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Original Research Article

Evaluation of a root extract gel from *Urtica dioica* (Urticaceae) as analgesic and anti-inflammatory therapy in rheumatoid arthritis in mice

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Abstract

Purpose: *To develop and characterize an herbal gel prepared from methanol root extract of Urtica dioica (Urticaceae) (Stinging nettle) for the treatment of arthritis in mice.*
Methods: *A methanol root extract from Urtica dioica was prepared, and a gel was then prepared using Carbopol 934. The prepared gel was subjected to various physical tests (color, appearance, pH, texture, viscosity) and in vivo evaluation, including primary skin irritation, analgesic, and anti-inflammatory tests, in arthritic mice and compared with 2 % indomethacin gel, which was used as standard.*
Results: *The prepared herbal gel was of light gray color with a smooth texture. It showed a pH of 7.1 and a viscosity of 21.2 cps. The gel exhibited pseudoplastic rheology, as evidenced by shear thinning with increased shear rate. It was non-irritating to the skin in primary skin irritation test in mice and showed 55.05 % inhibition of paw edema in a carrageenan-induced hind rat paw edema model, comparable to that of the standard gel (53.93 %), after 24 h. The gel showed 58.21 % analgesia, versus 61.19 % analgesia for the indomethacin gel standard in writhing test.*
Conclusion: *The topical gel from methanol root extract of U. dioica may be an efficacious and safe alternative to non-steroidal anti-inflammatory drugs in the treatment of rheumatoid arthritis but this requires further investigations to ascertain its safety and clinical efficacy.*

Keywords: *Rheumatoid arthritis, Urtica dioica, Stinging nettle, Anti-inflammatory activity, Analgesic activity, Herbal therapy*

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INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disorder characterized by pain in the joints and inflammation. Management of the moderate-to-severe chronic pain and inflammation is the major component of anti-arthritic therapy [1,2]. The management of pain and inflammation in RA involves two major types of drugs: those that can suppress the expression of tumor necrosis factor-α (and thus interleukin-1β, cyclooxygenase-2, and lipooxygenase) and those that can suppress the activation of NF-κB [3-5]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most widely prescribed drugs for the treatment of RA. However, oral NSAIDs are often needed at high dosing frequencies and show side effects, such as gastric irritation and

moderate plasma level fluctuations, which may lead to the chance of an overdose. Thus, to overcome these limitations and to improve bioavailability, prolong the effects, and minimize side effects, topical or transdermal delivery has been investigated for various NSAIDs [6-8].

Complementary and alternative medicines, in particular botanical medicines or herbal drugs, have long been used for their various therapeutic benefits. A variety of herbal drugs obtained from plants can suppress cell signaling intermediates mediating pain and inflammation in RA, such as various Chinese herbs (e.g., *Aconiti Radix*, *Fagopyrum cymosum*), *Curcuma longa*, and *Withania somnifera* [3,9-14].

Urtica dioica (UD, stinging nettle) is a member of the Urticaceae family. The leaf, flower, seed, and root of the nettle contain a variety of chemical constituents that have been used in various pathophysiological states. UD has been reported to be effective for arthritis, neuralgia, allergic rhinitis, and diabetes. Traditionally, it has been used for gout, hair loss, and mild bleeding [15-18].

Thus, the aim of the present study was to prepare and characterize of a topical gel-based methanol root extract of UD for *in vivo* analgesic and *in vivo* anti-inflammatory activities in adjuvant-induced arthritic rats.

EXPERIMENTAL

Materials

UD roots were collected from the herbal garden of Southern Medical University Affiliated People's Hospital of New District Longhua Shenzhen, Shenzhen, China. The roots were identified and authenticated. All other chemicals were of analytical grade.

Preparation of herbal gel

The roots were dried in the shade and made into a coarse powder. Root powder (1 kg) was extracted in a Soxhlet extraction apparatus using 700 mL petroleum benzene (60 – 80 °C), followed by consecutive extraction with 650 mL chloroform, and 500 mL methanol for 72 h using hot continuous successive extraction method. The solvents were evaporated under reduced pressure in a rotary vacuum evaporator, and the product was dried under vacuum. The dried extract 5 % (w/w) was added to Carbopol 2 % solution with continuous stirring (Table 1).

Table 1: Composition of herbal gel of Urtica

Ingredient	Content
UD	5 g
CP-934	3 %w/v
Sodium benzoate	0.5 %w/w
Distilled water qs to	100 mL

UD = dried methanol root extract of *Urtica dioica,* CP-934 = Carbopol-934

To this gel, sodium benzoate (0.5 % w/w) was added and mixed uniformly. The gel was then cooled to room temperature and stored in a cool dark place until use.

Physical evaluation

Color, appearance, texture, and pH were determined for the prepared gel.

Rheological studies

Rheology is an important parameter for gels. The viscosity of the herbal gel formulation was determined using a Brookfield viscometer DV II+Pro USA.

The change in viscosity with respect to angular velocity was observed.

