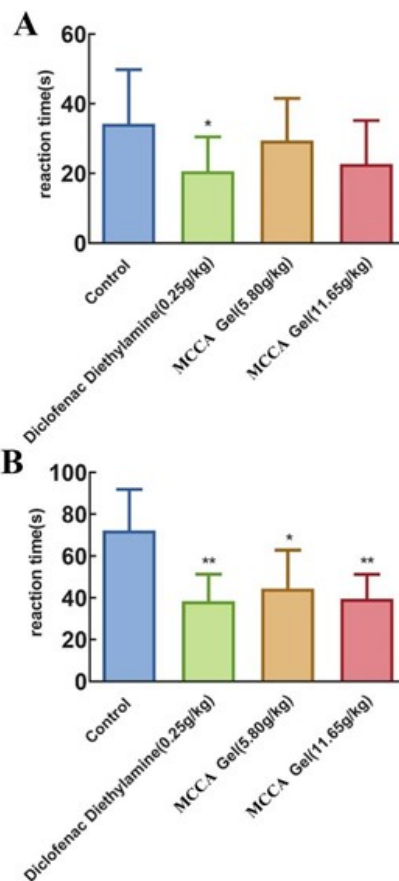


Fig. 8: Result of formalin experiment (A) first stage (0-5min) (B) Second stage (15-30min) (n=8, *P<0.05, **P<0.01, ***P<0.001, Diclofenac Diethylamine, MCCA Gel (5.80g/kg), MCCA Gel (11.65g/kg) separately compared with Control)

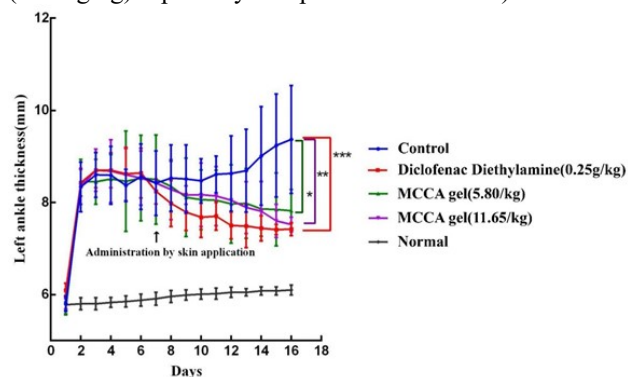


Fig. 9: Feet thickness of rats immunized with complete Freund's adjuvant (n=6, *P<0.05, **P<0.01, ***P<0.001, Diclofenac Diethylamine, MCCA Gel (5.80 g/kg), MCCA Gel (11.65 g/kg) separately.

The scoring of arthritic rats is shown in fig. 11. After successful modeling, the scores increased significantly. After a week, the score of the control group where the rats were applied with the appropriate amount of blank gel was almost unchanged, indicating that the disease process

was not changed. Versus control group, the score of the Diclofenac Diethylamine positive group decreased significantly ($P<0.001$). The score of the low- and high-dose MCCA Gel groups also decreased ($P<0.05$), which indicated that MCCA Gel had an inhibitory effect on arthritis. The posterior feet appearance images of rats were intuitive, as shown in fig. 12. After treatment, the posterior feet swelling of rats in the Diclofenac Diethylamine positive group and the MCCA Gel groups was reduced seen by the naked eye, which was consistent with the feet thickness measurement and inflammation.

The experiment results of inflammatory factor content in the plasma of rats are shown in fig. 13. Versus the model group, the performance of inflammatory factors such as IL-1 β , IL-6, TNF- α and PGE2 in the plasma of rats in the diclofenac diethylamine-positive group and the high-dose MCCA gel group was dramatically reduced. It showed that MCCA Gel may inhibit inflammation and inflammation-induced pain.