In vivo study

Healthy male albino mice (150-200 g) were used for the study. The mice were kept in cages under standard environmental light and temperature conditions. The mice were allowed free access to drinking water and a standard diet. After 2 days of acclimatization, mice were used for the study.

The *in vivo* animal study protocols were approved by the Institutional Ethical Committee of Southern Medical University, Guangzhou (Ref no. 2015/AH/XXII). All *in vivo* experiments were performed according to international guidelines (Directive 2010/63/EU) [19].

Primary skin irritation test

For the primary skin irritation test, healthy male mice were divided into three groups of six mice each. A 4 cm^2 area of the dorsal portion of all mice was shaved and wiped with surgical spirit.

At 12 h after shaving, liquid paraffin was applied to group I (control) using a cotton swab. To group II, herbal gel was applied. To group III (standard), 0.8 % (v/v) aqueous solution of formaldehyde (irritant) was applied. The application sites were observed for any erythema or edema on the skin surface at 2 days post-application.

In vivo anti-inflammatory study

The herbal gel was subjected to an in vivo anti-inflammatory study of complete Freund's adjuvant (CFA)-induced arthritis using the standard carrageenan-induced hind rat paw edema model. Three groups of six mice each were used for the study. At 12 h after shaving the dorsal surface of the mice, the study was started. To the control group (I), plain Carbopol gel was applied. The herbal gel was applied on the dorsal sides of the mice (200 mg/kg body weight) for group II. For the standard group, group III, indomethacin gel 2 % (10 mg/kg body weight) was applied. The prepared gels were applied once daily for 2 weeks. Arthritis was induced using CFA (0.1 mL of 0.1 % w/v suspension), which was injected into the left hind paw of the mice 30 min after application of the gels. The hind paw volume was measured immediately (0 h) and at different time intervals up to 24 h using a plethysmometer and was expressed as percent edema relative to the initial hind paw volume. Percent inhibition of edema was determined.

In vivo analgesic activity

Acute analgesia produced by the drugs was assessed by the acetic acid-induced writhing method in mice. Mice were divided into four groups of six mice each. The first group served as a control and received an appropriate volume of Carbopol plain gel only. To the test group II, the prepared herbal gel (0.5 g/kg) was applied on shaved skin of the dorsal surface. Group III (standard) was administered 2 % indomethacin gel. At 3 h after treatment, the mice were injected with 0.6 % (v/v) acetic acid solution (10 mL/kg) intraperitoneally. The total number of writhes, a parameter of chemically-induced pain (i.e., constriction of the abdomen, turning of the trunk, and extension of the hind legs), was counted for 15 min. The analgesic effect was expressed as the percent reduction in writhes in comparison with the control.

Statistical analysis

The results are expressed as mean ± standard deviation (SD). Statistical analysis was carried out using one-way analysis of variance (ANOVA) with Origin 9 software (Origin Labs., USA). $P < 0.05$ was considered to indicate statistical significance.

RESULTS

The herbal gel containing a methanol root extract of UD was formulated for use in patients with RA for prolonged relief. The physical properties were found to be satisfactory (Table 2).

Table 2: Physicochemical characteristics of herbal gel from *Urtica dioica*

Property	Characteristic
Color	Light gray
Texture	Smooth
Appearance	Translucent
pH	7.1
Viscosity	21.2 cps

The light gray-colored and smooth-textured gel showed a pH of 7.1 and a viscosity of 21.2 cps. The herbal gel exhibited pseudoplastic rheology, as evidenced by shear thinning with an increased shear rate. The pseudoplastic rheology was confirmed by a decrease in viscosity with increasing angular velocity (Fig. 1).

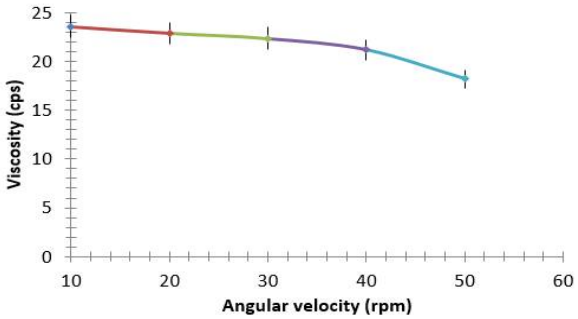

Figure 1: Rheological profile of the herbal gel

The gel was not irritating to the skin. It showed no erythema or edema at 48 h post-application of the gel in primary skin irritation test.

The anti-inflammatory activity of the prepared gel was evaluated using the standard carrageenan-induced hind rat paw edema model in CFA-induced arthritic mice (Table 3).

The results showed that the herbal gel induced a significant percent inhibition compared with the standard gel. The herbal gel showed 55.05 % inhibition of rat paw edema, comparable to that of the standard gel (53.93 %) after 24 h. It was evident that the formulation showed delayed percent inhibition activity due to the lag time taken for the drug to diffuse out of the gel. From the second hour, the herbal gel showed a higher percentage inhibition than that of the indomethacin gel (Fig. 2).

The gel showed excellent analgesic activity (Table 3). The gel showed 58.21 % analgesia, while the standard indomethacin gel showed 61.19 % analgesia. Thus, the analgesic effect was comparable to that of the standard.

Table 3: *In vivo* anti-inflammatory and analgesic activity of the herbal gel

Preparation	Anti-inflammatory activity		Analgesic activity	
	Hind paw volume (ml) [a, b]	***Inhibition of edema (%)***	***Writhes count*** [a]	***Analgesia (%)***
Control	0.89±0.02	-	67±2	-
Indomethacin gel (standard)	0.40±0.01 [c]	55.05	26±1 [c]	61.19
Herbal gel	0.41±0.01 [c]	53.93	28±2 [c]	58.21

[a] Mean ± SEM, $n = 6$; [b] Change in paw volume after carrageenan injection at 24 h; [c] $p < 0.05$, vs. control

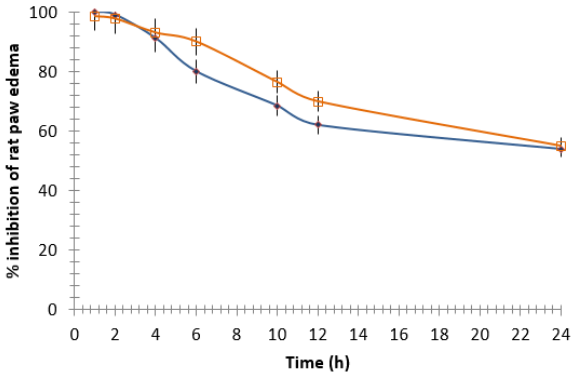

Figure 2. *In vivo* anti-inflammatory activity of herbal gel versus indomethacin gel in CFA-induced arthritis using the standard carrageenan-induced hind rat paw edema model. ***Note:*** indomethacin gel = □; herbal gel = ♦

DISCUSSION

For patients with RA, delivering drugs topically may not only increase patient compliance but also provide immediate and prolonged release of the drug [13]. The conventionally used NSAIDs in RA have also been developed into topical or transdermal forms for various benefits, including reduced adverse effects, reduced drug dosage, improved patient compliance, and prolonged drug release [6,8,20]. Herbal drugs have long been used in traditional medicine for various conditions of pain and inflammation including arthritis. Many previous studies have examined the use of herbal drugs in pain and inflammation management in RA [3,9-14]. In a recent study, a polyherbal gel was prepared using seeds of *Withania somnifera*, rhizomes of *Curcuma longa*, and gel from *Aloe vera* leaves. The prepared gel showed significant analgesic and anti-inflammatory activities, comparable to those of standard 2 % piroxicam gel [21].

Regarding the chemical composition, the stinging hairs of UD (stinging nettle) contain histamine, formic acid, acetylcholine, acetic acid, butyric acid, leukotrienes, 5 hydroxytryptamine, and other irritants [1-3]. The hydrophilic components of the nettle, including lectins and polysaccharides, appear to be important, particularly in prostate disease. Nettle root lignans, such as (-)-3,4-divanillyltetrahydrofuran, have been found to be effective in benign prostatic hyperplasia and other androgen- and estrogen-sensitive conditions. UD agglutinin (UDA), a heat- and acid-resistant lectin found primarily in its root, induces a pattern of T lymphocyte activity not seen with any other known plant lectin [22]. UDA appears to prevent the formation of a systemic lupus erythematosus-like condition in mice and has diverse antiviral effects *in vitro*.

In the present study, a root extract of UD was developed into a gel, and its anti-inflammatory activity was assessed. The present work dealt with the development of an herbal gel using methanol extract from roots of UD with Carbopol 934. The gel showed excellent analgesic and anti-inflammatory activities. Many previous studies have reported the potential anti-inflammatory and anti-arthritic activity of herbal drugs [3,23].

The anti-arthritic activity of UD has been reported to be due to components found in root extracts. In a previous study, a fraction of an aqueous extract containing polysaccharides showed potential and prolonged anti-inflammatory activity in the rat paw edema test. Polysaccharides isolated from this fraction stimulated T lymphocyte proliferation and influenced the complement system. Ethanol root extract has been investigated and showed potent human leukocyte elastase (HLE, an enzyme released by polymorphonuclear granulocytes that mediates the inflammatory process) suppressive activity [17,18,24].

Globally, many patients with RA have tried one or more complementary and alternative medicines (CAM), such as acupuncture, chiropractic, homeopathy, hypnotherapy, herbal drugs, and osteopathy. Of the CAMs, herbal drugs have long been used for various therapeutic benefits including in RA. They may be of potential benefit in patients with arthritis and can provide a safer treatment alternative with improved patient compliance (due to low

dose and frequency, avoidance of gastric irritation, and noninvasive nature).

CONCLUSION

The formulated UD root extract gel displayed analgesic and anti-inflammatory activities in mice comparable to those of the standard indomethacin gel. Thus, the test formulation has the potential to be developed clinically for the treatment of arthritis.

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