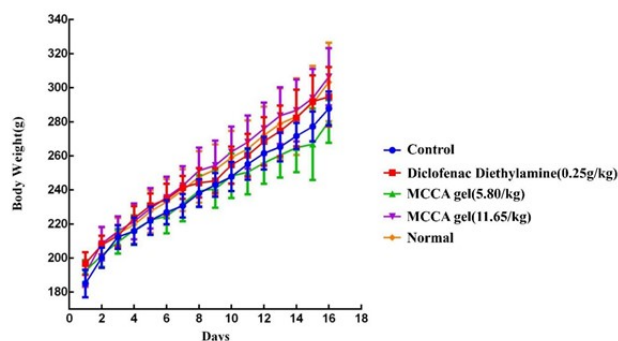


Fig. 10: Weight of rats immunized with complete Freund's adjuvant (n=6)

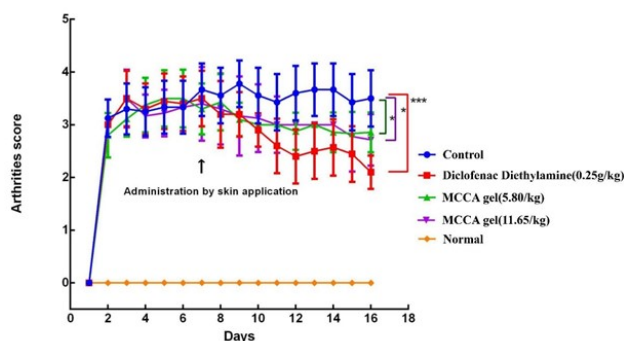


Fig. 11: The score for arthritic rats (n=6, * $P<0.05$, ** $P<0.01$, *** $P<0.001$, Diclofenac Diethylamine, MCCA Gel (5.80 g/kg), MCCA Gel (11.65 g/kg) separately compared with Control.

DISCUSSION

The development of a Chinese herbal gel containing three herbal extracts (*Cy*, *Cp*, and *Av*) with analgesic and anti-inflammatory activities is a promising alternative to

chemical analgesics that have adverse effects on the gastrointestinal and cardiovascular systems (Rengasamy 2021). It is important to note that while the MCCA gel showed promising results in preclinical studies, further research is necessary to determine its safety and efficacy in human subjects. Clinical trials should be conducted to investigate the pharmacokinetics, pharmacodynamics, and toxicity of the MCCA gel in order to evaluate its potential as a therapeutic agent. In addition, it is important to consider the variability of herbal extracts and the potential for interactions with other medications or medical conditions. Therefore, it is necessary to conduct rigorous studies to ensure the safety and efficacy of the MCCA gel before it can be used as a therapeutic agent.

The MCCA gel is a film-forming gel with high transdermal volume, indicating efficient drug delivery through the skin. This may reduce the need for oral medication and therefore minimize adverse effects associated with gastrointestinal and cardiovascular systems (Patil 2023). Although this study showed significant analgesic and anti-inflammatory activities of the MCCA gel, further research is needed to investigate the safety and efficacy of the MCCA gel in clinical trials. Clinical trials are essential to determine the optimal dosage and administration of the MCCA gel and to identify any potential adverse effects. It is also important to investigate the long-term effects of the MCCA gel to ensure its safety and effectiveness as a long-term treatment option. In conclusion, the MCCA gel containing *Cy*, *Cp* and *Av* extracts has potential as a safe and efficient analgesic preparation with minimal side effects. This study paves the way for the development of herbal-based analgesic and anti-inflammatory drugs, and further research is needed to fully explore their potential.

Table 2: Results of pain inhibition rate (n=8)

Group	Inhibition rate (%)
Control	—
Diclofenac Diethylamine (0.25 g/kg)	48.20
MCCA gel (5.80 g/kg)	47.24
MCCA gel (11.65 g/kg)	54.37

CONCLUSION

This study proposes a compound traditional Chinese medicine film-forming gel analgesic preparation - MCCA gel. It has a good ability to penetrate the skin and can quickly form a film. The results of the pain model and the rheumatoid arthritis model show that MCCA gel has a significant inhibitory effect on inflammatory pain. Based on the above results, MCCA gel is a promising new topical analgesic.

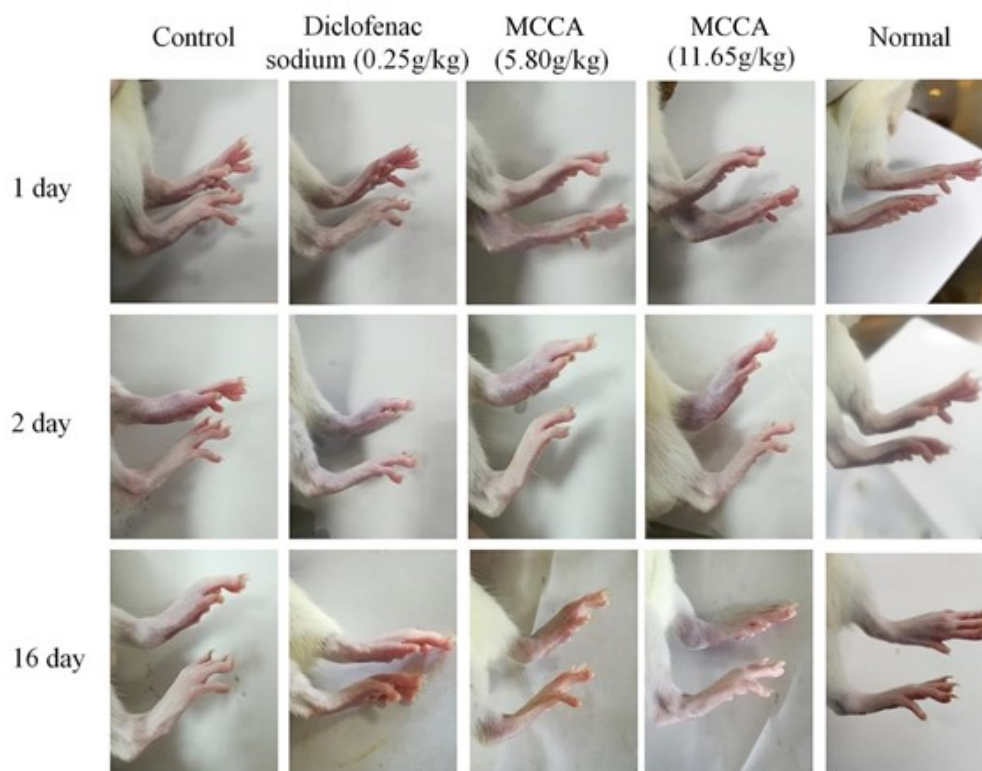


Fig. 12: Appearance Variations of rat hind feet

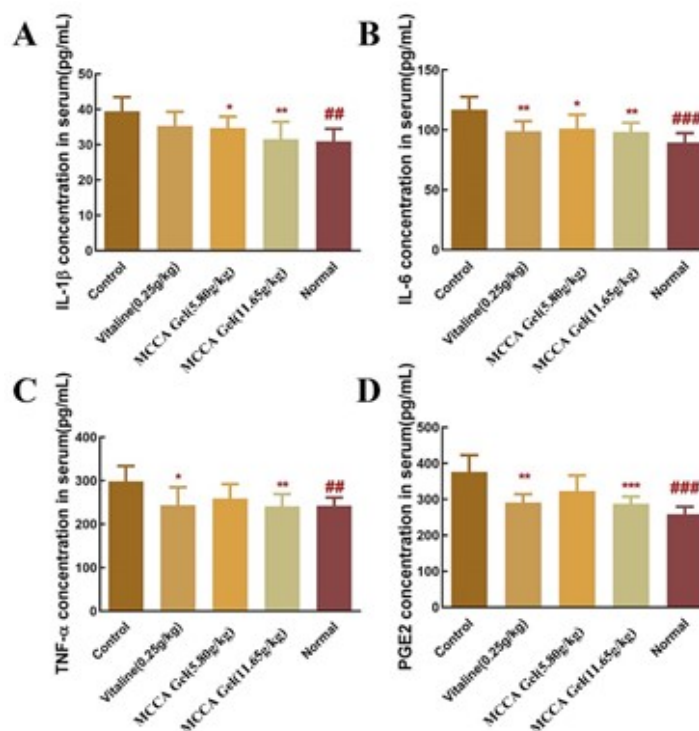


Fig. 13: Contents of IL-1 β (A) IL-6 (B), TNF- α (C) and PGE2 (D) in arthritic rats (n=6, *P<0.05, **P<0.01, ***P<0.001, Diclofenac Diethylamine, MCCA gel (5.80 g/kg), MCCA Gel (11.65g/kg) separately compared with Control

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